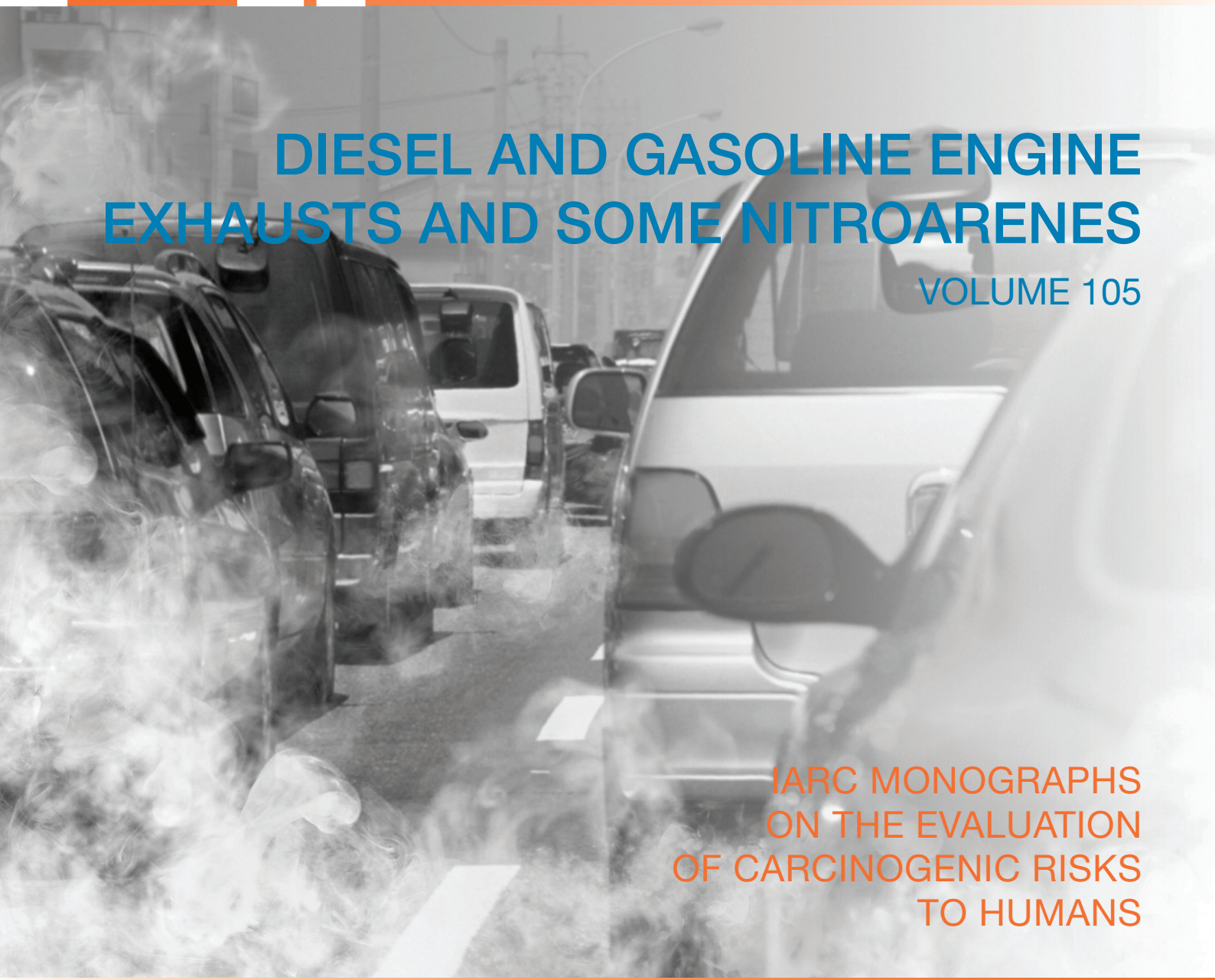
IARC MONOGRAPHS



DIESEL AND GASOLINE ENGINE EXHAUSTS AND SOME NITROARENES

VOLUME 105

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ON THE EVALUATION
OF CARCINOGENIC RISKS
TO HUMANS

International Agency for Research on Cancer

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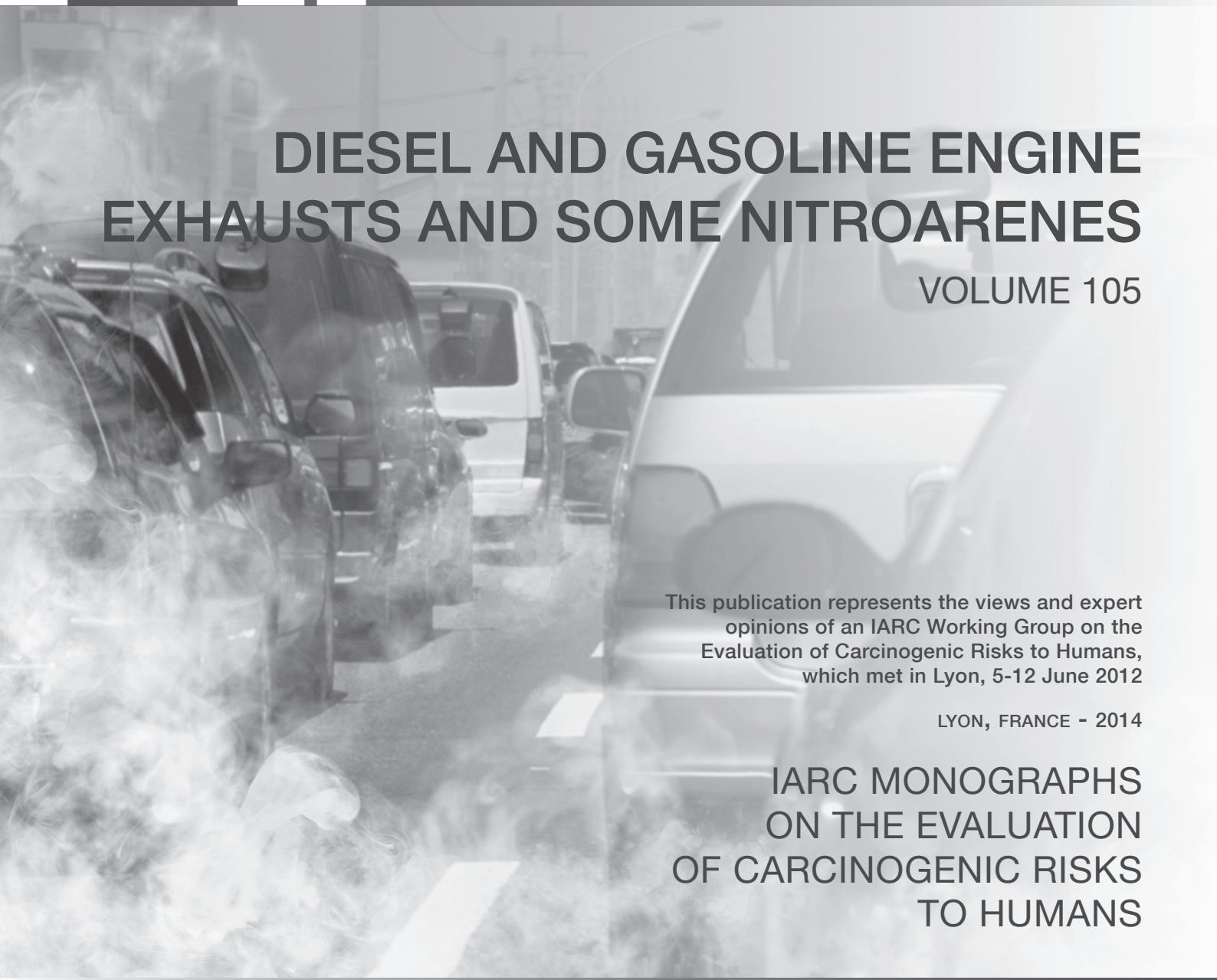

DIESEL AND GASOLINE ENGINE EXHAUSTS AND SOME NITROARENES

VOLUME 105

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, 5-12 June 2012

LYON, FRANCE - 2014

IARC MONOGRAPHS ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS

International Agency for Research on Cancer

World Health Organization

IARC MONOGRAPHS

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

This programme has been supported since 1982 by Cooperative Agreement U01 CA33193 with the United States National Cancer Institute, Department of Health and Human Services. Additional support has been provided since 1986 by the Health, Safety and Hygiene at Work Unit of the European Commission Directorate-General for Employment, Social Affairs and Equal Opportunities, and since 1992 by the United States National Institute of Environmental Health Sciences, Department of Health and Human Services. The contents of this volume are solely the responsibility of the Working Group and do not necessarily represent the official views of the U.S. National Cancer Institute, the U.S. National Institute of Environmental Health Sciences, the U.S. Department of Health and Human Services, or the European Commission Directorate-General for Employment, Social Affairs and Equal Opportunities.

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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. The *Monographs* evaluate cancer hazards, despite the historical presence of the word 'risks' in the title.

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

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

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

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

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

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[3] David B. Kittelson has received significant research funding from Caterpillar on the influence of biofuels on particulate emissions (ended in 2009); and from BP for methods of measuring ash in engine exhausts (current).

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[4] Martie van Tongeren has received significant research funding from Statoil, CONCAWE and CEFIC.
[5] Each Observer agreed to respect the Guidelines for Observers at IARC Monographs meetings. Observers did not serve as Meeting Chair or Subgroup Chair, draft any part of a Monograph, or participate in the evaluations. They also agreed not to contact participants before the meeting, not to lobby them at any time, not to send them written materials, and not to offer them meals or other favours. IARC asked and reminded Working Group Members to report any contact or attempt to influence that they may have encountered, either before or during the meeting.
[6] Observer for the IARC Review Stakeholder Group, USA. The IARC Review Stakeholder Group represents the AAM (Alliance of Automobile Manufacturers), ACEA (European Automobile Manufacturers Association), AECC (Association for Emissions Control by Catalyst), API (American Petroleum Institute), CONCAWE (Conservation of Clean Air Water and Environment, the oil companies European association for environment, health, and safety in refining and distribution), EMA (Truck and Engine Manufacturers of America), IPIECA (International Petroleum Industry Environmental Conservation Association), MECA (Manufacturers of Emission Controls Association), and OICA (International Organization of Motor Vehicle Manufacturers). John Gamble has received significant research funding from CONCAWE.

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[7] Dan Greenbaum is the President of the Health Effects Institute (HEI) which conducts research worldwide on the health effects of air pollution. The Institute's core funding comes in equal part from the United States Environmental Protection Agency and the makers of motor vehicles for sale in the USA.

[8] Observer for the IARC Review Stakeholder Group, USA (see footnote 6). Thomas Hesterberg is a full-time employee of Navistar, Inc., a manufacturer of diesel trucks and engines. He provided expert opinion to California Air Resources Board in 2010 regarding emissions from diesel engines.

[9] Observer for the Association of American Railroads (AAR). Timothy L. Lash served as a consultant to the diesel industry through Cambridge Environmental Inc.

[10] Observer for the IARC Review Stakeholder Group, USA (see footnote 6). Roger McClellan serves as a consultant for the Engine Manufacturers Association, Navistar International, Cummins Engine Co., Shell Exploration and Production Co., Union Pacific, and the American Petroleum Institute.

[11] Observer for the European Research Group on Environment and Health in the Transport Sector EUGT e.V. Peter Morfeld is a member of the Scientific Advisory Group of European Research Group on Environment and Health in the Transport Sector (EUGT); in addition, he has received significant research funding from EUGT.

[12] Dirk Pallapies holds small amounts of stock of Daimler-Benz AG and was employed until 2008 by BASF, a chemical company with business in trap technology, catalysts and additives for diesel and gasoline engines.

[13] Observer for the IARC Review Stakeholder Group, USA (see footnote 6). John C. Wall is Vice President – Chief Technical Officer of Cummins Inc., a manufacturer of diesel engines. He also holds stock and patents of Cummins Inc.

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[14] Dana Loomis consulted in a lawsuit involving exposure to diesel exhaust (ceased in 2011).
[15] Suzanne Moore holds significant stock of BHP Billiton Limited, a global natural resources company with business in oil and gas exploration, production, development and marketing.

PREAMBLE

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

A. GENERAL PRINCIPLES AND PROCEDURES

1. Background

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

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

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

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

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

2. Objective and scope

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

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

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

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

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

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

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

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

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

3. Selection of agents for review

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

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

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

4. Data for the *Monographs*

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

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

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

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

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

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

5. Meeting participants

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

(a) The Working Group

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

(b) Invited Specialists

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

(c) Representatives of national and international health agencies

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

(d) Observers with relevant scientific credentials

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

(e) The IARC Secretariat

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

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

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

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

6. Working procedures

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

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

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

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

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

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

B. SCIENTIFIC REVIEW AND EVALUATION

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

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

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

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

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

1. Exposure data

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

(a) General information on the agent

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

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

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

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

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

(b) Analysis and detection

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

(c) Production and use

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

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

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

(d) Occurrence and exposure

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

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

(e) Regulations and guidelines

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

2. Studies of cancer in humans

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

(a) Types of study considered

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

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

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

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

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

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

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

(b) Quality of studies considered

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

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

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

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

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

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

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

(c) Meta-analyses and pooled analyses

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

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

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

(d) Temporal effects

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

allow only indirect inferences about mechanisms of carcinogenesis.

(e) Use of biomarkers in epidemiological studies

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

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

(f) Criteria for causality

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

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

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

an assessment of the biological plausibility of epidemiological observations.

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

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

3. Studies of cancer in experimental animals

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

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

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

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

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

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

(a) Qualitative aspects

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

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

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

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

(b) Quantitative aspects

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

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

(c) Statistical analyses

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

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

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

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

4. Mechanistic and other relevant data

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

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

(a) Toxicokinetic data

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

(b) Data on mechanisms of carcinogenesis

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

(i) Changes in physiology

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

(ii) Functional changes at the cellular level

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

(iii) Changes at the molecular level

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

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

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

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

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

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

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

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

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

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

(c) Other data relevant to mechanisms

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

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

(d) Susceptibility data

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

(e) Data on other adverse effects

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

5. Summary

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

(a) Exposure data

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

(b) Cancer in humans

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

(c) Cancer in experimental animals

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

(d) Mechanistic and other relevant data

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

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

6. Evaluation and rationale

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

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

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

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

(a) Carcinogenicity in humans

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

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

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

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

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

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

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

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

(b) Carcinogenicity in experimental animals

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

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

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

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

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

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

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

(c) Mechanistic and other relevant data

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

physicochemical parameters and analogous biological agents.

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

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

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

(d) Overall evaluation

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

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

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

Group 1: The agent is carcinogenic to humans.

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

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

Group 2.

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

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

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

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

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

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

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

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

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

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

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

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

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

(e) Rationale

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

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GENERAL REMARKS

This one-hundred-and-fifth Volume of the *IARC Monographs* contains evaluations of the carcinogenic hazard to humans of exposure to diesel and gasoline engine exhausts and 10 nitroarenes that have been identified as components of these exhausts. This Volume is the fifth in a series of *Monographs* that have evaluated exposures related to air pollution. In 2004, an *IARC Monographs* Advisory Group recommended that the IARC develop such a series, in view of the probable widespread human exposure to and potential carcinogenicity of these compounds (Straif *et al.*, 2013). The agents and related exposures evaluated to date following this recommendation include non-heterocyclic polycyclic aromatic hydrocarbons (Volume 92; IARC, 2010a), some particles and fibres (Volume 93; IARC, 2010b), indoor air pollution (Volumes 95, 100E; IARC, 2010c, 2012), and bitumens, bitumen emissions and some heterocyclic polycyclic aromatic hydrocarbons (Volume 103; IARC, 2013).

Diesel and gasoline engine exhausts were evaluated previously in Volume 46 of the *IARC Monographs* (IARC, 1989). At that time, diesel engine exhaust was classified as *probably carcinogenic to humans (Group 2A)* on the basis of *limited evidence* from epidemiological studies in humans and *sufficient evidence* for the carcinogenicity in experimental animals of whole diesel engine exhaust and of extracts of particles from diesel engine exhaust. The evaluation of gasoline engine exhaust in 1989 was based on *inadequate evidence* in humans and *sufficient evidence* in experimental animals for the carcinogenicity of condensates or extracts of gasoline engine emissions, which resulted in a classification of gasoline engine exhaust as *possibly carcinogenic to humans (Group 2B)*. A series of 10 nitroarenes, all of which have been detected in diesel engine exhaust, was also reviewed. All of these nitroarenes, except for one (3-nitrobenzanthrone), were evaluated previously in Volume 46 of the *IARC Monographs* (IARC, 1989), when the Working Group classified six of them (1,3-dinitropyrene, 1,8-dinitropyrene, 6-nitrochrysene, 2-nitrofluorene, 1-nitropyrene and 4-nitropyrene) as *possibly carcinogenic to humans (Group 2B)* and three (3,7-dinitrofluoranthene, 3,9-dinitrofluoranthene and 1,3-dinitropyrene) as *not classifiable as to their carcinogenicity to humans* (Group 3).

A summary of the findings of this Volume appears in *The Lancet Oncology* (Benbrahim-Tallaa *et al.*, 2012).

Diesel and gasoline engine exhausts

Diesel-driven engines are used for on-road traffic (e.g. passenger cars, buses and heavy goods vehicles), off-road transport (e.g. trains and ships), (heavy) equipment in various industrial sectors (e.g. mining and construction) and in electricity generators, particularly in developing countries. Gasoline-driven engines are used for cars and hand-held equipment (e.g. chainsaws). Emissions from

these engines are complex and may vary widely in composition. The gas phase comprises carbon monoxide, nitrogen oxides and volatile organic compounds, such as benzene and formaldehyde. The particle fraction comprises elemental and organic carbon, ash, sulfate and metals. Polycyclic aromatic hydrocarbons and nitroarenes are distributed within the gas and the particle phases. The qualitative and quantitative composition of engine exhausts depends on the fuel, the type and age of the engine, the state of its tuning and maintenance, the use of an emission-control system and the pattern of use. The *Monograph* on diesel and gasoline engine exhausts is not a review of data on these individual compounds in engine exhausts, and covers only studies in which the whole exhaust or a major fraction of this exhausthas been investigated.

In anticipation of the availability of new epidemiological studies, an Advisory Group to the *IARC Monographs* Programme recommended in 1998 that diesel engine exhaust be treated as a high priority for re-evaluation because a large study by the US National Cancer Institute/National Institute for Occupational Safety and Health of occupational exposure to such emissions in underground miners was being performed at that time. The publication of the results of this study was delayed several times (Furlow, 2012), and the re-evaluation therefore was only undertaken after their publication in March 2012, together with updates of studies in the railroad and transport industry that included refined exposure assessments.

In evaluating the carcinogenic hazard of exposure to gasoline engine exhaust, the Working Group made attempts to disentangle their effects from those of diesel engine exhaust. Apart from those occupational environments in which diesel engine exhaust is the only or primary source of exposure (see above), many occupations involve exposures to a mixture of the two. Therefore, it is possible that a risk observed in association with exposure to gasoline engine exhaust is confounded by concomitant exposure to diesel engine emissions.

Every attempt was made to identify the exhaust tested in the cancer bioassays considered by the Working Group, taking into account the differences in the characteristics of exhausts from different types of motor (diesel/gasoline, light duty/heavy duty). When interpreting the results of studies of complex mixtures, it is important to characterize the exposures of the animals to the greatest extent possible. Therefore, with respect to experimental studies, several early bioassays in which the type of engine exhaust was not specified were not considered by the Working Group.

Diesel engine technology has changed over time in response to and in support of increasingly stringent regulations to control engine emissions. The stringency of these regulations has been increased most progressively for on-road light-duty vehicles, followed by heavy-duty on-road and off-road vehicles, and are at present largely absent for other heavy-duty engine applications, such as in trains and ships. Moreover, in many developing countries, emission standards are not enforced for either on-road or off-road use of diesel- or gasoline-driven engines. To meet the most stringent current emission-control regulations, diesel engines must be designed and constructed according to modern technology, which includes wall-flow particulate filters and diesel oxidation catalysts, in combination with the use of diesel fuel that has a very low sulfur content; the most rigorous technology measures will be required only after 2014. The new diesel engine technology has been shown to reduce particulate mass emissions by more than two orders of magnitude. Although the implications for carcinogenicity are not yet known, the 'new technology' diesel engines, due to their much lower emissions of particulate matter, will probably bring about an improvement with regard to public health. It should be noted that the human epidemiological studies reviewed in this *Monograph* were conducted before the introduction of the modern diesel engine technology.

Nitroarenes, components of diesel engine emissions

Biomonitoring studies conducted in workers and the general population showed that both are exposed to these substances (Scheepers *et al.*, 1994; Seidel *et al.*, 2002; Zwirner-Baier & Neumann, 1999). All of the nitro- and dinitroarenes (nitroarenes that carry one or two nitro groups) evaluated in this Volume of *Monographs* were genotoxic to different extents in a series of genetic toxicology assays. In general, the dinitroarenes exhibited greater genotoxic activity than the mononitroarenes, which is consistent with their tumorigenic activities. Although only a few studies were available, the metabolites of several nitroarenes have been found in urine samples collected from populations exposed to diesel engine exhaust. Two major metabolic pathways were identified for this group of compounds. First, the reduction of the nitro group to produce the hydroxylamine, which––either by itself or after conjugation to a sulfate by sulfotransferases or conjugation to an acetate by *N*-acetyltransferases––yields the ultimate carcinogenic aryl nitrenium ion that binds covalently to DNA to form adducts. In the case of dinitroarenes, only one nitro group is metabolically activated in this way. Second, ring oxidation to yield oxides, phenols and dihydrodiols, many of which are found as detoxification products conjugated to sulfate or glucuronic acid. Some nitroarenes formed products via both ring-oxidation and nitroreduction. Strong evidence of genotoxicity led to three nitroarenes being upgraded to *Group 2B* or *Group 2A*.

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DIESEL AND GASOLINE ENGINE EXHAUSTS

1. EXPOSURE DATA

Diesel and gasoline engine exhausts were evaluated by a previous IARC Working Group in 1988 (IARC, 1989a). New data have since become available, and these have been taken into consideration in the present evaluation.

1.1 Diesel and gasoline engines and the chemical composition of their exhausts

Diesel and gasoline engines are the major power sources used in motor vehicles. Both are internal, intermittent combustion engines but differ fundamentally in terms of the way in which their mixtures are prepared and ignition (for reviews, see Heywood, 1989; Stone, 1999; Majewski & Khair, 2006).

In diesel engines, air is introduced into the engine and heated by compression to temperatures in excess of 700 °K. The fuel is introduced into the combustion chamber by a high-pressure injection system and is mixed with the hot air until the fuel jet becomes sufficiently hot for auto-ignition to occur. The centre of this burning jet is very rich in fuel, which leads to the formation of elemental carbon (EC), partially burned fuel, polycyclic aromatic hydrocarbons (PAHs) and carbon monoxide (Flynn *et al.*, 1999). At the outer edges of the burning jet, excess air leads to high temperatures and the formation of nitrogen oxides.

In contrast, in port-fuel injection gasoline engines, fuel and air are mixed before entering the cylinder and the mixture of fuel and air is compressed. To prevent auto-ignition (knock), the compression ratio is much lower than that in a diesel engine, leading to slightly reduced efficiency. In a gasoline engine, the mixture is ignited with a spark, and a flame propagates across the combustion chamber. Premixing the fuel and air minimizes local fuel-rich conditions and normal gasoline combustion produces little soot. In contrast, high temperatures in the flame zone lead to the formation of carbon monoxide and nitrogen oxides, and flame quenching near the walls leads to the presence of unburned and partially oxidized hydrocarbons.

The fuels used in diesel and gasoline engines also differ. Diesel fuel is made up of petroleum fractions with a higher boiling range, has a higher density and contains approximately 13% more energy per unit volume of gasoline.

Hydrocarbon combustion by-products include nitrogen oxides, carbon monoxide, unburned and partially burned hydrocarbons, soot (mainly EC and particle-bound organic carbon) and some nitrated species. Engine exhaust also contains partially burned lubricating oil, and ash from metallic additives in the lubricating oil and wear metals. These combustion by-products represent thousands of chemical components present in the gas and particulate phases (Zaebst *et al.*, 1988); some specific chemical species and classes found in engine exhaust are listed in Table 1.1. Agents found in engine exhaust and evaluated by the IARC as group 2B, 2A or 1 are

Table 1.1 Some compounds and classes of compound in vehicle engine exhaust

Gas phase	Particulate phase
Acrolein	Heterocyclics and derivatives[a]
Ammonia	Hydrocarbons (C_{14}–C_{35}) and derivatives[a]
Benzene	Inorganic sulfates and nitrates
1,3-Butadiene	Metals (e.g. lead and platinum)
Formaldehyde	Polycyclic aromatic hydrocarbons and derivatives[a]
Formic acid	
Heterocyclics and derivatives[a]	
Hydrocarbons (C_1–C_{18}) and derivatives[a]	
Hydrogen cyanide	
Hydrogen sulfide	
Methane	
Methanol	
Nitric acid	
Nitrous acid	
Oxides of nitrogen	
Polycyclic aromatic hydrocarbons and derivatives[a]	
Sulfur dioxide	
Toluene	

[a] Derivatives include acids, alcohols, aldehydes, anhydrides, esters, ketones, nitriles, quinones, sulfonates, halogenated and nitrated compounds, and multifunctional derivatives.
From National Research Council (1983), Lies *et al.* (1986), Schuetzle & Frazier (1986), Carey (1987), Johnson (1988), Zaebst *et al.* (1988)

listed in Table 1.2. Diesel emission standards and diesel engine technology are closely linked: standards drive the technology and technology enables more stringent standards. The concentration of a chemical species in vehicle exhaust is a function of several factors, including the type and operating conditions of the engine, the compositions of the fuel and lubricating oil used and the presence of an emission control system (Johnson, 1988).

1.1.1 Diesel engine technology

(a) Historical and technical overview

Rudolf Diesel patented the Diesel engine in 1898. In the early part of the twentieth century, diesel engines were used mainly in marine applications, and were then installed in heavy goods vehicles (HGVs) in Europe in the 1920s. In the 1930s, manufacturers in the USA started to install diesel engines in commercial HGVs, buses and tracked vehicles. The first mass-produced diesel passenger car was introduced in Europe in 1936. Diesel engines had replaced steam power in railroad locomotives by the early 1950s, and had replaced gasoline engines in most HGVs by the 1960s. Today, diesel engines power all types of automotive vehicles: passenger cars (up to 50% of new car sales in some European countries), commercial vehicles, buses, industrial, agricultural and construction equipment, mine vehicles, locomotives, ships and many stationary power applications (Busch-Sulzer, 1913; Cummins, 1967; Hind, 1974; Cummins, 1993; IRSG, 2012a, b).

In a diesel engine, fuel is introduced into the engine by a high-pressure fuel injection system and mixes with air that has been heated by compression. Combustion begins when the fuel–air mixture becomes sufficiently hot for auto-ignition to occur. Common diesel engine configurations include indirect and direct

Table 1.2 Chemicals and metals found in diesel and gasoline engine exhaust and their evaluation by IARC

Agent	CAS No.	Evaluation	Volume (reference)
Metals			
Antimony compounds	1309-64-4 (Trioxide)	2B	47 (IARC, 1989b)
Arsenic and inorganic arsenic compounds	007440-38-2	1	100C (IARC, 2012a)
Beryllium and beryllium compounds	007440-41-7	1	100C (IARC, 2012a)
Cadmium and cadmium compounds	007440-43-9	1	100C (IARC, 2012a)
Chromium (VI)	018540-29-9	1	100C (IARC, 2012a)
Cobalt and cobalt compounds	007440-48-4	2B	52 (IARC, 1991)
Lead compounds	Inorganic/organic	2A/3	87 (IARC, 2006)
Nickel	Metallic/compounds	2B/1	100C (IARC, 2012a)
Organic chemicals			
1,3-Butadiene	106-99-0	1	100F (IARC, 2012b)
Acetaldehyde	75-07-0	2B	71 (IARC, 1999)
Benzene	71-43-2	1	100F (IARC, 2012b)
Bis(ethylhexyl)phthalate	117-81-7	2B	101 (IARC, 2012c)
Ethylbenzene	100-41-4	2B	77 (IARC, 2000)
Formaldehyde	50-00-0	1	100F (IARC, 2012b)
Propylene oxide	75-56-9	2B	60 (IARC, 1994)
Halogenated and other chemicals			
Dioxin/dibenzofurans	1746-01-6 (TCDD)	1	100F (IARC, 2012b)
Polycyclic aromatic hydrocarbons			
Benz[*a*]anthracene	56-55-3	2B	92 (IARC, 2010)
Benzo[*b*]fluoranthene	205-99-2	2B	92 (IARC, 2010)
Benzo[*k*]fluoranthene	207-08-9	2B	92 (IARC, 2010)
Benzo[*a*]pyrene	5-32-8	1	100F (IARC, 2012b)
Chrysene	218-01-9	2B	92 (IARC, 2010)
Dibenz[*a,h*]anthracene	53-70-3	2A	92 (IARC, 2010)
3,7-Dinitrofluoranthene	105735-71-5	2B	This volume
3,9-Dinitrofluoranthene	22506-53-2	2B	This volume
1,3-Dinitropyrene	75321-20-9	2B	This volume
1,6-Dinitropyrene	42397-64-8	2B	This volume
1,8-Dinitropyrene	42397-64-9	2B	This volume
Indeno[1,2,3-*cd*]pyrene	193-39-5	2B	92 (IARC, 2010)
Naphthalene	91-20-3	2B	82 (IARC, 2002)
3-Nitrobenzanthrone	17 117-34-9	2B	This volume
6-Nitrochrysene	7496-02-8	2A	This volume
2-Nitrofluorene	607-57-8	2B	This volume
1-Nitropyrene	5522-43-0	2A	This volume
4-Nitropyrene	57835-92-4	2B	This volume
Styrene	100-42-5	2B	82 (IARC, 2002)

TCDD, 2,3,7,8-tetrachlorodibenzodioxin

injection, and two- and four-stroke cycles (Heywood, 1989; Stone, 1999).

Indirect injection engines cost less and are less efficient than direct injection engines. Fuel is injected into a secondary chamber, where ignition takes place, and a jet of the partially burned fuel–air mixture is discharged into the main combustion chamber, where it is mixed with additional air and combustion is completed. This allows relatively fast and complete combustion without the need for a very high-pressure fuel injection system because most of the energy required for mixing is produced by the hot burning jet. However, significant energy loss occurs due to heat transfer and loss of pressure, which lead to higher fuel consumption. Indirect combustion engines dominated the diesel passenger car market until the mid-1990s and are still used in small engines, such as generator sets and auxiliary power units. Direct injection engines require higher-pressure fuel injection and more precise control of the fuel–air mixing process, but are considerably more fuel efficient. Nearly all modern heavy-duty vehicles are fitted with direct injection engines.

Two- and four-stroke cycles refer to the number of piston strokes required to complete an engine cycle. Two-stroke diesel engines are mechanically simpler but are more complex thermodynamically and aerodynamically than four-stroke engines. In a two-stroke engine, the four phases of an engine cycle (intake, compression, expansion and exhaust) require only one revolution, while two revolutions are required in a four-stroke engine. In a two-stroke engine, intake and compression take place in one stroke, and expansion, exhaust and the beginning of intake take place in the second stroke. Two-stroke diesel engines are generally more compact and have a better power-to-weight ratio than their four-stroke counterparts but are typically less efficient and their emissions are more difficult to control. Two-stroke engines came into general use in the 1930s, first in locomotives, then in military applications, generator sets, HGVs and buses (Sloan, 1964), and were widely used in HGVs and buses until the early 1990s, when it became apparent that increasingly stringent emission standards would be more difficult to meet than with four-stroke engines. However, they are still used to a great extent in large engines in rail, marine and stationary applications. Since the mid-1980s, stringent emission standards and highly competitive performance requirements have caused the design of on-road engines in developed countries to converge on a 'common diesel engine architecture' (IRSG, 2012a, b).

In this *Monograph*, diesel engines that are unregulated for particulate emissions are referred to as 'traditional technology diesel engines'; those that are fitted with wall-flow particulate filters and oxidation catalysts, and use ultra-low sulfur fuel are referred to as 'new technology diesel engines'; and those that fall in between the two are referred to as 'transitional diesel engines'. The following section focuses primarily on emission technology for heavy-duty and light-duty diesel engines in Europe and the USA.

(b) Traditional and transitional technology engines

Until the mid-1980s, a wide variety of diesel engine designs and technologies were available, including two-stroke and four-stroke combustion systems, two-valve and four-valve gas exchange systems (or side ports in the case of two-stroke engines), direct and indirect fuel injection systems, and turbocharged and naturally aspirated air induction systems. With increasingly stringent regulations on emissions (see Section 1.3), the industry converged on a common diesel engine architecture: four-stroke combustion, four-valve gas exchange, high-pressure direct fuel injection with electronic control and turbocharged air induction. Other technological changes have ensued: intake air cooling was introduced – first using engine cooling water, then air-to-air heat exchangers – to

produce lower peak combustion temperatures to reduce emissions of nitrogen oxides. In 2002, cooled exhaust gas recirculation was introduced an additional control for nitrogen oxides.

Fuel technology also changed when the sulfur content of fuel was reduced from up to 5000 ppm (0.5%) to 500 ppm to enable diesel engines to meet the particulate matter (PM) standards and to introduce cooled exhaust gas recirculation without unacceptable corrosion from sulfuric acid.

Diesel oxidation catalysts were introduced first (Volkswagen, 1989), then diesel exhaust particle filters (DPFs). The catalysts came into relatively wide use in light- and medium-duty applications in the 1990s, and are effective at reducing emissions of carbon monoxide, hydrocarbons and particle-bound organic carbon but have little influence on those of EC or nitrogen oxides.

(c) New technology engines: aftertreatment of diesel exhaust

The new PM standards for on-road heavy diesel equipment that were introduced in 2010 in the USA could not be achieved by new developments in combustion alone, and required exhaust aftertreatment from the installation of DPFs and diesel oxidation catalysts, and a reduction in the sulfur content of fuel to a maximum of 15 ppm.

The nitrogen oxide standard also introduced in 2010 led to the further integration of aftertreatment techniques in the form of selective catalytic reduction (SCR) or nitrogen oxide adsorber-based systems.

(i) Particle filtration

DPFs were first introduced into European passenger cars in 2000 (Salvat *et al.*, 2000) and in heavy-duty trucks and buses in the USA in 2007.

A variety of types of filter medium are available, including ceramic foams, sintered metal, and wound, knit and braided fibres (Majewski & Khair, 2006). Most of these filters have a qualitatively similar efficiency and differ mainly in durability, cost and packaging. The wall-flow filter is the most common for transportation applications, and comprises a honeycomb-like ceramic structure, the alternate passages of which are blocked. Wall-flow filters typically achieve removal efficiencies for diesel PM of more than 95%. In some applications, so-called partial flow filters are used (Mayer *et al.*, 2009), which have considerably lower collection efficiencies – typically less than 50% – and are designed for applications that have less stringent emission standards or for which emissions are already very low.

As the exhaust passes through the filter, the initial substrate is the filtration medium. However, as soot gradually fills the filter channels, the surface of the filter becomes covered with a layer of soot, which in turn serves as a very efficient filtration medium. The temperatures of diesel exhaust are typically too low for any significant oxidation by the oxygen contained therein. Soot must be removed from the filter by its periodical or continuous burning in a process called regeneration, which can be achieved in three ways: one active and two passive methods.

Passive regeneration is achieved by placing an oxidizing catalyst upstream from the filter or by adding a metallic catalyst (usually some combination of cerium, strontium and iron) to the fuel. Active regeneration systems are used under light load conditions, such as those encountered in congested urban traffic or during prolonged idling (Majewski & Khair, 2006; Twigg & Phillips, 2009). This usually involves spraying fuel onto an oxidizing catalyst upstream from the filter to raise the exhaust temperature to above ~600 °C to initiate oxidation of the collected soot. Active regeneration is initiated typically every few hours.

The efficiency of a freshly regenerated filter at the most penetrating (least efficient) size (100–300 nm) is approximately 90%. As the filter loads and a soot layer builds up on its surface, the efficiency across the size range approaches 100%.

DPFs remove most solid particles by filtration, as well as carbon monoxide and light and semi-volatile hydrocarbons by catalytic oxidation, and by the conversion of nitrogen monoxide to nitrogen dioxide and sulfur dioxide to sulfur trioxide and sulfuric acid. Nucleation mode particles (Kittelson, 1998; Kittelson *et al.*, 2006) formed by engines equipped with a catalysed DPF consist mainly of sulfuric acid or ammonium sulfate particles (Grose *et al.*, 2006).

(ii) Aftertreatment for nitrogen oxides

Diesel engines operate under oxidizing conditions, and the reduction of nitrogen oxides to elemental nitrogen is challenging. The two main types of control system for nitrogen oxides in diesel exhaust are SCR (Gekas *et al.*, 2002) and lean nitrogen oxide traps (Morita *et al.*, 2007) (for a review, see Majewski & Khair, 2006).

SCR is designed to reduce the emissions of nitrogen oxides by their reaction with a reductant over a catalyst to form elemental nitrogen. SCR systems may be used alone or integrated with a catalysed DPF (Cooper *et al.*, 2003; Servati *et al.*, 2005) to form a four-way catalyst (carbon monoxide, hydrocarbon, nitrogen oxides and PM). Ammonia may not be fully consumed in the SCR system, and an ammonia slip catalyst is therefore usually used to reduce ammonia emissions. However, these catalysts may produce emissions of other chemicals (Havenith & Verbeek, 1997).

A lean nitrogen oxide trap involves the storage of nitrogen oxides during lean operations and catalytic reduction and release of nitrogen during rich operations (Yezerets *et al.*, 2007). During lean operations, nitrogen monoxide is oxidized to form nitrogen dioxide by a platinum catalyst and is stored as nitrate on the surface. To reduce nitrogen oxides to nitrogen, all oxygen in the exhaust passing through the catalyst must be eliminated. This is accomplished by temporarily injecting fuel into the exhaust to consume the remaining excess oxygen or by operating the engine briefly in a fuel-rich mode. Similarly to SCR systems, these can be used alone or combined with a particle filter (Xu *et al.*, 2010).

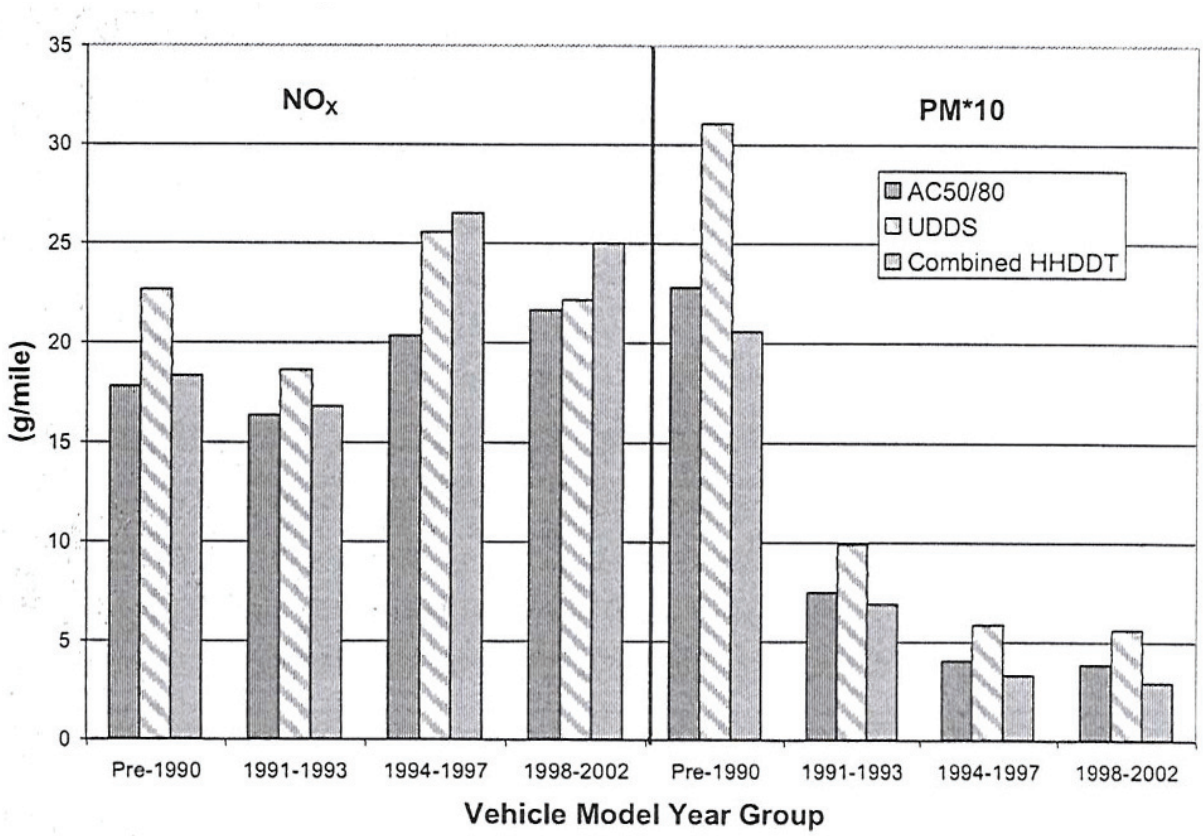
1.1.2 Levels of diesel engine exhaust emissions

(a) Traditional and transitional technology diesel engines

Clark *et al.* (2006) tested a fleet of heavy-duty diesel vehicles using three different driving cycles. Fig 1.1 shows the decreasing trend in PM emissions for vehicle models ranging from pre-1990 through 2002. A similar trend was also apparent from on-road tunnel data (Fig. 1.2; Gertler *et al.*, 2002). Measurements of PM emissions from heavy-duty vehicles in the Tuscarora tunnel were reduced by one order of magnitude between pre-1985 and 1999.

Operating conditions may influence PM emissions from traditional and transitional technology diesel engines. [Particulate mass emissions from new technology diesel engines are very low across the operating range because they are fitted with wall-flow particulate filters.] Clark *et al.* (2002) compared the relative effects of vehicle class and weight, simulated driving cycle, vehicle vocation [application] and driving activity, fuel, aftertreatment [catalytic converter], age [level of technology] and terrain on PM emissions from heavy-duty diesel vehicles (Fig. 1.3). PM emissions varied by 1500% due to differences in the driving cycle alone. Clark *et al.* (2006) tested a fleet of 25 heavy heavy-duty diesel vehicles using a chassis dynamometer and full flow dilution tunnel. Table 1.3 summarizes the influence of driving cycle on PM and hydrocarbon emissions from laden vehicle models from pre-1990 and from 1998 through 2002. The 'creep' cycle gave the highest PM and hydrocarbon emissions, while the cruise cycle gave the lowest PM emissions and the transient cycle gave the lowest hydrocarbon emissions. [The idle emissions are not directly comparable with the other cycles

Fig. 1.1 Trends in emissions of nitrogen oxides and particulate matter by model year group, measured on a chassis dynamometer with three different drive cycles



AC50/80, standard driving cycle; HHDDT, heavy heavy-duty diesel truck; NO_x, nitrogen oxides; PM, particulate matter; UDDS, urban dynamometer driving schedule
From Clark *et al.* (2006). Copyright © 2006 SAE International. Reprinted with permission.

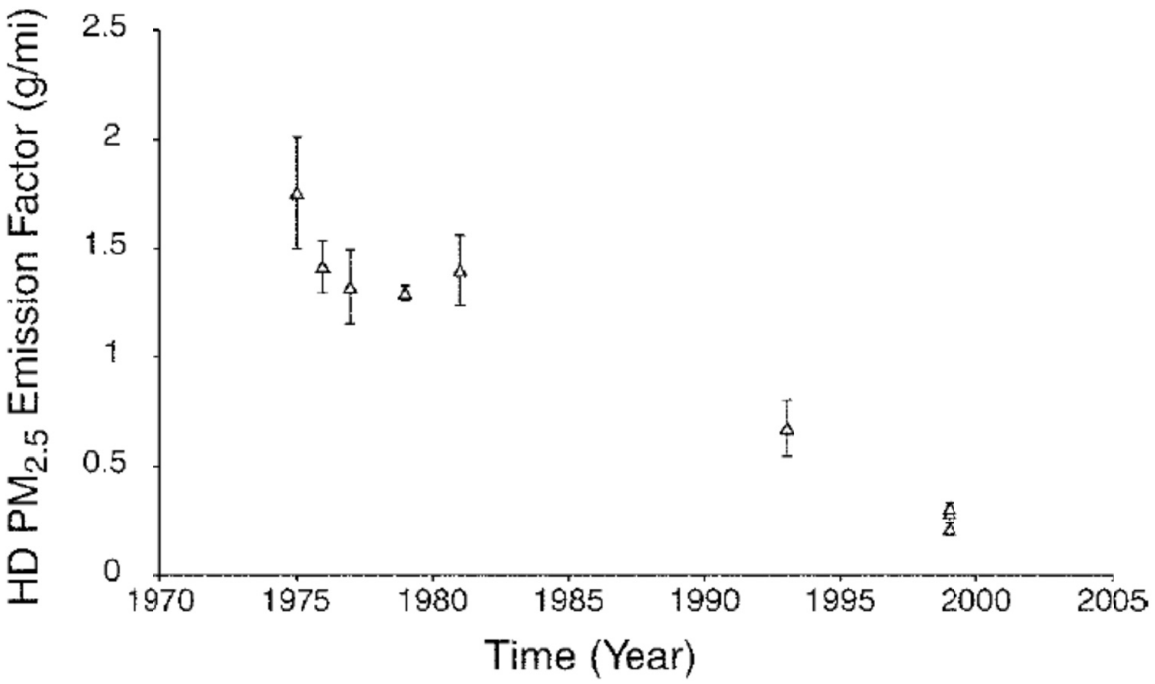
because they are measured in grams per second rather than grams per mile.]

Idle emissions are of particular interest in some exposure situations, e.g. for garage mechanics, and are influenced by engine technology and accessory load, especially air conditioning. Engines with electronic fuel injection systems produce substantially lower carbon monoxide, hydrocarbon and PM emissions but somewhat higher emissions of nitrogen oxides (Khan *et al.*, 2006, 2009).

Kweon *et al.* (2003) examined the impact of engine speed and load on particle-bound organic emissions. A Cummins N14-series single-cylinder research engine was run under the California Air Resources Board eight-mode test cycle. Under high load conditions, most of the particle-phase organic compounds were below the limit of detection in gas chromatography-mass spectrometry (GC-MS); in contrast, most of the 39 organic compounds quantified were detected under idling, light and medium load conditions, which are associated with lower exhaust temperatures. Fig 1.4 shows PAH emissions in relation to exhaust temperature for two speeds.

Kittelson *et al.* (2006) measured size distributions of PM for a variety of heavy-duty engines, both on-road and using engine and chassis dynamometers. Measurements were

Fig. 1.2 Heavy-duty vehicle particulate matter emission factor estimates measured on-road in the Tuscarora tunnel, USA



Note: the markers for 1999 include PM_{10}, $PM_{2.5}$, and $PM_{2.5}$ (reconstructed mass).
HD, heavy duty; PM, particulate matter
From Gertler *et al.* (2002). Reprinted with permission from the Health Effects Institute, Boston, MA.

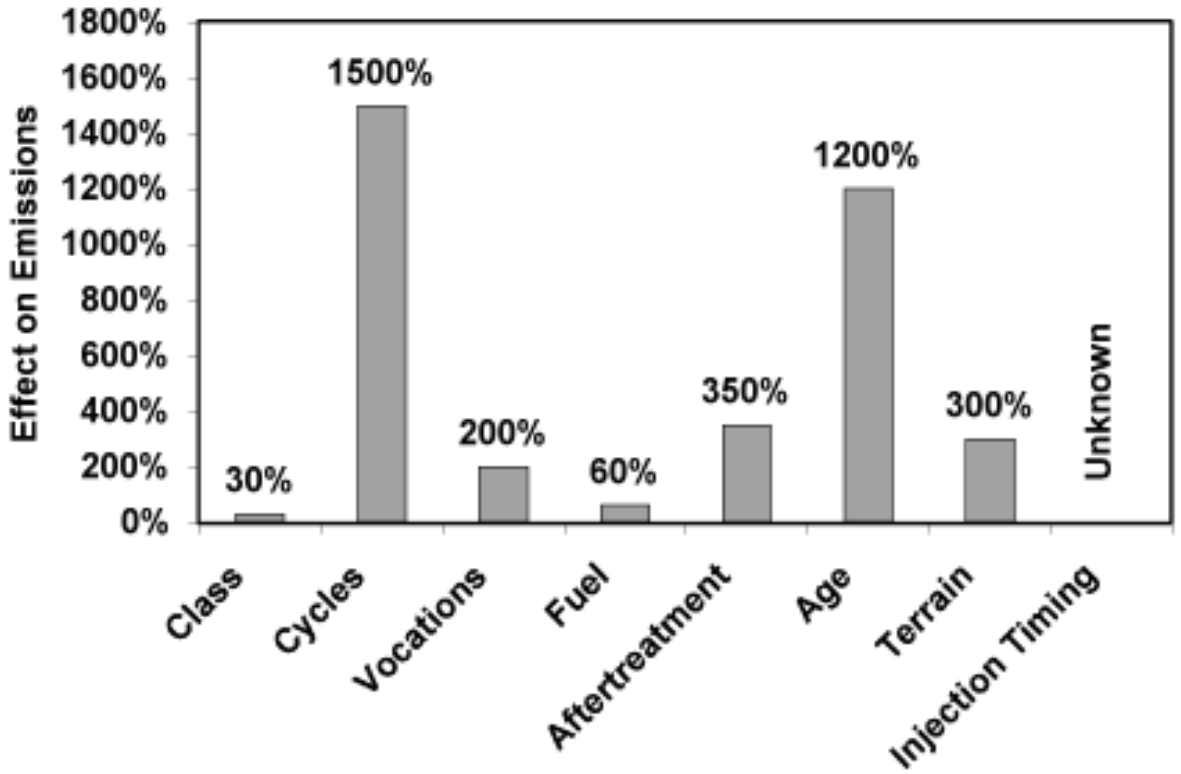
made using fuels with varying sulfur contents, and with and without a thermal denuder that was used to measure solid particles. Without the thermal denuder, the size distributions were nearly unimodal, whereas the thermal denuder revealed a bimodal structure. Except for the fuel with the highest sulfur content (325 ppm), the size distribution for the high-speed cruise condition showed a single mode – the accumulation mode centred at about 50 nm, which consisted mainly of carbonaceous soot particles. The PM concentration in this mode was nearly two orders of magnitude higher than that under idling conditions. Using fuel with the highest sulfur content, a nucleation mode was found, centred at about 10 nm. Although the formation of this mode was related to the sulfur content of the fuel, other work has shown that it consists mainly of heavy hydrocarbons, primarily from unburned lubricating oil (Sakurai *et al.*, 2003).

Table 1.3 Influence of operating cycle on particulate matter and hydrocarbon emissions by car model year

Driving cycle	Hydrocarbons		Particulate matter	
	Pre-1990	1998–2002	Pre-1990	1998–2002
Idle (g/s)	20.2	7.6	3.7	0.8
Creep (g/mile)	16.3	5.8	7.3	1.5
Transient (g/mile)	3.8	1.3	4.2	0.6
Cruise (g/mile)	1.2	0.4	2	0.2
Urban dynamometer driving schedule (g/mile)	3.2	0.8	3.1	0.6

Adapted from Clark *et al.* (2006)

Fig. 1.3 Relative impact of operating variables on particulate matter emissions



Parameters measured are: class (vehicle class and weight), cycle (simulated driving cycle), vocations (application and driving activity), fuel (type of diesel fuel), aftertreatment (catalytic converter), age (level of technology), terrain (driving terrain) and injection timing.
From Clark *et al.* (2002). Reprinted by permission of the publisher, Taylor & Francis Ltd, http://www.tandf.co.uk/journals/

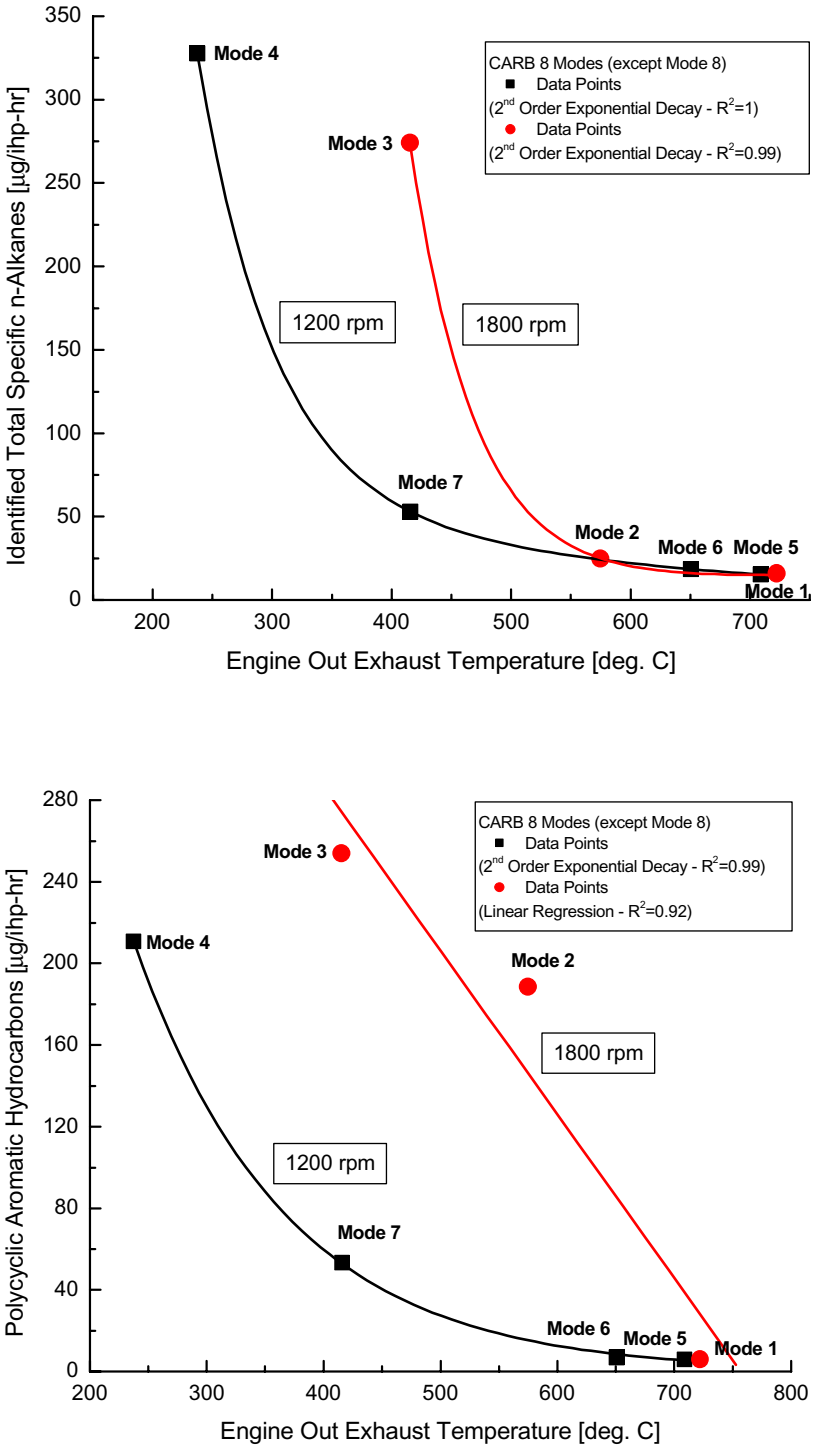
Fig. 1.5 shows a comparison of the average particle size distribution for 2007 model engines with and without regeneration, and for a 2004 model engine without a diesel oxidation catalyst or a DPF. Measurements for the 2007 engines were taken from the exposure chamber for 4-hour segments of the 16-hour cycle; the data were based on 19 repeats with regeneration and 29 repeats without regeneration. Measurements for the 2004 engine were taken from the full-flow constant volume sampling of the Federal Test Procedure (FTP) transient cycle, and were based on six repeats.

(b) New technology diesel engines

Several recent studies have reported on the changes in the composition of diesel exhaust linked to new technology. Emission trends in fluoranthene, pyrene, benzo[*a*]pyrene, benzo[*e*]pyrene and 1-nitropyrene with changing engine technology are shown in Fig. 1.6 as a fraction of the emissions from pre-1999 technology. All compounds showed a marked downward trend. Emissions from the 2000 and 2004 transitional technology engines represented only a fraction (maximum, 40%) of those from the traditional technology engines, and a further decrease occurred with the introduction of new technology engines fitted with catalysed DPFs.

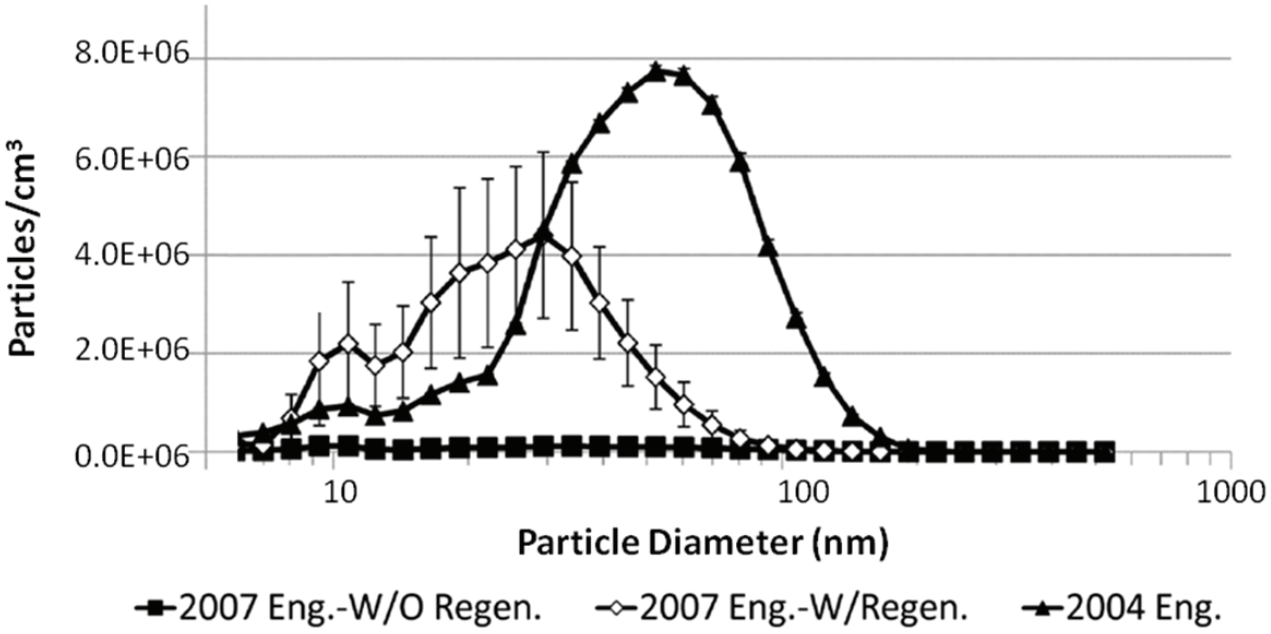
Hesterberg *et al.* (2011) published a comprehensive comparison of emissions from current

Fig. 1.4 Variations in total specific *n*-alkanes (top) and polycyclic aromatic hydrocarbons (bottom) versus engine-out exhaust temperature for CARB 8-mode test cycle



From Kweon *et al.* (2003). Copyright © 2003 SAE International. Reprinted with permission.

Fig. 1.5 Particle size distribution of a 2004 engine and 2007 engines with or without active regeneration



Geometric number mean diameter (GNMD) and geometric standard deviation (GSD):
2007 engine with regeneration: GNMD = 25 nm; GSD = 1.72
2007 engine without regeneration: GNMD = 40 nm; GSD = 1.95
2004 engine: GNMD = 46 nm; GSD = 1.90
From Khalek *et al.* (2011). Reprinted by permission of the publisher, Taylor & Francis Ltd, http://www.tandf.co.uk/journals/

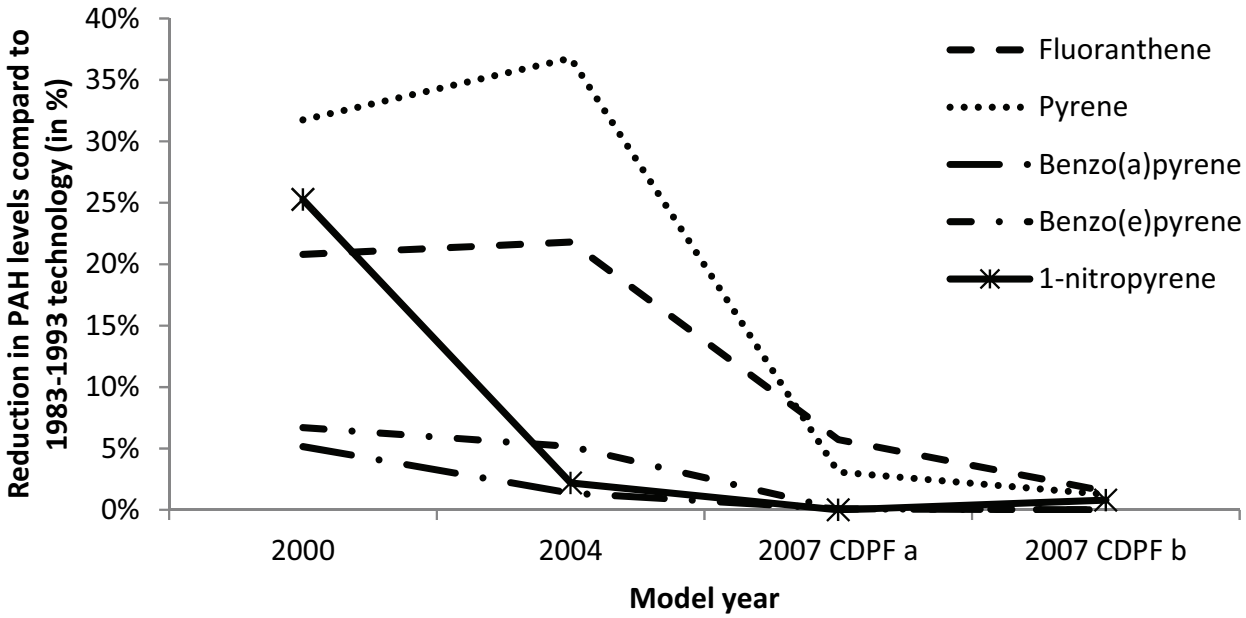
technology diesel engines fitted with and without advanced aftertreatment systems. Large reductions in total emissions of PM, sulfate/nitrate, hydrocarbons, EC and ash were observed. Total reductions in PM emissions of 99% were typical (Liu *et al.*, 2009).

PAH and nitro-PAH emissions have been studied extensively. The reduction in emission levels between transitional and new technology engines for various classes of compounds, for the 39 California Air Resources Board Toxic Air contaminants, and for PAHs and nitro-PAHs is shown in Table 1.4, Table 1.5, and Table 1.6, respectively. Studies generally showed large reductions in PAHs and nitro-PAHs in new technology diesel engines compared with traditional and transitional technology engines (52– > 99%) (Biswas *et al.*, 2009a, b; Khalek *et al.*, 2009, 2011; Liu *et al.*, 2010).

Some studies (Heeb *et al.*, 2008, 2010) showed that uncatalysed filters and filters that did not strongly oxidize carbon and nitrogen monoxides in the exhaust removed PAHs less efficiently than those that strongly oxidized carbon and nitrogen monoxides, with individual PAH removal efficiencies of 37–90% for the former and 75–98% for the latter.

Several recent studies that evaluated the formation of nitro-PAHs in DPFs produced conflicting results (Heeb *et al.*, 2008, 2010; Liu *et al.*, 2010; Khalek *et al.*, 2011). The level of 1-nitrophenanthrene increased by 20% for an uncatalysed DPF with fuel-borne catalysts in one study (Heeb *et al.*, 2008), and by 55 and 91% for low- and high-activity DPFs, respectively, in a later study by the same authors (Heeb *et al.*, 2010). In contrast, Liu *et al.* (2010) observed a 76% decrease in 1-nitrophenanthrene in 2004 model engines compared with 2007 model engines. The

Fig. 1.6 Trends in selected polycyclic aromatic hydrocarbon species with developing technology



Concentrations are plotted as a fraction of the emissions from a group of 1983–93 heavy-duty vehicles reported by Watson *et al.* (1998), except for 1-nitropyrene which was taken as the mid-point of the range of 1–4 mg/brake horse power–h given as typical of 1975–2000 engines by EPA (2002a). 2000 and 2007 CDPF b data from Khalek *et al.* (2011). 2004 and 2007 CDPF a data from Liu *et al.* (2010).

Table 1.4 Unregulated emissions for all 12 repeats of the 16-hour cycles for four 2007 ACES engines and for 2004 technology engines used in CRC E55/E59

Compound	2004 engines (average ± SD, mg/h)	2007 engines (average ± SD, mg/h)	2007 engines[a] (average ± SD, mg/bhp-h)	Average percentage reduction relative to 2004 technology engines
Single-ring aromatics	405.0 ± 148.5	71.6 ± 32.97	0.76 ± 0.35	82
PAHs	325.0 ± 106.1	69.7 ± 23.55	0.74 ± 0.25	79
Alkanes	1030.0 ± 240.4	154.5 ± 78.19	1.64 ± 0.83	85
Hopanes/steranes	8.2 ± 6.9	0.1 ± 0.12	0.0011 ± 0.0013	99
Alcohols and organic acids	555.0 ± 134.4	107.4 ± 25.4	1.14 ± 0.27	81
Nitro-PAHs	0.3 ± 0.0	0.1 ± 0.0	0.0065 ± 0.0028	81
Carbonyls	12 500.0 ± 3535.5	255.3 ± 95.2	2.68 ± 1.00	98
Inorganic ions	320.0 ± 155.6	92.3 ± 37.7	0.98 ± 0.40	71
Metals and elements	400.0 ± 141.4	6.7 ± 3.0	0.071 ± 0.032	98
Organic carbon	1180.0 ± 70.7	52.8 ± 47.1	0.56 ± 0.50	96
Elemental carbon	3445.0 ± 1110.0	22.6 ± 4.7	0.24 ± 0.05	99
Dioxins/furans	NA	$6.2 \times 10^{-5} \pm 5.2 \times 10^{-5}$	$6.6 \times 10^{-7} \pm 5.5 \times 10^{-7}$	99[b]

[a] Data shown in brake-specific emissions for completeness; no comparable data on brake-specific emissions were available
[b] Relative to 1998 technology engines
bhp, brake horse power; h, hour; NA, not applicable; PAHs, polycyclic aromatic hydrocarbons; SD, standard deviation
From Khalek *et al.* (2011)

Table 1.5 Average emissions of the 39 CARB toxic air contaminants for four 2007 ACES engines and for 1994–2000 technology engines[a]

TAC No.	Compound	1994–2000 technology engines[b] (mg/bhp-h)	2007 technology engines[c] (mg/bhp-h)	Percentage reduction
1	Acetaldehyde	10.3	0.61 ± 0.27	93
2	Acrolein	2.7	< 0.01	> 99
3	Aniline	NA	0.000150 ± 0.000075	NA
4	Antimony compounds	NA	< 0.001	NA
5	Arsenic	NA	< 0.0002	NA
6	Benzene	1.82	< 0.01	> 99
7	Beryllium compounds	NA	< 0.0003	NA
8	Biphenyl	NA	0.013780 ± 0.001716	NA
9	Bis(ethylhexyl)phthalate	NA	NR	NA
10	1,3-Butadiene	1.7	< 0.01	> 99
11	Cadmium	NA	< 0.00003	NA
12	Chlorine (chloride)	0.18	< 0.007	> 96
13	Chlorobenzene and derivatives	NA	NR	NA
14	Chromium compounds	NA	0.0007 ± 0.0003	NA
15	Cobalt compounds	NA	< 0.0001	NA
16	Cresol isomers	NA	0.02727 ± 0.01233	NA
17	Cyanide compounds	NA	< 0.05	NA
18	dl-*n*-Butylphthalate	NA	NR	NA
19	Dioxins and dibenzofurans	0.000066	0.00000066 ± 0.000000055	99
20	Ethyl benzene	0.49	0.05 ± 0.04	90
21	Formaldehyde	25.9	1.90 ± 1.01	94
22	Hexane	0.14	< 0.01	> 93
23	Inorganic lead	0.0009	< 0.0001	> 89
24	Manganese	0.0008	< 0.00022	> 73
25	Mercury	NA	< 0.00016	NA
26	Methanol	NA	0.07 ± 0.13	NA
27	Methyl ethyl ketone	NA	< 0.01	NA
28	Naphthalene	0.489	0.0982 ± 0.0423	80
29	Nickel	0.01	0.0002 ± 0.0001	98
30	4-Nitrobiphenyl	NA	< 0.00000001	NA

Table 1.5 (continued)

TAC No.	Compound	1994–2000 technology engines[b] (mg/bhp-h)	2007 technology engines[c] (mg/bhp-h)	Percentage reduction
31	Phenol	NA	0.00905 ± 0.00414	NA
32	Phosphorus	NA	0.0130 ± 0.0064	NA
33	POM, including PAHs and derivatives	See Table 1.6	See Table 1.6	See Table 1.6
34	Propionaldehyde	1.8	0.01	> 99
35	Selenium	NA	< 0.0001	NA
36	Styrene	0.73	< 0.01	> 99
37	Toluene	0.64	0.26 ± 0.28	59
38	Xylene isomers and mixtures	2.2	0.35 ± 0.10	85
39	*ortho*-Xylene	0.99	0.13 ± 0.07	87
40 and 41	*meta*- and *para*-Xylenes	1.21	0.20 ± 0.08	83

[a] Engines run over the Federal Test Procedure transient cycle for all 12 repeats of the 16-hour cycle.
[b] Data on standard deviations were not provided.
[c] The limit of detection is indicated by values marked ' < '.
bhp, brake horse power; h, hour; NA, not applicable; NR, not reported; PAHs, polycyclic aromatic hydrocarbons; POM, polycyclic organic matter; TAC, toxic air contaminant
From Khalek *et al.* (2011)

Table 1.6 Emissions of selected polycyclic aromatic hydrocarbon (PAH) and nitro-PAH compounds from 2000 and 2007 technology engines[a]

PAH/nitro-PAH	2007 technology engines[b] (mg/bhp-h)	2000 technology engines[b, c] (mg/bhp-h)	Percentage reduction
Naphthalene	0.0982 ± 0.0423	0.4829	80
Acenaphthylene	0.0005 ± 0.0005	0.0524	98
Acenaphthene	0.0004 ± 0.0001	0.0215	98
Fluorene	0.0015 ± 0.0009	0.0425	96
Phenanthrene	0.0077 ± 0.0025	0.0500	85
Anthracene	0.0003000 ± 0.0001000	0.0121	97
Fluoranthene	0.0006 ± 0.0006	0.0041	85
Pyrene	0.0005 ± 0.0004	0.0101	95
Benzo[*a*]anthracene	< 0.0000001	0.0004	> 99
Chrysene	< 0.0000001	0.0004	> 99
Benzo[*b*]fluoranthene	< 0.0000001	< 0.0003	> 99
Benzo[*k*]fluoranthene	< 0.0000001	< 0.0003	> 99
Benzo[*e*]pyrene	< 0.0000001	< 0.0003	> 99
Benzo[*a*]pyrene	< 0.0000001	< 0.0003	> 99
Perylene	< 0.0000001	< 0.0003	> 99
Indeno[1,2,3-*cd*]pyrene	< 0.0000001	< 0.0003	> 99
Dibenz[*a,h*]anthracene	< 0.0000001	< 0.0003	> 99
Benzo[*ghi*]perylene	< 0.0000001	< 0.0003	> 99
2-Nitrofluorene	0.0000036 ± 0.0000041	0.000065	94
9-Nitroanthracene	0.0000148 ± 0.0000213	0.0007817	98
2-Nitroanthracene	0.0000004 ± 0.0000009	0.0000067	94
9-Nitrophenanthrene	0.0000211 ± 0.0000209	0.0001945	89
4-Nitropyrene	< 0.0000001	0.0000216	> 99
1-Nitropyrene[d]	0.0000197 ± 0.0000243	0.0006318	97
7-Nitrobenz[*a*]anthracene[d]	0.0000002 ± 0.0000002	0.0000152	99
6-Nitrochrysene	< 0.00000001	0.0000023	> 99
6-Nitrobenzo[*a*]pyrene[d]	< 0.00000001	0.0000038	> 99

[a] Four 2007 ACES engines and some 2000 technology engines were run over the Federal Test Procedure transient cycle for all 12 repeats of the 16-hour cycles.
[b] The limit of detection is indicated by values marked '< '.
[c] Data on standard deviations were not provided
[d] Previous work showed artefact formation during filter collection
bhp, brake horse power; h, hour
From Khalek *et al.* (2011)

level of 2-nitrophenanthrene was also increased by 100% with DPFs in the first study (Heeb *et al.*, 2008), but was decreased by 34 and 81% for low- and high-activity DPFs, respectively (Heeb *et al.*, 2010), and by 91% for 2004 model engines compared with 2007 model engines (Liu *et al.*, 2010). The level of 9-nitrophenanthrene was increased in two studies (Heeb *et al.*, 2010; Liu *et al.*, 2010), whereas a 92% reduction was reported in another study (Khalek *et al.*, 2011). The concentration of 1-nitropyrene was shown to increase in one study (Heeb *et al.*, 2010), but two other studies reported decreases of 99 and 97%, respectively (Liu *et al.*, 2010; Khalek *et al.*, 2011).

[These differences were probably due to a combination of differences in the test cycle and sampling method. The studies by Liu *et al.* (2010) and Khalek *et al.* (2011) both used dilution sampling that is intended to approximate the atmospheric dilution process, while the studies by Heeb *et al.* (2008, 2010) used undiluted sampling of cooled exhaust gases with samples combined from a filter, a condenser and an XAD absorber.]

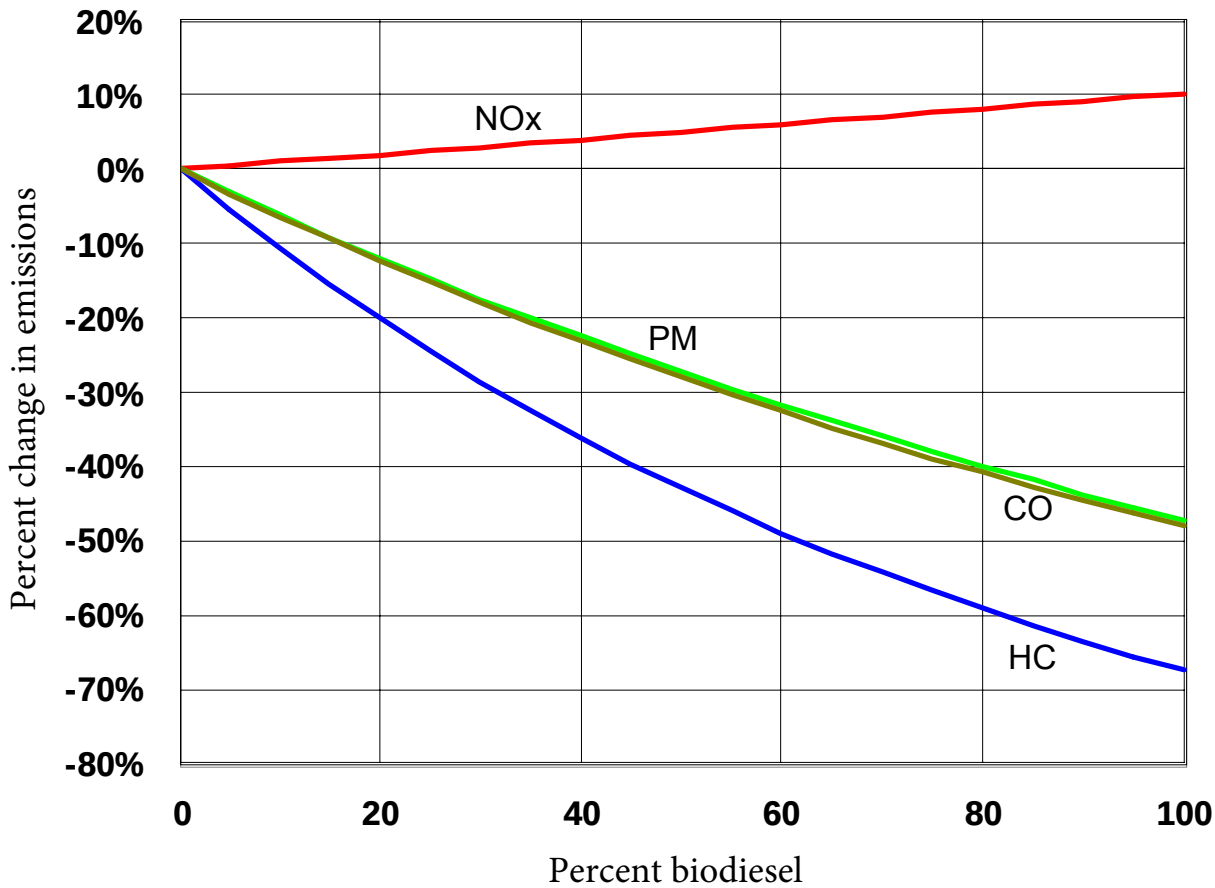
Some studies showed increases in dioxin/furan emissions with the use of copper fuel-borne catalyst materials, in particular in the presence of elevated levels of chlorine in the fuel (Mayer *et al.*, 2003; Heeb *et al.*, 2007); subsequent work by the same investigators (Wenger *et al.*, 2008) reported increases in copper fuel-borne catalysed systems but small absolute values except in one case using fuel with an extremely high chlorine content. Other studies (Table 1.4 and Table 1.5; Khalek *et al.*, 2011) reported a 99% reduction in dioxins/furans with catalysed DPF and SCR systems compared with transitional technology engines. In a comprehensive programme that tested two different 2010 new technology engines with a range of different exhaust aftertreatment configurations chosen for their potential for dioxin/furan formation, Liu *et al.* (2011) showed 60–80% reductions in dioxin/furan emissions for all aftertreatment configurations, and no impact of elevated levels of chlorine content. Dyke *et al.* (2007) reported an approximate 80% reduction in dioxin/furan emissions when a diesel oxidation catalyst was fitted.

Andersson *et al.* (2007) compared six conventional diesel vehicles with six DPF-equipped vehicles. Conventional vehicles emitted 300–700-fold more particles than DPF-equipped vehicles.

Although solid particle emissions are greatly reduced by DPFs, increases in total emissions have been reported with the use of DPFs. Sulfate and ammonium sulfate emissions may be the main constituents of nucleation mode particles, which are formed at higher exhaust temperatures and generally by more heavily catalysed systems (Herner *et al.*, 2011). Kittelson *et al.* (2008) observed the formation of a large nucleation mode and an increase in particle emissions downstream from a continuously regenerating trap (diesel oxidation catalyst upstream of an uncatalysed DPF) when exhaust temperatures exceeded about 350 °C. The DPF was very efficient at removing accumulation mode soot particles but, in the absence of a surface to adsorb the sulfuric acid, the vapours nucleated and grew into a large nucleation mode that contained very little mass but a large number of particles. No detectable nucleation mode formation took place when a catalysed continuously regenerating trap (diesel oxidation catalyst upstream from a catalysed DPF) was used.

Khalek *et al.* (2011) compared particle size and particle emissions from four new technology engines with those from a transitional technology engine, and did not observe the increase in total emissions described above. Total emissions were, respectively, 3.0×10^{14}, 3.3×10^{13} and 3.9×10^{12} particles/brake horse power (bhp)–h for the 2004 engine, the 2007 engines, including regeneration events, and the 2007 engines, not including the regeneration events. Thus, the total emissions were reduced by 89% and 88% of the remaining emissions were produced during the infrequent regeneration events.

Fig. 1.7 Impact of biodiesel blends on emissions



CO, carbon monoxide; HC, hydrocarbon; NO_x, nitrogen oxides; PM, particulate matter
From EPA (2002b)

1.1.3 Biodiesel

Biodiesel is usually a fatty acid methyl ester made by reacting vegetable oils or animal fats with methanol. The most common feedstocks are soya bean, rapeseed and palm oils, and various waste animal fats. In general, biodiesel does not significantly change engine efficiency but leads to slightly higher fuel consumption because of its lower energy content. It is usually used in blends with petroleum diesel, where for example B20 refers to a 20% biodiesel blend.

The US Environmental Protection Agency (EPA) published a comprehensive review of the performance and emissions of biodiesel blends (EPA, 2002b). The emissions are sensitive to engine specifications and aftertreatment systems, but general trends are observed. Fig 1.7 shows the average variation in emissions of nitrogen oxides, carbon monoxide, hydrocarbon and PM from a biodiesel blend fraction for soya methyl ester, based on many engine tests. Emissions may vary depending on feedstock.

Several studies of emissions from biodiesel blends have been reported (Sharp *et al.*, 2000; Turrio-Baldassarri *et al.*, 2004; Arapaki *et al.*, 2007; Cheung *et al.*, 2009; Karavalakis *et al.*, 2009a, b, 2010); most showed trends in regulated emissions similar to those described by the US EPA (2002b), while results for unregulated

emissions were mixed. Some showed increases in the levels of PAHs and derivatives with increasing blends, while others showed decreasing trends.

To resolve these inconsistencies, Eckerle *et al.* (2008) studied blends of B0 (i.e. pure conventional diesel fuel), B20 and B100 (pure biodiesel) and isolated the effects of changes in combustion due to the properties of the fuels and changes in emissions due to interactions with the calibration of the engine control system. Their experiments and analysis of combustion indicated little effect of biodiesel blend on combustion at higher loads; however, at lower loads, biodiesel tended to increase the emissions of nitrogen oxides.

A more significant and potentially confusing effect was the interaction between the volumetric energy content of the fuel and the engine control system. Biodiesel blends have a lower volumetric energy content compared with conventional diesel fuel. As a result, the engine requires a higher volumetric fuel flow of biodiesel to produce the same power as conventional diesel, and, depending on the specific engine control calibration, the levels of nitrogen oxides may increase or decrease as a result of this interaction. [The Working Group noted that this would explain to some extent the wide range of responses observed to the effects of biodiesel on emissions of nitrogen oxides that were dependent on the base properties of the fuels, engine operating conditions (especially load factor) and the means of control calibration.]

1.1.4 Spark ignition (gasoline) engines

(a) Historical perspective

Spark ignition engines have been and continue to be the main source of power for passenger cars, motorcycles and scooters, and for most small-scale on-road and off-road applications, e.g. all-terrain vehicles, snowmobiles, outboard motors, garden tractors and lawn mowers. The first operational four-stroke spark ignition engine was developed by Nicolaus Otto in 1876, and named the Otto Cycle. Unfortunately, his early design was unsuitable for automotive applications, and a more practical, single-cylinder automotive engine was developed in 1899 by Gottlieb Daimler (Amann, 1990).

The early development of spark ignition engines was hindered by destructive combustion knock, which limited the compression ratio and thus the power and efficiency of the engine. The discovery in 1921 by Charles Kettering of the anti-knock properties of tetraethyl lead opened the way to engines with higher compression ratios, which were more powerful and efficient. Tetraethyl lead was used as an anti-knock agent until growing concerns about engine emissions led to its gradual phase out (see below) (Amann, 1990).

The reduction of emissions from gasoline-fuelled passenger cars has for many years been focused on gaseous emissions, and led to major changes in spark ignition engine technology. The introduction of catalytic converters – oxidation catalysts in the mid-1970s followed by three-way catalysts in the early 1980s – allowed optimization of the engines for maximum performance while cleaning the exhaust with the catalyst (Twigg, 2011).

(b) Port-fuel injection engine technology

Spark ignition engines are designed to operate with well mixed homogeneous charges at either lean or chemically correct fuel–air mixture ratios. Until the mid-1980s, nearly all automotive spark ignition engines used carburettors (mechanical devices for mixing fuel and air in a predetermined ratio) to provide the fuel–air mixture. However, these were unable to provide the precise mixture control required to meet ever more stringent emission standards, and were replaced by port fuel injection systems (although carburettors are still used in off-road applications, especially in small engines). Today, nearly all passenger car spark ignition engines rely on the injection of fuel into the intake port and are therefore called port fuel injection (PFI)

engines. Well mixed homogeneous combustion under lean or chemically correct conditions does not lead to significant particle formation. Thus, particle emissions from PFI spark ignition engines are associated with conditions other than normal premixed combustion – for example, rich combustion during cold starts or at high loads. Engines with excessive oil consumption or an improper fuel–air mixture may also emit high levels of particles.

Regulatory efforts to reduce emissions from gasoline-fuelled vehicles preceded those for diesel-fuelled engines. Standards worldwide gradually reduced and finally eliminated the addition of tetraethyl lead to gasoline (Colucci, 2004; Twigg, 2005). The lower lead content had both direct and indirect impacts on gasoline engine emissions. In addition to decreasing the levels of lead in exhaust, the elimination of tetraethyl lead from gasoline was critical to the use of advanced aftertreatment technologies, such as three-way catalytic converters that reduce the emissions of carbon monoxide, nitrogen oxides and hydrocarbons in exhaust from gasoline-fuelled vehicles. The presence of even trace levels of lead in the exhaust poisoned the catalysts in exhaust treatment systems, and rendered them ineffective. By 2000, lead had been banned in gasoline in most countries around the world. Nevertheless, leaded fuel is still used in some light aircraft applications.

(c) Gasoline direct injection

The gasoline direct injection (GDI), a more fuel-efficient version of the gasoline engine (Maricq *et al.*, 2011; McMahon *et al.*, 2011), entered the marketplace in Japan and Europe in the 1990s and in the USA in 2003. Similarly to the diesel engine, GDI engines inject fuel directly into the combustion chamber (CARB, 2011). This system results in significant reductions in fuel consumption and provides greater power, but increases particle emissions to levels that are intermediate between PFI and diesel engines. In some cases, especially in the stratified charge mode, GDI engines can produce levels of PM emissions similar to those of diesel engines.

1.1.5 Levels of gasoline exhaust emissions

Several tests have been conducted on fleets of vehicles to determine emissions from representative on-road gasoline engines.

Cadle *et al.* (2001) tested a fleet of passenger car and light-duty goods vehicle (24 with normal and six with high emissions) model from years 1990–97 on a chassis dynamometer, using three test cycles and two fuels, one with ethanol (oxygenated) and one without ethanol. The test cycles were a cold-start (35 °F) FTP, a hot-start unified cycle and a hot-start REP05 cycle (high load, high speed cycle). Overall, average PM emissions from normal emitters running on oxygenated fuel were 6.1, 3.2 and 12.7 mg/mile for the FTP, unified cycle and REP05, respectively. Compositions of PM were determined for 12 vehicles (10 normal and two high emitters). For normal emitters, total carbon accounted for approximately 83 and 68% of the PM in the FTP and REP05 tests, respectively, and organic compounds accounted for 36 and 45% of the total carbon in the two tests. Chloride, nitrate and sulfate emission rates were measured, of which sulfate was the highest. These ions accounted for 2.1 and 10.5% of the PM mass in FTP and REP05, respectively. When the water associated with sulfuric acid was included, these compounds accounted for up to 20% of the PM in the REP05. The high particle number emissions observed during the REP05 may have been linked to these sulfuric acid emissions. Fourteen elements were measured using X-ray fluorescence and the major contributors were sulfur, silica, iron, magnesium and zinc. Total elements averaged 2.8 and 15.4% of the PM mass in FTP and REP05, and sulfur (also included in the sulfate mentioned above) was the major contributor. Emissions of PAHs were measured for 20 normal

vehicles and four with high emissions. The total PAH emissions were dominated by naphthalene and methylnaphthalene. The FTP PAH emission rates were ~6.5- and 2.3-fold higher than those from the REP05 for normal and high emitters, respectively. Total PAH emission rates were 1.55-fold higher with non-oxygenated than with oxygenated fuel. High emitters exhibited clearly elevated levels of light PAHs, but the pattern for larger four- and five-ring PAHs was less clear.

Zielinska *et al.* (2004) tested a set of models of passenger cars, sport utility vehicles and pick-up HGVs ranging from 1976 to 2000, including both normal and high emitters. Tests were carried out on a chassis dynamometer using the California unified cycle at 72 and 30 °F. Vehicles were classified as NG (new technology gasoline), G and G30 (gasoline, normal emitters at 72 and 30 °F), and WG and BG (gasoline, high-emitting white and black smokers). Average PM emission rates were 2.5, 8.7, 66 and 716 mg/mile for NG, G, BG and WG, respectively, and increased at low temperatures by a factor of about 4. PM emissions were almost entirely carbonaceous, but the ratio of EC to total carbon varied widely: G30 had the highest ratio of ~0.7, G and NG had ratios between 0.4 and 0.5, and WG had the lowest ratio of < 0.05 (presumably mostly lubricating oil). All cars emitted 5 mg/mile or less of total ions; for the G set, sulfate represented 10–20% of the PM emissions. The main elements measured were iron, silica and aluminium, presumably formed from dust ingested by the engine and from engine wear (iron and aluminium). Total elements accounted for 13% of the PM mass from G. Hopanes and steranes are species of high molecular weight and low vapour pressure that are found mainly bound to particles. These species are present mainly in the lubricating oil and are linked to the consumption of engine oil. Most of the PAHs were of low molecular weight and in the gas phase. Gasoline had the highest fraction of high-molecular-weight particle-bound PAHs. The highest total PAH emission rates were from WG, followed by BG and then G. PAH emissions were about 10-fold higher in the low-temperature tests (G30) than at normal temperatures (G). The PAH distributions were very similar to those in used (but not fresh) lubricating oils, which are believed to collect and store partial combustion products. Eight PAHs have been identified as known, probable or possible carcinogens by IARC and/or EPA: benz[*a*]anthracene, benzo[*a*]pyrene, benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, benzo[*k*]fluoranthene, chrysene, dibenz[*a,h*]anthracene and indeno[1,2,3-*cd*]pyrene. The Working Group calculated the emission levels of the sum of these PAHs, excluding chrysene and dibenz[*a,h*]anthracene: 12 and 140 µg/mile from G and G30, respectively; the highest were found from WG (400 µg/mile) and the lowest from NG (3 µg/mile).

Zielinska *et al.* (2004) also measured the levels of nine nitro-PAHs: 1-nitronaphthalene, 2-methyl-nitronaphthalene, methyl-nitronaphthalene, 9-nitroanthracene, 3-nitrofluoranthene, 1-nitropyrene, 7-nitrobenzo[*a*]anthracene, 6-nitrochrysene, and 6-nitrobenz[*a*]pyrene. The sum of their emission rates, excluding 2-methyl-nitronaphthalene and methyl-nitronaphthalene, was calculated by the Working Group. [These two species were excluded to report only those in common with the study by EPA (2008) described below.] Total emission rates of these nitro-PAHs were close to or below the limit of detection from NG [not reported] and were low from the other gasoline vehicles: [0.11], [0.27], [1.29] and [1.02] µg/mile from G30, G, BG and WG, respectively, and were not significantly influenced by temperature.

Fujita *et al.* (2007) tested 57 light-duty gasoline vehicles, two light-duty diesel vehicles and 30 medium- and heavy-duty goods vehicles and buses as part of the DOE Gasoline/Diesel Split Study. The objectives were to determine the relative contributions of gasoline and diesel vehicles to ambient fine PM and to identify suitable markers for diesel and gasoline exhaust. Gasoline

vehicle models ranged from earlier than 1980 to 1996 and more recent, and included six 'smoker' [vehicles with high emissions and visible smoke in the exhaust] models from 1969 to 1990. These vehicles were tested using modified cold- and hot-start unified cycles. The trucks included three weight categories (range of model years): seven light-heavy (1989–2000), seven medium-heavy (1988–99) and 16 heavy-heavy (1985–2001). The HGVs were tested using a city suburban and a highway driving cycle. A 1982 and a 1992 transit bus were also tested. PM mass, elements, ions, organic compounds, EC, PAHs, hopanes, steranes, alkanes and polar organic compounds were measured. Total $PM_{2.5}$ emissions of 1995–99 model gasoline vehicles were 3.7 mg/mile for warm starts and 8 mg/mile for cold starts. Average emissions of the entire gasoline fleet (all model years) were 16.9 and 27.2 mg/mile for hot and cold tests, respectively, and were highly skewed; the 10% that were the highest emitters were responsible for about two-thirds of the total emissions. PM emissions from 1997–2000 model light-heavy goods vehicles ranged from 150 to 230 on the city suburban cycle, and from 65 to 90 mg/mile on the highway cycle; for 1995–99 model medium-heavy goods vehicles, the emissions ranged from 70 to 450 and from 60 to 210 mg/mile on the city suburban and highway cycles, respectively; and for 1994–2001 model heavy-heavy goods vehicles, they ranged from 310 to 1130 and from 130 to 520 mg/mile on the city suburban and highway cycles, respectively. Average PM emissions for the HGV fleet were 404 and 187 mg/mile for the city suburban and highway tests, respectively, and were less skewed than those of gasoline vehicles. Emissions of non-carbonaceous materials from both gasoline and diesel engines were relatively small; silicon and ammonium sulfate were the dominant emissions. Elements present in lubricating oil (zinc, calcium and phosphorus) were present in all samples at very variable amounts, probably depending on the consumption of lubricating oil. The EC to total carbon fraction for heavy-duty vehicles ranged from 0.37 to 0.74 and was usually above 0.5; the fraction for gasoline engines was below 0.25 for all samples, except for high black carbon vehicles.

Both low- and high-emitter gasoline vehicles emitted larger amounts of the high-molecular-weight particulate PAHs, benzo[*ghi*]perylene, indeno[1,2,3-*cd*]pyrene and coronene, which have been identified as potential markers for gasoline emissions, than diesel vehicles. In contrast, diesel emissions contained higher levels of two- to four-ring semi-volatile PAHs. Total levels of carcinogenic PAHs [see above] were [140] and [180] µg/mile in combined city suburban and highway emissions from combined light-heavy and medium-heavy, and heavy-heavy diesel vehicles, respectively. Emissions of these substances from gasoline vehicles were lower, ranging from [2.6] to [13] µg/mile for low emitters after warm starts and smokers after cold starts. Similar compositions and amounts of hopanes and steranes were present in lubricating oil for both gasoline and diesel vehicles but were negligible in gasoline or diesel fuels. Total hopane and sterane emissions on a mass per mile basis were higher from diesel vehicles, but the relative contribution of hopanes and steranes to total carbon emissions was similar in diesel and gasoline exhausts. Thus, hopane and sterane emission levels were related to the rate of oil consumption. The three high-molecular-weight marker PAHs generated by combustion were found in used gasoline motor oil but not in fresh oil and were negligible in used diesel engine oil (Fujita *et al.*, 2007).

The Kansas City Light-Duty Vehicle Emission Study (EPA, 2008) was designed to evaluate the relative contributions of diesel and gasoline vehicles to $PM_{2.5}$, by taking comprehensive measurements of the emissions of PM, as well as other regulated and unregulated compounds, from a representative sample of light-duty gasoline-powered vehicles in the USA fleet (Fulper *et al.*, 2010). Vehicles were tested on a portable

chassis dynamometer under ambient temperature conditions. All tests were run using the LA92 unified cycle, and were conducted in two phases: a summer phase in 2004 with a fleet of 261 vehicles (80 HGVs and 181 passenger cars), and a winter phase in 2005 with a fleet of 235 vehicles (119 HGVs and 116 cars). Each class of vehicles was subdivided into four subgroups by model year: pre-1981 (as old as 1968), 1981–90, 1991–95 and 1996 and more recent (as new as 2004). The PM emissions ranged over more than three orders of magnitude, with a downward trend for more recent vehicles; however, even within a given model year subgroup, emissions ranged over up to two orders of magnitude. The median PM emissions from passenger cars in the summer tests were 45, 7, 5 and 2 mg/mile for the pre-1981, 1981–90, 1991–95 and post-1996 models, respectively. Median PM emissions from HGVs were typically higher by a factor of about two. Median emissions of carcinogenic PAHs (see above) from passenger cars in the summer tests were [120], [25], [15] and [3] μg/mile for the pre-1981, 1981–90, 1991–95 and post-1996 models, respectively. Nineteen nitro-PAHs were measured. Combined emission levels of the seven nitro-PAHs reported in the study by Zielinska *et al.* (2004) from cars in the summer tests were [0.23], [0.70], [0.11] and [0.00] μg/mile for the pre-1981, 1981–90, 1991–95 and post-1996 models, respectively.

Andersson *et al.* (2009) reported the chemical composition of PM from lean-burn GDI engines that meet Euro 4 standards: carbon, 55.8%; all hydrocarbons, 36%; sulfates, 2.2%; nitrate/nitrite, 4%; and water, 3%.

Khalek *et al.* (2010) tested a 2009 GDI engine using three commercially available fuels. Emissions ranged from 0.7 to 3.2 mg/km on the FTP cycle and from 1.3 to 12.8 mg/km on the more aggressive US06 cycle. Zhang & McMahon (2012) tested a fleet of nine 2007–10 model GDI light-duty vehicles and one 2009 model PFI vehicle, all of which met the California LEV II emission standard (see Section 1.3). The vehicles were tested on the FTP cycle and PM emissions ranged from 1 to 5.3 mg/km from the GDI vehicles and were 0.4 mg/km from the PFI vehicle.

1.1.6 Comparison of levels of emissions from current technology diesel and gasoline engines

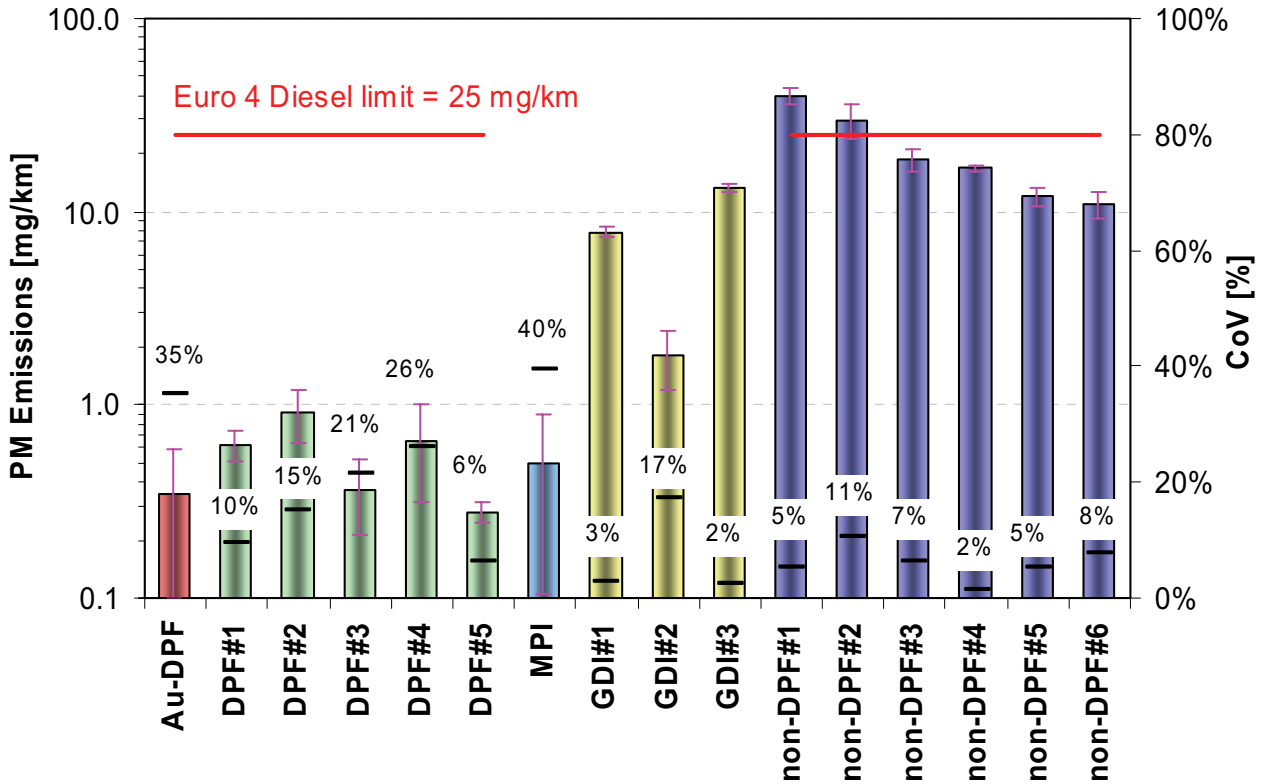
(a) Light-duty vehicles

Only a few studies have compared the emissions from current technology gasoline and diesel light-duty vehicles.

The bulk composition of PM emitted by GDI engines was similar to that of diesel engines without a DPF, i.e. mostly EC and hydrocarbons and other volatile compounds (Andersson *et al.*, 2009). At present, PM emissions from GDI engines are higher than those from PFI gasoline engines and diesel engines with a DPF.

In the 'PARTICULATES' programme, Samaras *et al.* (2005) tested nine diesel cars not fitted with a DPF that met the Euro-3 (model year, 2000) and earlier standards, five diesel cars fitted with a DPF that met the Euro-3+ standards (model year, 2000–05), six gasoline PFI engines that met the Euro-1 (model year, 1992), ultra-low emission vehicle and Euro-3 standards and five GDI cars that met the Euro-3 standards (see the Annex for a definition of the standards). PM emission levels of DPF-equipped vehicles were generally below 3 mg/km, although one vehicle emitted 7 mg/km. Those from GDI vehicles ranged from 4 to 11 mg/km and the majority of measurements were below 10 mg/km. Gasoline PFI vehicles emitted PM in the same range as diesel vehicles fitted with a DPF. The two older gasoline cars that met the ultra-low emission vehicle and Euro-1 standards emitted greater levels than the more recent models, but these were still below 2.5 mg/km. The authors questioned whether the methods used for the measurement of PM were appropriate for such ultra-low emitting vehicles. PAH measurements focused on the sum of 23 'particle-bound' PAHs. For the DPF-equipped

Fig. 1.8 Levels of particulate matter emission for diesel, diesel particle filter (DPF), port fuel-injected (PFI) gasoline and gasoline direct injection (GDI) vehicles measured in the PMP Programme



Au-DPF, golden vehicle (reference); DPF, diesel particle filter; GDI, gasoline direct injection; MPI, part-injected gasoline; PFI, port fuel-injected; PMP Programme, Particle Measurement Programme
Reprinted with permission from Andersson *et al.* (2007)

vehicles, the sum of PAHs ranged from 1.5 to 3 and from 0.2 to 0.6 µg/km during cold-start and motorway cycles, respectively. For gasoline vehicles, the sum of PAHs ranged from < 0.25 µg/km on the motorway cycle to 1.5 µg/km on a cold-start cycle. PAH emissions were very dependent on vehicle technology and driving cycle and increased significantly on cold-start cycles.

During the Particle Measurement Programme (Andersson *et al.*, 2007), a variety of modern vehicles (six conventional diesel, six diesel fitted with a DPF, one PFI gasoline and three GDI engines) was tested in multiple laboratories in Europe and Japan. The test fleet comprised predominantly Euro-4 vehicles, except for two vehicles that were tested in Japan with local calibrations. One of the DPF-fitted diesel vehicles (the so-called golden vehicle) was shipped from laboratory to laboratory while the other vehicles were provided locally. Fig 1.8 shows the average PM emissions on a test cycle that included cold start, urban, suburban and motorway components (New European Driving Cycle). PM emissions ranged from 11 to 40 mg/km for conventional diesel vehicles, from 0.3 to 0.9 mg/km for diesel vehicles with a DPF, and from 1.8 to 13.3 mg/km for GDI vehicles, and were 0.5 mg/km for the PFI gasoline vehicle.

Vouitsis *et al.* (2009) tested two Euro-4 passenger cars, one with a diesel and one with a

PFI gasoline engine. The diesel engine was tested under two configurations: the standard combination of an oxidizing catalyst and an SCR system for the control of nitrogen oxides, and a modified combination in which the oxidizing catalyst was replaced by a DPF. Total PM, carbon, elements, ions and PAHs were measured during four different driving cycles. Total PM ranged from 22 to 37 mg/km for the conventional diesel engine, from 1 to 5 mg/km for the DPF-fitted diesel engine and from 1 to 3 mg/km for the gasoline vehicle. The average chemical profile of the PM was: 97% carbon, 0.08% PAHs, 0.22% elements and 2.76% ions for the conventional diesel engine; 48% carbon, 0.22% PAHs, 1% elements and 51% ions for the DPF-fitted diesel engine; and 89% carbon, 0.26% PAHs, 0.86% elements and 10% ions for the gasoline engine. The principal ions were sulfate, nitrate and ammonium. Four of the six heavy PAHs discussed above (benz[*a*]anthracene, benzo[*a*]pyrene, benzo[*k*]fluoranthene and benzo[*b*]fluoranthene) were measured, the sum of which, depending on the operating cycle, ranged from not detected to 1.8 µg/km for the conventional diesel engine, from not detected to 0.1 µg/km for the DPF-fitted diesel engine and from not detected to 0.3 µg/km for the gasoline engine.

(b) Comparisons on the basis of fuel emissions

Comparisons of light-duty emissions with medium- and heavy-duty emissions that are measured in grams per mile, and heavy-duty emissions that are measured in grams per brake horse power-hour (g/bhp-h) are difficult. A further complication is that the various studies frequently used different test cycles. However, the conversion of emissions to a fuel-specific form – mass emissions per mass of fuel (g/kg_{fuel}) – allows some comparisons to be made. The conversion from grams per mile is:

$$\frac{g}{kg_{fuel}} = \frac{g}{mi} \times \frac{mi}{gallon} \times \frac{gallon}{kg_{fuel}} = 0.31 \frac{mi}{gallon} \times \frac{g}{mi}$$

for a typical fuel density of 0.85 kg/L.

Fuel economy varies with vehicle type from less than four miles per gallon (4 mpg) for some heavy-duty goods vehicles and buses to over 60 mpg for small light-duty vehicles. The conversion for heavy-duty vehicles is:

$$\frac{g}{kg_{fuel}} = \frac{g}{bhp-hr} \times \frac{bhp}{kW} \times \frac{kWh}{kg_{fuel}} = 1.34 \frac{1}{BSFC} \times \frac{g}{bhp-hr}$$

where 1.34 is the conversion from kilowatts (kW) to brake horse power (bhp) and BSFC is the engine brake-specific fuel consumption in kg_{fuel}/kW-h. BSFC values vary from > 1 kg_{fuel}/kW-h at very light loads to < 0.2 kg_{fuel}/kW-h for large diesel engines running at a high load.

The Working Group used the conversions to compare emissions from new technology diesel engines with those from gasoline engines (Table 1.7). [The Working Group noted that these data are for typical operating cycles and for vehicles with the latest technology for both diesel and gasoline engines. Values of miles per gallon and BSFC are the best estimates for a given test cycle and vehicle type when values are not provided by the authors.] The current heavy-duty PM emission standard converts to 34 mg/kg_{fuel}. The values for the four engines tested by Khalek *et al.* (2011) averaged 5 mg/kg_{fuel}; those for the vehicles tested by Herner *et al.* (2009) and Andersson *et al.* (2007) ranged from 2 to 17 mg/kg_{fuel}; and the vehicle retrofitted with a DPF in the study by Vouitsis *et al.* (2009) emitted 47 mg/kg_{fuel}.

For gasoline vehicles, the current light-duty PM emission standards in Europe and in the USA are 78 and 62 mg/kg_{fuel}, respectively. The estimate of emissions from the current in-use gasoline fleet (CARB, 2011) is 25 mg/kg_{fuel} and the emissions from the various fleets tested ranged

Table 1.7 Comparison of fuel-specific emissions of diesel engines and gasoline engines

Technology	Vehicle type	PM emission level			Assumed	High MW PAHs			1-Nitropyrene		
		(g/bhp-h)	(g/mile)	(g/kg fuel)	(mpg)	(ng/ bhp-h)	(ng/mile)	(ng/kg fuel)	(ng/ bhp-h)	(ng/mile)	(ng/kg fuel)
New technology diesel engines[a]											
2010 HDV diesel standard		0.01		0.034							
Khalek *et al.* (2011)		0.0014		0.005		0.5		2	20		70
Liu *et al.* (2010)						77		260	0.25		1
Pakbin *et al.* (2009)					8		5.6	7			
Heeb *et al.* (2008)								2			350
Herner *et al.* (2009)	High emitter		0.0140	0.017	6						
Herner *et al.* (2009)	Low emitter		0.0010	0.002	4						
Andersson *et al.* (2007)	Average		0.0010	0.010	35						
Vouitsis *et al.* (2009)	Average		0.0043	0.047	35		44	480			
Traditional diesel engines											
Wail & Hoekman (1984)		Unregulated[b]		NA	6	78 000		220 300			
PFI gasoline											
Current LDV standard (USA)			0.0100	0.062	20						
2009 Euro-5 (EU)			0.0072	0.078	35						
CARB (2011)	LDV fleet		0.0040	0.025	20						
Fujita *et al.* (2007)	Lowest emitter		0.0004	0.002	20		2600	16 000			
EPA (2008)	Latest LDV		0.0020	0.012	20		2700	17 000		0.7	2
Li *et al.* (2006)	SULEV		0.0002	0.001	20						
Andersson *et al.* (2007)	Average		0.0008	0.009	35						

Table 1.7 (continued)

Technology	Vehicle type	PM emission level			Assumed	High MW PAHs			1-Nitropyrene		
		(g/bhp-h)	(g/mile)	(g/kg fuel)	(mpg)	(ng/bhp-h)	(ng/mile)	(ng/kg fuel)	(ng/bhp-h)	(ng/mile)	(ng/kg fuel)
Vouitsis *et al.* (2009)	Average		0.0021	0.023	35		216	2400			
GDI											
Andersson *et al.* (2007)	Average		0.0123	0.134	35						
Zhang & McMahon (2012)	Average		0.0039	0.036	30						

[a] Assumed BSFC of 0.4 kg/kWh
[b] Actual BSFC of 0.475 kg/kWh
BSFC, brake-specific fuel consumption; EU, European Union; GDI, gasoline direct injection; HDV, heavy-duty vehicle; LDV, light-duty vehicle; mpg, miles per gallon; MW, molecular weight; NA, not applicable; PAHs, polycyclic aromatic hydrocarbons; PFI, port-fuel injection; PM, particulate matter; SULEV, super ultralow-emitting vehicle

from 1 to 23 mg/kg$_{fuel}$. The average value of PM emissions from the three GDI vehicles tested by Andersson *et al.* (2007) was 130 mg/kg$_{fuel}$, but engines have evolved rapidly, and the average level of emissions from nine GDI vehicle tested 5 years later by Zhang & McMahon (2012) was 36 mg/kg$_{fuel}$. Levels of PAHs and 1-nitropyrene were also converted into nanograms per kilogram of fuel. The level of high-molecular-weight particle-bound PAHs ranged from 2 to 480 and from 2400 to 17 000 ng/kg$_{fuel}$ for DPF-fitted diesel and PFI gasoline engines, respectively. Emissions of 1-nitropyrene ranged from 1 to 350 ng/kg$_{fuel}$ for DPF-fitted diesel engines, and those for the only gasoline engine measured were 2 ng/kg$_{fuel}$.

1.2 Sampling and analysis

Diesel and gasoline engine exhausts are complex mixtures of gaseous and PM species that require special methods for sampling and analysis. The gaseous species of interest are carbon monoxide, nitrogen oxides and various volatile and semi-volatile organic compounds; PM is a complex matrix in involving EC and organic carbon, various ions and metals.

This section reviews the methodologies of sampling and analysis for diesel and gasoline exhausts constituents, as well as the corresponding methodologies for monitoring ambient air and biomonitoring.

1.2.1 Sampling

(a) Experimental settings and testing

Sampling of engine exhaust is typically carried out in specialized laboratories where the engine (usually heavy-duty engines) or the vehicle (usually light-duty passenger vehicles) is operated under prescribed conditions while connected to a dynamometer which simulates the load on the engine. Heavy-duty vehicles can also be tested on chassis dynamometers, and an increasing number of studies have used transportable heavy-duty vehicle chassis dynamometers (Zhen *et al.*, 2009). The dynamometer drive and duty cycles used for the measurement of exhaust emissions generally attempt to replicate the main characteristics of the type of load that the engines may experience during 'real-life' use.

The raw exhaust is typically introduced into a constant volume sampling system with a dilution tunnel where it is diluted with air filtered by high-efficiency particulate filters at dilution ratios in the range of 10–100 to avoid condensation before PM and gaseous samples are collected from the diluted sample for analysis. Gaseous and PM species in engine exhaust can be sampled under either raw or dilute conditions. However, particulate sampling must be diluted even when the first sampling step is from undiluted exhaust. The dilution step for particulate sampling is to ensure that hydrocarbon vapours condense onto the particles to simulate ambient exposure conditions. When particulates reach the sample filters, the temperature must be 47 °C ± 5 °C (range, 42–52 °C) (IRSG, 2012a, b). Traditionally, diesel PM has been collected on filter media and then analysed to determine its chemical composition. The most commonly used method to determine the gravimetric PM mass is the sampling technique described by the EPA (2000, 2001) in the USA. Filter sampling is sensitive to thermodynamic and chemical phenomena, and is also accompanied by potential reactions of gases with the PM on the filter or with the filter medium during sampling and the absorption of water from humid air (IRSG, 2012a, b).

Gases (carbon monoxide and carbon dioxide, and nitrogen oxides) and hydrocarbon vapours in dilute exhaust are collected in tedlar bags or Summa canisters sequentially over the test phase(s) and analysed online by dedicated non-dispersive infrared (carbon monoxide and carbon dioxide), chemiluminescence (nitrogen oxides) or flame ionization detectors (total hydrocarbons), respectively. Corresponding measurements are made for the dilution air to correct for

any contribution to the diluted exhaust from that source. The dedicated analysers can also be used for taking instantaneous measurements during a test. Carbon dioxide is measured essentially to determine fuel consumption via a carbon balance and may be used to determine the dilution ratio. Volatile organic compounds collected in tedlar bags can later be analysed by GC-MS methods. Carbonyl compounds are absorbed in dinitrophenylhydrazine cartridges while gas- and particle-phase semivolatile PAH and nitro-PAH species are adsorbed onto different media for later chemical analysis.

The details of procedures for testing vehicles and engines are proscribed by various bodies in the USA (EPA, 2000, 2001) and in Europe. The test methods in Europe and in the USA are similar, although the European engine test cycles reflect road and use conditions that differ from those in the USA (CONCAWE, 2006a, b, c).

In contrast to the dilution approach, Heeb *et al.* (2007, 2008, 2010) described a special sampling procedure in which undiluted exhaust was sampled through a device comprising a probe, a cooler, a condensate separator, a filter stage and a two-stage adsorber unit. The procedure was used in the investigation of the secondary effects of 14 different DPFs with respect to PAHs, nitro-PAHs and dioxins/furans.

(b) Ambient air

Carbon monoxide and nitrogen oxides can be monitored continuously in ambient air. Hourly averages are typically recorded. Passive samplers are used for personal exposure in occupational settings (methods 6104 and 6604 for nitrogen oxides and carbon monoxide, respectively; NIOSH, 1994a, 1996). Analysis of organic compounds (volatile organic compounds and PAHs) in ambient air relies on methods similar to those for engine exhaust without dilution, and sampling is typically carried out using Summa canisters rather than tedlar bags.

PM mass concentration is typically determined by methods based on gravimetric filters that can be distinguished by size (e.g. PM_{10}, $PM_{2.5}$ and $PM_{1.0}$) when the sampling apparatus has size-selective inlets designed to exclude particles with aerodynamic diameters greater than the specified number (in micrometres). Compared with dilute exhaust, higher volumes of ambient air need to be sampled due to its lower concentrations. Several techniques provide continuous (real-time) PM concentrations in ambient air with appropriate calibration against reference methods.

1.2.2 Analysis

(a) Particulate matter

The mass concentration, size distribution and chemical composition of Particulate matter (PM) are all important factors from the perspective of health effects in humans. $PM_{2.5}$ has been widely used as a general marker of fine-particle air contaminants (see Section 1.4.2), but is less useful for traffic emissions due to the high background of materials related to regional emissions and because exhaust components comprise only a small fraction of the total mass.

Burtscher (2005) reviewed methods for the physical characterization of particulate emissions from diesel engines in terms of particle mass, number, surface area, density and structure. Particle mass and number counts, together with size distribution, are the more widely used measures. Particle number counts are determined by condensation particle counters. Coagulation, diffusional losses and the variation in lower size limit (between 3 nm and ~15 nm) of different condensation particle count models may account for variations in number counts (Burtscher, 2005). Systems to determine size distributions in the submicron range are based on mobility analysis or impaction – for example, the scanning mobility particle sizer (Wang & Flagan, 1990) or the electrical low pressure impactor (Keskinen

et al., 1992). Schlatter (2000) performed a round robin test of 11 scanning mobility particle sizer systems and showed differences of up to 10% in the particle size and 20% in the number concentration of diesel particles; these differences were attributed mainly to inaccurate flow controllers.

The transverse element oscillating microbalance can give mass concentrations in real time for both engine exhausts and ambient air that are in line with gravimetric methods. Ayers *et al.* (1999) reported ~30% lower values compared with gravimetric methods for ambient air measurements in four Australian cities, which they attributed to the loss of semi-volatile aerosol material from the heated sample filter employed on the microbalance. A calibration/correction model has been proposed to resolve this discrepancy (Green *et al.*, 2009).

Portable dust monitors for $PM_{2.5}$ and PM_{10} are used effectively to assess occupational and environmental exposure to aerosols. Cheng (2008) provided comparisons of measurements from gravimetric methods and the transverse element oscillating microbalance, with satisfactory results when appropriately calibrated.

PM in engine exhaust is a complex mixture of EC and organic carbon, metals and sulfate. EC and organic carbon are discussed in a separate section below.

The major elemental components can be analysed by X-ray fluorescence, a non-destructive measurement technique made directly on the filter. However, inductively coupled plasma-MS is necessary for trace and ultra-trace components; this destructive method requires acid digestion to dissolve the sample. Ion chromatography is used for inorganic and organic ionic species (nitrate, nitrite, sulfate, sulfite, elemental anions, ammonium, elemental cations, formate, acetate and oxalate). Capillary electrophoresis is used for the quantitation of organic acid ions and to confirm the results of inductively coupled plasma-MS.

(b) Elemental and organic carbon

The determination of EC and organic carbon components of PM is based on the principle of heating the filter in an inert atmosphere (helium) in which the organic carbon is expected to be removed from the PM on the filter by volatilization and pyrolysis, and then repeating the process in an oxidizing gas (oxygen–helium) in which EC is expected to be converted to carbon dioxide. Changes in the optical properties of PM on the filter, as determined by the reflection or transmittance of laser illumination, govern the temperature ramp and the switch between the inert and oxidizing gas environment. The fractions removed in subsequent temperature intervals are then termed sequentially.

Chow *et al.* (2001) compared the operational protocols used in the IMPROVE and National Institute of Occupational Safety and Health (NIOSH) networks/methodologies. Similarly, ten Brink *et al.* (2004) reported on the comparability of methods used in Europe to measure the concentration of aerosol–carbon. Four methods are available to measure EC/organic carbon, which differ in the times, temperatures and inert versus oxidative environments at different stages of the procedure. Two apply heat in a reduced atmosphere to remove organic carbon and one of these corrects for charring/pyrolysis of the sample with optical correction. The other two use a two-step oxidative method, with a first oxidation step at a lower temperature to remove organic carbon under oxidative conditions, and a second final oxidation step at a high temperature to oxidize EC. Differences between the methods of more than a factor of 3 were observed, but the daily EC concentrations over the sampling period were highly correlated ($r^2 > 0.9$).

(c) Black carbon

The terms ‘elemental carbon’, ‘soot’, ‘black carbon’ and ‘light-absorbing carbon’ in suspended particles are used loosely and often

interchangeably in the literature. The definition of EC is further complicated by the many different methods applied for its quantification, the most commonly applied being thermal evolution, light transmission or reflectance and solvent extraction. Chow *et al.* (2001) compared two studies that applied different protocols (developed for each of these methods) to the same samples with instruments calibrated against common standards. Results for total carbon were usually very consistent between protocols (typically within ~5%). Data for EC were often also reasonably comparable (within ~20% for diesel exhaust samples); however, differences by a factor of 2 or more were observed when ambient samples were measured with different protocols.

The aethalometer is one of the most frequently used techniques to measure real-time black carbon mass concentrations, especially for long-term background measurements. It is based on the attenuation of a broad-band light source (maximum wavelength, < 820 nm) due to particles accumulated on a quartz fibre filter. In one study at an Alpine site, EC and black carbon measurements were in good agreement (Lavanchy *et al.*, 1999).

Hitzenberger *et al.* (1999) compared one thermal and two optical methods that were applied to laboratory-generated aerosols that contained only black carbon. Differences in the measurement of black carbon of 20–30% were observed between the methods.

In the INTERCOM 2000 study (ten Brink *et al.*, 2004), black carbon was measured using three independent methods: the aethalometer, the integrating sphere technique and a third method that determines the difference in light reflection of loaded and unloaded filters. Similar to the observations on EC, the differences between the results for black carbon were considerable; however, the daily concentrations were highly correlated.

The comprehensive Carbonaceous Species Methods Comparison Study was carried out with the participation of 30 different research groups from the USA and Canada (Lawson & Hering, 1990). Special sampling artefact studies were also performed simultaneously with side-by-side ambient sampling to assess the relative magnitude of absorption, volatilization or chemical reaction artefacts under field conditions.

(d) Polycyclic aromatic hydrocarbons (PAHs) and nitro-PAHs

Nitro-PAHs are difficult to analyse because they are typically present at low concentrations (typically low or subpicograms per cubic metre in air) and in combination with significant concentrations of other compounds that may interfere with the analysis. Before detection by chemical analysis, these substances were identified and quantified by fractionation of diesel exhaust particulate extracts and subsequent testing in the *Salmonella typhimurium* mutagenicity (Ames) assay (Gibson, 1983; Arey *et al.*, 1988; Kinouchi *et al.*, 1988; Hayakawa *et al.*, 1995). The involvement of nitro compounds was suggested by the appearance of strong direct mutagenicity, which was reduced by addition of a liver enzyme microsome fraction and also by the use of bacterial strains with a low or lack of nitroreductase activity for a comparison with mutagenic activity in the parent strains. Strains with enhanced nitroreductase activity were introduced for the sensitive detection of nitrated mutagens (Scheepers *et al.*, 1991).

The chemical analysis of nitro-PAHs has been reviewed extensively (IPCS, 2003). Zielinska & Samy (2006) reviewed many of these considerations for analysis in air, most methods of which collect the aerosol onto filters (Teflon-coated glass fibre or quartz fibre filters) and sorbents (e.g. XAD-4). The PAH or nitro-PAH species captured on the filter or sorbent media is recovered by solvent extraction. The solvents are typically used in a specialized (Soxhlet, microwave extraction, supercritical fluid extraction or ultrasonication) apparatus and comprise hexane,

toluene, dichloromethane, acetone and methanol, used individually or in combination.

Most methods require extensive solvent clean-up of the extracts before analysis. Extraction methods may include Soxhlet, sonication or pressurized fluid extraction. Common approaches include GC-MS and liquid chromatography-MS. However, several other chromatographic methods have been used successfully, many of which required chemical modification (derivatization) of the nitro-PAHs before analysis to enhance affinity for specialized detectors, such as electron capture or fluorescence (Zielinska & Samy, 2006). Immunoaffinity methods have also been used with some success (Zühlke *et al.*, 1998). Negative ion chemical ionization-GC-MS has historically been the method of choice because it provides enhanced sensitivity and selectivity compared with many other methods. Liquid chromatography-MS has not been used as extensively as other techniques.

Pandey *et al.* (2011) reviewed the techniques for the determination of airborne PAHs in the gas and particle phases (Fig. 1.9 and Fig. 1.10), while the review by Zielinska & Samy (2006) focused specifically on nitro-PAHs (Table 1.8). Analytical procedures for the individual nitro-PAHs reviewed in this Volume are presented in their respective *Monographs* and are not discussed further here.

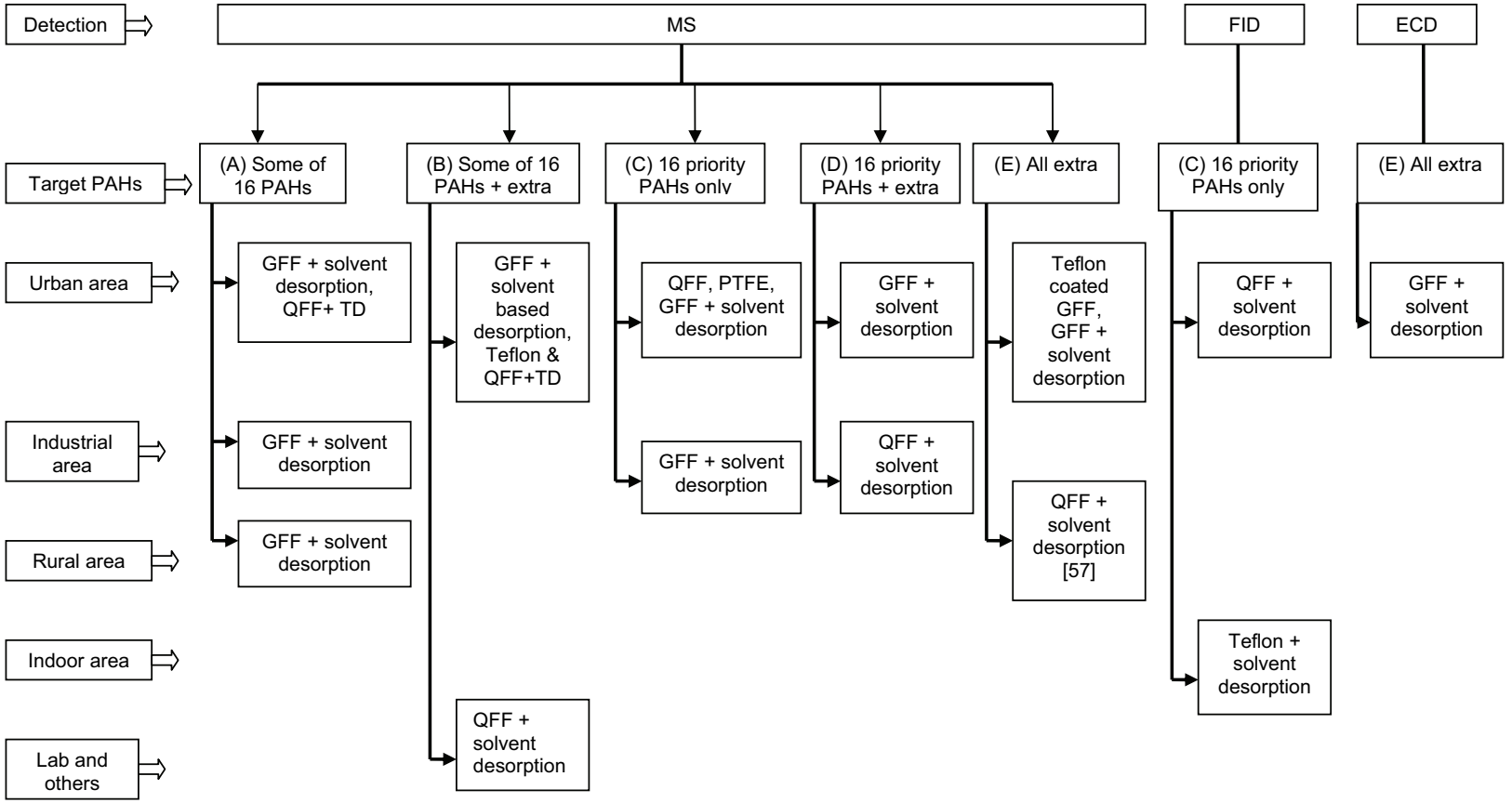
The high-efficiency recovery of all the target species from the filter and sorbent media is an important consideration in these techniques and has been an even greater challenge for nitro-PAH species. Recovery of the target species from the sampling medium is quantified by adding an internal standard (e.g. triphenylene and *para*-quaterphenyl; Schauer *et al.*, 2004) or deuterated nitroarenes that have the same recovery rates as the non-deuterated analogues (Zielinska & Samy, 2006). Albinet *et al.* (2006) reported recovery rates of nitro-PAHs for the whole analytical procedure in the range of 14% (1-nitronaphthalene) to 84% (7-nitrobenz[*a*]anthracene), while those of oxy-PAHs ranged from 5% (1-naphthaldehyde) to 83% (benzo[*b*]fluorenone).

Castells *et al.* (2003) used a two-step supercritical fluid extraction procedure to remove non-polar compounds, such as aliphatic hydrocarbons, using pure carbon dioxide as the extraction fluid in the first step, and extract nitro- and oxy-PAHs with toluene–carbon dioxide in the second, with total recoveries of 68–93% (Table 1.9). The second step represents one- third to half of the total recovery. Total 1-nitropyrene recovery was 72%, with 42% from the second step. An earlier study (Paschke *et al.*, 1992) compared the extraction of PAHs and nitro-PAHs from diesel exhaust particulates and diesel soot using supercritical chlorodifluoromethane, carbon dioxide and carbon dioxide with added modifiers. Supercritical fluid extraction with pure chlorodifluoromethane yielded the highest recovery (117 ± 7%) of 1-nitropyrene and carbon dioxide modified with toluene yielded 97 ± 7%.

'Reduction' refers to the process by which nitro-PAH species are reduced to their respective amino-PAHs or other derivatives; this is required for sensitive detection because the parent nitro-PAHs exhibit only very weak fluorescence and chemiluminescence signals (Schauer *et al.*, 2004). 'On-line' and 'off-line' methods exist for this reduction process; the first requires an additional column packed with a reduction agent before the analysis and the second is achieved using a titanium (III) citrate complex as the reducing agent in a hydrochloric acid medium (Table 1.9).

Although the derivatization process is used mostly for high-performance liquid chromatography (HPLC)-fluorescence detection and HPLC-circular dichroism systems, Brichac *et al.* (2004) reported an application of derivatization with a gas chromatography-electron capture detector (GC-ECD). The measurements of nitro-PAHs in ambient PM involved their reduction to the corresponding amino-PAHs with sodium borohydride, followed by derivatization with

Fig. 1.9 Plot of all possible combinations of sampling and detection methods of polycyclic aromatic hydrocarbons in the particle phase



ECD, electron capture detector; FID, flame ionization detector; GFF, glass fibre filter; MS, mass spectroscopy; PAH, polycyclic aromatic hydrocarbons; PTFE, polytetrafluoroethylene filter; QFF, quartz fibre filter; TD, thermal desorption

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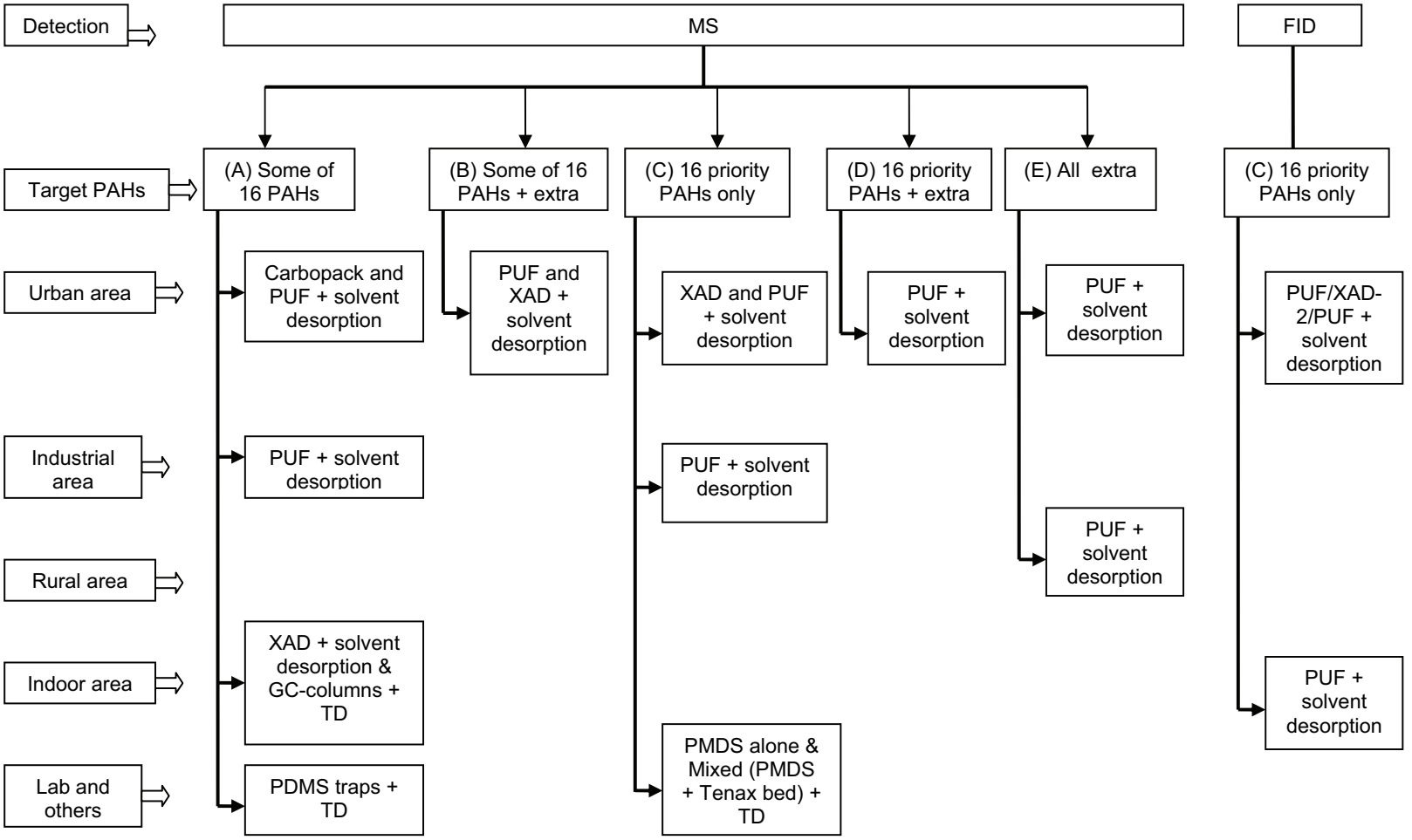
Fig. 1.10 Plot of all possible combinations of sampling and detection methods of polycyclic aromatic hydrocarbons in the vapour phase



FID, flame ionization detector; GC, gas chromatography; MS, mass spectroscopy; PAH, polycyclic aromatic hydrocarbons; PDMS, polydimethylsiloxane foam; PTFE, polytetrafluoroethylene filter; PUF, polyurethane foam; TD, thermal desorption; XAD, polymeric resin
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Table 1.8 Examples of the detection ranges for nitro-polycyclic aromatic hydrocarbons (N-PAHs) associated with particulate matter analysed by gas chromatography/mass spectrometry with various detectors

N-PAH[a]	Detection mode	Extraction technique	Detection range	Sampling environment	Reference
1-NP, 3-NF, 3-NPh, 6-NC, 1,3-DNP, 1,8-DNP	NICI	SE	5–80 ng/m^3, sum for all six species	Industrial workplaces	Chaspoul *et al.* (2005)
1-NP, 2-NP, 7-NBA, 6-NC, 1,3-DNP, 1,6-DNP, 1,8-DNP, 6-NBP, 2-NF, 3-NF, 9-NA, 9-NPh, 2-NFl	NICI	PFE	< 2–282 ng/g	Urban dust (SRM 1649a)	Bamford *et al.* (2003)
19 N-PAHs (see reference for details)	NICI	SE	< 0.1–99 pg/m^3	Urban/suburban (ambient)	Bamford & Baker (2003)
1-NP	EI	SFE	24–104 pg/m^3	Urban (ambient)	Castells *et al.* (2003)
1-NN+2-NN, 14 total MNN	EI	SE	10–1906 pg/m^3	Urban (ambient)	Gupta *et al.* (1996)
1-NN+2-NN	NICI	SE	202–494 pg/m^3	Urban (ambient)	Reisen *et al.* (2003)

[a] 1,3-DNP, 1,3-dinitropyrene; 1,6-DNP, 1,6-dinitropyrene; 1,8-DNP, 1,8-dinitropyrene; MNN, methylnitronaphthalenes; 9-NA, 9-nitroanthracene; 7-NBA, 7-nitrobenz[*a*]anthracene; 6-NBP, 6-nitrobenzo[*a*]pyrene; 7-NBaA, 7-nitrobenz[*a*]anthracene; 6-NC, 6-nitrochrysene; 2-,3-NF, 2- or 3-nitrofluoranthene; 2-NFl, 2-nitrofluorene; 1-,2-NN, 1- or 2-nitronaphthalene; 3-NPh, 3-nitrophenanthrene; 9-NPh, 9-nitrophenanthrene; 1-NP, 1-nitropyrene; 2-NP; 2-nitropyrene
EI, electron ionization/impact; NICI, negative ion chemical ionization; PFE, pressurized fluid extraction; SE, Soxhlet extraction; SFE, supercritical fluid extraction; SRM, standard reference material
From Zielinska & Samy (2006)

heptafluorobutyric anhydride. This procedure adds a polyfluorinated group to the amino-PAH, thus enhancing the sensitivity of electron capture detector analysis by approximately 10-fold in comparison with the GC-ECD analysis of underivatized nitro-PAHs. Murahashi *et al.* (2003a) used a GC-MS method that does not require derivatization (Table 1.10).

(e) Carbon monoxide

Non-dispersive infrared detection is the basis for instruments used to quantify both carbon monoxide and carbon dioxide in exhaust and ambient air, and is a well established method that is widely used.

Gasoline engines typically emit more carbon monoxide per unit quantity of fuel than diesel engines. Thus, carbon monoxide levels have been used as an indicator of emissions from light-duty passenger vehicles. For example, ventilation systems in closed underground garages are typically triggered by carbon monoxide sensors.

NIOSH method 6604 (NIOSH, 1996) describes the analysis of passive sampler media for the measurement of carbon monoxide concentrations in ambient air and occupational settings.

(f) Nitrogen oxides

Chemiluminescent analysers are the principal analytical technology available for determining levels of nitric oxide emissions from combustion sources. Nitric oxide in a gas sample reacts with ozone, which is prepared by an ozone generator in a reaction chamber. The chemiluminescence generated in this reaction is detected with a photomultiplier. The intensity of nitric oxide is in direct proportion to its concentration in a wide range. By switching a three-way valve, the sample gas is passed through a converter to convert nitrogen dioxide to nitric oxide;

Table 1.9 Recoveries obtained for two-step supercritical fluid extraction of spiked[a] urban aerosol samples

Compound	Spiked samples			Non-spiked samples
	Total recovery (%)	RSD (%) (n = 3)	Second SFE step[b] (%)	Second SFE step[b]
1,4-Naphthoquinone	84	4	25	ND
2-Methyl-1-nitronaphthalene	68	6	29	ND
2-Nitronaphthalene	70	7	26	ND
9-Fluorenone	76	7	25	28
Acenaphthenequinone	86	3	27	ND
9,10-Anthraquinone	70	7	33	37
1,5-Dinitronaphthalene	89	8	29	ND
2-Methyl-9,10-anthraquinone	78	3	28	26
2-Nitrofluorene	93	5	40	ND
9-Nitroanthracene	89	6	32	ND
Benzanthrone	70	5	37	35
Benz[*a*]anthracene-7,12-dione	78	5	46	48
1-Nitropyrene	72	7	42	39

[a] Spiking conditions: 0.5 mL dichloromethane, incubation time 2 hours
[b] Percentages of the total amount extracted
ND, not detected; RSD, relative standard deviation; SFE, supercritical fluid extraction
From Castells *et al.* (2003)

the concentration of nitrogen oxides (nitric oxide and nitrogen dioxide) can be obtained by measuring the intensity of the chemiluminescence (Bionda *et al.*, 2004). The concentration of nitrogen dioxide in the sample gas can be obtained by calculating the difference between the two measured values. The method is very sensitive and selective for the quantification of nitrogen oxides. Chemiluminescent analysers are usually calibrated with a blend of nitric oxide in a nitrogen balance.

NIOSH method 6014 describes the analysis of passive sampler media for the measurement of concentrations of nitric oxide and nitrogen dioxide in ambient air and occupational settings (NIOSH, 1994a).

Nitric oxide has been used as a marker for HGV exhaust emissions (see Section 1.4.2).

(g) Volatile organic compounds

Volatile organic compounds are typically analysed by GC-MS, although a preconcentration step (by an automated cryogenic concentrator interfaced to the gas chromatograph) is required to achieve adequate detection limits with flame ionization detection. Heavier hydrocarbons (C2–C10) in diesel exhaust are captured on Tenax, solvent extracted and then analysed by GC-flame ionization detection. Aldehydes and ketones are trapped on dinitrophenylhydrazine cartridges. The hydrazones formed on the cartridge are then analysed by HPLC. These methods provide quantitative analysis for about 100 compounds.

1.2.3 Biomarkers of exposure

Sensitive analytical techniques are required to assess the uptake by tissues of chemicals from exhaust emissions.

Table 1.10 Examples of limits of detection (LOD) (per sample) for nitro-polycyclic aromatic hydrocarbon (nitro-PAH)-associated particulate matter analysed by high-performance liquid chromatography with various reduction techniques and detectors

nitro-PAH	LOD (pg) S/N 3	Reduction technique	Detection method	Reference
1-NP, 2-NFl, 6-NC	46, 41, 584	Titanium(III) citrate	FD	Brichac *et al.* (2004)
1-NP, 1,3-DNP, 1,6-DNP, 1,8-DNP	2.20, 1.03, 1.61, 1.71	Electrochemical	FD	Kuo *et al.* (2003)
1-NN, 2-NN, 2-NFl, 9-NPhe, 1-NP, 6-NC, 6-NBaP, 9-NA, 3-NF, 1,6-DNP, 7-NBaA, 1,3-DNP	6–100 (4–80)	Pt (Pt/Rh)	FD	Schauer *et al.* (2004), see also Tejada *et al.* (1986)
3-NBA	2	Raney nickel/ hydrazine	FD	Murahashi *et al.* (2003a)
3-NBA, 2-NTP, 1-NP, 1,8-DNP, 6-NC, 2-NF, 3-NF	5.50, 11.0, 2.0, 6.0, 17.0, 10.0, 3.0	Pt/Rh	CD	Inazu *et al.* (2004)
1-NP, 1,3-DNP, 1,6-DNP, 1,8-DNP	100, 3, 6, 3	Pt/Rh	CD	Hayakawa *et al.* (2001)
1,8-DNN, 9-NA, 3-N-9-Fl, 2,2-DNBphe, 1-NP, 2,7-DNFl, 2,7-DN-9-Fl	1–10	None	MS-NICI	Bonfanti *et al.* (1996)
2-NN, 9-NA, 1-NP	300–1600	Electrochemical	ED	Galceran & Moyano (1993)

CD, chemiluminescence detection; 2,7-DNFl, 2,7-dinitrofluorene; 2,7-DN-9-Fl, 2,7-dinitro-9-fluorene; 2,2-DNBphe, 2,2′–dinitrobiphenyl; 1,8-DNN, 1,8-dinitronaphthalene; 1,3-DNP, 1,3-dinitropyrene; 1,6-DNP, 1,6-dinitropyrene; 1,8-DNP, 1,8-dinitropyrene; ED, electrochemical detection; FD, fluorescence detection; MS, mass spectrometry; 9-NA, 9-nitroanthracene; 7-NBaA, 7-nitrobenz[*a*]anthracene; 3-NBA, 3-nitrobenzanthrone; 6-NBaP, 6-nitrobenzo[*a*]pyrene; 6-NC, 6-nitrochrysene; 2-,3-NF, 2- or 3-nitrofluoranthene; 2-NFl, 2-nitrofluorene; 3-N-9-Fl, 3-nitro-9-fluorene; NICI, negative ion chemical ionization; 1-,2-NN, 1- or 2-nitronaphthalene; 9-NPhe, 9-nitrophenanthrene; 1-NP, 1-nitropyrene; 2-NTP, 2-nitrotriphenylene; Pt/Rh, platinum/rhodium; S/N, signal-to-noise ratio
From Zielinska & Samy (2006)

(a) Biomarkers of exposure to diesel engine exhaust

Organic constituents associated with the particulate phase of diesel exhaust have been proposed as chemical markers of exposure. The measurement of these biomarkers in human body fluids requires sophisticated and ultrasensitive analytical methods. Some of the methods that have been developed to achieve this are discussed below.

(i) Protein and DNA adducts

Nielsen *et al.* (1996) used GC-MS to detect hydroxyethylvaline adducts in haemoglobin as a biomarker to assess exposure to diesel exhaust in garage workers. In addition, they used P1 nuclease extraction of DNA adducts and a butanol method to extract polar DNA adducts from peripheral blood lymphocytes, and detected these adducts using ^{32}P-postlabelling.

Zwirner-Baier & Neumann (1999) detected several nitro-PAH metabolites after hydrolysis of haemoglobin using liquid chromatography equipped with negative ion chemical ionization and MS. [The Working Group noted that this method is based on the hydrolysis of haemoglobin adducts before detection of the free metabolites. It is not certain whether the entire quantity of each of the retrieved (free) metabolites originates from covalent binding to haemoglobin.]

(ii) Urinary metabolites

1-Hydroxypyrene, a urinary metabolite of pyrene, was used as a marker for exposure to diesel exhaust in garage workers (Nielsen *et al.*, 1996). Using GC-MS, Seidel *et al.* (2002) found elevated urinary excretion of 1-Hydroxypyrene

and hydroxylated phenanthrene metabolites in salt miners operating diesel-powered equipment. 1-Hydroxypyrene may not be sufficiently specific because it may originate from other combustion sources. A more specific marker for diesel exhaust is 1-nitropyrene (Schuetzle & Perez, 1983; Scheepers *et al.*, 1995a). Urinary metabolites of 1-nitropyrene were measured in humans exposed to diesel exhaust using immunoassays (Scheepers *et al.*, 1994, 1995b). This method is very sensitive but cross-reactivity with other PAH metabolites was reported (Scheepers *et al.*, 1995b). A more specific approach to the analysis of urinary metabolites is HPLC with fluorescence detection.

(b) Biomarkers of exposure to gasoline engine exhaust

Carbon monoxide and lead are the most frequently used biomarkers of exposure to gasoline exhaust emissions (see Section 1.4.1(b)).

(i) Carbon monoxide

Exposure to carbon monoxide can readily be detected using spectrometric principles in exhaled air and also as carboxyhaemoglobin in the blood (Lee *et al.*, 1994; Gourdeau *et al.*, 1995). [The Working Group noted that direct-reading spectrometric instruments may not have the accuracy of most off-line analytical methods used for routine analysis in hospitals.]

(ii) Lead and platinum

The measurement of metals such as lead and platinum in blood or urine requires sensitive inductively coupled plasma-MS methods, which are well established (Iavicoli *et al.*, 2004; Ghittori *et al.*, 2005; Heitland & Köster, 2006).

(iii) Volatile organic compounds

Using GC-MS, organic constituents of gasoline exhaust emissions can be detected with high specificity and sensitivity. Benzene, toluene, ethylbenzene, xylene isomers and other volatile organic compounds have been measured in exhaled air (Jo & Song, 2001), blood (White *et al.*, 1995; Romieu *et al.*, 1999) and urine (Ghittori *et al.*, 2005).

1.3 Regulations and guidelines

1.3.1 Structure and progression of emission standards

There is a strong link between emission standards and engine technology: standards drive the technology and technology enables more stringent standards. Increasing environmental concerns over the past two decades have resulted in regulatory action around the globe to introduce successively more rigorous emission standards. The technological developments and their impact on the characteristics of engines and of vehicle emissions are presented in Section 1.1.

Standard values for different categories of engines and vehicles from several countries are presented in detail in the Annex. The Section below gives an overview of the structure and development of the standards and their penetration into the fleet.

Except for the USA, most countries worldwide have adopted European Union standards, occasionally with small local modifications and at a rate dictated by local economics and other factors. Different test cycles are used by Europe and the USA and across different applications. Emissions are expressed in a variety of units depending upon vehicle type – usually in grams per mile (g/mi) or per kilometre (g/km) when the entire vehicle is tested, and g/bhp-h or per kilowatt-h (g/kW-h) when only the engine is tested. Normally, the engine is tested for heavier commercial and industrial applications, and the vehicle for passenger cars and light goods vehicles. Table 1.11 gives a very approximate relationship between these units. For PM, standards based on both mass and number are shown. The engine or vehicle operating test cycle, the protocol used to measure emissions and the absolute number of

Table 1.11 Equivalent emission standards

Mass emissions					Number emissions			
Heavy-duty vehicles								
g/kWh	g/bhp-h	g/km	g/mile	g/kg fuel	#/kWh	#/bhp-h	#/km	#/mile
0.013	[a]0.0100	0.018	0.029	–	2.7×10^{12}	2.0×10^{12}	3.6×10^{12}	5.8×10^{12}
Light-duty vehicles								
–	–	0.0030	0.0048	0.054	–	–	[a]6.0×10^{11}	9.6×10^{11}

[a] Current standards
bhp, brake horse power; h, hour
Compiled by the Working Group

the emission standard are important to establish the stringency of the standard (see Section 1.2 for details of some of the measurement protocols).

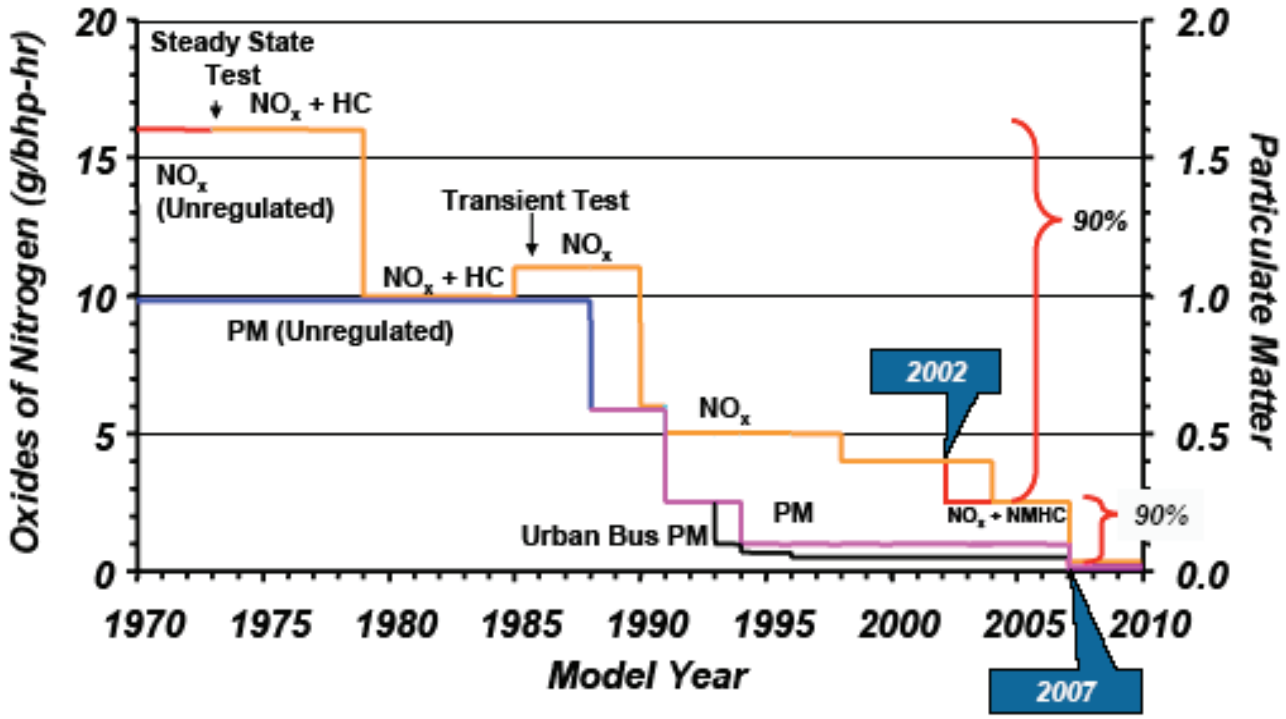
Standards are becoming increasingly more stringent throughout the world. As an example, Fig. 1.11 shows the downward stepwise trend of heavy-duty engine emission standards in the USA, starting from the first Californian standards in 1969. From the unregulated state in the early 1980s to 2010, permitted levels for nitrogen oxides, nitrogen oxides plus hydrocarbons, nitrogen oxides plus non-methane hydrocarbons, and PM have decreased progressively over time, with particulate and nitrogen oxide emission standards being reduced by more than two orders of magnitude.

Particle number standards are more stringent than mass standards, and are intended to result in more effective particulate control technologies. The current European Union light-duty mass emission standard is 4.5 mg/km and the number (#) standard is 6×10^{11} #/km. If one assumes typical diesel particle size distribution with a geometric standard deviation (σ_g) of 1.8, a geometric mean diameter of 60 nm and an effective particle density of 0.6 g/cm^3, this number standard corresponds to approximately 0.19 mg/km. If one assumes larger and more dense particles, with a geometric standard deviation of 2, a geometric mean diameter of 100 nm and a density of 1 g/cm^3, the number standard corresponds to 2.4 mg/km, i.e. about half of the mass standard.

1.3.2 Representative emission standards worldwide

To illustrate the evolution of engine and vehicle emission standards, trends for key categories of diesel-powered mobile sources for several countries are summarized in Fig. 1.12, Fig. 1.13, and Fig. 1.14: on-highway heavy-duty diesel engines, diesel passenger cars and non-road heavy-duty diesel engines. For simplicity, key changes are indicated in terms of US and European standards, even when some countries have their own local equivalents. Each figure indicates the time of introduction of the first emission standard for PM: for on-highway heavy-duty engines, Euro-1/US-1998; for passenger cars, Euro-1/USA Tier 1; and for non-road engines, Tier 1. Before these standards were introduced, particulate emissions from diesel engines were not regulated and the engines were of 'traditional technology' (grey area on the left of each chart). An intermediate level was implemented for on-highway heavy-duty diesel engines (Euro-4/USA-1994), representing a reduction of approximately one order of magnitude in PM and nitrogen oxides from unregulated levels, and corresponding to 'transitional technology' (see Section 1.1). The next levels of standards, which result in a reduction of two orders of magnitude in PM from unregulated levels and are expected to force the use of 'new technology diesel engines' are: on-highway heavy-duty engines, Euro-6/USA-2010; passenger car, Euro-5b/USA Tier 2,

Fig. 1.11 Historical trend in emission standards for heavy-duty engines in the USA



bhp, brake horse power; HC, hydrocarbon; NMHC, non-methane hydrocarbons; NO_x, nitrogen oxides; PM, particulate matter
From Department of Energy (2006)

with particle number standard; and non-road engines, Tier 4.

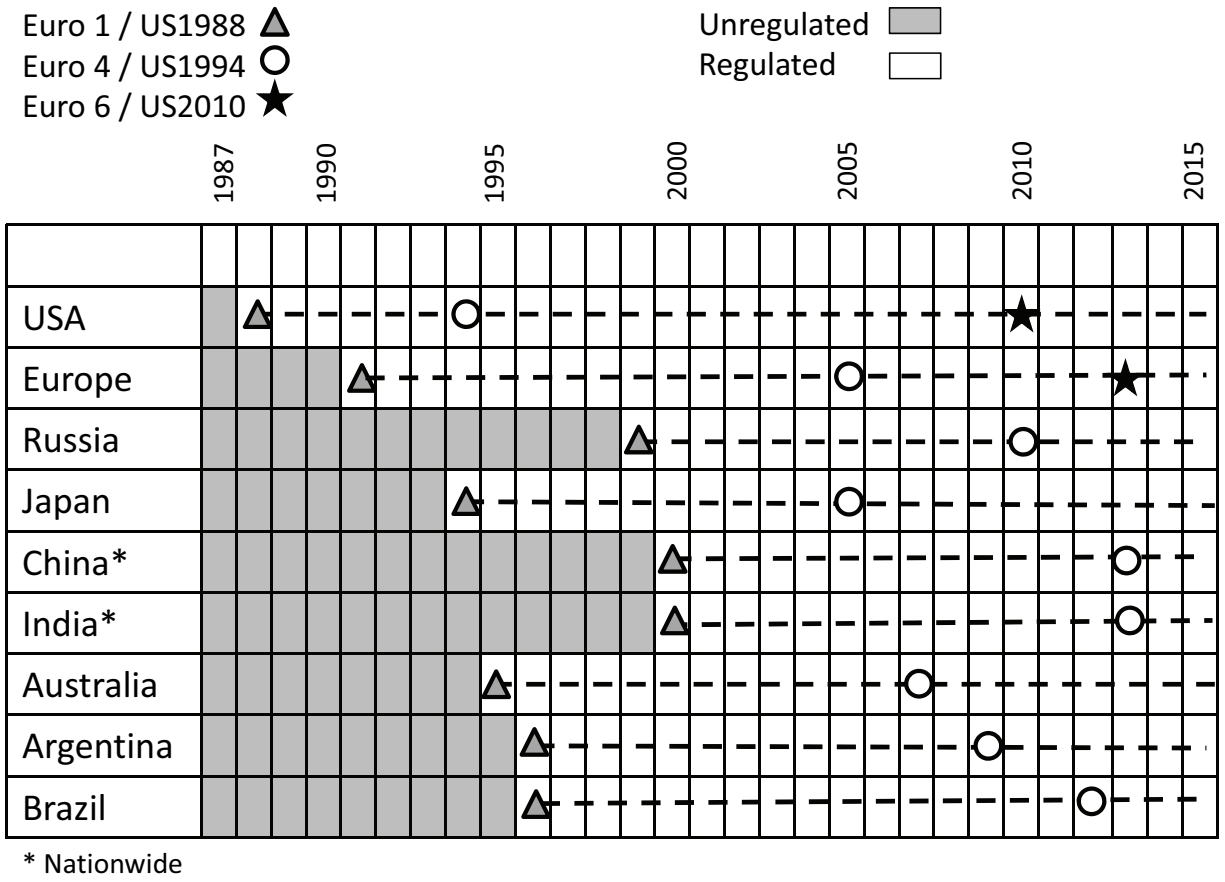
These charts also illustrate the strategy of regulatory agencies to introduce the most stringent emission controls first in on-road applications, and then in non-road and industrial applications at a later date: non-road heavy-duty applications, followed by high- and very low-horse power non-road applications, followed by locomotive and marine engines. Consequently, PM emissions from many non-road applications worldwide are still uncontrolled today.

The rate of development and implementation of technology in any country or market is governed by local regulatory standards for tailpipe emissions and fuel quality. Most countries have implemented at least basic regulations for PM for heavy-duty and light duty on-highway vehicles, although many developing countries lag behind with regard to the technology of heavy-duty and light-duty vehicles. Also, engines in non-road vehicles, especially higher horse power engines in ships and locomotives worldwide, including those in Europe and the USA, are still unregulated for PM emissions.

1.3.3 Availability of engines with new emission control technologies in the fleet/temporal impact of new emission standards

When new emission standards are implemented, all new products must comply but products already in use are generally not required to be upgraded (or 'retrofitted') to the new standard. The exception is a change in fuel standards. Thus, engines that meet new emission standards penetrate the fleet only as fast as new vehicles are

Fig. 1.12 Implementation schedule of on-road heavy-duty diesel emission standards in selected countries



Compiled by the Working Group

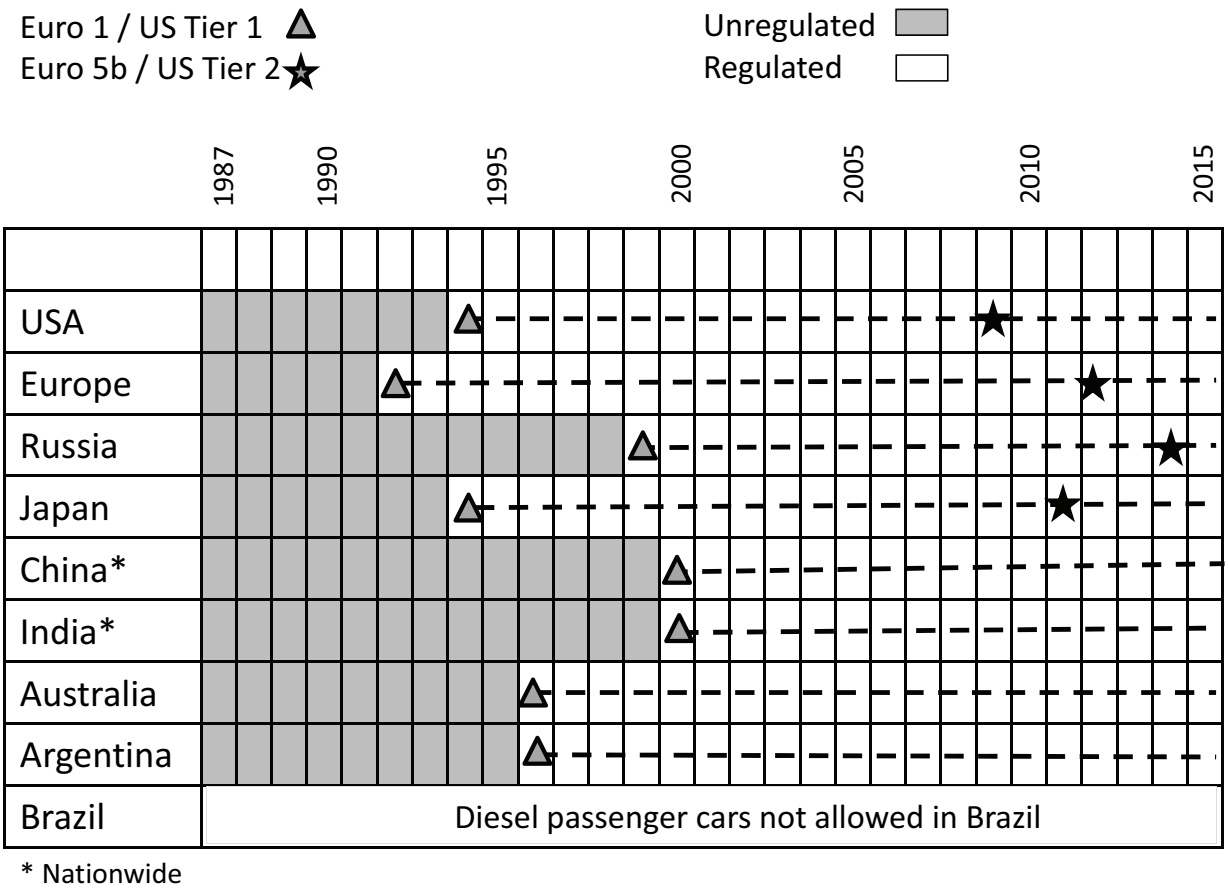
purchased to replace those already in use. For on-highway heavy-duty engines, this transition is slightly accelerated because the highest-annual-mileage applications, generally larger long-haul HGVs, favour most recent vehicles powered by the most recent engines. The US EPA models total mileage accumulation by age of the vehicle for the purposes of emission inventory calculations. The heavy-duty mileage accumulation rates used in the EPA model MOBILE5b indicate that half of the total fleet mileage accumulation is accounted for by vehicles that are 6 years old or more recent, and new vehicles account for 10 times the annual mileage of the oldest vehicles in the fleet (Kremer, 1999).

1.3.4 In-use emission verification programmes

In addition to the emission standards to which new products must comply, several in-use verification tests and practices are applied worldwide to ensure continued compliance with emission standards in service.

Many countries in Europe, 33 states and local jurisdictions in the USA, Japan and other developed and developing countries have implemented inspection and maintenance programmes. To maintain on-road vehicle registration, vehicles are required to pass emission verifications at annual or biannual intervals. The US EPA has also incorporated in-use monitoring

Fig. 1.13 Implementation schedule of passenger car diesel emission standards in selected countries



Compiled by the Working Group

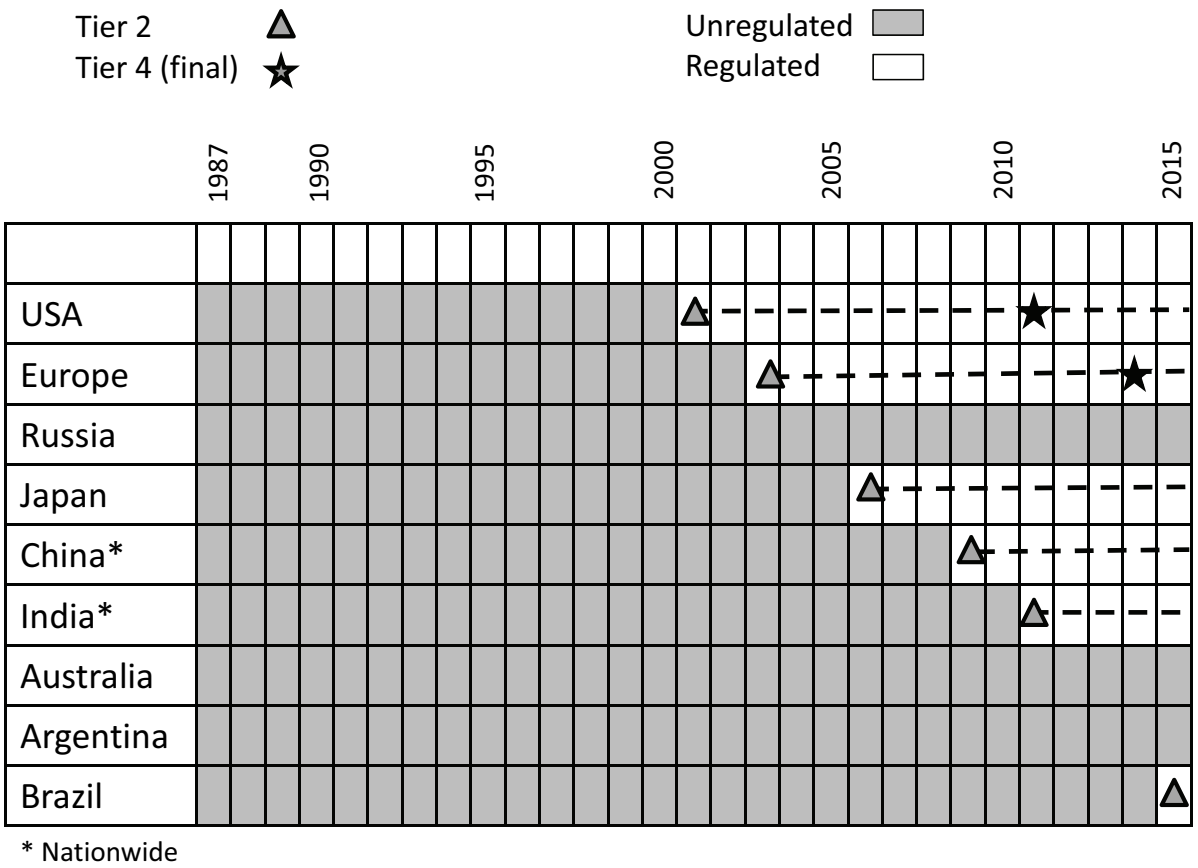
of on-highway heavy-duty vehicle emissions through a programme that requires manufacturer-run in-use testing to validate compliance with not-to-exceed limits (http://www.epa.gov/otaq/regs/hd-hwy/inuse/420f05021.htm). For Euro VI regulations, European commercial vehicle and engine manufacturers must demonstrate in-use conformity with these emission standards.

1.4 Occurrence and exposure

1.4.1 Occupational exposure of workers predominantly exposed to diesel exhaust

It has been estimated that 1.4 million workers in the USA and 3 million workers in Europe are occupationally exposed to diesel engine exhaust (Lewtas & Silverman, 2010). Exposure to diesel engine exhaust through the use of diesel equipment occurs in many different occupational settings, including those in the mining, railroad, construction and transportation industries. Exposures to diesel engine exhaust in

Fig. 1.14 Implementation schedule of off-road heavy-duty diesel emission standards in selected countries



Compiled by the Working Group

occupational and traffic settings are the result of vehicle operations and vehicle mix, and the composition and particle size characteristics of diesel exhaust are known to change depending on the load, fuel–air ratio and exhaust temperature, as summarized in Table 1.12. This affects the quantities of PAHs, EC and hydrocarbons in the particles emitted from the engine, which can influence the qualitative and quantitative nature of exposures in various occupational settings as shown in Table 1.13.

An overview of the levels of exposure to diesel engine exhaust is presented in Table 1.14, Table 1.15, Table 1.16, and Table 1.17 and is an update of the review published by Pronk *et al.* (2009). The exposure levels are summarized by on-road and off-road equipment, and major occupational settings. Data on the components of diesel engine exhaust, such as EC, nitrogen oxides and carbon monoxide are summarized below. Because the exposure data were mainly collected between 1990 and 2005, these measurements predominantly reflect the use of traditional and transitional diesel engines.

(a) On-road vehicles

The conversion of HGVs to diesel engines began in the 1950s and from the 1960s and 1970s predominantly diesel-powered heavy-duty vehicles were sold. In contrast to Europe, where about

Table 1.12 Approximate characteristics[a] of particle emissions defined by diesel engine operating conditions for an uncontrolled diesel engine

Engine operation[b]			Characteristics[b]			
Load	**Fuel–air ratio**	**Exhaust Temperature**	**PAH, EC and HC**	**Particulate HC droplets**	**Soluble organic fraction**	**Ratio PAH/surface (ng/mm²)[c]**
Idle, low and medium load (< 50% max); high acceleration	42–60	Low (< 250 °C)	Highest% PAHs, HC covers EC surface	Higher MW; heavy oil emissions; major nuclei fraction	High (> 40%)	< 0.3
High load (> 50% max) and speed; moderate acceleration	25–30	High (~500 °C)	Low total PAHs and HC; HC partially covers EC surface	Lower MW; limited oil emissions; no nuclei fraction	Low (< 20%)	1–10

[a] These approximate characteristics apply to sustained conditions, not to rapid changes, such as slowing down to a stop after a period of cruising with a heavy load because the exhaust system will remain hot for a period of time.
[b] From Kweon *et al.* (2003) and Kittelson *et al.* (2006)
[c] From Bukowiecki *et al.* (2002)
EC, elemental carbon; HC, hydrocarbon; MW, molecular weight; PAH, polycyclic aromatic hydrocarbon

one-third of all new passenger cars have diesel engines, only very few new passenger cars or taxis in the USA are diesel-fuelled (Lloyd & Cackette, 2001). Workers in occupations that involve the driving, maintenance and unloading of diesel HGVs and, to a lesser extent, diesel cars can be exposed to diesel exhaust. Table 1.14 summarizes the reported levels of exposure to EC, carbon monoxide, nitric oxide and nitrogen dioxide for workers exposed to diesel engine exhaust from on-road vehicles, by agent and by activity.

(i) HGV drivers

Studies performed mainly in the 1990s and 2000s showed that HGV drivers were exposed on average to levels of EC ranging from 1 to 22 μg/m³ [weighted arithmetic mean (AM), 2 μg/m³], and levels of nitric oxide and nitrogen dioxide were around 0.2–0.3 and 0.03–0.04 ppm, respectively.

Several factors appear to influence the levels of exposure of drivers to diesel engine exhaust. For instance, long-haul drivers tended to have lower levels of exposure than local drivers (Garshick *et al.*, 2002). In addition, a comparison of two cross-sectional exposure surveys in the HGV transport industry indicated a two- to threefold decline in the levels of exposure to EC between the 1980s and 2001–05 (Zaebst *et al.*, 1991; Davis *et al.*, 2007; Fig. 1.15). Furthermore, a positive association was found between the age of the vehicle and the levels of EC. This increase in exposure was attributed to a higher seepage of diesel engine exhaust in older HGV cabins due to leaks in their rubber seals (Davis *et al.*, 2007).

(ii) HGV/bus mechanics

Mechanics of heavy-duty diesel equipment (e.g. HGVs and buses) were exposed to levels of EC that were generally higher than those of drivers. Average levels of exposure to EC ranged between 4 and 39 μg/m³ [weighted AM, 29 μg/m³].

(iii) Bus garage and other bus workers

Few studies have addressed exposure to diesel engine exhaust for bus garage and other bus workers. In a study by Ulfvarson *et al.* (1987), levels of carbon monoxide, nitric oxide and nitrogen dioxide in a bus garage varied from 1.7 to 24, 0.3 to 1.0 and 0.2 to 1.1 ppm, respectively.

(iv) Firefighters

Firefighters are frequently exposed to diesel engine exhaust and other combustion products (Froines *et al.*, 1987). Exposures can occur during

Table 1.13 Exposure scenarios, size distributions and composition of diesel emissions under operating conditions in common work settings

	Engine temperature and operation	Exposure scenarios	Particle size characteristics	Composition
Off-road activities				
Maintenance shops for railroads and trucks	Cold engines	Briefly move vehicles in/out of shop; emissions into confined space; slow removal by ventilation	High agglomeration; considerably reduced nuclei and surface area; most in accumulation mode	Lower EC and very high OC from lubricating oils
Railroad operations and exposures of crews	Hot engines; continuous engine operations; frequent idling and steady speed under loads	Emissions into the environment by leading locomotive(s); exposure intensity defined by downwind proximity to source(s)	Low agglomeration Idling: high nuclei level and PM Steady speed: low/no nuclei, reduced surface.	 Higher EC and very high OC Moderate EC and lower OC from lubricating oils
Underground mining	Hot engines; steady use, frequent stops and idling, and hard acceleration under load. Fixed engines: hot; steady use	Exposure intensity defined by proximity to vehicles – haulage trucks, loaders; and fixed engines – generators, large equipment; moderate to fast removal by ventilation	High agglomeration; no nuclei and lower surface area.	Higher EC and lower OC from lubricating oils
Above-ground mining	Hot engines; steady use: haulage trucks – limited stops and hard acceleration under load; frequent idling and low load return trips. Fixed engines: hot; steady use	Brief exposure to occasional exhaust from preceding trucks or nearby heavy equipment	Idle: high nuclei level and PM Steady speed: up-hill, low nuclei and low hydrocarbons down-hill, high nuclei and high hydrocarbons	High EC and OC High EC and low OC High EC and OC
On-road vehicle operations (exposure from preceding vehicles)				
City driving	Hot; frequent stops and hard acceleration under load; frequent of idling	Exposure from preceding vehicles depends on traffic density and proximity	Moderate agglomeration; Idle and high acceleration: high nuclei level and PM Steady speed: low nuclei and accumulation mode (depends on proximity).	 High EC and OC Moderate EC and low OC
Highway driving	Hot engine; usually heavy load; rare idling or acceleration	Exposure from preceding vehicles depends on traffic density and proximity	Low agglomeration; low nuclei and accumulation mode (depends on proximity)	High EC and low OC

EC, elemental carbon; OC, organic carbon; PM, particulate matter

Table 1.14 Measurements of occupational exposure to diesel exhaust from on-road vehicles: elemental carbon (µg/m³), and carbon monoxide, nitric oxide and nitrogen dioxide (ppm)

Description	Agent	Duration (h)	No.	AM (SD)	GM (GSD)	Location	Year	Reference
Drivers								
Truck – local	EC_S	> 4	56	5 (0.9)	0.9 (4.0)	USA	1980s	Zaebst *et al.* (1991)
Truck – local	EC_S	> 4	576[a]	2 (2.3)	1 (2.8)	USA	2001–05	Davis *et al.* (2007)
Truck – local	EC_R	> 4	5	7[b]	6 (1.6)	USA	1999	Garshick *et al.* (2002)
Truck – local	EC_{NR}	> 4	4 [a]	5 (0.1)	5 (1.0)	USA	1985	NIOSH (1986)
Truck – long haul	EC_S	> 4	72	5 (0.4)	0.4 (3.8)	USA	1980s	Zaebst *et al.* (1991)
Truck – long haul	EC_S	> 4	349[a]	1 (0.8)	1 (2.3)	USA	2001–05	Davis *et al.* (2007)
Truck – long haul	EC_R	> 4	5	5[b]	4 (2.0)	USA	1999	Garshick *et al.* (2002)
Truck – long haul	EC_{NR}	> 4	4[a]	22 (13.2)	19 (2.0)	USA	1985	NIOSH (1986)
HGV	EC_I	1- > 4	3	10 (6.0)	9 (1.8)	USA	1992	NIOSH (1993)
Bus	EC_R	> 4	5	10[b]	9 (1.3)	Estonia	2002[c]	Boffetta *et al.* (2002)
Bus	EC_R	> 4	39	2.0 (1.3)	1.4 (3.3)	USA	2002[c]	Ramachandran *et al.* (2005)
Bus	EC_I	> 4	4	2 > LOD: 11–20		USA	1998	NIOSH (1998)
Bus and HGV[d]	EC_I	> 4	20	11[b]	6 (2.9)	Sweden	2002–04	Lewné *et al.* (2007)
Taxi[d]	EC_I	> 4	8	8[b]	7 (1.6)	Sweden	2002–04	Lewné *et al.* (2007)
Mechanics								
Truck	EC_S	> 4	80	27 (4.1)	4 (12.1)	USA	1980s	Zaebst *et al.* (1991)
Truck	EC_R	> 4	10	4[b]	4 (1.6)	USA	1999	Garshick *et al.* (2002)
Ambulance depot	EC_R	> 4	3	31	29 (1.6)	United Kingdom	2000[c]	Groves & Cain (2000)
Bus	EC_R	> 4	53	39	31 (2.1)	United Kingdom	2000[c]	Groves & Cain (2000)
Bus	EC_R	> 4	15	39[b]	38 (1.3)	Estonia	2002[c]	Boffetta *et al.* (2002)
HGV/bus (+inspection)	EC_I	> 4	40	21[b]	11 (3.2)	Sweden	2002–04	Lewné *et al.* (2007)
Bus	EC_I	> 4	4	ND	ND	USA	1998	NIOSH (1998)
Others								
Firefighter	EC_I	> 4	27	24 (max)		USA	2002[c]	Roegner *et al.* (2002)
Firefighter	EC_I	> 4	18	40 (20.3)	35 (1.7)	USA	1995[c]	Echt *et al.* (1995)
Firefighter	EC_I	> 4	12	10 (max)		USA	1997	NIOSH (1998)
Firefighter	EC_I	< 1	8	ND	ND	US	1998	NIOSH (1998)
Service worker bus	EC_I	> 4	4	2 > LOD: 0.3–15		USA	1998	NIOSH (1998)
Vehicle testing	EC_R	> 4	11	11	11 (1.8)	United Kingdom	2000[c]	Groves & Cain (2000)
Car park attendant (booth)	EC_R	> 4	34[a]	1.1 (0.6)	1.1 (1.8)	USA	2002[c]	Ramachandran *et al.* (2005)

Table 1.14 (continued)

Description	Agent	Duration (h)	No.	AM (SD)	GM (GSD)	Location	Year	Reference
Others								
Bus garage	CO			1.7–24			1987[c]	Ulfvarson *et al.* (1987)
Driver								
Truck – local	NO	> 4	4[a]	0.23 (0.05)	0.22 (1.3)	USA	1985	NIOSH (1986)
Truck – long haul	NO	> 4	4[a]	0.27 (0.10)	0.25 (1.5)	USA	1985	NIOSH (1986)
Others								
	NO			0.3–1.0			1987[c]	Ulfvarson *et al.* (1987)
Driver								
Taxi[d]	NO_2	> 4	12	0.03[b]	0.02 (0.7)	Sweden	2002–04	Lewné *et al.* (2007)
Bus and HGV[d]	NO_2	> 4	30	0.03[b]	0.03 (0.7)	Sweden	2002–04	Lewné *et al.* (2007)
HGV	NO_2	> 4	40	0.04 (0.02)		Sweden	1997–99	Lewné *et al.* (2006)
Taxi	NO_2	> 4	20	0.03 (0.01)		Sweden	1997–99	Lewné *et al.* (2006)
Bus	NO_2	> 4	42	0.03 (0.01)		Sweden	1997–99	Lewné *et al.* (2006)
Mechanics								
HGV/bus (+inspection)	NO_2	> 4	60	0.05[b]	0.05 (0.9)	Sweden	2002–04	Lewné *et al.* (2007)
Bus	NO_2		232	0.24 (0.26)		USA	1987[c]	Gamble *et al.* (1987)
Others								
Bus	NO_2		232	0.2–1.1		Sweden	1987[c]	Ulfvarson *et al.* (1987)

[a] Area sample representative of personal exposure
[b] AM estimated from GM and GSD or from range
[c] Year of publication, year of sampling not available
[d] Mostly diesel powered vehicles

AM, arithmetic mean; CO, carbon monoxide; EC, elemental carbon; EC_I, inhalable; EC_{NR}, not reported; EC_R, respirable; EC_S, submicron; GM, geometric mean; GSD, geometric standard deviation; h, hour; HGV, heavy-goods vehicle; LOD, limit of detection; ND, Not detected; NO, nitrogen oxide; NO_2, nitrogen dioxide; SD, standard deviation

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Table 1.15 Measurements of occupational exposure to diesel exhaust in the mining industry: elemental carbon (µg/m³), and carbon monoxide, nitric oxide and nitrogen dioxide (ppm)

Description	Agent	Duration (h)	No.	AM (SD)	GM (GSD)	Location	Year	Reference
Underground								
Production (NM)	EC_R		6[a]	148 (136)	85 (3.5)	United Kingdom	2004[b]	Leeming & Dabill (2004)
Production (NM)	EC_R	> 4	343	202 (32–144)	111 (1.4–4.8)	USA	2002[b]	Cohen *et al.* (2002)
Production (NM)	EC_S	> 4	38	219 (65–193)		USA	1997[b]	Stanevich *et al.* (1997)
Production (C)	EC_R	> 4	4	241[c]	202 (1.8)	Estonia	2002[b]	Boffetta *et al.* (2002)
Production (M)	EC_R	> 4	15	637 (75–508)		USA	1999	McDonald *et al.* (2002)
Production (NR)	EC_I	< 1–4	12	538 (512)		USA	2007[b]	Burgess *et al.* (2007)
Maintenance (NM)	EC_S	> 4	8	53 (46)		USA	1997[b]	Stanevich *et al.* (1997)
Maintenance (NM)	EC_R	> 4	269	144 (17–462)	66 (1.7–4.6)	USA	2002[b]	Cohen *et al.* (2002)
Mining, NS (C)	EC_R		7[a]	66 (28)	62 (1.5)	United Kingdom	2004[b]	Leeming & Dabill (2004)
Mining, NS (M)	EC_{NR}		27	27		Sweden	2006[b]	Adelroth *et al.* (2006)
Mining, NS (NM)	EC_R	> 4	779	[135] (40–384)		USA	1998–2001	Coble *et al.* (2010)
Surface								
Production/maintenance (NM)	EC_R	> 4	164	13 (2–89)	2 (1.8–6.2)	USA	2002[b]	Cohen *et al.* (2002)
Production/maintenance (NM)	EC_S	> 4	23	23 (15–54)		USA	1997[b]	Stanevich *et al.* (1997)
Production/maintenance (NM)	EC_R	> 4	265	3.5		USA	1998	Coble *et al.* (2010)
Underground								
Production (NM)	CO	1- > 4	5	2.0 (0.6)	1.9 (1.4)	USA	1991	NIOSH (1991)
Mining, NS (NR)	CO	< 4	21	12.4 (0–23)		USA	1978	Holland (1978)
Mining, NS (NM)	CO		≥ 5[a, d]	8.9		USA	1976–77	Attfield (1978)
Mining, NS (M)	CO		≥ 5[a, d]	6.1		USA	1976–77	Attfield (1978)
Underground								
Mining, NS (NI)	NO	< 4	10	10.3 (0.4–57)		USA	1978	Holland (1978)
Production (NM)	NO	> 4	9	14.7 (2.8)	14.5 (1.2)	USA	1991	NIOSH (1991)
Production (NM)	NO	> 4	7	4.2 (1.7)	3.9 (1.5)	USA	1991	NIOSH (1991)
Production (NM)	NO	> 4	6	4.7 (1.0)	4.6 (1.2)	USA	1991	NIOSH (1993)

Table 1.15 (continued)

Description	Agent	Duration (h)	No.	AM (SD)	GM (GSD)	Location	Year	Reference
Mining, NS (M)	NO	> 4	54[a]	11.0 (5.7)		USA	1988	NIOSH (1992)
Mining, NS (M)	NO	> 4	25	0.7 (0.6)		USA	1988	NIOSH (1991, 1992)
Mining, NS (NM)	NO	> 4	666	[0.9] (0.2–1.5)		USA	1998–2001	Coble *et al.* (2010)
Surface								
Production/maintenance (M)	NO	> 4	12	0.3 (0.2)		USA	1988	NIOSH (1992)
Production/maintenance (NM)	NO	> 4	225	[0.07] (0.02–0.11)		USA	1988	Coble *et al.* (2010)
Underground								
Production (NM)	NO_2	> 4	9	2.9 (0.5)	2.9 (1.2)	USA	1991	NIOSH (1991)
Production (NM)	NO_2	> 4	7	0.8 (0.4)	0.7 (1.6)	USA	1991	NIOSH (1991)
Production (NM)	NO_2	> 4	6	0.7 (0.1)	0.7 (1.1)	USA	1991	NIOSH (1993)
Mining, NS (NR)	NO_2	< 4	29	0.8 (0–5.5)		USA	1978	Holland (1978)
Production (NM)	NO_2		183	1.9 (1.6)		USA	1978[b]	Gamble *et al.* (1978)
Production (C)	NO_2	> 4	41	0.2[c]	0.1 (1.5–2.8)	USA	1976–80	Wheeler *et al.* (1981)
Production (C)	NO_2	> 4	76	0.2 (0.1–0.1)		USA	1982[b]	Reger *et al.* (1982)
Production (M)	NO_2		29	0.2		Sweden	2006[b]	Adelroth *et al.* (2006)
Production (M)	NO_2	> 4	54[a]	1.5 (0.9)		USA	1988	NIOSH (1992)
Production (M)	NO_2	> 4	25	5.5 (3.9)		USA	1988	NIOSH (1991)
Mining, NS (C)	NO_2	> 4	60	0.2 (0.1)		USA	1982[b]	Ames *et al.* (1982)
Mining, NS (NM)	NO_2	> 4	689	[0.3] (0.1–0.6)		USA	1998–2001	Coble *et al.* (2010)
Surface								
Production/maintenance (M)	NO_2	> 4	12	0.04 (0.03)		USA	1988	NIOSH (1992)
Production/maintenance (NM)	NO_2	> 4	233	[0.04] (0.01–0.06)		USA	1988	Coble *et al.* (2010)

[a] Area sample representative of personal exposure
[b] Year of publication, year of sampling not available
[c] AM estimated from GM and GSD or from range
[d] At least five samples for all jobs combined in the study

AM, arithmetic mean; C, coal; CO, carbon monoxide; EC, elemental carbon; EC_I, inhalable; EC_{NR}, not reported; EC_R, respirable; EC_S, submicron; GM, geometric mean; GSD, geometric standard deviation; M, metal; NM, non-metal; NO, nitrogen oxide; NO_2, nitrogen dioxide; NR, not reported; NS, job not specified; SD, standard deviation

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Table 1.16 Measurements of occupational exposure to diesel exhaust in the railroad industry: elemental carbon (µg/m³), and carbon monoxide, nitric oxide and nitrogen dioxide (ppm)

Description	Agent	Duration (h)	No.	AM (SD)	GM (GSD)	Location	Year	Reference
Train crew								
Driver, assistant, shunter driver	EC_R	> 4	19	20 (18.7)	16 (2.0)	Russian Federation	2002[a]	Boffetta *et al.* (2002)
Hostler	$EC_{R/I}$	> 4	5	4 (1.3)	3 (1.5)	Canada	1999–2000	Verma *et al.* (2003)
Engineer/driver, conductor/ trainman	$EC_{R/I}$	> 4	76[b]	5 (1.1–15.8)	3 (1.5–3.5)	Canada	1999–2000	Verma *et al.* (2003)
Non-operating crew trailing locomotive	EC_I	> 4	47[b]	10 (12)	6	Canada	2003	Seshagiri (2003)
Engineer's operating console	EC_I	1- > 4	49[b]	6	4 (3)	USA	1996–98	Liukonen *et al.* (2002)
Maintenance								
Rolling equipment	$EC_{R/I}$	> 4	48	5 (4.9–8.8)	3 (2.4–2.7)	Canada	1999–2000	Verma *et al.* (2003)
Rolling equipment	EC_R	> 4	64	39	17 (1.9)	United Kingdom	2000[a]	Groves & Cain (2000)
Train crew								
Non-operating crew trailing locomotive	CO	> 4	280[b]	4.50 (max)		Canada	2003	Seshagiri (2003)
Locomotive and caboose	CO	> 4	16[b]	< 1		USA	1974–76	Hobbs *et al.* (1977)
Train crew								
Non-operating crew trailing locomotive	NO	> 4	46[b]	1.13 (0.87)	0.82	Canada	2003	Seshagiri (2003)
Locomotive	NO	> 4	9[b]	0.55		Canada	1996	Verma *et al.* (1999)
Locomotive and caboose	NO	> 4	16[b]	0.23		USA	1974–76	Hobbs *et al.* (1977)

Table 1.16 (continued)

Description	Agent	Duration (h)	No.	AM (SD)	GM (GSD)	Location	Year	Reference
Maintenance								
Rolling equipment	NO	> 4	18	0.26		Canada	1996	Verma *et al.* (1999)
Train crew								
Non-operating crew trailing locomotive	NO_2	> 4	181[b]	0.3 (max)		Canada	2003	Seshagiri (2003)
Locomotive on board	NO_2	> 4	9[b]	0.05		Canada	1996	Verma *et al.* (1999)
Locomotive and caboose	NO_2	> 4	16[b]	0.03		USA	1974–76	Hobbs *et al.* (1977)
Maintenance								
Rolling equipment	NO_2	> 4	18	0.10		Canada	1996	Verma *et al.* (1999)

[a] Year of publication, year of sampling not available

[b] Area sample representative of personal exposure

AM, arithmetic mean; CO, carbon monoxide; EC, elemental carbon; EC_I, inhalable; EC_R, respirable; $EC_{R/I}$, respirable/inhalable; GM, geometric mean; GSD, geometric standard deviation; NO, nitrogen oxide; NO_2, nitrogen dioxide; SD, standard deviation

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Table 1.17 Measurements of occupational exposure to diesel exhaust from other off-road vehicles: elemental carbon (µg/m³), and carbon monoxide, nitric oxide and nitrogen oxide (ppm)

Description	Agent	Duration (h)	No.	AM (SD)	GM (GSD)	Location	Year	Reference
Construction								
Tunnel	EC_I	> 4	10	314[a]	163 (1.5–3.0)	Norway	1996–99	Bakke *et al.* (2001)
	EC_I	> 4	12	132[a]	87 (2.5)	Sweden	2002–04	Lewné *et al.* (2007)
Heavy/highway	EC_R	> 4	261	13	8 (2.7)	USA	1994–99	Woskie *et al.* (2002)
Above-ground	EC_I	> 4	22	13[a]	8 (2.8)	Sweden	2002–04	Lewné *et al.* (2007)
Electric utility installation	EC_I	> 4	120	4		USA	1996–97	Whittaker *et al.* (1999)
Tunnel	CO	> 4	78	9[a]	5.7 (1.5–2.6)	Norway	1996–99	Bakke *et al.* (2001)
	CO	> 4	52	5 (3.7)		Sweden	1991[b]	Ulfvarson *et al.* (1991)
Electric utility installation	CO	> 4	27	1 (0.6–0.6)		USA	1996–97	Whittaker *et al.* (1999)
Tunnel	NO	> 4	53	2.6 (1.5)		Sweden	1991[b]	Ulfvarson *et al.* (1991)
Electric utility installation	NO	> 4	27	0.2 (0.2–0.4)		USA	1996–97	Whittaker *et al.* (1999)
Tunnel	NO_2	> 4	18	0.22[a]	0.19 (0.58)	Sweden	2002–04	Lewné *et al.* (2007)
	NO_2	> 4	82	0.86[a]	0.54 (1.5–4.5)	Norway	1996–99	Bakke *et al.* (2001)
	NO_2	> 4	53	0.88 (0.68)		Sweden	1991[b]	Ulfvarson *et al.* (1991)
Above-ground	NO_2	> 4	33	0.02[a]	0.02 (1.06)	Sweden	2002–04	Lewné *et al.* (2007)
Electric utility (outside)	NO_2	> 4	24	0.32 (0.2–0.2)		USA	1996–97	Whittaker *et al.* (1999)
Dock/distribution								
Dock worker	EC_S	> 4	54	24 (0.4–2.5)	2 (1.3–27.2)	USA	1991[b]	Zaebst *et al.* (1991)
	EC_S	> 4	≥ 5[c]		7	USA	1990	Zaebst *et al.* (1992)
Fork-lift truck	EC_R	> 4	39[d]	36[a]	27	United Kingdom	2004[b]	Wheatley & Sadhra (2004)
Dock worker	EC_R	> 4	27	122	66 (3.3)	United Kingdom	2000[b]	Groves & Cain (2000)
	EC_R	> 4	12	9[a]	7 (2)	USA	1999	Garshick *et al.* (2002)
	EC_I	> 4	5	4 (1.8)	4 (1.5)	USA	1992	NIOSH (1993)
	NO_2	> 4	≥ 5[c]		0.18	USA	1990	Zaebst *et al.* (1992)

Table 1.17 (continued)

Description	Agent	Duration (h)	No.	AM (SD)	GM (GSD)	Location	Year	Reference
Airline personnel								
Baggage and screening	EC_I	> 4	72	11 (5.4)		USA	2004	NIOSH (2005)
	CO	> 4	61	2.4[a]		USA	2004	NIOSH (2005)
Mechanics and refuelers	CO	> 4	10	5 (1.5)	4.7 (1.3)	USA	1992	NIOSH (1994b)
Baggage and screening	NO	> 4	40	0.13 (0.07)		USA	2004	NIOSH (2005)
	NO_2	> 4	40	0.12 (0.07)		USA	2004	NIOSH (2005)
Loading/unloading ships								
Marine terminal	EC_I	> 4	168	6 (0.9–9.0)		USA	2003–05	NIOSH (2006)
Ferry	EC_R	> 4	20	49	37 (2.5)	United Kingdom	2000[b]	Groves & Cain (2000)
Marine terminal	CO	> 4	60	2.5		USA	2003–05	NIOSH (2006)

[a] AM estimated from GM and GSD or from range
[b] Year of publication, year of sampling not available
[c] At least five samples for all jobs combined in the study
[d] Area sample representative of personal exposure

AM, arithmetic mean; CO, carbon monoxide; EC, elemental carbon; EC_I, inhalable; EC_R, respirable; EC_S, submicron; GM, geometric mean; GSD, geometric standard deviation; NO, nitrogen oxide; NO_2, nitrogen dioxide; SD, standard deviation

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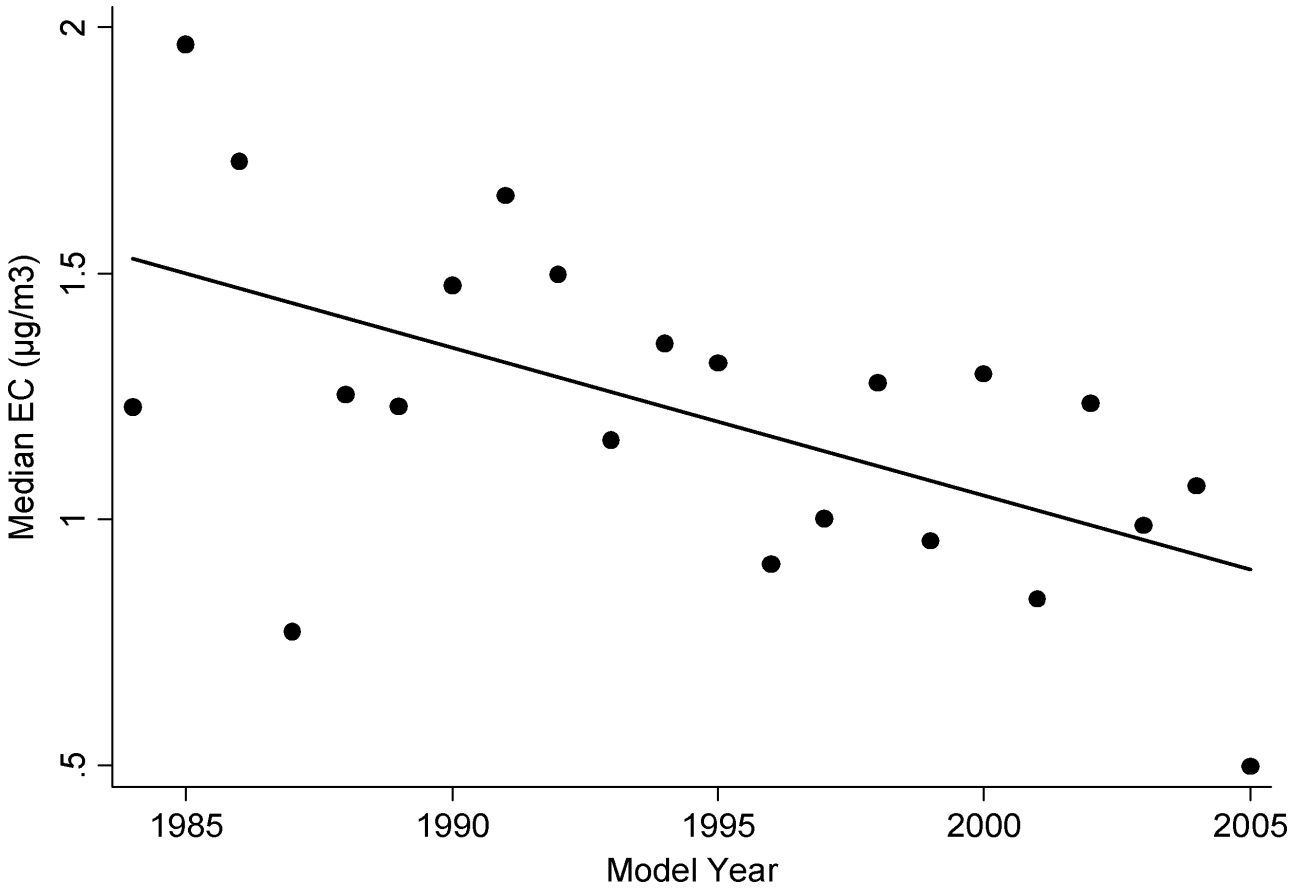
Fig. 1.15 Median levels of exposure to elemental carbon in truck cabins for pick-up and delivery drivers, by model year



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EC, elemental carbon

a response to an incident and in the fire station, and those reported varied considerably between studies. Echt *et al.* (1995) reported average levels of 40 µg/m³ EC, while others reported maximum levels of 24 µg/m³, 10 µg/m³ or non-detectable levels (Roegner *et al.*, 2002; Pronk *et al.*, 2009).

(v) Others

Other occupations with exposure to diesel engine exhaust from on-road vehicles include vehicle testing, parking attendant, toll booth worker, transport terminal worker and traffic police officer. Reported exposures to EC were mostly < 10 µg/m³ (see Table 1.14).

(b) Off-road vehicles

Exposure to exhaust emissions from off-road vehicles can occur in many different industries and occupations (see Table 1.15). The major industries are mining, railroad transport, construction and loading/unloading operations.

(i) Mining

Mining operations can be carried out on the surface or underground. In surface (open-pit) mining, the ore is mined using large excavating equipment, such as load and dump vehicles, drills, graders and utility trucks, and transported using very large dumper trucks.

Diesel equipment in underground mining may include vehicles for the transportation of personnel, haulage trucks, load and dumper vehicles, drills, graders and utility trucks. The first diesel engine-powered vehicles in underground mines were used in Germany in 1927 (Kaplan, 1959). In the 1970s, the use of diesel engines in underground mines greatly increased in the USA. In 1998, the Mine Safety and Health Administration estimated that 18% of the 971 underground coal mines and 78% of the 261 underground metal/non-metal mines in the USA used diesel engines (Pronk *et al.*, 2009).

In addition to underground mining operations, diesel equipment is also used in some surface operations. These include bulldozers for cleaning stockpiles, trucks for the transportation of ore and tailings, and forklift trucks for loading/unloading.

Other potential exposures in mines include airborne particles, from blasting, and the mining and transport of the ore, and gases, such as carbon monoxide and sulfur dioxide. Some other exposures that may occur in mines, depending on the ores extracted, have been evaluated in previous *Monographs*; these include radon (IARC, 2012d), silica, nickel, chromium and asbestos (IARC, 2012a).

Table 1.15 summarizes the levels of exposure to EC, carbon monoxide, nitric oxide and nitrogen dioxide reported for workers exposed to diesel engine exhaust in the mining industry.

(ii) Underground production/mining

Production workers include miners involved in drilling, blasting and hauling ore. Reported levels of exposure to EC varied from 148 to 637 μg/m^3 [weighted AM, 135 μg/m^3], average levels of nitric oxide varied from < 1 to 15 ppm [weighted AM, 1.8 ppm] and average levels of nitrogen dioxide ranged from 0.2 to 5.5 ppm [weighted AM, 1.9 ppm].

A large exposure survey among non-metal miners in seven mines showed that, in general, the highest levels of exposure to diesel engine exhaust were for miners working in the active face area. Workers in the haulage or travel ways were generally lower exposed than workers at the face. Workers in the underground maintenance shop and offices had the lowest levels of exposure, most probably due to the proximity of these areas to the fresh-air intake shafts (Coble *et al.*, 2010).

(iii) Underground maintenance

Maintenance workers include those in the maintenance shops and underground warehouses, and those involved in the maintenance of travel and haulage routes. The studies that reported the exposure to EC of underground maintenance workers indicated levels between 53 and 144 μg/m^3 [weighted AM, 141 μg/m^3].

(iv) Surface production

Reported levels of exposure on the surface were much lower than those reported underground. Workers involved in surface activities had levels of exposure to EC between 3.5 and 23 μg/m^3 [weighted AM, 8 μg/m^3]. Levels of nitric oxide varied from 0.07 to 0.3 ppm [weighted AM, 0.08 ppm], while concentrations of 0.04 ppm nitrogen dioxide were observed.

(v) Railroad transportation

The use of diesel engines in railroad locomotives was first introduced into Canada and the USA in 1928 and into Germany in 1932 (Garshick *et al.*, 1988), and diesel engines largely replaced steam engines between 1945 and the 1960s (Pronk *et al.*, 2009). In the 1960s, second-generation diesel locomotives were introduced that were reported to be cleaner (Woskie *et al.*, 1988). Table 1.16 and Fig. 1.16 summarize the levels of exposure reported in the railroad industry.

(vi) Train crews

A typical train crew comprises a conductor and an engineer and occasionally includes brakers/switchers for local or yard jobs. Several studies measured the exposures of train personnel

to diesel engine exhaust. Average levels of exposure to EC varied from 4 to 20 μg/m³ [weighted AM, 8 μg/m³], and concentrations of nitric oxide and nitrogen dioxide ranged from 0.2 to 1.1 and from 0.03 to 0.3 ppm, respectively.

The location of the exhaust stack in relation to the cabin (either in front of or behind the cabin) has been shown to be an important determinant of exposure. In addition, significantly increased levels of exposure to diesel engine exhaust have been reported in the cab when the windows were open compared with when they were closed.

(vii) Maintenance

The levels of exposure of train maintenance personnel to diesel engine exhaust have been reported to be in the same range as those of train crews, with levels of exposure to EC varying from 5 to 39 μg/m³ [weighted AM, 24 μg/m³], and those to nitric oxide and nitrogen dioxide being around 0.3 and 0.1 ppm, respectively.

Woskie *et al.* (1988) conducted an industrial hygiene survey of the US railroad industry. Personal exposure to respirable particles was measured and then corrected for the estimated contribution of cigarette-smoke particulates. Similar to the measurements of EC, these results indicated that the highest exposures occurred for hostlers (yard workers who move trains and rail cars, sometimes using specialized locomotives), electricians, machinists and mechanics. Lower exposures were reported for clerks.

(viii) Construction

Exposure to diesel engine exhaust within the construction industry varies widely (Table 1.17). Many studies have been carried out on exposure during tunnel construction, and indicated that the average levels of exposure to EC were between approximately 100 and 300 μg/m³ [weighted AM, 215 μg/m³]. Similarly, high levels of nitric oxide and nitrogen dioxide were reported (2.6 and 0.22–0.88 ppm, respectively). Construction workers above ground are exposed to much lower levels of diesel engine exhaust, with measured levels of generally around 10 μg/m³ [weighted AM, 13 μg/m³] of EC, 0.2 ppm of nitric oxide and 0.02–0.32 ppm of nitrogen dioxide.

Blute *et al.* (1999) reported several determinants of exposure to diesel engine exhaust at highway construction sites. Regression analyses indicated higher exposures to EC for enclosed versus open worksites, cranes versus other types of diesel-powered equipment, proximity within 10 feet of diesel equipment and greater numbers of diesel sources.

(ix) Loading/unloading operations

Loading and unloading activities can be performed at transport terminals and docks, and on board ships and aircraft.

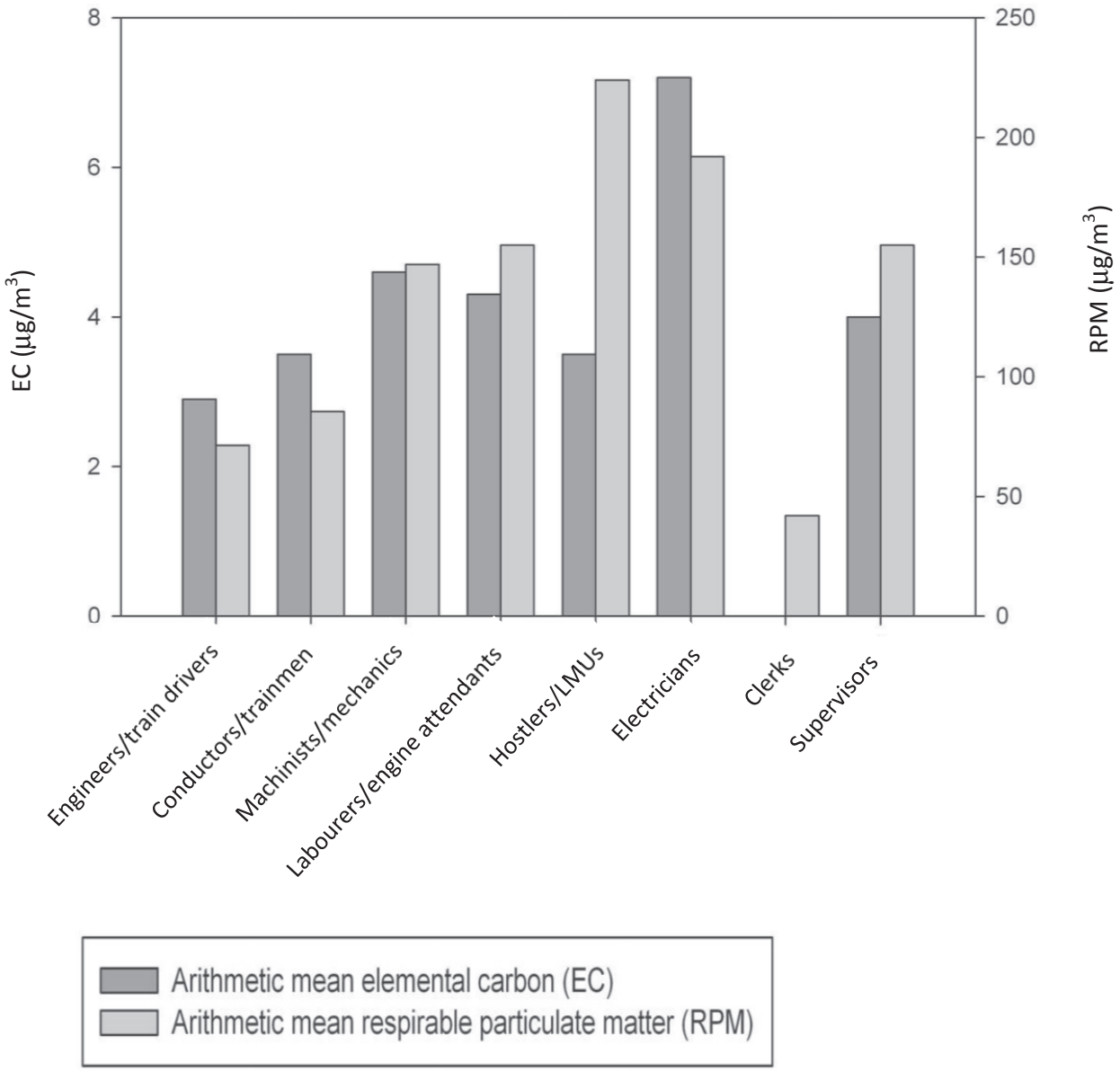
Several studies have measured the average levels of exposure to EC among dock workers (see Table 1.17), and reported that these varied from 4 to 122 μg/m³ [weighted AM, 43 μg/m³]. The sources of exposure for dock workers include diesel-powered forklift trucks and on-road HGVs. Studies at docks where only non-diesel-powered forklifts were used reported low levels of exposure to EC (< 5 μg/m³), indicating that the major contributors to the exposure of dock workers were diesel-powered forklifts (Zaebst *et al.*, 1991).

Similarly, a large variation in exposure to diesel engine exhaust has been reported for people involved in the loading/unloading of ships, for whom levels of EC were reported to range from 6 to 49 μg/m³ [weighted AM, 11 μg/m³].

Little information was available on the exposure of workers who unload baggage from airplanes. The limited data indicated that levels of exposure were around 10 μg/m³ for EC and around 0.1 ppm for nitric oxide and nitrogen dioxide (Schauer, 2003). However, the contribution of jet fuel to the measured levels of EC cannot be excluded.

Fig. 1.17 summarizes measurements of EC, nitric oxide and nitrogen dioxide by major industry/job based on weighted averages. Miners

Fig. 1.16 Average personal exposures to elemental carbon and respirable particulate matter by job title among railroad workers



LMU, labourer moving unit
Compiled by the Working Group

(when mines use diesel engines) and tunnel construction workers have the highest exposures, with average levels of EC above 100 μg/m³. Dock workers, diesel mechanics and maintenance personnel are exposed to average levels of 20–40 μg/m³. Train crews, construction workers and workers involved in unloading airplanes and ships are exposed to levels of ~10 μg/m³ EC. Professional drivers have the lowest average exposure to levels of ~2 μg/m³. However, variations within job titles can be large, and these relative rankings can therefore differ according to specific situations.

1.4.2 Occupational exposure of workers predominantly exposed to gasoline engine exhaust

In occupational settings, gasoline engine exhaust is produced by spark-ignition engines. Four-stroke gasoline-powered engines are mostly used for the propulsion of road vehicles, ranging from motorcycles to small lorries (see Section 1.1.4). Exposures to emissions from road vehicles can occur in occupations such as border inspectors, car mechanics, office workers, car park attendants, professional drivers, service station attendants, shopkeepers, street workers, tollbooth workers and (traffic) policemen.

In addition to road traffic-related sources, gasoline exhaust may also be emitted by engines used in small electric power generators and in small portable equipment (power output, 2–7 kW) used to power chain saws, leaf blowers, hedge trimmers, brush cutters and clearing saws. During these applications, gasoline exhaust may be emitted in the breathing zone of some workers, such as loggers.

(a) Markers of exposure

For exposure assessment, some chemical components, such as inorganic compounds (metals) or some specific organic compounds, are fairly specific for emissions from gasoline engines. Some of these chemical markers of gasoline exhaust exposure are discussed in more detail below.

(i) Lead

Tetraethyl lead was used as an anti-knock agent in gasoline fuel but, during combustion, more than 90% was converted to and emitted as inorganic lead. Within a few hours to days, alkyl lead vapour emitted from the tailpipe is decomposed to lead oxides (IARC, 2006). The use of lead in gasoline was phased out to facilitate the introduction of exhaust catalysts and, in most parts of the world, this use of tetraethyl lead was abolished in the 1990s (IARC, 2006).

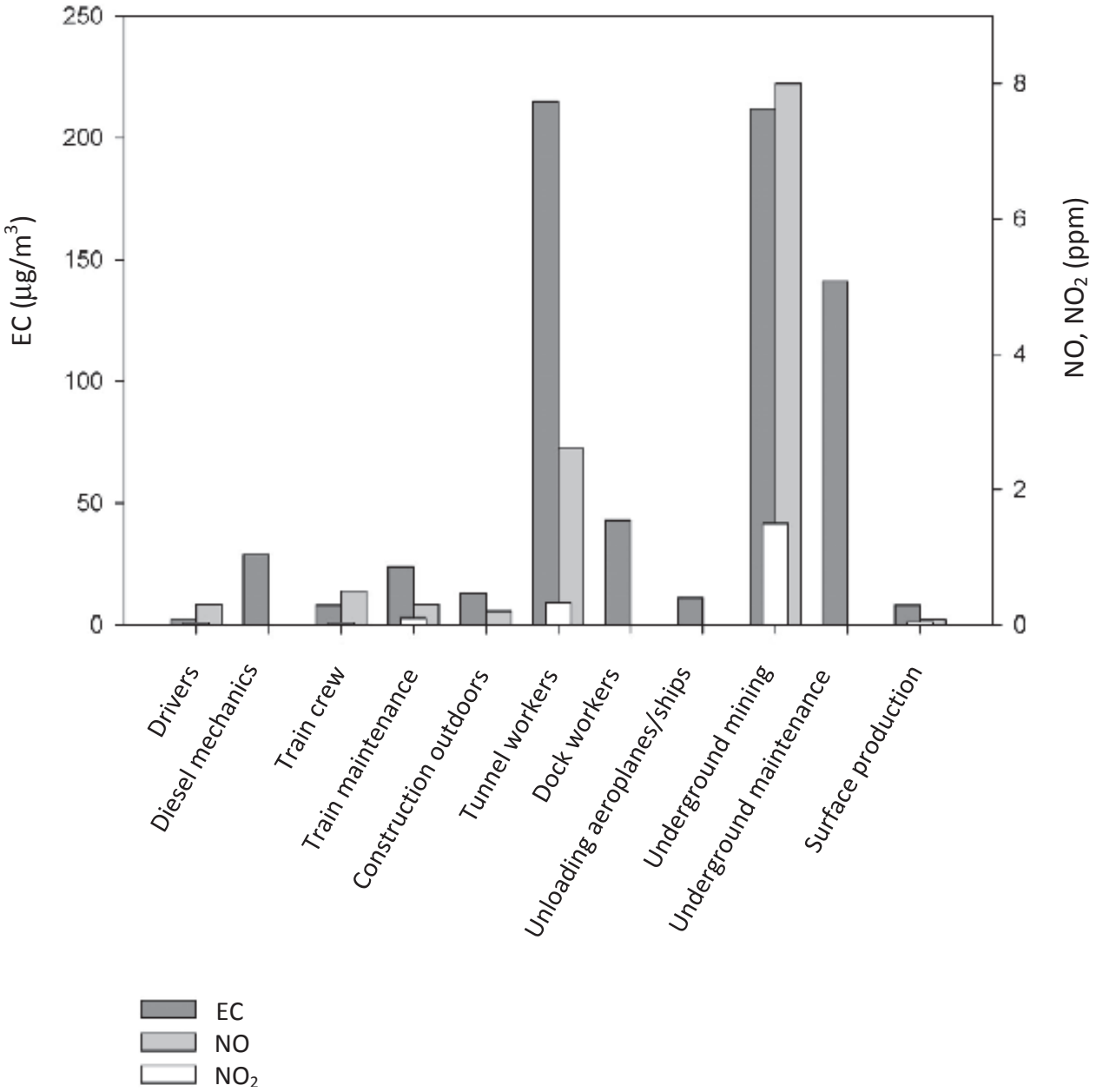
Lead is an important marker to reconstruct historical exposure to gasoline fuel and gasoline exhaust emissions, and appears to be related to exposures to both gasoline vapours and engine exhaust emissions over time (Anttila *et al.*, 1995). Occupational exposure to lead derived from gasoline exhaust emissions is summarized in Table 1.18 and Table 1.19. For exposure assessment in epidemiological studies, blood lead values are a reliable index for long-term exposure to lead. However, the interpretation of airborne lead exposure in relation to lung cancer is complicated due to the particle distribution of lead in airborne dust (Anttila *et al.*, 1995).

(ii) Carbon monoxide

Carbon monoxide in ambient air is a marker for occupational exposures to gasoline exhaust emissions. Especially in outdoor workplaces, ambient carbon monoxide is mostly derived from gasoline-powered engines from road traffic or other uses. In indoor environments, other (incomplete) combustion sources may contribute to elevated concentrations, including tobacco smoking.

Biomonitoring methods can be used to estimate internal exposure. Carbon monoxide can be monitored by determination of the percentage of carboxyhaemoglobin in blood and that of carbon

Fig. 1.17 Average personal exposures to elemental carbon, and nitric oxide and nitrogen dioxide by major occupational group predominantly exposed to exhaust from diesel engines



Compiled by the Working Group
EC, elemental carbon; NO, nitrogen oxide; NO_2, nitrogen dioxide

Table 1.18 Exposure to components from gasoline engine exhaust (mean ± standard deviation)

Type of source	Conditions	CO (ppm)	VOC (ppm)	Benzene (μg/m³)	Formal-dehyde (μg/m³)	MtBE (μg/m³)	1,3-Butadiene (μg/m³)	NO_2 (ppb)	Respirable dust (μg/m³)	Lead in respirable dust (μg/m³)	Reference
Car mechanics											
Emissions and contaminants from exhaust systems	Indoor exposure in garage A ('small')	–	–	–	–	–	–	–	–	53.2 (n = 1)[a] [21.7 ± 11.3] (n = 8)[b]	Chambers *et al.* (1984)
	Indoor exposure in garage B (100 m²)	–	–	–	–	–	–	–	–	[59.8 ± 31.8] (n = 4)[a] [43.7 ± 24.3] (n = 12)[b]	
	Indoor exposure in garage C (85 m²)	–	–	–	–	–	–	–	–	[21.7 ± 12.3] (n = 3)[a] [17.5 ± 5.9] (n = 4)[b]	
Car exhaust emissions	Workers at 7 garages and automobile firms in Stamford, CT, USA (n = 37)		0.003–0.053	–	–	108–43 464	–	–	–	–	White *et al.* (1995)
Car exhaust emissions	496 car mechanics working in 76 dealer workshops in Quebec, Canada, during the winter of 1991–92 and 1992–93	< 25 (28.8%) 25–35 (30.7%) 36–50 (19.3%) > 51 (21.2%)	–	–	–	–	–	–	–	–	Gourdeau *et al.* (1995)
Car exhaust emissions	Indoor exposure in garages in South Wales, United Kingdom (n = 16)		[0.419 (0.070–1280)]	[3.4 (6–9.3)]			–				Parry *et al.* (1997)
Commuting office workers											
Car exhaust emissions	Officers of South Wales Constabulary, United Kingdom, not considered to be exposed during work hours	–	[0.0589 (0.014–0.336)]	[11 (7–42)]	–						Parry *et al.* (1997)

Table 1.18 (continued)

Type of source	Conditions	CO (ppm)	VOC (ppm)	Benzene (μg/m³)	Formaldehyde (μg/m³)	M*t*BE (μg/m³)	1,3-Butadiene (μg/m³)	NO_2 (ppb)	Respirable dust (μg/m³)	Lead in respirable dust (μg/m³)	Reference
Loggers											
Chain saw (two-stroke engine)	Felling, limbing, buckling and manual skidding in snow-free conditions in a thick and dense mixed and pine forest stand at wind speed < 3 m/s and temperature –3 to –8 °C	[29 (36–38)] (*n* = 4)[d]	15 (7–40) mg/m³	[700 (300–1800)] (*n* = 28)	[80 (40–200)] (*n* = 12)	–	–	–	–	0.8 (0.5–10) (*n* = 7)	Nilsson *et al.* (1987)
	Felling, limbing, buckling and manual skidding in 50–90-cm deep snow in a sparse pine forest stand at wind speed < 3 m/s and temperature –16 to +1 °C	[17 (8.6–20)] (*n* = 4)	19 (3–74) (*n* = 42) mg/m³	[600 (100–2400)] (*n* = 42)	[80 (20–100)] (*n* = 25)	–	–	–	–	2 (0.4–4) (*n* = 14)	
	Felling only in a thick pine forest stand in 80–100-cm deep snow; wind speed < 4 m/s and temperature –1 to +1 °C	[32 (4–73)] (*n* = 7)	22.0 (9–69) (*n* = 8) mg/m³	[700 (300–2,300)] (*n* = 8)	[90 (70–100)] (*n* = 8)	–	–	–	–	–	
	Felling, limbing, buckling and manual skidding in a thick pine forest stand in 80–100-cm deep snow; wind speed < 4 m/s and temperature –1 to +1 °C	[12 (4–21)] (*n* = 18)	8.0 (2–17) (*n* = 16) mg/m³	[300 (70–600)] (*n* = 16)	[70 (30–90)] (*n* = 18)	–	–	–	–	–	
Chain saw (two-stroke engine)	Logging during 36 working periods of 2 hours at a snow depth of 80–100 cm in the county of Västerbotten, Sweden; wind speed < 4 m/s and temperature –2 to +3 °C	17 (4–73) (*n* = 23)	12 (2–69) (*n* = 23) mg/m³	–	[63 (28–130)] (*n* = 23)	–	–	–	–	–	Hagberg *et al.* (1985)

Table 1.18 (continued)

Type of source	Conditions		CO (ppm)	VOC (ppm)	Benzene (µg/m³)	Formal-dehyde (µg/m³)	MtBE (µg/m³)	1,3-Butadiene (µg/m³)	NO_2 (ppb)	Respirable dust (µg/m³)	Lead in respirable dust (µg/m³)	Reference
Car park attendantsc												
Emissions from private cars	Eight-story parking garage for 1400 cars at a hospital and university, Baltimore, MD, USA	Wk d (71 cars/h)	2.6	–	2.7	–	7.4	0.5	–	–	–	Kim *et al.* (2007)
		Wk-ends (6 cars/h)	1.2	–	0.3		0.4	0.2	–	–	–	
Professional drivers												
Traffic emissions	Professional drivers (n = 250)		71.2 ± 8.1	–	–	–	–	–	–	–	–	Jovanović *et al.* (1999)
Service station workers												
Traffic emissions	Pavia, Lombardy, Italy (n = 26)		–	–	38.81 ± 46.26	10.38 ± 5.49	174.04 ± 258.72	–	–	–	–	Ghittori *et al.* (2005)
Traffic emissions	Mexico City, Mexico (n = 24)		–	–	330 (130–770)	–	–	–	–	–	–	Romieu *et al.* (1999)
Shop keepers												
Road traffic	Polishers and repairmen working 10.11 ± 1.44 h/d in shoe stalls in Seoul, South-Korea (n = 32), indoor		–	–	732 ± 1640[d]	–	–	–	57.4 ± 17.1	127.7 ± 44.7	–	Bae *et al.* (2004)
	Outdoor (n = 32)		–	–	8 ± 20	–	–	–	58.1 ± 23.2	138.7 ± 43.6	–	
Street workers												
Traffic emissions	Mexico City, Mexico (n = 6)		–	–	62 (49–180)	–	–	–	–	–	–	Romieu *et al.* (1999)

Table 1.18 (continued)

Type of source	Conditions	CO (ppm)	VOC (ppm)	Benzene (μg/m³)	Formaldehyde (μg/m³)	MtBE (μg/m³)	1,3-Butadiene (μg/m³)	NO_2 (ppb)	Respirable dust (μg/m³)	Lead in respirable dust (μg/m³)	Reference
Toll booth workers											
Road traffic city	Bridge in New York, USA (n = 440)	63 (217)[e]	7.9 (29.6)[e]	–	61 (196)[e]	–	–	70 (320)[e]	64	10.6	Ayres *et al.* (1973)
Road traffic on three locations in Boston, MA, USA (n = 174)	August 1972	16.0 (4.0–> 130)	6.1 (2.9–> 50)	–	–	–	–	67 (15–220)	90 (< 20–200)	12.9 (1.5–35.5)	Burgess *et al.* (1977)
	March 1973	15.4 (3.0–> 100)	5.7 (3.2–16.6)	–	–	–	–	81 (10–290)	105 (65–150)	12.9 (1.0–33.2)	
	August 1973	33.7 (11.0–> 100)	19.8 (3.9–24.5)	–	–	–	–	100 (< 5–215)	100 (60–105)	15.6 (7.4–36.3)	
	Feb—March 1974	24.3 (6.0–81)	10.1 (7.0–13.0)	–	–	–	–	38 (< 5–119)	105 (55–160)	7.7 (1.0–17.8)	
Baltimore Harbor Tunnel in the summer of 2001;400–5900 vehicles/h and 72 000 vehicles/d	Inside toll booth (average over three shifts)	–	–	4.12 (0.29–14.9)	–	18.8 (0.50–43.8)	1.60 (0.23–8.09)	–	–	–	Sapkota *et al.* (2005)
	Inside toll booth (morning shift)			6.70 ± 1.31			2.91 ± 0.90				
	Inside toll booth (afternoon shift)			3.21 ± 1.20			0.91 ± 0.40				
	Inside toll booth (night shift)			2.42 ± 1.42			0.92 ± 0.51				
	Outside toll booth (average over three shifts)	–	–	13.3 (0.73–35.0)	–	39.6 (0.17–121)	7.24 (0.23–20.5)	–	–	–	
	Outside toll booth (morning shift)			19.8 ± 2.81			10.7 ± 3.20				
	Outside toll booth (afternoon shift)			14.9 ± 4.70			7.23 ± 1.72				
	Outside toll booth (night shift)			4.90 ± 2.21			3.71 ± 0.91				

Table 1.18 (continued)

Type of source	Conditions		CO (ppm)	VOC (ppm)	Benzene (µg/m³)	Formal-dehyde (µg/m³)	M*t*BE (µg/m³)	1,3-Butadiene (µg/m³)	NO_2 (ppb)	Respirable dust (µg/m³)	Lead in respirable dust (µg/m³)	Reference
Road traffic Klang Valley Kuala Lumpur, Malaysia (n = 90)	Toll booths with air conditioning system	Mon-Sun: 1597 (752–6333) vehicles/d in morning shift	20 (< 34)	–	–	–	–	–	–	–	–	Niza & Jamal (2007)
		Mon-Sun: 1778 (700–3269) vehicles/d in evening shift	30 (< 61)									
Traffic policemen												
Traffic emissions	Wuhan, China		–	–	–	–	–	–	–	–	5.2 ± 2.4 (n = 36)	Zhang *et al.* (1994)

Concentrations expressed as time-weighted average exposures in the breathing zone (unless otherwise indicated); range of exposure concentrations in parentheses

[a] Air samples collected in breathing zone

[b] Air samples collected from fixed locations

[c] Air concentrations determined from fixed locations in parking garages; this study was not performed with the explicit aim of assessing exposure of parking attendants

[d] The authors noted that benzene was presumably derived from indoor sources (such as solvents, polish and adhesives) but not from traffic or smoking, since shop keepers did not smoke during business hours.

[e] Average per 30-day period (maximum hourly reading)

–, not determined; CO, carbon monoxide; d, day; M*t*BE, methyl *tertiary* butyl ether; NO_2, nitrogen dioxide; VOC, volatile organic compounds; wk, week

Table 1.19 Biomonitoring of exposures to carbon monoxide and lead in occupations that entail exposure to gasoline engine exhaust (mean ± standard deviation unless otherwise indicated)

Reference	Type of source	Conditions		COHb (%)		CO in end-exhaled air (ppm)		Blood lead (µg/dL)
				Nonsmokers	Smokers	Nonsmokers	Smokers	
Border inspector								
Cohen *et al.* (1971)	Road traffic at border San Ysidro, CA, USA-Mexican Border	All of three shifts	Pre-shift	[1.4 ± 0.61] (n = 9)[a]	[4.8 ± 1.1] (n = 11)[a]	5.7 ± 1.05 (n = 9)	25.5 ± 4.01 (n = 11)	-
			Post-shift	[3.6 ± 1.0] (n = 9)[a]	[6.4 ± 1.3] (n = 11)[a]	18.6 ± 3.27 (n = 9)*	35.0 ± 4.93 (n = 11)	
		Night and morning shift	Pre-shift	[1.4 ± 0.58] (n = 7)[a]	[3.7 ± 0.83] (n = 6)[a]	5.0 ± 0.90 (n = 7)	19.2 ± 2.34 (n = 6)	
			Post-shift	[4.0 ± 1.1] (n = 7)1[a]	[7.5 ± 1.6] (n = 6)[a]	21.0 ± 3.70 (n = 7)*	41.8 ± 6.77 (n = 6)**	
Car mechanic								
Tola *et al.* (1976)	Exhaust emissions from repaired cars in Finland (n = 165)	In 13 workplaces; highest exposures occurred during cleaning the motor with compressed air		–	–	–	–	27 (10–80)[b]
Gourdeau *et al.* (1995)	Exhaust emissions from repaired cars in Canada (n = 496)	Indoor exposure in 76 repair shops involving 287 non-smoking mechanics		< 3.5 (28.6%) 3.5–5.0 (26.8%) 5.1–7.5(32.0%) > 7.6 (12.6%)	–	–	–	–
White *et al.* (1995)	Stamford, CT, USA	Workers at 7 garages and automobile firms (n = 37)	Men	1.05 (n = 12)	3.80 (n = 8)	–	–	–
			Women	0.90 (n = 1)	–		–	–
Commuting office worker								
White *et al.* (1995)	Stamford, CT, USA	Workers at 7 garages and automobile firms in Stamford Connecticut, US (n = 37)	Men	0.60 (n = 7)	2.6 (n = 1)	–	–	–
			Women	0.70 (n = 4)	4.80 (n = 2)	–	–	–
Car park attendant								
Johnson *et al.* (1975)	Parking cars in a garage in Houston, TX, USA	Indoor exposure to slow moving traffic (n = 36)		–	–	–	–	28.3 ± 10.33
Professional driver								
Suzuki (1990)	Bandung, Indonesia	Drivers of minibuses who work 12 h/d (n = 22)		–	–	–	–	2.5 ± 1.7[c]
Khan *et al.* (1995)	Pakistan	Employees of the governmental transport service in the city of Abbottabad (n = 36)		–	–	–	–	51.06[c, d]

Table 1.19 (continued)

Reference	Type of source	Conditions	COHb (%)		CO in end-exhaled air (ppm)		Blood lead (µg/dL)
			Nonsmokers	Smokers	Nonsmokers	Smokers	
Jovanović *et al.* (1999)	Russian Federation	Professional drivers exposed to road traffic (n = 250)	[8.9 ± 1.7] (n = 97)[e]	[24.6 ± 3.2] (n = 153)[e]	–	–	–
Zhou *et al.* (2001)	Shanghai, China	Taxi and bus drivers (n = 164)	–	–	–	–	10.9 ± 0.13
Service station worker							
Tola *et al.* (1976)	Traffic emissions and gasoline fuel	Ten different workplaces in Finland entailing exposure to fuel (n = 76)	–	–	–	–	20 (11–40)
Hunaiti *et al.* (1995)		Bus drivers in Jordan (n = 47)	–	–	–	–	7.6
Naeher *et al.* (2004)		Summer 2002 in Trujillo, Peru (n = 17)	–	–	–	–	2.8 ± 1.1[f]
Ghittori *et al.* (2005)		Pavia, Lombardy, Italy (n = 26)	–	–	–	–	7.0 ± 1.72 (4.0–10.0)
Zhang *et al.* 1994	Gasoline depot workers	Wuhan, China (n = 227)	[1.67 ± 0.79][a] [1.76 ± 0.76][a]				7.28 ± 2.13 7.85 ± 1.97
Shop keeper							
Khan *et al.* (1995)	Traffic emissions	8–10 h/d along busy road in the city of Abbottabad, Pakistan (n = 36)	–	–	–	–	52.10
Street worker							
Nordman & Hernberg (1975)	Primarily from automobile exhaust (86 men)	Street sweepers in Finland	–	–	–	–	13.3 (7–29)
Naeher *et al.* (2004)	Summer 2002 Trujillo, Peru	Street vendors (n = 3) Newspaper vendors (n = 3)	– –	– –	– –	– –	2.8 ± 0.8[f] 3.9 ± 1.5[f]
Toll booth worker							
Ayres *et al.* (1973)	Road traffic in New York city, NY, USA (n = 619)	Bridge Tunnel Square	2.12 ± 1.08 2.93 ± 1.36 1.94 ± 0.62	3.90 ± 2.13 5.01 ± 2.25 3.84 ± 2.06	– – –	- – –	28 ± 10 (n = 360)
Burgess *et al.* (1977)	Road traffic on three locations in Boston, MA, USA (n = 172)	Tunnel location 1 Alsston Location 2 Weston Location 3	[4.88 ± 1.48][g] [3.82 ± 1.4][g] [2.78 ± 1.3][g]	[8.20 ± 3.26][g] [7.82 ± 4.76][g] [6.02 ± 2.9][g]	26.9 ± 9.9 (n = 37) 21.6 ± 9.5 (n = 22) 16.4 ± 9.0 (n = 10)	43.5 ± 18.8 (n = 56) 41.6 ± 26.3 (n = 20) 32.6 ± 17.0 (n = 24)	30.84 ± 14.98 (n = 57) 35.11 ± 17.14 (n = 28) 37.75 ± 15.72 (n = 24)

Table 1.19 (continued)

Reference	Type of source	Conditions		COHb (%)		CO in end-exhaled air (ppm)		Blood lead (µg/dL)
				Nonsmokers	Smokers	Nonsmokers	Smokers	
Kocasoy & Yalin (2004)	Istanbul Bosphorus Bridge, Turkey	Cashiers on working in 3 shifts of 5–6 h; slowly moving road traffic on bridge	Pre-shift	0.67 ± 0.90 (n = 236)	1.99 ± 1.84 (n = 295)	4.09 ± 3.52 (n = 236)	12.13 ± 10.99 (n = 295)	–
			Post-shift	1.25 ± 1.03 (n = 236)	3.23 ± 2.19 (n = 295)	7.67 ± 6.35 (n = 236)	20.09 ± 13.61 (n = 295)	
			Increase	0.58 (n = 236)	1.24 (n = 295)	3.58 (n = 236)	7.96 (n = 295)	
Niza & Jamal (2007)	Road traffic Klang Valley, Kuala Lumpur, Malaysia	Toll booths with functioning air conditioning system; samples collected Mon-Sun: 1597 (752–6339) vehicles/d in the morning shift		1.0 (ND–18.4) (n = 66)	–	–	–	–
Traffic controller and traffic policeman								
Johnson *et al.* (1975)	Traffic emissions Houston, TX, USA	On foot patrol (number not specified)		–	–	–	–	23.1 ± 9.21
Nordman & Hernberg (1975)	Primarily automobile exhaust	(n = 28)		–	–	–	–	13.5 (9–20)[b]
Suzuki (1990)	Traffic emissions, Indonesia	Working 6 h/d on the road in Bandung, Indonesia (n = 24)		–	–	–	–	3.1 ± 1.8
Kamal *et al.* (1991)	Traffic emissions, Egypt	(n = 126)		–	–	–	–	29.2 ± 7.5
Biava *et al.* (1992)	Traffic emissions, Milan, Italy	Pre-shift		1.5 (n = 79)	2.6 (n = 79)	-	-	Low 14.2 ± 5.9
		Post-shift		2.5 (n = 79)	4.3 (n = 79)			Middle 15.3 ± 6.8
		Increase		1.0 (n = 79)	1.7 (n = 79)			High 16.2 ± 6.9
Zhang *et al.* (1994)	Traffic emissions Wuhan, China	Traffic policemen, pre-shift (n = 36)		[1.17 ± 0.72][a]				4.36 ± 1.71
		Traffic policemen, post-shift (n = 36)		[1.25 ± 0.71][a]				4.81 ± 1.64
Khan *et al.* (1995)[f]	Traffic emissions, Abbottatabad, Pakistan	8–10 h/d along busy road (n = 36)		–	–	–	–	53.43
Potula & Hu (1996)[f]	Traffic emissions, India	(n = 88)		–	–	–	–	11.2 (0.5–40.2)

Table 1.19 (continued)

Reference	Type of source	Conditions	COHb (%)		CO in end-exhaled air (ppm)		Blood lead (µg/dL)
			Nonsmokers	Smokers	Nonsmokers	Smokers	
Bono *et al.* (2007)	Traffic emissions, Torino, Italy	(*n* = 228)	0.6 ± 0.3 (*n* = 176)	2.4 ± 1.8[h] (*n* = 52)	–	–	–
Iavicoli *et al.* (2004)	Traffic emissions, Rome, Italy	Pre-shift (*n* = 161) Post-shift (*n* = 161)	–	–	–	–	–

* Increase from pre- to post-shift (in all three shifts) $P < 0.01$; afternoon value not reported in abstract
** Increase from pre- to post-shift (only in evening and night shifts) $P < 0.02$
[a] Calculated by Working Group using [%COHb] = 0.43+0.17·[CO] (Cohen *et al.*, 1971)
[b] Median (range)
[c] A positive trend with exposure classification (low – middle – high) was found for male but not female traffic wardens (data not presented).
[d] The value for the group of professional drivers, shop keepers and traffic policemen (*n* = 118) was 52.20 ± 2..88 µg/dL, which was higher than that for controls living in a rural area ($P < 0.001$), see text for further details.
[e] % COHb calculated by the Working group from mmol/L, assuming a concentration of 10 mmol/L haemoglobin (for men and women drivers)
[f] Geometric mean and geometric standard deviation
[g] Calculated from alveolar CO concentrations using the equation of Ringold *et al.* (1962)
[h] COHb was 4.8% for policemen who spent more than 7 days outdoors.
CO, carbon monoxide; COHb, carboxyhaemoglobin; d, day; h, hour; ND, not detected

monoxide in alveolar air [the last fraction of the exhaled (end-exhaled) air is usually collected to avoid dilution by the air volume, which does not reach the region of the lung where gas is exchanged with blood]. These parameters of internal exposure are highly correlated (r ~0.99) (Cohen *et al.*, 1971). The background level of carboxyhaemoglobin is substantially higher in smoking than in nonsmoking workers and is dependent on the number of cigarettes smoked and on the time since the last cigarette was smoked. Occupational exposure to carbon monoxide is usually assessed in smokers and nonsmokers separately, and by pre- and post-shift collection of blood or exhaled air. An increase in the percentage of carboxyhaemoglobin over the shift is often correlated to the ambient concentrations of carbon monoxide (Hagberg *et al.*, 1985). Fig 1.18 shows a comparison of exposure to carbon monoxide for the main occupational groups discussed in this section, and Fig. 1.19 shows the corresponding blood values of carboxyhaemoglobin, expressed as a percentage for smokers and nonsmokers.

(iii) Volatile organic compounds

Some volatile organic compounds are highly specific for gasoline engine exhaust, such as methyl *tertiary* butyl ether and 1,3-butadiene. Benzene, toluene, ethylbenzene and xylene have been studied extensively in relation to exposure to traffic emissions. Tobacco smoking leads to important co-exposures to benzene in some workers (White *et al.*, 1995). Occupational exposures to toluene and xylenes can also occur through work-related contact with products such as adhesives, paint, ink and cleaning solvents (Jo & Song, 2001). Fig 1.20 shows concentrations of benzene measured during personal air sampling by job title.

(iv) Formaldehyde and nitrogen oxides

Formaldehyde and nitrogen oxides are often used in the characterization of gasoline exhaust emissions, but these substances are also formed in diesel engines or may originate from alternative sources, such as building materials, or the use of preservative solutions, such as formalin.

(v) Particles

Compared with traditional diesel engines, spark-ignition engines only emit relative small amounts of PM (Murahashi *et al.*, 2003b), parent PAHs and PAH-derivatives such as alkylated, oxygenated and nitrated PAHs (Alsberg *et al.*, 1985; Scheepers & Bos, 1992). Particles emitted from gasoline engines are usually condensates of incompletely combusted fuel constituents and some metal oxides formed by the corrosion of engines and tailpipes.

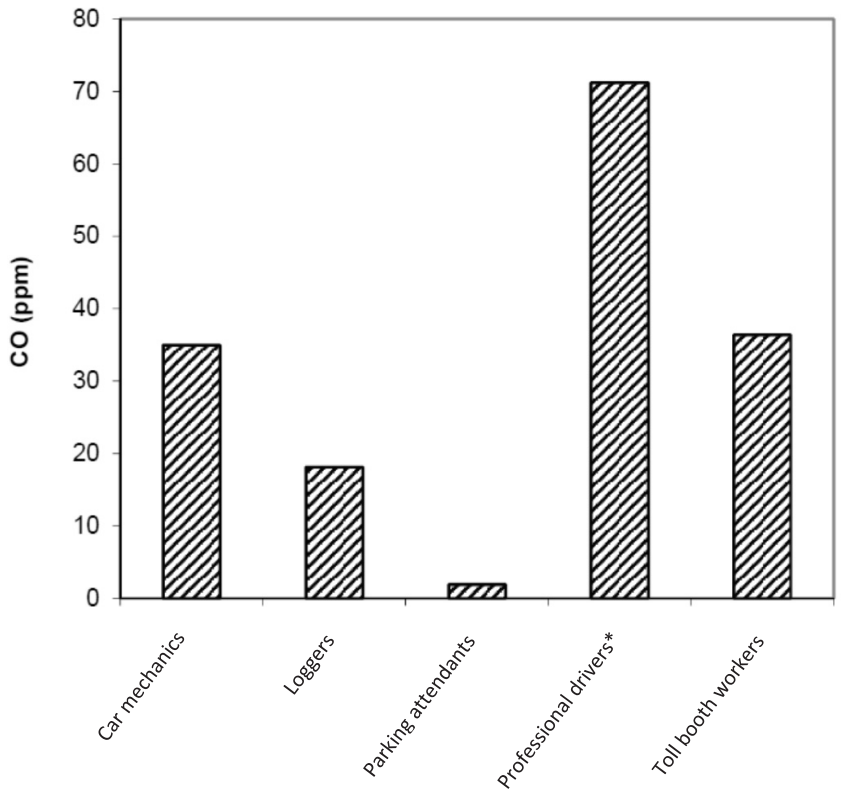
(b) Occupational exposures

Job titles associated with indoor and outdoor exposure to exhaust emissions from gasoline-powered engines are reviewed in this section, and the data are presented in Table 1.18, Table 1.19, Table 1.20, and Table 1.21.

(i) Border inspectors

Cohen *et al.* (1971) studied 26 workers at the border inspection station of San Ysidro, California, which is located at the USA–Mexican border (Table 1.19). Exposure assessment was performed by measuring carbon monoxide in exhaled air after the breath had been held, as described by Jones *et al.* (1958). The percentage of carboxyhaemoglobin was determined from the breath concentrations using a regression equation with a correlation coefficient of 0.99 and an intercept at zero exposure to carbon monoxide of 0.43% carboxyhaemoglobin (see the footnote in Table 1.19). In nonsmoking inspectors (in all shifts), the mean concentration of carbon monoxide increased from pre-shift to post-shift by a factor of 3.3 ($P < 0.01$). For smokers, post-shift carbon monoxide levels were increased but this difference was not statistically significant. However, for combined night and morning shifts, the increase in nonsmokers was fourfold

Fig. 1.18 Personal air concentrations of carbon monoxide by occupational group predominantly exposed to exhaust from gasoline engines



*Exposure estimated based on 250 samples using a hand-operated pump colorimetric analysis (Jovanović *et al.*, 1999)
CO, carbon monoxide
Compiled by the Working Group

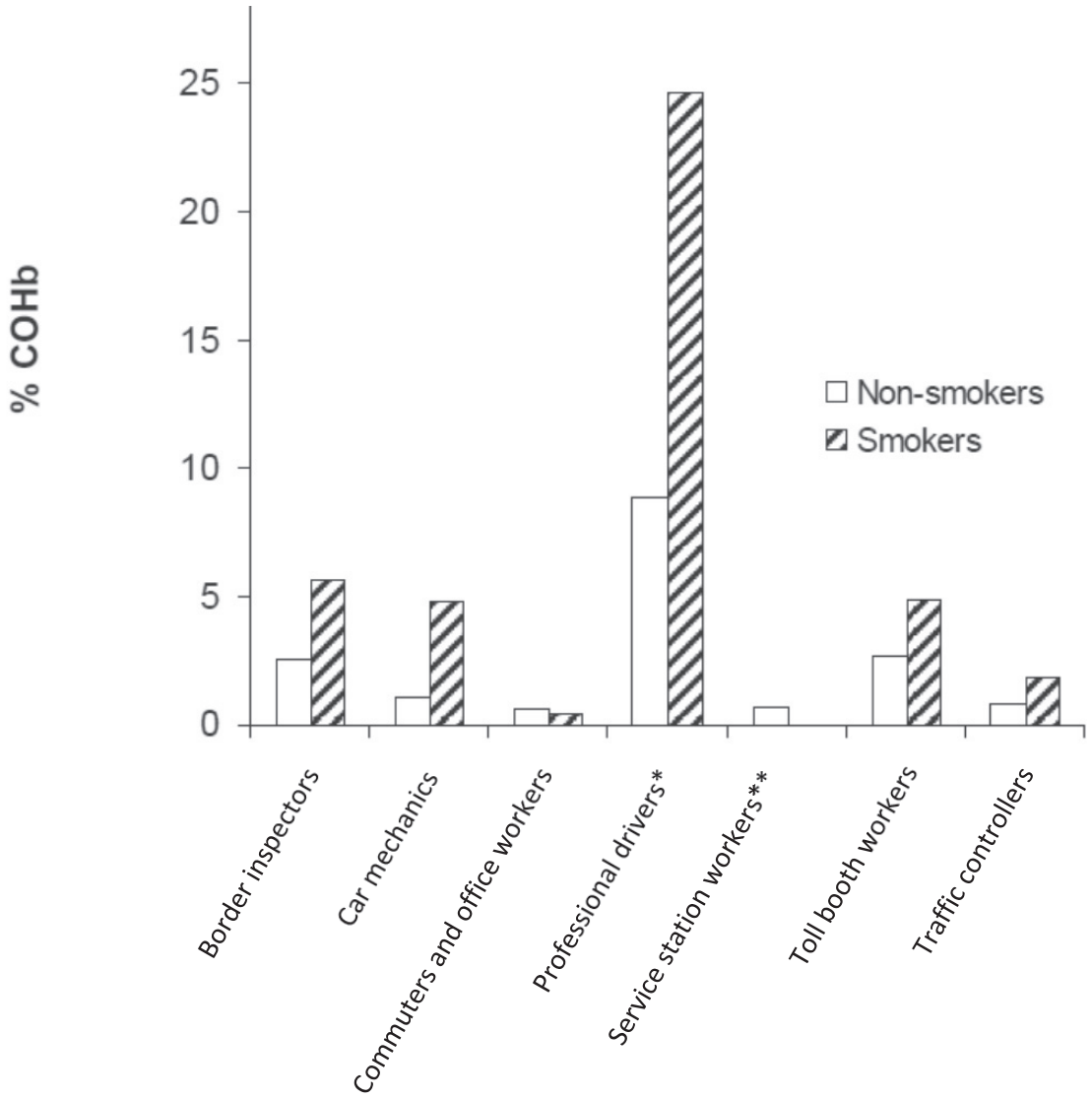
($P < 0.01$) and, for this subgroup, a statistically significant increase was also observed in smokers ($P < 0.02$). In nonsmoking and smoking control subjects (office clerks), no increase was observed (data not presented).

(ii) Office workers

Workers in offices may be exposed to chemicals derived from gasoline-powered road traffic, especially if their workplace is ventilated (either forced or naturally) with unfiltered outdoor air. In Taipei, Taiwan, China, Chan & Lin (1994) showed that differences in exposure to volatile organic compounds can occur, depending on the type of organization and type of office facilities used (see Table 1.20). Office workers are often assumed to have low exposures, but some data suggest they can experience exposures to volatile organic compounds from road traffic comparable with those of traffic policemen, car park attendants and store keepers (Romieu *et al.*, 1999; Jo & Song, 2001).

Office workers commuting to and from work typically have no direct contact with engine exhausts and consequently receive an exposure that is determined by the general air quality, derived primarily from pollution by road traffic. White *et al.* (1995) studied 14 commuters and assessed both environmental exposure by air

Fig. 1.19 Weighted percentage (%) of carboxyhaemoglobin by job title in workers predominantly exposed to exhaust from gasoline engines



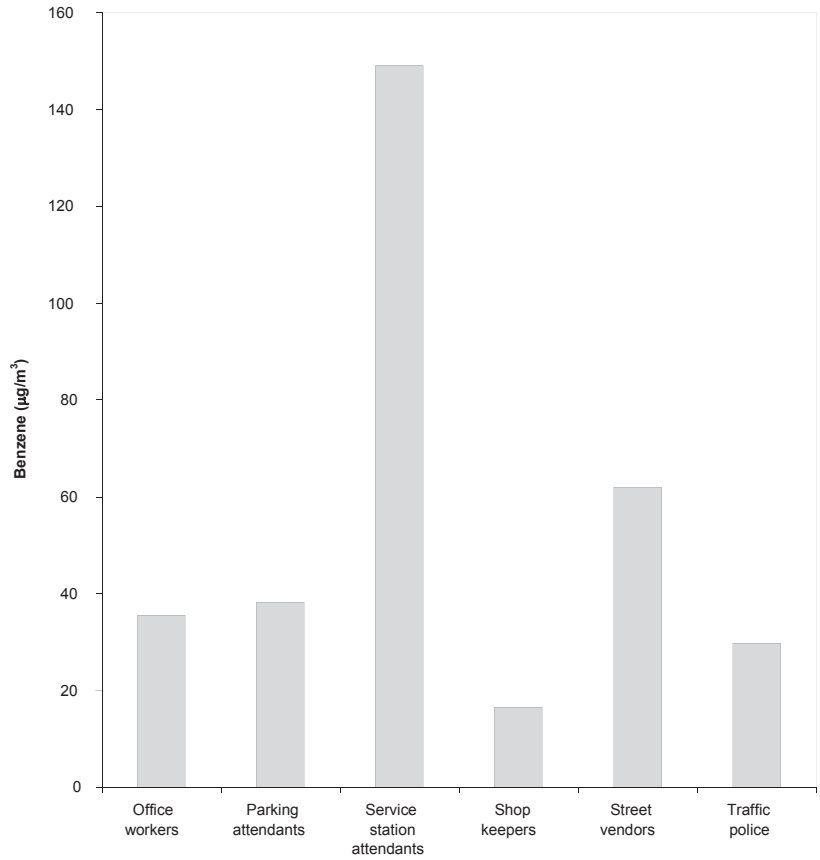
* Calculated from mmol/L assuming a concentration of 10 mmol/L haemoglobin (Jovanović *et al.*, 1999).
** The study of service station workers reported results from three nonsmokers only (White *et al.*, 1995).
COHb, carboxyhaemoglobin
Compiled by the Working Group

sampling and internal exposure by biological monitoring (see Table 1.21). In comparison with car mechanics, the median blood levels of methyl *tertiary* butyl ether of commuting office workers were an order of magnitude lower while the median blood levels of benzene were only 40% lower for nonsmoking men. The percentage of carboxyhaemoglobin of commuting office workers was in the normal range and more than 50% lower than the blood value of car mechanics (see below).

(iii) Car mechanics

Mechanics who work in garages have higher exposures to gasoline engine exhaust than outdoor workers. In most of the smaller

Fig. 1.20 Mean personal air sampling of benzene by job title in workers predominantly exposed to exhaust from gasoline engines



[The Working Group noted that the exposure of service station attendants was probably caused primarily by evaporation from fuels and only to a limited extent from exhaust fumes.]
Compiled by the Working Group

workshops, mechanical general or local exhaust ventilation is rarely available (Chambers *et al.*, 1984). Following the introduction of unleaded gasoline, exposure to lead in repair shops should have been reduced substantially, but no studies were found to confirm this.

Exposures to carbon monoxide from gasoline engine exhaust vary widely among car mechanics. In a study of 287 nonsmoking car mechanics in Canada, approximately 45% of the participants had carboxyhaemoglobin values higher than 5.0% (Gourdeau *et al.*, 1995). A study in Connecticut, USA, showed that the percentage of carboxyhaemoglobin was much lower and did not exceed the range observed in office workers (White *et al.*, 1995). Relatively low exposures to volatile organic compounds and benzene were reported for 16 car mechanics in garages in South Wales, United Kingdom by Parry *et al.* (1997), although these exposures were still much higher than those of office workers (threefold for benzene and about 10-fold for total aromatic compounds and volatile organic compounds).

Table 1.20 Concentrations of volatile organic compounds in occupations with exposure to gasoline engine exhaust (mean ± standard deviation)

Reference	Type of source, location	Conditions	Compound	Personal air concentration during shift (μg/m³)		End-exhaled air concentration (μg/m³)		Benzene metabolites (μg/g creatinine)	
				Smoker	Non-smoker	Pre-shift (n = 8–10)	Post-shift (n = 8–10)	SPMA	MA
Office worker									
Chan & Lin (1994)	Air pollution mainly from traffic sources in Taipei, Taiwan, China	Office of governmental agency (n = 10)	Benzene Toluene Ethylbenzene m/p-Xylene o-Xylene	25 ± 8 125 ± 99 16 ± 11 30 ± 21 20 ± 14		–	–	–	–
		Office of private company (n = 12)	Benzene Toluene Ethylbenzene m/p-Xylene o-Xylene	17 ± 8 150 ± 106 11 ± 7 25 ± 28 14 ± 8		–	–	–	–
		Office of research institute (n = 10)	Benzene Toluene Ethylbenzene m/p-Xylene o-Xylene	61 ± 29 79 ± 30 13 ± 4 23 ± 8 15 ± 5		–	–	–	–
Car park attendant									
Jo & Song (2001)	Four underground parking garages and four parking garages on ground floor in the city of Taegu, Republic of Korea	Working 8–12 h/d in the garage	Benzene Toluene Ethylbenzene p-Xylene m-Xylene o-Xylene	38.1 ± 19.6 130 ± 57.6 10.6 ± 2.8 9.5 ± 4.3 7.5 ± 3.7 17.2 ± 8.8	24.6 ± 10.3 119 ± 74.9 9.2 ± 4.8 8.9 ± 4.4 5.9 ± 3.7 17.3 ± 9.0	20.5 ± 11.4 56.5 ± 26.7 5.4 ± 2.4 5.2 ± 2.4 3.7 ± 2.6 8.2 ± 3.9	24.0 ± 11.1 61.4 ± 21.4 6.2 ± 2.8 6.4 ± 3.1 4.6 ± 2.6 10.6 ± 4.9	–	–

Table 1.20 (continued)

Reference	Type of source, location	Conditions	Compound	Personal air concentration during shift (µg/m³)		End-exhaled air concentration (µg/m³)		Benzene metabolites (µg/g creatinine)	
				Smoker	Non-smoker	Pre-shift (n = 8–10)	Post-shift (n = 8–10)	SPMA	MA
Service station attendant									
Jo & Song (2001)	Five service station located in the city of Taegu, Republic of Korea	Working 6–8 h/d at the service station	Benzene Toluene Ethylbenzene p-Xylene m-Xylene o-Xylene	84.4 ± 33.2 141 ± 18.2 12.9 ± 3.4 14.6 ± 6.5 14.0 ± 4.9 26.5 ± 8.6	72.1 ± 16.0 126 ± 42.0 12.1 ± 5.9 13.1 ± 6.3 13.6 ± 6.8 24.0 ± 11.1	26.6 ± 14.5 40.2 ± 17.5 6.1 ± 2.8 6.0 ± 2.6 3.5 ± 2.0 8.9 ± 3.8	41.0 ± 14.7[a] 63.6 ± 18.8[a] 10.3 ± 4.1[a] 11.0 ± 6.1[a] 6.6 ± 2.8[a] 16.1 ± 5.9[a]	–	–
Service station workers									
Ghittori *et al.* (2005)	Traffic emissions in Pavia, Lombardy, Italy	(n = 26)	Benzene	38.81 ± 46.26 (1.71–178.28)	-	-		2.36 ± 1.82 (0.30–6.90)	96.57 ± 53.44 (26.0–215)
Shop keeper									
Jo & Song (2001)	Roadside store near main street with heavy traffic in Taegu, Republic of Korea	Working 9–11 h/d in the store	Benzene Toluene Ethylbenzene p-Xylene m-Xylene o-Xylene	22.4 ± 7.3 167 ± 230 5.1 ± 3.4 4.3 ± 2.3 3.8 ± 3.1 7.9 ± 5.1	16.7 ± 4.9 176 ± 193 3.9 ± 2.2 3.6 ± 2.6 3.3 ± 2.4 7.5 ± 5.0	16.1 ± 8.2 48.3 ± 16.9 3.1 ± 0.9 2.7 ± 1.1 2.8 ± 1.1 4.9 ± 2.1	16.8 ± 7.6 83.6 ± 48.2[a] 3.7 ± 1.0 3.1 ± 1.3 2.7 ± 0.7 5.2 ± 1.8	–	–
	Store near underground entrance or exit in Taegu, Republic of Korea	Working 9–11 h/d in the store	Benzene Toluene Ethylbenzene p-Xylene m-Xylene o-Xylene	23.8 ± 10.4 175 ± 96.7 7.8 ± 5.4 4.9 ± 3.4 4.6 ± 2.4 11.1 ± 5.2	19.9 ± 15.3 197 ± 213 7.2 ± 4.6 5.2 ± 2.1 4.2 ± 1.6 9.2 ± 4.5	18.8 ± 8.9 52.6 ± 17.7 5.2 ± 1.9 4.4 ± 1.6 2.9 ± 1.4 6.7 ± 3.0	19.3 ± 6.5 93.1 ± 44.7[a] 5.3 ± 1.7 4.5 ± 1.5 2.9 ± 1.3 7.0 ± 2.5	–	–

Table 1.20 (continued)

Reference	Type of source, location	Conditions	Compound	Personal air concentration during shift (µg/m³)		End-exhaled air concentration (µg/m³)		Benzene metabolites (µg/g creatinine)	
				Smoker	Non-smoker	Pre-shift (*n* = 8–10)	Post-shift (*n* = 8–10)	SPMA	MA
Bae *et al.* (2004)	Polishers and repairmen shoe stalls in Seoul, South-Korea (*n* = 32), outdoor	working 10.11 ± 1.44 h/d in the store (*n* = 32)	Benzene Toluene p-Xylene o-Xylene	8 ± 20 (1–42) 160 ± 120 (33–504) 182 ± 120 (58–431) 139 ± 80 (52–229)		-	-	–	–
Traffic policeman									
Jo & Song (2001)	Traffic emissions in Taegu, Republic of Korea	6–8 h/d standing in dense motor vehicle traffic	Benzene Toluene Ethylbenzene p-Xylene m-Xylene o-Xylene	35.3 ± 14.8 114 ± 57.9 7.8 ± 4.7 6.3 ± 2.5 4.7 ± 2.0 11.1 ± 3.7	24.2 ± 10.0 125 ± 61.9 7.7 ± 4.2 8.4 ± 4.9 5.4 ± 3.7 13.4 ± 8.8	16.8 ± 7.8 27.1 ± 16.2 3.2 ± 1.6 3.0 ± 1.5 1.9 ± 0.9 4.6 ± 2.1	24.9 ± 11.5 46.8 ± 26.4[a] 4.8 ± 1.9[a] 4.9 ± 1.8[a] 2.9 ± 1.1[a] 7.3 ± 2.9[a]	–	–

[a] Statistically significant increase from pre- to post-shift level

d, day; MA, t,t-muconic acid; m-, *meta*; o-, *ortho*; p-, *para*; SPMA, S-phenylmercapturic acid

Table 1.21 Concentrations of volatile organic compounds in occupations with exposure to gasoline engine exhaust (median and range)

Reference	Job title	Location	Conditions	Compound	Breathing zone concentration (µg/m³)	Blood concentration (µg/L)	
						Pre-shift	Post-shift
White *et al.* (1995)	Car mechanic	Stamford, CT, USA	Post-shift samples collected at 7 garages and automobile firms	Benzene (*n* = 12)*a*	–		0.19 (0.11–0.98)[a]
				M*t*BE (*n* = 21)	–		1.73 (0.17–36.7)
				TBA (*n* = 21)	–		15 (2–50)[b]
	Commuting office worker		Samples collected after arriving at work during the shift in the morning	Benzene (*n* = 7)[a]	–		0.12 (0.10–0.20)[a]
				M*t*BE (*n* = 14)	–		0.11 (< 0.05–2.60)
				TBA (*n* = 14)	–		2.5 (0.5–9)[b]
	Service station attendant		Post-shift samples collected at 7 garages and automobile firms	Benzene (*n* = 3)[a]	–		0.36 (0.32–0.47)[a]
				M*t*BE (*n* = 3)	–		15 (7.6–28.9)
				TBA (*n* = 3)	–		80 (20–90)[b]
Romieu *et al.* (1999)	Service station attendant	Downtown Mexico City, Mexico	(*n* = 23–25)	Benzene	330 (130–770)	0.63 (0.26–2.3)	0.42 (0.13–1.4)
				Ethylbenzene	90 (61–1,400)	0.35 (0.12–1.4)	0.37 (0.12–7.8)
				o-Xylene	100 (65–1,900)	0.39 (0.16–1.2)	0.45 (0.15–6.3)
				m/p-Xylene	290 (180–5,800)	1.4 (0.50–4.7)	1.3 (0.36–16)
				Toluene	610 (410–1,300)	1.3 (0.44–4.1)	1.2 (0.34–4.7)
				M*t*BE	–	7.7 (2.2–48)	6.8 (0.22–25)
				Styrene	–	0.029 (0.022–0.045)	0.024 (0.020–0.093)
	Office worker		(*n* = 7–10)	Benzene	39 (32–67)	0.17 (0.12–0.23)	0.14 (0.12–0.20)
				Ethylbenzene	18 (12–22)	0.12 (0.071–0.18)	0.076 (0.045–0.11)
				o-Xylene	23 (16–28)	0.15 (0.081–0.31)	0.10 (0.073–0.21)
				m/p-Xylene	60 (44–80)	0.55 (0.37–0.81)	0.39 (0.19–0.73)
				Toluene	250 (20–7,100)	0.71 (0.30–1.4)	0.61 (0.38–7.4)
				M*t*BE	–	0.26 (0.22–0.97)	0.24 (0.16–0.57)
				Styrene	–	0.025 (0.022–0.049)	0.023 (0.022–0.027)
	Street vendor		Entire day outdoors (*n* = 5–6)	Benzene	62 (49–180)	0.30 (0.20–0.68)	0.22 (0.14–0.33)
				Ethylbenzene	29 (20–35)	0.13 (0.096–0.31)	0.12 (0.054–0.18)
				o-Xylene	6.0 (2.0–44)	0.18 (0.13–0.30)	0.15 (0.083–0.20)
				m/p-Xylene	95 (71–120)	0.75 (0.41–1.1)	0.53 (0.25–0.70)
				Toluene	170 (110–210)	1.8 (0.39–5.4)	0.51 (0.32–4.6)
				MtBE	–	0.47 (0.23–0.80)	0.33 (0.20–0.37)
				Styrene	–	0.028 (0.025–0.18)	0.025 (0.022–0.073)

[a] Nonsmokers

[b] Approximations of values taken from graphs (no table available)

m/p-, *meta-/para-*; M*t*BE, methyl *tertiary* butyl ether; o-, *ortho-*; TBA, tertiary butyl alcohol

(iv) Loggers

Loggers perform a range of tasks, including felling trees, limbing, bucking and manual skidding of trunks, although some of these may be performed mechanically and felling remains the main task (Nilsson *et al.*, 1987). Much of the exposure is dependent on the type, maintenance and operational conditions of the two-stroke engines used in the chain saw. Worn-out chain saw engines may cause increased exhaust emissions. The carburettor settings of the engine can also influence the composition of the exhaust; a lean setting (low fuel–air ratio) increases the emission of aldehydes and nitrogen oxides, whereas a rich setting (high fuel–air ratio) increases carbon monoxide and hydrocarbon emissions (Nilsson *et al.*, 1987).

The work of loggers is particularly strenuous because of the heavy physical workload, high levels of exposure to noise and also high levels of exposure to exhaust emissions. Loggers have high exposures to volatile organic compounds, benzene and formaldehyde. Exposures to carbon monoxide were low, which may be related to the carburettor settings on the chain saw engines (Table 1.18). The highest exposures were observed for loggers who were specifically performing felling operations. In 80–100-cm deep snow, loggers performing felling tasks only were exposed to more than twofold higher exhaust emissions compared with loggers performing all tasks using the same carburettor adjustment on their chain saws and under similar snow conditions. The estimated peak exposures during logging were 1.6 mg/m^3 of aldehydes, 320 ppm of carbon monoxide and 0.2 mg/m^3 of nitrogen oxides. The value for carbon monoxide was close to the short-term exposure levels of 400 ppm [calculated from milligrams per cubic metre by the Working Group] reported in the field (Bünger *et al.*, 1997). The maximum carboxyhaemoglobin level of German loggers exceeded the biological exposure index of the American Conference of Governmental Industrial Hygienists of 3.5%, 2–3 h after the start of the shift, when workers were paid on a piecework basis (Bünger *et al.*, 1997).

(v) Car park attendants

As shown in Table 1.18, the mean levels of exposure to carbon monoxide, benzene and methyl *tertiary* butyl ether of attendants at a university hospital car park in Baltimore, USA, were 2.6 ppm, 2.7 μg/m^3 and 7.4 μg/m^3, respectively, on weekdays (72 cars/h), compared with 1.2 ppm, 0.3 μg/m^3 and 0.4 μg/m^3 at the weekend (6 cars/h), respectively (Kim *et al.*, 2007). Jo & Song (2001) studied the exposure of car park attendants in ground-level and underground parking garages in Taegu, Republic of Korea (see Table 1.20). All garages used mechanical ventilation systems and ground-level garages also benefited from natural ventilation in the entrance/exit area. Exposure to benzene, toluene, ethylbenzene, *meta*-xylene, *para*-xylene and *ortho*-xylene was measured by the collection of personal air samples. In addition, before and after shift (of 8–12 hours), alveolar air samples were collected to determine the concentrations in alveolar air. The mean and median breath concentrations of all components tended to increase across the shift, but no statistically significant differences were observed. Personal air samples were collected from four smoking and four nonsmoking car park attendants (Table 1.20). Both breath and personal air concentrations of benzene, toluene, ethylbenzene, *meta*-xylene, *para*-xylene and *ortho*-xylene were approximately twofold higher in attendants who worked in underground car parks compared with those who worked at ground-level, possibly due to the additional natural ventilation in the entrance/exit area. Personal measurements of concentrations in workplace air correlated significantly with post-shift concentrations in alveolar air for all substances except *meta*-xylene (Spearman correlation coefficients varied from 0.76 to 0.84). Mean and median levels of alveolar

air concentrations of benzene, toluene, ethylbenzene, *meta*-xylene, *para*-xylene and *ortho*-xylene were twofold higher in car park attendants compared with the levels reported in a control group of college students (results not presented).

Johnson *et al.* (1975) reported elevated blood lead values of 28.3 ± 10.3 µg/dL in car park attendants in Houston, TX, USA.

(vi) Professional drivers

Drivers represent a heterogeneous group of workers, including taxi drivers and drivers of lorries and buses, who spend most of their time in a vehicle driving mostly in urban areas and on highways (Table 1.18 and Table 1.19). Exposure to carbon monoxide and the percentage of carboxyhaemoglobin were clearly elevated in 250 drivers studied by Jovanović *et al.* (1999) but it is uncertain whether such exposures were derived from exhaust emissions from their own vehicle, which can enter into the interior of the vehicle due to a leaking exhaust system, or from emissions from other road vehicles, which enter the interior of the vehicle through the windows or the ventilation system. [The Working Group noted that the exposure values for carbon monoxide reported in this study were exceptionally high but were nevertheless consistent with the high reported values of carboxyhaemoglobin.] Studies of blood lead levels in professional drivers in China, Indonesia and Pakistan have also indicated elevated exposure originating from gasoline engine exhaust (Suzuki, 1990; Khan *et al.*, 1995; Zhou *et al.*, 2001).

(vii) Service station attendants

Attendants of gasoline stations are primarily exposed to volatile organic compounds from gasoline vapours and also from exhaust emissions from the vehicles of customers and from passing road traffic. These sources of exposure have been investigated in two studies that used both personal air sampling and biological monitoring (Romieu *et al.*, 1999; Ghittori *et al.*, 2005).

Romieu *et al.* (1999) reported the breathing zone concentrations of several PAHs and some chemicals specific for gasoline such as methyl *tertiary* butyl ether. Mexican fuel contains approximately 5% of methyl *tertiary* butyl ether, which resulted in higher blood levels of this compound compared with those of benzene, toluene, ethylbenzene and xylene (see Table 1.21), whereas the benzene content of Mexican fuel is lower (1.5–2%) than that of European gasoline (2–6%) (Romieu *et al.*, 1999). About half of the population smoked, but the blood levels of volatile organic compounds and chemical markers for smoking, such as 2,5-dimethylfuran, were similar in smokers and nonsmokers, suggesting that the data reflected primarily exposure to volatile organic compounds derived from gasoline fuel and exhaust. Overall the levels of exposure to benzene, toluene, ethylbenzene and xylene reported in this study appeared to be higher than those in other available studies (Moolenaar *et al.*, 1994). [The Working Group noted that the higher exposures in the study in Mexico were probably due to high traffic density and the use of old vehicles with poor emission control technology. The median concentrations of markers of exposure to volatile organic compounds in end-of shift blood samples appeared to be somewhat lower than those in pre-shift samples, which may be related to the collection of the pre-shift sample more than half an hour after the start of the shift. Long-term exposure may also contribute to enhanced pre-shift values due to bioaccumulation because of incomplete metabolism/excretion between exposures.]

Ghittori *et al.* (2005) performed a detailed study of service station attendants in Pavia, Italy, who were stratified into three subgroups: cashiers, self-service attendants and fuel dispensers (in Table 1.18, Table 1.19, and Table 1.20, only results of the entire group are presented). Mean exposure to methyl *tertiary* butyl ether in air was significantly higher for fuel dispensers (401.75 ± 302.75 µg/m^3) than for cashiers

(16.28 ± 7.47 μg/m³) and self-service attendants (19.66 ± 5.79 μg/m³). This was reflected in the significantly higher values of methyl *tertiary* butyl ether in the urine of fuel dispensers (3.0 ± 3.2 μg/L) compared with cashiers (0.10 ± 0.06 μg/L) and self-service attendants (0.74 ± 1.69 μg/L). This suggests that primarily fuel-related exposure occurred, although levels of formaldehyde were slightly lower in fuel dispensers (no statistically significant difference with the other job titles). Fuel dispensers also had significantly higher exposure to benzene (88.56 ± 47.64 μg/m³) than cashiers (4.38 ± 2.08 μg/m³) and self-service attendants (19.66 ± 5.79 μg/m³). This pattern was also reflected in the values of urinary excretion of the benzene metabolites, S-phenylmercapturic acid and *t,t*-muconic acid.

Exposures to carbon monoxide were not studied extensively in service station attendants but appeared to be relatively low (White *et al.*, 1995). Except for one report of elevated blood lead (Tola *et al.*, 1976), exposure to lead also appeared to be low relative to other job titles (Hunaiti *et al.*, 1995; Ghittori *et al.*, 2005). Urinary platinum concentrations of 73.8 ± 79.9 ng/L were reported in service station workers in Italy (Ghittori *et al.*, 2005).

(viii) Shop keepers

Jo & Song (2001) studied exposure to traffic emissions by persons tending a shop, either underground or at the street level, in a busy sector of Taegu, Republic of Korea. Vehicle emissions were the primary source of the exposure of shop attendants to ethylbenzene, and *ortho*-, *meta*- and *para*-xylene (Table 1.20) because outdoor and indoor concentrations were found to be in the same range in stores tended by both smoking and nonsmoking shop keepers. No information was provided on the type of ventilation or the distance of these shops from the road. Exposure of store keepers to benzene, toluene, ethylbenzene and xylene was two- to fourfold higher than that reported for a control group of college students (results not presented). A statistically significant increase in the mean and median concentrations of toluene in alveolar air from pre-shift to post-shift was observed in both underground and ground-level shops. Workers in shoe stalls in Seoul, Republic of Korea, were exposed to an average concentration of 8 μg/m³ of benzene (Bae *et al.*, 2004).

[The Working Group noted that, for print shops, the post-shift increase in toluene concentration could be explained by the presence of toluene in the adhesives used for bookbinding. This may also explain the somewhat higher alveolar air levels of toluene observed in keepers of book stores compared with those of keepers of cosmetic and flower shops. This interpretation of the results on toluene is supported by the finding of higher indoor than outdoor levels of toluene in print shops and book shops. Because of the apparent lack of indoor sources of toluene, it may be assumed that, for keepers of flower and cosmetic shops, vehicle emissions were the primary source of exposure to toluene. The concentrations of benzene in alveolar air were higher in smokers, suggesting that exposure from traffic may be masked by active smoking.]

Thirty-six shop keepers who worked 8–10 hours per day along the main roads of Abbottabad, Pakistan had mean blood lead values of 52.10 μg/dL (Khan *et al.*, 1995), which were similar to those of policemen who worked in the same city (see Section 1.4.2 (b, xi)).

(ix) Street vendors and street sweepers

Very limited data were available on street vendors and street sweepers in cities with high road traffic intensity, such as Mexico City (Romieu *et al.*, 1999). Reported exposures to benzene were higher for street vendors than for office workers but much lower than those of service station attendants and also lower than those of shop keepers who worked in outdoor locations (Table 1.18, Table 1.19, and Table 1.20). Street sweepers in Finland had intermediate

exposure to lead at a time when tetraethyl lead was still added to gasoline (Tola *et al.*, 1976).

(x) Toll booth workers

The exposure of attendants in toll booths at bridges and tunnels is primarily outdoor and depends on the local infrastructure and weather conditions. Exposure patterns primarily reflect traffic density, weather conditions and emissions during the acceleration of vehicles. One study found that air levels of aldehydes, lead, total hydrocarbons, nitrogen oxides and carbon monoxide followed a similar pattern during the day, suggesting that carbon monoxide could be a useful exposure indicator for toll booth workers (Ayres *et al.*, 1973). The levels of exposure to nitric oxide, respirable PM and lead of toll booth workers were found to be consistently elevated at three different locations near Boston, MA, USA (Burgess *et al.*, 1977). [The Working Group did not take into account lead in hair levels reported by Burgess *et al.* (1977) because of uncertainty whether this represents real uptake or contamination due to external deposition of dust.]

No difference was noted between exposures to carbon monoxide observed in the USA in 1973 (Ayres *et al.*, 1973) and in Boston, USA (Burgess *et al.*, 1977). More recently reported exposures for toll booth workers in Turkey (Kocasoy & Yalin, 2004) and Malaysia (Niza & Jamal, 2007) were in a similar range. These results suggest that control technologies combined with reduced carbon monoxide emissions from car exhaust over a period of three decades have led to substantially lower exposures to carbon monoxide in nonsmokers (but less striking decreases in smokers). Reduction in exposure can also be achieved by ventilation, irrespective of high traffic density and/or high emission levels. Sapkota *et al.* (2005) demonstrated exposures to benzene, methyl *tertiary* butyl ether and 1,3-butadiene two- to fourfold lower in toll booths compared with levels outside the booth (Table 1.18). This was achieved by the supply of filtered air through a set of pre-filters and a box filter as part of an air conditioning unit, which in part recirculated the air.

(xi) Traffic policemen

Exposure to emissions from road traffic has been studied extensively among policemen and similar occupations. Biava *et al.* (1992) observed a small cross-shift increase of the percentage of carboxyhaemoglobin in nonsmoking and smoking traffic wardens in Milan, Italy. Zhang *et al.* (1994) reported similar findings in Wuhan, China (Table 1.19). The contribution of ambient exposure to carbon monoxide on carboxyhaemoglobin levels was significantly higher only in nonsmokers compared with policemen who predominantly worked indoors. In Torino, Italy, Bono *et al.* (2007) also reported relatively low carboxyhaemoglobin values (except for a subgroup of police officers who worked on the street for shifts of more than 7 hours).

The mean and median concentrations of benzene, toluene, ethylbenzene and xylene in alveolar air increased from pre-shift to post-shift in policemen in Taegu, Republic of Korea (Jo & Song, 2001). This increase was statistically significant ($P < 0.05$) for all substances except benzene. Traffic police spent 6–8 hours in dense motor vehicle traffic. No differences in concentrations in the alveolar air between smokers and nonsmokers were observed, suggesting that smoking habits did not mask possible exposure to emissions from road traffic. Concentrations of benzene, toluene, ethylbenzene and xylene in post-shift alveolar air samples correlated significantly with concentrations obtained by personal air sampling (Spearman correlations ranging from 0.65 to 0.86). Mean and median levels of benzene, toluene, ethylbenzene and xylene in alveolar air were up to twofold higher than those reported for a control group of college students (results not presented). One study (Iavicoli *et al.*, 2004) provided data on urinary platinum levels in traffic policemen: the reported pre- and

post-shift concentrations of 4.43 ± 2.34 and 4.63 ± 2.47 ng/L, respectively, were markedly lower than those reported for service station workers (Ghittori *et al.*, 2005).

High mean blood lead levels of 52.2 (± 2.88) µg/dL were reported in policemen who worked 8–10 hours per day in Abbottabad, Pakistan (Khan *et al.*, 1995). The mean levels in traffic policemen (53.43 µg/dL) were similar to those observed in shop keepers (52.10 µg/dL) and in employees of the governmental transport service (51.06 µg/dL), and were twofold higher ($P < 0.001$) than those observed in 36 controls from a rural area (24.06 ± 1.70 µg/dL). The elevated exposure to lead in policemen was also reflected in a higher level of urinary 5-amino-levulinic acid dehydrase of 3.68 ± 0.2 mg/dL, compared with 0.8 ± 0.04 mg/dL in the control group ($P < 0.001$).

1.4.3 Exposure of the general population

Exposures of the general population to traffic emissions are a function of proximity to fresh traffic emissions and the presence of aged traffic emissions in regional pollutants. During air transport, atmospheric processes change the contaminants: particles agglomerate, rain out and settle out according to size, chemical reactions affect some components and weather processes remove others (Lippmann *et al.*, 2003).

(a) Exposure from traffic

Source apportionment can be conducted to determine the contribution of traffic to a complex mixture of air pollution (Schauer *et al.*, 1996; Schauer & Cass, 2000; Schauer *et al.*, 2002; Schauer, 2003; Zheng *et al.*, 2005; Zhao *et al.*, 2006; Hopke, 2008; Brinkman *et al.*, 2009). Sources emit correlated sets of individual pollutants in a pattern that is more or less unique. The ability to distinguish sources depends on the components being measured [large numbers of elements can be measured by X-ray fluorescence or neutron activation analysis] plus EC, organic carbon, $PM_{2.5}$ and perhaps ultrafine particles. Table 1.22 summarizes the data of Viana *et al.* (2008) in European source apportionment studies. They noted that overlap among source component emissions and variability in the pattern for a given set of sources, such as for diesel and gasoline engines, may occur and the results may therefore be difficult to interpret. One limitation of the application of organic tracers is that a large sample is required and a very sophisticated analysis must be performed using extremely sensitive equipment. In addition, environmentally stable tracers must be used, wherever possible, to avoid problems with changes during transport which may blur source assignments. Several studies have been conducted in cities and regions around the world, and these are presented in Table 1.23 to illustrate the findings and relative importance of traffic sources in a variety of urban backgrounds.

In urban areas, the $PM_{2.5}$ ranged from 9.5 µg/m³ in Corpus Christi, TX, USA (Karnae & John, 2011) to 231 µg/m³ in Delhi, India (Chowdhury *et al.*, 2007). The corresponding contributions from traffic were about 10% for Corpus Christi and 21% and 9% for diesel and gasoline vehicles, respectively, in Delhi (Table 1.23). Beijing, China, had high $PM_{2.5}$ levels (39–167 µg/m³) with a low contribution from traffic (7% ± 3%) (Zheng *et al.*, 2005), but vehicle ownership has increased rapidly in China. Developing countries have fewer engine emission controls and subsequently may have higher emission levels and exposures depending on the country and traffic density.

(b) Other sources

Source apportionment analyses have been used to determine the amount of emissions from ships in marine ports, from aircraft near airports and from the use of diesel generators.

Table 1.22 Characteristics of the four main source categories identified from the European studies

PM	Source signatures	No. of studies	Source contribution range (%)	Trend	Source interpretations
Source interpretations provided by the different authors					
PM_{10}	C, Fe, Ba, Zn, Cu	23/28	1 (Areao, PT)	Dependent on volume and mixture of traffic emissions	Traffic
$PM_{2.5}$	C, Fe, Ba, Zn, Cu, Pb	19/20	55 (Milan, IT) 5 (Birkenes, NO) 49 (Barcelona, ES)		Vehicle exhaust
PM_{10}	Al, Si, Ca, Fe	24/28	5 (Duisburg, DE)	Marked North to South gradient	Mineral matter
$PM_{2.5}$	Al, Si, Ca, Fe, K	19/20	37 (Huelva, ES) 5 (Helsinki, FI) 30 (Huelva, ES)		City dust
PM_{10}	Na, Cl, Mg	20/28	< 5 inland sites	Gradient from Atlantic coastal sites to continental inland sites	Marine aerosol
$PM_{2.5}$	Na, Cl, Mg	15/20	88 (Areao, PT) < 5 inland sites 15 (Areao, PT)		Sea spray
PM_{10}	V, Ni, SO^{2-}_4	21/28	8 (Barcelona, ES) 37 (Florence, IT)	No clear trend	Oil combustion
$PM_{2.5}$	V, Ni, SO^{2-}_4	16/20	11 (Amsterdam, NL) 43 (Barcelona, ES)		Industry
PM_{10}	SO^{2-}_4, NO^{-}_3, NH^{+}_4	9/28	12 (Cork, IE) 46 (Milan, IT)	No clear trend	Secondary aerosols Regional background
$PM_{2.5}$	SO^{2-}_4, NO^{-}_3, NH^{+}_4	10/20	17 (Huelva, ES) 57 (Duisburg, DE)		Long-range support
Signatures reported for specific sources identified at single locations					
Source signatures	*Location*	*Source interpretation*	*Study*		
Cu, K, Ca	Estarreja, PT	Copper smelter	Pio *et al.* (1989)		
Cr, Ni, Cu	Estarreja, PT	Oil combustion	Pio *et al.* (1989)		
As, Cu, Pb, Ba, Sb	Huelva, ES	Copper smelter	Alastuey *et al.* (2006), Querol *et al.* (2007a)		

Table 1.22 (continued)

PM	Source signatures	No. of studies	Source contribution range (%)	Trend	Source interpretations
Cr, Ni, Mo, Cu, Co, As	Llodio, ES	Pigment production	Viana *et al.* (2006)		
As, Pb, Zn, Zr, Tl, Cs	L'Alcora, ES	Ceramic frit production	Cruz-Minguillón *et al.* (2007)		
Mn, Zn, Pb, Cd	Llodio, ES; Alasua, ES	Metallurgy	Viana *et al.* (2006), Zabalza *et al.* (2006)		
Si, Al, Fe, Li, Sc	L'Alcora, ES	Ceramic production	Querol *et al.* (2007b)		
K, As	Copenhagen, DK	Biomass burning	Andersen *et al.* (2007)		
K, C, O_3	Monagrega, ES	Biomass burning	Rodríguez *et al.* (2004)		

Al, aluminium; As, arsenic; Ba, barium; C, carbon; Ca, calcium; Cd, cadmium; Cl, chlorine; Co, cobalt; Cr, chromium; Cs, caesium; Cu, copper; Fe, iron; K, potassium; Li, lithium; Mg, magnesium; Mn, manganese; Mo, molybdenum; Na, sodium; NH^+_4, ammonium; Ni, nickel; NO^-_3, nitrate; O_3, ozone; Pb, lead; PM, particulate matter; Sb, antimony; Sc, scandium; Si, silica, SO^{-2}_4, sulfate; Tl, thallium; V, vanadium; Zn, zinc; Zr, zirconium

DE, Germany; DK, Denmark; ES, Spain; FI, Finland; IE, Ireland; IT, Italy; NL, Netherlands; NO, Norway; PT, Portugal

Table 1.23 Occurrence of diesel and gasoline vehicle exhaust in measurements of urban particulate matter ($PM_{2.5}$) or ultrafine particles

Country, region	Type of sample location	$PM_{2.5}$ concentration ($\mu g/m^3$)	Gasoline exhaust (%)	Diesel exhaust (%)	Ship exhaust (%)	Main monitor activities	References
USA							
Seattle, WA	Urban background or rural	8.97 – 11.57	13 – 31	2 – 9	4–6	3 city, 1 rural, 1 port sites	Kim & Hopke (2008)
Kalmiopsis, OR	Remote location	3.4 ± 3.07	1.9	1.4	–	1 remote site	Hwang & Hopke (2007)
Corpus Christi, TX	Urban background and port	9.45	Traffic 9.7		4.6	1 city site	Karnae & John (2011)
St. Louis, MO	Urban background	16.4	21.1	5.0	–	2 city sites	Lee & Hopke (2006)
New York City area, NY	Urban background	10.7 ± 8.7–13.9 ± 9.0	8–22	3–15	–	3 city sites, 2 suburban	Qin *et al.* (2006)
Raleigh and Chapel Hill, NC	Personal, indoor and outside residences	Personal, 23.0 Indoors, 19.1 Outdoors, 19.3 Ambient, 19.2	Traffic Personal, 10.0 Indoors, 9.4 Outdoors, 17.2 Ambient, 19.4		–	38 suburban homes	Williams *et al.* (2003), Zhao *et al.* (2006)
Los Angeles area, CA	Urban background	Azusa, 14–20 Los Angeles, 14–24	Traffic, 40–50%		–	2 sites	Lough *et al.* (2006)
Los Angeles port area, CA	Ship emissions	10.2 ± 3	–		< 5	4 urban 1 on pier	Arhami *et al.* (2009)
Seattle, WA	Ship emissions	8.05	–		1–2	1 site	Wu *et al.* (2009)
Los Angeles airport, CA	Aircraft emissions	UFP counts 11 900–28 500 during take-off	–		–	Site at blast fence	Zhu *et al.* (2011)
Asia							
Lahore, Pakistan	Urban background	190.5 ± 90.5	7.7 (2-cycle engine)	28.3	–	1 city site	Raja *et al.* (2010)
Delhi, Mumbai, Kolkata, India	Urban background	Delhi, 230.9 Mumbai, 88.9 Kolkata, 304.5 Chandigarth, 29.2	9 6 11 16	21 10 16 13	–	3 city sites, 1 background	Chowdhury *et al.* (2007)[a]

Table 1.23 (continued)

Country, region	Type of sample location	$PM_{2.5}$ concentration ($\mu g/m^3$)	Gasoline exhaust (%)	Diesel exhaust (%)	Ship exhaust (%)	Main monitor activities	References
Beijing, China	Urban background	39–167	Traffic, 7 ± 3		–	4 city monitors 1 background	Zheng *et al.* (2005)
Europe							
Erfurt, Germany	Urban background	19.2 (% of $PM_{2.8}$)	Traffic: 4% local, 39% remote		–	1 city site	Yue *et al.* (2008)
Prague, Czech Republic	Urban background	8473 (total particle count)	34.2 (% of total count)	37.8 (% of total count)	–	1 city site	Thimmaiah *et al.* (2009)
Several European countries	Urban background	NR	Traffic 12–35		–	48 city sites, 8 countries	Viana *et al.* (2008)
Canary Islands, Spain	Ship emissions	UFP count $35–50 \times 10^3$/cm	–		65–70	1 near water front	Gonzalez *et al.* (2011)

[a] Values include some two-cycle engine exhausts

NR, not reported; UPF, ultrafine particles

(i) Ships

Kim & Hopke (2008) carried out studies in Seattle, WA, USA, to determine the $PM_{2.5}$ emissions from ships. They had access to a source profile for oil combustion but not for ship emissions, although they knew that ships burn a low-grade residual oil (No. 6 or Bunker C) that contains significant concentrations of vanadium, nickel and sulfur. At three of their sampling sites, analysis of wind patterns back tracked the source locations of vanadium, nickel and sulfate pollutants suggested that 'oil combustion' in the harbour was the source that contributed to ship emissions, and accounted for 4–6% of the $PM_{2.5}$.

Arhami *et al.* (2009) investigated the impact of ship activity in the Port of Los Angeles, CA, USA. Because of the heavy highway traffic in the area of the port, diesel vehicle emissions were elevated. The ship emissions from 'oil combustion' were clearly identified by the vanadium, nickel and sulfate correlations in the quasi-ultrafine particles, but were only a minor source of EC and organic carbon.

Gonzalez *et al.* (2011) conducted a study to determine the contribution of ship emissions to ultrafine particles in the port city of Santa Cruz de Tenerife, Canary Islands, Spain. Changes in the wind direction during the day permitted an assessment of the ship component associated with high levels of sulfur dioxide and ultrafine particles. They estimated that ship exhaust accounted for 65–70% of the ultrafine particles, which was in the range of 35–50 × 10^3 particles/cm^3. Because the sampling site was 1 km or more away from the ships, samples taken in closer proximity might demonstrate higher exposures, but few residences were closer.

In summary, the impact of ship emissions on exposures in port areas appears to be small compared with that of diesel vehicles in traffic. However, emissions from ships are a major source of ultrafine particles with significant sulfate nuclei.

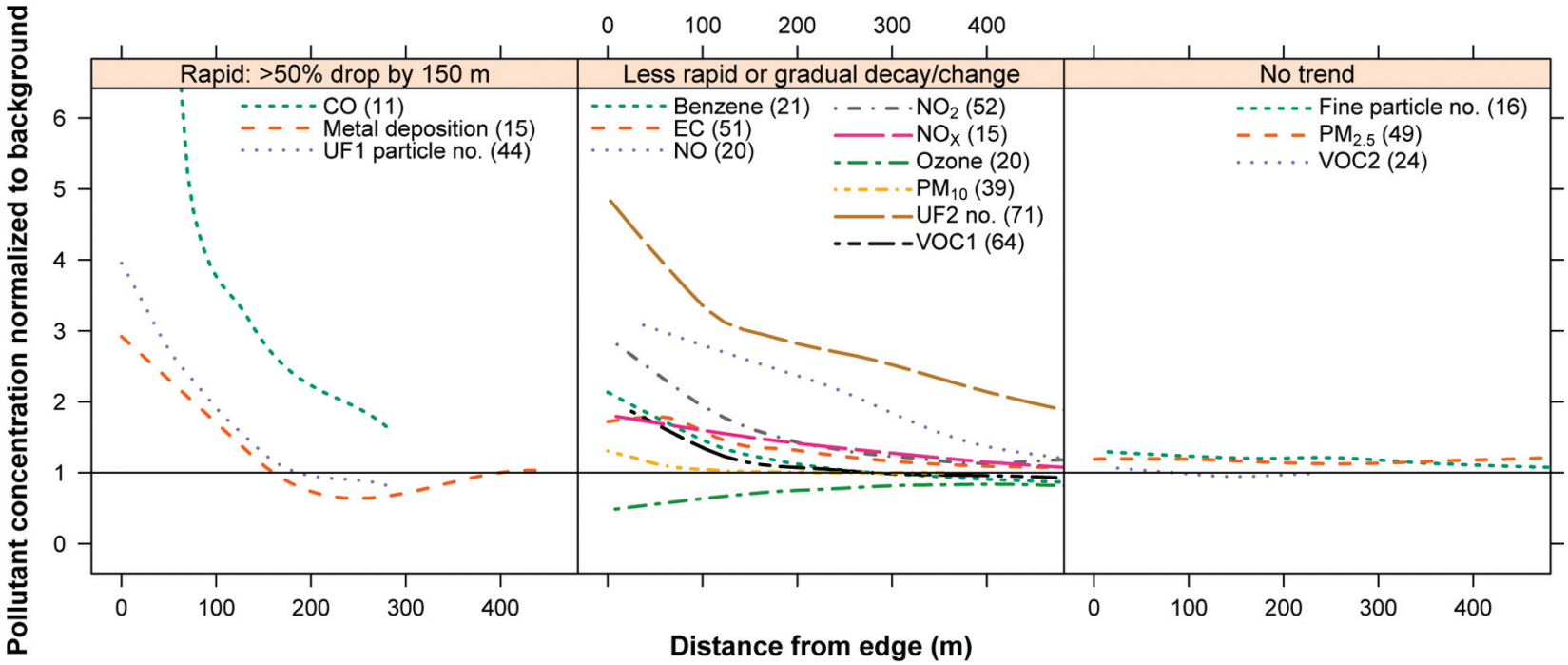
(ii) Diesel generators

In developing countries and locations where electrical supplies are unreliable or lacking, people often rely on small to medium-sized stationary diesel generators to provide electricity. Because these generators have limited emission controls, they can be major sources of exposure to diesel exhaust. In experimental studies, emissions were strongly influenced by engine load, and higher loads produced lower PM emissions. In some areas, waste cooking oil is mixed with diesel fuel (25–75%) and used as a biodiesel for the generators (Betha & Balasubramanian, 2011; Valente *et al.*, 2012). With 50% cooking oil, the emissions of carbon monoxide and hydrocarbons were increased on average by 20.1% and 23.5%, respectively, over a range of engine loads. Although several laboratory tests have been carried out, no reports have been made of exposure concentrations during the home use of generators.

(c) Roadside exposures

Karner *et al.* (2010) summarized 41 studies that assessed the decline in roadside pollutant levels at a distance from roadways. The common markers of traffic were investigated and listed by decreasing number of studies reporting data for EC, $PM_{2.5}$, PM_{10}, number of ultrafine particles, nitrogen dioxide, nitric oxide, nitrogen oxides, carbon monoxide, benzene, sulfur, organic carbon, ozone, volatile organic compounds, number of fine particles, metal deposition, and carbon dioxide. The majority of studies assessed the first eight pollutants, and most investigated emissions from major highways or freeways. Wind speed and direction, and night-time atmospheric stability had considerable effects on the rate of decline in concentrations moving downwind of roadways. During low wind speeds or calm conditions, such as in the evening or at night, emissions accumulated in a wide area surrounding the roadways with little or no

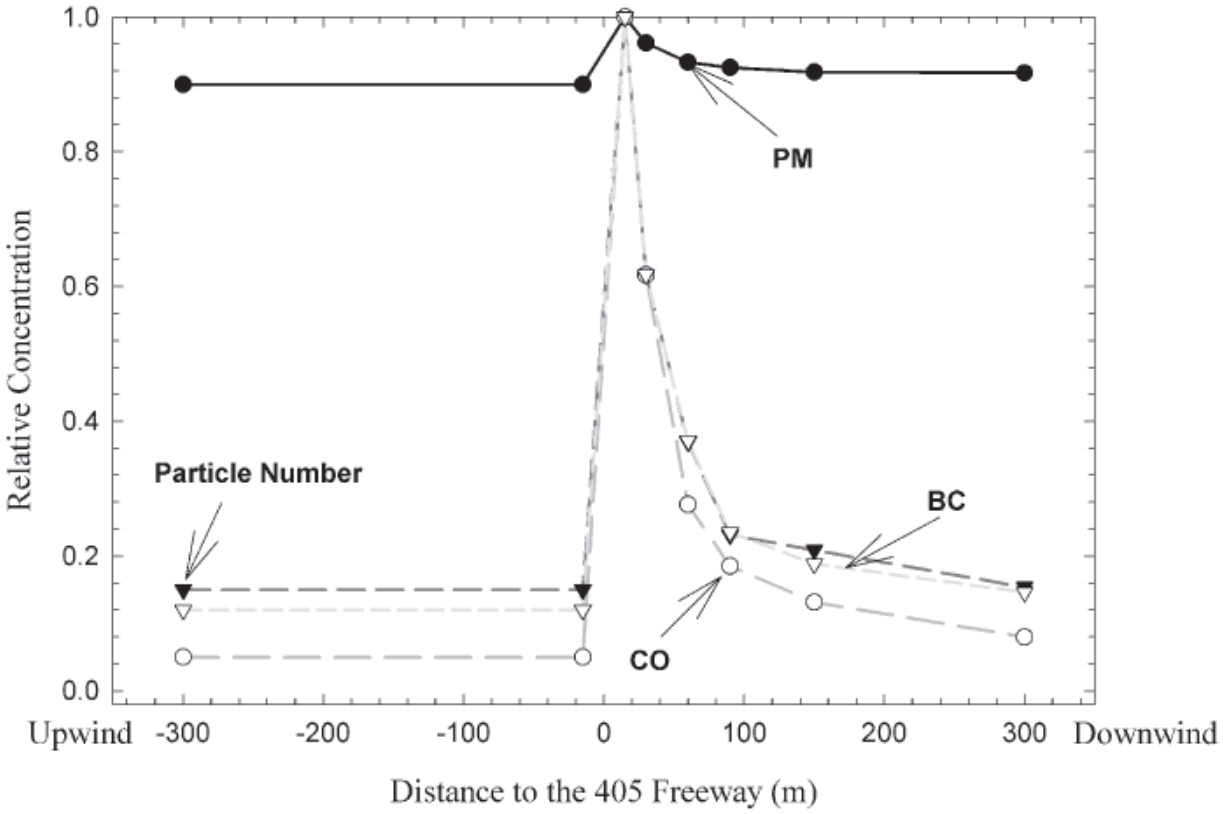
Fig. 1.21 Locally smoothed regression of background-normalized pollutant concentrations at a distance from edge of the road



The horizontal line indicates background concentration. A Loess smoother (α = 0.75; degree = 1) is fitted to each pollutant which is placed into one of three groups. The regression sample size (n) is given in parentheses after each pollutant.

CO, carbon monoxide; EC, elemental carbon; NO, nitric oxide; NO_2, nitric dioxide; No_x, nitric oxides; PM, particulate matter; UF1, UF2, number of particles with data collection beginning at 3 nm and 15 nm, respectively; VOC1, 8 volatile organic compounds whose concentrations varied with distance from road; VOC2, 4 volatile organic compounds whose concentrations did not vary with distance from road

Fig. 1.22 Relative mass and number of ultrafine particles, and black carbon and carbon monoxide concentrations to downwind distance from a freeway



BC, black carbon; CO, carbon monoxide
From Zhu *et al.* (2002). Reprinted by permission of the publisher, Taylor & Francis Ltd, http://www.tandf.co.uk/journals

gradients, and during high wind speeds they were rapidly diluted.

Fig. 1.21 shows smoothed trends of pollutant concentrations with distance from the roadway (Karner *et al.*, 2010). Concentrations of carbon monoxide and metals and the number of ultrafine particles > 3 nm declined sharply to near background levels by about 300 m from the road. The number of ultrafine particles > 15 nm also declined rapidly but did not reach background levels. In contrast, $PM_{2.5}$, EC and most other contaminants showed either moderate declines or no decline with distance.

Zhu *et al.* (2002) studied the decline in $PM_{2.5}$, ultrafine particles, black carbon and carbon monoxide with distance from a freeway. Fig 1.22 shows a rapid exponential decay in the concentrations of ultrafine particles, black carbon and carbon monoxide, but only a slight change in $PM_{2.5}$. Concentrations reached background levels at a shorter distance (within 100 m) compared with the studies reviewed by Karner *et al.* (2010).

A source apportionment study was conducted in Los Angeles, CA, USA, in 2005 to determine how well this approach can distinguish between diesel and gasoline emissions (Phuleria *et al.*, 2007). Samples were collected from one site along a freeway that did not allow HGVs and was assumed to characterize emissions from purely gasoline vehicles, and from one site

Table 1.24 Mean concentrations of the meteorological and bulk-chemical parameters measured near CA-110 and I-710 in Los Angeles, USA

Parameters	CA-110[a]				I-710[b]			
	Freeway		Background		Freeway		Background	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
CO_2 (ppm)	476	39	427	44	430	28	383	10
Temperature (°C)	21.6	3.0	21.3	3.4	18.9	2.9	18.0	2.2
Humidity (%)	50.7	14.2	50.1	15.3	46	11.8	45.3	7.0
$PM_{2.5}$ (μg/m³)	20.0	11.2	15.7	5.6	15.4	5.1	12.0	6.0
EC (μg/m³)	1.8 (9%)	1.2	1.4 (8.9%)	0.9	3.3 (21%)	0.6	0.7 (5.8%)	0.3
OC (μg/m³)	14.9 (74%)	5.2	11.4 (73%)	6.6	6.9 (45%)	1.8	5.4 (45%)	1.6

[a] Only cars are allowed on CA-110.
[b] A combination of cars and trucks travel on I-710, with an average of 17% heavy-duty vehicles.
CO_2, carbon dioxide; EC, elemental carbon; OC, organic carbon; PM, particulate matter; SD, standard deviation
From Phuleria *et al.* (2007)

next to a freeway with HGV traffic (~17% heavy-duty vehicles). Table 1.24 shows that, compared with background, the $PM_{2.5}$ was higher near the freeway with only car traffic, while the percentages of EC and organic carbon were similar. In contrast, the area near HGV traffic had a much higher percentage of EC (21%) than other sites. The percentage of organic carbon was the same at all sites. Fig 1.23 shows a comparison of the two highways for two types of organic tracers, the 15 hopanes and steranes from motor oil and 15 PAHs from combustion with a wide range of molecular weights. The comparison shows clear distinctions between the patterns ('fingerprints') for the two freeways with their different sources. The car traffic was evident from the hopanes–steranes and heavy PAHs, whereas the diesel emissions were evident from the EC and lighter PAHs. It is important to note that tracer patterns were quantitatively related to the amount of vehicle emissions.

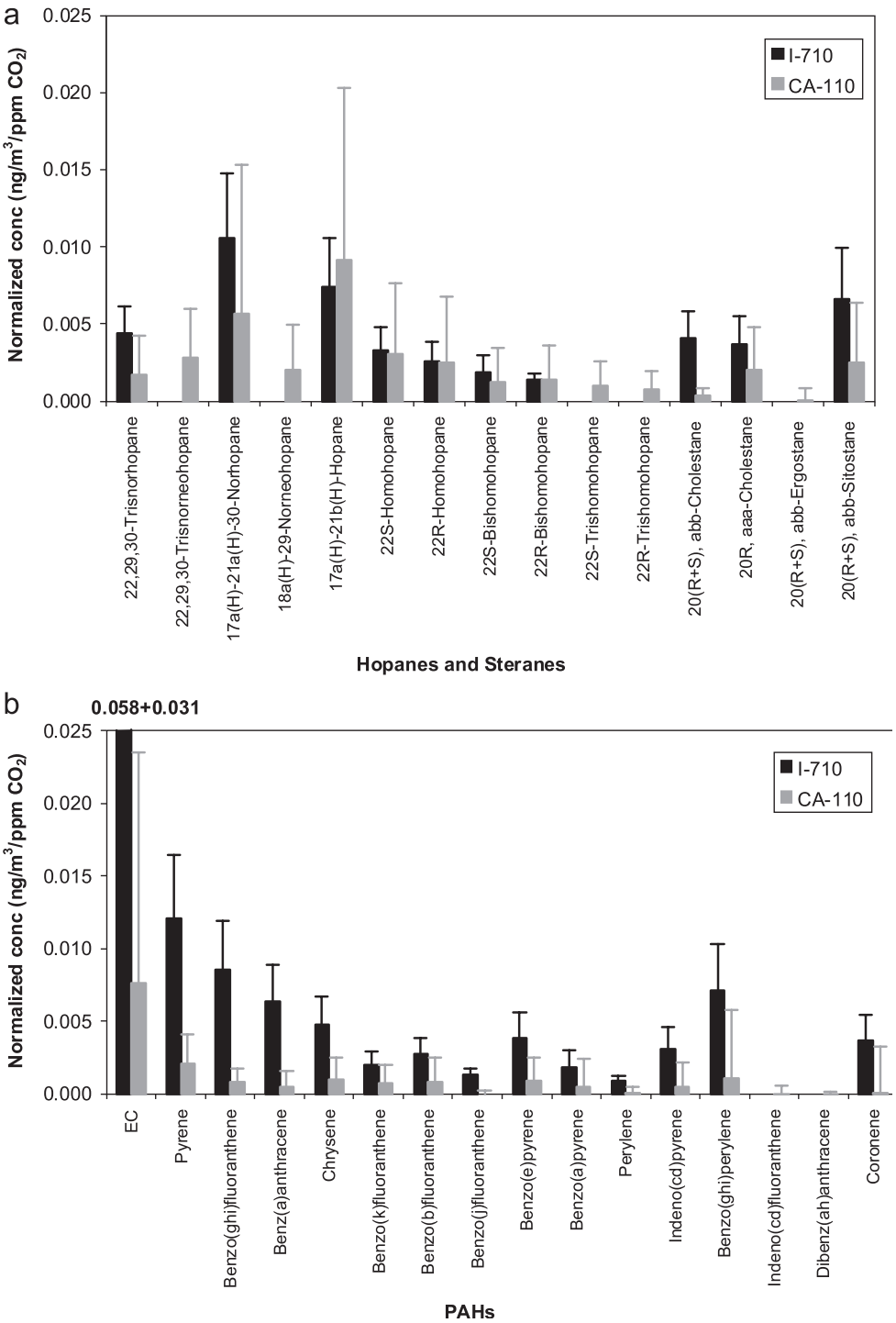
(d) Exposure of children to traffic emissions

Exposures of children to traffic emissions are a strong function of the location of their homes and schools downwind from major highways. Exposure is strongly modified by the prevailing wind directions and speed, distance from the roadways, and the type and density of the traffic. Those who ride school buses are exposed to traffic emissions that depend on the route taken and on the age, engine and fuel type of the bus, and the seal on the engine compartment relative to the passenger compartment. Finally, exposures during walking or cycling are dependent on proximity to heavy traffic and the route taken, with the highest exposures occurring by the roadside.

Several studies have investigated exposures of schoolchildren in large cities (Janssen *et al.*, 2001; Behrentz *et al.*, 2005; Wichmann *et al.*, 2005; Van Roosbroeck *et al.*, 2006; Wu & Batterman, 2006; Weichenthal *et al.*, 2008; Ashmore & Dimitroulopoulou, 2009; Branis *et al.*, 2009; Lin & Peng, 2010; Zhang & Zhu, 2010; Habil & Taneja, 2011).

Van Roosbroeck *et al.* (2007) conducted a study in the Netherlands to validate the use of proximity of a school to heavily travelled roads as a proxy for 'high exposure' situations for epidemiological studies. The investigators carried out personal sampling over a 9-month period to define long-term exposures. Exposures at schools near a very busy freeway were mainly increased, but not those at schools near a ring road.

Fig. 1.23 Comparison of measured (a) hopanes and steranes (normalized to carbon dioxide) and (b) polycyclic aromatic hydrocarbons and elemental carbon (normalized to carbon dioxide) between CA-110 and I-710 in particulate matter ($PM_{2.5}$) size mode



Error bars represent standard error.
CO_2, carbon dioxide; EC, elemental carbon; PAH, polycyclic aromatic hydrocarbon
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Another study (Van Roosbroeck *et al.*, 2006) sought to determine whether a positive answer to 'living near a motorway' on a time activity questionnaire was associated with increased personal exposure of children to black carbon and nitrogen oxides. Children living near a busy motorway had a 35% higher exposure to black carbon ('soot') compared with children living further away from the motorway, even when they attended the same school. The children living near the motorway also had increased exposure to nitric oxide, nitrogen dioxide and nitrogen oxides (14% higher) than children living further away from the motorway. These findings supported the validity of the questionnaire approach.

Janssen *et al.* (2001) reported a study of air contaminants from traffic in 24 schools in the Netherlands as a function of traffic density, distance from a heavily travelled motorway and percentage of time the school was downwind from the motorway. They found that traffic counts for HGVs, but not cars, were related to black carbon. Both indoor and outdoor concentrations of black carbon declined with increasing distance from the road, and the average concentration was about 20% higher indoors compared with outdoors, possibly because indoor samples were only collected when schools were open during the day, whereas outdoor samples were collected around the clock, including low ambient concentrations at night and on weekends. The percentage of time that the school was downwind from the motorway significantly increased the levels of black carbon, nitrogen dioxide and benzene indoors, but not those of benzene and $PM_{2.5}$ outdoors. The level of nitrogen dioxide showed no gradient with distance.

Two studies examined exposures of children while riding school buses in Los Angeles, CA, USA (Behrentz *et al.*, 2005; Sabin *et al.*, 2005). Sabin *et al.* (2005) collected samples and made real-time measurements during 24 morning and afternoon commutes on two bus routes running from South to West Los Angeles, plus seven runs on a rural/suburban route. Mean concentrations of diesel vehicle-related pollutants showed a wide range: 0.9–19 μg/m^3 for black carbon, 23–400 ng/m^3 for particle-bound PAHs and 64–220 μg/m^3 for nitrogen dioxide. These air contaminants were significantly lower inside a compressed natural gas-powered bus compared with conventional diesel buses, although the compressed natural gas bus emitted higher concentrations of formaldehyde. Both studies reported higher concentrations of exhaust-related pollutants when the windows were closed, in part because the exhaust of the bus entered the cabin (Behrentz *et al.*, 2004; Sabin *et al.*, 2005). The levels of black carbon, particle-bound PAHs, benzene and formaldehyde pollutants were also higher on urban bus routes than on the rural/suburban route, and were also substantially higher inside the bus relative to urban background measurements. Driving behind other diesel vehicles contributed to high exposure within the buses. $PM_{2.5}$ showed smaller effects between open/closed window conditions and between bus routes (Sabin *et al.*, 2005).

(e) In-vehicle exposures while driving and commuting

Time spent in vehicles can contribute a large proportion of total exposure to vehicle exhaust (Fruin *et al.*, 2004, 2008). Fruin *et al.* (2008) measured on-road exposures in Los Angeles, CA, USA, extensively. An electric car outfitted with sampling devices was used to minimize the contributions of the sampling platform to the exposures (Westerdahl *et al.*, 2005; Fruin *et al.*, 2008). They used real-time monitors for $PM_{2.5}$, particle counts by size, including ultrafine particles, nitric oxide, nitrogen dioxide, black carbon, particle-bound PAHs, carbon monoxide and carbon dioxide. Sampling frequencies ranged from 2 seconds up to 1 minute. They drove on two routes: a freeway route and an arterial street route. While driving, they videotaped the traffic

in and then performed analyses to determine the type of vehicle followed for each 5-minute interval, vehicle speed, acceleration, road type, traffic density, fraction of HGVs among all vehicles and the number of leading and surrounding vehicles during acceleration. In-cabin exposures on Los Angeles freeways were dominated by diesel truck emissions, including ultrafine particles, nitric oxide, black carbon and PAHs bound to ultrafine particles. Table 1.25 summarizes the median concentrations and interquartile ranges for each of the contaminants in different road and traffic settings. In dense traffic on the freeways, the time from emission to entering the passenger compartments of nearby vehicles was very short (Fruin *et al.*, 2008). Exposure concentrations were proportional to the density of HGVs, but not to the total volume of vehicles. Automobiles did not contribute significantly to the variability of freeway pollutants. However, on arterial streets with heavy traffic with mixed vehicles and frequent traffic lights, the emissions were dominated by those from groups of cars accelerating powerfully after having stopped at the lights. Powerful acceleration can overload the catalytic exhaust pollution control, allowing considerably more emissions (Fruin *et al.*, 2008). The close grouping and limited ventilation of the area can lead to brief accumulation of the emissions. High-speed acceleration does not produce the same accumulation because vehicles are more widely spread out and surrounded by high-velocity air flows. Table 1.26 gives the explanatory strength (R^2) for the predictive variables and each of the contaminant measures.

Apte *et al.* (2011) measured in-vehicle concentrations of $PM_{2.5}$, black carbon and ultrafine particles in open-sided auto-rickshaws that carry one to four people in New Delhi, India. They are powered by a 5–6-kW four-stroke engine that burns compressed natural gas. Because of the arrangement of the exhaust system, self-pollution is unlikely. Ambient sampling to measure the urban background was conducted in an affluent area 200–250 m away from the nearest heavily travelled road. Sampling was carried out inside the auto-rickshaw for about 3 h per trip to measure potential exposures of occupants: concentrations for about 60 trips averaged 190 μg/m^3 for $PM_{2.5}$, 42 μg/m^3 for black carbon and 280 × 10^3 particles/cm^3 for ultrafine particles; all three pollutants had similar geometric standard deviations of ~1.3. The corresponding geometric mean values for the urban background were 130 μg/m^3 for $PM_{2.5}$, 12 μg/m^3 for black carbon and 35 × 10^3 particles/cm^3 for ultrafine particles. The in-vehicle and background levels were much higher than those reported for vehicles in other megacities.

Passenger and driver exposures inside vehicles on heavily travelled roads are consistently higher than community-wide measurements of urban background. Time activity diaries are important to determine the relative contribution of high commuting exposures to the overall exposure of a subject. Fruin *et al.* (2008) estimated daily exposure to ultrafine particles by typical activities in common micro-environments (Table 1.27). On average, while only 1 hour was spent on freeways and arterial roads, it accounted for 36% of the total exposure.

(f) Penetration of traffic emissions into homes and buildings

Children and adults spend a large amount of their time indoors at home or at work. Therefore, the amount of contaminants from traffic that enter indoor spaces is important. Indoor:outdoor ratios of concentrations of ultrafine particles have been measured in Los Angeles (Sarnat *et al.*, 2006) and Boston (Levy *et al.*, 2002), USA. Fruin *et al.* (2008) collected information on indoor:outdoor ratios to estimate indoor exposures in Los Angeles. Gotschi *et al.* (2002) determined the penetration of $PM_{2.5}$ and black carbon in homes in Athens (Greece), Basel (Switzerland), Helsinki (Finland) and Prague (Czech Republic), and found that levels of $PM_{2.5}$ and black carbon

Table 1.25 Average median and interquartile range (P25%, P75%) of pollutant concentrations by road segment or location for four days in Spring, 2003

Location	UFP (1000s/cm³)	$PM_{2.5}$ (μg/m³)	NO (ppb)	BC (μg/m³)	CO (ppm)	CO_2 (ppm)	*n*
Long Beach residential	26 (25, 28)	17 (15, 20)	17 (14, 21)	1.5 (1.1, 1.6)	0.13 (0.10, 0.18)	400 (390, 420)	329
Pasadena residential	13 (12, 15)	7.9 (7.3, 8.8)	16 (14, 19)	0.74 (0.39, 0.89)	0.13 (0.10, 0.27)	560 (540, 580)	87
Start of USC	43 (33, 53)	45 (44, 46)	59 (50, 66)	3.3 (2.6, 4.3)	0.54 (0.50, 0.58)	540 (540, 560)	41
End of USC	32 (31, 34)	23 (22, 24)	35 (32, 37)	2.2 (2.0, 2.6)	0.26 (2.4, 3.9)	580 (540, 620)	84
Arterial roads north of USC	33 (24, 53)	23 (20, 26)	79 (45, 120)	1.5 (1.2, 1.8)	1.8 (1.1, 2.5)	710 (690, 750)	117
CA-110N (≈3500 TrPD)	47 (33, 69)	25 (21, 29)	170 (120, 240)	2.4 (1.5, 3.6)	2.3 (1.8, 2.9)	820 (760, 860)	376
I-10E (≈10 000 TrPD)	130 (95, 200)	110 (60, 820)	280 (210, 350)	13 (11, 20)	2.7 (2.3, 4.7)	930 (900, 980)	111
I-710S (≈25 000 TrPD)	190 (150, 240)	54 (44, 60)	390 (330, 470)	12 (10, 16)	1.9 (1.6, 2.3)	850 (790, 910)	500

BC, black carbon; CO, carbon monoxide; CO_2, carbon dioxide; *n*, 4-day total number of 10-s average in data set; NO, nitric oxide; PM, particulate matter; TrPD, average number of diesel-powered trucks per day (CalTrans, 2004); UFP, ultrafine particles; USC, University of Southern California

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were lowest in Helsinki, moderate in Basel and higher in Athens and Prague (see Table 1.23). Spearman correlation coefficients for indoor versus outdoor levels were higher for black carbon (range, 0.57–0.86) than for $PM_{2.5}$ (range, 0.05–0.69). In linear regression models, outdoor levels explained clearly the greater variation in indoor black carbon (86%) than in indoor $PM_{2.5}$ (59%), indicating the greater ease with which soot particles penetrate indoors compared with larger particles of $PM_{2.5}$.

Zhao *et al.* (2006) examined the indoor (personal and residential) and outdoor (residential and ambient) concentrations of $PM_{2.5}$ components in low- and moderate-income residences in Raleigh and Chapel Hill, NC, USA, and reported data for PM mass, EC, organic carbon and 19 elements. Traffic emissions were identified as one of the components across all four types of environment: 10.0% of personal exposure; 9.4% of residence indoors; 17.2% of residence outdoors; and 19.4% of ambient outdoors. They found that the relative contribution of indoor and outdoor sources to indoor $PM_{2.5}$ were a strong function of the air exchange rate. When the rate was > 1.1 per hour, the contributions were equal but, when the air-exchange rate was < 0.3 per hour, then only about 25% of particles were from outdoors. Williams *et al.* (2003) studied the PM data for the same population, and reported that the mean personal exposure was 23.0 μg/m³, while measured indoor, outdoor and ambient (at a central site) levels were 19.1, 19.3 and 19.2 μg/m³, respectively. Mean personal $PM_{2.5}$ exposures were only weakly correlated with ambient $PM_{2.5}$ concentrations (r = 0.39).

Arhami *et al.* (2010) investigated the penetration of air contaminants into the residences of three retirement communities in the San Gabriel Valley and one in Riverside, CA, USA. During 2005–2007, they looked at the indoor:outdoor values for $PM_{0.25}$, ultrafine particles, EC, organic carbon, selected PAHs, hopanes and steranes, *n*-alkanes, *n*-alkanoic acids, vanadium and nickel. Traffic emissions were found to make up 24–47% of the ultrafine particle mass. Heavy-duty

Table 1.26 Coefficients of determination (R^2) for freeway concentrations by predictive variable[a]

Predictor (No. of labels)	PB-PAH	Ln(UFP)	NO	Ln(BC)	Ln(CO)	CO_2	NO_2
Road + direction (17)	0.65	0.66	0.56	0.69	0.31	0.18	0.16
Truck density (5)	0.67	0.58	0.57	0.64	0.096	0.066	0.14
Hour of the day (wind speed) (9)	0.26	0.26	0.24	0.26	0.11	0.55	0.017 ($P = 0.33$)
Vehicle followed (6)	0.23	0.20	0.18	0.18	0.24	0.084	0.072
Speed (6)	0.14	0.19	0.23	0.18	0.11	0.053	0.097
Overall congestion (5)	0.21	0.14	0.15	0.14	0.23	0.081	0.051
Day (4)	NA	0.095	0.19	0.09	0.15	0.56	0.072

[a] $P < 0.0001$ unless otherwise noted in parentheses

Road categories: parked (freeway shoulder or residential), arterial, arterial intersection, freeway, freeway entrance, or freeway exit. Direction categories: the relative wind direction to the flow of traffic, and wind speed. Truck density categories: NA, none, 1 or 2 oncoming trucks, ≈5% trucks, > 10% trucks and > 20% trucks. Hour of the day categories: wind speed changes each hour, increasing through the day. Overall congestion: NA, low, medium (many vehicles but free flowing) high (reduced speeds).

BC, black carbon; CO, carbon monoxide; CO_2, carbon dioxide; Ln, Log-normal distribution; NO, nitric oxide; NO_2, nitrogen dioxide; PB-PAH, particle-bound polycyclic aromatic hydrocarbons; UFP, ultrafine particles

vehicles (large HGVs) comprised 10–15% of all vehicles. [The authors did not distinguish the vehicles by fuel type, but heavy-duty vehicles were predominantly diesel-powered and light-duty vehicles were predominantly gasoline-powered cars.] The air exchange rates for the dwellings were relatively low (0.2–0.4 per hour). The indoor:outdoor ratios for ultrafine particles were 0.63–1.07, and those for PAHs, hopanes and steranes were close to 1, indicating the potential impact of outdoor sources, including motor vehicle emissions. The *n*-alkanes and *n*-alkanoic acids had indoor:outdoor ratios much larger than 1, indicating significant indoor sources, such as cooking, tobacco smoking and candle burning. The finding of a large contribution of ultrafine particles from traffic sources was consistent with the measurements of organic carbon at the same sites (Polidori *et al.*, 2007). [The Working Group noted some uncertainty in the estimates of the vehicular fraction of particles, because light-duty vehicles in the study area may have been older than the average for the area that produced the source profile and might therefore have higher emissions.]

Studies in the Netherlands have shown that black carbon is a better marker of personal or indoor versus outdoor exposures than nitric oxide, nitrogen dioxide or nitrogen oxides (Wichmann *et al.*, 2005; Van Roosbroeck *et al.*, 2006). In addition, the outdoor exposures to black carbon close to busy urban streets compared with those near quiet urban streets were 29% higher for adults ($n = 16$ days; $P < 0.01$); a similar difference was seen for black carbon in outdoor versus indoor locations (25% higher; $n = 25$; $P < 0.05$). These differences remained for the indoor values even after adjustment for cooking and unvented heating devices (Wichmann *et al.*, 2005). Wind direction had considerable effects on the personal exposures of children to black carbon: when winds blew > 50% of the time from the busy streets towards their homes, exposure to black carbon increased by 54%. Nitrogen dioxide and nitrogen oxides showed smaller differences of 4% and 21%, respectively, and nitric oxide showed a larger difference (73%), but none of these were statistically significant (Van Roosbroeck *et al.*, 2006). These findings were broadly consistent with several earlier studies using area samplers.

Table 1.27 In-vehicle contributions to total exposures to ultrafine particles based on typical micro-environmental concentrations and activity time

Micro-environment	Time	Duration (h)	UFP concentration (1000s/cm^3)	Source of concentration estimate (low and high conditions)	Fraction of total exposure (%)
Home	7.15 pm–6.45 am	11.5	7.6[a] (7.1[b], 8.9[c])	Outdoor concentration × I/O for seasonal AER[a] (Cyrys *et al.*, 2004) (summer[b], winter[c])	27 (25, 29) [d]
Home, breakfast	6.45–7.15 am	0.5	20 (0, 25)	Average, during operation of tea kettle, toaster oven (Wallace, 2006) (no cooking, frying eggs)	3.1 (0, 3)
Arterial roads	7.15–7.45 am	0.5	58 (41, 76)	Morning measurements from arterial loop, 4/14/03, calm conditions (lower IQR, upper IQR)	8.8 (6, 11)
Freeways	7.45–8.00 am	0.25	204 (126, 253)	9–10 am average, LA, 5% trucks (2.5% trucks, 10% trucks)	16 (10, 19)
Workplace, office	8.00 am–12.00 pm, 1.00–5.00 pm	8	5.3 (1.3, 11)	Average I/O ratio for public places with no cooking times, outdoor annual average (Levy *et al.*, 2002) (lowest I/O value, highest I/O value)	13 (4, 23)
Outdoors	12.00–1.00 pm	1	21 13, 27)	LA annual average, four locations (Singh *et al.*, 2006) (June average, January average)	6.4 (4, 8)
Arterial roads	5.00–5.30 pm	0.5	33 (24, 53)	4-day average, arterial roads North of USC (lower IQR, upper IQR)	5.0 (4, 8)
Freeways	5.30–5.45 pm	0.25	90	4–5 pm average, 5% truck	6.8
Home, dinner	5.45–7.15 pm	1.5	33 (0, 50)	Two gas burners and oven (Wallace, 2006) (no cooking, deep frying)	15 (0, 21)
Average daily exposure		24	14 (8.0, 19) [d]	Time-weighted average (weighted average of low values, weighted average of high values)	
In-vehicle fraction		1.5			36 (45)

[a] Outdoor annual LA average concentration times I/O = 0.42 for an AER = 1.33/h (four evening hours) and outdoor annual average times I/O = 0.33 for an AER = 0.91/h (7.5 night hours) (Cyrys *et al.*, 2004)

[b] January average times I/O = 0.33 for AER = 0.91/h (Cyrys *et al.*, 2004).

[c] June average times I/O = 0.78 for AER = 3.4/h (four evening hours) (open windows) and June average times I/O = 0.42 for AER = 1.3/h (7.5 night hours) (Cyrys *et al.*, 2004; Singh *et al.*, 2006; Wallace, 2006)

[d] Upper and lower estimates given in parentheses

AER, air exchange rate (air changes per hour); I/O, indoor-to-outdoor ratio with no indoor sources; LA, Los Angeles; UFP, ultrafine particles; USC, University of Southern California

(g) *Time trends in exposures to traffic emissions*

Air pollution levels have declined over time in the USA and western Europe where major industrial sources, such as coal-fired power plants, steel and other metal production, and chemical manufacturing, have been controlled. Emissions from the large numbers of homes in the cities have also declined, because the heating of homes and buildings has become more efficient and has switched from coal and fuel oil to natural gas and electricity. As a result of these changes and emission controls on vehicles, the nature of air pollution in cities has altered. Although considerable efforts have been made to control and reduce vehicle exhaust from diesel and gasoline engines, the number of vehicles has increased steadily, causing city-wide pollutants to plateau and limiting the effectiveness of individual vehicle reductions. The control activities have also altered the composition of source emissions. For example, reductions in particle mass to meet PM_{10} limitations have tended to reduce the number of larger particles because they contain most of the mass. However, this has not reduced the emissions of smaller particles, which also have much more surface area per gram, to the same extent.

Davis *et al.* (2006) used a historical data set of coefficient of haze measurements covering 1971–2003 for 25 community locations across New Jersey, USA, to determine the time course and determinants of air pollution levels (Davis *et al.*, 2010). Levels of EC were highly correlated with coefficient of haze and both are indicators of diesel vehicle emissions. This data set was analysed with additional annual data on the statewide unemployment and county level markers of activity in the transport industry, which is sensitive to fluctuations in the national economy. Economic indicators of the business activity of the transport industry were used to indicate that local variations in activity affected the community level of coefficient of haze. The analysis showed that 50% of the variability in coefficient of haze was associated with changes in shipping activity. Strengthening Federal regulations also produced a downward trend in the levels of coefficient of haze.

Kirchstetter *et al.* (2008) also examined a long time course of coefficient of haze to determine the historical trends of diesel exposures at 11 locations in the San Francisco Bay Area, USA. The data covered the period from the late 1960s to the early 2000s. The values for coefficient of haze were found to be highly correlated ($R^2 = 0.96$) with the black carbon estimates of the aethalometer. While the use of diesel fuel in the Bay area over this period, as indicated by data on fuel tax, increased by a factor of approximately 6, concentrations of black carbon were seen to decrease by a factor of about 3 over the same period. An analysis indicated that diesel emission factors decreased by a factor of approximately 10 as a result of changes in fuel composition, engine design and emission controls.

Shen *et al.* (2011) performed an analysis of global PAH emissions from motor vehicles. Emissions have declined because of controls applied to vehicles in developed countries. However, since the 1990s, they have begun to increase again because of the increase in vehicles with few controls in the developing world, and especially in China.

Firdaus & Ahmad (2011) reported on trends in air pollution in Delhi, India, where air pollution has steadily worsened due to increased motor vehicle traffic. The number of motor vehicles has increased 19-fold from 1974 to 2005. The number of private cars increased by nearly 10% per year in 1991–97, but the annual growth rate declined to under 5% in 2010. Levels of nitrogen dioxide increased for most years during 1987–2005, and levels of total suspended PM also increased steadily over that period. The level of respirable particles increased from 1998 to 2002, after which it declined until 2006. Pollution controls

implemented since 1996 are thought to have mitigated increasing pollution levels (Firdaus & Ahmad, 2011).

Sawyer (2010) reviewed the status of vehicle emissions, their controls and future trends. In the USA, the emissions per vehicle of $PM_{2.5}$ and nitrogen oxides have declined steadily since the 1970s. However, the distance travelled by on-road vehicles has steadily increased, which has partially offset the reduction in emission levels.

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2. CANCER IN HUMANS

2.1 Introduction

2.1.1 General aspects

Diesel and gasoline engine exhausts have been evaluated previously in the *IARC Monographs* (IARC, 1989). Since that time, a large number of cohort and case–control studies have been published on the topic, many of which provided high-quality data on exposure and potential confounding factors. General aspects on the methodological advantages and limitations of the various study designs are discussed briefly below, including a discussion of developments in the methods of exposure assessment and problems regarding bias and confounding.

Occupational cohort studies typically provide reasonably accurate data on current exposure levels, but frequently lack information on historical exposure levels and other risk factors, such as tobacco smoking habits. Over the past few decades, the quality of cohort studies has been further improved by the inclusion of more detailed data on exposure and more advanced exposure modelling, and some now incorporate individual data on tobacco smoking. However, full lifetime occupational histories are often unavailable, which reduces the ability to adjust the findings for occupational exposures incurred during employment outside the industry under study. Comparisons are made between groups with different levels of exposure, either within or outside the study population. In general, the use of an internal unexposed group gives more valid risk estimates than that of an external group, because less bias is introduced from the incomparability of lifestyle-associated factors, such as smoking, and the general health status of the exposed and unexposed groups. Studies that investigate exposure–response associations with measures of exposure to diesel exhaust (e.g. duration, average exposure and/or cumulative exposure) have been given greater weight in the evaluation of carcinogenicity.

A specific group of cohort studies are based on record linkage, and link routinely collected population data on occupational titles to national registers of cancer incidence or mortality. These studies usually provide very crude data on exposure that typically consist of a job title in a specific year, no data on tobacco smoking and no lifetime occupational histories, and are consequently usually viewed as generating hypotheses. This type of study has been considered to give only supportive evidence for the present evaluation.

Finally, some cohort studies use proportionate mortality ratios, a methodology that is applied when data are available on deaths for those employed at a specific industry, but not for the total population at risk, and the distribution of causes of death in the study population is compared with that of an external comparison group. Proportionate mortality ratio studies may give biased results, because it is not possible to assess whether an observed excess in the proportion of deaths from a specific cause is due to a true excess of risk or a reduced risk of deaths for

other causes. For this reason, and in view of the large number of high-quality studies available, proportionate mortality ratio studies have not been considered for the present evaluation.

Case–control studies are sometimes considered to be sensitive to inaccurate recall of previous exposures, with the potential for biased findings, which is a particular concern when information on exposures is based on self-assessment. This potential bias is largely reduced when exposure assessment is based on the assignment of exposures from a lifetime occupational history by an expert assessment method or the application of a job–exposure matrix (JEM), or combinations of the two. The strength of case–control studies is that researchers can obtain detailed individual data on tobacco smoking and other potential risk factors, as well as lifetime occupational histories, from the study subjects. These studies may be based on the general population or be nested within defined occupational cohorts. A potential problem in the former is that detailed exposure data are rarely available, resulting in potential non-differential exposure misclassification, which tends to attenuate the observed risks. A design that incorporates the advantages of detailed data on exposure and individual data on risk factors is the nesting of a case–control study within a cohort. This type of study may provide very high-quality information on the association of occupational exposures with cancer. Finally, case–control studies may recruit controls directly from the general population or from individuals with diagnoses other than that under study. Controls recruited randomly from the population are considered to reflect the prevalence of exposure in the study population more accurately than hospital controls, and afford less potential for bias.

For the present evaluation, the Working Group identified three broad types of study, in order of increasing quality: (1) studies that reported risk for cancer by occupation, with no reference to whether or not the occupation indicated exposure to motor exhaust. Because the predictive value of an occupational title may differ considerably between study settings and countries, such studies have not generally been included for the evaluation of lung cancer. However, for other cancer sites, for which fewer data were available, such studies have been included; (2) studies that reported risk for cancer on the basis of occupational titles, in which the titles were used to indicate potential or definite exposure to diesel or gasoline exhaust. Frequently, duration of employment in such jobs was used as a proxy for quantitative exposure. All such pertinent studies have been reviewed; (3) studies that aimed to assess individual exposure to motor exhaust (diesel, gasoline or both) quantitatively through measurements, modelling, expert assessments, JEMs or other means. In many cases, these studies investigated the intensity and duration of exposure, in addition to cumulative exposure, and had access to full occupational and smoking histories. These studies have been given the greatest weight in the present evaluation.

2.1.2 Aspects of exposure assessment methods

Diesel exhaust is a complex mixture of variable characteristics and several markers of exposure have been used, including polycyclic aromatic hydrocarbons (PAHs), nitrogen dioxide, nitrogen oxides, particulate matter (PM) and elemental carbon (EC). Any proxy of diesel exhaust may or may not reflect accurately one of its underlying carcinogenic components. For instance, in the studies reviewed that investigated proximity to diesel emission sources, EC was a good marker for diesel exhaust. However, this does not imply that EC is, or that it accurately reflects, the causal agent. Therefore, the association between such markers and the true substances of etiological interest may differ to some extent by time and place.

The present evaluation covers both diesel and gasoline exhaust. Many occupations that entail exposure to engine exhausts, for example professional drivers and some garage workers, involve exposure to a mixture of the two exhausts. Cohort studies that more specifically addressed exposure to diesel exhaust were based on railroad workers, miners and bus garage employees. Cohorts of professional drivers often comprised individuals with combined exposure to diesel and gasoline exhausts. Several population-based case–control studies that used expert assessment or JEMs to classify exposure presented separate risk estimates for each of the two exhausts. However, in population-based studies, few occupations generally entailed exposures to specific types of engine exhaust and few individuals incurred very high exposures. Some collinearity may have also arisen between exposures to diesel and gasoline engine exhausts. The main body of available evidence was related to diesel exhaust, while the data that specifically addressed the risk of cancer from exposure to gasoline exhaust among individuals with no concurrent exposure to diesel exhaust were very limited.

2.1.3 Studies of environmental air pollution

Several studies showed associations between lung cancer and ambient air pollution. In addition, exposure to specific components of air pollution, for example $PM_{2.5}$, has been linked to lung cancer (Samet & Cohen, 2006; EPA, 2009). Ambient air pollution comprises emissions from vehicles fuelled by diesel and gasoline, but also those from a variety of other sources and processes, including industrial air pollution. At present, it is very difficult to assess the specific contributions of these sources to the observed cancer risk. These studies have not been included in the review, because they would contribute little information in addition to the studies reviewed here.

2.2 Cohort studies

See Table 2.1

2.2.1 Railroad workers

Howe *et al.* (1983) conducted a cohort study of 43 826 male pensioners of the Canadian National Railway Company who had retired before 1965 and who were alive at the start of that year, as well as those who retired between 1965 and 1977. The cause of death of 17 838 pensioners who died between 1965 and 1977 was ascertained by computerized record linkage to the Canadian national mortality database. A total of 76 different occupations were represented in the cohort. Experts classified each occupation in terms of exposure to diesel fumes (unexposed, possibly exposed and probably exposed), coal dust (unexposed, possibly exposed and probably exposed) and any other fumes or substances. The analyses compared the mortality of different groups of railroad workers with that of the Canadian population and then compared those who presumably incurred higher exposures with those who presumably incurred lower exposures, by calculating their standardized mortality ratios (SMRs). A total of 933 lung cancer deaths resulted in an overall standardized mortality ratio of 1.06. A comparison of the possibly and probably exposed with the unexposed (239 lung cancer deaths) provided a relative risk (RR) for lung cancer of 1.20 ($P = 0.013$; 407 lung cancer deaths) and 1.35 ($P < 0.001$; 279 lung cancer deaths), respectively. No data were available on tobacco smoking habits. In the same study, the authors found a standardized mortality ratio of 1.03 for urinary bladder cancer based on 175 cases, although no internal analyses were performed for this cancer. Exposures to coal dust and diesel overlapped and similar standardized mortality ratios were reported for both exposures. The deaths of those who retired before 1950, and were hence exposed primarily to coal dust, were too

Table 2.1 Selected cohort studies of cancer in railroad workers, bus garage workers, professional drivers, miners, heavy equipment operators and other workers exposed to diesel exhaust

Reference Location, follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of exposed cases	Relative risk (95% CI)	Covariates Comments
Railroad workers							
Howe *et al.* (1983) Canada, 1965–77	43 826 retired railroad workers	Review of job titles by experts employed by railroad	Trachea, bronchus and lung			**SMR**	Men; Canadian mortality records; no adjustment for tobacco smoking
				All railroad workers	933	1.06 ($P > 0.05$)	
				Possibly exposed	407	1.20 ($P = 0.013$)	
				Probably exposed	279	1.35 ($P < 0.001$)	
						P for trend < 0.001	
				Possibly exposed	333	1.21	Excluded asbestos- exposed workers
				Probably exposed	256	1.33	
						P for trend < 0.001	
			Urinary bladder	All railroad workers	175	1.03 ($P > 0.05$)	
Boffetta *et al.* (1988) American Cancer Society Cohort, 1982–84	461 981	Self-reported job	Lung	Railroad workers	14	1.59 (0.94–2.69)	Men aged 40–79 yr in 1982; adjusted for age and tobacco smoking; 4–10-yr age strata and 5 strata of smoking
Garshick *et al.* (2004) US Railroad Workers cohort, 1959–96	54 973	Industrial hygiene review of yearly job title and exposure sampling	Lung	Based on job as train crew in 1959; referents: clerks and signal maintainers	4351		Update of Garshick *et al.* (1988); attained age, calendar yr, time on and off work; white men aged 40–64 yr in 1959 with 10–20 yr of employment; indirect adjustment for smoking: adjusted HR, 1.17–1.27
				Age (yr)		**HR**	
				40–44	884	1.49 (1.30–1.70)	
				45–49	732	1.37 (1.18–1.58)	
				50–54	456	1.39 (1.18–1.64)	
				55–59	286	1.34 (1.09–1,64)	
				60–64	121	0.99 (0.75–1.30)	

Table 2.1 (continued)

Reference Location, follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of exposed cases	Relative risk (95% CI)	Covariates Comments
Garshick et al. (2004) US Railroad Workers cohort, 1959–96 (cont.)				**Train crew work (yr; 5-yr lag)**			
				0– < 5	391	1.41 (1.24–1.61)	
				5– < 10	484	1.39 (1.23–1.56)	
				10– < 15	618	1.51 (1.35–1.68)	
				15– < 20	707	1.33 (1.19–1.49)	
				≥ 20	204	1.31 (1.10–1.56)	
				Any exposure (5-yr lag)		1.40 (1.30–1.51)	
Garshick *et al.* (2006) US Railroad Workers cohort, 1959–96	39 388	Industrial hygiene review of job history and exposure sampling	Lung (162)	**Train crew work (yr; 5-yr lag)**	Total 4055		Subset of Garshick *et al.* (2004); attained age, calendar yr, time on and off work, tobacco smoking (pack–yr); white men aged 40–59 yr in 1959 with 10–20 yr of employment; adjusted for smoking by imputation from Garshick *et al.* (1987)
				Unexposed	895	1.0	
				0– < 5	330	1.31 (1.12–1.51)	
				5– < 10	449	1.23 (1.08–1.39)	
				10– < 15	615	1.23 (1.10–1.38)	
				15– < 20	707	1.16 (1.03–1.30)	
				≥ 20	204	1.22 (1.02–1.47)	
				Any exposure	22 305	1.22 (1.12–1.32)	
						Unadjusted for smoking	
				0– < 5	330	1.44 (1.25–1.67)	
				5– < 10	449	1.36 (1.20–1.55)	
				10– < 15	615	1.36 (1.22–1.52)	
				15– < 20	707	1.28 (1.14–1.43)	
				≥ 20	204	1.32 (1.11–1.58	
				Any exposure	22 305	1.35 (1.24–1.46)	

Table 2.1 (continued)

Reference Location, follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of exposed cases	Relative risk (95% CI)	Covariates Comments
Laden *et al.* (2006) US Railroad Workers cohort, 1959–96	52 812	Industrial hygiene review of job history and exposure sampling	Lung	**Train crew work (yr; 5-yr lag)**			Subset of Garshick *et al.* (2004); attained age, calendar yr, time on and off work; white men aged 40–64 yr in 1959 with 10–20 yr of employment
				Hired 1939–44			
				Unexposed	687	1.0	
				0– < 10	211	1.19 (1.00–1.41)	
				10– < 15	330	1.28 (1.11–1.47)	
				15– < 20	454	1.37 (1.21–1.55)	
				20– < 25	577	1.37 (1.21–1.54)	
				≥ 25	355	1.16 (1.00–1.34)	
				Hired 1945–49			
				Unexposed	229	1.0	
				0– < 10	32	1.15 (0.77–1.70)	
				10– < 15	66	1.49 (1.11–1.99)	
				15– < 20	103	1.89 (1.48–2.40)	
				20– < 25	119	1.83 (1.45–2.32)	
				≥ 25	124	1.78(1.39–2.28)	
				Intensity (yr)			
				Hired 1939–44			
				Unexposed	687	1.0	
				Quintile 1	326	1.26 (1.09–1.46)	
				Quintile 2	394	1.37 (1.17–1.51)	
				Quintile 3	417	1.33 (1.17–1.50)	
				Quintile 4	427	1.28 (1.13–1.45)	
				Quintile 5	363	1.24 (1.08–1.43)	
				Hired 1945–49			
				Unexposed	229	1.0	
				Quintile 1	80	1.63 (1.24–2.14)	
				Quintile 2	98	1.54 (1.21–1.96)	
				Quintile 3	93	2.02 (1.58–2.59)	
				Quintile 4	76	1.50 (1.14–1.96)	
				Quintile 5	97	1.81 (1.39–2.35)	

Table 2.1 (continued)

Reference Location, follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of exposed cases	Relative risk (95% CI)	Covariates Comments
Guo *et al.* (2004a) Finland, records from 1971–95	Finnish working population, census data in 1970; 67 121 men	Longest held job in 1970 census	Lung	Locomotive driver	85	**SIR** 0.63 (0.51–0.78)	All working Finns born after 1906; linkage to Finnish Cancer Registry; indirect adjustment for tobacco smoking
Guo *et al.* (2004b) Finland, 1971–95			Urinary bladder	Locomotive driver	22	**SIR** 0.85 (0.53–1.28)	Same census linkage study as Guo *et al.* (2004a); cases in men
Bus garage workers							
Gustavsson *et al.* (1990) Stockholm, Sweden, mortality, 1952–86; cancer incidence, 1958–84	695 bus garage workers, 1945–70	JEM for diesel exhaust exposure; intensity × duration score	Lung	Entire cohort	17	**SMR** 1.22 (0.71–1.96)	Expected numbers based on Stockholm rates; occupationally active men; no information on tobacco smoking; exposure to asbestos assessed; no effect of exposure on lung cancer
	Nested case–control study: 20 incident cases/120 controls			**Diesel exhaust score**			Controls matched on age (± 2 yr)
						OR	
				0–10	5	1.00 (referent)	
				10–20	2	1.27 (0.21–7.72)	
				20–30	3	1.56 (0.34–7.16)	
				> 30	10	2.63 (0.74–9.42)	
Professional drivers							
Bus drivers							
Balarajan & McDowall (1988) London, United Kingdom, 1950–84	3392 professional drivers	Job title in 1939 census and still alive in 1950	Lung	Bus and coach drivers	18	**SMR** [1.42] ($P > 0.05$)	Expected based on England and Wales rates; no adjustment for tobacco smoking
			Urinary bladder		1	[0.58] ($P > 0.05$)	

Table 2.1 (continued)

Reference Location, follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of exposed cases	Relative risk (95% CI)	Covariates Comments
Soll-Johanning *et al.* (1998) Denmark, 1943–92	15 249 male and 958 female bus and tramway workers	Job title obtained from population register; ever employed as an urban bus driver or tramway worker, 1900–94	Lung	**Employed > 3 mo**		**SIR**	Similar to slightly greater smoking rates compared with Copenhagen * All Denmark ** Copenhagen only
				Men*	473	1.6 (1.5–1.8)	
				Men**	390	1.2 (1.1–1.3)	
				Women*	15	2.6 (1.4–4.3)	
				Men–30 yr since first employed			
				< 0.25	9	1.1	
				0.25– < 1.5	57	2 ($P < 0.0001$)	
				1.5– < 4.0	48	1.7 ($P < 0.0001$)	
				5.0–14	70	2.1 ($P < 0.0001$)	
				≥ 15	188	1.5 ($P < 0.0001$)	
			Urinary bladder	**Employed > 3 mo**			* All Denmark ** Copenhagen only
				Men*	177	1.4 (1.2–1.6)	
				Men**	165	1.1 (0.9–1.3)	
Soll-Johanning *et al.* (2003) Denmark, 1943–92	153 cases, 255 controls; all men	Job title from population register	Lung	**Duration of employment (no lag)**			Nested case–control study from the cohort of Soll-Johanning *et al.* (1998); tobacco smoking estimated from spouse, converted to pack–years in 7 categories; cases and controls aged < 85 yr; matched to 1–4 controls by yr of birth and vital status, free of urinary bladder, lung or other cancer, or non-malignant lung disease
				< 3 mo	5	0.74 (0.23–2.39)	
				3 mo– < 3 yr	29	1.0 (referent)	
				2– < 10 yr	54	1.26 (0.69–2.28)	
				10– < 20 yr	22	1.39 (0.69–2.81)	
				≥ 20 yr	43	0.63 (0.32–1.14)	
				Duration of employment (10-yr lag)			
				< 3 mo	4	0.50 (0.14–1.81)	
				3 mo– < 3 yr	27	1.0 (referent)	
				2– < 10 yr	45	1.03 (0.54–1.95)	
				10– < 20 yr	22	1.34 (0.65–2.77)	
				≥ 20 yr	43/139 (cases/controls)	0.54 (0.28–1.03)	

Table 2.1 (continued)

Reference Location, follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of exposed cases	Relative risk (95% CI)	Covariates Comments
Soll-Johanning et al. (2003) Denmark, 1943–92 (cont.)	84 cases, 255 controls; all men		Urinary bladder	**Duration of employment (no lag)**			
				< 3 mo	3	2.00 (0.27–10.92)	
				3 mo– < 3 yr	13	1.0 (referent)	
				2– < 10 yr	20	1.18 (0.47–2.96)	
				10– < 20 yr	15	1.24 (0.46–3.33)	
				≥ 20 yr	33	1.13(0.47–2.68)	
				Duration of employment (10-yr lag)			
				< 3 mo	1	1.21 (0.12–12.34)	
				3 mo– < 3 yr	11	1.0 (referent)	
				2– < 10 yr	17	1.23 (0.46–3.30)	
				10– < 20 yr	15	1.61 (0.57–4.55)	
				≥ 20 yr	33	1.28 (0.52–3.13)	
Guo *et al.* (2004a) Finland, records from 1971–95	Finnish working population, census data in 1970; 667 121 men	Longest job held in 1970 census	Lung	Bus drivers	253	**SIR** 0.89 (0.78–1.00)	Indirect adjustment for tobacco smoking
Guo *et al.* (2004b) Finland, 1971–95			Urinary bladder	Bus drivers	75	**SIR** 1.29 (1.02–1.62)	Same cohort as Guo *et al.* (2004a); indirect adjustment for tobacco smoking
Petersen *et al.* (2010) Denmark, 1979–2003	2037 male Danish urban bus drivers employed in 1978	Bus company records in 3 largest cities; mailed questionnaire	Lung	Bus drivers	100	**SIR** 1.2 (1.0–1.4)	City-specific expected lung cancer rates; age, calendar time, city of employment, bus route, tobacco smoking; smoking history, details of work history from questionnaire
				≥ 15 yr employment		1.3 (1.0–1.8)	
				Per yr employment	100	**IRR** 1.00 (0.98–1.03)	
				< 15 yr	49	1.0	
				15–24 yr	24	0.89 (0.59–1.48)	
				≥ 25 yr	25	0.95 (0.55–1.63)	

Table 2.1 (continued)

Reference Location, follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of exposed cases	Relative risk (95% CI)	Covariates Comments
Petersen et al. (2010) Denmark, 1979–2003 (cont.)						*P* for trend = 0.79	
				10-yr lag			
				< 15 yr		1.00	
				15–24 yr		0.9 (0.5–1.4)	
				≥ 25 yr		0.8 (0.5–1.4)	
			Urinary bladder	Bus drivers		**SIR**	
					69	1.6 (1.2–2.0)	
				≥ 15 yr employment	69	1.8 (1.2–2.5)	
						IRR	
				Per yr employment		1.02 (0.99–1.05)	
				< 15 yr	34	1.00	
				15–24 yr	17	1.11 (0.60–2.03)	
				≥ 25 yr	18	1.31 (0.70–2.48)	
						P for trend = 0.40	
				10- yr lag			
				< 15 yr		1.00	
				15–24 yr		1.6 (0.9–2.8)	
				≥ 25 yr		1.4 (0.7–2.6)	
Heavy goods vehicle (HGV) and other drivers							
Boffetta et al. (1988) American Cancer Society Cohort, 1982–84	476 648	Self-reported job	Lung	All HGV drivers	48	1.24 (0.93–1.66)	Age and tobacco smoking, including pipe/cigar only; mortality in men aged 40–79 yr in 1982; any work as HGV driver
				Reporting exposure to diesel		1.22 (0.77–1.95)	
				Not reporting exposure to diesel		1.19 (0.74–1.89)	
				1–15 yr exposure to diesel	6	0.87 (0.33–2.25)	
				≥ 16 yr exposure to diesel	12	1.33 (0.64–2.75)	

Table 2.1 (continued)

Reference Location, follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of exposed cases	Relative risk (95% CI)	Covariates Comments
Balarajan & McDowall (1988) London, United Kingdom, 1950–84	3392 professional drivers	Job title in 1939 census and still alive in 1950; professional drivers identified from NHS Central Registry	Lung			**SMR**	Observed and expected based on England/Wales rates; men only; no adjustment for tobacco smoking
				Taxi drivers	30	0.86 ($P > 0.05$)	
				HGV drivers	280	1.59 ($P < 0.05$)	
			Urinary bladder	Taxi drivers	5	1.21 ($P > 0.05$)	
				HGV drivers	19	1.06 ($P > 0.05$)	
Gubéran *et al.* (1992) Geneva, Switzerland, 1949–1986 for mortality, 1970–1986 for incidence	6630 drivers holding a licence in 1949–61	Occupation on driver's licence	Lung	Professional drivers, 15 yr latency	77 deaths	**SMR** 1.50 (1.23–1.81)	Expected based on male mortality rates for Switzerland Expected based on mean incident rates for men in Geneva 1970–75, 1976–81, 1982–86
					64 incident cases	**SIR** 1.61 (1.29–1.98)	
				Mortality (time from first exposure in yr)			Men born 1900 or later; limited comparison of smoking rates with other men
				0–14	2	0.67	
				15–24	11	1.18	
				24–34	24	1.3	
				35–44	21	1.35	
				≥ 45	21	2.59	
						P for trend = 0.02	
				Non-professional drivers, 15-yr latency			
				Less exposure group	126	1.21 (1.03–1.40)	
					97 incident cases	1.15 (0.97–1.37)	
				More exposure group	24 deaths	1.32 (0.91–1.85)	
					24 incident cases	1.61 (1.11–2.27)	

Table 2.1 (continued)

Reference Location, follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of exposed cases	Relative risk (95% CI)	Covariates Comments
Gubéran et al. (1992) Geneva, Switzerland, 1949–1986 for mortality, 1970–1986 for incidence (cont.)			Urinary bladder	Professional drivers, 15-yr latency	13 incident cases	**SIR** 1.25 (0.74–1.99)	
Hansen (1993) Denmark, 1970–80	14 225 HGV drivers and 43 024 unskilled labourers as reference	Job in 1970 census	Lung	HGV drivers	76	**SMR** 1.60 (1.26–2.00)	No direct control for tobacco smoking, but similar rates among HGV drivers and referents from population survey
			Urinary organs	HGV drivers	11	0.98 (0.49–1.75)	
Järvholm & Silverman (2003) Sweden, to 1995	Swedish construction workers (389 000 total), 1971–92: 6364 male HGV drivers; 110 984 carpenters and electricians as referents	Job recorded at examination	Lung	HGV drivers	57 deaths	**SMR** 1.37 (1.04–1.78)	Tobacco smoking (never, current, former, unknown), age at diagnosis or death, calendar time; national industrial health service health examination in 1971–92; smoking history from first examination; general population SIR and SMR not adjusted for smoking
					61	**SIR** 1.29 (0.99–1.65)	
					General population referents		Age- and calendar time-adjusted
					57 deaths	**SMR** 1.18 (0.89–1.53)	
					61	**SIR** 1.14 (0.87–1.46)	
Guo *et al.* (2004a) Finland, records in 1971–95	Finnish working population, census data in 1970; 667 121 men	Longest job held in 1970 census	Lung	HGV drivers	620	**SIR** 1.13 (1.04–1.22)	Indirect adjustment for tobacco smoking

Table 2.1 (continued)

Reference Location, follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of exposed cases	Relative risk (95% CI)	Covariates Comments
Guo *et al.* (2004b) Finland, 1971–95			Urinary bladder	HGV drivers	144	**SIR** 1.01 (0.85–1.19)	Same cohort as Guo *et al.* (2004a); men only; indirect adjustment for tobacco smoking
Laden *et al.* (2007) USA, 1985–2000	54 319, including 36 299 union drivers	Job title and industrial hygiene survey	Lung			**SMR**	Survey of current workers in 2003 indicated similar birth cohort-specific tobacco smoking rates compared with US men; results by job title presented only in figures
				All workers	769	1.04 (0.97–1.12)	
				Drivers	NR	1.10 (1.02–1.19)	
				Loading dock workers	NR	1.10 (0.94–1.30)	
			Urinary bladder	All workers	29	0.80 (0.56–1.15)	
Garshick *et al.* (2008) USA, 1985–2000	31 135 union drivers	Job title and industrial hygiene survey	Lung (underlying or secondary cause)			**Change in HR (%) per yr increase**	Age, calendar yr, time period of hire, attained age in 1985, time on and off work, race, census region; men only; indirect adjustment for tobacco smoking using smoking rates from 2003 worker survey
				Long-haul drivers	323	2.5 (0.2–4.9)	
				Pick-up and delivery drivers	233	3.6 (1.2–6.1)	
				Dock workers	205	3.4 (0.8–6.0)	
				Combination workers (local drivers/dock workers)	150	4.0 (1.5–6.6)	
						HR for > 1 yr work	
				Mechanics	38	0.95 (0.66–1.38)	
				Hostlers	29	0.99 (0.68–1.45)	
				Clerks	15	0.55 (0.32–0.95)	
						HR for 20 yr work	
				Long-haul drivers	323	1.65 (1.04–2.62)	
						1.40 (0.88–2.24)	Smoking-adjusted

Table 2.1 (continued)

Reference Location, follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of exposed cases	Relative risk (95% CI)	Covariates Comments
Garshick et al. (2008) USA, 1985–2000 (cont.)				Pick-up and delivery drivers	23	2.04 (1.28–3.25)	
						2.21 (1.38–3.52)	Smoking-adjusted
				Dock workers	205	1.94 (1.18–3.18)	
						2.02 (1.23–3.33)	Smoking-adjusted
				Combination workers (local drivers/dock workers)	150	2.20 (1.35–3.61)	Smoking-adjusted
						2.34 (1.42–3.83)	
Birdsey *et al.* (2010) USA, 1989–2004	156 241 members of US HGV owners/ operators trade association	None	Lung	Trade association membership	557	**SMR** 1.00 (0.92–1.09)	Expected deaths from US population; 94% men; only 32% had more than 9 yr of follow-up; overall SMR, 0.76, suggesting healthy-worker effect; no adjustment for tobacco smoking
			Bladder and other urinary	Trade association membership		0.93 (0.62–1.34)	
Garshick *et al.* (2012) USA, 1985–2000	31 135 union drivers	Job title and industrial hygiene survey, workers employed ≥ 1 yr in 1985	Lung	**Cumulative exposure (µg/m3–mo)**			Age, calendar year, time period of hire, attained age at study entry, race, census region; men only; mechanics excluded
				No lag			
				< 530	153	Reference	
				530– < 1061	193	1.13 (0.90–1.42)	
				1061– < 2076	202	1.13 (0.87–1.46)	
				≥ 2076	193	1.02 (0.76–1.36)	
				Adjusted for duration			
				< 530		Reference	
				530– < 1061		1.25 (0.99–1.71)	
				1061– < 2076		1.30 (0.99–1.72)	
				≥ 2076		1.24 (0.89–1.71)	
				5-yr lag			
				< 371	122	Reference	
				371– < 860	191	1.18 (0.92–1.52)	
				860– < 1803	202	1.17 (0.88–1.55)	
				≥ 1803	226	1.19 (0.86–1.63)	

Table 2.1 (continued)

Reference Location, follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of exposed cases	Relative risk (95% CI)	Covariates Comments
				Adjusted for duration			
Garshick et al. (2012) USA, 1985–2000 (cont.)				< 371		Reference	
				371– < 860		1.31 (1.01–1.71)	
				860–1803		1.38 (1.02–1.87)	
				≥ 1803		1.48 (1.05–2.10)	
				10-yr lag			
				< 167	112	Reference	
				167– < 596	179	1.06 (0.80–1.40)	
				596– < 1436	202	1.05 (0.77–1.45)	
				≥ 1436	248	1.12 (0.78–1.61)	
				Adjusted for duration			
				< 167		Reference	
				167– < 596		1.17 (0.88–1.57)	
				596– < 1436		1.26 (0.90–1.78)	
				≥ 1436		1.41 (0.78–1.61)	
				Average exposure, 5-yr lag (µg/m3)			
				< 3.6	146	Reference	
				3.6– < 5.4	211	1.15 (0.93–1.43)	
				5.4– < 7.9	221	1.11 (0.89–1.39)	
				≥ 7.9	163	1.11 (0.87–1.43)	
				Adjusted for duration			
				< 3.6		Reference	
				3.6– < 5.4		1.15 (0.93–1.43)	
				5.4– < 7.9		1.12 (0.89–1.40)	
				≥ 7.9		1.13 (0.88–1.44)	

Table 2.1 (continued)

Reference Location, follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of exposed cases	Relative risk (95% CI)	Covariates Comments
Miners							
Boffetta *et al.* (1988) American Cancer Society Cohort, 1982–84	476 648	Self-reported job	Lung	Miners	15	2.67 (1.63–4.37)	Age and tobacco smoking, including pipe/cigar only; mortality in men aged 40–79 yr in 1982; any work as a miner
Guo *et al.* (2004a) Finland, records in 1971–95	Finnish working population in 1970 census; 667 121 men	Longest job held in 1970 census	Lung			**SIR**	Indirect adjustment for tobacco smoking
				Metal ore miners	36	3.26 (2.28–4.51)	
				Non-metal ore miners	181	1.85 (1.59–2.14)	
				Other miners	70	1.73 (1.35–2.19)	
Guo *et al.* (2004b) Finland, 1971–95			Urinary bladder			**SIR**	Same cohort as Guo *et al.* (2004a); men only; indirect adjustment for tobacco smoking
				Non-metal ore miners	22	1.16 (0.73–1.76)	
Neumeyer-Gromen *et al.* (2009) Former East Germany, 1970–2001	5862 former East German potash miners employed after 1969	Industrial hygiene job review and total carbon measurements	Lung (mortality)			**SMR**	Follow-up of Säverin *et al.* (1999); expected based on male population of former East Germany; median duration of exposure, 14.9 yr; mean follow-up, 26 yr since hire
				All workers	61	0.73 (0.57–0.93)	
			Urinary bladder (mortality)	All workers	8	0.80 (0.40–1.60)	
			Lung (mortality)	> 4.9 [mg/m^3]–yr	61	1.28 (0.61–2.71)	Age, tobacco smoking (nonsmoker, smoker, missing); men; data on smoking available from medical examinations
				Exposure quintiles [mg/m3]–yr			Age, smoking, duration of follow-up
				< 1.29	9	1.0	
				1.29– < 2.04	11	1.13 (0.46–2.75)	
				2.04– < 2.73	17	2.47 (1.02–6.02)	
				2.73– < 3.90	9	1.50 (0.56–4.04)	

Table 2.1 (continued)

Reference Location, follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of exposed cases	Relative risk (95% CI)	Covariates Comments
Neumeyer-Gromen et al. (2009) Former East Germany, 1970–2001 (cont.)	3335			> 3.90	15	2.28 (0.87–5.97) *P* for trend = 0.09	
				Subcohort with more accurate exposure data			
				> 4.9 [mg/m³]–yr	37	1.50 (0.66–3.43)	Age, smoking (nonsmoker, smoker, missing)
Attfield *et al.* (2012) USA, depending on mine, through 1997	12 315 non-metal miners in 8 mines 1947–67	Modelled estimate of respirable EC underground; no historical measurements for surface miners				**SMR**	SMRs calculated using state-specific rates Exposures to radon, asbestos and silica low; no effect on results of exposure to EC
			Lung (mortality)	Ever underground	122	1.21 (1.01–1.45)	
				Surface only	81	1.33 (1.06–.66)	
			Urinary bladder (mortality)	Ever underground		0.69 (0.23–1.62)	
				Surface-only		1.68 (0.72–3.30)	
		Cumulative EC, 15-yr lag	Lung (mortality)	**Ever underground (µg/m3–yr)**		**HR**	Unadjusted for tobacco smoking; similarly elevated risks with average EC exposure in underground workers
				< 108	30	1.0	
				108– < 445	31	1.50 (0.86–2.62)	
				445– < 946	30	2.17 (1.21–3.88)	
				≥ 946	31	2.21 (1.19–4.09)	
				Excluding persons with < 5 yr work, limiting exposure to < 1280 µg/m³–yr	79	4.06 (2.11–7.83) per 1 000 µg/m³–yr ($P < 0.001$)	
				Surface only (µg/m3–yr)		**HR**	Unadjusted for tobacco smoking
				0– < 0.70	19	1.00	
				0.70– < 4.6	20	1.28 (0.64–2.58)	
				4.6– < 14	19	0.73 (0.35–1.53)	
				≥ 14	20	1.00 (0.44–2.28)	

Table 2.1 (continued)

Reference Location, follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of exposed cases	Relative risk (95% CI)	Covariates Comments
Attfield et al. (2012) USA, depending on mine, through 1997 (cont.)				Excluding persons with < 5 yr work	57	1.02 (1.00–1.03) per μg/m³–yr (P = 0.026)	
		Average EC intensity, 15-yr lag		0– < 0.57	19	1.00	
				0.57– < 0.91	18	1.71 (0.82–3.58)	
				0.92– < 1.4	21	2.22 (1.01–4.90)	
				≥ 1.4	20	2.56 (1.09–6.03)	
		Cumulative EC, 15-yr lag		**Entire cohort (μg/m3–yr)**		**HR**	Unadjusted for tobacco smoking; adjusted for work location: above or underground
				0– < 02.5	50	1.00	
				2.5– < 56	50	0.55 (0.35–0.85)	
				56– < 583	50	1.03 (0.60–1.77)	
				> 583	50	1.39 (0.78–2.48)	
		Average EC intensity, 15-yr lag		**Entire cohort (μg/m3)**			
				0– < 0.86	50	1.00	
				0.86– < 5.2	50	1.13 (0.72–1.76)	
				5.2– < 60	50	1.98 (1.12–3.52)	
				≥ 60	50	1.57 (0.86–2.86)	
Silverman *et al.* (2012) USA, depending on mine, through 1997	Non-metal miners in 8 mines, nested case-control study, 1947–67; 198 cases and 562 controls	See Attfield *et al.* (2012) Cumulative EC, no lag	Lung	**Ever underground (μg/m3–yr)**		**OR**	Tobacco smoking status; history of respiratory disease; previous history of a high-risk job
				< 298	29	1.00	
				298– < 675	29	1.45 (0.68–3.11)	
				675– < 1465	29	1.81(0.84–3.89)	
				≥ 1465	29	1.93 (0.90–4.15)	P for trend = 0.123
				< 81	29	1.00	
				81– < 325	29	2.46 (1.01–6.01)	
				325– < 878	29	2.41(1.00–5.82)	
				≥ 878	29	5.10 (1.88–13.87)	P for trend = 0.004

Table 2.1 (continued)

Reference Location, follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of exposed cases	Relative risk (95% CI)	Covariates Comments
Silverman et al. (2012) USA, depending on mine, through 1997 (cont.)				**Surface only (μg/m3–yr)**		**OR**	
				0– < 0.6	13	1.00	
				0.60– < 0.9	13	3.98 (0.69–23.02)	
				0.9– < 1.4	13	0.76 (0.12–4.98)	
				≥ 1.4	14	0.42(0.05–3.59)	*P* for trend = 0.117
				All workers		**OR**	
				< 3	49	1.00	
				3– < 72	50	0.74 (0.40–1.38)	
				72– < 536	49	1.54 (0.74–3.20)	
				≥ 536	50	2.83 (1.28–6.26)	*P* for trend = 0.001
		Cumulative EC, 15-yr lag, ≥ 2 pack/day smoker		< 8 μg/m³–yr	19	26.79 (6.15–116.63)	Adjusted for location, history of respiratory disease; previous history of a high-risk job
				< 8–304 μg/m³–yr	15	22.17 (4.84–101.65)	
				≥ 304 μg/m³–yr	10	17.38 (3.48–86.73)	
		Cumulative EC, 15-yr lag, < 1 pack/day smoker		< 8 μg/m³–yr	10	6.25 (1.42–27.60)	
				< 8–304 μg/m³–yr	10	7.42 (1.62–34.00)	
				≥ 304 μg/m³–yr	15	16.35 (3.45–77.63)	

Table 2.1 (continued)

Reference Location, follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of exposed cases	Relative risk (95% CI)	Covariates Comments
Other exposed workers							
Heavy equipment operators							
Wong *et al.* (1985) USA, 1964–1978	34 156 members of a construc-tion union for at least 1 yr	Job category review	Lung	All members	309	**SMR** 0.99 (0.88–1.10)	Expected deaths from USA age-, sex-, cause-specific rates; men only; no direct information on tobacco smoking; survey of only 107 members with similar rates to USA
				Membership			
				< 5 yr	10	0.45	
				5–9 yr	25	0.75	
				10–14 yr	53	1.08	
				15–19 yr	58	1.02	
				≥ 20 yr	163	1.07	
				Retired at/after age 65 yr and early retirees	86	1.30 (1.04–1.61)	
		Categorization of job title provided by union based on proximity		High		0.94	Not clear if longest job held was used to categorize exposure to diesel exhaust
				Low		0.86	
				Unknown		0.67	
				No history		1.19 ($P < 0.05$)	
			Urinary bladder	All members	27	1.18 (0.78–1.72)	
Boffetta *et al.* (1988) American Cancer Society Cohort, 1982–84	476 648	Self-reported job	Lung	Heavy equipment operators	5	2.60 (1.12–6.06)	Age and tobacco smoking; mortality in men aged 40–79 yr in 1982; any work as a heavy equipment operator

Table 2.1 (continued)

Reference Location, follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of exposed cases	Relative risk (95% CI)	Covariates Comments
Järvholm & Silverman (2003) Sweden, to 1995	Swedish construction workers (389 000 total), 1971–92: 14 364 operators of heavy construction vehicles; 110 984 carpenters and electricians as referents	Job recorded at examination	Lung (162)	Heavy construction vehicle operators	49 deaths	**SMR** 0.83 (0.61–1.09)	Lung cancer rates from Swedish cancer and death registries; tobacco smoking (never, current, former, unknown), age at diagnosis or death, calendar time; national industrial health service examination 1971–92; tobacco smoking history from first examination
					61	**SIR** 0.87 (0.66–1.11)	
				Use of a cabin on construction vehicle		**SIR**	
				Never	10	0.86 (0.4–1.6)	
				Sometimes	37	0.71 (0.5–1.0)	
				Always	7	0.50 (0.2–1.0)	
						P for trend < 0.001	
Guo *et al.* (2004a) Finland, records in 1971–95	Finnish working population in 1970 census; 667 121 men	Longest job held on 1970 census	Lung	Forklift drivers	80	**SIR** 0.91 (0.72–1.13)	Indirect adjustment for tobacco smoking
				Excavation machine operators	76	1.12 (0.88–1.40)	
				Road building machine operators	121	1.07 (0.89–1.28)	
				Construction machine operators	104	1.13 (0.92–1.37	
Guo *et al.* (2004b) Finland, 1971–95			Urinary bladder	Forklift drivers	19	**SIR** 1.07 (0.65–1.67)	Same cohort as Guo *et al.* (2004a); indirect adjustment for tobacco smoking
				Excavation machine operators	18	1.10 (0.65–1.74)	
				Road building machine operators	23	1.04 (0.66–1.57)	
				Construction machine operators	19	0.93 (0.56–1.46)	

Table 2.1 (continued)

Reference Location, follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of exposed cases	Relative risk (95% CI)	Covariates Comments
Dock workers							
Gustafsson *et al.* (1986) Sweden, 1961–80	6071 Swedish dock workers	None	Lung	Cohort membership	70 deaths; 86 incident cases	**SMR** 1.32 (1.05–1.66) **SIR** 1.68 (1.36–2.07)	Lung cancer deaths and cases linked to national cancer and mortality register; expected rates from county/metro area of workers; diesel HGVs first in ports in the late 1950s, increased use in the 1960s; no information on tobacco smoking
Emmelin *et al.* (1993) Sweden, 1950–74	Nested case–control study in update of Swedish dock worker cohort: 50 cases/154 controls	Duration of use of diesel equipment and indices related to fuel consumption, 2-yr lag	Lung	**Yr of work since diesel introduced**			Lung cancer deaths and cases linked to national cancer and mortality register; adjusted for tobacco smoking and exposure variables; controls matched on port and date of birth; results presented with 90% CI
				Low/nonsmoker	9	1.00	
				Medium	27	1.8 (0.5–6.6)	
				High	14	2.9 (0.6–14.4)	
				Fuel use index			
				Low	10	1.00	
				Medium	25	1.5 (0.5–4.8)	
				High	15	2.9 (0.7–11.5)	
				Exposure time			
				Low/non-smoker	12	1.00	
				Medium	19	2.7 (0.6–11.3)	
				High	19	6.8 (1.3–34.9)	
Guo *et al.* (2004a) Finland, records in 1971–95	Finnish working population in 1970 census; 667 121 men	Longest job held on 1970 census	Lung	Stevedores	236	**SIR** 1.32 (1.16–1.50)	Indirect adjustment for tobacco smoking
Guo *et al.* (2004b) Finland, 1971–95			Urinary bladder	Stevedores	31	**SIR** 0.95 (0.65–1.35)	Same cohort as Guo *et al.* (2004a); men only; indirect adjustment for tobacco smoking

Table 2.1 (continued)

Reference Location, follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of exposed cases	Relative risk (95% CI)	Covariates Comments
Occupations entailing exposure to diesel exhaust							
Boffetta *et al.* (1988) American Cancer Society Cohort, 1982–84	476 648	Self-reported exposure to diesel exhaust at work in 1982	Lung	Any exposure 1–15 yr ≥ 16 yr	174	1.18 (0.97–1.44) 1.05 (0.80–1.39) 1.21 (0.94–1.56)	Age strata, tobacco smoking, other occupational exposures; mortality in men aged 40–79 yr in 1982
Van Den Eeden & Friedman (1993) California, USA, 1964–88	160 230	Questionnaire; self-reported exposure in past and previous to past yr	Lung	Exposure in past yr Exposure in past yr and before	1 662	1.13 (0.93–1.36) 1.02 (0.81–1.29)	Age, gender, education, race and tobacco smoking; health plan participants, aged 18–79 yr, free of cancer at time of examination
			Urinary bladder	Exposure in past yr Exposure in past yr and before	650	1.17 (0.86–1.59) 1.16 (0.81–1.67)	
Boffetta *et al.* (2001) Sweden, 1971–89	Men, 28 million PY; women, 15 million PY	Linkage to Swedish Cancer Environment Register for job and industry title from 1960 census; JEM	Lung	Any diesel exposure Unexposed Low probability Medium probability High probability	6 266 17 979 2 222 1 881 1 841	**SIR** 1.09 (1.06–1.12) 1.0 (reference) 1.1 (1.04–1.13) 0.90 (0.86–0.94) 1.2 (1.10–1.21)	Expected based on Swedish rates; age, calendar period, region and urban/rural residence; rates for men shown; few cases in women; no information on tobacco smoking
			Urinary bladder	Unexposed Low probability Medium probability High probability	12 287 1 380 1 220 1 069	1.0 (reference) 0.99 (0.94–1.05) 0.84 (0.79–0.89) 0.98 (0.92–1.04)	
Zeegers *et al.* (2001) Netherlands, 1986–92	58 279 from 204 municipal population registries	JEM from job history	Urinary bladder	No exposure Low Medium High	428 35 31 32	1.0 1.00 (0.65–1.54) 0.96 (0.60–1.53) 1.17 (0.74–1.84)	Age, other occupational exposures, intensity and duration of cigarette smoking

Table 2.1 (continued)

Reference Location, follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of exposed cases	Relative risk (95% CI)	Covariates Comments
Guo *et al.* (2004a) Finland, records in 1971–95	Finnish working population in 1970 census; 667 121 men	JEM using longest held job in 1970 census, 20-yr lag	Lung	None Lowest Middle Highest	26 723 2 436 758 120	1.0 (reference) 0.98 (0.94–1.03) 1.04 (0.97–1.12) 0.95 (0.83–1.10)	Asbestos, quartz dust, SES, age, time period; indirect adjustment for tobacco smoking
Guo *et al.* (2004b) Finland, 1971–95		JEM using longest held job in 1970 census, 20-yr lag	Urinary bladder	None None Lowest Middle Highest	6 026 5 872 493 200 78	1.0 (reference) 1.0 (reference) 1.00 (0.91–1.11) 0.95 (0.83–1.10) 0.97 (0.77–1.21)	Same cohort as Guo *et al.* (2004a); adjusted for tobacco smoking, SES, age and time period; men only

CI, confidence interval; EC, elemental carbon; HR, hazard ratio; ICD, International Classification of Diseases; IRR, incidence rate ratio; JEM, job–exposure matrix; mo, months; NHS, national health service; NR, not reported; OR, odds ratio; PY, person–years; SES, socioeconomic status; SIR, standardized incidence ratio; SMR, standardized mortality ratio; yr, year

few for a meaningful analysis. [Although exposure to coal dust has not been proven to be associated with the risk for lung cancer, it is probable that workers who incurred this exposure also had concurrent exposure to coal combustion before conversion to diesel-powered locomotives. Therefore, although these results are consistent with an increased risk of lung cancer attributable to exposure to diesel exhaust, it is possible that coal combustion products contributed to this risk.]

Boffetta *et al.* (1988) examined the relationship between lung cancer and occupational exposure to diesel exhaust using data from a prospective mortality study including more than 1.2 million American men and women that was begun in 1982 by the American Cancer Society. Volunteers from across the USA were enrolled by completing a questionnaire that included information on tobacco smoking, current and last jobs, and the job held for the longest period, other exposures and self-reported exposure to diesel exhaust. An assessment of mortality up to September 1984 was conducted in men aged 40–79 years at enrolment. Of these, 2973 men reported working in the railroad industry during any period of their life. Their relative risk for lung cancer was 1.59 (95% confidence interval [CI], 0.94–2.69; based on 14 deaths), compared with men who did not report working in the railroad industry and who reported no exposure to diesel exhaust, after adjusting for age and smoking. Similar results were obtained for those who reported railroad work as their principal occupation. The risk for lung cancer among railroad workers who reported exposure to diesel exhaust was not stated (approximately 50% of persons who had railroad work as their principal occupation reported exposure to diesel exhaust). [The Working Group found some indication of an increased risk of lung cancer, although this was weakened by the use of self-reporting to assess exposure.]

Nokso-Koivisto & Pukkala (1994) studied 8391 members of the Finnish Locomotive Drivers' Association and determined their cancer incidence between 1953 and 1991 by linkage to the Finnish Cancer Registry. The standardized incidence ratio (SIR) was 0.86 (95% CI, 0.75–0.97; 236 cases) for lung cancer, 1.08 (95% CI, 0.80–1.43; 48 cases) for urinary bladder cancer, 1.25 (95% CI, 0.88–1.17) for kidney cancer and 1.12 (95% CI, 0.92–1.32) for prostate cancer. [These locomotive drivers had also been exposed to asbestos while in training, to coal combustion products (in the 1950s) and to diesel exhaust thereafter. However, in the Finnish railroad industry, much overlap occurred between the periods of steam, diesel and electric locomotive use. Because of the lack of specific information that linked job titles and duties to periods of diesel exhaust use, and in the absence of an internal comparison group, this study was regarded as uninformative in relation to associations between cancer and exposure to diesel exhaust.]

Garshick *et al.* (2004) studied mortality from lung cancer in 54 973 railroad workers in the USA between 1959 and 1996 (38 years). This study was an update of an earlier study of the same cohort (Garshick *et al.*, 1988), and a pilot study by Schenker *et al.* (1984). The cohort comprised a sample of men aged 40–64 years and with 10–20 years of railroad service in 1959. Work histories and death follow-up were extended to 1996. The sample comprised approximately 75% of subjects in diesel exhaust-exposed jobs (engineers, conductors, brakemen, hostlers and shop workers) and 25% in jobs with low or no exposure (ticket agents, clerks and signal maintainers), determined from job categories in 1959. Exposure assignment was validated by an industrial hygiene review of current and historical jobs and work practices and measurement of exposure in current workers (Woskie *et al.*, 1988a, b). By 1959, the railroad industry in the USA had largely converted from coal-fired to diesel-powered locomotives and, in this

study, exposure to diesel exhaust was considered to have begun in 1959. Work histories were obtained from the US Railroad Retirement Board, and mortality was ascertained using Railroad Retirement Board, Social Security and Health Care Financing Administration records. Cause of death was obtained from the National Death Index and death certificates. A total of 43 593 deaths occurred, including 4351 from lung cancer. Analyses consisted of internal comparisons (using workers with low or no exposure as the referents). Efforts were made to adjust for a healthy-worker survivor effect by including a variable in the models for total years worked and also terms for time off work.

Compared with workers with low and no exposure, exposed workers had a relative risk for mortality from lung cancer of 1.40 (95% CI, 1.30–1.51), using a 5-year lag, which did not increase with increasing number of years worked in these jobs. The increased risk was associated with all groups stratified by age in 1959, with the exception of the oldest group (aged 60–64 years). Indirect adjustment for tobacco smoking using the methods of Schlesselman and Axelson (Schlesselman, 1978; Axelson & Steenland, 1988), based on job-specific smoking information from a survey among 547 railroad workers in 1982 and an accompanying case–control study (Garshick *et al.*, 1987; Larkin *et al.*, 2000), suggested some positive confounding which would account for a decrease of about 10–20% in exposed versus unexposed railroad workers. Adjustment using these estimated effects of smoking resulted in rate ratios of 1.17–1.27 for exposed versus unexposed railroad workers. [The Working Group noted that mechanics did not have an elevated risk of lung cancer, probably due to exposure misclassification, because this job title included workers with and without repair shop-related exposures to diesel exhaust.]

An additional approach to adjust for cigarette smoking (Garshick *et al.*, 2006) used information on age- and job-specific cigarette smoking histories available from a previous case–control study of railroad workers based on US Railroad Retirement Board records (Garshick *et al.*, 1987). Data on cause of death, birth cohort, and age- and job-specific smoking habits were used to simulate the smoking behaviour of 39 388 deceased railroad workers. Unadjusted for smoking, the risk of lung cancer among exposed workers with a 5-year lag was 1.35 (95% CI, 1.24–1.46), and an excess risk remained after adjustment (1.22; 95% CI, 1.12–1.32).

To improve the estimation of historical exposures during the transition from steam to diesel locomotives (starting in 1945 and during the decade of the 1950s), yearly locomotive rosters from builders' records and company-specific information were obtained starting in 1945 (Laden *et al.*, 2006). Although only information on the last railroad worked was available in the computerized database, a review of a sample of work history records of railroad employers that were available on paper indicated that the majority of workers (95%) did not change railroads during their careers. The rosters were used to determine the make, model and horse power (hp) of each locomotive in service for 93% of the eligible cohort (52 812 subjects). From this information, the number and type of locomotives that were diesel-fuelled were calculated annually for each railroad. An estimate of the relative amount of PM produced by diesel locomotives (in grams of particulate per hour) for a given railroad in a given year was estimated using Environmental Protection Agency emission factors and information on engine horse power. The probability of diesel exposure per year per railroad (diesel fraction) was then calculated using the year-specific ratios of grams of particulate until this value became constant or the railroad was known to be 100% diesel-powered. Years of exposure to diesel exhaust were calculated by weighting yearly months worked with a specific railroad and the yearly diesel fraction, which allowed the estimation of years of exposure for each worker

before 1959 in contrast to the previous analysis (Garshick *et al.*, 2004), in which exposure was considered to start in 1959. Among workers hired in 1945–49, who started work when diesel locomotives were introduced into the industry, the relative risk of lung cancer with a 5-year lag for any exposure was 1.77 (95% CI, 1.50–2.09) and, for workers hired in 1939–44, was 1.30 (95% CI, 1.19–1.43; *P* for interaction = 0.003). There was evidence of an exposure–response relationship with years of exposure duration that plateaued at 15 years and was not present for workers hired before 1945. Compared with no exposure, the relative risks for exposure for 0– < 10 years, 10– < 15 years, 15– < 20 years, 20– < 25 years and ≥ 25 years were 1.15 (95% CI, 0.77–1.70), 1.49 (95% CI, 1.11–1.99), 1.89 (95% CI, 1.48–2.40), 1.83 (95% CI, 1.45–2.32) and 1.78 (95% CI, 1.39–2.28), respectively, for workers hired in 1945–49. For workers hired before 1945, these were 1.19 (95% CI, 1.00–1.41), 1.28 (95% CI, 1.11–1.47), 1.37 (95% CI, 1.21–1.55), 1.37 (95% CI, 1.21–1.54) and 1.16 (95% CI, 1.00–1.34), respectively. Another more complex metric was calculated as a measure of exposure intensity. Railroad-specific emission estimates based on emission factors and engine horse power were converted to intensity categories using the overall distribution to group values below the median, at the median and above the median level (score 1, 2 or 3). For each cohort member, the year-specific intensity score was multiplied by months of work in each year and by diesel fraction to obtain an index of cumulative exposure to diesel exhaust, or 'intensity–years'. Using this exposure metric, the risk of lung cancer was significantly elevated in all exposure quintiles compared with unexposed workers, but with no evidence of a greater risk with greater exposure.

In a case–control study of railroad workers in the USA that used the same source of work records and directly adjusted for smoking behaviour (Garshick *et al.*, 1988), the magnitude of the lung cancer risk estimate was similar to that reported in the cohort studies described by Garshick *et al.* (2004) and Laden *et al.* (2006).

[This series of studies of railroad workers includes some of the stronger epidemiological studies, due to the well defined linkage between job titles and exposure to train and diesel exhaust, the availability of yearly job titles for a large number of workers, sufficient latency for the development of lung cancer, and consideration of smoking behaviour. In the report of Laden *et al.* (2006), there was indication of an exposure–response relationship with duration based on estimates of years of work with diesel locomotives after 1945 but not with a cumulative exposure metric. The Working Group noted that the calculation of the metric for intensity score of exposure involved much uncertainty, which limited its interpretation with regard to trends in risk based on exposure intensity.]

Guo *et al.* (2004a) linked the occupation held for the longest period in the 1970 census to data in the Finnish Cancer Registry from 1971 up to 1995. A JEM was used to estimate exposures to gasoline and diesel exhaust and non-occupational risk factors, including tobacco smoking, based on job descriptions (see Section 2.2.5). Expected cancer cases were calculated using national rates and adjusted for smoking, where relevant. The standardized incidence ratio for lung cancer in locomotive engineers was not elevated (SIR, 0.63; 95% CI, 0.51–0.78; 85 cases). Guo *et al.* (2004b) used the same data (Guo *et al.*, 2004a) to study incident cancers other than of the lung in locomotive engineers. The standardized incidence ratio for urinary bladder cancer in locomotive engineers was 0.85 (95% CI, 0.53–1.28; 22 cases), and ratios for oesophageal cancer (SIR, 0.93; 95% CI, 0.30–2.17; five cases), kidney cancer (SIR, 1.11; 95% CI, 0.71–1.66; 24 cases) and leukaemia (SIR, 0.84; 95% CI, 0.38–1.59; nine cases) were not elevated. [The Working Group noted that these studies were limited due to the lack of detailed information on work history.]

2.2.2 Bus garage workers

Gustavsson *et al.* (1990) studied mortality from, and incident cases of, lung cancer in a cohort of 695 Stockholm (Sweden) bus mechanics, servicemen and hostlers who had worked for at least 6 months from 1945 to 1970. Mortality was assessed from 1952 to 1986 and incidence from 1958 to 1984. Diesel-powered buses were first introduced in the 1930s in Stockholm and all buses were diesel-fuelled after 1945. A JEM was designed by industrial hygienists to categorize the intensity of exposure to diesel exhaust and asbestos by work period and specific workplace. Specific exposure measurements were limited, and relative exposures were therefore estimated on the basis of work practice, number and characteristics of buses, and garage ventilation. Intensity was estimated over six levels, starting at zero, and cumulative exposure was calculated (intensity × years) for each period and summed. A nested case–control study was performed to match each incident lung cancer case (n = 20) to six controls by age. Relative to the lowest category of cumulative exposure to diesel exhaust, an increased risk was observed with increasing categories of the exposure index: 10–20–relative risk, 1.27 (95% CI, 0.21–7.72; two cases); 20–30–relative risk, 1.56 (95% CI, 0.34–7.16; three cases); and > 30–relative risk, 2.63 (95% CI, 0.74–9.42; 10 cases). The relative risk per unit of a continuous diesel exhaust 'score' was 1.37 (95% CI, 0.91–2.07). No evidence of an exposure–response relationship with asbestos score was observed. Compared with mortality rates of other occupationally active men, the standardized mortality ratio was 1.22 (95% CI, 0.71–1.96; 17 deaths) for lung cancer and 1.23 (95% CI, 0.45–2.68; six deaths) for haematopoietic cancer. Compared with the Stockholm general population, the standardized mortality ratio was 1.93 (95% CI, 0.53–4.94; four deaths) for oesophageal cancer, 0.97 (95% CI, 0.32–2.27; five deaths) for stomach cancer and 0.52 (95% CI, 0.01–2.88; one death) for urinary bladder cancer. [The Working Group noted that no information on tobacco smoking was available. However, confounding by smoking based on diesel exposure category was improbable. The major limitation of this study was the small number of cases, which limited statistical inferences.]

2.2.3 Professional drivers

(a) Bus drivers

Balarajan & McDowall (1988) used the National Health Service Central Register to identify 3392 men, who were employed as professional drivers, and were required to hold a professional licence, in London (United Kingdom) according to the 1939 census, and were alive in January 1950. Mortality compared with the general population was subsequently assessed from 1 January 1950 until the end of 1984. Among bus drivers, the risk for lung cancer (SMR, 1.42 [95% CI, 0.84–2.24]; 18 deaths) was not significantly elevated. [The Working Group calculated the exact 95% confidence intervals that were not provided by the authors.] No significant increase in risk of mortality was observed for cancer of the stomach (SMR, 1.68; 95% CI, 0.72–3.31; eight deaths) or for urinary bladder cancer (SMR, 0.58; 95% CI, 0.015–3.23; one death). No deaths from leukaemia or other lymphatic neoplasms occurred. A significantly elevated standardized mortality ratio was found for bronchitis, emphysema and asthma (SMR, 1.66; 95% CI, 1.06–2.47; 24 deaths). [The Working Group noted that no information was available regarding tobacco smoking, and the relationship between occupational title and the specific periods of work with exposure to diesel exhaust was not described. These drivers were unlikely to have been exposed to appreciable levels of diesel exhaust before the 1950s, when diesel engines were introduced.]

Soll-Johanning *et al.* (1998) conducted a retrospective cohort study of 18 174 bus drivers and tramway employees in Copenhagen (Denmark)

who were employed 1900–94. In Copenhagen, the first diesel-powered buses were introduced in 1936, but, during the Second World War, all buses were fuelled with gasoline. Diesel-powered buses gradually replaced gasoline-powered models after that time, and, in the 1960s, they also replaced the trams. Cancer rates were compared with those of the general population of Denmark by linkage to the Danish Cancer Registry and National Death Index to identify cancers that occurred after 1943. Among male workers employed for 3 months or longer, the standardized incidence ratio was 1.6 (95% CI, 1.5–1.8; 473 cases) for lung cancer, 1.0 (95% CI, 0.8–1.3; 82 cases) for stomach cancer, 1.2 (95% CI, 1.0–1.5; 105 cases) for rectal cancer, 1.4 (95% CI, 1.0–1.9; 39 cases) for laryngeal cancer, 1.6 (95% CI, 1.3–1.6; 83 cases) for kidney cancer, 1.4 (95% CI, 1.2–1.6; 177 cases) for urinary bladder cancer, 1.9 (95% CI, 1.2–2.8; 22 cases) for pharyngeal cancer and 1.1 (95% CI, 0.8–1.5; 46 cases) for leukaemia. In women, the standardized incidence ratio for lung cancer was 2.6 (95% CI, 1.5–4.3; 15 cases). In both men and women, a greater risk of lung cancer was observed with longer time since first employment. No trend in lung cancer risk was found based on periods of predominantly gasoline or diesel vehicle use, and the risks were similarly elevated for workers who started before, at the onset, or during the use of diesel buses. [The Working Group noted that no information on specific exposures or tobacco smoking was available and the periods of diesel and gasoline emissions overlapped. Compared with other men in Copenhagen, the smoking rates among the bus drivers were slightly higher during some time periods, suggesting the possibility of some confounding by smoking.]

A nested case–control study (Soll-Johanning *et al.*, 2003) was conducted with 153 cases of lung cancer and 84 cases of urinary bladder cancer included in the cohort of Copenhagen bus drivers and tramway employees. The cases and controls or next of kin were interviewed regarding tobacco smoking history. Deaths from cancer or non-neoplastic respiratory disease were excluded from the control group and cases and controls were matched on date of birth. Both 10-year lag and no lag models, based on duration of employment, were assessed, adjusting for smoking history in seven categories based on pack–years. No consistent increase in lung cancer risk was observed based on categories of duration of employment in either lag model. The risk, although not statistically significant, increased with greater number of years of employment, but then decreased after > 20 years. With a 10-year exposure lag, there was a suggestion of an increased risk for urinary bladder cancer in persons with 10– < 20 years of work (relative risk, 1.61; 95% CI, 0.57–4.55).

In the cohort study described in Section 2.2.1, Guo *et al.* (2004a) reported on lung cancer risk in male bus drivers who had exposure to both gasoline and diesel exhausts; the standardized incidence ratio was not significantly elevated (SIR, 0.89; 95% CI, 0.78–1.00; 253 cases). [The Working Group noted that this study was limited due to the lack of detailed work histories relating to exposures to exhaust and information on tobacco smoking.]

In a separate report, Guo *et al.* (2004b) presented data for other cancers in bus drivers. The risk was elevated for urinary bladder cancer (SIR, 1.29; 95% CI, 1.02–1.62; 75 cases), oesophageal cancer (SIR, 1.10; 95% CI, 0.60–1.85; 14 cases), kidney cancer (SIR, 1.29; 95% CI, 1.00–1.64; 67 cases) and leukaemia (SIR, 1.04; 95% CI, 0.68–1.51; 27 cases). [The Working Group noted that this study was limited due to the lack of detailed work histories relating to exposures to exhaust and information on tobacco smoking.]

Petersen *et al.* (2010) studied the cancer incidence in a cohort, established in 1978, of 2037 male Danish urban bus drivers over a 25-year period of follow-up (1979–2003). In 1978, public bus drivers in the three largest cities of Denmark received a mailed questionnaire on occupational

history, information regarding bus routes and tobacco smoking habits. Information on incident cases of cancer up to 2003 was obtained by linkage to the Danish Cancer Registry. In analyses comparing external rates for men in the three cities, the standardized incidence ratios were 1.2 (95% CI, 1.0–1.4; 100 cases) for lung cancer and 1.6 (95% CI, 1.2–2.0; 69 cases) for urinary bladder cancer; no cancer at other sites had an elevated risk. The risk for urinary bladder cancer was increased for drivers employed for 15 years or longer (SIR, 1.81; 95% CI, 1.2–2.5) and for drivers with less than 15 years of employment (SIR, 1.5; 95% CI, 1.0–2.1). The standardized incidence ratio for lung cancer was also marginally increased for bus drivers with 15 years or more of employment (SIR, 1.3; 95% CI, 1.0–1.8). A Cox regression model was used to assess the relationship between risk and duration of employment. After adjustment for smoking, city of employment and usual type of bus route operated (urban versus rural), in addition to age and calendar time, each additional year of employment as a bus driver was associated with slightly, non-significantly increased risks for bladder cancer (RR, 1.02; 95% CI, 0.99–1.05). No overall increased risk was observed for lung cancer (RR, 1.00; 95% CI, 0.98–1.03). In a comparison of drivers employed for 15–24 and 25 years or longer with those with those employed for less than 15 years, the relative risks were 1.1 and 1.3, respectively, for bladder cancer and 0.9 and 1.0, respectively, for lung cancer. No change in the estimates was found in a 10-year lag model. [The Working Group noted that these data indicated that, when adjusted for smoking and other risk factors and using an internal comparison group, there was little to no increased risk for urinary bladder or lung cancer in bus drivers with increasing duration of employment. This finding contrasted with the elevated risks for bus drivers suggested by the standardized incidence ratio results reported. Adjustment for the type of bus route (urban versus rural) may have limited the ability to demonstrate an effect of occupational exposure to diesel exhaust.]

(b) Heavy goods vehicle and other drivers

In the USA, Menck & Henderson (1976) reviewed 2161 death certificates that reported lung cancer (trachea, bronchus and lung) in white men, aged 20–64 years, in 1968–70 and all 1777 incident cases of lung cancer in white men of the same age reported to the Los Angeles County Cancer Surveillance Program in 1972–73. These mortality and morbidity data were pooled because of the high accuracy of lung cancer death ascertainment and high mortality. Information on either occupation or industry was not available for 1911 subjects. The population at risk by age group, occupation and industry was estimated from a '1-in-50' sample of Los Angeles County white men, aged 20–64 years, obtained from the 1970 census for Los Angeles. Expected deaths and expected incident cases were calculated for each specific occupation, assuming that the age-specific rates of cancer in each occupation were the same as those for all occupations. The standardized mortality ratio was 3.44 ([95% CI, 2.18–5.16]; 16 deaths, seven incident cases) among taxi drivers and 1.65 ([95% CI, 1.35–1.99]; 58 deaths, 51 incident cases) among heavy goods vehicle (HGV) drivers. [The Working Group calculated the exact 95% confidence intervals, which were not provided by the authors. This study was limited by the absence of data and the use of the occupation recorded on the death certificate as a proxy for exposure.]

In the study by Boffetta *et al.* (1988) described in Section 2.2.1, 9738 men stated their main occupation as an HGV driver, of whom 47% reported exposure to diesel exhaust, 33% reported no such exposure and 20% did not respond. Among the 16 208 HGV drivers, based on any past employment in this occupation, the relative risk for lung cancer was 1.24 (95% CI, 0.93–1.66; 48 deaths) compared with other men who were not HGV drivers and who reported no employment in a

job that entailed exposure to diesel exhaust, after controlling for age and tobacco smoking. No difference in relative risks for lung cancer was observed between HGV drivers who reported exposure to diesel (RR, 1.22; 95% CI, 0.77–1.95; 18 deaths) and those who reported no exposure to diesel (RR, 1.19; 95% CI, 0.74–1.89; 18 deaths). However, in a direct comparison between drivers exposed and those not exposed to diesel exhaust, a suggestion of a positive trend with duration was found for the diesel-exposed HGV drivers (duration 1–15 years–RR, 0.87; 95% CI, 0.33–2.25; six exposed deaths; duration > 16 years–RR, 1.33; 95% CI, 0.64–2.75; 12 exposed deaths). [The Working Group noted that this study was limited by the small number of HGV drivers who reported exposure, and categorization of exposure by self-reporting.]

In the study by Balarajan & McDowall (1988) described in Section 2.2.2, a significant excess of lung cancer was observed among HGV drivers (SMR, 1.59 [95% CI, 1.41–1.79]; 280 deaths), but not among taxi drivers (SMR, 0.86 [95% CI, 0.58–1.23]; 30 deaths). [The Working Group calculated the exact 95% confidence intervals which were not provided by the authors.] Among HGV drivers, the standardized mortality ratio for cancer of the stomach was 1.41 ([95% CI, 1.11–1.77]; 73 deaths), but was not significantly elevated for urinary bladder cancer (SMR, 1.06; 19 deaths), leukaemia (SMR, 1.02; nine deaths) or other lymphatic neoplasms (SMR, 0.92; 12 deaths). Among taxi drivers, no significant increase in risk of mortality was found for cancer of the stomach (SMR, 0.68; eight deaths), urinary bladder cancer (SMR, 1.21; five deaths), leukaemia (SMR, 1.64; three deaths) or other lymphatic neoplasms (SMR, 1.61; four deaths). [The Working Group noted that the interpretation of this study was limited by the lack of information on tobacco smoking.]

Rafnsson & Gunnarsdóttir (1991) identified 868 HGV drivers and 726 taxi drivers in Reykjavik (Iceland) in 1951 from union records and assessed mortality up to 1988. The standardized mortality ratio was 2.14 (95% CI, 1.37–3.18; 24 deaths) for HGV drivers and 1.39 (95% CI, 0.72–2.43; 12 deaths) for taxi drivers. The risk for HGV drivers was also evaluated on the basis of duration of employment and no variation was found.

Gubéran *et al.* (1992) conducted a cohort mortality and cancer morbidity study of 6630 drivers from the Canton of Geneva (Switzerland) who were licensed from 1949 to 1961. The exposed group was compared with the general population of Geneva. The drivers were distributed into three groups: (1) professional drivers (n = 1726), (2) non-professional drivers 'more exposed' to exhaust gas and fumes (this group included occupations such as vehicle mechanic, policeman and road sweeper; n = 712), and (3) non-professional drivers 'less exposed' (comprising all other occupations; n = 4192). The cohort was followed up from 1949 to 1986 for mortality and from 1970 to 1986 for cancer morbidity. With a 15-year latency, significant excesses for lung cancer mortality (SMR, 1.50; 90% CI, 1.23–1.81; 77 deaths) and morbidity (SIR, 1.61; 90% CI, 1.29–1.98; 64 cases) were observed among professional drivers, and the risk of lung cancer increased significantly with time from first exposure. Among non-professional drivers, no significant excess risk was found except for lung cancer mortality among the 'less exposed' group (SMR, 1.21; 90% CI, 1.03–1.40), and for the incidence of lung cancer among the 'more exposed' group (SIR 1.61; 90% CI, 1.11–2.27). For 15 years of latency, the authors reported a standardized mortality ratio of 1.43 (95% CI, 0.80–2.36; 11 deaths) and a standardized incidence ratio of 1.25 (95% CI, 0.74–1.99; 13 cases) for urinary bladder cancer. An excess of mortality and morbidity from rectal cancer (SMR, 2.58; 95% CI, 1.62–3.92, 16 deaths; SIR, 2.00; 95% CI, 1.27–3.00; 17 cases) and stomach cancer (SMR, 1.79; 95% CI, 1.17–2.63; 19 deaths; SIR 2.33; 95% CI, 1.56–3.36; 21 cases) was also observed. No significantly increased risk was

found for leukaemia or lymphatic cancers, prostate cancer or cancer at other sites. [The Working Group noted that this study was limited by a lack of specific information on tobacco smoking, and uncertainty on the extent to which these broad occupational titles indicated exposure to diesel exhaust.]

Hansen (1993) identified a cohort of 14 225 HGV drivers and 43 024 other workers based on occupations reported in the 1970 Danish census. The group of non-HGV drivers included unskilled labourers from other industries with no exposure to combustion products. Up to 1980, 627 drivers and 3811 non-drivers died. The risk for mortality from lung cancer was significantly elevated (SMR, 1.60; 95% CI, 1.26–2.00; 76 cases), whereas mortality from urinary bladder cancer (SMR, 0.87; 95% CI, 0.32–1.89) and cancer of the blood and lymph-forming tissues (ICD-8 200–209; SMR, 1.26; 95% CI, 0.78–1.92; 21 deaths) was not increased. [The Working Group noted that the interpretation of these results was limited by the lack of information on specific exposures and tobacco smoking. However, the use of a blue-collar, non-HGV driver comparison group was liable to reduce possible confounding by smoking.]

Järvholm & Silverman (2003) analysed a computerized register of Swedish construction workers (389 000 workers) who participated in health examinations to assemble a cohort of male HGV drivers (n = 6364) and drivers of heavy construction vehicles (see Section 2.2.5). Carpenters/electricians constituted the reference group (n = 119 984). Workers were identified from health examinations in 1971–92 and were linked to the Swedish National Cancer Registry and National Death Registry, from which cases of lung cancer were ascertained up to 1995. For the analysis, data were stratified into never, former and current smokers, on the basis of tobacco smoking habits recorded at the baseline or when available. HGV drivers had an increased risk of lung cancer after adjusting for smoking (SIR, 1.29; 95% CI, 0.99–1.65; 61 cases); it was not possible to conduct an analysis based on duration and latency. In the same cohort, a significant excess of prostate cancer was also found among HGV drivers (SIR, 1.24; [95% CI, 1.04–1.48]; 124 cases). No significant excess of cases of cancer at other sites was observed, including laryngeal cancer (SIR, 1.25; seven cases), urinary tract cancer [probably bladder] (SIR, 0.72; 26 cases), nasopharyngeal cancer (SIR, 0.82; 12 cases), stomach cancer (SIR, 1.23; 27 cases), rectal cancer (SIR, 1.46; 35 cases), kidney cancer (SIR, 1.12; 23 cases), and lymphoma and leukaemia (SIR, 1.21; 53 cases). [The Working Group noted that the interpretation was limited by the lack of specific information on exposure to diesel exhaust. The strengths of the study were the adjustment for smoking and the use of another blue-collar comparison group, which was liable to reduce possible confounding.]

In the study by Guo *et al.* (2004a) described in Section 2.2.1, among male HGV drivers who were exposed to both gasoline and diesel exhaust, the risk of lung cancer was elevated (SIR, 1.13; 95% CI, 1.04–1.22; 620 cases); the standardized incidence ratio for taxi drivers was 1.10 (95% CI, 0.96–1.26; 209 cases). In a separate report, Guo *et al.* (2004b) presented data for other cancers among male HGV drivers. The risk of leukaemia (SIR, 1.29; 95% CI, 1.02–1.60; 82 cases) was significantly elevated, but not that of kidney cancer (SIR, 1.00; 95% CI, 0.84–1.19; 131 cases), oesophageal cancer (SIR, 1.10; 95% CI, 0.76–1.54; 34 cases) or urinary bladder cancer (SIR, 1.01; 95% CI, 0.85–1.19; 144 cases). In male taxi drivers, the risk of kidney cancer was significantly elevated (SIR, 1.39; 95% CI, 1.06–1.79; 61 cases), but not that of oesophageal cancer (SIR, 1.28; 95% CI, 0.70–2.15; 14 cases), urinary bladder cancer (SIR, 1.06; 95% CI, 0.80–1.38; 55 cases) or leukaemia (SIR, 1.09; 95% CI, 0.70–1.62; 24 cases). [The Working Group noted that these studies were limited by the lack of detailed work histories.]

A cohort was assembled from the records of four transport companies in the USA, from which 54 319 male workers employed in 1985 were identified (Laden *et al.*, 2007). Cause-specific mortality was assessed up to 2000 using the National Death Index; 769 deaths from lung cancer were ascertained. Standardized mortality ratios and 95% confidence intervals were calculated for the entire cohort and by job title, using US mortality rates as the referent. Rates of lung cancer were elevated among all drivers (SMR, 1.10; 95% CI, 1.02–1.19) and loading dock workers (SMR, 1.10; 95% CI, 0.94–1.30). The standardized mortality ratio for urinary bladder cancer was 0.80 (95% CI, 0.56–1.15; 29 deaths) for the entire cohort, with similar estimates for drivers and non-drivers.

Garshick *et al.* (2008) conducted an internal analysis in the same cohort, based on employment records for 31 135 male workers, aged 40 years and over, with at least 1 year of employment as of 1985. Exposure to engine exhaust was estimated for eight job categories (long haul driver, pick-up and delivery driver, loading dock worker, combined driver and dock worker, mechanic, hostler in the vehicle yard, clerk and other) through an industrial hygiene review of previous exposures and current measurements of work-shift exposures to EC. Time-varying cumulative years of work in each of the categories were calculated. Group level adjustment for cigarette smoking was carried out using the methods of Schlesselman and Axelson (Schlesselman, 1978; Axelson & Steenland, 1988). Job-specific information on the distribution of smoking habits was obtained from a survey of 11 986 workers that included all clerks and a random sample of active and retired workers from three of transport companies who contributed to the cohort. In the assessment of mortality from lung cancer, all eight job-specific exposure variables were included in Cox regression models to adjust the risk for lung cancer for different jobs held throughout a worker's career. The healthy-worker survivor effect was controlled for using variables for duration of employment and time since leaving work. Hazard ratios (HRs) for lung cancer were elevated in workers who held jobs associated with regular exposure to vehicle exhaust, including long-haul drivers (HR, 1.15, 95% CI, 0.92–1.43; 323 deaths), pick-up and delivery drivers (HR, 1.19; 95% CI, 0.99–1.42; 233 deaths), dock workers (HR, 1.30; 95% CI, 1.07–1.58; 205 deaths) and combination dock workers/drivers (HR, 1.40; 95% CI, 1.12–1.73; 150 deaths). No excess risk was seen in the other job groups. The risk of mortality increased linearly with years of employment in long-haul drivers, pick-up and delivery, dock workers and combination dock workers/drivers. Estimates of risk for 20 years of employment in a job versus no employment in the job, adjusted for smoking, were 1.40 (95% CI, 0.88–2.24) for long-haul drivers, 2.21 (95% CI, 1.38–3.52) for pick-up and delivery drivers, 2.20 (95% CI, 1.23–3.33), for dock workers and 2.34 (95% CI, 1.42–3.83) for combination drivers/dock workers. Risks not adjusted for smoking were slightly greater than the adjusted estimates for long-haul drivers and slightly lower for pick-up and delivery drivers and for dock workers. [The Working Group noted that this study had particular strengths because detailed historical work records were available, exposures in each job were supported by an industrial hygiene review and exposure measurements, and variation in smoking behaviour by job was considered. Compared with the standardized mortality ratio analysis of the same cohort by Laden *et al.* (2007), greater risks were observed in this study that used an exposure assessment and an internal comparison of risks based on job titles. Although the risks for lung cancer in mechanics, who had greater current and historical exposures, was not elevated, it was noted that the number of mechanics was relatively small (6% of the cohort) and contributed relatively few cases of lung cancer (n = 38). The Working Group also noted that there was no consensus on the optimal method to adjust for

the healthy-worker survivor bias, and that the applied adjustment probably did not negate the positive findings.]

Birdsey *et al.* (2010) conducted a cohort mortality study of independent HGV owners/operators using files from a trade association. The 156 241 subjects were members of the trade association between 1989 and 2004, and mortality was assessed using the National Death Index up to 2004. Indirect adjustment was made for tobacco smoking. Most of the cohort (86%) was aged 25–54 years at entry into the study. No excess mortality from lung cancer (SMR, 1.00; 95% CI, 0.92–1.09; 557 deaths), urinary bladder cancer (SMR, 0.93; 95% CI, 0.62–1.34; 29 deaths) or any other type of cancer was observed. [The Working Group noted that the interpretation of this study was limited by the lack of individual information on smoking and information on how association membership served as a surrogate for exposures to exhaust.]

Garshick *et al.* (2012) investigated the risk for lung cancer in the cohort of Laden *et al.* (2007) in relation to a reconstruction of occupational exposure to EC. An exposure assessment was conducted in 2001–06 by collecting more than 4000 cross-shift samples of EC measured in PM ≤ 1.0 μm diameter at representative transport terminals. Separate exposure models were constructed for drivers and terminal workers. Historical trends in the ambient levels of EC in terminals were modelled on the basis of historical trends available for 1971–2000 in the coefficient of haze, a measurement of PM based on optical density that is highly predictive of ambient EC. A 1988–89 assessment of EC in the same industry that used the same methodology as the current assessment was used to calibrate model estimates. Historical data on jobs and terminal-specific monthly EC concentrations determined by the exposure model were summed by year for 1971–2000 to estimate cumulative exposure (μg/m^3–months) for individuals. Job-specific EC values before 1971 (8% of total exposure time) were assigned values for 1971 exposures because data on coefficient of haze were not available to estimate background. Combination workers were assumed to spend 50% of their time as a pick-up and delivery driver and 50% as a dock worker. When adjusted for race, calendar year and census region, with a 5-year lag, mortality from lung cancer was elevated for the upper three cumulative EC quartiles compared with the lowest quartile, but the differences were not statistically significant (HR, 1.17–1.19 for 5-year lagged exposures excluding mechanics). However, the risk for lung cancer was inversely associated with the total duration of employment. The association of lung cancer with cumulative exposure to EC was stronger after adjustment for duration of employment, and when mechanics were excluded. The job duties of mechanics changed over time and their exposures were intermittent. The risks for 5- and 10-year lagged exposures increased with each cumulative exposure quartile when mechanics were excluded, resulting in estimated hazard ratios of 1.48 (95% CI, 1.05–2.10) and 1.41 (95% CI, 0.95–2.11) for the highest versus lowest quartiles of 5- and 10-year lagged exposures, respectively, when adjusted for duration of employment. Associations were weaker for average exposure to EC. In addition, adjusting for duration of employment, a linear exposure–response relationship was suggested when cumulative EC was used as a continuous covariate and splines were incorporated into the models. For each 1000 μg/m^3–months of cumulative EC, based on a 5-year exposure lag, the hazard ratio was 1.07 (95% CI, 0.99–1.15) with a similar association for a 10-year exposure lag (HR, 1.09; 95% CI, 0.99–1.20). [The Working Group noted that this analysis provided similar results to those of Garshick *et al.* (2008), who did not use quantitative exposure measurements. An additional strength was a comprehensive exposure assessment and the development of exposure models that were linked to accurate historical job title records and incorporated

historical trends in background exposures. Although uncertainty is inherent when estimating historical exposures, systemic bias was improbable. It was not possible to adjust directly for tobacco smoking, but previous adjustment in the same cohort revealed little difference in the risk for lung cancer with or without adjustment. A possible interaction between average exposure and duration of employment on mortality from lung cancer may explain some of the apparent paradoxes of the results, such as the observation that cumulative exposure, adjusted for duration of employment, had a greater effect while average exposure, adjusted for duration of employment, did not. The study provided evidence for an association between sources of EC (predominantly diesel) and the risk for lung cancer.]

2.2.4 Miners

In the cohort described in Section 2.2.1, Boffetta *et al.* (1988) studied 2034 men who reported working as a miner on the basis of any past employment in this occupation. The age- and tobacco smoking-adjusted relative risk for lung cancer was 2.67 (95% CI, 1.63–4.37; 15 deaths) compared with other men who did not report working as a miner and who reported no exposure to diesel exhaust. [The Working Group noted that this study was limited by the small number of miners and the lack of information regarding specific exposures to exhaust. Only 14% of miners reported exposure to diesel exhaust; these miners may also have been exposed to other lung carcinogens, such as silica or radon.]

In the cohort described in Section 2.2.1, Guo *et al.* (2004a) reported that male miners in three different occupational categories had an elevated risk of lung cancer. These included mine and quarry work involving metal ore (SIR, 3.26; 95% CI, 2.28–4.51; 36 cases), mine and quarry work involving non-metal ore (SIR, 1.85; 95% CI, 1.59–2.14; 181 cases) and other unspecified mine and quarry work (SIR, 1.73; 95% CI, 1.35–2.19; 70 cases). All three groups were classified by an expert review as having been exposed to diesel exhaust but not gasoline exhaust. In a separate report, Guo *et al.* (2004b) presented data for other cancers in male miners. In non-metal ore miners and quarry workers, the risks were elevated for leukaemia (SIR, 2.31; 95% CI, 1.39–3.61; 19 cases), oesophageal cancer (SIR, 1.74; 95% CI, 0.70–3.58; seven cases), kidney cancer (SIR, 0.88; 95% CI, 0.47–1.50; 13 cases) and urinary bladder cancer (SIR, 1.16; 95% CI, 0.73–1.76; 22 cases). Too few cases occurred in other mining groups to carry out a meaningful assessment. [The Working Group noted that these studies were limited due to the lack of detailed work histories and information on tobacco smoking. In particular, for lung cancer, these miners may have had confounding exposures to other substances, such as silica and radon.]

Neumeyer-Gromen *et al.* (2009) updated the mortality from lung cancer in a cohort of 5862 German underground potash miners, first described by Säverin *et al.* (1999), from 1970 up to 2001. Diesel equipment was introduced into potash mines in 1969 and, in 1991, the mines were closed. Tobacco smoking histories were available from medical and personnel records for 80% of the cohort. Estimates of diesel exposure were obtained in 1992, and expressed as total carbon in respirable dust. Because technology had not changed, these levels were assumed to be representative of previous exposure and were used for its categorization. The overall standardized mortality ratio was not elevated for lung cancer (SMR, 0.73; 95% CI, 0.57–0.93; 61 deaths) or urinary bladder cancer (SMR, 0.80; 95% CI, 0.40–1.60; 8 deaths). Using Cox regression modelling, the smoking-adjusted relative risk in the highest category of exposure dichotomized at 4.90 mg/m^3–years was 1.28 (95% CI, 0.61–2.71; 61 cases). In a subgroup of 3335 workers who had worked underground for at least 10 years, the age- and smoking-adjusted relative risk was 1.50 (95% CI, 0.66–3.43; 37 cases). Adjusting for smoking

resulted in higher risk estimates. In a model that further adjusted for time since hire and calendar year, the relative risk in the entire cohort was 2.53 (95% CI, 1.13–5.69) and that in the subcohort of workers who had worked for more than 10 years after 1969 was 3.30 (95% CI, 1.30–8.37). Using time since first hire as the time variable in a Cox regression analysis to account for duration of employment, and adjusting for age and smoking, a non-significant trend (P = 0.19) in risk for mortality from lung cancer was observed with greater exposure in the entire cohort (RR, 1.81; 95% CI, 0.92–3.58; and 1.59; 95% CI, 0.75–3.40; for the second and third tertiles, respectively). A non-significant increased trend (P = 0.17) in risk of was also found within the subcohort, for which more accurate information on exposure was available. [The Working Group noted that, although the power of the study was limited by the sample size, one of its strengths was that the effects of smoking were considered together with quantitative estimates of exposure based on measurements. The study also used an internal comparison group. Another strength in the design was the control of confounding for other mining-related occupational risk factors for lung cancer, because exposure to radon, silica dust, asbestos and heavy metals were not significant in potash mining. This study was supportive of an effect of exposure to diesel exhaust on the risk of lung cancer.]

Attfield *et al.* (2012) studied the mortality of a cohort of 12 315 blue-collar workers who were employed in one of eight non-metal mines in the USA for at least 1 year after diesel equipment had been introduced. The mines were selected to minimize exposures to silica, radon and asbestos. Detailed work histories were abstracted from company records and mortality was assessed up to 1997. The dates of the introduction of diesel equipment ranged from 1947 to 1967. Historical estimates of exposure to respirable EC were constructed on the basis of personal measurements taken in the mines in 1998–2001, which were extrapolated retroactively based on a model using diesel exhaust-related determinants, including diesel engine horse power and ventilation rates, and historical measurements of carbon monoxide. The modelled trends in concentrations of CO for previous years were then used to adjust the 1998–2001 levels of exposure to respirable EC to estimate historical annual concentrations of respirable EC for each job. Estimates of exposure to silica, asbestos, respirable dust, radon and other PAHs were also made. Estimates of exposure to diesel exhaust in surface workers were based on measurements made in 1998–2001 and no reconstruction of historical exposure was carried out. The mean exposure of surface workers only was 1.7 μg/m^3 and that of the ever-underground miners was 128.2 μg/m^3. Standardized mortality ratios using external referents were determined from state-specific mortality rates, and their calculation was limited to persons employed since 1960 (12 270 subjects), because state-specific rates were not available for earlier years. The standardized mortality ratio for lung cancer was 1.26 (95% CI, 1.09–1.44) for the complete cohort, 1.21 (95% CI, 1.01–1.45) for workers involved in any underground work and 1.33 (95% CI, 1.06–1.66) for workers involved exclusively in surface work. In the complete cohort, standardized mortality ratios were 1.09 (95% CI, 0.58–1.86) for urinary bladder cancer, 1.18 (95% CI, 0.76–1.74) for leukaemia, 0.98 (95% CI, 0.54–1.64) for kidney cancer, 1.12 (95% CI, 0.76–1.60) for pancreatic cancer and 0.85 (95% CI, 0.60–1.16) for prostate cancer. Mortality from oesophageal cancer was significantly elevated in all workers (SMR, 1.83; 95% CI, 1.16–2.75). In an internal analysis of the entire cohort, adjustment for the location of work (ever surface or underground) and a 15-year lag for cumulative exposure resulted in relative risks by quartile of 1.0, 0.55 (95% CI, 0.35–0.85), 1.03 (95% CI, 0.60–1.77) and 1.39 (95% CI, 0.78–2.48). In an analysis of surface workers only, the corresponding relative risks by quartiles of cumulative exposure

were 1.0, 1.28 (95% CI, 0.64–2.58), 0.73 (95% CI, 0.35–1.53) and 1.00 (95% CI, 0.44–2.28). The relative risks for average exposure among surface workers were 1.0, 1.71 (95% CI, 0.82–3.58), 2.22 (95% CI, 1.01–4.90) and 2.56 (95% CI, 1.09–6.03). In an analysis of ever underground miners only, the relative risks by quartile of cumulative exposure were 1.0, 1.50 (95% CI, 0.86–2.62), 2.17 (95% CI, 1.21–3.88) and 2.21 (95% CI, 1.19–4.09), and those by quartile of average exposure were 1.0, 1.73 (95% CI, 0.99–3.05), 2.11 (95% CI, 1.14–3.90) and 1.86 (95% CI, 0.98–3.52). Further analysis of underground miners with ≥ 5 years of exposure showed similar patterns (test for trend using log cumulative exposure as a continuous variable, $P = 0.015$). [The Working Group noted that the log cumulative exposure best fit the exposure–response curve over the entire range. In addition, all the internal analyses of surface workers involved groups with very small ranges of exposure. Moreover, no historical exposure assessments were available for surface workers, which further limited the interpretation of the analyses of surface workers alone.]

In a nested case–control study of the above cohort of miners, Silverman *et al.* (2012) obtained histories of tobacco smoking, occupation and previous respiratory disease by interview for 198 lung cancer cases and 562 control subjects. Controls were assigned by random sampling from all members of the cohort who were alive before the day the case subject died and were matched on birth year, gender, ethnicity and mine. Analyses of trend in risk by level of respirable EC were adjusted for smoking, previous respiratory disease and a history of jobs that entailed a high risk of lung cancer. Smoking adjustments included separate terms for levels of smoking of surface and underground miners, to take into account that the differences in the odds ratios (ORs) for levels of smoking between these two groups. In analyses of surface and underground miners combined, the odds ratios by quartile of cumulative exposure (15-year lag) were 1.0, 0.74 (95% CI, 0.40–1.38), 1.54 (95% CI, 0.74–3.20) and 2.83 (95% CI, 1.28–6.26; P for trend = 0.001). Corresponding odds ratios for average exposure (15-year lag) were 1.0, 1.11 (95% CI, 0.59–2.07), 1.90 (95% CI, 0.90–3.99) and 2.28 (95% CI, 1.07–4.87; P for trend = 0.062). Analyses restricted to surface miners gave odds ratios by quartile of cumulative exposure (15-year lag) of 1.0, 3.98 (95% CI, 0.69–23.02), 0.76 (95% CI, 0.12–4.98) and 0.42 (95% CI, 0.05–3.59; P for trend = 0.12), and corresponding odds ratios for average exposure (15-year lag) of 1.0, 4.38 (95% CI, 0.56–34.24), 5.67 (95% CI, 0.77–42.06) and 1.31(95% CI, 0.14–12.01; P for trend = 0.66). For underground miners, odds ratios by quartile of cumulative exposure (15-year lag) were 2.46 (95% CI, 1.01–6.01), 2.41 (95% CI, 1.00–5.82) and 5.10 (95% CI, 1.88–13.87; P for trend = 0.004), and the corresponding odds ratios for average exposure (15-year lag) were 1.0, 1.04 (95% CI, 0.45–2.43), 2.19 (95% CI, 0.87–5.53) and 5.43 (95% CI, 1.92–15.31; P for trend = 0.001). Analysis of the interaction between cumulative exposure to respirable EC and smoking showed an increased risk with increased cumulative exposure (15-year lag) for both never smokers and ever smokers (P for interaction = 0.09). Among never smokers, the odds ratio increased with increasing cumulative exposure to respirable EC from 1.47 (95% CI, 0.29–7.50; four exposed cases) for tertile 2 up to 7.30 (95% CI, 1.46–36.57; seven exposed cases). The trend of increasing risk with increased cumulative diesel exposure was attenuated in heavy smokers. [The Working Group noted that the exposure assessment methodology applied in the National Institute of Occupational Safety and Health/National Cancer Institute (NIOSH/NCI) studies was of high quality and used an established approach for occupational cohort studies. Because surface workers formed a group that had very low exposures, no significant trends were observed with increasing exposure. Consequently, the Working Group focused primarily on the combined analyses of surface

and underground miners, in which the surface workers formed part of the low-exposure group. The Working Group gave the results of the case–control greater weight than those of the cohort because the former included an adjustment for tobacco smoking. The research team appeared to have used the available data most effectively. However, in any such study, uncertainties exist that may result in measurement error, and, although historical exposures might have been over- or underestimated, this could have affected the risk per unit exposure but not the pattern of the exposure–response relationship.]

2.2.5 Other groups exposed to vehicle exhausts

(a) Heavy equipment operators

Wong *et al.* (1985) conducted a cohort mortality study of 34 156 men who had been members of a heavy construction equipment operators union for at least 1 year between 1964 and 1978. The mortality experience of the cohort was compared with that of white men in the USA. Historical environmental measurements were not available, and only partial work histories were accessible for some cohort members. Mortality from respiratory cancer for the whole cohort was similar to that expected (SMR, 0.99; 95% CI, 0.88–1.10), with no trend by duration of union membership. A significant excess of mortality from lung cancer was found among the 4075 retirees, after excluding early retirees who were thought potentially to have retired due to illness (SMR, 1.30; 95% CI, 1.04–1.61). The standardized mortality ratio for urinary bladder cancer was 1.18 (95% CI, 0.78–1.72), with no trends by duration or latency. Data on cigarette smoking were available for a small sample of 107 workers, and did not indicate any difference in smoking habits between the cohort and the general population. [The Working Group noted that the main limitation of this study with regard to diesel exhaust was the lack of any information documenting such an exposure; in general, it is not known to what degree heavy equipment operators have appreciable exposure to diesel exhaust.]

In the study described in Section 2.2.1, Boffetta *et al.* (1988) studied 855 men who reported working as heavy equipment operators. The age- and tobacco smoking-adjusted relative risk for lung cancer was 2.60 (95% CI, 1.12–6.06) compared with men who reported no such employment and no exposure to diesel exhaust. [The Working Group noted that this study was limited by the small number of heavy equipment operators included and the categorization of exposure from self-reporting. Less than half (46%) of the heavy equipment operators reported exposure.]

In the study described in Section 2.2.3, Järvholm & Silverman (2003) found that 14 364 heavy equipment operators had a lower incidence of lung cancer than a reference group of electricians/carpenters after adjusting for tobacco smoking (SIR, 0.87; 95% CI, 0.66–1.11; 61 cases; smoking-adjusted SMR, 0.83; 95% CI, 0.61–1.09; 49 cases) compared with the referents. The cohort of heavy equipment operators also showed no significant excess of cancer at other sites, including the prostate (SIR, 0.93; 116 cases), larynx (SIR, 1.03; nine cases), urinary tract [probably bladder] (SIR, 1.15; 61 cases), nasopharynx (SIR, 0.75; 18 cases), stomach (SIR, 1.05; 32 cases), rectum (SIR, 0.82; 29 cases), kidney (SIR, 0.74; 24 cases), and lymphoma and leukaemia (SIR, 1.08; 78 cases). [The Working Group noted that the interpretation was limited by the lack of specific information on exposure to diesel exhaust.]

In the cohort study described in Section 2.2.1, Guo *et al.* (2004a) carried out a separate analysis in other male exhaust-exposed workers, including forklift drivers and three categories of heavy equipment operators, none of whom had a significantly elevated risk of lung cancer. The standardized incidence ratios ranged from 0.89 to 1.13 with 36–121 cases in each occupational

group. Guo *et al.* (2004b) presented data for cancers at other sites in these workers. Neither forklift drivers nor the three categories of heavy equipment operators had a significantly increased risk of urinary bladder cancer, oesophageal cancer or leukaemia: the standardized incidence ratios ranged from 0 to 1.32 with 0–23 cases in each occupational group. However, the risk for kidney cancer in this group was significantly elevated (SIR, 1.65; 95% CI, 1.11–2.36). [The Working Group noted that these studies were limited by the lack of detailed information on exposures and tobacco smoking.]

(b) Dock workers

Gustafsson *et al.* (1986) compared the incidence of cancer among male Swedish dock workers with that of the Swedish male population. Diesel trucks were introduced into Swedish ports in the late 1950s and became prevalent during the 1960s. The cohort comprised 6071 men first employed at the beginning of 1961, and mortality and lung cancer incidence were assessed from 1961 to 1981. In the cohort, 70 deaths from and 86 cases of lung cancer occurred. The risk for lung cancer was significantly increased (SMR 1.32; 95% CI, 1.05–1.66; SIR, 1.68; 95% CI, 1.36–2.07). The standardized mortality and standardized incidence ratios, respectively, were 0.98 (95% CI, 0.83–1.15) and 1.06 (95% CI, 0.89–1.27) for digestive cancer, 1.10 (95% CI, 0.85–1.42) and 0.97 (95% CI, 0.80–1.17) for urogenital cancer, 0.71 (95% CI, 0.46–1.10) and 0.86 (95% CI, 0.60–1.23) for all leukaemias, and 1.23 (95% CI, 0.91–1.66) and 1.22 (95% CI, 0.91–1.64) for stomach cancer. [The Working Group noted that the interpretation of the elevated standardized mortality ratios was limited by the lack of information on tobacco smoking and on the extent of occupational exposures to exhaust.]

Emmelin *et al.* (1993) conducted a nested case–control study on and assessed the exposure of subjects in the above Swedish cohort study (Gustafsson *et al.*, 1986), and included 6573 men employed as dock workers for at least 6 months in 1950–74 in 15 Swedish ports where data for exposure assessment were available. Up to four referents were matched by port and date of birth to each of 50 eligible cases that had occurred between 1960 and 1982. Information on tobacco smoking was obtained from living referents, next of kin and from interviews with retired workers. No measurements of exposure to exhaust were available, and exposure was therefore estimated on the basis of annual fuel consumption divided by the number of employees in the same port in that year. 'Exposed time' was an indicator variable that indicated exposure in a year when the annual fuel consumption per person exceeded the lower quartile of the overall distribution of all years and ports. Exposure was categorized as time worked with diesel equipment ('machine time'), cumulative fuel consumption (in person–L) and exposed time (three classes per category), with a 2-year exposure lag, and 90% confidence intervals were calculated. The three exposure metrics gave largely similar results, and the greatest odds ratios were found for persons with the highest exposure after adjusting for smoking. The inclusion of smoking in the regression models increased the effects of exposure: for machine time, the odds ratio for medium exposure was 1.8 (90% CI, 0.5–6.6) and that for high exposure was 2.9 (90% CI, 0.6–14.4). Similar odds ratios were obtained for fuel consumption. For exposed time, the odds ratio for medium exposure was 2.7 (90% CI, 0.6–11.3) and that for high exposure was 6.8 (90% CI, 1.3–34.9) compared with low exposure/nonsmokers. Further analyses for exposed time with smoking reclassified as ever/never resulted in an odds ratio of 1.7 for medium exposure and 4.6 for high exposure. [The Working Group noted that confidence intervals were not provided, nor were odd ratios provided for the other exposure metrics. The interpretation was limited by the classification of smoking into two categories, the imprecise results attributable to small sample size, and the

imprecise categorization of exposure, but the results were nevertheless suggestive of an association between exposure and the risk for lung cancer. The Working Group also noted that there may have been some exposure to asbestos during work in ports but this would probably not fully explain the observed associations.]

In the cohort study described in Section 2.2.1, Guo *et al.* (2004a) reported that stevedores, who were classified as having exposure to both diesel and gasoline exhausts, had an elevated risk of lung cancer (SIR, 1.32; 95% CI, 1.16–1.50). Guo *et al.* (2004b) presented data for cancers at other sites and reported that stevedores had an elevated risk for oesophageal cancer (SIR, 2.03; 95% CI, 1.11–3.40) but no increased risk for urinary bladder cancer (SIR, 0.95; 95% CI, 0.65–1.35), kidney cancer or leukaemia. [The Working Group noted that this study was limited by the lack of detailed work histories, and information on exposures and smoking.]

(c) Occupations entailing exposure to diesel exhaust based on expert reviews or self-reporting

In the American Cancer Society cohort, Boffetta *et al.* (1988) also reported results based on self-reporting of exposure to diesel exhaust. Among the 378 622 subjects who provided information regarding self-reported exposure to diesel exhaust, 174 cases of lung cancer occurred among those who stated that they had been exposed. In this group, the relative risk for lung cancer, adjusted for tobacco smoking and other occupational exposures (asbestos, coal, coal tar and gasoline exhaust), was 1.18 (95% CI, 0.97–1.44,) for all men with self-reported exposure to diesel exhaust. Based on duration of exposure, the adjusted relative risk for lung cancer was 1.05 (95% CI, 0.80–1.39) for men with 1–15 years of exposure, with a suggestion of a positive trend for men with 16 or more years of exposure (RR, 1.21; 95% CI, 0.94–1.56). The relative risks for other cancers based on self-reported exposure to diesel exhaust, adjusted for smoking and other occupational exposure, were not significantly elevated: 1.21 for multiple myeloma (14 deaths), 1.29 for all leukaemias (17 deaths), 1.39 for pancreatic cancer (27 deaths) and 1.04 for urinary bladder cancer (13 deaths). Analysis of urinary bladder cancer by duration of exposure showed no trend (1–15 years exposure: RR, 1.43; 95% CI, 0.61–2.33; > 15 years of exposure: RR, 0.94; 95% CI, 0.35–2.51). [The Working Group noted that the reliance on self-reported exposure to diesel exhaust may have led to misclassification and reduced the ability to detect an effect of exposure.]

Van Den Eeden & Friedman (1993) studied 160 230 members of a health maintenance organization who self-reported whether they had been exposed to engine exhausts in the past year or more than 1 year previously during the years 1964–72; 3% reported exposure in both periods. Follow-up for cancer incidence was up to 1988, during which time 1662 lung cancers and 650 urinary bladder cancers were observed. After adjusting for tobacco smoking and other covariates, the rate ratios for those exposed in the past year and more than 1 year previously were 1.02 (95% CI, 0.81–1.29) for lung cancer and 1.16 (95% CI, 0.81–1.67) for urinary bladder cancer. [The Working Group noted that this study did not specify whether the exhaust was from diesel engines; reliance on self-reported exposure to engine exhausts was a limitation.]

Boffetta *et al.* (2001) investigated the risk of cancer among workers exposed to diesel exhaust by linking the occupations and industries reported in the 1960 census with the Swedish Environment Register III that contains nationwide data on cancer incidence and mortality for 1971–89. Using a JEM, exposures were graded on the basis of intensity and probability of exposure to diesel exhaust. A total of 28 million person-years of observation were accrued, 28% of which was related to persons classified as exposed. In men, increasing risks for lung cancer were observed with increasing exposure intensity: the

relative risks were 0.95 (95% CI, 0.92–0.98), 1.1 (95% CI, 1.08–1.21) and 1.3 (95% CI, 1.26–1.42) for low, medium and high intensity of exposure, respectively. Corresponding results for probability of exposure were 1.1 (95% CI, 1.04–1.13), 0.90 (95% CI, 0.86–0.94) and 1.2 (95% CI, 1.10–1.24). Fewer cases (n = 57) and less exposure occurred in women, and the risk for lung cancer was not increased. Among men exposed to diesel exhaust, the standardized incidence ratio for urinary bladder cancer was 1.00 (95% CI, 0.97–1.03), with no trends by intensity or probability. For men with a high probability of exposure, the standardized incidence ratios were 1.1 (95% CI, 0.96–1.28) for laryngeal cancer, 0.98 (95% CI, 0.92–1.04) for urinary bladder cancer, 1.0 (95% CI, 0.96–1.14) for kidney cancer, 1.1 (95% CI, 0.99–1.21) for oral and pharyngeal cancer, 1.1 (95% CI, 1.05–1.20) for stomach cancer and 1.1 (95% CI, 0.99–1.19) for pancreatic cancer. A significantly increased risk of oral/pharyngeal (SIR, 1.64) and cervical (SIR, 1.48) cancers was observed among women, with a suggestion of a dose–response relationship. [The Working Group noted that the lack of work histories, which prohibited analyses by duration and latency, and the lack of smoking data were factors that weakened this study.]

In a population-based study in the Netherlands, Zeegers *et al.* (2001) studied 58 279 men who answered a questionnaire on occupation in 1986 and were followed up for the incidence of urinary bladder cancer until 1992. Experts using a JEM assigned the levels of exposure to diesel exhaust as none, low, medium and high to each subject. Using a case–cohort approach (532 cases, 1630 non-cases), the relative risks for urinary bladder cancer, after adjustment for smoking, demographics and other occupational exposures, were 1.00 (95% CI, 0.65–1.54), 0.96 (95% CI, 0.60–1.53) and 1.17 (95% CI, 0.74–1.84) for low, medium and high exposure, respectively, relative to no exposure (P for trend = 0.76).

Lee *et al.* (2003) analysed the risk for multiple myeloma in relation to diesel exposures in a large Swedish cohort of construction workers who were followed up from 1971 to 1999. By linkage with the Swedish National Cancer Registry, 446 cases of primary multiple myeloma were identified. A JEM was developed to classify exposure to occupational agents, including diesel exhaust, using a 1971–76 survey of occupational exposures in the construction industry and nitric oxide as a marker of exposure to diesel exhaust. However, few occupations were considered to entail exposure to diesel exhaust in the original survey: drivers, earthmoving, mountain and asphalt workers, as well as some repair and concrete workers, were classified as occupationally exposed to diesel exhaust. Tobacco smoking status, body mass index and age, as well as socioeconomic status and other occupational exposures, were considered as potential confounders. Among diesel-exposed workers, the adjusted relative risk for multiple myeloma was 1.3 (95% CI, 1.00–1.77). No evidence of a dose–response was found. [The Working Group noted that the lack of information on duration of exposure, as well as the overall low levels of exposure to diesel exhaust in exposed cohort members, were limitations of the study.]

In a study described in Section 2.2.1, Guo *et al.* (2004a) also used a Finnish JEM (FINJEM) to estimate exposures to gasoline and diesel exhaust based on job reviews, and to assign exposure to diesel exhaust to different jobs reported in the census. In FINJEM jobs were classified as diesel-exposed when nitrogen dioxide had been found in surveys, and as gasoline-exposed when carbon monoxide had been found. The overall relative risk for lung cancer was 0.99 (95% CI, 0.96–1.03) among men and 1.22 (95% CI, 0.85–1.73) among women estimated to have been exposed to diesel exhaust, and 1.05 (95% CI, 1.01–1.09) among men and 1.61 (95% CI, 1.23–2.1) among women estimated to have been exposed to gasoline exhaust. The Finnish occupational exposure database included occupation, gender, age and period-specific tobacco smoking

rates that were used to adjust for smoking in the analysis of lung cancer risk. [The Working Group noted that the authors also developed estimates of cumulative exposures to diesel and gasoline exhausts, but these were of doubtful validity due to the lack of work histories.] Guo *et al.* (2004b) presented risks for other cancers, based on JEM-estimated exposure to diesel and gasoline exhausts. No overall risk for exposure to either diesel or gasoline exhaust was presented. No consistent increased risk for leukaemia, kidney cancer, urinary bladder cancer or oesophageal cancer was observed based on estimated cumulative exposure to either diesel or gasoline exhaust. Vasama-Neuvonen *et al.* (1999) reported similar findings for ovarian cancer in the same registry. [The Working Group noted that these studies were limited due to the lack of detailed work histories and information on tobacco smoking.]

Tarvainen *et al.* (2008) studied cancers of the mouth and pharynx using the same Finnish Cancer Registry data and assessed the risk of cancer based on categories of exposure to diesel and gasoline engine exhausts. For exposure to diesel exhaust, the risks for the lowest, mid-and highest levels of exposure were 1.26 (95% CI, 1.04–1.53), 1.15 (95% CI, 0.83–1.55) and 1.62 (95% CI, 0.99–2.50), respectively. For exposure to gasoline exhaust, the corresponding risks were 1.28 (95% CI, 1.07–1.52), 1.37 (95% CI, 1.02–1.80) and 1.43 (95% CI, 0.62–2.82), respectively, suggesting an association with exposure. [The Working Group noted that this study was of limited value due to the lack of lifetime work histories and information on smoking.]

Boers *et al.* (2005) studied 1386 cases of prostate cancer and 2335 subcohort members from a prospective cohort of 58 279 subjects in the Netherlands. Cancer incidence was determined by linkage to nine regional cancer centres and assessed up to 1995. Information on diet and job history was retrieved from self-administered questionnaires completed at baseline in a previous cohort study. Occupational histories were assessed by experts to categorize exposure to pesticides, PAHs, diesel exhaust, metal dust and metal fumes. No association was found between exposure to diesel exhaust and prostate cancer (RR, 0.81; 95% CI, 0.62–1.06) in the highest tertile compared with unexposed subjects.

Bender *et al.* (1989) studied 4849 men with 1 or more years of experience as a highway maintenance worker for the Minnesota Department of Transportation (USA) and who had worked at least 1 day after 1 January 1945. Mortality was assessed up to 31 December 1984. The standardized mortality ratio was 0.69 (95% CI, 0.52–0.90) for lung cancer and 1.09 (95% CI, 0.56–1.90; 12 deaths) for urinary bladder cancer. [The Working Group noted that this study was limited by the lack of details regarding job history.]

2.3 Case–control studies

2.3.1 Cancer of the lung

See Table 2.2

Coggon *et al.* (1984) carried out a case–control study based on information from death certificates for England and Wales. Cases were 598 men, aged less than 40 years, who died from bronchial carcinoma during 1975–79, and controls were 1180 men who died from causes other than lung cancer, matched to the cases on age, year of death and area of residence. The most recent full-time occupation was mentioned on the death certificate. A JEM was developed for nine occupational exposures, including diesel exhaust, and jobs were classified as entailing any or high exposure. Any exposure to diesel exhaust was associated with an increased risk of lung cancer (RR, 1.3; 95% CI, 1.0–1.6), whereas the risk for high exposure to diesel exhaust was lower (RR, 1.1; 95% CI, 0.7–1.8). [The Working Group noted that the study demonstrated an increased risk of lung cancer associated with exposure to diesel exhaust, although no dose–response was indicated. The lack of full-time occupational

Table 2.2 Case–control studies of lung cancer and exposure to engine exhausts

Reference Location, period	Total No. of cases/ controls	Source of controls	Exposure assessment	Exposure categories	No. of exposed cases	Relative risk (95% CI)	Covariates Comments
Coggon *et al.* (1984) England and Wales, 1975–79	598 men aged < 40 yr/1180 men matched for age, yr of death, area of residence; based on death certificates	Deaths from causes other than lung cancer	JEM developed and applied to the occupation on death certificates	**Diesel fumes**			Bronchial carcinoma; age, yr of death, sex and area of residence; no data on tobacco smoking available
				Any exposure	172	1.3 (1.0–1.6)	
				High exposure	32	1.1 (0.7–1.8)	
Garshick *et al.* (1987) USA, 1981–82	1256/2385 individually matched (age, time of death) excluding deaths from cancer, accidents and suicide; nested within a cohort of male railway workers	Railway worker cohort identified from Railway Retirement Board	Industrial hygiene sampling survey used to classify job tasks as exposed or unexposed to DME; diesel–yr calculated for job tasks after 1959	**Age ≤ 65 yr**			Age, asbestos and tobacco smoking
				0–4 diesel–yr	NR	1.00	
				5–19 diesel–yr	NR	1.02 (0.72–1.45)	
				> 20 diesel–yr	117	1.64 (1.18–2.29)	
				Age > 65 yr			
				0–4 diesel–yr	NR	1.00	
				5–19 diesel–yr	NR	0.95 (0.79–1.13)	
				> 20 diesel–yr	26	0.94 (0.56–1.59)	
Hayes *et al.* (1989) USA, 1976–83	2291 men/2570; pooled analysis of 3 NCI-organized studies	Hospital and population	Full occupational histories used to classify duration of jobs in motor exhaust-exposed occupations	No exposure	1567	1.00	Birth cohort, cigarette use and study area; underlying 3 studies not summarized individually here, hence no overlap
				< 10 yr	362	1.0 (0.9–1.2)	
				> 10 yr	348	1.3 (1.1–1.5)	
Boffetta *et al.* (1990) (includes previous study reported by Hall & Wynder, 1984) USA, six cities, 1977–87	2584 men (primary lung cancer)/5099 men	Hospital, selected among patients with non-tobacco-related diseases	Occupations classified as low probability of (reference group), possible (19 occupations) and probable exposure (13 occupations); interviews	Possible exposure	240	0.92 (0.76–1.10)	Age, hospital, yr of interview, tobacco smoking habits, race, education and exposure to asbestos; only usual occupation available for cases in 1977–85; men only
				Probable exposure	210	0.95 (0.78–1.16)	
				Duration of probable exposure (1985–87 only)			
				1–15 yr	4	0.52 (0.15–1.86)	
				16–30 yr	15	0.70 (0.34–1.44)	
				≥ 31 yr	17	1.49 (0.72–3.11)	
						P for trend = 0.18	

Table 2.2 (continued)

Reference Location, period	Total No. of cases/ controls	Source of controls	Exposure assessment	Exposure categories	No. of exposed cases	Relative risk (95% CI)	Covariates Comments
Steenland *et al.* (1990) USA, 1982–83	994 deceased teamsters' union members with ≥ 20 yr of membership/1085	Union members deceased in the same period, excluding deaths from lung cancer, urinary bladder cancer and motor vehicle accidents	Mail and telephone interviews with next of kin; additional job information obtained from union records	**Long-haul (yr after 1959)**			Age, tobacco smoking, asbestos and other jobs with potential exposure to diesel exhaust
				1–11	162	1.08 (0.68–1.70)	
				12–17	228	1.41 (0.90–2.21)	
				≥ 18	213	1.55 (0.97–2.47)	
						P for trend = 0.04	
				Short-haul (yr after 1959)			
				1–11	36	1.11 (0.61–2.03)	
				12–17	37	1.15 (0.63–2.43)	
				≥ 18	40	1.79 (0.94–3.42)	
Steenland *et al.* (1998) (re-analysis of Steenland *et al.*, 1990)	994/1085	See Steenland *et al.* (1990)	Estimates of exposure to EC (marker of DME) obtained from exposure measurements in 1990 and modelling of historical levels	**Quartiles of cumulative exposure (EC–years; no lag)**			Age, race, tobacco smoking and asbestos
				0–174		1.20 (0.79–1.81)	
				174–268		1.16 (0.77–1.75)	
				268–360		1.39 (0.91–2.11)	
				≥ 360		1.72 (1.11–2.64)	
						P for trend = 0.023	
Swanson *et al.* (1993) Detroit metropolitan area, USA, 1984–87	3792 male incident/1996 colon cancer	Registry	Duration of employment based on lifetime occupational histories	**HGV drivers**		**OR**	White men only; age and tobacco smoking; separate ORs estimated for black men; potential confounding from socioeconomic factors possible due to use of carcinogen-free occupations as unexposed, but would not affect dose–response
				0 yr	88	1.00	
				1–9 yr	78	1.4 (0.8–2.4)	
				10–19 yr	38	1.6 (0.8–3.5)	
				≥ 20 yr	121	2.5 (1.4–4.4)	
				LGV drivers			
				0 yr	88	1.00	
				1–9 yr	46	1.7 (0.9–3.3)	
				≥ 10 yr	36	2.1 (0.9–4.6)	
				Garage and service station workers			
				0 yr	88	1.00	
				1–9 yr	47	2.2 (1.1–4.4)	
				≥ 10 yr	7	2.3 (0.5–10.8)	

Table 2.2 (continued)

Reference Location, period	Total No. of cases/ controls	Source of controls	Exposure assessment	Exposure categories	No. of exposed cases	Relative risk (95% CI)	Covariates Comments
Brüske-Hohlfeld *et al.* (1999) (same study base as Jahn *et al.*, 1999) Germany	3498 male incident/3541	Population	Personal interview on occupational history and tobacco smoking habits; exposure to DME from expert assessment	Ever exposed	716	1.43 (1.23–1.67)	Age, area, tobacco smoking and exposure to asbestos
				Duration of DME exposure (yr)			
				> 0–3	132	1.28 (0.95–1.73)	
				3–0	155	1.21 (0.91–1.61)	
				10–20	165	1.84 (1.34–2.52)	
				20–30	148	1.62 (1.16–2.24)	
				≥ 30	116	1.35 (0.95–1.93)	
Gustavsson *et al.* (2000) Stockholm County, Sweden, 1985–90	1042 male incident/2365	Population	Mailed questionnaire and supplementary telephone interview with study subjects or next of kin; quantitative DME exposure from expert assessment of lifetime occupa-tional history	**DME (mg–yr/m3 of NO2)**			Age, year of diagnosis, tobacco smoking, residential radon, residential air pollution and other occupational exposure to lung carcinogens
				Unexposed	842	1.00	
				> 0–0.53	29	0.65 (0.40–1.04)	
				0.54–1.41	54	1.13 (0.77–1.66)	
				1.42–2.37	45	1.05 (0.70–1.60)	
				≥ 2.38	72	1.63 (1.14–2.33)	
				Mixed exhaust (gasoline/diesel) (mg–yr/m3 of CO)			
				Unexposed	833	1.00	
				> 0–13.5	19	0.43 (0.25–0.74)	
				13.6– 8.8	47	1.10 (0.74–1.65)	
				38.9–113.6	78	1.32 (0.92–1.90)	
				≥ 113.7	65	1.09 (0.74–1.61)	
Richiardi *et al.* (2006) Turin, Italy, 1991–92	595 incident > 76 yr of age/845	Population	Interviewed on life time occupational history; supplementary questionnaires evaluated for DME exposure by industrial hygienist	**Cumulative exposure (intensity yr, h/wk)**			Age, cigarette consumption, exposure to list A occupations and educational level; environmental exposure to motor exhaust not included
				No exposure	436	1.00	
				I tertile (< 440)	53	1.01 (0.66–1.56)	
				II tertile (440–519)	51	0.99 (0.64–1.53)	
				III tertile (≥ 2520)	55	0.86 (0.56–1.31)	

Table 2.2 (continued)

Reference Location, period	Total No. of cases/ controls	Source of controls	Exposure assessment	Exposure categories	No. of exposed cases	Relative risk (95% CI)	Covariates Comments
Tse *et al.* (2011) Hong Kong Special Administrative Region SAR, China, 2004–06	132 nonsmoking men/536 nonsmokers	Population	Lifetime occupational history (job title, job task) obtained from personal interview; exposure to 16 occupational agents, including diesel exhaust	Diesel exhaust, any exposure (self-assessed)	6	4.17 (1.3–13.5)	Age, place of birth, education level, residential radon, history of lung diseases, cancer in relatives and intake of meat; reference group never exposed to confirmed or suspected human carcinogens
				Gasoline exhaust	7	1.52 (0.51–4.53)	
Villeneuve *et al.* (2011) Eight Canadian provinces, 1994–97	1681 male incident aged > 40 yr/2053	Population	Self-administered questionnaires inquiring about lifetime occupational and residential history	**Diesel exhaust (cumulative)**			Age, province, cigarette pack–yr, secondhand smoke, silica (yes/no) and asbestos
				Lowest tertile	267	0.93 (0.75–1.17)	
				Middle tertile	307	1.03 (0.83–1.29)	
				Highest tertile	326	1.12 (0.89–1.40)	
						P for trend = 0.07	
				Gasoline exhaust (cumulative)			
				Lowest tertile	261	0.92 (0.74–1.14)	
				Middle tertile	351	1.08 (0.88–1.33)	
				Highest tertile	314	1.11 (0.88–1.39)	
						P for trend = 0.68	
Olsson *et al.* (2011) Europe and Canada	13 304 men and women/16 282; pooled from 11 studies	Mixed	Lifetime occupational histories; population JEM developed to assign no, low or high exposure to DEM	**Cumulative exposure to DME (unit–yr)**			Age, sex, study, ever employed in other occupation noted to be at high risk for lung cancer, pack–yr and time since quitting smoking; partial overlap with Brüske-Hohlfeld *et al.* (1999), Gustavsson *et al.* (2000), Richiardi *et al.* (2006) and Pintos *et al.* (2012)
				Never	7676	1.00	
				< 6	1269	0.98 (0.89–1.08)	
				6–17.33	1325	1.04 (0.95–1.14)	
				17.34–34.5	1440	1.06 (0.97–1.16)	
				> 34.5	1594	1.31 (1.19–1.43)	
						P for trend < 0.0	
				Low levels of DME (yr of exposure)			
				0	7676	1.0	
				1–10	1576	1.00 (0.92–1.09)	
				11–20	785	0.98 (0.88–1.10)	
				1–30	660	1.03 (0.91–1.17)	
				> 30	1246	1.17 (1.07–1.29)	
						P for trend < 0.01	

Table 2.2 (continued)

Reference Location, period	Total No. of cases/ controls	Source of controls	Exposure assessment	Exposure categories	No. of exposed cases	Relative risk (95% CI)	Covariates Comments
				High levels of DME (yr of exposure)			
Olsson et al. (2011) Europe and Canada (cont.)				0	7676	1.0	
				1–10	858	1.28 (1.14–1.45)	
				11–20	228	1.21 (0.98–1.51)	
				21–30	149	1.52 (1.15–2.02)	
				> 30	126	1.45 (1.07–1.96)	
						P for trend < 0.01	
Pintos *et al.* (2012) Montreal, Canada, Study I 1979–86, Study II 1996–2001 (expansion and re-analysis of Parent *et al.*, 2007)	1593/1427	Population	Interview with subjects or proxies on lifetime occupational history; exposure intensity to diesel exhaust and 293 other factors were coded by expert assessment	**Study I**			Adjusted for age, ethnicity, level of education, SES, tobacco smoking, respondent status and occupational carcinogens; men only
				Ever exposed	165	1.48 (1.0–2.1)	
				Non-substantial	97	1.25 (0.8–1.9)	
				Substantial	68	2.00 (1.1–3.6)	
				Study II			
				Ever exposed	298	1.30 (1.0–1.7)	
				Non-substantial	224	1.21 (0.9–1.6)	
				Substantial	74	1.74 (1.1–2.8)	
				Pooled			
				Ever exposed	463	1.34 (1.1–1.7)	
				Non-substantial	309	1.21 (1.0–1.5)	
				Substantial	75	1.80 (1.3–2.6)	
Parent *et al.* (2007) Montreal, Canada, 1979–85 (expansion of Siemiatycki *et al.*, 1988)	857 men aged 35–70 yr/1349 cancer and 533 population	Population and cancer patients	Interview with subjects or proxies on lifetime occupational history; exposure intensity to diesel exhaust, gasoline exhaust and other factors coded by expert assessment	**Diesel exhaust (population controls)**			Age, SES, ethnicity, self/ proxy respondent, tobacco smoking, asbestos and crystalline silica; men only
				Any exposure	166	1.2 (0.8–1.8)	
				Non-substantial	92	1.1 (0.7–1.7)	
				Substantial	74	1.6 (0.9–2.8)	
				Gasoline exhaust (population controls)			
				Any exposure	380	0.8 (0.6–1.1)	
				Non-substantial	110	0.9 (0.6–1.3)	
				Substantial	270	0.8 (0.6–1.1)	

CI, confidence interval; CO, carbon monoxide; DME, diesel motor exhaust; EC, elemental carbon; HGV, heavy goods vehicle; JEM, job–exposure matrix; LGV, light goods vehicle; NCI, National Cancer institute; NO_2, nitrogen dioxide; NR, not reported; OR, odds ratio; SES, socioeconomic status; wk, week; yr, year

histories may have reduced the precision of the exposure classification, and the absence of data on tobacco smoking could have resulted in confounding.]

Garshick *et al.* (1987) conducted a nested case–control study of lung cancer among a cohort of male railroad workers in the USA. Study subjects were identified from approximately 650 000 members of the US Railroad Retirement Board, which kept a register of virtually all railroad workers with 10 years of service or more. All deaths from lung cancer between March 1981 and February 1982 were identified; two controls per case were selected from deaths from causes other than cancer, accidents or suicide and were matched to cases on age and date of death. Tobacco smoking habits were obtained from questionnaires completed by the next of kin. Exposure to diesel exhaust was assessed on the basis of yearly job title after 1959, and validated through an industrial hygiene sampling survey and a review of job titles. During the 1950s, the railroad industry in the USA converted from coal-fired to diesel-powered locomotives; starting in 1959, workers were classified as exposed or unexposed and years of work in a job associated with exposure to diesel exhaust were summarized. Odds ratios were estimated after adjustment for age, tobacco smoking and exposure to asbestos. The risk for lung cancer was significantly increased in cases aged < 65 years at the time of death with ≥ 20 diesel–years of exposure (OR, 1.64; 95% CI, 1.18–2.29). No increased odds ratio was noted for those aged ≥ 65 years at the time of death, when the prevalence of exposure to diesel exhaust was believed to have been lower because fewer had worked for ≥ 20 years after 1959. A sensitivity analysis gave no indication that residual confounding from exposure to asbestos could explain the positive findings. [The Working Group noted that this study, based on a large data set, used yearly job records to classify exposure and was nested within the retrospective cohort mortality study that was conducted by the same authors using US Railroad Retirement Board records (Garshick *et al.*, 2004). The positive findings were probably not caused by bias or confounding (see Table 2.2).]

Hayes *et al.* (1989) reported the findings of a pooled analysis of three US NCI-organized case–control studies that used both population-based and hospital-based designs and were conducted in Florida in 1976–79 (Blot *et al.*, 1982), Louisiana in 1979–83 (Correa *et al.*, 1984) and New Jersey in 1980–81 (Schoenberg *et al.*, 1987). The original studies were not reviewed by the Working Group, and hence there was no overlap. A total of 2291 male cases of lung cancer and 2570 controls were identified. Full occupational histories and tobacco smoking habits were obtained by interview with study subjects or next of kin. Occupations were coded from the work histories and classified in relation to exposure to motor exhaust. Jobs coded as exposed to motor exhaust were HGV drivers, heavy equipment operators, bus drivers, taxi drivers, other groups of drivers and HGV mechanics. [The Working Group noted that the coding did not specifically address exposure to diesel or gasoline exhaust.] Odds ratios were estimated according to the duration of exposure to motor exhaust. The odds ratio, adjusted for birth cohort, study area and smoking, was 1.0 (95% CI, 0.9–1.2) for < 10 years of exposure versus no exposure and 1.3 (95% CI, 1.1–1.5) for > 10 years of exposure. Findings were consistent among the three study populations. An analysis limited to study subjects who were interviewed directly showed an odds ratio of 1.5 (95% CI, 1.2–1.9) in the group with a duration of > 10 years of exposure. Restriction of the analysis to those who had never smoked gave an odds ratio in the group with > 10 years of exposure of 1.7 (95% CI, 0.6–4.5; eight cases). [The Working Group noted that this study was based on full occupational histories; the positive findings could not be explained by uncontrolled confounding or bias. The study assessed exposure to mixed motor exhaust, but diesel motor

exhaust was probably the predominant source of exposure in most of the jobs coded as exposed.]

A hospital-based case–control study carried out in 18 hospitals in six cities in the USA aimed primarily at investigating the health effects of tobacco smoking (Boffetta *et al.*, 1990) included cases and controls reported in an earlier study (Hall & Wynder, 1984). Cases were 2584 men diagnosed with primary lung cancer, interviewed in 1977–87, and matched to 5099 controls selected from male patients without tobacco-related diseases on age, year of interview and hospital. Occupations were classified according to the probability of exposure to diesel exhaust (low, possible and probable). Self-assessed exposures were also recorded. After adjustment for tobacco smoking, the odds ratio for lung cancer was not related to the probability of exposure to diesel exhaust or the duration of probable exposure. However, duration of exposure was only available for a subset of study subjects, giving wide confidence intervals (*P* for trend with duration not significant). [The Working Group noted that this study provided limited data on occupations, which may have led to a misclassification of exposure.]

Steenland *et al.* (1990) reported a case–control study of men who had been members of the US Teamsters' union for more than 20 years and who had died in 1982–83. Among these, 994 had died from lung cancer and 1085 controls were selected from Teamsters' union members who had died during the same period from causes excluding lung cancer, urinary bladder cancer and motor vehicle accidents. Data on tobacco smoking and jobs driving diesel or gasoline HGVs were obtained from the next of kin, and additional data on long-haul/short-haul driving was obtained from the Teamsters' union. Odds ratios were adjusted for age, tobacco smoking, exposure to asbestos and other jobs with potential exposure to diesel exhaust. Based on the information from union records, a positive trend in the risk for lung cancer was observed with duration of long-haul HGV driving. The duration of short-haul driving after 1959 gave increasing odds ratios with increasing duration of exposure, but the trend was not statistically significant. Driving diesel-powered vehicles for > 35 years was associated with a significantly increased risk for lung cancer (OR, 1.89; 95% CI, 1.04–3.42) and driving gasoline-powered vehicles for > 35 years was also associated with an increase (OR, 1.34; 95% CI, 0.81–2.22). Shorter durations gave lower odds ratios for both diesel and gasoline vehicles. [The Working Group noted that this study, which was based on detailed work histories and adjusted for tobacco smoking habits, showed positive evidence of an increased risk for lung cancer after a long duration of employment in jobs that entailed exposure to diesel exhaust. No data were available on the intensity of exposure.]

A re-analysis of the study by Steenland *et al.* (1990) was carried out (Steenland *et al.*, 1998). Quantitative group-based exposure estimates were applied to the study subjects, using EC as a marker of exposure, and were based on an industrial hygiene survey of the HGV transport industry performed in 1990 (Zaebst *et al.*, 1991). Earlier exposure levels were modelled on the basis of available exposure measurements and changes in fuels, motors and traffic intensity. The robustness of historical exposure assessments were tested in a sensitivity analysis. Odds ratios were adjusted for age, race, tobacco smoking and exposure to asbestos. The risk for lung cancer increased (although not monotonically) with cumulative exposure to EC, showing a statistically significant trend ($P = 0.023$). The odds ratio in the highest quartile of cumulative exposure versus no exposure was 1.72 (95% CI, 1.11–2.64). The use of alternative assumptions for time trends in past exposure levels did not change the conclusions. [The Working Group noted that this study was based on a large data set, and demonstrated a positive and significant association between cumulative dose of diesel motor exhaust and the risk for lung cancer. The general

pattern of trends of exposure to EC, particularly a consistent decrease after the 1970s, was similar in the studies of Steenland *et al.* (1998) and Garshick *et al.* (2012) of the HGV industry. The Working Group also noted that Garshick *et al.* (2012) calibrated their exposure estimates to those of Zaebst *et al.* (1991), which, to a large extent, were the basis of the exposure estimation in Steenland *et al.* (1998).]

Swanson *et al.* (1993) conducted a population-based case–control study in the Detroit metropolitan area (USA). Incident cases of histologically confirmed cancer, aged 40–84 years in 1984–87, were identified from a cancer surveillance programme, including 3792 cases of lung cancer and 1966 colon cancer controls. Lifetime occupational and tobacco smoking histories were obtained by telephone interview with the subjects or surrogates. Odds ratios were estimated by occupational group, which defined a set of occupations with little or no exposure to carcinogens as unexposed, and were adjusted for age, pack–years of smoking and race. Results were presented separately for black and white men. Among white men, the odds ratio was significantly increased among long-term (> 20 years) HGV drivers and was raised among long-term (> 10 years) lighter vehicle drivers. The number of cases among black men was smaller and the odds ratios were not significant, but were elevated for those employed long-term. Short- and long-term white male garage and service station workers had non-significantly increased odds ratios; again, the numbers of similarly employed black men were very low. [The Working Group noted that the study was based on occupational titles only and no estimates of exposure to motor exhaust were used. The classification of nearly carcinogen-free occupations as unexposed may have introduced a systematic difference between the exposed and unexposed, resulting in biased risk estimates.]

Brüske-Hohlfeld *et al.* (1999) reported the risk for lung cancer among men in relation to exposure to diesel motor exhaust from two pooled case–control studies in Germany. One study identified cases of lung cancer in the Bremen and Frankfurt/Main areas in 1988–93 and the other study covered parts of Nordrhein-Westfalen, Rheinland-Pfalz and Bayern, the Saarland, Thüringen and Sachsen in 1990–96. A total of 3498 male incident cases of lung cancer and 3541 population controls were interviewed in person regarding occupational history and tobacco smoking habits. Special questionnaires were used to assemble information on specific job tasks. Exposure to diesel motor exhaust was investigated by expert assessment. The findings were adjusted for age, region, smoking and exposure to asbestos. The risk for lung cancer among those ever exposed to diesel motor exhaust was significantly elevated (1.43; 95% CI, 1.23–1.67), and increased, although not monotonically, with duration of exposure. The odds ratio was higher among those most recently exposed. An analysis by job titles showed a significantly increased odds ratio for professional drivers, those in other traffic-related jobs and heavy equipment operators. For professional drivers, the increased risk was found only in West Germany (OR, 1.44; 95% CI, 1.18–1.76) and not in East Germany (OR, 0.83; 95% CI, 0.60–1.14). [The Working Group noted that this study was based on detailed occupational data including information on job tasks, as well as detailed data on smoking. A low response rate among controls in one part of the study may have contributed to the discrepant results found for drivers in former West and East Germany.]

Gustavsson *et al.* (2000) conducted a population-based case–control study in Stockholm County, Sweden, of 1042 male cases of lung cancer diagnosed in 1985–90, who were identified from the regional cancer registry, and 2364 frequency-matched controls from the general population. Lifetime occupational and residential histories and information on tobacco smoking were obtained from a postal questionnaire supplemented by a telephone interview

with study subjects or next of kin. Occupational exposures were assessed by an occupational hygienist, who coded the intensity and probability of exposure to diesel motor exhaust and seven other occupational exposures. The odds ratio for cumulative exposure to diesel exhaust, adjusted for age, year of diagnosis, tobacco smoking, residential radon, residential exposure to traffic-generated air pollution and exposure to asbestos, was significantly increased in the highest quartile of cumulative exposure (OR, 1.63; 95% CI, 1.14–2.33). No increased risk for lung cancer was noted for exposure to vehicle exhaust in general (mixed diesel and gasoline exhaust). [The Working Group noted that this study was based on full occupational histories and lifetime data on smoking. It is improbable that the positive finding for diesel exhaust was caused by bias or confounding.]

A population-based case–control study of lung cancer conducted in the Turin area, Italy, was reported by Richiardi *et al.* (2006). Incident cases of primary lung cancer among men and women aged < 76 years (n = 595) were identified from hospitals in the city. Controls (n = 845) were randomly selected from population registers, and were frequency-matched for sex and 5-year age group. Subjects were interviewed regarding tobacco smoking habits, lifetime occupational history and exposure to secondhand smoke. Interviewers administered supplementary questionnaire modules for subjects in occupations with potential exposure to diesel exhaust. Questionnaires were evaluated for exposure to diesel exhaust by an industrial hygienist, who assessed the probability, intensity and daily frequency of exposure. Odds ratios were adjusted for age, gender, cigarette smoking, exposure to other occupational lung carcinogens and level of education, and were estimated for any exposure, for duration, intensity and probability of and for cumulative exposure to diesel exhaust; no indications of increasing risk for any of these parameters were observed. Analyses by job group and histological types of lung cancer showed no evidence of increased risk in association with exposure to diesel exhaust. [The Working Group noted that data on environmental exposure to motor exhaust, which may be substantial in the study area, were not available. The lack of such data may possibly have reduced the ability of the study to detect an association with occupational exposure. This study was later expanded and re-analysed using a JEM in a pooled, multicentre study (Olsson *et al.*, 2011).]

Tse *et al.* (2011) carried out a study on occupational exposures and the risk for lung cancer among nonsmoking men in Hong Kong Special Administrative Region (China). Histologically confirmed cases of primary lung cancer among Chinese men, aged 35–79 years, were identified from the largest oncology centre in Hong Kong Special Administrative Region during 2004–06 (response rate, 96%). Community controls were randomly selected from same districts as the cases (response rate, 48%) and frequency-matched by age. The analysis among lifelong nonsmokers included 132 cases and 536 controls with lifetime occupational histories (jobs and job tasks) obtained by personal interview. Regular exposure (at least once a week for at least 6 months) to specific agents or groups of agents at each workplace was determined based on a list of confirmed or suspected human carcinogens, including diesel exhaust and 13 other occupational agents. Odds ratios were adjusted for age, place of birth, level of education, residential radon, history of lung diseases, cancer in first-degree relatives and intake of meat. Only six cases were exposed to diesel exhaust which gave an odds ratio of 3.47 (95% CI, 1.08–11.14) when those not exposed to diesel exhaust were defined as unexposed, and 4.17 (95% CI, 1.28–13.53) when those not exposed to any of the agents were defined as unexposed. No increased risk was noted for exposure to gasoline exhaust, based on seven cases. A positive association was noted for exposure to silica and work as a painter but not for exposure to

asbestos. [The Working Group noted that one strength of this study was the analysis of lifelong nonsmokers, which considerably reduced the risk of residual confounding from smoking. Although the odds ratio for exposure to diesel exhaust was significantly increased, the confidence interval was wide because of the small numbers. The low response rate among controls may have biased the risk estimates. Self-assessed exposures may have resulted in misclassification, although a positive bias appeared to be improbable because no increased risks were noted for most of the other agents studied.]

A population-based case–control study of lung cancer among men aged > 40 years in eight Canadian provinces (excluding Montreal) was reported by Villeneuve *et al.* (2011). Living incident cases of lung cancer were identified from cancer registries in British Columbia, Alberta, Saskatchewan, Manitoba, Ontario, Nova Scotia, Newfoundland and Prince Edward Island from 1994 to 1997, and age- and sex-matched controls were identified from the population. Self-administered postal questionnaires provided information on lifetime occupational and residential histories. Response rates were 64% for cases and 61% for controls. Occupation and industry codes were assigned by two hygienists, who also coded exposure (concentration, frequency and reliability; four levels for each) to diesel and gasoline emissions, asbestos and crystalline silica. Odds ratios were estimated adjusting for age, province, cigarette pack–years, secondhand smoke, silica and asbestos. For cumulative exposure to diesel emissions, the odds ratios across the increasing tertiles of cumulative exposure were 0.93, 1.03 and 1.12 (P = 0.07). A stronger trend with cumulative exposure was observed for squamous cell (P = 0.04) and large cell carcinoma (P = 0.02) of the lung. The odds ratio for exposure to diesel exhaust was significantly increased for large cell carcinoma in the upper tertile of cumulative exposure to diesel exhaust (OR, 1.68). (A positive association was noted for exposure to silica and work as a painter but not for exposure to asbestos; OR, 1.03–2.74; 36 cases). No consistent indications of increased risk were observed with exposure to gasoline exhaust. [The Working Group noted that the findings suggest that exposure to diesel engine emissions increased the risk of lung cancer, particularly for squamous and large cell carcinoma subtypes. This study used detailed data on occupational exposures and tobacco smoking and a weak positive association with cumulative exposure to diesel exhaust was noted.]

Olsson *et al.* (2011) reported a pooled re-analysis of 11 case–control studies from 41 study centres in 12 European countries and Canada. The study base partially overlapped with other studies reported by Brüske-Hohlfeld *et al.* (1999), Gustavsson *et al.* (2000), Richiardi *et al.* (2006) and Pintos *et al.* (2012), and included 13 304 cases (10 812 men and 2492 women) and 16 282 controls (13 031 men and 3251 women). For most centres, controls were recruited from the population, but some used hospital controls. Lifetime occupational and smoking histories were taken from the original studies. Work histories were recoded to a common classification (ISCO-68), and smoking histories were harmonized to enable a pooled analysis. A population JEM was developed (independently from earlier exposure assessments in the participating studies) to assign no, low or high exposure to diesel motor exhaust (representing relative intensities of 0, 1 and 4) for every work period for all study subjects. Cumulative lifetime exposure to diesel motor exhaust was expressed as unit–years. Odds ratios were adjusted for age, sex, study, occupational exposure to known carcinogens (based on A-list jobs; Mirabelli *et al.*, 2001), and pack–years of and time since quitting smoking. The odds ratio for lung cancer was significantly increased in the highest quartile of cumulative exposure to diesel exhaust, with evidence of a dose–response trend (P < 0.01). Subgroup analyses of workers never employed in jobs involving exposure to known

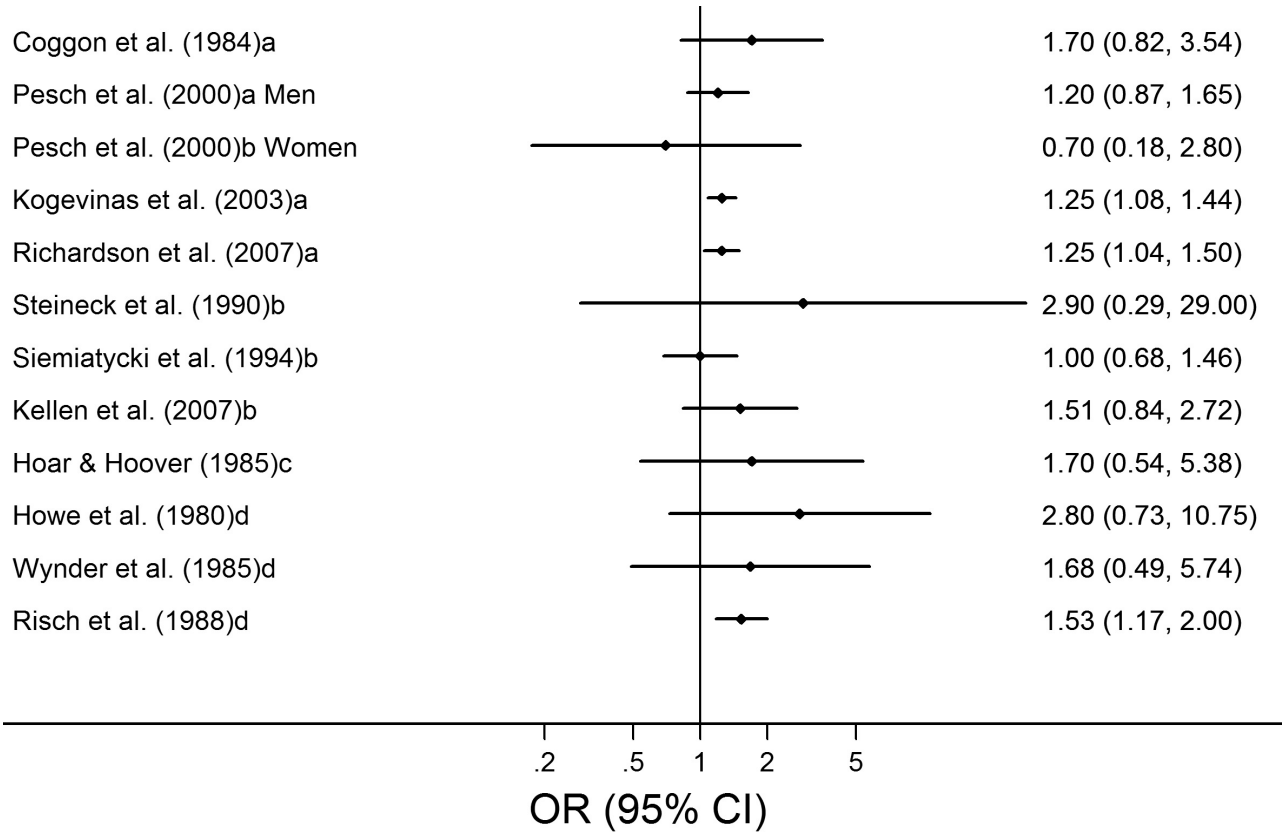
carcinogens showed similar results. Analysis of risk among never-smokers also showed an increased odds ratio in the highest quartile of cumulative exposure to diesel exhaust (OR, 1.26; 95% CI, 0.90–1.78). A positive and significant trend with duration of exposure was observed among those exposed to low ($P < 0.01$) and high levels ($P < 0.01$) of diesel motor exhaust. The risk estimates were similar for population-based and hospital-based case–control studies, and no significant heterogeneity for the effect of diesel motor exhaust in the highest quartile of cumulative exposure was found across studies ($P = 0.29$). [The Working Group noted that this study was based on a very large data set with detailed information on job histories and smoking habits. Exposure was coded in a standardized manner by the application of a JEM, although temporal changes in exposure were not taken into account. Although this study base partially overlapped with some earlier publications, these present findings were considered to be independent in so far as a new exposure assessment was used. The association between exposure to diesel motor exhaust and the risk for lung cancer reported here probably could not be explained by bias or confounding.]

Pintos *et al.* (2012) reported an expansion (Montreal II) of an earlier case–control study from the Montreal area, Canada (Siemiatycki *et al.*, 1987; Parent *et al.*, 2007; Montreal I), and covered the findings from Montreal I and Montreal II, as well as those from the pooled data set. Cancer cases were identified from area hospitals and population controls were recruited from electoral lists. Subjects or proxies were interviewed regarding lifetime occupational history, including data on work site and work tasks, and several other risk factors, including tobacco smoking. Exposure to diesel exhaust and several other occupational exposures were coded by expert assessment which was improved in relation to the method employed in the original study (Siemiatycki *et al.*, 1987). Odds ratios were adjusted for age, ethnicity, level of education, socioeconomic status, smoking, respondent status and occupational carcinogens. Study I (sampled in 1979–86) comprised 857 cases and 533 population controls; study II (sampled in 1996–2001) comprised 736 cases and 894 population controls, all of whom were men. An increased odds ratio of 1.34 (95% CI, 1.1–1.7) was found for subjects ever-exposed versus those never-exposed to diesel motor exhaust; for substantial cumulative exposure, the odds ratio was 1.80 (95% CI, 1.3–2.6). The findings were essentially similar for study I and study II when analysed separately. An analysis of risk in relation to histological type of lung cancer showed that the risks were more pronounced for squamous cell carcinomas (OR, 2.09; 95% CI, 1.3–3.2) for ever exposure to substantial levels of diesel exhaust but were lower for adenocarcinomas (OR, 1.17; 95% CI, 0.7–1.9). An intermediate risk was reported for small cell carcinomas (OR, 1.52; 95% CI, 0.8–2.7). A previous analysis based on the subjects in study I (Parent *et al.*, 2007) investigated the risk of lung cancer in relation to diesel exhaust and gasoline exhaust. No excess risk was found with exposure to gasoline exhaust. [The Working Group noted that this large study showed an increased risk of lung cancer after occupational exposure to diesel motor exhaust. A detailed exposure assessment and adjustment for potential confounders was applied, and the findings were internally consistent between the two sampling periods. The study showed a stronger effect for squamous cell carcinoma than for other histological types.]

2.3.2 Cancer of the urinary bladder

Numerous case–control studies that evaluated the risk for urinary bladder cancer and potential occupational exposure to diesel or motor exhaust were identified. The major limitation of the studies reviewed was the small number with well characterized exposure to

Fig. 2.1 Case–control studies of urinary bladder cancer with odds ratios for the highest exposure to diesel exhaust



a Job–exposure matrix
b Expert assessment
c Proxy
d Combined jobs
CI, confidence interval; OR, odds ratio

diesel or gasoline exhaust. A few studies used JEMs or experts to assess exposure, while others provided risk estimates for exposure to diesel exhaust for combined occupations based only on job titles or proxy exposure, and a large number of studies reported risk estimates for occupations that had potential for exposure to diesel exhaust. These studies are discussed in Section 2.3.2(a–c), and are summarized in Table 2.3. Odds ratios are plotted in Fig. 2.1. The use of expert or JEM assessment of exposure to diesel exhaust increased the probability of distinguishing exposed workers from unexposed workers, and these studies were most influential in the evaluation of risks for urinary bladder cancer. Studies that used job titles alone as a surrogate for exposure to diesel exhaust were less informative with regard to specific exposure to diesel or gasoline exhaust; however, some studies that provided a more focused analysis of HGV drivers were given more weight. Studies that reported risk estimates for specific occupations are described by occupation in Table 2.4, and the findings across studies are briefly discussed in Section 2.3.2(d). The odds ratios for urinary bladder cancer by occupational title are plotted in Fig. 2.2–2.7.

Table 2.3 Case–control studies of urinary bladder cancer and exposure to engine exhausts

Reference Location, period	Total No. of cases/ controls	Source of controls	Exposure assessment	Exposure categories	No. of exposed cases	Relative risk (95% CI)	Covariates Comments
Job–exposure matrix							
Coggon *et al.* (1984) England and Wales, 1975–79	291/638	Death certificates, including deaths from other cancers	JEM from occupational codes on death certificates	**Diesel fumes** All occupations (versus no exposure) Occupations with high exposure	 69 19	 1.0 (0.7–1.3) 1.7 (0.9–3.9)	Men under 40 yr, controls matched by date of birth; no information on tobacco smoking, only most recent full-time job on death certificate
Dolin & Cook-Mozaffari (1992) United Kingdom, 1965–80	2457	External controls	Death certificates, JEM to identify jobs with exposure to diesel exhaust	Occupations with low exposure	125	**SMR** 1.06 (0.88–1.26)	Age and degree of urbanization; men aged 25–64 yr; no information on tobacco smoking
Pesch *et al.* (2000) Germany, 1991–95	1035/4298	Population	In-person interview using structured questionnaire (JEM, JTEM)	**Exhaust JEM** *Men* Medium High Substantial *Women* Medium High Substantial **Self-assessed** *Men* Medium High Substantial	 157 173 57 21 18 2 38 74 19	**OR** 1.0 (0.8–1.3) 1.3 (1.0–1.6) 1.2 (0.9–1.7) 1.3 (0.7–2.2) 1.0 (0.6–1.8) 0.7 (0.2–3.2) 0.6 (0.4–0.9) 1.0 (0.8–1.3) 0.8 (0.5–1.4)	Urothelial cancer (urinary bladder: 90% men, 84% women); tobacco smoking, study centre and age; controls matched by region, sex and age ORs for women < 1.0, small number of exposed cases
Kogevinas *et al.* (2003) Europe, 1976–96	3346/6840 matched on age and geographical area	Population and hospital	JEM	Highest tertile of exposure to diesel exhaust (compared with unexposed)	NR	~1.25 (~1.05–1.4) estimated from graph	Age, tobacco smoking and study centre; pooled analysis of 13 studies; men aged 30–79 yr

Table 2.3 (continued)

Reference Location, period	Total No. of cases/ controls	Source of controls	Exposure assessment	Exposure categories	No. of exposed cases	Relative risk (95% CI)	Covariates Comments
Richardson *et al.* (2007) British Columbia, Canada, 1983–90	1062/8057	Other cancers except lung from cancer registry	NOES–JEM	**Diesel exhaust**			
				No cumulative exposure		1.0 (reference)	Tobacco smoking, alcohol consumption and questionnaire respondent; same cancer registry database as Band *et al.* (2005)
				Ever exposure	605	1.18 (0.95–1.37)	
				Cumulative exposure			
				Low	NR	1.14 (0.95–1.37)	
				Medium	NR	1.17 (0.97–1.40)	
				High	NR	1.25 (1.04–1.49)	
						P for trend = 0.01	
Expert assessment							
Steineck *et al.* (1990) Stockholm, Sweden, 1985–87	256/287	Population	Postal questionnaire and interview, expert classification of exposure to diesel or petrol exhaust	**Diesel exhausts**			Urothelial cancer and/or squamous cell carcinoma of lower urinary tract; yr of birth and tobacco smoking; men born 1911–45; participation rate: 80% cases, 79% controls
				Exposed	25	1.7 (0.9–3.3)	
				Annual dose			
				Low	NR	1.3 (0.6–3.1)	
				Moderate	NR	2.2 (0.7–6.6)	
				High	NR	2.9 (0.3–30.0)	
				Petrol exhausts			
				Ever exposed	24	1.0 (0.5–1.9)	
				Annual dose			
				Low	NR	0.6 (0.3–1.3)	
				Moderate	NR	1.4 (0.5–3.7)	
				High	NR	3.9 (0.4–35.5)	
				Diesel and petrol exhausts			Moderate/high annual dose
				None	247	1.0 (reference)	
				Diesel only	4	1.1 (0.3–4.3)	
				Petrol only	6	1.0 (0.3–3.4)	
				Both	7	7.1 (0.9–58.8)	Yr of birth and tobacco smoking
				Both	7	5.1 (0.6–43.6)	Yr of birth, tobacco smoking and benzene

Table 2.3 (continued)

Reference Location, period	Total No. of cases/ controls	Source of controls	Exposure assessment	Exposure categories	No. of exposed cases	Relative risk (95% CI)	Covariates Comments
Siemiatycki *et al.* (1994) Montreal area, Canada, 1979–86	484/1879 cancer and 533 population	Other cancers and population	In-person (some proxy) interview with detailed job history, semiquantitative exposure assessment by experts	**Diesel engine exhaust**			Age, ethnicity, smoking, SES, regular coffee consumption and respondent status (self or proxy)
				Non-substantial	46	1.3 (0.9–1.8)	
				Substantial	32	1.0 (0.7–1.5)	
				Confidence			
				Probable	28	1.0 (0.7–1.6)	
				Definite	50	1.2 (0.9–1.7)	
				Concentration			
				Low	27	1.2 (0.8–1.9)	
				Medium/high	51	1.1 (0.8–1.50	
				Frequency			
				Low	48	1.1 (0.8–1.5)	
				Medium/high	30	1.3 (0.8–1.9)	
				Duration (yr)			
				1–10	22	1.2 (0.7–1.9)	
				≥ 11	56	1.3 (0.8–1.5)	
Kellen *et al.* (2007) Belgium, 1999–2004	200/385	Population	Structured interview, expert assessment	**Diesel**	144	1.0 (reference)	Transitional cell carcinoma; age, sex and tobacco smoking; same population as Reulen *et al.* (2007)
				No exposure			
				Low	20	0.8 (0.4–1.57)	
				High	56	1.51 (0.85–2.75)	
						P for trend = 0.25	
				Ever exposure			Sex and age
				Never smoked	2	1.24 (0.30–5.03)	
				Ever smoked	54	1.34(0.83–2.16)	
Self-reported assessment							
Howe *et al.* (1980) Canada, 1974–76	480 men, 152 women/480 men, 152 women	Population	In-person interviews; occupational history coded according to the 1971 Canadian census, more details collected on a-priori suspect industries, exposure to fumes, dust or chemicals	Diesel and traffic fumes	11	2.8 (0.8–11.8)	Work in a-priori suspect industries considered; control matched by sex, province and age
				Railroad workers	9	9.0 (1.2–394.5)	

Table 2.3 (continued)

Reference Location, period	Total No. of cases/ controls	Source of controls	Exposure assessment	Exposure categories	No. of exposed cases	Relative risk (95% CI)	Covariates Comments
Hoar & Hoover (1985) New England, USA, 1975–79	325/673	Population	In-person interviews with next of kin; lifetime occupation	**Exposure to diesel, any occupation**		**OR**	Coffee drinking, tobacco smoking and education considered but did not change crude OR in duration analysis; controls matched on state, sex, age, race, yr of death, county or residence and age at death
				Never	197	1.0 (reference)	
				Ever	26	1.5 (0.8–2.8)	
				1–19 yr	5	0.9 (0.3–2.8)	
				20–29 yr	5	2.1 (0.5–8.6)	
				30–39 yr	6	3.2 (0.8–13.7)	
				> 40 yr	7	1.7 (0.5–5.0)	
						P for trend = 0.024	
Risch *et al.* (1988) Canada, 1979–82	826/792	Population	In-person interview using structured questionnaire; occupational history	**Occupation/industry: job entailing contact with diesel or traffic fumes**			Control for lifetime tobacco smoking; other factors considered include SES, marital status, educational group, urban versus rural residence and birth place; controls matched by birth yr, age and area of residence; response rate: 67% cases, 53% controls
				Men	309		
				Ever employed		1.53 (1.17–2·00)	
				Employed 8–28 yr before diagnosis		1.69 (1.24–2.31)	
				Trend with duration		1.23 (1.08–1.41)	
				Women	19		
				Ever employed	NR	0.62 (0.23–1.57)	
				Employed 8–28 yr in past	NR	0.49 (0.10–2.11)	
				Trend with duration		0.83 (0.27–2·50)	
Wynder *et al.* (1985) USA (American Health Foundation Study), 1981–83	194/582	Hospital; men with non-tobacco-related diseases	Interview with structural questionnaire, exposure to diesel exhaust classified by: (1) jobs with probable exposure, (2) high, moderate or minimal occupations by % of workers exposed to diesel exhaust	No exposure to diesel exhaust	178	1.0 (reference)	Age, smoking habit and SES; controls matched by age, race, yr of interview and hospital (% of workers exposed to diesel exhaust)
				Probable exposure			
				Minimal (< 10%)	189	1.0 (reference)	
				Moderate (10–19%)	1	0.16	
				High (≥ 20%)	4	1.68 (0.49–5.73)	

Table 2.3 (continued)

Reference Location, period	Total No. of cases/ controls	Source of controls	Exposure assessment	Exposure categories	No. of exposed cases	Relative risk (95% CI)	Covariates Comments
Iyer *et al.* (1990) USA (American Health Foundation Study), NR	136/272	Hospital; non-tobacco-related diseases	Lifetime and self-reported occupational history	No exposure to diesel	95	1.0 (reference)	Primary cancer; educational status and tobacco smoking; controls matched by age, race, sex, yr of interview and hospital
				Any exposure to diesel (possible, probable or self-reported)	41	1.24 (0.77–2.00)	
				Main occupation with exposure to diesel			
				Possible	19	1.11 (0.6–2.08)	
				Probable	13	0.86 (0.41–1.81)	

CI, confidence interval; JEM, job–exposure matrix; JTEM, job-task–exposure matrix; NOES, National Occupational Exposure Survey; NR, not reported; OR, odds ratio; SES, socioeconomic status; SMR, standardized mortality ratio; yr, year

Table 2.4 Case–control studies of urinary bladder cancer and exposure to engine exhaust estimated from job title

Reference Location, period	Total No. of cases/ controls	Source of controls	Exposure assessment	Exposure categories	No. of exposed cases	Relative risk (95% CI)	Covariates Comments
Steenland *et al.* (1987) Ohio, USA, 1960–82	731/1275	Death certificates	Occupation and industry from death certificates and city directories	**Occupation as HGV driver**			Mortality study
				< 20 years	49	1.06 (0.89–1.26)	
				> 20 years	1	12.00 (2.29–62.9)	
				Railroad industry			
				< 20 years	10	0.42 (0.23–0.78)	
				> 20 years	19	2.21 (1.21–4.03)	
Siemiatycki *et al.* (1994) Montreal area, Canada, 1979–86	484/1879 cancer and 533 population	Other cancers except lung and kidney and population	Interview with detailed job history, semiquantitative exposure assessment by experts	**Occupation as HGV driver** *Exposure duration*			Age, ethnicity, tobacco smoking, SES, regular coffee consumption and respondent (self or proxy)
				< 10 yr	25	1.1 (0.7–1.8)	
				≥ 10 yr	26	1.2 (0.8–1.9)	
Colt *et al.* (2004) New Hampshire, USA, 1994–98	424/645	Population	In-person interview, occupational history, SOC codes	Male tractor-trailer drivers	47	2.4 (1.4–4.1)	Primary cancers, aged 25–74 yr, identified from cancer registry; age and tobacco smoking
				< 5 yr	19	1.5 (0.8–3.1)	
				≥ 5 yr	28	4.0 (1.8–8.7)	
						P for trend, 0.0003	
				HGV drivers	3	0.8 (0.2–3.5)	
				LGV drivers	27	1.3 (0.7–2.3)	
				Bus drivers	5	0.5 (0.2–1.6)	
				Taxicab drivers/ chauffeurs	8	0.8 (0.3–2.3)	
				Female auto mechanics	17	Reported as < 1.3 (NS)	
				Garage or gas station workers	19	1.7 (0.8–3.4)	
				< 5 yr	14	1.4 (0.6–2.9)	
				≥ 5 yr	5	6.3 (0.7–54.8)	
						P for trend, 0.07	

Table 2.4 (continued)

Reference Location, period	Total No. of cases/ controls	Source of controls	Exposure assessment	Exposure categories	No. of exposed cases	Relative risk (95% CI)	Covariates Comments
Colt *et al.* (2011) New England, USA, 2001–04	1158/1402	Population	In-person interview, occupational history, SOC codes	**Men only** Tractor-trailer drivers	54	0.9 (0.6–1.3)	Cases identified from hospital and state registries and records, including carcinoma *in situ*; age, race, ethnicity, tobacco smoking and employment in other high-risk occupations; participation rate, 65%
				HGV drivers	57	1.3 (0.9–2.0)	
				LGV drivers	43	0.8 (0.5–1.2)	
				Taxicab drivers/ chauffeurs	23	1.1 (0.6–2.0)	
				Vehicle/mobile equipment mechanics/repairers	119	1.5 (1.1–2.0)	
				Automobile mechanics	59	1.6 (1.05–2.4)	
				< 5 yr	25	1.4 (0.7–2.5)	
				5– < 15 yr	17	1.6 (0.7 –3.4)	
				≥ 15 yr	17	2.1 (0.98–4.6)	
						P for trend, 0.03	
Dolin & Cook-Mozaffari (1992) United Kingdom, 1965–80	2457	External	Death certificates; JEM to identify jobs with exposure to diesel exhaust			**SMR**	Death certificate study; no information on tobacco smoking
				HGV driver	92	1.08 (0.88–1.32)	
				HGV driver's mate	1	0.95 (0.03–5.31)	
				Railroad industry	74	2.22 (1.77–2.79)	
				Railway engine driver	13	1.61 (0.85–2.75)	
				Railway shunter	4	1.49 (0.40–3.82)	
				Railway signallman	5	1.46 (0.47–3.39)	
				Railway guard	4	2.58 (0.70–6.61)	
				Railway lengthman	11	1.47 (0.73–2.63)	
				Bus driver	14	0.81 (0.44–1.36)	
				Taxi driver	18	1.24 (0.73–1.36)	
Coggon *et al.* (1986) United Kingdom, 1975–80	179/NR	Hospital pathology and cancer registry files; other cancers	Postal questionnaire	HGV drivers (ever versus never employed)	NR	1.6 (1.0–2.4)	Urinary bladder and renal pelvis; age, tobacco smoking, county and source of interview; men aged 18–54 yr

Table 2.4 (continued)

Reference Location, period	Total No. of cases/ controls	Source of controls	Exposure assessment	Exposure categories	No. of exposed cases	Relative risk (95% CI)	Covariates Comments
Silverman *et al.* (1986) USA, 1977–78 (National Bladder Cancer Study)	1909/3569 (white men)	Population	Interviewer-administered questionnaire	**Truck driver or delivery man**			Age and tobacco smoking; controls matched for age and geographic location; response rate: 75% cases, 83–84% controls
				Usual	99	1.5 (1.1–2.0)	
				Ever	488	1.3 (1.1–1.4)	
				Duration of employment (yr)			
				< 5	208	1.1 (NR)	
				5–9	102	1.3 (NR)	
				10–14	58	1.7 (NR)	
				15–24	59	2.2 (NR)	
				≥ 25	54	1.1 (NR)	
						P for trend, < 0.001	
				Duration of employment (yr) among drivers first employed > 50 yr before diagnosis			
				5–9	74	1.2 (NR)	
				10–14	32	1.4 (NR)	
				15–24	33	2.1 (NR)	
				≥ 25	22	2.2 (NR)	
						P for trend, < 0.0001	
				Bus driver			
				Usual	9	1.5 (0.6–3.9)	
				Ever	49	1.3 (0.9–1.9)	
				Duration of employment (yr)			
				< 5	21	1.3	
				5–9	11	1.2	
				≥ 10	16	1.3	
						P for trend, 0.2	
				Taxicab driver/chauffeur			
				Usual	10	6.3 (1.6–29.3)	
				Ever	77	1.6 (1.2–2.2)	
				Duration of employment (yr)			
				< 5	44	1.9	
				5–9	14	1.0	
				≥ 10	16	2.0	

Table 2.4 (continued)

Reference Location, period	Total No. of cases/ controls	Source of controls	Exposure assessment	Exposure categories	No. of exposed cases	Relative risk (95% CI)	Covariates Comments
Iyer *et al.* (1990) USA (American Health Foundation Study), NR	136/272	Hospital; non-tobacco-related diseases	Lifetime and self-reported occupational history	HGV driver	4	0.48 (0.15–1.56)	Primary cancer; educational status and tobacco smoking; controls matched by age, race, sex, yr of interview and hospital
Hoar & Hoover (1985) New England, USA, 1975–79	325/673	Population	In-person interviews with next of kin; lifetime occupation	**HGV driver**		**OR**	Coffee drinking, tobacco smoking and education considered but did not change crude OR in duration analysis; controls matched on state, sex, age, race, yr of death, county of residence and age at death
				Ever	35	1.5 (0.9–2.6),	
				1–4 yr	9	1.4 (0.6,3.3)	
				5–9 yr	12	2.9 (1.2–6.7)	
				> 10 yr	11	1.8 (0.8–4.1)	
						P for trend, 0.006	
				Reported exposure to diesel fuel	NR	1.8 (0.5–7.0)	
				Not reported exposure to diesel fuel	NR	1.5 (0.8–2.7)	
				Calendar year first employed			
				< 1929	10	1.2 (0.5–2.6)	
				1930–49	18	2.6 (1.3–5.1)	
				> 1950	6	1.4 (0.5–4.1	

Table 2.4 (continued)

Reference Location, period	Total No. of cases/ controls	Source of controls	Exposure assessment	Exposure categories	No. of exposed cases	Relative risk (95% CI)	Covariates Comments
Silverman *et al.* (1983) Detroit, USA, 1977–78 (National Bladder Cancer Study)	303/296	Population	Interviewer-administered questionnaire; details on HGV driving	HGV driver	42	2.1 (NR)	Lower urinary tract (95% bladder); age and tobacco smoking; white men; age-matched controls
				Duration of employment (yr)			
				< 10	23	1.4 (NR)	
				≥ 10	16	5.5 (NR)	
						P for trend, 0.004	
				Yr employment started			
				1910–29	8	1.6 (NR)	
				1930–39	12	2.0 (NR)	
				1940–49	10	1.5 (NR)	
				1950–69	7	6.5 (NR)	
						P for trend, 0.03	
				Ever operated a vehicle with diesel engine			
				No	21	1.4 (0.7–2.9)	
				Yes	13	11.9 (2.3–61.1)	One exposed control
						P for trend, 0.014	
Brownson *et al.* (1987) Missouri, USA, 1984–86	823 white men/2469	Cancer registry; other patients without smoking-related diseases	Registry records, occupation coded using 1980 US census code	**Occupation**	18	**OR**	Age, tobacco smoking and alcohol use; age-matched controls
				HGV drivers		1.2 (0.7–2.1)	
				Industry	17	1.2 (0.7–2.2)	
				Transport service			
				Railroads	18	1.3 (0.7–2.3)	
Kogevinas *et al.* (2003) Europe, 1976–96	3346/6840	Population and hospital	Occupational history, job codes	**Occupations**			Age, tobacco smoking and study centre; controls never employed in a-priori suspect occupation
				Railway engine drivers/ firemen	34	1.41(0.87–2.28)	
				Railway brakemen, signallmen and shunters	18	1.43(0.77–2.63)	
				Motor vehicle mechanics	108	1.16(0.90–1.50)	
				Automobile mechanics	78	1.38(1.02–1.87)	

Table 2.4 (continued)

Reference Location, period	Total No. of cases/ controls	Source of controls	Exposure assessment	Exposure categories	No. of exposed cases	Relative risk (95% CI)	Covariates Comments
Band *et al.* (2005) British Columbia, Canada, 1983–90	1129/14 334	Cancer registry; other cancers except lung	Self-administered questionnaire, SOC and SIC codes	**Usual occupation**			Smoking (yr), alcohol consumption and proxy response; controls matched on age and yr of diagnosis; 65% response rate
				HGV driver			
				Usual	44	1.21 (0.92–1.60)	
				Ever	145	1.20 (1.02–1.41)	
				Railroad transport			
				Usual	11	1.01 (0.59–1.75)	
				Ever	26	1.11 (0.77–1.59)	
				Locomotive operations			
				Usual	8	1.84 (0.92–3.68)	
				Ever	13	1.26 (0.75–2.12)	
				Taxi drivers/chauffeurs			
				Usual	6	1.82 (0.85–3.90)	
				Ever	27	1.17 (0.82–1.66)	
				Motor vehicle mechanics (8581)			
				Usual	29	1.37 (0.97–1.94)	
				Ever	75	1.49 (1.20–1.86)	
				Usual industry			
				HGV transport			
				Usual	33	1.38 (0.99–1.99)	
				Ever	92	1.28 (1.05–1.56)	
				Rail transport			
				Usual	28	0.83 (0.59–1.17)	
				Ever	71	0.93 (0.74–1.15)	
				Taxicab service			
				Usual	6	1.94 (0.90–4.20)	
				Ever	22	1.02 (0.70–1.51)	
				Gasoline service			
				Usual	6	1.69 (0.79–3.61)	
				Ever	38	1.75 (1.28–2.38)	

Table 2.4 (continued)

Reference Location, period	Total No. of cases/ controls	Source of controls	Exposure assessment	Exposure categories	No. of exposed cases	Relative risk (95% CI)	Covariates Comments
Zheng *et al.* (2002) Iowa, USA, 1986–89	1452/2434	Population	Postal questionnaire (some telephone interviews)	**Occupation**			*In situ* and invasive cancers; age, tobacco smoking (lifetime) and first-degree relative with bladder cancer; cases identified from state registry; controls matched by gender and age
				Automobile mechanic			
				All	44	1.6 (1.0–2.6)	
				Duration of employment			
				< 10 yr	8	1.4 (0.5–3.7	
				≥ 10 yr	36	1.7 (1.0–2.8)	
				Garage/service station–related			
				All	27	1.7 (0.9–3.1)	
				Duration of employment			
				< 10 yr	10	1.8 (0.7–4.8)	
				≥ 10 yr	17	1.6 (0.8–3.5)	
				Supervisors: transport and material moving			
				All	11	6.5 (1.4–29.9)	
				Duration of employment			
				< 10 yr	1	NR	
				≥ 10 yr	10	6.0 (1.3–28.2)	
				Material-moving equipment operators			
				All	26	1.9 (1.0–3.6)	
				Duration of employment			
				< 10 yr	6	1.9 (0.5–7.3)	
				≥ 10 yr	20	1.9 (0.9–3.9)	
				Industries			
				Automotive dealer/service station			
				All	54	1.6 (1.0–2.4)	
				Duration of employment			
				< 10 yr	15	1.4 (0.7–3.0)	
				≥ 10 yr	39	1.7 (1.0–2.7)	
				Railroad transport (40)			
				All	33	1.4 (0.8–2.3)	
				Duration of employment			
				< 10 yr	4	0.6 (0.2–2.0)	
				≥ 10 yr	29	1.7 (1.0–3.1)	

Table 2.4 (continued)

Reference Location, period	Total No. of cases/ controls	Source of controls	Exposure assessment	Exposure categories	No. of exposed cases	Relative risk (95% CI)	Covariates Comments
Samanic *et al.* (2008) Spain, 1988–2000	1159/1231	Hospital	In-person interview, SOC and SIC codes	**Occupation**			White cases of carcinoma or carcinoma *in situ*; age, hospital region, tobacco smoking and ever employed in high-risk occupation; controls matched by age at diagnosis/interview, sex, race/ethnicity and hospital
				HGV driver			
				Overall	89	0.91 (0.67–1.25)	
				0– < 10 yr	41	0.83 (0.50–1.36)	
				≥ 10 yr	56	0.97 (0.66–1.43)	
				Railroad transport			
				Overall	11	1.04 (0.40–2.69)	
				0– < 10 yr	4	0.62 (0.15–2.55)	
				≥ 10 yr	7	1.56 (0.43–5.70)	
				Bus driver			
				Overall	11	0.75 (0.32–1.73)	
				0– < 10 yr	8	1.42 (0.47–4.34)	
				≥ 10 yr	3	0.31 (0.08–1.24)	
				Taxi driver			
				Overall	37	1.14 (0.69–1.90)	
				0– < 10 yr	16	0.74 (0.35–1.54)	
				≥ 10 yr	23	1.68 (0.83–3.37)	
				Industry			
				Local urban transport			
				Overall	33	0.67 (0.42–1.10)	
				0– < 10 yr	15	0.72 (0.35–1.48)	
				≥ 10 yr	18	0.64 (0.34–1.22)	
				Auto repair services/garages			
				Overall	55	1.21 (0.81–1.81)	
				0– < 10 yr	29	1.32 (0.75–2.35)	
				≥ 10 yr	26	1.11 (0.64–1.94)	
Risch *et al.* (1988) Canada, 1979–82	826/792	Population	In-person interview using structured questionnaire; occupational history	**Railway occupations**			Lifetime tobacco smoking; other factors considered included SES, marital status, educational group, urban versus rural residence and birth place; controls matched by age and area of residence; response rate: 67% cases, 53% controls
				Ever employed	113	1.07 (0·71–1.61)	
				Employed 8–28 yr in past	NR	1.17 (0.65–2·12)	
				Trend with 10 years duration	NR	1·09 (0.87–1.36)	

Table 2.4 (continued)

Reference Location, period	Total No. of cases/ controls	Source of controls	Exposure assessment	Exposure categories	No. of exposed cases	Relative risk (95% CI)	Covariates Comments
Burns & Swanson (1991) Detroit, USA, 1973–91	2160/3979	Cancer registry; colon cancers	Telephone interview with subject or surrogate; occupations coded by duties rather than job title	**Usual occupation** Railroad worker **Usual industry** Railroads Automobile repair	 12 12	 0.7 (0.2–2.4) 0.7 (0.3–1.4) 0.5 (0.2–1.1)	Cigarette smoking, gender, race and age at diagnosis; white and black men and women; response: 94% cases, 95% controls
Decouflé *et al.* (1977) USA, 1956–65	6434 men/ NR	Hospital non-cancer	Structured questionnaire	Ever employed as HGV driver Locomotive engineers/ firemen Bus driver Taxicab drivers and chauffeurs	26 8 4 5	1.66 ($P > 0.05$) 1.65 ($P > 0.05$) 2.78 (NR) 1.70 ($P > 0.05$)	Age; clerical workers were the unexposed referent group
Dryson *et al.* (2008) New Zealand, 2003–04	213/471	Population	In-person interview	HGV or tanker drivers Bus drivers Car, taxi and light van operators Road freight transport industry	16 7 12 12	1.36 (0.60–5.09) 1.69 (0.55–5.26) 0.73 (0.33–1.60) 1.65 (0.61–4.47)	Cases (aged 25–70 yr) identified from cancer registry; age, gender, Maori ethnicity and tobacco smoking; SES semi-Bayes methods adjusted for multiple comparisons; response rate: 64% cases, 48% controls
Silverman *et al.* (1989a) USA, 1977–78 (National Bladder Cancer Study)	2100/3874 (white men)	Population	Interviewer-administered questionnaire	Railroad worker Auto mechanic (HGV transport industry)	57 11	1.3 (0.9–2.0) 10.2 (2.1–68.6)	Same population as Silverman *et al.* (1986); tobacco smoking; controls matched by age
Silverman *et al.* (1989b) USA 1977–78 (National Bladder Cancer Study)	126/383 (non-white men)	Population	Interviewer-administered questionnaire	Taxicab driver/ chauffeur Auto mechanic Garage worker/gas pump attendant	10 6 6	1.3 (0.5–3.2) 1.4 (0.4–4.4) 1.6 (0.5–4.5)	Population included in Smith *et al.* (1985); tobacco smoking and high-risk occupations

Table 2.4 (continued)

Reference Location, period	Total No. of cases/ controls	Source of controls	Exposure assessment	Exposure categories	No. of exposed cases	Relative risk (95% CI)	Covariates Comments
Schifflers *et al.* (1987) Belgium, 1984–85	74/203	Population	In-person interviews using standard form, analysis on jobs considered a-priori as hazardous	HGV/engine drivers	10	2.15 (0.88–5.29)	Tobacco use; controls matched for yr of birth and sex
Brooks *et al.* (1992) Missouri, USA, 1984–88	1415/NR	Cancer registry; case/case analysis	Registry records			**OR**	Age and tobacco smoking; same population as Brownson *et al.* (1987); ORs for high-grade tumours
				Truck driver	14	2.7	
				Vehicle mechanic	5	1.2	
Gaertner *et al.* (2004) Canada, 1994–97	887/2847	Population	Mailed questionnaires	**Men only**			Cases identified from cancer registry; age, race, tobacco smoking, province, several dietary factors and employment in suspect industries
				HGV driver	68	1.23 (0.88–1.75)	
				> 1–5 yr	19	1.14 (0.63–2.04)	
				> 5–15 yr	16	1.50 (0.73–3.10)	
				> 15 yr	33	1.19 (0.74–1.91)	
						P for trend, 0.25	
				Auto mechanic	36	1.69 (1.02–2.82)	
				> 1–5 yr	15	1.37 (0.66–2.83)	
				> 5–15	9	1.93 (0.76–4.88)	
				> 15	12	2.48 (0.97–6.34)	
						P for trend, 0.01	
				Gas station attendant	13	0.65 (0.33–1.32)	
Reulen *et al.* (2007) Belgium, 1996–2004	202/390	Population	In-person interviews	Male motor vehicle mechanics	5	0.6 (0.2–1.9)	Men and women (aged 40–96 yr) with transitional cell carcinoma identified by cancer registry (same population as Kellen *et al.*, 2007); age, sex, tobacco smoking and educational status; very low response rate: 26% controls, 9% cases

Table 2.4 (continued)

Reference Location, period	Total No. of cases/ controls	Source of controls	Exposure assessment	Exposure categories	No. of exposed cases	Relative risk (95% CI)	Covariates Comments
Smith *et al.* (1985) USA, 1977–78 (National Bladder Cancer Study)	2108/4046 (black and white men)	Population	Interview with structured questionnaire	**Auto and HGV mechanics**			Age, education and coffee consumption; controls: unexposed, never worked in occupations with similar exposures as mechanics or with suspected chemical exposures
				Smokers	NR	1.21 (0.90–1.63)	
				Duration (yr)			
				1–5	NR	1.53 (1.07- 2.20)	
				6–10	NR	0.74 (0.43–1.28)	
				11–20	NR	1.13 (0.67–1.92)	
				21–29	NR	2.77 (1.34- 5.71)	
				≥ 30	NR	1.19 (0.66–2.13)	
				Nonsmokers	NR	1.33 (0.77–2.31)	
				Duration (yr)			
				1–5	NR	1.48 (0.73–2.75)	
				6–10	NR	1.29 (0.54–3.11)	
				11–20	NR	0.73 (0.21–2.53)	
				21–29	NR	0.51 (0.06–4.11)	
				≥ 30	NR	2.13 (0.78–5.80)	
Schoenberg *et al.* (1984) New Jersey, USA, 1978–79	658/1258	Population	In-person interviews using structured questionnaires	Taxicab drivers	25	1.36 (0.79–2.34)	White men (aged 21–84 yr) with carcinoma; age and duration of smoking; part of the in the National Bladder Cancer Study
				Motor vehicle mechanics	55	1.26 (0.87–1.84)	
				Duration (yr)			
				< 2	NR	2.96 ($P < 0.05$)	
				2–4.9	NR	0.82	
				5–9.9	NR	1.34	
				10–19.9	NR	0.77	

Table 2.4 (continued)

Reference Location, period	Total No. of cases/ controls	Source of controls	Exposure assessment	Exposure categories	No. of exposed cases	Relative risk (95% CI)	Covariates Comments
Schoenberg et al. (1984) New Jersey, USA, 1978–79 (cont.)				≥ 20	NR	2.31	
						P for trend, 0.63	
				Garage and/or gas station workers	43	2.35 (1.47–3.78)	
				Age at first exposure (yr)			
				≤ 25	NR	2.15 ($P < 0.05$)	
				26–40	< 5	5.44	
				≥ 41	< 5	4.75	
						P for trend, 0.25	
				Latency (yr)			
				< 20	NR	3.06 ($P < 0.05$)	
				20–39	NR	3.16 ($P < 0.05$)	
				≥ 40	NR	1.66	
						P for trend = 0.29	
				Duration (yr)			
				< 2	NR	1.58	
				2–4.9	NR	5.02 ($P < 0.05$)	
				5–9.9	NR	4.08	
				≥ 20	NR	0.63	
						P for trend, 0.45	
Cassidy *et al.* (2009) Texas, USA, 1999–NR	604/604	Hospital; non-cancer	Interviews with structured questionnaire; OCC and SIC codes	**Occupations**	37	1.43 (0.81–2.53)	Age, sex and tobacco smoking; controls matched to cases by age, gender and ethnicity; participation rate: 92% cases, 75% controls
				Motor freight			
				< 10 yr	22	1.49 (0.72–3.11)	
				≥ 10 yr	15	1.30 (0.57–3.01)	
				Automobile mechanic	23	1.05 (0.54–2.04)	
				< 10 yr	13	1.26 (0.50–3.14)	
				≥ 10 yr	10	0.87 (0.34–2.23)	

Table 2.4 (continued)

Reference Location, period	Total No. of cases/ controls	Source of controls	Exposure assessment	Exposure categories	No. of exposed cases	Relative risk (95% CI)	Covariates Comments
Cassidy et al. (2009) Texas, USA, 1999–NR (cont.)				**Industry**	36	1.17 (0.66–2.07)	
				Motor freight transportation/ warehousing			
				< 10 yr	18	1.18 (0.54–2.60)	
				≥ 10 yr	18	1.16 (0.52–2.58)	
				Railroad transport	13	1.46 (0.56–3.75)	
				< 10 yr	7	1.39 (0.40–4.87)	
				≥ 10 yr	6	1.54 (0.37–6.42)	
				Automotive repair, services/ parking	19	0.80 (0.40–1.61)	
				< 10 yr	8	0.65 (0.24–1.73)	
				≥ 10 yr	11	0.99 (0.37–2.67)	
				Automotive dealers/ gasoline service station	37	0.98 (0.58–1.64)	
				< 10 yr	22	0.81 (0.43–1.53)	
				≥ 10 yr	15	1.47 (0.59–3.63)	
Steineck *et al.* (1990) Stockholm, Sweden, 1985–87	256/287 (men)	Population	Postal questionnaire and interview, expert classification exposure to diesel or petrol exhaust	**Occupation**			Urothelial cancer and/or squamous cell carcinoma in the lower urinary tract; yr of birth and tobacco smoking; born 1911–45; participation rate: 80% cases, 79% controls
				Petrol station/auto repair	17	1.2 (0.6–2.8)	
				Railroad	8	0.4 (0.2–1.0)	
Wynder *et al.* (1985) USA, six cities, 1981–83	194/582	Hospital; non-tobacco-related diseases	Interview with structured questionnaire; a-priori high-exposure jobs and percentage of workers exposed	Railroad worker	2	2.0 (0.34–11.61)	Men only; age, tobacco smoking and SES; controls matched by age, race, yr of interview and hospital
				Bus/HGV driver	10	0.9 (0.44 –1.87)	
				Heavy equipment mechanic	2	0.75 (0.16–3.53)	
				Warehouse materials handler	2	0.85 (0.18–4.14)	

CI, confidence interval; HGV, heavy goods vehicle; JEM, job–exposure matrix; LGV, light goods vehicle; NR, not reported; NS, not significant; OCC, occupational codes; OR, odds ratio; SES, socioeconomic status; SIC, standard industrial classification; SMR, standardized mortality ratio; SOC, standard occupational classification; yr, year

(a) Studies that used job–exposure matrices to assess exposure to diesel exhaust

Two death certificate studies, a case–control study (Coggon *et al.*, 1984) and a record linkage study (Dolin & Cook-Mozaffari, 1992) were identified that used a crude JEM to identify certain occupations and industries that entailed exposure to diesel exhaust. The study by Coggon *et al.* (1984), described previously in Section 2.3.1, included 291 men who died from urinary bladder cancer (underlying cause of death) and 638 aged-matched controls identified from death certificates collected in England and Wales between 1975 and 1979 for all men aged 40 years or younger. The odds ratios for exposure to diesel exhaust were 1.0 (95% CI, 0.7–1.3; 68 exposed cases) for all occupations and 1.7 (95% CI, 0.9–3.3; 19 exposed cases) for occupations with high exposure. Dolin & Cook-Mozaffari (1992) reported findings for 2457 men, aged 25–64 years, who died from urinary bladder cancer during 1965–80 in selected regions in England and Wales. The standardized mortality ratio for exposure to 'diesel fuel/fumes' in occupations in which most workers had low exposure was 1.06 (95% CI, 0.88–1.26; 125 deaths). No occupations were considered to have high exposure to diesel fuel or fumes. [The Working Group noted that, although Dolin & Cook-Mozaffari (1992) used the term 'diesel fuel/fumes', the occupations identified by the JEM (HGV, bus and taxi drivers and HGV drivers' mates) were those associated with exposure to diesel exhaust. The preceding two studies were not very informative for an evaluation of cancer risk because of inaccuracies and incompleteness of occupational history on the death certificates (on which only the most recent full-time occupation was listed) and the lack of information on tobacco smoking or other potential confounders. In the study of Coggon *et al.* (1984), the subjects were young and thus the latency period was inadequate to evaluate cancer risk.]

A multicentre population-based case–control study was conducted between 1991 and 1995 in Germany to evaluate risks from specific occupational exposures (Pesch *et al.*, 2000). The study population consisted of 1035 incident cases of urothelial cancer (predominantly of the urinary bladder) and 4298 controls randomly selected from resident registries and matched to cases by region, sex and age. Information on exposures and other risk factors was obtained by in-person interviews using structured questionnaires with lists of specific agents. Exposure to specific substances was assessed using a JEM and a job-task–exposure matrix, which assigned probability and intensity of exposure to each job title or task. Among men, the odds ratio (adjusted for age, tobacco smoking and study centre) for the association of bladder cancer with exposure to 'exhaust' (as classified by the JEM) was 1.3 (95% CI, 1.0–1.6) for high exposure and 1.2 (95% CI, 0.9–1.7) for substantial exposure. No excess risk of urinary bladder cancer was found for self-assessed exposure to 'traffic exhaust' among men or women or for JEM-assessed exposure to exhaust in women. [The Working Group noted that this study did not distinguish between exposure to gasoline or other types of exhaust and exposure to diesel exhaust. Documentation on the source of identification of the cases was incomplete, and it was unclear whether case ascertainment was complete for the five geographical areas.]

Kogevinas *et al.* (2003) combined data from 11 case–control studies of urinary bladder cancer conducted between 1976 and 1999 in six European Countries, including three studies from Germany (Claude *et al.*, 1988; Pohlabeln *et al.*, 1999; Pesch *et al.*, 2000), two studies each from France (Cordier *et al.*, 1993; Hours *et al.*, 1994), Italy (Vineis & Magnani, 1985; Porru *et al.*, 1996) and Spain (González *et al.*, 1989; Serra *et al.*, 2000), and one study each from Greece (Rebelakos *et al.*, 1985) and Denmark (Jensen *et al.*, 1987). With the exceptions of Jensen *et al.*, (1987), which provided additional information on

duration of employment as an HGV driver, and Pesch *et al.* (2000), which used a different JEM to assess exposure to exhaust, these studies were not reviewed independently because their findings were captured in the pooled analyses. The analysis included 3346 male incident cases and 6840 male controls, aged 30–79 years. Controls from populations and hospitals were matched to the cases on age and geographical area. Information on every job held for more than 6 months was available from 10 studies and for the longest-held job from one study. The FINJEM was applied to evaluate exposure to diesel exhaust. Risk estimates were calculated by job duration, calendar year of first employment and age at diagnosis for all occupations with at least 10 subjects; individuals never employed in the occupation or any of the a-priori defined high-risk occupations were used as the unexposed population. Odds ratios were adjusted for age, tobacco smoking and study centre. Associations with diesel exhaust were presented in graphical form (Fig. 2.1), which showed an odds ratio of approximately 1.25 (95% CI, ~1.05–1.4) for urinary bladder cancer among subjects with the highest tertile of exposure to diesel exhaust compared with the unexposed; odds ratios were less than 1.0 for medium and low exposure to diesel exhaust. [The Working Group noted that this was one of the more informative case–control studies for evaluating the potential risk for urinary bladder cancer from exposure to diesel exhaust because it was large and used high-quality exposure assessment. The number of exposed subjects in each diesel exhaust exposure category was not reported. The study provided some evidence for an association between exposure to diesel exhaust and the risk of urinary bladder cancer.]

Richardson *et al.* (2007) investigated the risks of exposure to specific chemicals using the same cancer registry database as an earlier study in British Columbia, Canada, which found an association between several occupations with potential exposure to diesel engine emissions and an excess incidence of urinary bladder cancer (Band *et al.*, 2005). Self-administered questionnaires were completed by 15 463 men (response rate, 60.1%), aged 20 years or older, diagnosed with cancer from 1983 to 1990. Complete occupational histories were available for 1062 cases of urinary bladder cancer (94% transitional cell carcinoma) and 8057 cancer controls, matched to cases by year of birth and year of diagnosis. Exposure to specific chemical agents, classified by IARC as definite or probable urinary bladder carcinogens, was assessed using the National Occupational Exposure Survey JEM, which predicts the probability of exposure to a specific substance in a specific job, based on walk-through assessments in a stratified sample of workplaces within the USA during 1981–83. Odds ratios were estimated after matching for age and year of diagnosis and adjusting for ethnicity, years of smoking, alcohol consumption and questionnaire responder. The odds ratios for urinary bladder cancer for ever and high cumulative exposure to diesel exhaust were 1.18 (95% CI, 1.04–1.35; 604 exposed cases) and 1.25 (95% CI, 1.04–1.49), respectively; a significant exposure–response trend was observed for cumulative exposure (P for trend = 0.01). [The Working Group noted that the advantages of this study were the use of a JEM to assess exposure specific for diesel exhaust and adequate power to evaluate an exposure–response relationship; however, the exposure assessment was not calendar year-specific and was based on occupational data collected over a 3-year period in a different country. Despite its limitations, the study added some support for an association between urinary bladder cancer and exposure to diesel exhaust.]

(b) Studies that used expert assessments or self-reporting to estimate exposure to diesel exhaust

(i) Expert assessments

A population-based case–control study of 256 male incident cases of urothelial cancer and/or squamous cell carcinoma of the lower urinary tract and 287 controls, selected randomly from registers, was conducted in Stockholm, Sweden, during 1985–87 (Steineck *et al.*, 1990). Information on all occupations and industries for all jobs held was obtained by postal questionnaires and in person. Men were classified as being exposed or unexposed to 38 agents or groups of substances and were assigned to categories of annual dose (low, moderate or high) by an industrial hygienist who was blinded to case–control status. Exposure after 1981 was ignored in the analyses. An elevated risk was found for ever exposure to diesel exhaust (RR [adjusted for year of birth and smoking], 1.7; 95% CI, 0.9–3.3; 25 exposed cases); the risks increased with increasing annual dose, but the estimates were imprecise and not statistically significant, and no trend test was performed. A similar pattern of increasing risk with increasing annual dose was found for exposure to petrol exhausts. The joint effects from exposure to diesel and petrol exhausts were calculated (RR, 7.1; 95% CI, 0.9–58.8; seven exposed cases); the magnitude of the relative risk decreased to 5.1 (95% CI, 0.6–43.6) after further adjustment for exposure to benzene. [The Working Group noted that the advantages of this study were the use of experts to assess exposure to diesel and petrol (i.e. gasoline) exhausts using lifetime occupational data, including the exposure period; however, the assignment of annual dose of exposure to exhaust appeared to be mainly based on job title. The ability to evaluate exposure–response relationships was limited because of the relatively small numbers of exposed cases and the inability to distinguish reliably between exposure to diesel exhaust and exposure to gasoline exhaust.]

Siemiatycki *et al.* (1994) investigated occupational risk factors in a population-based case–control study in Montreal, Canada, comprising 484 men with histologically confirmed urinary bladder cancer, 1879 cancer controls (excluding lung cancer) and 533 population controls. The cases and cancer controls were identified from major hospitals between 1976 and 1986. Occupational histories, including a detailed description of lifetime jobs held by the subject, and information on potential confounders were obtained from in-person (82%) or proxy interviews. A team of experts and hygienists translated this information into exposure to specific substances and characterized suspected exposures for each job. Using a-priori criteria related to the certainty of exposure, exposure duration and frequency × concentration scores, individuals were classified into three exposure groups: no exposure, non-substantial exposure and substantial exposure; lifetime prevalence of exposure to diesel exhaust was estimated to be 15%. Odds ratios for urinary bladder cancer in relation to exposure to diesel exhaust, adjusted for age, family income, ethnicity, cumulative tobacco smoking index, coffee consumption and respondent status (self or proxy), were 1.3 (95% CI, 0.9–1.8) for non-substantial exposure and 1.0 (95% CI, 0.7–1.5) for substantial exposure to diesel engine emissions. Risk estimates, adjusted for non-occupational factors, ranged from 1.1 to 1.3 (statistically non-significant) among individuals in the highest categories of certainty, duration and frequency of exposure to diesel exhaust. An earlier report of this study population, which analysed 486 cases of urinary bladder cancer and 2196 cancer controls, did not find an association between the risk for urinary bladder cancer and any exposure to diesel exhaust (OR adjusted for age, socioeconomic status, ethnic group, cigarette smoking and blue-/white-collar job history, 1.0; 95% CI, 0.8–1.2; 82 exposed cases) or any

exposure to gasoline exhaust (adjusted OR, 1.0; 95% CI, 0.9–1.1; 208 exposed cases) (Siemiatycki *et al.*, 1988). [The Working Group noted that the 1994 study was more informative for the current evaluation because of the attempts made to evaluate exposure specific to diesel exhaust.]

A Belgian case–control study included 200 cases selected from the Limburg Cancer Registry, diagnosed between 1991 and 2004 with histologically confirmed transitional cell carcinoma of the urinary bladder, and 385 population controls (Kellen *et al.*, 2007). Lifetime occupational history was obtained from in-person interviews using a structured questionnaire. Two occupational hygienists, blinded to case–control status, assigned individuals into three categories (no, low and high) of probability of cumulative exposure to diesel exhaust. Compared with individuals with no exposure to diesel exhaust, the odds ratios, adjusted for tobacco smoking, age and sex, were 0.80 (95% CI, 0.40–1.57) for low and 1.51 (95% CI, 0.85–2.75) for high cumulative probability of exposure to diesel exhaust (*P* for trend = 0.25). Odds ratios were similar for smokers and nonsmokers and no effect modification was observed for several cytochrome P450 polymorphisms. [The Working Group noted that an advantage of this study was the use of detailed lifetime occupational information to calculate the probability of cumulative exposure. However, it was not clear whether the statistical power was adequate to evaluate exposure–response relationships; the Working Group also noted that only two cases of urinary bladder cancer who had never smoked were ever exposed to diesel exhaust.]

(ii) Self-reporting

Howe *et al.* (1980) conducted a population-based case–control study of urinary bladder cancer among 480 pairs of men and 180 pairs of women between 1974 and 1976, and age- and sex-matched controls from three regions in Canada. Subjects were interviewed in person to obtain information on lifestyle habits and occupational history, including duration of employment in every job, details on a-priori suspected occupations and ever exposure to fumes, dusts or specific substances. The odds ratio was 2.8 (95% CI, 0.8–11.8; 11 exposed cases) for ever exposure to diesel and traffic fumes in non-a-priori suspected industries. [The Working Group noted that this study was not considered to be very informative because the exposure assessment was purely based on self-reported job titles, and the small number of exposed cases affected the precision of the odds ratios.]

Hoar & Hoover (1985) evaluated mortality from urinary bladder cancer in a case–control study comprising 325 cases and 673 controls who died in New Hampshire and Vermont (USA) during 1975–79. In-person interviews were conducted with the next of kin and provided information on lifetime occupations, demographics, and medical and lifestyle factors. Cigarette smoking, coffee consumption, education and age at death were considered in all analyses. The odds ratio for exposure to diesel exhaust in any occupation (as reported by the next of kin) was 1.5 (95% CI, 0.8–2.8). A significant duration–response (*P* for trend = 0.024) was observed reaching a threefold excess risk for 30–39 years of employment in these jobs; the odds ratio decreased among men employed for 40 years or longer. [The Working Group noted that this study was limited because exposures were reported only by the next of kin, the specificity of the assessment for exposure to diesel exhaust was unclear and the study was based on mortality rather than incidence.]

Risch *et al.* (1988) conducted a population-based case–control study in Edmonton, Calgary, Toronto and Kingston, Canada, from 1979 to 1982. Cases of histologically confirmed urinary bladder cancer, aged 35–79 years, were identified from hospital, medical and cancer institute records, and a tumour registry. Controls were randomly selected from population lists

and matched to cases by sex, age and area of residence. Analyses were performed on 826 cases and 792 controls who completed interviews, during which structured questionnaires were used to obtain information on 26 occupations and exposures to 18 substances. Among men, elevated risks of urinary bladder cancer, adjusted for year of birth and lifetime tobacco smoking, were observed for ever employment (OR, 1.53; 95% CI, 1.17–2.00), employment for 8–18 years before diagnosis (OR, 1.69; 95% CI, 1.24–2.31) and for every 10 years of duration of employment (OR, 1.23; 95% CI, 1.08–1.41) in jobs that entailed contact with 'diesel or traffic fumes'. [The Working Group noted that no information was provided on which occupations were considered to entail exposure to diesel exhaust; this study has limited utility for the evaluation of the risk for cancer.]

Data from the American Health Foundation hospital-based, case–control study of tobacco-related neoplasms were used in two analyses of the relationship between exposure to diesel and traffic fumes and the risk for urinary bladder cancer (Wynder *et al.*, 1985; Iyer *et al.*, 1990). Both analyses included cases of histologically confirmed urinary bladder cancer diagnosed from 1981 to 1983 and controls with non-tobacco-related diseases (both malignant and non-malignant) at 18 hospitals located in six cities in the USA. [The Working Group was unsure whether the study populations overlapped.] The first analysis by Wynder *et al.* (1985) included 194 male cases and 582 controls matched by age, race, hospital and year of interview, and the second analysis by Iyer *et al.* (1990) included 136 cases and 272 controls matched for sex, age, race, hospital and year of interview. Occupational histories were obtained by in-person interviews using a structured questionnaire. In the first analysis, occupational exposure to diesel exhaust fumes was assessed for specific occupations (titles for usual employment) defined as entailing probable high exposure and for occupations with high, moderate and minimal probable exposure to diesel exhaust, based on the percentage of employees in a given occupation entailing exposure to diesel exhaust. The odds ratios were 1.0 or less for all specific occupations related to diesel exhaust, except for railroad workers (OR, 2.0; 95% CI, 0.34–11.61; two exposed cases) (Wynder *et al.*, 1985). The odds ratio for combined occupations with high probable exposure was 1.68 (95% CI, 0.49–5.73; four exposed cases). In the second analysis, occupations were grouped into low (referent), possible and probable categories of exposure to diesel exhaust according to an a-priori list of job titles. Self-reported exposure to diesel exhaust was also considered. The odds ratio was 1.24 (95% CI, 0.77–2.00; 41 exposed cases) for any exposure to diesel exhaust (including possible or probable exposure classified by job title and self-reported exposure). The odds ratios for possible and probably exposure considered separately were equivalent to unity or below. [The Working Group noted that both studies were limited by small numbers of cases and controls in the categories or occupations with a higher probability of exposure. Although these studies reported risk estimates for different categories of probable exposure to diesel exhaust, the categories appeared to be based on job titles only.]

Dolin & Cook-Mozaffari (1992) did not report an odds ratio because the only available estimate was for low exposure to diesel exhaust, and Pesch *et al.* (2000) reported no odds ratio because the authors reported results for 'exhaust' in general and not diesel exhaust.

(c) Studies that reported risk estimates from job titles

Numerous studies of occupation and the risk for urinary bladder cancer have found that reported risk estimates from job titles – either specific occupations or industries with potential exposure to diesel and/or engine exhaust – and comprised the following:

Population-based studies: these included a series of reports that analysed data from the US National Bladder Cancer Study in New Hampshire (Colt *et al.*, 2004), New England (Colt *et al.*, 2011) and Iowa (Zheng *et al.*, 2002), studies from Canada (Gaertner *et al.*, 2004), Limburg, Belgium (Kellen *et al.*, 2005; Reulen *et al.*, 2007) and New Zealand (Dryson *et al.*, 2008) with a similar study design, in which regional or local cancer registries were used to identify cases and matched controls were selected from the same geographical regions, a matched population-based case–control study in two industrial regions in Belgium (Schifflers *et al.*, 1987) and a case–control study in Copenhagen and surrounding areas (Jensen *et al.*,1987).

The US National Bladder Cancer Study was a population-based case–control study comprising all histologically confirmed cases of carcinoma of the urinary bladder diagnosed from 1978 to 1979 identified from 10 cancer registries that participated in the US NCI Surveillance, Epidemiology, and End Results Program. Findings that focused on occupations involving motor vehicles were reported separately for white men (Silverman *et al.*, 1989a), non-white men (Silverman *et al.*, 1989b) and white women (Silverman *et al.*, 1990). Silverman *et al.* (1986) reported findings that focused on motor-related occupations in white men (1909 cases and 3565 controls), and Smith *et al.* (1985) reported findings specific for automobile and HGV mechanics for all men (2108 controls and 4046 cases). Two studies reported findings separately by individual regions: Silverman *et al.* (1983) for men in Detroit and Schoenberg *et al.* (1984) for white men in New Jersey. [Although the latter four populations were included in the larger studies, they are included in the tables because they provided more detailed analyses.]

Studies with hospital or other cancer controls: these included three studies that identified cases of urinary bladder cancer and controls with other cancers from cancer registries in Missouri, USA (Brownson *et al.*, 1987; Brooks *et al.*, 1992), Detroit, USA (Burns & Swanson, 1991), British Columbia, Canada (Band *et al.*, 2005), and the United Kingdom (limited to men aged 18–54 years) (Coggon *et al.*, 1986), a study that used cancer and non-cancer controls (oral cancer or diseases) in Bombay, India (Notani *et al.*, 1993), and four studies that used non-cancer controls in La Plata, Argentina (Iscovich *et al.*, 1987), Spain (Spanish Bladder Study) (Samanic *et al.*, 2008), the USA (Decouflé *et al.*, 1977) and Texas, USA (Cassidy *et al.*, 2009). Brooks *et al.* (1992) reported risks for invasive urinary bladder cancer using the Detroit population.

Mortality studies: two studies that measured mortality were identified, including a study that used city directories and death certificates as a source for occupational information in Ohio, USA (Steenland *et al.*, 1998), and one that provided detailed analyses of HGV drivers in New Hampshire and Vermont, USA (Hoar & Hoover, 1985). For cancers that have higher survival rates, such as urinary bladder cancer, studies that report mortality are less informative than those that report incidence, because mortality studies overlook cases of cancer that do not result in death.

Several other studies of occupations with potential exposure to motor exhaust were identified, but were not reviewed because either the numbers of exposed cases were small (Bonassi *et al.*, 1989; Ahmad & Pervaiz, 2011) or no formal analyses of risk estimates were performed (Tola *et al.*, 1980; Yaris *et al.*, 2006).

The studies on occupational titles were the least informative to evaluate risks specific for exposure to gasoline or diesel exhaust, because job titles alone are a crude surrogate of exposure. Diesel engines were introduced into the workplace at various rates and at different times, and thus the confidence that the individual workers in the study were actually exposed to diesel exhaust was low. Other limitations included potential confounding from co-exposures to

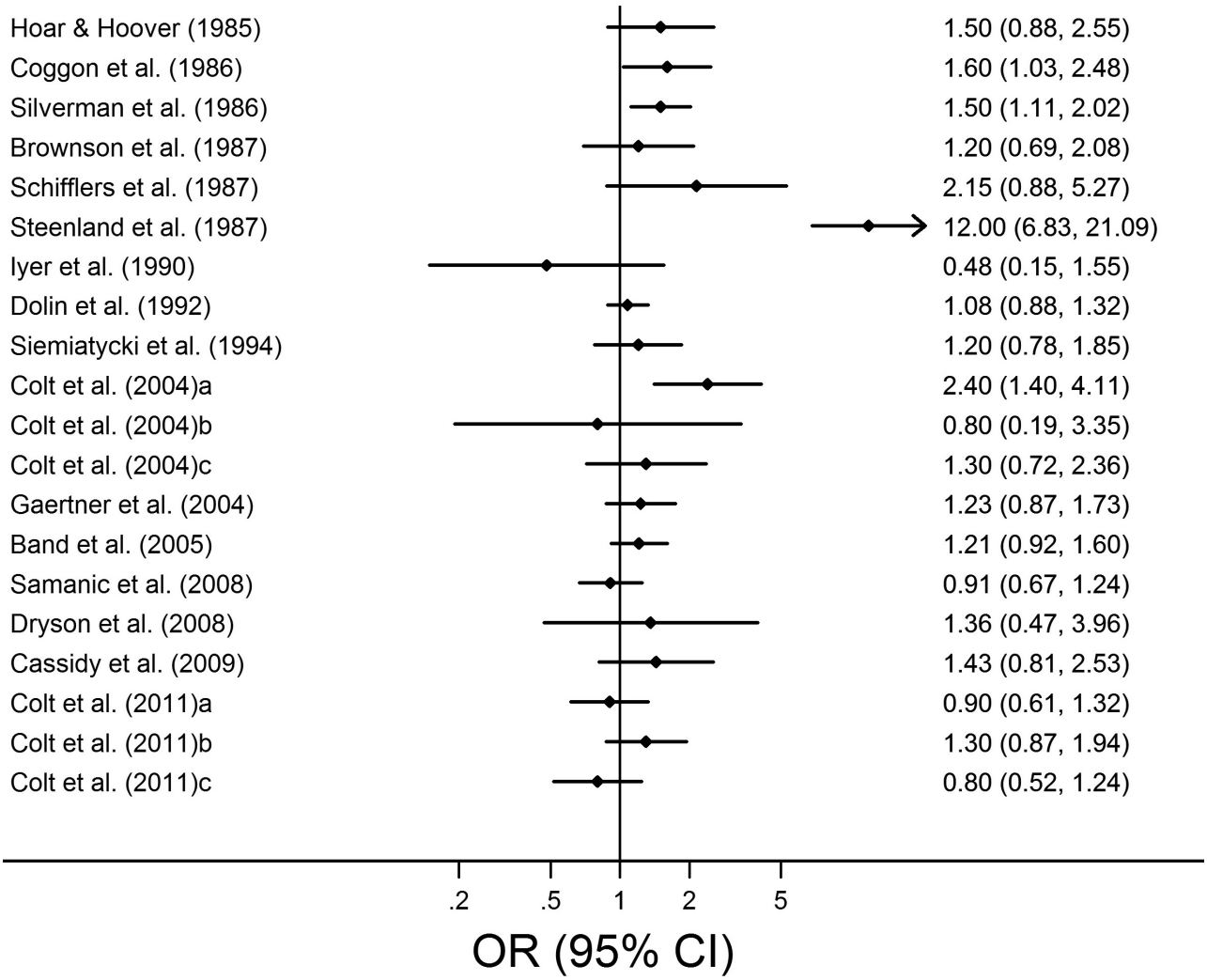
other occupational substances, multiple comparisons and insufficient statistical power because of small numbers of exposed cases and controls. In general, most analyses were restricted to men, because few women were employed in occupations with potential exposure to diesel or gasoline engine exhausts. Because of these limitations, the methods and findings are presented in Table 2.4 and are not reviewed individually in the text; the odds ratios for ever or usual employment are plotted in Fig. 2.2–2.7 and the findings are briefly summarized across studies for each specific occupation with potential for exposure to diesel exhaust. The studies in the figures are organized chronologically by study period. Occupations or industries that were thought to entail potential contact with diesel or gasoline exhaust included railroad workers, HGV drivers, bus drivers, taxicab drivers, automobile mechanics, gasoline station attendants and garage workers. Of these, HGV drivers, railroad workers and bus drivers were considered to have the highest probable exposure to diesel exhaust.

(i) HGV drivers

Case–control studies reporting risk estimates for urinary bladder cancer and employment as an HGV driver are described in Table 2.4 and a summary of the odds ratios for ever or usual exposure are depicted in Fig. 2.2. Steenland *et al.* (1987) and Siemiatycki *et al.* (1994) did not present findings for ever employment, and those for the longest duration of employment are indicated in the graph. Multiple odds ratios are plotted from two studies (Colt *et al.*, 2004, 2011) because they reported odds ratios for different types of HGV driver, such as tractor-trailer, light and heavy. Of the 21 odds ratios reported for employment as an HGV driver (Fig. 2.2), 15 were greater than 1.10 (ranging from 1.2 to 2.4 for ever or usual employment), one was between 1.01 and 1.1 (Dolin & Cook-Mozaffari, 1992) and five were below 1.0. The 12-fold elevated odds ratio plotted in the graph was for employment of more than 20 years and was based on six exposed cases (Steenland *et al.*, 1987). Four of the elevated odds ratios were statistically significant (Coggon *et al.*, 1986; Silverman *et al.*, 1986; Steenland *et al.*, 1987; Colt *et al.*, 2004; tractor-trailer HGV drivers). Two of the odds ratios that were below 1.0 were based on estimates that used fewer than five exposed cases (Iyer *et al.*, 1990; Colt *et al.*, 2004; HGV drivers). Four of the seven independent studies that evaluated duration of employment found higher risks with longer duration of employment as an HGV driver (Hoar & Hoover, 1985; Silverman *et al.*, 1986; Steenland *et al.*, 1987; Colt *et al.*, 2004).

Two studies provided more detailed analysis of HGV drivers in an attempt to evaluate risks from potential exposure to diesel or motor exhaust and thus were somewhat more informative for an evaluation of cancer risks: (1) an analysis of 300 cases and 296 controls from Detroit, one of the 10 geographical areas in the US National Bladder Cancer Study (Silverman *et al.*, 1983), and a case–control study of mortality from urinary bladder cancer comprising deceased cases and controls from New Hampshire and Vermont, USA (Hoar & Hoover, 1985). Silverman *et al.* (1983) was not plotted in Fig. 2.2 because the same population was included in Silverman *et al.* (1986). In the Detroit study (Silverman *et al.*, 1983), a significant trend in risk was found with duration of employment (P for trend < 0.004) and year of starting employment (P for trend = 0.03). Diesel fuel became more prevalent in the USA after 1950, and the risk for urinary bladder cancer was highest among men who started employment between 1950 and 1969 (RR [adjusted for age and smoking], 6.5; CI not reported; seven exposed cases). In an analysis of duration of employment as an HGV driver that was restricted to the period 1950–78 and controlled for duration before 1950, relative risks increased with increasing duration of employment, and reached 2.6 for a duration of 5 or more years compared with never having worked as an HGV driver from 1950 to 1978. No association between the risk for urinary bladder

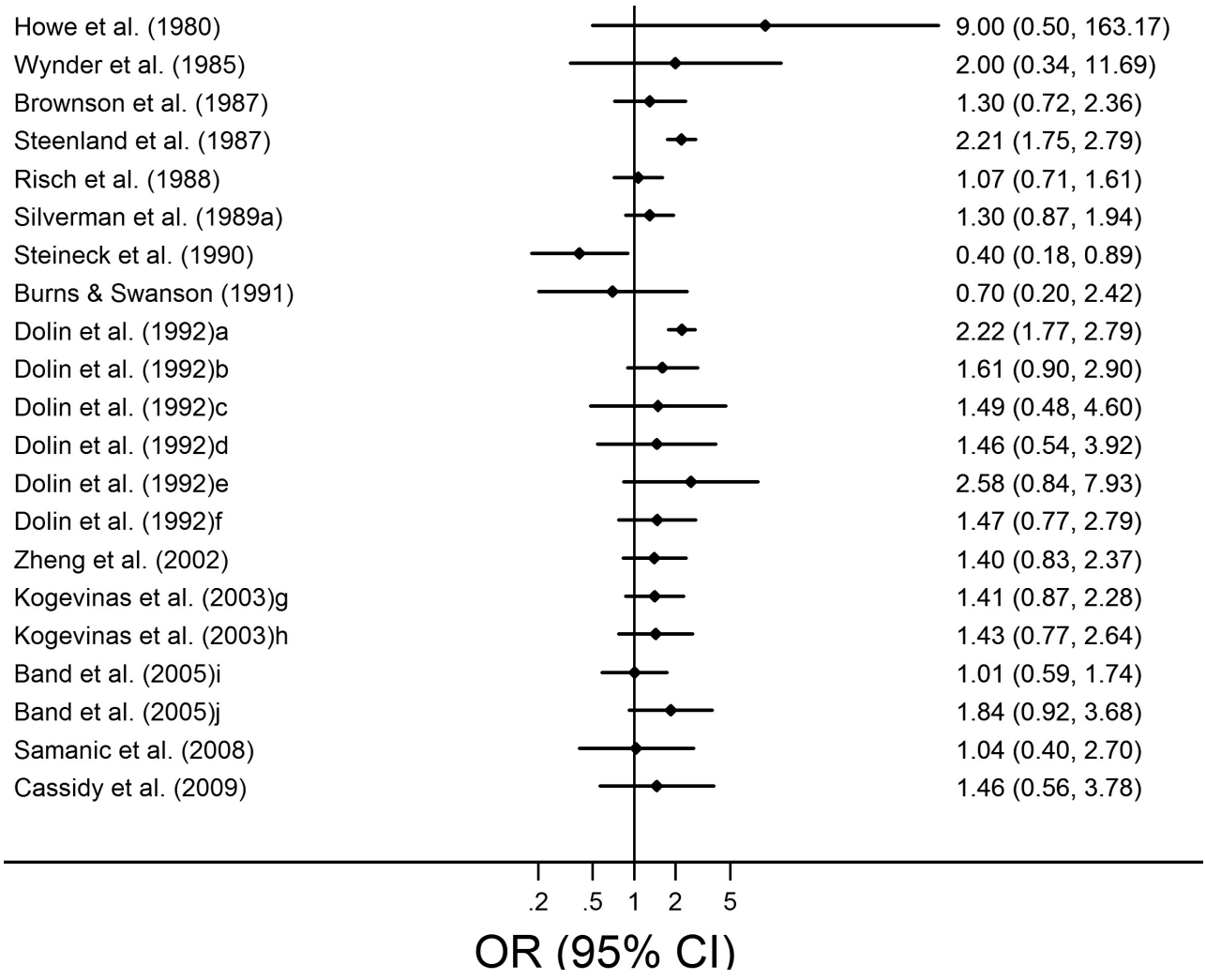
Fig. 2.2 Case–control studies of urinary bladder cancer that reported risk estimates for ever or usual exposure as a heavy goods vehicle driver



a Tractor trailer
b Heavy goods vehicle
c Light goods vehicle
CI, confidence interval; OR, odds ratio

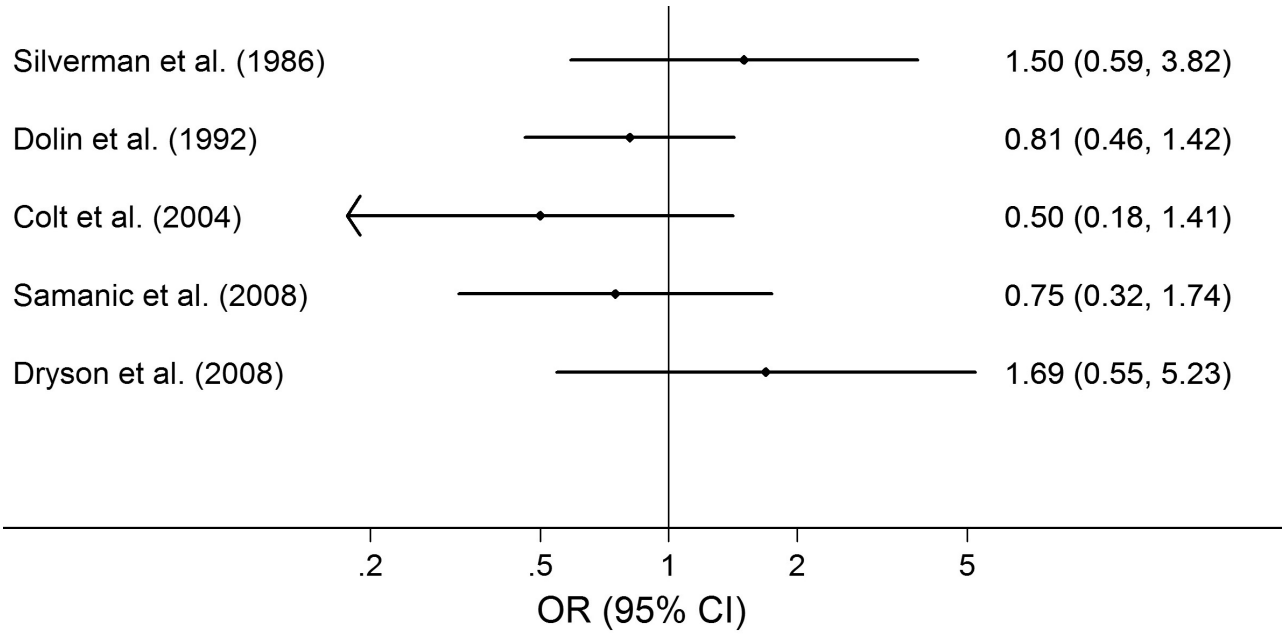
cancer and duration of employment as an HGV driver before 1950 was observed in an analysis that adjusted for duration between 1950 and 1978 (CI not reported; 14 exposed cases). Among HGV drivers, the risk for those who reported operating a vehicle with a diesel engine was 11.9 (95% CI, 2.3–61.1; 13 exposed cases) compared with 1.4 (95% CI, 0.7–2.9; 21 exposed cases) for never having operated a vehicle with a diesel engine. In addition, an interaction between employment as an HGV driver and cigarette smoking was observed ($P = 0.04$). [The Working Group noted that this study provided some evidence that the elevated odds ratio observed for HGV drivers may be due (or at least in part) to exposure to diesel exhaust.]

Fig. 2.3 Case–control studies of urinary bladder cancer that reported risk estimates for ever or usual exposure as a railroad worker



a Railroad industry
b Engine driver
c Shunter
d Signalman
e Guard
f Lengthman
g Driver
h Brakeman
i Railroad transport industry
j Locomotive operation
CI, confidence interval; OR, odds ratio

Fig. 2.4 Case–control studies of urinary bladder cancer that reported risk estimates for ever or usual exposure as a bus driver



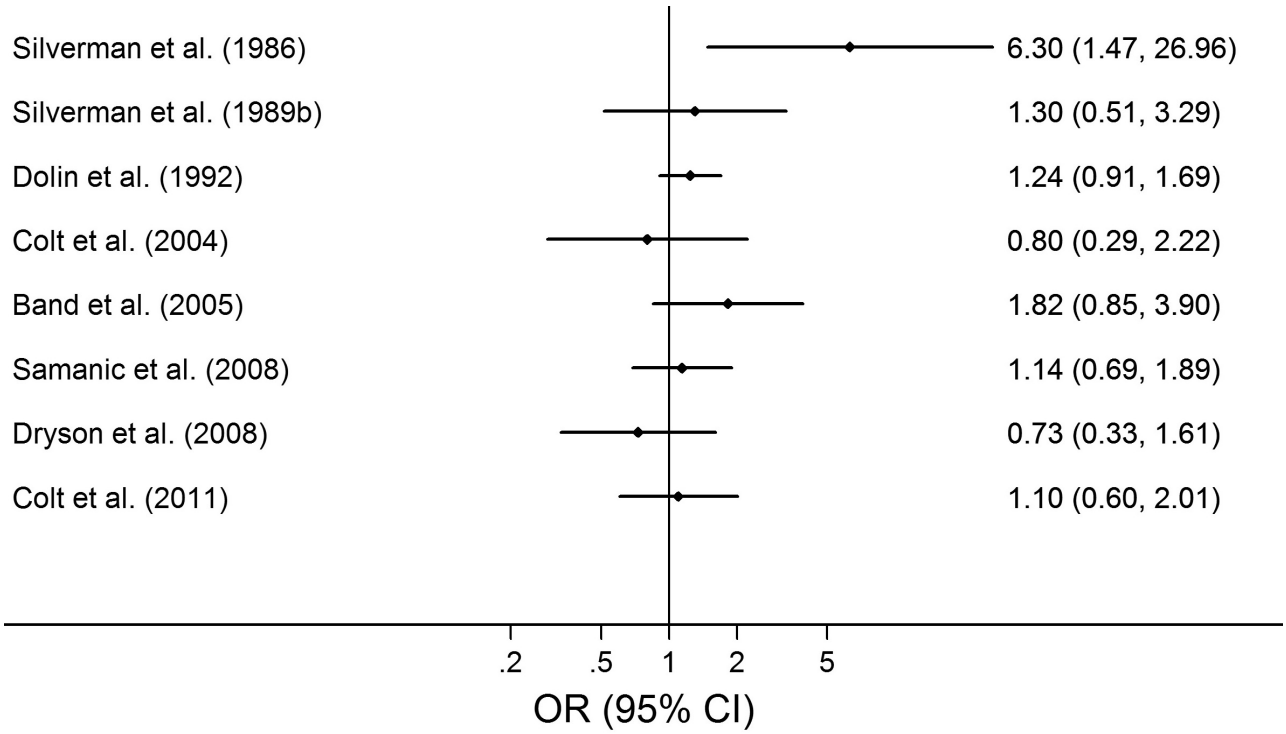
CI, confidence interval; OR, odds ratio

In the mortality study of Hoar & Hoover (1985), the odds ratio for any employment as an HGV driver was 1.5 (95% CI, 0.9–2.6) and the number of years of driving an HGV was associated with mortality from urinary bladder cancer (*P* for trend = 0.006). The highest risk was observed for driving HGVs for 5–9 years (OR, 2.9; 95% CI, 1.2–6.7), but decreased for driving for more than 10 years (OR, 1.8; 95% CI, 0.8–4.1). Among HGV drivers, the odds ratio [adjusted for age and county of residence] when next of kin reported exposure to diesel exhaust was 1.8 (95% CI, 0.5–7.0) compared with 1.5 (95% CI, 0.8–2.7) for those with no report of exposure to diesel exhaust. In an analysis by year of first employment, the highest odds ratio was observed among HGV drivers who were first employed from 1930 to 1949 (OR, 2.6; 95% CI, 1.3–5.1), but was lower for first employment after 1950 (OR, 1.4; 95% CI, 0.5–4.1). Among all occupations that reported exposure to diesel exhaust, a significant duration–response trend (*P* for trend = 0.024) was seen, reaching a threefold excess risk for 30–39 years of employment and a decrease in risk among men employed for 40 years or more. Cigarette smoking, coffee consumption, education and age at death were consid ered in all analyses. [The Working Group noted that a limitation of this study was that mortality from rather than incidence of urinary bladder cancer was assessed. Some analyses were also limited by small numbers of exposed cases.]

(ii) Railroad workers

Case–control studies that reported risk estimates for urinary bladder cancer and employment as a railroad worker are described in Table 2.4 and a summary of the odds ratios for ever or usual exposure is depicted in Fig. 2.3. Multiple odds ratios are plotted from the studies by Dolin & Cook-Mozaffari (1992), Kogevinas *et al.* (2003) and Band *et al.* (2005) because they reported risks for different types of railroad worker; the risk estimate for Steenland *et al.* (1987) was for

Fig. 2.5 Case–control studies of urinary bladder cancer that reported risk estimates for ever or usual exposure as a taxi driver



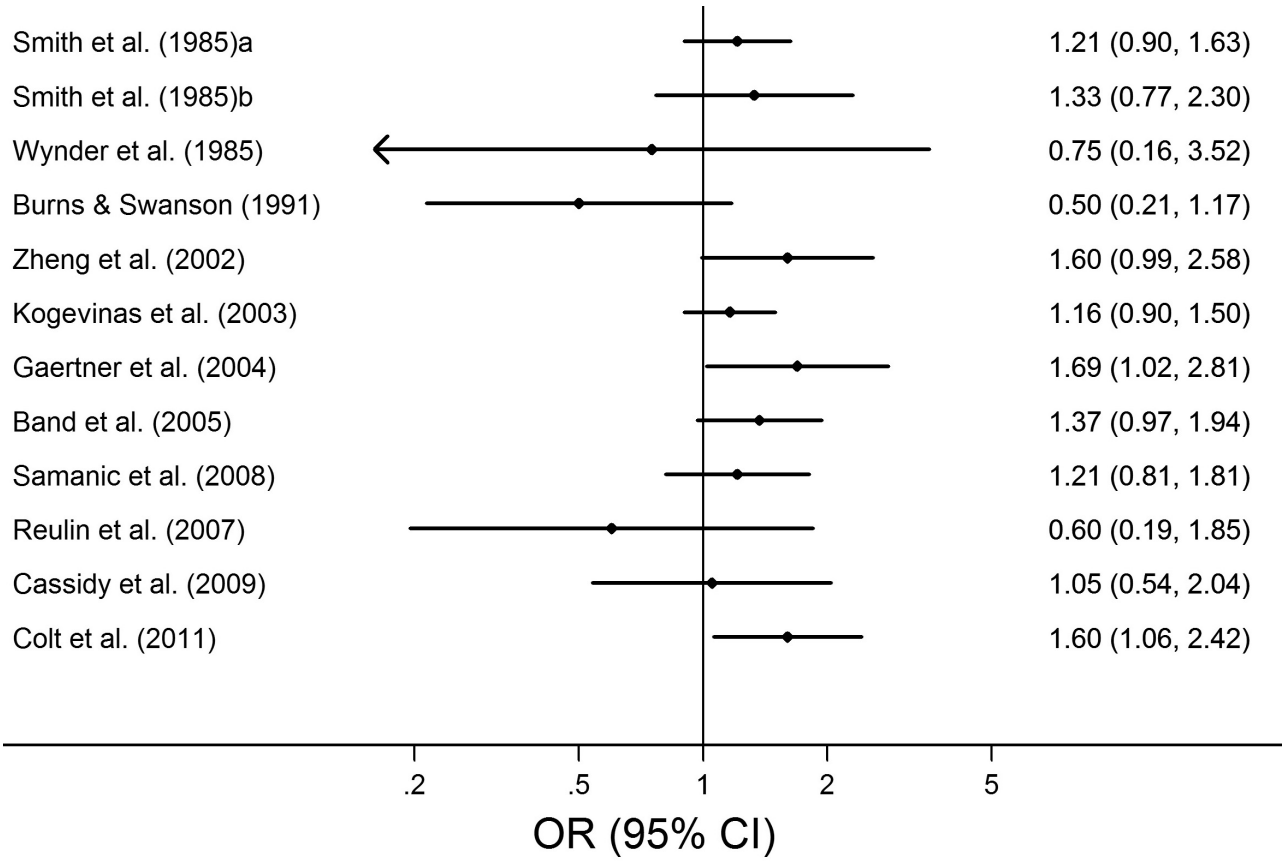
CI, confidence interval; OR, odds ratio

20 years of employment. Of the 22 odds ratios reported for employment as a railroad worker (Fig. 2.3), 17 were greater than 1.10 (ranging approximately from 1.3 to 9), three were between about 1.01 and 1.10 and two were below 1.0. No information on tobacco smoking was available in the study by Dolin & Cook-Mozaffari (1992). Only three of the odds ratios were statistically significant. Two of the three studies that stratified by duration of employment found higher risks with longer employment as a railroad worker (Steenland *et al.*, 1987; Zheng *et al.*, 2002; Samanic *et al.*, 2008); a fourth study reported an odds ratio of 1.09 (95% CI, 0.87–1.36) for every 10 years of employment (Risch *et al.*, 1988).

(iii) Bus and taxi drivers

Case–control studies that reported risk estimates for urinary bladder cancer and employment as bus or taxi driver are summarized in Table 2.4, Fig. 2.4, and Fig. 2.5. Of the six odds ratios reported for employment as a bus driver (Fig. 2.4), three were greater than 1.0, and none was statistically significant (Decouflé *et al.*, 1977; Silverman *et al.*, 1986; Dryson *et al.*, 2008). No association with duration of employment was found in the two studies in which it was evaluated. For taxicab drivers (including chauffeurs), risk estimates were reported separately for white and non-white males in the US National Bladder Cancer Study (Silverman *et al.*, 1986, 1989a, b). The majority of the odds ratios (from seven of the nine studies) were greater than 1.0 (ranging from 1.1 to 6.3), one of which was statistically significant (white men; Silverman *et al.*, 1986). Both of the studies that evaluated duration of employment found indications of a higher risk among workers with the longest employment as a taxicab driver (Silverman *et al.*, 1986; Samanic *et al.*, 2008).

Fig. 2.6 Case–control studies of urinary bladder cancer that reported risk estimates for ever or usual exposure as a motor vehicle mechanic



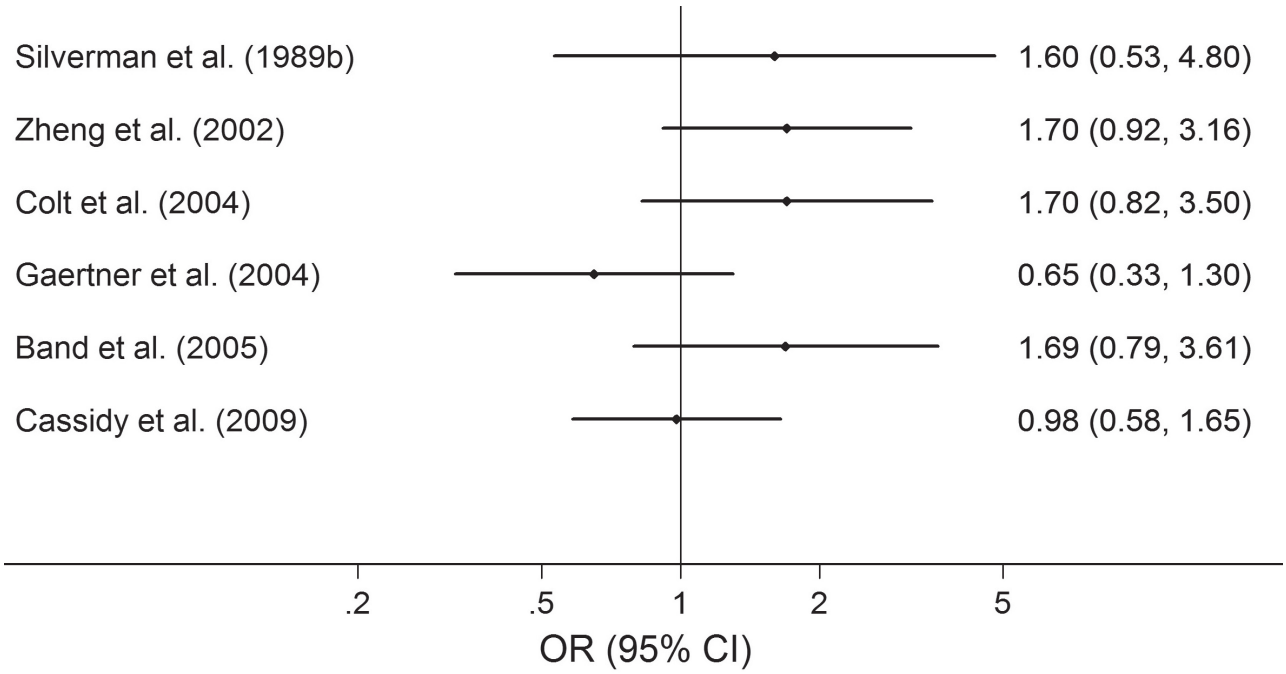
a Smokers
b Nonsmokers
CI, confidence interval; OR, odds ratio

(iv) Motor vehicle mechanics, gasoline station and garage workers, and other occupations

For employment as a motor vehicle mechanic, eight studies reported odds ratios greater than 1.1, three of which were statistically significant (Zheng *et al.*, 2002; Kogevinas *et al.*, 2003; Gaertner *et al.*, 2004); three studies reported odds ratios below 1.0, and one reported an odds ratio of 1.05 (see Table 2.4 and Fig. 2.6). Findings were reported separately for vehicle mechanics in the US National Bladder Study (Smith *et al.*, 1985); risks were higher among workers with a longer duration of employment in three of six studies in which it was evaluated (Smith *et al.*, 1985 (both smokers and nonsmokers); Gaertner *et al.*, 2004; Colt *et al.*, 2011).

Of the six studies that reported risk estimates for garage workers and/or gasoline station attendants, four reported odds ratios greater than 1.1; none was statistically significant (see Table 2.4 and Fig. 2.7). Two of the four studies that evaluated duration of employment found higher risks among workers with a longer duration (Colt *et al.*, 2004; Cassidy *et al.*, 2009). A study of one of the geographical regions in one of the 10 areas that contributed to the US National Bladder Cancer study did not find an employment duration–response relationship (Schoenberg *et al.*, 1987).

Fig. 2.7 Case–control studies of urinary bladder cancer that reported risk estimates for ever or usual exposure as a garage worker



CI, confidence interval; OR, odds ratio

Zheng *et al.* (2002) found elevated risks for employment as a transport or material-moving supervisor (OR, 6.5; 95% CI, 1.4–29.9), which they stated were occupations associated with exposure to diesel exhaust (see Table 2.4). No elevated risk for urinary bladder cancer was found for ever employment as a warehouse materials handler in the American Health Foundation study, which was also an occupation that the authors stated was associated with exposure to diesel exhaust (Wynder *et al.*, 1985).

2.3.3 Cancer at other and multiple sites

See Table 2.5

A study by Decouflé *et al.* (1977) of cancer and occupation included cancer cases and hospital controls admitted to a large hospital in Buffalo, NY, USA, from 1956 to 1965. Ever employment in an occupation and duration of employment of at least 5 years were analysed on the basis of personal interviews. A large number of different occupations and cancer sites were evaluated, using clerical occupations as an unexposed comparison group. For employment as an HGV or tractor driver, relative risks [CI not provided] of 1.53 (29 exposed cases; $P > 0.05$) for laryngeal cancer, 0.60 (24 exposed cases; $P = 0.04$) for colon/rectal cancer and 0.63 (23 exposed cases; $P > 0.05$) for lymphoma were reported. For cancers at other sites, numbers were generally low and the risks were close to unity. [The Working Group noted that the report included many comparisons, lacked detailed descriptions of occupations and information on confounders, and was of limited value for the evaluation of exposure to exhaust.]

In a large population-based case–control study in Canada (Siemiatycki *et al.*, 1988), the associations between 10 types of engine exhaust and combustion products and cancers at 12 sites

Table 2.5 Case–control studies of other and multiple sites of cancer and exposure to engine exhaust

Reference, Location, period	Total No. of cases/ controls	Source of controls	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of exposed cases	Relative risk (95% CI)	Covariates Comments
Decouflé *et al.*, (1977), Buffalo, USA, 1956–65	13 949/NR	Hospital	Personal interviews; ever employment, duration	Larynx Lymphoma Colon/ rectum	HGV and tractor drivers (ever employment)	29 23 24	1.53 ($P > 0.05$) 0.63 ($P > 0.05$) 0.60 ($P = 0.04$)	Clerical occupations as comparison group, response rate not specified
Siemiatycki *et al.* (1988) Montreal, Canada, 1979–85	3276/3276	Hospital; other cancers	Interviewer-administered standardized questionnaire; detailed lifetime job history; expert assessment	12 sites Colon Rectum Kidney	 Any exposure to diesel exhaust Long-term high exposure to diesel exhaust Long-term high exposure to gasoline exhaust Occupation as driver (bus, HGV, taxi)	 68 30 36 34 24	**90% CI** 1.3 (1.1–1.6) 1.7 (1.2–2.5) 1.6 (1.1–2.3) 1.4 (1.0–2.0) 1.5 (1.0–2.2)	Men only; age, SES, tobacco smoking, ethnic group and blue-/white-collar job history; response rate: 82%; cases in one analysis served as controls for other comparisons
Goldberg *et al.* (2001) Montreal, Canada, 1979–85	497/1514 and 553	Hospital (cancers at other sites) and population	Interviewer-administered standardized questionnaire; detailed lifetime job history; expert assessment	Colorectum	**Diesel engine exhaust (frequency, level, duration, confidence in assessment)** Non-substantial Substantial	 45 35	 1.2 (0.8–1.8) 1.6 (1.0–2.5) (all controls) 2.1 (1.1–3.7) (population controls)	Same population as Siemiatycki *et al.* (1988); age, SES, tobacco smoking, ethnic group and other non-occupational factors
Fang *et al.* (2011) British Columbia, Canada, 1983–90	1155/registry (other cancer cases)	Hospital	Self-administered questionnaire, lifelong occupational history	Colon	**Occupation and industry titles (ever versus never)** Taxi driver/ chauffeur HGV driver Bus driver Locomotive operator	 30 124 20 8	 1.54 (1.01–2.25) 1.08 (0.88–1.33) 0.84 (0.52–1.36) 0.71 (0.33–1.54)	Marital status, education, tobacco smoking, alcohol consumption and respondent (self/ proxy); response rate: 65.4% cases, 60% controls; controls (excluding lung and rectum) matched on age and yr of diagnosis

Table 2.5 (continued)

Reference, Location, period	Total No. of cases/ controls	Source of controls	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of exposed cases	Relative risk (95% CI)	Covariates Comments
Seidler *et al.* (1998) Frankfurt and Hamburg, Germany, NR	192/210	Hospital	Self-administered questionnaire, lifelong occupational history, JEM	Prostate	**Diesel engine exhaust (intensity, probability and duration)**			Age, tobacco smoking and region; response rate: 71% cases, 55% controls
					0 dose–yr	118	1.0 (reference)	
					> 0–25 dose–yr	53	1.1 (0.7–1.8)	
					> 25 dose–yr	17	3.7 (1.4–9.8)	
Fritschi *et al.* (2007) Western Australia, 2001–02	606/471	Population	Self-administered questionnaire, telephone interview, lifelong occupational history, expert assessment	Prostate	**Diesel engine exhaust (substantial, non-substantial and unexposed)**			Age; response rate: 57% cases, 37% controls
					Substantial	36	1.07 (0.67–1.72)	
					Non-substantial	213	0.92 (0.71–1.19)	
Santibañez *et al.* (2010) Eastern Spain, 1995–99	161/455	Hospital	Interviewer-administered standardized questionnaire; JEM	Pancreas	Employment as HGV/LGV driver	5	3.46 (1.01–11.83)	Sex, age, province, level of education, alcohol consumption and tobacco smoking; response rate: 81% cases, 99.6% controls; 60% of cases histologically confirmed
					Low exposure to diesel exhaust	14	1.49 (0.72–3.08)	
					High exposure to diesel exhaust	8	1.88 (0.72–4.90)	
					Low exposure to gasoline exhaust	11	1.38 (0.62–3.07)	
					High exposure to gasoline exhaust	8	1.85 (0.71–4.80)	
Brown *et al.* (1988) Texas, USA, 1975–80	183/250	Population	Interviewer-administered standardized questionnaire; occupational history; expert assessment	Larynx	Exposure to diesel/gasoline fumes	79	1.5 (1.0–2.26)	Men only; tobacco smoking and alcohol consumption; response rate: 70% cases, 61–86% various controls; deceased cases and respective controls included

Table 2.5 (continued)

Reference, Location, period	Total No. of cases/ controls	Source of controls	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of exposed cases	Relative risk (95% CI)	Covariates Comments
Muscat & Wynder (1995) New York, USA, 1985–92	235/205	Hospital	Interviewer-administered standardized questionnaire; detailed history for six main jobs	Larynx	Employment in job with substantial exposure to diesel exhaust	36	0.96 (0.5–1.8)	Men only; tobacco smoking and alcohol consumption (alcohol not used for adjustment); response rate: 90% cases and controls; tobacco smoking and alcohol consumption high in case patients, lower in controls
					Automobile mechanic	13	1.30 (0.4–4.1)	
					Self-reported exposure to diesel exhaust	13	1.47 (0.5–4.1)	
					Self-reported exposure to diesel fumes	17	6.4 (1.8–22.6)	
Elci *et al.* (2003) Istanbul, Turkey, 1979–84	940/1519	Hospital	Interviewer-administered standardized questionnaire; detailed lifetime occupational history; expert assessment	Larynx	**Diesel exhaust** Ever	297	1.5 (1.3–1.9)	Age, tobacco smoking and alcohol consumption; response rate unclear; crude adjustment for major risk factors
					Intensity			
					Low	161	1.5 (1.1–1.8)	
					Medium	91	1.7 (1.2–2.3)	
					High	45	1.6 (1.0–2.4)	
					Diesel exhaust probability			
					Low	92	1.6 (1.2–2.2)	
					Medium	148	1.5 (1.1.-1.9)	
					High	57	1.6 (1.1.-2.4)	
					Gasoline exhaust Ever	220	1.6 (1.3–2.0)	
					Intensity			
					Low	141	1.5 (1.2–2.0)	
					Medium	78	1.8 (1.3–2.5)	
					High	NR	NR	
					Probability			
					Low	86	1.6 (1.1–2.2)	
					Medium	131	1.7 (1.3–2.2)	
					High	3	0.7 (0.2–2.9)	

Table 2.5 (continued)

Reference, Location, period	Total No. of cases/ controls	Source of controls	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of exposed cases	Relative risk (95% CI)	Covariates Comments
Karunanayake *et al.* (2008) Canada, six provinces, 1991–94	513/1506	Population	Standardized written questionnaire, lifetime occupational history	NHL (200–202)	Exposure to diesel engine exhaust fumes (ever/never)	183	1.33 (1.03–1.67)	Age and province; self-reported exposure, no detailed exposure assessment
					Longest held occupation			
					Driver	27	1.45 (0.88.2.37)	
Flodin *et al.* (1987) Sweden, 1973–83	131/431	Population	Postal questionnaire, lifetime occupational history	Multiple myeloma (203)	Occupational exposure to engine exhaust	35	**OR** 2.3 (1.3–4.7)	Crude OR, no major change after adjustment for tobacco smoking and X-ray treatment; population not well described; unclear adjustment; exposure self-reported
Boffetta *et al.* (1989) USA, 1982–86 (American Cancer Society (ACS) Study)	282/1128	Nested in ACS study	Self-administered questionnaire at base-line, self-assessment	Multiple myeloma	Diesel exhaust	14	1.40 (0.70–2.70)	Age, sex, ACS division, race, education, diabetes, X-ray treatment, farming, and exposure to pesticides and herbicides; incident cases only used for risk analysis
					Gasoline exhaust	14	0.9 (0.5–1.6)	
					HGV driver	3	4.0 (0.9–17.6)	
					Railroad worker	3	6.0 (1.3–28.9)	
					Auto mechanic	3	0.9 (0.2–3.5)	
Eriksson & Karlsson (1992) Sweden, 1982–86	275/275	Population	Self-administered questionnaire, self-assessment	Multiple myeloma	Engine exhausts	61	1.11 (0.60–2.05)	Age, sex, county and vital status; participation rate: 97%
Heineman *et al.* (1992) Denmark, 1970–84	1098/4169	Population	Employment data from registries; expert assessment	Multiple myeloma	**Engine exhausts**			Men only; age and 'other agents'
					Possible exposure	125	1.3 (1.0–1.6)	
					< 5 yr, no lag	52	1.5 (1.0–2.3)	
					< 5 yr, 10-yr lag	56	1.5 (1.0–2.3)	
					≥ 5 yr, no lag	76	1.3 (1.0–1.9)	
					≥ 5 yr, 10-yr lag	73	1.5 (0.9–2.7)	
					Probable exposure	89	1.2 (0.9–1.6)	
					< 5 yr, no lag	25	1.2 (0.7–2.1)	
					< 5 yr, 10-yr lag	94	1.0 (0.6–1.7)	
					≥ 5 yr, no lag	52	1.2 (0.8–1.9)	
					≥ 5 yr, 10-yr lag	19	1.0 (0.5–2.0)	

Table 2.5 (continued)

Reference, Location, period	Total No. of cases/ controls	Source of controls	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of exposed cases	Relative risk (95% CI)	Covariates Comments
Pottern *et al.* (1992) Denmark, 1970–84	1010/4040	Population	Employment data from registries, and expert assessment	Multiple myeloma	**Exhaust gases** Possible exposure Probable exposure	 8 4	 1.4 (0.6–3.2) 1.6 (0.4–5.5)	Women only; same study as Heineman *et al.* (1992); age
Demers *et al.* (1993) USA, 1977–81	692/1683	Population	Interviews	Multiple myeloma	Motor vehicle operators Rail and water transport workers	76 4	1.2 (0.8–1.6) 0.3 (0.1–1.1)	Age, sex, race and study area; response rate: 89% cases, 83% controls; 32% of case interviews with proxy
Flodin *et al.* (1988) Sweden, 1973–83	111/431	Population	Postal questionnaire, life-time occupational history	Chronic lymphocytic leukaemia (204)	Occupational exposure to engine exhaust	31	**OR** 2.5 (1.5–4.0)	Crude OR, see Flodin *et al.* (1987)
Lindquist *et al.* (1991) Sweden, 1980–83	125/125	Population	Interviewer-administered questionnaire, detailed life-time occupational history	Acute leukaemia	Occupation as professional driver Rally driver (hobby)	18 3	3.0 (1.1–9.2) 3.0 (NR)	Solvents, radiation treatment, tobacco smoking, sex and age; response rate: > 90% cases and controls; exposure of drivers to petroleum products [not further specified]
Clavel *et al.* (1995) France, 1980–90	291/541	Hospital	Postal questionnaire, life-time occupational history	Hairy cell leukaemia	Self-reported occupational exposure to engine exhaust	43	1.6 (1.0–2.6)	Men only; response rate: 61% cases, 58% controls; only live cases included; retrospective case ascertainment; control for confounders not reported
Blair *et al.* (2001) USA, 1980–83	513/1087	Population	Interviews; JEM for selected exposures	Leukaemia (ICD-9: 204–208)	LGV drivers **Motor vapour and exhausts** Low High	13 187 60	3.4 (1.4–8.4) 1.0 (0.8–1.2) 1.3 (0.9–1.9)	Age, residence state, proxy interview, education, pesticides, hair dyes, tobacco smoking and first- degree relative with lymphatic or haematopoietic tumour; response rate: 86% cases, 77–79% controls

Table 2.5 (continued)

Reference, Location, period	Total No. of cases/ controls	Source of controls	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of exposed cases	Relative risk (95% CI)	Covariates Comments
Seniori Costantini *et al.* (2001) Italy, 1991–93	2737/1779	Population	Interviewer-administered questionnaire; expert assessment	Hematolymphopoietic neoplasms (200–208)				Age, sex and area; all newly diagnosed cases among residents aged 20–74 yr; response rate: 81% cases, 74% controls; job-specific results reported for men only
				NHL (ICD-9: 200, 202, 204.1)	Material-handling and related equipment operators	43	1.2 (0.8–2.0)	
					Transport operators	74	0.9 (0.7–1.3)	
				Hodgkin lymphoma (ICD-9: 201)	Material-handling and related equipment operators	5	1.0 (0.4–2.7)	
					Transport operators	10	0.8 (0.4–1.7)	
				Leukaemia (ICD-9: 204–208)	Material-handling and related equipment operators	25	2.5 (1.4–4.5)	
					Transport operators	34	1.1 (0.7–1.7)	
				Multiple myeloma (ICD-9: 203)	Material-handling and related equipment operators	3	0.6 (0.2–1.9)	
					Transport operators	7	0.5 (0.2–1.1)	
Adegoke *et al.* (2003) Shanghai, China, 1987–89	486/502	Population	Interview; self-reported; JEM	Leukaemia (ICD-9: 204–208)	Water and rail transport	11	0.5 (0.2–1.1)	Age, sex and income
					Auto and truck drivers	8	0.8 (0.3–2.0)	

Table 2.5 (continued)

Reference, Location, period	Total No. of cases/ controls	Source of controls	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of exposed cases	Relative risk (95% CI)	Covariates Comments
Wong *et al.* (2010) Shanghai, China, 2003–07	722/1444	Hospital	Interviewer-administered questionnaire; detailed lifetime occupational history; expert assessment	Acute myeloid leukaemia (205)	Occupational exposure to diesel or gasoline engine exhaust	2	0.57 (0.12–2.57)	Sex, age, education, alcohol, rural residence and other occupational factors (not used in analysis presented in this table); response rate: 97% cases; exposure assessment not detailed for exhausts and other exposures
					Exposure to diesel fuel (all acute myeloid leukaemia)	36	1.23 (0.89–1.81)	
West *et al.* (1995) United Kingdom, NR	400/400	Hospital	Interviewer-standardized questionnaire; self- and expert assessment	Myelodys-plasia	**Exhaust gases**			Age, sex, area of residence, hospital and yr of diagnosis; response rate: 63% cases
					10 h low exposure	79	1.26 (0.86–1.86)	
					> 50 h, > medium intensity	31	1.72 (0.93–3.20)	
					> 2500 h, > medium intensity	22	1.57 (0.77–3.23)	

ACS, American Cancer Society; CI, confidence interval; h, hour; HGV, heavy goods vehicle; ICD, International Classification of Diseases; JEM, job–exposure matrix; LGV, light goods vehicle; NHL, non-Hodgkin lymphoma; NR, not reported; OR, odds ratio; SES, socioeconomic status; yr, year

(oesophagus, stomach, colon, 'rectosigmoid', rectum, pancreas, lung, prostate, bladder, kidney, skin melanoma and non-Hodgkin lymphoma) were evaluated for 3726 male cancer patients, aged 35–70 years, diagnosed in any of the 19 participating hospitals in Montreal and interviewed (response rate, 82%). For each cancer site, patients with cancers at other sites comprised the control group. The interview elicited a detailed job history, and a team of chemists and industrial hygienists translated each job into a list of potential exposures (Gérin *et al.*, 1985). The probability of exposure ('possible', 'probable' and 'definite'), the frequency of exposure (< 5, 5–30 and > 30% working time) and the level of exposure (low, medium and high) were estimated. After stratifying for age, socioeconomic status, ethnic group, cigarette smoking and blue-/white-collar job history, elevated odds ratios were seen for exposure to diesel engine exhaust and colon cancer (OR, 1.3; 90% CI, 1.1–1.6 for any exposure; OR, 1.7; 90% CI, 1.2–2.5 for long-term, high-level exposure), for long-term high-level exposure to gasoline engine exhaust and cancer of the rectum (OR, 1.6; 90% CI, 1.1–2.3) and kidney cancer (OR, 1.4; 90% CI, 1.0–2.0) and for bus, HGV and taxi drivers and rectal cancer (90% CI, 1.5; 1.0–2.2). [The Working Group noted that the study included numerous comparisons and used 90% confidence intervals; at the 95% level, most of the intervals would have included unity. Thus, this study was considered to give weak evidence of an association between cancers of the colon, rectum and kidney with exposure to engine exhaust.]

In the framework of the previous Canadian multisite population-based case–control study of occupational exposures and risks for various cancers (Siemiatycki *et al.*, 1988), Goldberg *et al.* (2001) assessed the associations of colon cancer with diesel engine emissions, and many other occupational exposures, in 497 male case patients compared with 1514 other cancer patients (excluding lung and peritoneal cancer, and cancers possibly associated with known risk factors for colon cancer and other cancers of the digestive tract) and 533 population controls. Exposures were assessed as described above (Siemiatycki *et al.*, 1988). The results differed according to the control group: when the pooled group of cancer and population controls was used, the odds ratio for substantial exposure to diesel engine exhaust was 1.6 (95% CI, 1.0–2.5), whereas the risk increased to 2.1 (95% CI, 1.1–3.7) when only the population-based controls were used. [The Working Group noted that the reasons for these differences were not discussed, but the use of cancer controls was a potential source of bias. Multivariate models were adjusted for an extended list of risk factors including socioeconomic status, tobacco smoking and body mass index, but no detailed dietary factors or physical activity.]

Another Canadian study obtained information on lifetime occupational history through a questionnaire from male cancer patients, aged 20 years and over, registered by the British Columbia Cancer Registry between 1983 and 1990. A case–control study was conducted on 1155 cases of colon cancer and 7752 cases of other cancers matched on age and year of diagnosis as controls (Fang *et al.*, 2011). Occupations and industries were coded according to Canadian and international standard classifications. Having ever/never been employed in a specific occupation or industry, as well as the usual occupation or industry of employment, were analysed for all of the 597 occupational titles and 1104 industry titles used in Canada, but results were only reported for those that concerned at least five cases. The analyses showed elevated risks for colon cancer (OR, 1.54; 95% CI, 1.01–2.25) for ever employment as a taxi driver/chauffeur, while other occupational titles, including bus drivers, HGV drivers and locomotive operators, showed no association. [The Working Group noted that no specific assessment of exposure to diesel or gasoline exhaust was carried out. The large number of

statistical comparisons and the crude exposure assessment were among other limitations of this study, which contributed minimally to an assessment of engine exhausts.]

The association between prostate cancer and occupational exposure to diesel fuels and fumes was studied in a case–control study in Germany of 192 histologically confirmed cases and 210 controls with negative biopsies assembled in one university hospital (Frankfurt) and two urology practices (Frankfurt, Hamburg) (Seidler *et al.*, 1998). Lifelong job titles and frequency of exposure to occupational agents were assessed through a self-administered questionnaire, which also included questions on alcohol and coffee consumption, education and other issues. The Pannett JEM, which lists exposures to approximately 50 agents according to probability and intensity of exposure, was used for exposure estimation. A further JEM was used in a second analysis that focused only on those exposures that were significantly associated with prostate cancer in the first evaluation. In a multivariate logistic regression analysis, exposure to diesel fuels and fumes for more than 25 dose–years was significantly associated with the risk for prostate cancer (OR, 3.7; 95% CI, 1.4–9.8; 17 cases, six controls). The odds ratio for up to 25 years of exposure was close to unity. When only subjects with a high probability of exposure were considered as exposed, no cases were observed in the ≥ 25 dose–years category and an odds ratio of close to unity was found for prostate cancer and exposure to diesel fuels and fumes in the lower dose–years category. [The Working Group noted that the study, in particular the subgroup analyses, had low statistical power to detect elevated risks. While exposure to diesel fuel and fuel vapours appeared to be assessed, the occupations considered to entail exposure to these agents included drivers of road transport vehicles, motor mechanics and automobile engineers, deck and engine-room ratings, barge- and boatmen, drivers of other motor vehicles, firemen and railway engineers. Although these occupations mostly entailed exposure to exhausts to a varying extent, the exposure assessment was relatively weak, being based on job titles and external JEMs.]

In an Australian case–control study, 606 patients with prostate cancer identified through the Western Australian Cancer Registry and 471 population controls were studied with regard to occupational exposures, including diesel fumes (Fritschi *et al.*, 2007). The response rate was 57% for cancer cases and 37% for controls. Information on lifetime occupation was obtained through written questionnaires and telephone interviews on job-specific modules for a set of 14 pre-specified jobs. Using information on probability, level and duration, exposure was classified by an occupational hygienist as substantial, non-substantial or unexposed. The odds ratio for the risk of prostate cancer in association with exposure to diesel fumes was 1.07 (95% CI, 0.67–1.72) for those with substantial exposure compared with the unexposed. [The Working Group noted that this study reported data for exposure to 'diesel fumes' rather than diesel exhaust and the extent to which these categories overlap was not clear. The low response rates among both eligible cases and controls were a further limitation of this study.]

The association between pancreatic cancer and exposure to diesel and gasoline exhaust fumes was investigated in a hospital-based case–control study of occupational exposures and gastrointestinal cancers in Spain (Santibañez *et al.*, 2010). Occupational exposures among 161 cases (96 men and 65 women) and 455 frequency-matched controls with non-cancer diseases were assessed on the basis of information from interviews about the two main jobs held and through the application of the FINJEM for 21 specific chemical substances and other exposures. Only ~60% of cases were histologically confirmed. Male patients with ductal adenocarcinoma of the pancreas were more frequently employed as

HGV and light goods vehicle drivers (OR, 3.46; 95% CI, 1.01–11.83). For male cases in the highest tertile of exposure to diesel engine exhaust, the odds ratio was 1.88 (95% CI, 0.72–4.90), calculated from a multivariate model with adjustment for confounding by age, sex, hospital province, alcohol consumption, tobacco smoking and educational status. The corresponding odds ratio for exposure to gasoline engine exhaust was 1.85 (95% CI, 0.71–4.80). [The Working Group noted that the study had a high proportion of direct interviews with case patients. Some known risk factors for pancreatic cancer, such as diabetes and obesity, were not controlled for in the analyses. The sample size and number of exposed cases were small, and multiple comparisons were made, which hampered the interpretation of the positive findings.]

A case–control study of laryngeal cancer included 183 male patients from 56 hospitals on the Texas Gulf Coast (USA), diagnosed between 1975 and 1980, and 250 controls frequency-matched for age group, vital status, ethnicity (all white) and area of residence, recruited through various population sources and records (Brown *et al.*, 1988). Information on employers, job titles and duties was collected by interview. Exposure data were categorized into industrial and occupational categories and potential exposure to specific agents was coded by an industrial hygienist. In the agent-specific analyses, results were given for potential exposure to diesel/gasoline fumes. The risks for laryngeal cancer were non-significantly elevated for the occupational category of drivers. [The Working Group noted that it was unclear whether the target exposure was fumes of diesel fuel or diesel exhaust, and the study was therefore regarded as uninformative with regard to engine exhausts.]

The risk from occupational exposure to diesel fumes and exhaust was investigated in a hospital-based case–control study of 235 male cases of laryngeal cancer and 205 control patients frequency-matched for age, hospital and year of interview (Muscat & Wynder, 1995). Control patients had malignancies such as prostate cancer and lymphoma or various non-malignant diseases. The response rate was 90% for the eligible study subjects who were approached. Detailed data were collected on tobacco smoking, alcohol consumption, lifetime occupational history and self-reported exposure to occupational agents, including diesel exhaust and fumes. Jobs with known substantial exposure to diesel exhaust (HGV drivers, mine workers, fire-fighters and railroad workers) were analysed jointly and yielded a smoking-adjusted odds ratio of 0.96 (95% CI, 0.5–1.8). Self-reported exposure to diesel exhaust had no significant association with laryngeal cancer (OR, 1.47; 95% CI, 0.5–4.1), with an exposure prevalence of 5.5% for cases and 4.4% for controls. Work as an automobile mechanic yielded an odds ratio of 1.3 (95% 0.4–4.1) for laryngeal cancer. [The Working Group noted that the exposure assessment in this study was crude and, overall, the level of detail was limited; the interpretation of the results was hampered by low numbers, and residual confounding from tobacco smoking and alcohol consumption was possible.]

A larger hospital-based case–control study included 940 male cases of laryngeal cancer and 1519 controls from a referral hospital in Istanbul, Turkey (Elci *et al.*, 2003). Controls were patients with other cancers, including Hodgkin lymphoma, soft tissue sarcoma and non-melanoma skin cancer, and several non-cancer diseases. Based on a standardized personal interview, occupations and industries were coded using standard classification schemes, and exposure intensity and probabilities for diesel exhaust, gasoline exhaust and other agents were assigned by an industrial hygienist. Analyses were adjusted for age and ever consumption of alcohol and tobacco. For ever exposure to diesel exhaust, the odds ratio was 1.5 (95% CI, 1.3–1.9). Analyses that used exposure intensity and those that used exposure probability both

showed elevated odds ratios for laryngeal cancer associated with exposure to diesel exhaust; the highest risks, by a small margin, were found for the subgroup of supraglottic laryngeal cancers. When the odds ratios were adjusted for smoking pack–years, positive dose–response trends were observed for all laryngeal cancers and supraglottic cancers. For gasoline exhaust, the results were similar to those for diesel exhaust, with an odds ratio of 1.6 (95% CI, 1.3–2.0) for all laryngeal cancers. However, no cases occurred in the high-exposure intensity group exposed to gasoline exhaust. Adjustment for alcohol and tobacco consumption did not alter the results. [The Working Group noted that the strengths of this study included the comparatively large number of cases and expert assessment of exposure to diesel and gasoline exhausts. The use of hospital controls and the rather superficial assessment of potential confounders, as well as the absence of information on duration of exposure, were limitations of the study.]

A Canadian case–control study investigated the risk of non-Hodgkin lymphoma associated with various occupational exposures (Karunanayake *et al.*, 2008) in 513 male patients, diagnosed between 1991 and 1994, in six provinces and 1506 population controls frequency-matched by age. A postal questionnaire providing information on all jobs held for more than 1 year was the basis for the occupational exposure assessment. Exposure to diesel exhaust fumes showed a statistically significant odds ratio of 1.33 (95% CI, 1.03–1.67). Exposure to diesel fuel or exhaust was evaluated as a confounder in a previous analysis based on the same cases and controls, with similar results (McDuffie *et al.*, 2002). [The Working Group noted that the large number of exposures studied and the multiple tests performed, as well as the limitations of an approach that measured exposures by self-reporting, led to cautious interpretation of these results.]

In a hypothesis-generating case–control study in Sweden, Flodin *et al.* (1987) analysed the association between occupation and the risk for multiple myeloma. Cases were diagnosed between 1973 and 1983 and still alive in 1981–83, and controls were drawn randomly from population registers; 131 cases and 431 controls were available for analysis. Information on occupational history, X-ray treatment and tobacco smoking habits were obtained by a mailed questionnaire. The crude odds ratio for occupational exposure to engine exhaust was 2.3 (95% CI, 1.4–3.7); this association remained significant after adjusting for confounding variables. [The Working Group noted that a limitation of this study was the unclear descriptions of the population and the control for confounding. Exposure to engine exhausts was self-reported and not further defined by the authors, which limited the informativeness of the study.]

Boffetta *et al.* (1989) studied multiple myeloma in a case–control study nested in the American Cancer Society Prevention Study II. Study participants who had died of multiple myeloma (282 cases) were each matched to four randomly selected controls. Occupational information on current job, last occupation if retired, and any other job held for the longest period of time were assessed from a written questionnaire completed at enrolment into the cohort. Specific exposures in the workplace to any of 12 groups of substances, including diesel and gasoline exhausts, were also assessed by questionnaire. The odds ratio for exposure to diesel engine exhaust was 1.4 (95% CI, 0.7–2.7; 14 exposed incident cases, 43 controls) and that for gasoline exhaust was 0.9 (95% CI, 0.5–1.6; 14 cases). An elevated odds ratio of 6.0 (95% CI, 1.3–28.9; three cases, two controls) was found for main occupation as a railroad worker. [The Working Group noted that incident cases were enrolled and the exposure was assessed prospectively, thus eliminating recall bias; however, self-reporting of workplace exposures was a limitation. The result

for railroad workers was based on a very small number of cases, and may be a chance finding.]

Eriksson & Karlsson (1992) investigated occupational risk factors, including engine exhaust, in 275 cases of multiple myeloma (43% deceased) and an equal number of matched population controls in Sweden. Jobs held and tasks associated with exposure to specific chemicals were assessed from mailed questionnaires, together with information on tobacco smoking habits and various other exposures. No increases in risk were found for occupation as a railroad worker or motor vehicle driver. Exposure to engine exhaust was assessed based on work with tractors, power saws or as a driver. The odds ratio for exposure to engine exhaust was 1.11 (95% CI, 0.60–2.05) in a multivariate analysis. [The Working Group noted that the exposure assessment was limited with regard to engine exhausts, being based on occupational titles, and did not differentiate between diesel and gasoline exhausts.]

Two reports from Denmark described the results of a large population-based case–control study on occupational exposures and multiple myeloma. Heineman *et al.* (1992) reported 1098 male cases diagnosed between 1970 and 1984 and 4169 age-matched male controls. Pottern *et al.* (1992) performed an analysis of 1010 women with multiple myeloma and 4040 age-matched female controls. Cases were identified from the Danish Cancer Registry, and controls were drawn from the population registry. Information on employment was retrieved from the pension fund and occupational titles were taken from tax forms held at the population registry. This information was used to assess exposure to 20 broad categories of substances and 27 specific substances by industrial hygienists. Pottern *et al.* (1992) reported odds ratios of 1.4 (95% CI, 0.6–3.2; eight cases) for possible exposure to exhaust and 1.6 (95% CI, 0.4–5.5; four cases) for probable exposure to exhaust among women. Among men, the odds ratios were 1.3 (95% CI, 1.0–1.6; 125 cases) for possible and 1.2 (95% CI, 0.9–1.6; 89 cases) for probable exposure to engine exhaust. Lagging of exposure and stratification by duration of exposure (< 5 years versus ≥ 5 years) showed some evidence of a dose–response for duration of exposure, but the risks were lower for those probably exposed compared with those possibly exposed. When men with at least 5 years of employment were considered separately, exposure to engine exhaust with a 10-year lag was associated with elevated risks for possible exposure, but no elevation for probable exposure in either category of exposure duration, showing no evidence of a dose–response in this analysis. [The Working Group noted that the overall number of cases was relatively large in these studies, but few cases were available for specific comparisons. Occupational information was not reported for 10% of male cases (15% of controls) and 40% of female cases (36% of controls). It was not clear which occupations among women entailed exposure to engine exhaust. No information on confounders beyond the matching factors and other jobs was available, and exposure misclassification may have been a problem in these studies due to the sources of information used.]

Demers *et al.* (1993) conducted a case–control study of 692 cases of multiple myeloma and 1683 population controls recruited between 1977 and 1981 in Washington, Atlanta, Utah and Detroit, USA. Lifetime work histories were collected during personal interviews. Results were reported by occupation and industry group. Rail and water transport workers had an odds ratio of 0.3 (95% CI, 0.1–1.1) and motor vehicle operators had an odds ratio of 1.2 (95% CI, 0.8–1.6). No elevations in risk were found for other occupational groups with some potential for exposure to engine exhaust. [The Working Group noted that the study was limited by the use of occupational/industry titles, and the large number of risk estimates presented. No specific results pertaining to engine exhaust were provided.]

In a Swedish study that used the same source of cases, the same set of 431 controls and the

same methods as those described in Flodin *et al.* (1987), Flodin *et al.* (1988) investigated the association between risk and occupational exposures in 111 cases of chronic 'lymphatic' (lymphocytic) leukaemia. The crude odds ratio for occupational exposure to engine exhausts was 2.5 (95% CI, 1.5–4.0); the association remained significant after adjustment for confounding variables.

In another study in Sweden, 125 patients with acute leukaemia and the same number of population controls were included in a study of the association between exposure to petroleum products and leukaemia (Lindquist *et al.*, 1991). Cases were diagnosed between 1980 and 1983 in five participating Swedish hospitals. Personal interviews to obtain information on environmental and professional exposures to petroleum products, including gasoline and diesel fuels and exhausts, and a checklist of occupations were conducted by one investigator. For the occupational group of professional drivers and rally drivers combined, an odds ratio of 3.0 (95% CI, 1.2–8.4; 21 cases) was estimated. Adjustment for exposure to solvents, radiation treatment and tobacco smoking did not change the results. [The Working Group noted that the study used a very short minimum duration of exposure (1 month) and relied on a crude exposure assessment based on occupational titles. Types of exhaust could not be evaluated separately, and the study therefore contributed little to an assessment of the risks of exposure to exhaust.]

Cases of hairy cell leukaemia were recruited from 18 hospitals in France in a hospital-based case–control study to investigate the risks associated with occupational exposures and tobacco smoking (Clavel *et al.*, 1995). Living cases first diagnosed between 1980 and 1990 were included, and controls were retrospectively selected from admission records mostly from orthopaedic and rheumatology departments in the same hospitals. Two controls per case were matched by date of birth, gender, area of residence and date of admission. The analysis set included 229 live male patients (425 controls) and 62 live female patients (116 controls). The response rate was 61% for cases and 58% for controls. Exposure to engine exhaust was associated with a risk for leukaemia (OR, 1.6; 95% CI, 1.0–2.6) among men. Results for exposure to exhaust for women were not reported. [The Working Group noted that exposure to gasoline and diesel exhaust could not be separated further in the study. The exclusion of deceased cases could have led to survivor bias. In addition, the choice of hospital controls was a limitation of this study.]

Blair *et al.* (2001) carried out a study of 513 cases of leukaemia and 1087 frequency-matched population controls in Iowa and Minnesota, USA, in 1980–83. The main focus was to evaluate the risks associated with agriculture. Personal interviews were conducted to obtain lifetime occupational histories, and selected exposures were evaluated using a JEM. Next of kin were interviewed as surrogates for ~40% of cases who were deceased or too ill to be interviewed (similar for controls). Increases in the risk for leukaemia were associated with driving light goods vehicles (OR, 3.4; 95% CI, 1.4–8.4; 13 exposed cases). Exposure to motor vapours and exhausts was associated with odds ratios of 1.3 (95% CI, 0.9–1.9) in the group considered to be highly exposed and 1.0 (95% CI, 0.8–1.2) in the 'low' exposure group. Analyses by different histological types also showed no significant increase in risk associated with these exposures. [The Working Group noted that the study used a detailed exposure assessment; however, information on exposure to engine exhausts was limited. Potential confounders, such as age, type of interview and education, were adjusted for. The number of cases of different histological types of leukaemia for specific exposures was small.]

Seniori Costantini *et al.* (2001) studied occupational risk factors for haematolymphopoietic malignancies in Italy among 2737 cases diagnosed between 1991 and 1993 (652 cases of leukaemia) and 1779 population controls from 12 areas,

who were interviewed to obtain information on occupational history and specific exposures. The results were reported by occupation. Groups with potential exposure to exhaust included transport operators (OR for leukaemia, 1.1; 95% CI, 0.7–1.7) and material handling and related equipment operators, dockers and freight handlers (OR for leukaemia, 2.5; 95% CI, 1.4–4.5). No significant risks were noted for these occupations in relation to non-Hodgkin lymphoma, multiple myeloma or Hodgkin lymphoma. [The Working Group noted that no specific information regarding exposures to exhaust was presented, and that the occupational groupings were rather broad.]

A population-based case–control study from Shanghai, China, included 486 cases of leukaemia and 502 controls (Adegoke *et al.*, 2003). Information was collected on jobs held and exposure to certain agents, but not specifically to engine exhausts. No increases in risk were seen for occupations that potentially involved some exposure to exhaust (water and rail transport, and drivers). The odds ratio for automobile and HGV drivers was 0.8 (95% CI, 0.3–2.0; eight exposed cases). [The Working Group noted that the exposure assessment in this study was crude.]

Acute myeloid leukaemia subtypes and their association with specific occupational and environmental exposures were investigated in a hospital-based case–control study of 722 case patients and 1444 age- and gender-matched controls from 29 hospitals in Shanghai, China (Wong *et al.*, 2010). Cases and controls were diagnosed in the period 2003–07; controls with malignant or non-malignant diseases of the lymphatic or haematopoietic system were not eligible. The participation rate was ~97% for cases but was not reported for the control group. Personal interviews were conducted to obtain data on occupational exposures and employment history. These data were categorized according to the Chinese standard classification system, and exposure assessment was performed by experts familiar with workplace exposures in Shanghai. Among the large number of risk estimates reported, exposure to diesel or gasoline engine exhaust was very rare and the odds ratio for acute myeloid leukaemia was 0.57 (95% CI, 0.12–2.75; two exposed cases). [The Working Group noted that there was concern about the large number of associations investigated, as well as the choice of hospital controls. Only two cases had been exposed to engine exhaust, which limited the interpretation of the results.]

West *et al.* (1995) studied 400 cases of myelodysplasia and an equal number of hospital or outpatient controls in Wales, Wessex and Yorkshire, United Kingdom [exact period of recruitment not specified]. A lifetime occupational history and exposure to specific chemicals or potential hazards were elicited by questionnaires and interviews. Occupational histories and self-reported exposures were reviewed by hygiene and chemical experts. For exposure to exhaust gases, an odds ratio of 1.26 (95% CI, 0.86–1.86; 63 exposed cases) was estimated. For reported exposures of > 2500 hours and greater than medium intensity, the odds ratio was 1.57 (95% CI, 0.77–3.23), and was slightly lower than that for the group with less intense exposure. [The Working Group noted that the study used an exposure assessment that relied mainly on self-reporting with some further review by experts. Some details, including the statistical analysis, were not adequately described, and potential confounders related to occupation and lifestyle were not assessed. No evidence of a dose–response relationship and no significant increases in risk were found seen for myelodysplasia.]

2.4 Meta-analyses

2.4.1 Cancer of the lung

Bhatia *et al.* (1998) conducted a meta-analysis of occupational exposure to diesel exhaust and the risk for lung cancer using data from 23 case–control and cohort studies that met the

inclusion criteria of adequate latency – at least 10 years from first exposure to diesel exhaust to the end of follow-up (for at least some of the subjects) – and for which work with diesel engine equipment could be confirmed or inferred. Studies of miners were excluded because of potential confounding by exposure to carcinogens such as radon and silica for certain types of miner. Tobacco smoking-adjusted risk estimates were used whenever available and, when risks were reported by duration of exposure, the longest duration category was used in the analysis. Standardized mortality ratios for lung cancer in cohort studies with external referent groups were adjusted by the all-cause standardized mortality ratio to account for the healthy-worker effect. Combined estimates were calculated using a fixed-effects model for all studies and for subsets of studies grouped by (1) study design, (2) type of comparison group for cohort studies, (3) adjustment for tobacco smoking and (4) occupation, including railroad workers, equipment operators, HGV drivers and bus drivers. Confidence intervals were adjusted for heterogeneity. A random-effects model was not used because the underlying assumption that interstudy variance was constant was unlikely to be met. The relative risk for all studies was 1.33 (95% CI, 1.24–1.44); and similar results were obtained when studies were stratified by study design (case–control and cohort) or adjustment for smoking (e.g. a significant 30% excess risk was found for studies that adjusted for smoking and for studies that did not adjust for smoking; see Table 2.6). Summary estimates were higher for cohort studies that used an internal comparison group (RR, 1.43; 95% CI, 1.29–1.58) than for those that used an external comparison group (RR, 1.22; 95% CI, 1.04–1.44). Estimates also varied among occupational groups. Evidence for heterogeneity was found for all studies combined, all cohort studies and all case–control studies, but was reduced after stratification by occupation. Greater heterogeneity was observed for cohort studies that used external comparisons compared with those that used internal comparisons. Six of seven studies (all using internal referent groups) that evaluated duration of exposure found a positive exposure–response relationship. Funnel chart analysis showed little evidence of publication bias (Bhatia *et al.*, 1998).

Another meta-analysis of lung cancer and occupational exposure to diesel exhaust (Lipsett & Campleman, 1999) included studies that reported risk estimates and confidence intervals or adequate data to obtain them, had an adequate latency for the development of lung cancer after the onset of exposure (≥ 10 years) and adequate case ascertainment, and were independent. Studies of miners were excluded because of potential confounding by exposure to carcinogens such as radon, arsenic and silica. Risk estimates for the highest level or duration of exposure for occupations with the most specific exposure to diesel and adjustment for tobacco smoking were used when available. A random-effects model was designed for the meta-analyses, which were based on 39 effect estimates from 30 studies. Subset analyses were performed for studies that could be grouped in relation to several characteristics, including study design, adjustment for tobacco smoking, occupational category, referent group, latency, duration of exposure, selection methods, year of publication, covariates controlled for in the analysis and the presence of a healthy-worker effect. Sensitivity and influence analyses were also conducted. Similar to that reported by Bhatia *et al.* (1998), the overall summary risk estimate was 1.33 (95% CI, 1.21–1.46), which showed significant heterogeneity. Increased risk estimates and lower heterogeneity were observed for studies that adjusted for smoking (RR, 1.43 versus 1.25 for studies that did not adjust for smoking), had a lower risk of selection bias (i.e. did not show evidence of a healthy-worker effect; RR, 1.52 versus 1.06 for cohort studies with clear evidence of a healthy-worker effect) and used internal controls (RR, 1.48 versus 1.14

Table 2.6 Meta-analyses of cohort and case–control studies of cancer and exposure to engine exhaust

Reference	Organ site	Exposure	No. of estimates	Relative risk (adjusted 95% CI)	Heterogeneity *P*-value	Covariates Comments
Bhatia *et al.* (1998)	Lung	All studies	29	1.33 (1.24–1.44)	58	Fixed-effects model; χ^2 for heterogeneity
		All exposed occupations	24	1.37 (1.27–1.49)	48.4	
		Railroad workers	6	1.44 (1.30–1.60)	5.6	
		Equipment operators	3	1.11 (0.89–1.38)	4.3	
		HGV drivers	10	1.49 (1.36–1.65)	9.8	
		Bus workers	5	1.24 (0.93–1.64)	14.8	
		Tobacco smoking-adjusted	16	1.35 (1.20–1.52)	23.4	
		Not adjusted for tobacco smoking	13	1.33 (1.20–1.47	34.5	
Lipsett & Campleman (1999)	Lung	All studies	39	1.33 (1.21–1.46)	0.001	Random-effects model
		Railroad workers	6	1.45 (1.08–1.93/)	< 0.001	
		Heavy equipment operators /dock workers	4	1.28 (0.99–1.66)	0.046	
		HGV drivers	9	1.47 (1.33–1.63)	0.398	
		Mechanics/garage workers	6	1.35 (1.03–1.78)	0.010	
		Professional drivers/ transportation operatives	6	1.45 (1.31–1.60)	0.716	
		Diesel exhaust	5/5	0.97 (0.95–1.00)	0.585	
Boffetta & Silverman (2001)	Urinary bladder	Heavy equipment operators	5	1.37 (1.05–1.81)	0.6	Only studies with ≥ 5 yr latency; fixed-effects model; evidence of publication bias for all studies and HGV and bus drivers
		HGV drivers	15	1.17 (1.06–1.29)	0.3	
		Bus drivers	10	1.33 (1.22–1.45)	0.4	
		JEM for diesel	10	1.13 (1.00–1.27)	0.3	
		Any exposure to diesel exhaust	12	1.23 (1.12–1.36)	0.5	
		High exposure to diesel exhaust	12	1.44 (1.18–1.76)	0.1	
Manju *et al.* (2009)	Urinary bladder	**HGV drivers**				Case–control studies with no adjustment for tobacco smoking excluded; fixed-effects model
		Overall	17	1.18 (1.09–1.28)	0.25	
		Yr of publication				
		1977–87		1.30 (1.16–1.46)		
		1998–2008		1.20 (1.00–1.40)		
		Bus drivers				
		Overall	9	1.23 (1.06–1.44)	0.25	

Table 2.6 (continued)

Reference	Organ site	Exposure	No. of estimates	Relative risk (adjusted 95% CI)	Heterogeneity *P*-value	Covariates Comments
		Yr of publication				
Manju et al. (2009) (cont.)		1977–87		1.30 (1.10–1.53)		
		1998–2008		1.21 (0.72–2.01)		
		Railroad worker				
		Overall	14	1.20 (1.02–1.41)	0.31	
		Yr of publication				
		1977–87		1.33 (0.98–1.54)		
		1998–2008		1.25 (0.96–1.61)		
		Other motor vehicle drivers	10	1.11 (0.99–1.23)	0.11	
Reulen *et al.* (2008)	Urinary bladder	**All studies**				Random-effects model
		Car, taxi and van drivers	9	1.20 (1.03–1.39)	NR	
		Bus drivers	8	1.29 (1.08–1.53)	NR	
		HGV drivers	15	1.18 (1.06–1.33)	NR	
		Motor vehicle drivers	32	1.11 (1.06–1.17)	NR	
		Adjusted for tobacco smoking				
		Car, taxi and van drivers	NR	1.20 (0.99-1.46)	NR	
		Bus drivers	NR	0.96 (0.65-1.42)	NR	
		HGV drivers	NR	1.18 (1.06-1.31)	NR	
		Motor vehicle drivers	NR	1.11 (1.04-1.18)	NR	
		Not adjusted for tobacco smoking				
		Car, taxi and van drivers	NR	1.27 (0.83-1.93)		
		Bus drivers	NR	1.39 (1.22-1.58)		
		HGV drivers	NR	1.45 (0.83-2.53)		
		Motor vehicle drivers	NR	1.14 (1.02-1.27)		

CI, confidence interval; HGV, heavy goods vehicle; JEM, job–exposure matrix; NR, not reported; yr, year

for studies that used national rates). Among the studies that adjusted for smoking, some evidence of an exposure–response relationship with duration of exposure was found; a higher summary estimate was observed for ≥ 10 years of duration (RR, 1.64 versus 1.39 for < 10 years of duration). Sensitivity or influence analyses did not substantially alter the results. In meta-regression analyses of the case–control studies, higher risks were reported in studies that were published after 1989 compared with those published before 1989. No systematic relationship was observed between study size and the magnitude of risk in funnel plot analyses.

Mahjub & Sadri (2006) conducted a meta-analysis of case–control studies of lung cancer that evaluated occupational or environmental pollutants, including exposure to motor and diesel exhaust. Case–control studies included in the analysis were published in English during 1990–2006 and focused on environmental or occupational exposures. Risk estimates from 12 studies were summarized using a random-effects model. Summary risk estimates were 1.42 (95% CI, 1.26–1.59) for exposure to 'motor and diesel exhaust'. [The Working Group noted that the restricted focus of this meta-analysis limited its usefulness to address cancer hazard identification for exposure to diesel exhaust.]

Tsoi & Tse (2012) conducted a systematic review of studies of professional drivers potentially exposed to diesel exhaust that were published in English from 1996 to 2011 and reported data on risks for lung cancer. The review included 19 cohort and case–control studies (seven historical cohorts, one prospective cohort and 11 case–control studies) of workers who were bus, taxi, HGV and mixed or unspecified drivers. The combined relative risk was 1.21 (95% CI, 1.10–1.32; *P* for heterogeneity < 0.01) for all studies and 1.22 (95% CI, 1.09–1.36; *P* for heterogeneity = 0.02) for higher-quality studies. Funnel plot analysis revealed no evidence of publication bias. [The Working Group noted that this meta-analysis provided little new information regarding exposure to diesel exhaust and the risk for lung cancer because it focused on professional drivers and did not consider exposure assessment in the quality evaluation.]

2.4.2 Cancer of the urinary bladder

Boffetta & Silverman (2001) conducted a meta-analysis of exposure to diesel exhaust and the risk for urinary bladder cancer. The analysis included studies of exposure to diesel exhaust based on JEMs or expert assessments of individual occupational histories for five occupational groups: railroad workers, bus garage maintenance workers, HGV drivers, bus drivers and operators of heavy construction machines. Only studies with at least 5 years between the first exposure to diesel engines or equipment and the development of urinary bladder cancer were included in the final analysis, which included seven cohort studies, 16 case–control studies and six studies of routinely collected data. Most of the case–control studies adjusted for tobacco smoking. Risk estimates were not calculated for all studies combined or for studies of railroad workers because of heterogeneity (*P* for heterogeneity = 0.002 and 0.02, respectively), or for garage workers because the number of studies was too small. An indication of an excess risk of urinary bladder cancer was found for the other occupational groups (OR, 1.13–1.37). Evidence of publication bias was found for studies of bus and HGV drivers, and for the entire set of studies. Although studies that did not adjust for smoking were included in the analysis, in general, those that adjusted for smoking did not have lower risk estimates, and smoking did not explain the heterogeneity of the results. The authors also combined eight relative risks for high exposure to diesel exhaust, most of which were for the longest duration of employment in a specific occupation with potential exposure to diesel exhaust. The summary relative risks were 1.23 (95% CI,

1.12–1.36) for any exposure and 1.44 (95% CI, 1.18–1.76) for high exposure to diesel exhaust.

The risk of urinary bladder cancer among motor vehicle drivers was evaluated in a meta-analysis of data reported in 30 studies, including 27 case–control studies and three cohort studies, published from 1977 to 2008 (Manju *et al.*, 2009). Studies excluded from the analysis were case–control studies that did not adjust for tobacco smoking and cohort studies that did not provide numbers of observed and expected cases. For cohort studies, a pooled observed/expected ratio for urinary bladder cancer was calculated by summarizing the observed and expected numbers of events across studies. No overall summary estimate was calculated for case–control studies because of significant heterogeneity in the results. However, summary odds ratios and corresponding 95% confidence intervals were calculated as a weighted average of odds ratios for occupational groups and subsets of studies stratified by publication date. The pooled observed/expected ratio for all cohort studies of motor vehicle drivers and railroad workers was 1.08 (95% CI, 1.00–1.17). Increased risks were observed for each of the occupational groups, and there was some evidence that risks were lower for HGV, bus drivers, and railroad workers in more recent publications (1998–2008) than in earlier publications (1977–87). Funnel chart analysis suggested some evidence of publication bias; however, some studies were excluded from the analysis because of the unavailability of case numbers.

Reulen *et al.* (2008) combined data from 130 studies (66 cohort and 64 case–control studies) that evaluated occupation and the risk for urinary bladder cancer. Occupations were coded and grouped together using the International Classification of Occupations. For each occupation, a random-effects model was used to calculate summary relative risks and 95% confidence intervals. Statistically significant summary relative risks were observed for several occupations, which have been associated with potential exposure to diesel or motor exhaust (see Table 2.6). Stratified analyses were performed for study design (cohort versus case–control), tobacco smoking status and publication date. Summary relative risks (for diesel-related occupations) did not significantly differ when stratified by study design (cohort versus case–control) or smoking status; however, the summary relative risks for HGV and bus drivers were somewhat higher in studies that did not control for smoking compared with those that did. The authors also stratified by the time period during which the study was conducted (before 1980, 1980–90 and after 1999); no significant linear trends were observed, although risk estimates were generally lower for studies of all occupations, except motor vehicle drivers, conducted after 1990. [The Working Group noted that this study calculated risk estimates for many different types of occupation, of which only a few were associated with potential exposure to diesel fuel; thus the specificity of exposure was limited and multiple comparisons may have been a concern.]

2.4.3 Cancer of the pancreas

A meta-risk ratio of pancreatic cancer with exposure to diesel exhaust was calculated as part of a larger meta-analysis of pancreatic cancer and exposure to 23 chemicals present in the workplace (Ojajärvi *et al.*, 2000). Relative risks for exposure to diesel exhaust or job titles with verified exposure to diesel exhaust from seven studies were combined using a simple random-effects model. When available, relative risks that were adjusted for confounders and social class, and had a latency period closest to 20 years were used in the analysis. No excess risk for pancreatic cancer was found for exposure to diesel exhaust (meta-risk ratio, 1.0; 95% CI, 0.9–1.20).

2.4.4 Summary of meta-analyses

The meta-analyses found consistent elevated risk estimates for occupations associated with exposure to diesel exhaust and lung cancer and urinary bladder cancer, but not for pancreatic cancer, although fewer data were available for the latter. The analyses for lung cancer by Bhatia *et al.* (1998), Lipsett & Campleman (1999) and Tsoi & Tse (2012), and those for urinary bladder cancer by Boffetta & Silverman (2001) found significant heterogeneity for the overall results, which was explained in part by the type of occupation. (Boffetta & Silverman, 2001) did not calculate an overall risk estimate because of the significant heterogeneity.) The major limitation of the meta-analyses was the paucity of data relating job titles to exposure to diesel exhaust, and the inclusion of studies with varying quality of exposure information. Moreover, most of the individual studies included in the analyses evaluated risks for occupations and not for exposure to diesel exhaust, and the most recent meta-analyses (Reulen *et al.*, 2008; Manju *et al.*, 2009) included only studies that evaluated risk according to occupation.

A further limitation of the earlier studies was their inclusion of subjects who worked before or at the beginning of the diesel era or workers with a latency period that was inadequate to attribute any increases in lung cancer to exposure to diesel exhaust.

Most of the meta-analyses included studies that did not adjust for tobacco smoking, but the analyses were stratified by this factor. In general, meta-risk estimates for studies that did not adjust for smoking were similar to those that did and meta-risk estimates for lung cancer from cohort studies that used internal comparison groups, which should have reduced the potential for confounding from smoking, were higher than the those from studies that used an external comparison group (Bhatia *et al.*, 1998; Lipsett & Campleman, 1999).

2.5 Studies of childhood cancer

See Table 2.7

Studies have been carried out to examine hypotheses that the risk of childhood cancer is associated with exposure to engine exhausts, including parental exposure that results in germ cell mutations, direct intrauterine exposure of the fetus or early postnatal exposure. A common limitation of these studies was the non-specific assessment of exposure, which was often based on records of parental job titles without a more specific assessment of exposure to engine exhaust. Studies have also been conducted on traffic-related air pollution and childhood cancer, but these were not considered to be informative and are not reviewed here.

In a case–control study in Québec, Canada (Fabia & Thuy, 1974), occupation of the father at the time of birth was ascertained from the birth certificates of 386 children who had died from malignant disease before the age of 5 years in 1965–70. The study included 772 control children whose birth registration immediately preceded or followed that of the case in the official records. The occupation of the father was not known for 30 cases or 56 controls. Paternal occupation was recorded as motor vehicle mechanic or service station attendant for 29 (7.5%) cases and 29 (3.8%) controls and as driver for 19 (4.9%) cases and 49 (6.4%) controls. [Odds ratios were not reported, but crude odds ratios calculated by the Working Group were 2.1 (95% CI, 1.2–3.4) for mechanics/service station attendants and 0.76 (95% CI, 0.4–1.3) for drivers. The Working Group noted that no cancer-specific analyses were conducted and the study was limited in terms of details of exposure.]

In a case–control study in Finland (Hakulinen *et al.*, 1976), all 1409 incident cases of cancer in children under 15 years of age reported to the Finnish Cancer Registry in 1959–68 were ascertained. Paternal occupation was obtained from antenatal clinic records for the first trimester of

Table 2.7 Studies of childhood cancer and exposure to engine exhaust

Reference Location, period	Total No. of cases/controls	Source of controls	Exposure assessment	Organ site	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Fabia & Thuy (1974) Quebec, Canada, 1965–70	386/772	Population	Occupation of father on birth certificate	All childhood cancers	**Registered occupation**		**Crude OR**	Controls matched by birth date, no exposure details; specific sites/cancer types not assessed; ORs NR: crude ORs calculated by the Working Group
					Motor vehicle mechanic/service station attendant	29	[2.1 (1.2–3.4)]	
					Driver	19	[0.76 (0.4–1.3)]	
Hakulinen *et al.* (1976) Finland, 1959–68	852/852	Population	Occupation of father on antenatal records		**Registered occupation**			Only 60% of cases with suitable information; controls matched by birth date and district; no details of exposure
				Leukaemia (age < 5 yr)	Driver	14	0.74 (0.34–1.6)	
				Leukaemia and lymphoma	Driver	35	1.06 (0.63–1.8)	
				Brain tumour (age < 5 yr)	Driver	4	0.17 (0.0–1.4)	
				Brain tumour	Driver	16	0.67 (0.29–1.5)	
Kantor *et al.* (1979) Connecticut, USA, 1935–73	149/149	Population	Occupation of father on birth certificate	Wilms tumour	**Registered occupation**		**Crude OR**	Controls matched by sex, race, birth yr; no details of exposure; ORs NR; crude ORs calculated by the Working Group
					Driver	8	[2.1 (0.6–6.7)]	
					Motor vehicle mechanic	6	[6.2 (0.8–49.8)]	
					Service station attendant	3	NR (no exposed controls)	
Kwa & Fine (1980) Massachusetts, USA, 1947–57 and 1963–67	692/1384	Population	Occupation of father on birth certificate		**Registered occupation**		**Crude OR**	Controls matched by birth date; ORs NR: crude ORs calculated by the Working Group; no details of exposure
				Leukaemia/ lymphoma	Mechanic/service station attendant	21 deaths	[1.1 (0.7–1.5)]	
				Neurological	Mechanic/service station attendant	6 deaths	[1.02 (0.4–2.4)]	
				Urinary tract	Mechanic/service station attendant	4 deaths	[2.9 (1.0–8.9)]	

Table 2.7 (continued)

Reference Location, period	Total No. of cases/controls	Source of controls	Exposure assessment	Organ site	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Zack *et al.* (1980), Houston, TX, USA, 1976–77	296/566/838/465	Hospital/ parents/ siblings and neighbourhood	Personal interview with parents on job history in yr before birth	All childhood cancers	**Paternal occupation as mechanic/service station attendant/driver**			No exposure details, choice of controls not clear; OR calculated by the Working Group from Table 1 in the publication; details of maternal occupation NR
					Control uncles	12	[0.79 (0.38–1.63)]	
					Neighbourhood controls	12	[0.92 (0.40–2.17)]	
					Hospital controls	12	[0.59 (0.28–1.23)]	
Hemminki *et al.* (1981), Finland, 59–1975	948/1892	Population	Occupation of parents from on welfare centre records		**Registered occupation as driver**			Extension of Hakulinen *et al.* (1976)
				Leukaemia		96	1.50 ($P < 0.10$)	
				Brain		84	0.92 ($P > 0.10$)	
				All cancers		303	1.25 ($P < 0.10$)	
Gold *et al.* (1982), Maryland, USA, 1965–74	43 leukaemia, 70 brain tumour/43 and 70 population, 43 and 70 hospital	Population and hospital	Personal interview with mother		**Paternal occupation as mechanic/service station attendant/driver before birth of index child**			Information for occupation before birth missing for 16–40% of cases; controls matched on sex, race, age and date of diagnosis (cancer controls only); ORs and 95% CIs NR.
				Leukaemia		3	$P < 0.05$ for cancer or population controls	
				Brain tumour		3	$P > 0.05$ for cancer and population controls	
Vianna *et al.* (1984), New York, USA, 1949–78	60 leukaemia, 103 brain tumour/60 and 103	Population	Personal interview with mother	Leukaemia	**Paternal job group with presumed exposure to exhaust (≥ 1 yr before birth)**			Controls matched on birth yr, sex, race and county; jobs grouped according to presumed exposure level
					High exposure		2.4 [1.1–3.7]	
					Low exposure		1.3 [0.8–2.1]	
Wilkins & Sinks (1984), Columbus,Ohio, USA, 1950–1981	62/124	Population	Occupation of father on birth certificate	Wilms tumour	Paternal occupation as mechanic/service station attendant/ driver/metal worker	5	1.37 (0.59–3.11) compared with 2 controls	Controls matched on sex, race birth yr and county; incomplete records of paternal occupation; only 62 pairs for analysis

Table 2.7 (continued)

Reference Location, period	Total No. of cases/controls	Source of controls	Exposure assessment	Organ site	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
van Steensel-Moll *et al.* (1985), Netherlands, 1973–80	519/507	Population	Postal questionnaire on occupations before and during pregnancy	Acute lymphatic leukaemia	**Hydrocarbon-related occupations OR**			Matching on age, gender place of residence, social class and birth order; response rate: 88% cases, 66–67% controls; hydrocarbon-related group included broad range of occupations
					Mother	7	2.5 (0.7–9.4)	
					Father	37	1.0 (0.6–1.7)	
					Exposure to exhaust during pregnancy			
					Mother	4	NR	
					Father	89	1.3 (0.8–1.9)	
Cordier *et al.* (2001), Australia, Canada, France, Israel, Italy, Spain, USA, 76–1994 (differed by centre)	1218/2223	Population	Personal interview with parents on occupational history 5 yr before diagnosis; JEM	Brain	Paternal occupation as driver	127	1.3 (1.0–1.7)	Centre, age of child, yr of birth and gender; response rate: 75% cases and controls
					Paternal occupation as mechanic	45	1.5 (1.0–2.3)	
McKinney *et al.* (2003), United Kingdom, 1991–96	3838/7629	Population	Personal interview with parents including full occupational history; JEM	Leukaemia	**Periconceptual exposure to exhaust**			Age of child, gender and region of residence; response rate: 87% cases, 64% controls
					Mother	9	1.58 (0.74–3.40)	
					Father	147	1.33 (1.09–1.61)	
					Periconceptual occupation as driver			
					Mother	5	1.48 (0.53–4.13)	
					Father	121	1.36 (1.10–1.68)	
				Central nervous system	**Periconceptual exposure to exhaust**			
					Mother	3	1.33 (0.40–4.42)	
					Father	47	1.08 (0.79–1.47)	
					Periconceptual occupation as driver			
					Mother	1	0.85 (0.11–6.50)	
					Father	36	1.04 (0.73–1.48)	

Table 2.7 (continued)

Reference Location, period	Total No. of cases/controls	Source of controls	Exposure assessment	Organ site	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Reid *et al.* (2011) Australia, 2003–06	511/2071	Population	Parental occupation, job-specific modules; expert assessment	Acute lympho-blastic leukaemia	**Prenatal and postnatal paternal/maternal exposure**			Sex (child), age at diagnosis, socioeconomic status, smoking, alcohol consumption, age (mother) and state
					Moderate/substantial pre-birth exhaust exposure combined			
					Mother	17	1.97 (0.99–3.90)	
					Diesel only	14	2.06 (0.97–4.39)	
					Petrol only	7	1.59 (0.58–4.41)	
					Father	127	1.37 (1.01–1.86)	
					Diesel only	101	1.24 (0.91–1.69)	
					Petrol only	48	1.20 (0.82–1.77)	

CI, confidence interval; JEM, job–exposure matrix; NR, not reported; OR, odds ratio; yr, year

pregnancy. After excluding twins and cases for which the paternal occupation was unobtainable, 852 cases were available for analysis. For each case, a child whose date of birth was immediately before that of the case and who had been born in the same maternity welfare district was chosen as a control. Leukaemias and lymphomas (339 pairs; 158 under 5 years of age), brain tumours (219 pairs; 77 under 5 years of age) and other tumours (294 pairs; 160 under 5 years of age) were analysed separately; analyses were carried out for the whole group and for children under 5 years of age at the time of diagnosis. Paternal occupation as a motor vehicle driver was not more frequent in any group of cases than in controls: the odds ratio for paternal occupation as a driver was 0.74 (95% CI, 0.34–1.6) for leukaemia in children under 5 years, 1.1 (95% CI, 0.63–1.8) for leukaemia and lymphoma in the whole group, 0.17 (95% CI, 0.00–1.4; four cases) for brain tumours in children under 5 years and 0.67 (95% CI, 0.29–1.5) for brain tumours in the whole group. [The Working Group noted that the study had several limitations: only 60% of cases were available for analysis due to the lack of records or information on paternal occupation, the exposure assessment was crude and the odds ratios were not adjusted for other risk factors.]

In a case–control study in Connecticut, USA (Kantor *et al.*, 1979), paternal occupation was ascertained from birth certificates for all 149 cases of Wilms tumour (aged 0–19 years) reported to the Connecticut Tumor Registry in 1935–73 and for 149 controls selected from State Health Department files and matched for sex, race and year of birth. The paternal occupation was recorded as driver for eight cases and four controls [OR, 2.1; 95% CI, 0.6–6.7], as motor vehicle mechanic for six cases and one control [OR, 6.2; 95% CI, 0.8–49.8] and as service station attendant for three cases and no control. [Odds ratios were not reported; crude odds ratios were calculated by the Working Group. The Working Group noted that the number of exposed cases in this study was small, the exposure assessment was crude and other risk factors were not taken into account. The study was therefore judged to be not very informative.]

In a case–control study of the association between paternal occupation and childhood cancer (Kwa & Fine, 1980), 692 children born in 1947–57 or 1963–67 and who had died of cancer before the age of 15 years in Massachusetts, USA, were identified from the National Center for Health Statistics. Two controls were selected from the registry of births for each case – one born immediately before the case and the other immediately after. Paternal occupation was taken from birth certificates and classified into one of nine categories on the basis of the type of chemical exposures involved. Mechanic/service station attendant was recorded as the paternal occupation for 21 cases of leukaemia/lymphoma [OR, 1.1; 95% CI, 0.7–1.5], six cases of neurological cancer [OR, 1.02; 95% CI, 0.4–2.4], four cases of urinary tract cancer [OR, 2.9; 95% CI, 1.0–8.1], four cases of all other cancers [OR, 0.93; 95% CI, 0.34–2.6] and 61 controls. In the children of fathers who were motor vehicle drivers, no excess of leukaemia/lymphoma, neurological cancer, urinary tract cancer or all other cancers was observed. [The Working Group noted that, as in the other studies with a similar approach, the nature of the exposure assessment allowed only limited inference of an association of parental exposures to engine exhaust with subsequent childhood cancer. In addition, no risk factors other than date of birth were taken into account in the analysis.]

In a case–control study of associations between childhood cancer and parental occupation (Zack *et al.*, 1980), the parents of 296 children with cancer followed at a haematology clinic in Houston, Texas, USA, from March 1976 to December 1977 and three sets of controls were interviewed to obtain demographic information and job history from the year preceding the birth of the child until the diagnosis of cancer. The

first set of controls comprised 283 fathers and stepfathers and 283 mothers and stepmothers of children without cancer in the same clinic; the second set comprised siblings of the parents of the case (413 uncles and 425 aunts), matched by age and number of children; and the third set was selected from among residents in the neighbourhood of the cases (228 fathers and 237 mothers). The proportion of cases with paternal occupation as a motor vehicle mechanic, service station attendant or driver did not differ substantially from that in any control group (crude OR, 0.59; 95% CI, 0.28–1.23 in comparison with fathers' siblings; 0.79; 95% CI, 0.38–1.63 in comparison with the fathers of cancer cases; and 0.92; 95% CI, 0.40–2.17 in comparison with neighbourhood fathers). [The Working Group noted that the limitations of the study included unclear selection criteria for cases and controls and a lack of control for confounders. The completeness of the data on the occupations of mothers was also unclear.]

Hemminki *et al.* (1981) obtained data from the Finnish Cancer Registry for children under 15 years of age with a cancer that was diagnosed in 1959–75 and on parental occupation (see the study of Hakulinen *et al.*, 1976), and included approximately twice the number of cases as the earlier study. Data were analysed separately for the periods 1959–1968 and 1969–1975. The odds ratio for the father of a child with leukaemia in 1969–1975 being a professional driver was 1.25 ($P < 0.10$) for all childhood cancers, 1.5 ($P < 0.10$) for leukaemia and 0.92 ($P > 0.10$) for brain tumours. Odds ratios for leukaemia and all cancers were somewhat higher when only occupations in the later period were considered. Detailed data were not reported for maternal occupations. [The Working Group noted that the exposure information was limited to occupational titles and that a large number of occupations were assessed; thus, multiple comparisons were a limitation. The interpretation of this study was further limited by the lack of information on potential confounders.]

Associations between paternal occupation and childhood leukaemia (43 cases) and brain tumour (70 cases) were investigated in a case–control study in Maryland, USA (Gold *et al.*, 1982). Children and adolescents under the age of 20 years with leukaemia (diagnosed in 1969–74) or brain tumours (diagnosed in 1965–74) were ascertained in the Baltimore area from hospital records, death certificates, hospital tumour registries and from the pathology, radiotherapy and clinical oncology records of 21 Baltimore hospitals. Two control groups were included: one comprised children with no malignant disease, selected from birth certificates at the Maryland State Health Department and matched for sex, date of birth and race; the other group comprised children with malignancies other than leukaemia or brain cancer, matched for sex, race, date of diagnosis and age at diagnosis. Information on occupational exposures of both parents before the birth of the child and between the birth and diagnosis was collected from interviews with the mothers. Odds ratios and 95% confidence intervals were not reported. The paternal occupational category that included driver, motor vehicle mechanic, service station attendant or railroad worker was not more frequent for children with leukaemia or brain tumours compared with the population control children, but was for leukaemia cases in comparison with the cancer controls (six exposed cases, no exposed controls; $P < 0.05$). [The Working Group noted that the small numbers involved, the choice of cancer controls and the lack of control for potential confounders were limitations of the study. In addition, the occupations included in the motor vehicle-related group represented a somewhat diverse group of occupations with different potential exposure to exhaust.]

In a case–control study of childhood leukaemia in the USA (Vianna *et al.*, 1984), children born in 1949–78 who were diagnosed with

acute leukaemia during the first year of life and reported to the Tumor Registry of the New York State Health Department or with neuroblastoma up to 12 years of age at diagnosis were identified. Using information from birth certificates, two sets of controls were selected: the primary control group was matched by year of birth, sex, race and county of residence; the second group was additionally matched for age of the mother and birth order of the child. Information on parental age, race, education and occupation, and medical, obstetrical and therapeutic histories were obtained by telephone interviews with the mothers. A similarly designed case–control study of 103 children diagnosed with neuroblastoma before the age of 13 years was conducted simultaneously to assess data quality, but few details were reported. Of the 65 eligible cases of leukaemia, 60 were finally included in the analysis, each with two controls. The odds ratio for acute leukaemia for children with 'high' presumed paternal exposure to motor exhaust fumes (service station attendants, automobile or HGV repairmen and aircraft maintenance personnel) was 2.4 [95% CI, 1.1–3.7] in comparison with the main control group and 2.5 [95% CI, 1.2–5.3] in comparison with the second control group. For 'lower' presumed exposure (taxi drivers, travelling salesmen, HGV or bus drivers, railroad workers, toll-booth attendants, highway workers and police officers), the odds ratio was 1.3 [95% CI, 0.8–2.1] in comparison with the first control group and 3.4 [95% CI, 1.4–10.2] in comparison with the second. Data were not tabulated for the study of neuroblastoma, but no significant difference was found in the number of fathers who had had 'high' or 'moderate' exposure. [The Working Group noted that, as a limitation, the categorization of exposures as 'high' and 'lower' on the basis of the jobs listed appeared questionable, because jobs classified as having low exposure to one type of exhaust may have high exposure to another type. No potential confounders beyond the matching factors were controlled for in the analysis.]

In a case–control study of paternal occupation and Wilms tumour (Wilkins & Sinks, 1984), 105 patients were identified through the Columbus (OH, USA) Children's Hospital Tumor Registry during the period 1950–81. For each case, two controls were selected from Ohio birth certificate files: the first control series was individually matched for sex, race and year of birth, and the second series was additionally matched for mother's county of residence when the child was born. Due to changes in birth certification, the study included only the 62 cases and their matched controls for which paternal occupation was recorded. The crude odds ratio for Wilms tumour in children with paternal occupation as motor vehicle mechanic, service station attendant or driver/heavy equipment operator was 1.37 (95% CI, 0.59–3.11) compared with both groups of controls combined. [The Working Group noted that the study was limited with regard to the small number of exposed cases, crude exposure assessment and the lack of control for confounders other than demographic characteristics.]

Using a nationwide registry of childhood leukaemia, a case–control study including 519 cases of childhood acute lymphatic leukaemia (period of diagnosis, 1973–80) and 507 population-based controls matched for year of birth, gender and place of residence was conducted in the Netherlands (van Steensel-Moll *et al.*, 1985). A postal questionnaire provided information on parental occupations and selected exposures before and during pregnancy. The response rate was 88% for parents of cases and 66% for those of controls. Four mothers of cases compared with no control mother indicated exposures to exhausts during pregnancy, while 89 case fathers and 70 control fathers reported such exposure (OR, 1.3; 95% CI, 0.8–1.9). [The Working Group noted that this study relied on job titles and self-reporting for exposure assessment, with the

potential for misclassification and low specificity. Exposures to diesel and gasoline exhausts were not separated.]

In a joint analysis of case–control studies of childhood brain cancer that formed part of an international study coordinated by the IARC, 1218 cases and 2223 controls were analysed with regard to parental occupation (Cordier *et al.*, 2001). All studies were population-based, but the upper age of inclusion varied from up to 15 years in European and Australian centres to up to 19 years in Israel and centres in the USA. The overall response rate among cases was 75%. Population-based controls were either individually or frequency-matched to cases, depending on the centre, with a 75% response rate. Exposure assessment was based on parental interviews and coding occupations and industries according to international standard classifications. For paternal occupation during the 5-year period before birth, the odds ratio was 1.3 (95% CI, 1.0–1.7) for occupation as a driver and 1.5 (95% CI, 1.0–2.3) for all childhood brain tumours combined. Maternal occupations related to motor vehicles were not associated with an increased risk, but some indication of a positive association was observed with employment in a motor vehicle-related industry during pregnancy. [The Working Group noted that this study was notable for its large size and improved methods relative to most of the earlier studies. However, no specific information was available on exposure to engine exhausts and the numbers of mothers in exposed occupations was small.]

Different diagnostic groups of childhood cancer and their association with 31 categories of parental occupation were investigated in the framework of the United Kingdom Childhood Cancer Study (McKinney *et al.*, 2003). In total, 3838 cases, including 1737 cases of leukaemia (1461 acute lymphoblastic leukaemia) and 687 cases of central nervous system cancer, and 7629 randomly selected controls (1:2 matched by sex, age and area) participated. The response rate was 87% for case and 64% for control parents. Personal interviews including a complete occupational history were conducted with each parent; surrogate information was collected mainly from spouses if one parent could not be contacted. Occupations were classified according to standard schemes, and further categorized into 31 occupational exposure groups using a JEM. Paternal exposures to exhaust fumes and inhaled particulate hydrocarbons, as well as occupation as a driver showed small but statistically significant associations with childhood leukaemia and the subgroup of acute lymphoblastic leukaemia. The odds ratio for exhaust fumes was 1.33 (95% CI, 1.09–1.61) for leukaemia and slightly lower for acute lymphoblastic leukaemia. The odds ratio for occupation as driver and leukaemia was 1.36 (95% CI, 1.10–1.68). The risks for maternal exposures and leukaemia were non-significantly elevated. The number of cases of central nervous system cancer with exposed mothers was very small and the odds ratios were not notably elevated for fathers employed as drivers or with exposure to exhaust. [The Working Group noted a substantial overlap between the three different indicators of exposure (exhaust, inhaled particulate hydrocarbons and occupation as a driver). Specific exposure to diesel or gasoline exhaust was not assessed in the study.]

A case–control study of children with acute lymphoblastic leukaemia was conducted in Australia (Reid *et al.*, 2011). Parents of 511 cases (response rate, 80%; diagnosis in 2003–06) and of 2071 population controls (response rate, 70%) completed a written questionnaire and were interviewed in greater detail if they had worked in one of 14 occupations. Information on parental exposure to specific chemicals, including exhausts, during different time periods around the birth of the children was assessed through the use of job-specific modules; probability, frequency and intensity of exposure was estimated by an expert industrial hygienist, and grouped exposure variables were created. The odds ratio was 2.06 (95%

CI, 0.97–4.39) for maternal exposure to moderate/high levels of diesel exhaust any time before the child's birth and 1.59 (95% CI, 0.58–4.41) for maternal exposure to petrol exhaust before birth. Paternal pre-birth exposure to diesel exhaust was associated with an increased odds ratio of 1.24 (95% CI, 0.91–1.69); for paternal pre-birth exposure to moderate/substantial petrol exhaust, the odds ratio was 1.20 (95% CI, 0.82–1.77). [The Working Group noted that this study provided improved exposure assessments in relation to most of the earlier studies and showed some evidence of an increased risk for acute lymphoblastic leukaemia associated with prenatal parental exposure to exhausts. However, the numbers of exposed cases were small in some of the exposure categories.]

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3. CANCER IN EXPERIMENTAL ANIMALS

3.1 Diesel engine exhaust

The whole diesel exhaust used in the studies evaluated here was generated from fuels and diesel engines produced before the year 2000. Exhaust from these engines include three basic components: elemental carbon particles in respirable clusters; organic matter adsorbed onto the surface of the carbon particles, which is readily extractable with organic solvents; and a mixture of gas and vapour phases that include volatile organic compounds. Many studies have been carried out using several animal species to evaluate the potential carcinogenicity of exposure to whole exhaust and to the components of exhaust from diesel engines. The studies were considered under three subcategories: (i) whole diesel engine exhaust; (ii) gas-phase diesel engine exhaust (with particles removed); and (iii) diesel engine exhaust particles or extracts of diesel engine exhaust particles.

Animal bioassays conducted with different diesel engine exhausts have been reviewed previously in the *IARC Monographs* (IARC, 1989). This section provides a summary of these and a detailed review of more recent studies.

3.1.1 Mouse

See Table 3.1

(a) Inhalation

Groups of 96 female NMRI mice, aged 8–10 weeks, were exposed to clean air (control), or filtered or unfiltered exhaust from a 1.6-L diesel engine (Volkswagen ; operated to simulate average urban driving) for 19 hours a day, 5 days a week for life (up to 120 weeks). The unfiltered and filtered exhausts were diluted 1:17 with air, and the unfiltered exhaust contained 4.24 mg/m^3 of particles. Levels of nitrogen dioxide and nitrogen oxides were 1.5 ± 0.3 and 11.4 ± 2.1 ppm in whole exhaust and 1.2 ± 0.26 and 9.9 ± 1.8 ppm in filtered exhaust, respectively. Exposure to total diesel exhaust and filtered diesel exhaust significantly increased the number of animals with lung adenocarcinomas to 13 out of 76 (17%) and 18 out of 93 (19%), respectively, as compared with 2 out of 84 (2%) controls; no increase was seen in the numbers of animals with lung adenomas (Heinrich *et al.*, 1986a). [The incidence of lung tumours in 'historical' controls in this laboratory was reported to be 32% in untreated controls and 12.5% in inhalation controls (exposed to clean air only) (Heinrich *et al.*, 1986b).]

Two groups of 225 newborn male and female C57BL/N and 205 newborn male and female ICR mice each were exposed to either clean air (control) or diesel exhaust (from a 269-cm^3 displacement, small diesel engine run at idling speed), diluted 1:2–1:4 with clean air to give concentrations of 2–4 mg/m^3 of particulate matter and 2–4 ppm of nitrogen dioxide, for 4 hours a day, 4 days a week, starting within

Table 3.1 Studies of the carcinogenicity of diesel engine exhaust in mice

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
NMRI (F) Up to 120 wk (lifetime) Heinrich *et al.* (1986a)	Inhalation Clean air (control), filtered or unfiltered exhaust from a 1.6-L displacement diesel engine (operated to simulate average urban driving), diluted 1:17 with air, containing 4.24 mg/m^3 particles, and 1.5 ± 0.3 ppm NO_2, 11.4 ± 2.1 ppm NO_x (whole exhaust) and 1.2 ± 0.26 ppm NO_2, 9.9 ± 1.8 ppm NO_x (filtered exhaust); 19 h/d, 5 d/wk Groups of 96, aged 8–10 wk	Lung (adenoma): 9/84 (11%), 11/93 (12%), 11/76 (14%) Lung (adenocarcinoma): 2/84 (2%), 18/93 (19%)*, 13/76 (17%)*	*$P < 0.05$ compared with controls	Well conducted study; incidence of lung tumours in 'historical' controls in this laboratory reported to reach 32% in untreated controls and 12.5% in inhalation controls (exposed to clean air only) (Heinrich *et al.*, 1986b)
C57BL/6N and ICR (M, F) Up to 28 mo Takemoto *et al.* (1986)	Inhalation Clean air (control) or exhaust from a 269-cm^3 small diesel engine diluted 1:2–1:4 with clean air (2–4 mg/m^3 particulate matter, 2–4 ppm NO_2), 4 h/d, 4 d/wk for up to 24 mo; survivors held untreated for up to additional 4 mo Groups of 225 newborn C57BL/6N and 205 newborn ICR	C57BL/N Lung (adenocarcinoma): M+F–0/51, 5/150 (3%) Lung (adenoma): M+F–1/51 (2%), 12/150 (8%) ICR Lung (adenocarcinoma): 1/60, 4/56 (7%) Lung (adenoma): 6/60 (10%), 10/56 (18%)	NS	Daily duration and frequency of exposures were short
NMRI (F) Up to 23 mo Heinrich *et al.* (1995)	Inhalation Clean air (control), or filtered or unfiltered exhaust from two 40-kW 1.6-L diesel engines (VW; operated either according to the US 72 cycle or under constant load conditions), diluted 1:15 and 1:9 with clean air (average diesel soot particle concentrations, 4.5 and 7.0 mg/m^3, respectively); particle-free diesel exhaust diluted 1:15 with clean air and the particles removed by a heated filter system (whole exhaust: 0.5–0.6 ppm NO_2, 4.1–4.3 ppm NO_x; filtered exhaust: 0.5 ppm NO_2, 2.7 ppm NO_x), 18 h/d, 5 d/wk for 13.5 mo; kept untreated for up to additional 9.5 mo Groups of 80, aged 7 wk, exposed to (A) clean air, (B) diesel exhaust (7.0 mg/m^3 diesel soot); groups of 120, aged 8–10 wk, exposed to (C) clean air, (D) diesel exhaust (4.5 mg/m^3 diesel soot) or (E) filtered exhaust (particle-free)	Lung (adenoma): A, 25%; B, 21.8%; C, 25%; D, 18.3%, E, 31.7% Lung (adenocarcinoma): A, 15.4%; B, 15.4%; C, 8.8%; D, 5%; E, 15%	NS	Well conducted study; results, summarized in a general manner, no numbers of lung tumours given; pathology for organs other than the lung NR

Table 3.1 (continued)

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
C57BL/6N (F) Up to 30 mo Heinrich *et al.* (1995)	Inhalation Clean air (control), filtered or unfiltered exhaust from two 40-kW 1.6-L diesel engine (VW; operated either according to the US 72-cycle or under constant load conditions), diluted 1:15 with clean air (average diesel soot particles, 4.5 mg/m^3); particle-free diesel exhaust diluted 1:15 with clean air and particles removed by a heated filter system (whole exhaust: 0.5 ppm NO_2, 4.1 ppm NO_x; filtered exhaust: 0.5 ppm NO_2, 2.7 ppm NO_x); 18 h/d, 5 d/wk for 24 mo; kept untreated for up to additional 6 mo. Groups of 120, aged 7 wk	Lung (all tumours): 5.1%, 8.5%, 3.5%	NS	Well conducted study; results, summarized in a general manner, no numbers of lung tumours given; pathology for organs other than the lung NR
CD-1 (M, F) 24 mo Mauderly *et al.* (1996)	Inhalation Clean air (control), exhaust generated from a 1980 model 5.7-L V8 engine (operated according to US FTP cycles), concentrations reported as dilution of whole exhaust: measured soot of 0.35, 3.5 or 7.0 mg/m^3 (0.1 ± 0.1, 0.3 ± 0.2 and 0.7 ± 0.5 ppm NO_2, respectively); 7 h/d, 5 d/wk Numbers NR, aged 17 wk	Lung (bronchiolo-alveolar adenoma): M+F–10/157 (6%), 16/171 (9%), 8/155 (5%), 10/186 (5%) Lung (bronchiolo-alveolar carcinoma): M+F–9/157 (6%), 6/171 (3%), 7/155 (4%), 4/186 (2%)	NS	Well conducted study
C57BL/6N (F) 18 mo Kunitake *et al.* (1986)	Subcutaneous injection 0 (control), 10, 25, 50, 100, 200 or 500 mg/kg bw residue from dichloromethane extract of diesel particles from a V6 11-L heavy-duty diesel engine exhaust in olive oil containing 5% DMSO; once/wk for 5 wk Groups of 15–50, aged 6-wk	Soft-tissue (malignant fibrous histiocytoma): 0/38, 0/15, 1/15 (7%), 2/14 (14%); 3/30 (10%), 1/15 (7%), 5/22 (23%)*	*$P < 0.01$, Fisher's exact	

Table 3.1 (continued)

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
ICR and C57BL (M, F) 24 mo Kunitake *et al.* (1986)	Subcutaneous injection 0 (control), 2.5, 5 or 10 mg/mouse of residue from dichloromethane extract of diesel particles from a V6 11-L heavy-duty diesel engine exhaust in olive oil containing 5% DMSO; once 24 h after birth Groups of 12–36 newborn	ICR strain Liver (hepatoma): M–2/14 (14%), 0/13, 6/30 (20%), 3/12 (25%) F–0/16, 1/18 (6%), 2/36 (6%), 1/12 (8%) Lymphoma: M–2/14 (14%), 0/13, 4/30 (13%), 4/12 (33%) F–1/16 (6%), 1/18 (6%), 3/36 (8%), 1/12 (8%) Lung (all tumours [NR]): M–2/14 (14%), 4/13 (30%), 7/30 (23%), 2/12 (17%) F–3/16 (19%), 0/18, 5/36 (14%), 0/12	[NS]	Experiment with newborn C57BL mice performed only with 0 and 5 mg/mouse; the authors reported no increase in tumour incidence in treated mice versus controls
ICR (M) 12 mo Ichinose *et al.* (1997)	Intratracheal instillation 0 (control), 0.05, 0.1 or 0.2 mg/mouse diesel exhaust particles from exhaust emission of a diesel engine (2740 cm^3 exhaust volume, operated at 1500 rpm and 10 torque), collected on a glass filter and suspended in sterile 50 mM phosphate-buffered 0.9% saline (pH 7.4) containing 0.05% Tween 80; once/wk for 10 wk Groups of 120, aged 4 wk	Lung (adenoma): 18/116 (16%), 30/120 (25%), 31/119 (26%), 28/117 (24%) Lung (adenocarcinoma): 1/116 (1%), 6/120 (5%), 5/119 (4%), 5/117 (4%) Lymphoma: 13/116 (11%), 17/120 (14%), 17/119 (14%), 23/117 (20%)	NS	Short exposure period; high spontaneous lung tumour incidence; designed to investigate the involvement of oxygen radicals in lung carcinogenesis induced by diesel exhaust particles, i.e. the relationship between lung tumour response and formation of 8-OH-dG in lung DNA
SENCAR (M, F) 52 wk Nesnow *et al.* (1983)	Skin application 0 (control), 0.1, 0.5, 1.0, 2.0, or 4.0 mg/ mouse particulates from emissions of a 1973 Nissan Datsun 220C diesel engine, collected on Teflon-coated fibreglass filters, extracted with dichloromethane removed by evaporation, dissolved in 0.2 mL acetone, once/wk (4.0 mg dose given as 2.0 mg twice/wk) on shaved dorsal surface for 50–52 wk Groups of 40 M and 40 F, aged 7–9 wk	Skin (squamous cell carcinoma): M–0%, 0%, 0%, 0%, 0%, 3% F–0%, 0%, 0%, 0%, 0%, 5%	NS	Short duration of exposure

Table 3.1 (continued)

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Initiation-promotion				
ICR (F) [presumed to be 29 wk] Kunitake *et al.* (1986)	Skin application 0 (control), 0.5, 1.5 or 4.5 mg tar from a V6 11-L heavy-duty diesel engine exhaust in 0.1 mL acetone to shaved back, every other day for 20 days; 1 wk later, 2.5 µg TPA in 0.1 mL acetone, 3 × /wk for 25 wk Groups of 50, aged 8–9 wk	Skin (papilloma): 0/50, 0/49, 1/48, 4/50 Skin ('cancer'): 0/50, 0/49, 0/48, 0/50	[NS]	Study poorly reported, with short duration of treatment and implied lack of observation time after treatment
SENCAR (M, F) Up to 26 wk Nesnow *et al.* (1982a, b, 1983)	Skin application 0 (control), 0.1, 0.5, 1.0, 2.0 or 10.0 mg/mouse particulates from emissions of (A) a 1973 Nissan Datsun 220C, (B) a 1978 Oldsmobile 350, (C) a prototype VW turbo-charged Rabbit or (D) a 1972 heavy-duty Caterpillar 3304 diesel engine collected on Teflon-coated fibreglass filters, extracted with dichloromethane removed by evaporation, dissolved in acetone, 0.2 mL once (10 mg given as 5 × 2 mg/d) to shaved dorsal surface; 1 wk later, 2.0 µg TPA in 0.2 mL acetone twice/wk for up to 25 wk; B[*a*]P used as a positive control (highest dose, 101 µg/mouse) Groups of 40, aged 7–9 wk	Diesel engine A Skin (papilloma/mouse): M–0.08, 0, 0.34, 0.38, 1.1, 5.5 F–0.05, 0.03, 0.39, 0.53, 1.6, 5.7 Skin (squamous cell carcinoma): M–0/37 versus 12/38 (31%) high-dose F–1/38 versus 14/38 (36%) high-dose Diesel engines B, C and D Skin (papilloma/mouse): M+F– 0.1–0.5 compared with 0.05–0.08 in TPA controls B[a]P (101 µg/mouse) Skin (papilloma/mouse): M–10.2 F–7.9 Skin (squamous cell carcinoma): M–30% F–25%	[$P < 0.001$] [$P < 0.001$]	

Table 3.1 (continued)

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Administration with known carcinogens				
NMRI (F) Up to 120 wk (lifetime) Heinrich *et al.* (1986a)	Intratracheal instillation/inhalation Instillations of 50 or 100 µg B[*a*]P for 20 and 10 wk, respectively, or 50 µg DB[*a*,*h*]A for 10 wk, followed by inhalation exposure to clean air (control), or filtered or unfiltered exhaust from a 1.6-L displacement diesel engine (VW; operated to simulate average urban driving), diluted 1:17 with air (4.24 mg/m^3 particles; whole exhaust: 1.5 ± 0.3 ppm NO_2, 11.4 ± 2.1 ppm NO_x; filtered exhaust: 1.2 ± 0.26 ppm NO_2, 9.9 ± 1.8 ppm NO_x), 19 h/d, 5 d/wk Groups of 64, aged 8–10 wk	20 B[*a*]P instillations induced a 71% lung tumour rate while 20 B[*a*]P instillations plus total diesel exhaust gave a 41% rate; results not reproduced in the group receiving the same total dose of B[*a*]P in 10 installations	NS	Authors reported that the various treatments with carcinogens gave no consistent results; no other data on tumours were reported
NMRI (F) 6 mo Heinrich *et al.* (1986a)	Subcutaneous injection/inhalation 5 or 10 µg DB[*a*,*h*]A 24– 48 h after birth followed by inhalation of clean air (control), or filtered or unfiltered exhaust from a 1.6-L displacement diesel engine (operated to simulate average urban driving), diluted 1:17 with air (4.24 mg/m^3 particles; whole exhaust: 1.5 ± 0.3 ppm NO_2, 11.4 ± 2.1 ppm NO_x; filtered exhaust: 1.2 ± 0.26 ppm NO_2, 9.9 ± 1.8 ppm NO_x), 19 h/d, 5 d/wk Groups of 96 newborn	Lung tumour rate: DB[*a*,*h*]A–5 µg, 46%; 10 µg, 81% DB[*a*,*h*]A + unfiltered exhaust–10 µg, 63%*	*Significant decrease	Animals exposed for only 6 mo; authors reported that the various treatments with carcinogens gave inconsistent and erratic results; incidence of tumours not reported

B[*a*]P, benzo[*a*]pyrene; bw, body weight; d, day; DB[*a*,*h*]A, dibenz[*a*,*h*]anthracene; DMSO, dimethyl sulfoxide; F, female; FTP, Federal Test Procedures; h, hour; M, male; mo, month; NO_2, nitrogen dioxide; NO_x, nitrogen oxides; NR, not reported; NS, not significant; rpm, revolutions per minute; 8-OH-dG, 8-hydroxydeoxyguanosine; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; VW, Volkswagen; wk, week

24 hours of birth, for up to 24 months; survivors were maintained with no further treatment for up to an additional 4 months. A low, not significantly increased incidence of lung adenoma and/or adenocarcinoma was observed in mice of both strains exposed to diesel exhaust. Other tumours were observed in the liver, mammary gland and haematopoietic system (malignant lymphomas), but their incidence did not differ statistically significantly between the exposed and control groups (Takemoto *et al.*, 1986). [The Working Group noted that the duration and frequency of the daily exposures were short.]

Groups of 80 female NMRI mice, aged 7 weeks, were exposed to clean air (control) or diesel exhaust from a 1.6-L diesel engine (VW; operated according to the US 72-cycle or under constant load conditions) for 18 hours a day, 5 days a week for 13.5 months and then maintained with no further treatment for up to an additional 9.5 months. The exhaust was diluted 1:9 with air, and the unfiltered exhaust contained 7.0 mg/m^3 of particles. Levels of 0.5–0.6 ppm of nitrogen dioxide and 4.1–4.3 ppm of nitrogen oxides were found in the whole exhaust. Additional groups of 120 female NMRI mice, aged 8–10 weeks, or female C57BL/6N mice, aged 7 weeks, were exposed to either clean air (control), diesel exhaust containing 4.5 mg/m^3 of particles (0.5–0.6 ppm of nitrogen dioxide and 4.1–4.3 ppm of nitrogen oxides) or particle-free diesel exhaust for 18 hours a day, 5 days a week for either 13.5 months and then maintained without treatment for up to an additional 9.5 months (NMRI mice) or for 24 months and then maintained without treatment for up to an additional 6 months (C57BL/6N mice). The exhaust from the engine was diluted 1:15 with clean air, and the particles were removed by a heated filter system. Exposure to total or filtered diesel exhaust did not cause any increase in the number of animals with lung tumours (Heinrich *et al.*, 1995). [The study was well conducted, but the results were summarized in general manner. Histopathology for organs other than the lung and the lung tumour incidence were not reported.]

Groups of male and female CD1 mice [number unspecified], aged 17 weeks, were exposed to either clean air (control) or diesel engine exhaust generated from a 1980 model 5.7-L V8 engine operated according to US Federal Test Procedures (FTP) cycles at concentrations (reported as a dilution of the whole exhaust to measured soot concentrations) of 0.35, 3.5 or 7.0 mg/m^3 with levels of nitrogen dioxide of 0.1 ± 0.1, 0.3 ± 0.2 and 0.7 ± 0.5 ppm, respectively, for 6 hours a day, 5 days a week for 24 months. Exposure to diesel exhaust did not affect survival or body weight and did not increase the incidence of bronchiolo-alveolar adenoma and/or carcinoma (Mauderly *et al.*, 1996).

(b) Subcutaneous administration

Groups of 15–50 female C57BL/6N mice, aged 6 weeks, received subcutaneous injections into the intrascapular region of 10, 25, 50, 100, 200 or 500 mg/kg body weight (bw) of residue from a dichloromethane extract of diesel particles (collected from the exhaust of a V6 11-L heavy-duty diesel engine) suspended in olive oil containing 5% dimethyl sulfoxide (DMSO) once a week for 5 weeks. A control group of 38 mice received injections of the vehicle only. The animals were killed 18 months after the beginning of the experiment. The first tumours were palpated at week 47 (25 mg/kg bw), week 30 (50 mg/kg bw), week 27 (100 mg/kg bw) and week 39 (200 and 500 mg/kg bw) in the five treated groups. A significant increase in the incidence of subcutaneous tumours, diagnosed as malignant fibrous histiocytomas, was observed in 5 out of 22 mice that receiving the 500-mg/kg bw dose ($P < 0.01$) in comparison with controls (0 out of 38) (Kunitake *et al.*, 1986).

In a second experiment, groups of 12–36 newborn male and female ICR mice received a single subcutaneous injection of 0, 2.5, 5 or 10 mg of residue from a dichloromethane extract

of diesel particles (collected from the exhaust of a V6 11-L heavy-duty diesel engine) suspended in olive oil containing 5% DMSO 24 hours after birth. The surviving animals were killed after 24 months. The incidence of lymphoma in male mice that received 10 mg of residue/mouse (4 out of 12 mice) was slightly increased compared with controls (2 out of 14 [not significant]). Overall, no statistically significant increase in the incidence of any tumours or of total tumours was observed in any of the treated groups. The authors reported that they also injected newborn C57BL mice with doses of 0 and 5 mg/mouse and observed no increase in the incidence of tumours in treated animals compared with controls (Kunitake *et al.*, 1986). [The Working Group noted that the study was limited by the small number of animals used.]

(c) Intratracheal instillation

Groups of 120 male ICR mice, aged 4 weeks, received intratracheal instillations 0, 0.05, 0.1 or 0.2 mg of diesel exhaust particles (obtained from the exhaust emission generated by a diesel engine, with a 2740-cm^3 exhaust volume, operated at 1500 revolutions per minute (rpm) and 10 torque, and collected on a glass filter) suspended in sterile 50 mM phosphate-buffered 0.9% saline (pH 7.4) containing 0.05% Tween 80 once a week for 10 weeks and were then killed 12 months after the first injection. A non-significant increase in the incidence of lymphoma was observed in the high-dose group compared with controls (23 out of 117 versus 13 out of 116) (Ichinose *et al.*, 1997). [The Working Group noted the high incidence of spontaneous lung tumours in control animals and the short observation period. This study was designed to investigate the role of oxygen radicals in lung carcinogenesis induced by diesel exhaust particles, i.e. the relationship between lung tumour response and the formation of 8-hydroxydeoxyguanosine in lung DNA, which explained the short exposure period.]

(d) Skin application

Groups of 40 male and 40 female SENCAR mice, aged 7–9 weeks, received topical 0.2-mL applications of extracts of particles obtained from the emissions of a 1973 Nissan Datsun 220C diesel engine that were collected on Teflon-coated fibreglass filters, extracted with dichloromethane and then dissolved in acetone to give doses of 0, 0.1, 0.5, 1.0, 2.0 or 4.0 mg/mouse once a week (the 4.0-mg dose was given as two applications a week) to the shaved dorsal skin for 50–52 weeks. At that time, squamous cell carcinomas of the skin had developed in 3% of males and 5% of females given the 4.0-mg dose, which was not statistically significant compared with controls (Nesnow *et al.*, 1983). [The Working Group noted the short duration of exposure.]

(e) Initiation–promotion studies

Groups of 40 male and 40 female SENCAR mice, aged 7–9 weeks, received a single topical 0.2-mL application of extracts of particles obtained from the emissions of (A) a 1973 Nissan Datsun 220C, (B) a 1978 Oldsmobile 350, (C) a prototype VW turbo-charged Rabbit or (D) a 1972 heavy-duty Caterpillar 3304 diesel engine that were collected on Teflon-coated fibreglass filters, extracted with dichloromethane and then dissolved in acetone to give doses of 0, 0.1, 0.5, 1.0 or 2.0 mg/mouse to the shaved dorsal skin; a 10-mg/mouse dose was administered as five daily applications of 2 mg. One week later the mice received topical applications of 2.0 µg of 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in 0.2 mL of acetone twice a week for up to 25 weeks. Benzo[*a*]pyrene was used as a positive control. The sample from engine A produced a dose-related increase in the incidence of skin papillomas, with 5.5 and 5.7 papillomas/mouse at the highest dose; 12 out of 38 (31%) males [$P < 0.001$] and 14 out of 38 (36%) females [$P < 0.001$] treated with the highest dose developed squamous cell carcinomas of the skin compared with 0 out of 37

male and 1 out of 38 female controls. Responses of 0.1–0.5 papillomas/mouse were observed after treatment with samples from engines B, C and D, compared with 0.05–0.08 papillomas/mouse in TPA-treated controls (Nesnow *et al.*, 1982a, b, 1983).

Three groups of 50 female ICR mice, aged 8–9 weeks, received topical applications of extracts of diesel particles (collected from the exhaust of a V6 11-L heavy-duty displacement diesel engine) dissolved in acetone onto the shaved back skin every other day for 20 days (total doses: 5, 15 or 45 mg/animal). A further group of 50 mice treated with acetone only served as controls. Beginning 1 week after the last application of diesel extract, each animal received applications of 2.5 µg of TPA in 0.1 mL of acetone three times a week for 25 weeks [duration of the study presumed to be ~29 weeks]. No skin 'cancer' was found in the treated or control groups; skin papillomas were observed in 1 out of 48 and 4 out of 50 animals in the 15- and 45-mg dose groups, respectively (Kunitake *et al.*, 1986). [The Working Group noted that the limitations of the study included the short duration of treatment and the implied lack of observation time after treatment.]

(f) Administration with known carcinogens

Groups of 64 female NMRI mice, aged 8–10 weeks, received intratracheal instillations of 50 or 100 µg of benzo[*a*]pyrene once a week for 20 or 10 weeks, respectively, or 50 µg of dibenz[*a,h*]anthracene (DB[*a,h*]A) for 10 weeks, followed by exposure to clean air (control), or filtered or unfiltered exhaust from a 1.6-L diesel engine (VW; operated to simulate average urban driving) for 19 hours a day, 5 days a week for life (up to 120 weeks). The unfiltered and filtered exhausts were diluted 1:17 with air, and the resulting whole exhaust contained 4.24 mg/m^3 of particles. Levels of nitrogen dioxide and nitrogen oxides were 1.5 ± 0.3 and 11.4 ± 2.1 ppm in whole exhaust and 1.2 ± 0.26 and 9.9 ± 1.8 ppm in filtered exhaust, respectively. The authors reported that the various treatments with carcinogens gave no consistent results and stated that the 20 instillations of benzo[*a*]pyrene induced a 71% lung tumour rate while the 20 instillations of benzo[*a*]pyrene plus exposure to total diesel exhaust produced a rate of only 41% that was not reproduced in the group that received the same total dose of benzo[*a*]pyrene in 10 instillations. No other data on tumours were reported (Heinrich *et al.*, 1986a).

In another experiment, groups of 96 newborn female NMRI mice received an initial subcutaneous injection of 5 or 10 µg of DB[*a,h*]A between 24 and 48 hours after birth followed by exposure to clean air (control), or filtered or unfiltered exhaust from a 1.6-L diesel engine (VW; operated to simulate average urban driving) for 19 hours a day, 5 days a week for up to 6 months. The unfiltered and filtered exhausts were diluted 1:17 with air, and the resulting whole exhaust contained 4.24 mg/m^3 of particles. Levels of nitrogen dioxide and nitrogen oxides were 1.5 ± 0.3 and 11.4 ± 2.1 ppm in whole exhaust and 1.2 ± 0.26 and 9.9 ± 1.8 ppm in filtered exhaust, respectively. The authors reported that subcutaneous injection of the low dose of DB[*a,h*]A resulted in 46% of lung tumour-bearing animals and no significant variation was observed after exposure to diesel exhaust. The high dose of DB[*a,h*]A resulted in 81% of lung tumour-bearing animals, which was significantly reduced to 63% by exposure to whole diesel exhaust (Heinrich *et al.*, 1986a). [The Working Group noted that, although this type of inhibitory effect is not uncommon, the data obtained in this study were inconsistent and appeared to be erratic. The Working Group also noted that the animals were only exposed for 6 months and that the incidence of tumours was not reported.]

3.1.2 Rat

See Table 3.2

Table 3.2 Studies of the carcinogenicity of diesel engine exhaust in rats

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Wistar (M) Up to 20 mo Karagianes *et al.* (1981)	Inhalation Clean air (control), 6.6 ± 1.9 mg/m^3 or 14.9 ± 6.2 mg/m^3 coal dust or exhaust from a 3-cylinder, 43-hp diesel engine run to simulate operating patterns of such engines in mines containing 8.3 ± 2.0 mg/m^3 soot or 8.3 ± 2.0 mg/m^3 diesel soot plus 5.8 ± 3.5 mg/m^3 coal dust, 6 h/d, 5 d/wk for up to 20 mo Groups of 24, aged 18 wk	No increase in the incidence of lung tumours in treated or control animals	NS	Study limited by very small number of animals exposed for 20 mo because groups of six rats per exposure group were killed after 4, 6, 16 and 20 mo of exposure, and use of male animals only.
Wistar (F) Up to 140 wk (lifetime) Heinrich *et al.* (1986a)	Inhalation Clean air (control), or filtered or unfiltered exhaust from a 1.6-L displacement diesel engine (operated to simulate average urban driving), diluted 1:17 with air (4.24 mg/m^3 particles; whole exhaust: 1.5 ± 0.3 ppm NO_2, 11.4 ± 2.1 ppm NO_x; filtered exhaust: 1.2 ± 0.26 ppm (2.4 ± 0.5 mg/m^3) NO_2, 9.9 ± 1.8 ppm NO_x), 19 h/d, 5 d/wk Groups of 96, aged 8–10 wk	Lung (bronchiolo-alveolar adenoma): 0/96, 0/92, 8/95 (8%)* Lung (squamous cell tumour): 0/96, 0/92, 9/95 (9%, mainly benign)** Lung (all tumours): 0/96, 0/96, 15/95 (16%)***	*[$P = 0.003$] **[$P = 0.002$] ***[$P < 0.0001$]	

Table 3.2 (continued)

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
F344/Jcl (M, F) 30 mo Ishinishi *et al.* (1986)	Inhalation Exhaust from (A) a light-duty 1.8-L, 4 cylinder diesel engine (particle concentrations, 0 (control), 0.11, 0.41, 1.08 or 2.32 mg/m^3; NO_2 concentrations, 0, 0.08, 0.26, 0.70 or 1.41 ppm; NO_x concentrations, 0, 1.24, 4.06, 10.14 or 20.34 ppm) or (B) a heavy-duty 11-L, 6 cylinder diesel engine (particle concentrations, 0 (control), 0.46, 0.96, 1.84 or 3.72 mg/m^3; NO_2 concentrations, 0, 0.46, 1.02, 1.68 or 3.00 ppm; NO_x concentrations, 0, 6.17, 13.13, 21.67 or 37.45 ppm), diluted ~10–15 times in volume with conditioned air, 16 h/d, 6 d/wk Groups of 64 M and 59 F, aged 5 wk	Exhaust A Lung (adenoma): M–0/64, 0/64, 1/64 (2%), 0/64, 0/64 F–1/59 (2%), 1/59 (2%), 0/61, 0/59, 1/60 (2%) Lung (carcinomas, all): M–2/64 (3%), 1/64 (2%), 0/64, 3/64 (5%), 2/64 (3%) F–1/59 (2%), 1/59 (2%), 0/61, 2/59 (3%), 0/60 Exhaust B Lung (adenoma): M–0/64, 0/64, 0/64, 0/64, 0/64 F–0/59, 0/59, 0/61, 0/59, 0/60 Lung (carcinomas, all): M–0/64, 1/64 (2%), 0/64, 3/64 (5%), 5/64 (8%) F–1/59 (2%), 0/59, 0/61, 1/59 (2%), 3/60 (5%)	See comments.	The incidence of lung carcinomas in high-dose M and F (combined) was significantly different ($P < 0.05$) from that in M and F controls (combined). Lung carcinomas were diagnosed as adenocarcinoma, squamous cell carcinoma or adenosquamous carcinoma
F344 (F) Up to 30 mo Iwai *et al.* (1986)	Inhalation Clean air (control), or filtered or unfiltered exhaust from a 2.4-L diesel truck engine diluted with conditioned air (particle concentration, 4.9 ± 1.6 mg/m^3; NO_2 concentration, 1.8 ± 1.8 ppm; NO_x concentration, 30.9 ± 10.9 ppm), 8 h/d, 7 d/wk for 24 mo and then held unexposed for up to 6 mo Groups of 24, aged 7 wk	Lung (adenoma or carcinoma combined): 1/22 (5%), 0/16, 8/19 (42%)* Lung (all carcinomas): 0/22, 0/16, 5/19 (26%) ** Spleen (lymphoma, with or without leukaemia): 2/24 (8%), 9/24 (37%)**, 6/24 (25%)	*$P < 0.01$ **$P < 0.05$	Small number of exposed animals; lung tumours (in animals surviving ≥ 2 years) in the group exposed to whole diesel exhaust were 3 adenomas, 1 adenocarcinoma, 2 adenosquamous carcinomas, 1 squamous cell carcinoma and 1 large cell carcinoma; lung tumour in the control group was 1 adenoma
F344 (M, F) 24 mo Lewis *et al.* (1986, 1989)	Inhalation Clean air (control), 2 mg/m^3 coal dust, exhaust from a 7.0-L Caterpillar Model 3304 diesel engine diluted 1:27 with clean air (particle concentration, 2 mg/m^3; NO_2 concentration, 1.5 ± 0.5 ppm; NO_x concentration, 8.7 ± 3.6 ppm), or 1 mg/m^3 coal dust plus 1 mg/m^3 diesel exhaust particles, 7 h/d, 5 d/wk Groups of 216 M and 72 F, aged 8–10 wk	Lung (adenoma): M+F–3/180 (2%), 7/175 (4%), 7/177 (4%), 7/171 (4%) Lung (carcinoma): M+F–3/180 (2%), 0/182, 1/183 (1%), 0/178	NS	

Table 3.2 (continued)

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
F344 (M, F) Up to 30 mo Mauderly *et al.* (1986, 1987)	Inhalation Clean air (control), or diesel engine exhaust from a 1980 model 5.7-L V8 engine (operated according to US FTP cycles); concentrations reported as a dilution of whole exhaust to measured soot concentrations (0, 0.35, 3.5 or 7.0 mg/m^3; 0, 0.1 ± 0.1, 0.3 ± 0.2 and 0.7 ± 0.5 ppm NO_2, respectively), 7 h/d, 5 d/wk Groups of 221–230 M and F, aged 17 wk	Lung (total tumours): 0.9%, 1.3%, 3.6%*, 12.8%* Lung (bronchiolo-alveolar adenoma): 0%, 0%, 2.3%*, 0.4% Lung (adenocarcinoma and squamous cell carcinoma, combined): 0.9%, 1.3%, 0.5%, 7.5%* Lung (squamous cysts): 0%, 0%, 0.9%, 4.9%	*$P < 0.05$	Squamous cysts were mostly benign tumours
F344 (F) Up to 24 mo Takemoto *et al.* (1986)	Inhalation Clean air (control) or exhaust from a 269-cm^3 small diesel engine diluted 1:2–1:4 with clean air (particulate matter, 2–4 mg/m^3; NO_2, 2–4 ppm), 4 h/d, 4 d/wk for up to 24 mo Groups of 20–26, aged 5 wk	No lung tumours observed in either the 12 controls or 15 treated rats that survived 18–24 mo	NS	Small group sizes and short exposure durations
F344 (M, F) Up to 30 mo Brightwell *et al.* (1989)	Inhalation Conditioned air (control), (A) unfiltered exhaust emission from a VW Rabbit 1.5-L diesel engine, diluted with a constant volume of 800 m^3 of air (high dose), further diluted 1:3 (mid-dose) or 1:9 (low dose) in air (particle concentrations, 0.7, 2.2 or 6.6 mg/m^3, respectively; 0.9–2.8 ppm NO_x; 0.7–7 ppm NO_2) or (B) exhaust emission (A) passed through a stationary filtration system to remove 99.97% of the mass of particles, diluted 1:3 in air; 16 h/d, 5 d/wk for 2 yr and survivors maintained for an additional 6 mo; 8 animals per sex killed at 6, 12, 18 and 24 mo Treated groups of 72 M and 72 F, aged 6–8 wk; and control groups of 144 M and 144 F	Exhaust (A) Lung (all tumours): M–2/134 (1%), 1/72 (1%), 3/72 (4%), 16/71 (23%)* F–1/126 (1%) (C), 0/71, 11/72 (15%)*, 39/72 (54%)* Exhaust (B) No increase in the incidence of respiratory tract tumours	[*$P < 0.0001$] NS	Lung tumour incidence not reported by type; highest incidence seen in high-dose rats after the end of exposure (24–30 mo): 12/27 (M), 24/25 (F); malignant tumours: 10/27 (M), 19/25 (F); authors stated that the study showed a large and statistically significant increase in incidence of lung tumours in exposed F344 rats but provided no statistics. Tumours were mainly adenoma squamous cell carcinoma, adenocarcinoma, and mixed adenoma/adenocarcinoma/squamous cell carcinoma, and one mesothelioma

Table 3.2 (continued)

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
F344 (M, F) Duration NR Takaki *et al.* (1989)	Inhalation Exhaust from a 1.8-L light-duty diesel engine diluted with filtered conditioned air (particle concentrations, 0 (control), 0.1, 0.4, 1.1 or 2.3 mg/m^3) or from an 11-L heavy-duty diesel engine diluted with filtered conditioned air (particle concentrations, 0 (control), 0.5, 1.0, 1.8 or 3.7 mg/m^3), 16 h/d, 6 d/wk Groups of 64 M and 59 F, aged 5 wk	Light duty Lung (carcinoma): M+F–3/123 (2%), 2/123 (2%), 0/125, 5/123 (4%), 2/124 (2%) Lung (adenoma) M+F–1/123 (1%), 1/123 (1%), 1/125 (1%), 0/123, 1/124 (1%) Heavy duty Lung (carcinoma): M+F–1/123 (1%), 1/123 (1%), 0/125, 4/123 (3%), 8/124 (1%) Lung (adenoma): M+F–0/123, 0/123, 0/125, 0/123, 0/124	NS	Study published as a poster presentation; lack of some experimental details; carcinomas were adenocarcinoma, adenosquamous carcinoma or squamous cell carcinoma.
F344 (M, F) Up to 30 mo (lifetime) Mauderly *et al.* (1994); Nikula *et al.* (1995)	Inhalation Conditioned air (control), aerosolized carbon black or diesel exhaust from two 1988 Model LH6 General Motors 6.2-L V8 diesel engines, diluted in filtered conditioned air (particulate concentrations, 2.5 or 6.5 mg/m^3; NO_2, 0.73 or 3.78 ppm; NO_x, 8.79 or 23.45 ppm), 16 h/d, 5 d/wk for 24 mo and then held for up to 6 mo Groups of approximately 100, aged 7–9 wk	Carbon black Lung (bronchiolo alveolar adenocarcinoma): M–1/109 (1%), 1/106 (1%), 1/106 (1%) F– 0/105, 6/107 (6%)*, 20/105 (19%)** Lung (squamous cell and adenosquamous carcinoma): M–control, 1/109 (1%), 0/106, 3/106 (3%) F–0/105, 0/107, 2/105 (2%) Lung (bronchiolo-alveolar adenoma): M–1/109 (1%), 1/106 (1%), 0/106 F–0/105, 2/107 (2%), 13/105 (12%)***	[*$P < 0.03$; **$P < 0.0001$; ***$P < 0.001$]	

Table 3.2 (continued)

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
F344 (M, F) Up to 30 mo (lifetime) Mauderly et al. (1994); Nikula et al. (1995) (cont.)		Diesel exhaust Lung (bronchiolo alveolar adenocarcinoma): M–1/109 (1%), 1/105 (1%), 3/106 (3%) F–0/105, 3/105 (3%), 19/106 (18%)** Lung (squamous cell and adenosquamous carcinoma): M–1/109 (1%), 2/105 (2%), 2/106 (2%) F–0/105, 1/105 (1%), 2/106 (2%) Lung (bronchiolo-alveolar adenoma): M–1/109 (1%), 2/105 (2%), 4/106 (4%) F–0/105, 5/105 (5%), 19/106 (18%)**		
Wistar [Crl:(W1) BR] (F) Up to 30 mo Heinrich *et al.* (1995)	Inhalation Clean air (control) or exhaust from two 40-kW 1.6-L diesel engine (VW; operated either according to the US 72 cycle or under constant load conditions), diluted 1:80, 1:27 or 1:9 with clean air (average diesel soot particles, 0.8, 2.5 or 7.0 mg/m^3; 0.3–3.8 ppm NO_2; 4.7–33.1 ppm NO_x), 18 h/d, 5 d/wk for 24 mo and kept untreated for up to an additional 6 mo Groups of 100–220, aged 7 wk	Lung (all tumours): 1/217, 0/198, 11/200 (6%), 22/100 (22%) Lung (bronchiolo-alveolar adenoma): 0/217, 0/198, 2/200 (1%), 4/100 (4%)* Lung (adenocarcinoma): 1/217 (0.5%), 0/198, 1/200, 5/100 (5%)** Lung (squamous cell carcinoma): 0/217, 0/198, 0/200, 2/100 (2%) Lung (benign squamous cell tumour): 0/217, 0/198, 7/200 (4%)*, 14/100 (14%)***	*$P < 0.01$ **$P < 0.05$ *** $P < 0.001$	

Table 3.2 (continued)

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
F344 (F) Up to 30 mo Iwai *et al.* (1997)	Inhalation *Experiment 1* Clean air (control) or filtered or unfiltered exhaust from a 2.4-L diesel engine diluted 1:8 with conditioned air (particle concentration, 9.4 mg/m^3; 1.8 ppm NO_2) either directly or after particle exclusion through a HEPA filter, 8 h/d, 7 d/wk for 24 mo and survivors held unexposed for up to 6 mo. Groups of approximately 120 (clean air or filtered exhaust) and 24 (unfiltered exhaust), aged 8 wk	Lung (all tumours): 5/121 (4%), 4/108 (4%), 8/19 (42%)*	*$P < 0.01$	Results of study poorly reported; small number of animals exposed to unfiltered exhaust; lung tumours were mainly bronchiolo-alveolar adenoma and adenocarcinoma
	Experiment 2 Clean air (control) or filtered or unfiltered exhaust from a 2.4-L diesel engine diluted with conditioned air (particle concentration, 3.2 mg/m^3; 1.8 ppm NO_2), 8 h/d, 6 d/wk for 24 mo and then held unexposed for 6 mo Groups of approximately 120 (clean air or filtered exhaust) and 48 (unfiltered exhaust), aged 8 wk	Lung (all tumours): 5/121 (4%), 4/108 (4%), 5/43 (12%)*	*$P < 0.01$	Results of study poorly reported; lung tumours were mainly bronchiolo-alveolar adenoma and adenocarcinoma
	Experiment 3 Clean air (control) or filtered or unfiltered exhaust from a 2.4-L diesel engine run on commercial light oil, diluted with conditioned air (particle concentration, 5.1 mg/m^3; 1.8 ppm NO_2), 18 h/d, 3 d/wk for 24 mo and then held unexposed for 6 mo Groups of approximately 120 (clean air or filtered exhaust) and 96 (unfiltered exhaust), aged 8 wk	Lung (all tumours): 5/121 (4%), 4/108 (4%), 40/96 (42%)*	*$P < 0.01$	Results of study poorly reported; lung tumours were mainly bronchiolo-alveolar adenoma and adenocarcinoma

Table 3.2 (continued)

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
F344 (F) 30 mo Iwai *et al.* (2000)	Inhalation Clean air (control) or exhaust from a light-duty diesel engine (operating at 1050 rpm), diluted with filtered conditioned air (particle concentration, 3.5 ± 1.4 mg/m^3; 1.3 ± 1.0 ppm NO_2; 34.5 ± 10.8 ppm NO_x), 17 h/d, 3 d/wk for 3, 6, 9 or 12 mo, moved to a clean air room after each exposure period and maintained in clean air until end of 30 mo Control group of 50 and exposure groups of 48, aged 8 wk	Lung (all tumours): 1/48 (2%), 0/48, 6/43 (14%), 19/47 (40%)*, 10/44 (23%)**	*$P < 0.001$ **$P < 0.01$	Incidence of lung tumour types not reported; histological types of the lung tumours were bronchiolo-alveolar adenoma in 14 rats and adenocarcinoma in 22 rats, which were the major types observed, and squamous cell carcinoma in 3 rats, adenosquamous carcinoma in 1 rat and sarcoma in 1 rat
Wistar (M, F) 30 mo Stinn *et al.* (2005)	Inhalation (nose-only) Clean air (control) or unfiltered exhaust from a 1.6-L displacement VW diesel engine (operated under US FTP protocol), diluted 1:5 with air (3 mg/m^3 (low dose, obtained by further dilution) or 10 mg/m^3 (high dose) particles; 7 (low dose) or 23 (high dose) ppm NO; 9 (low dose) or 28 (high dose) ppm NO_x), 6 h/d, 7 d/wk for 24 mo Groups of 99 M and 99 F, aged 40 d	Lung (bronchiolo-alveolar adenoma): M–2/50 (4%), 3/50 (6%), 8/49 (16%)* F–0/51, 5/50 (10%)*, 21/51 (41%)* Lung (squamous cell carcinoma): M–0/50, 0/50, 1/49 (2%) F–0/51, 0/50, 4/51 (8%) Lung bronchiolo-alveolar carcinoma): M–0/50, 0/50, 3/49 (6%) F–0/51, 0/50, 1/51 (2%) Lung (all tumours): M–2/50 (4%), 9/50 (18%)*, 17/49 (35%)* F–0/51, 14/50 (28%)*, 29/51 (57%)*	*$P < 0.0$	

Table 3.2 (continued)

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Osborne-Mendel (F) Up to 140 wk Grimmer *et al.* (1987)	Intrapulmonary implantation Vehicle alone (control) or condensate from exhaust generated by a diesel passenger car (3.0-L, Daimler-Benz 300D), separated into hydrophilic (6.7 mg) and hydrophobic (20 mg) fractions (hydrophobic fraction separated by column chromatography into several subfractions): (A) non-aromatic compounds plus PAHs with two and three rings (19.22 mg), (B) PAHs with four or more rings (0.21 mg), (C) polar PAHs (0.29 mg), and (D) nitro-PAHs (0.19 mg) or a hydrophobic fraction reconstituted from subfractions A–D (19.9 mg) in beeswax:trioctanoin (1:1) Groups of 35, aged 3 mo	Lung (bronchiolo-alveolar adenoma): 1/35 (3%; control), 1/35 (3%; hydrophilic), 1/35 (3%; reconstituted hydrophobic) Hydrophobic fraction: Lung (squamous cell carcinoma): 0/35 (control), 5/35 (14%; hydrophobic)*, 6/35 (17%; PAHs 4–7 rings)*, 1/35 (3%; nitro-PAHs), 7/35 (20%; reconstituted hydrophobic)*	*[$P < 0.05$]	Study very poorly reported; details of results, including pathology and dosing regimen not clear; results summarized in a general manner and difficult to interpret
Wistar Crl:(WI) BR (F) Up to 800 d Dasenbrock *et al.* (1996)	Intratracheal instillation 0 (control) or 0.2–0.3 mL of particle suspensions from the 1:10 diluted exhaust of a 1.6-L VW diesel engine (Golf/Passat) out of a dilution tunnel on cellulose nitrate filters (pore size, 5 µm): A, diesel particles (total dose, 15 mg); B and C, diesel particles extracted with toluene (total doses, 15 and 30 mg); D, extracted diesel particles coated with B[*a*]P (total dose, 15 mg including 170 µg B[*a*]P); or E and F, B[*a*]P (total dose, 15 and 30 mg) in 0.25% Tween 80 saline, once/wk for 16–17 wk and observed for up to 800 d Groups of 50–52, aged 7 wk	Lung (cystic keratinizing epithelioma): 0/47, 8/48 (17%; A)*, 1/48 (2%; B), 8/48 (17%; C)*, 3/48 (6%; D), 3/48 (6%; E), 22/47 (47%; F)** Lung (bronchiolo-alveolar adenoma): 0/47, 0/48 (A), 1/48 (2%; B), 1/48 (2%; C), 0/48 (D), 2/48 (4%; E), 0/47 (F) Lung (squamous cell carcinoma): 0/47, 0/48 (A), 0/48 (B), 0/48 (C), 0/48 (D), 8/48 (17%; E)*, 38/47 (81%; F)** Lung (bronchiolo-alveolar carcinoma): 0/47, 0/48 (A), 0/48 (B), 2/48 (4%; C), 1/48 (2%; D),1/48 (2%; E), 2/47 (4%; F)	*$P < 0.01$, **$P < 0.001$	Early mortality (less than 50% survival after 2 yr) may have affected the outcome of the study

Table 3.2 (continued)

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
F344 (F) Up to 30 mo Iwai *et al.* (1997)	Intratracheal instillation 0.2 mL of 5 mg/[mL] suspension of particles from exhaust of a 2.4-L diesel engine (collected on each stage of a middle volume Andersen's sampler), suspended in a 0.05% Tween 80 or DMSO phosphate buffer (pH 7.4) solution, once/wk for 2–10 wk (total doses, 2, 4, 8 and 10 mg) and observed for up to 30 mo Groups of at least 50, aged 8 wk	Lung (all tumours): 6% (2% malignant), 20% (13% malignant), 43% (34% malignant), 74% (48% malignant)	NR	Study poorly described and designed, and results poorly reported; unclear whether there was a control group. No individual data on lung tumour incidence in exposed groups. Lung tumours were mainly bronchiolo-alveolar adenoma and adenocarcinoma
Wistar (HsdCpb:WU) (F) Up to 30 mo Pott & Roller (2005); Mohr *et al.* (2006)	Intratracheal instillation 0 (control) or 2.5 mg diesel soot suspended in 4.1 µL 0.5% Tween 80 phosphate buffer solution, once/wk for 3 wk, 3 mg suspended in 8.1 µL, once/wk for 5 wk, or 6 mg suspended in 16.2 µL, once/wk for 5 wk Groups of 48, aged 8–9 wk	Lung (adenocarcinoma or squamous cell carcinoma): 0/46, 1/45 (2%), 5/47 (11%), 6/45 (13%)* Lung (adenoma or epithelioma): 0/46, 1/45 (2%), 7/47 (15%)**, 12/45 (27%)***	[* $P = 0.03$, ** $P = 0.01$, *** $P = 0.0005$]	Control group not treated concurrently
Administration with known carcinogens				
F344 (F) Up to 24 mo Takemoto *et al.* (1986)	Intraperitoneal injection/inhalation Clean air (control) or exhaust from a 269 cm^3 small diesel engine, diluted 1:2 to 1:4 with clean air (D; particulate matter concentration, 2–4 mg/m^3; 2–4 ppm NO_2), 4 h/d, 4 d/wk for up to 24 mo; 1 mo later, two groups injected with 1 g/kg DiPN once/wk for 3 wk Groups of 20–35, aged 5 wk	Lung (carcinoma): 0/12, 0/15 (D), 7/18 (39%; D+DiPN), 4/21 (19%; DiPN) Lung (adenoma): 0/12, 0/15 (D), 12/18 (67%; D+DiPN), 10/21 (48%; DiPN)	NS (D+DiPN versus DiPN)	Small group size

B[*a*]P, benzo[*a*]pyrene; d, day; DiPN, *N*-nitrosodiisopropanolamine; DMSO, dimethyl sulfoxide; F, female; FTP, Federal Test Procedures; h, hour; HEPA, high-efficiency particulate air; M, male; mo, month; NO, nitrogen oxide; NO_2, nitrogen dioxide; NO_x, nitrogen oxides; NR, not reported; NS, not significant; PAHs, polycyclic aromatic hydrocarbons; VW, Volkswagen; wk, week; yr, year

(a) Inhalation

Groups of 24 Wistar male rats, aged 18 weeks, were exposed for 6 hours a day, 5 days a week for 20 months to one of five experimental atmospheres: clean air (control); 8.3 ± 2.0 (standard deviation) mg/m^3 of soot from diesel exhaust; 8.3 ± 2.0 mg/m^3 of soot from diesel exhaust plus 5.8 ± 3.5 mg/m^3 of coal dust; 6.6 ± 1.9 mg/m^3 of coal dust; or 14.9 ± 6.2 mg/m^3 of coal dust. The diesel exhaust was produced by a three-cylinder, 43-brake horse power (hp) diesel engine. The fuel injection system of the engine was modified to simulate the operating patterns of such engines when used in mines and was operated on a variable duty cycle (dilution, approximately 35:1). Six rats per group were killed after 4, 8, 16 and 20 months of exposure, and grossly visible lesions were examined histopathologically. Significant non-neoplastic lesions were restricted primarily to the respiratory tract and increased in severity with duration of exposure. In the six rats examined from each group after 20 months of exposure, two bronchiolar adenomas [bronchiolo-alveolar adenomas] were observed: one in the group exposed to diesel exhaust only and one in the group exposed to diesel exhaust and coal dust. No tumours were observed in controls or in the two groups exposed to coal dust only (Karagianes *et al.*, 1981). [The Working Group noted the limited number of rats examined (24 rats per group, only six of which were exposed for 20 months) and the use of male animals only.]

Groups of 96 female Wistar rats, aged 8–10 weeks, were exposed to clean air (control) or to filtered or unfiltered exhaust from a 1.6-L displacement diesel engine (operated to simulate average urban driving) diluted 1:17 with air for 19 hours a day, 5 days a week for life (up to 140 weeks). The unfiltered exhaust contained 4.24 mg/m^3 of particles; the levels of nitrogen dioxide and nitrogen oxides were 1.5 ± 0.3 and 11.4 ± 2.1 ppm in whole exhaust and 1.2 ± 0.26 and 9.9 ± 1.8 ppm in filtered exhaust, respectively. A significantly increased incidence of lung tumours [$P < 0.0001$] (identified histologically as eight bronchiolo-alveolar adenomas and nine squamous cell tumours [mainly benign]) was observed in rats exposed to unfiltered diesel exhaust (15 out of 95 (18%) versus 0 out of 96 controls). No lung tumours were reported in rats exposed to clean air or filtered exhaust (Heinrich *et al.*, 1986a).

Groups of 64 male and 59 female Fischer 344Jcl rats, aged 5 weeks, were exposed to diesel exhaust from either a light-duty 1.8-L displacement, four-cylinder engine (particle concentrations of 0.11, 0.41, 1.08 or 2.32 mg/m^3; nitrogen dioxide concentrations of 0.08, 0.26, 0.70 or 1.41 ppm; and nitrogen oxide concentrations of 1.24, 4.06, 10.14 or 20.34 ppm) or a heavy-duty 11-L displacement, six-cylinder engine (particle concentrations of 0.46, 0.96, 1.84 or 3.72 mg/m^3; nitrogen dioxide concentrations of 0.46, 1.02, 1.68 or 3.00 ppm; and nitrogen oxide concentrations of 6.17, 13.13, 21.67 or 37.45 ppm) for 16 hours a day, 6 days a week for up to 30 months. Separate control groups for the light-duty and heavy-duty engine exhaust-treated animals were exposed to clean air. The incidence of malignant lung tumours, diagnosed as adenocarcinoma, squamous cell carcinoma or adenosquamous carcinoma, was 5 out of 64 (8%) high-dose (3.72 mg/m^3) males and 3 out of 60 (5%) high-dose females exposed to heavy-duty engine diesel exhaust compared with 0 out of 64 control males and 1 out of 59 (2%) control females. The lung tumour incidence in males and females combined (6.5%) differed significantly ($P < 0.05$) from that in controls (0.8%). The incidence of malignant lung tumours in the groups exposed to 1.84 mg/m^3 heavy-duty engine diesel exhaust was 3 out of 64 (5%) males and 1 out of 59 (2%) females [and did not differ statistically significantly from that in the controls.] No statistically significant increase in the incidence of lung tumours was noted in the groups exposed to light-duty diesel engine exhaust (Ishinishi *et al.*, 1986).

Groups of 24 female Fischer 344 rats, aged 7 weeks, were exposed to clean air (control), diluted diesel exhaust or diluted filtered diesel exhaust for 8 hours a day, 7 days a week for 24 months, at which time some rats were killed and the remainder was returned to clean air for 6 months of observation. The diesel exhaust, produced by a 2.4-L small truck diesel engine, was diluted 1:10 in clean air and contained 4.9 ± 1.6 mg/m^3 of particles, 1.8 ± 1.8 ppm of nitrogen dioxide and 30.9 ± 10.9 ppm of nitrogen oxides. The incidence of lung tumours in the group exposed to whole diesel exhaust, with (n = 5) or without (n = 14) a subsequent observation period of 6 months, was 8 out of 19 (42%; three adenomas, one adenocarcinoma, two adenosquamous carcinomas, one squamous cell carcinoma and one large cell carcinoma) and was significantly higher (all tumours, $P < 0.01$; malignant tumours, $P < 0.05$) than that in the control group (1 (adenoma) out of 22, 5%). No lung tumours were observed in the group exposed to filtered exhaust (0 out of 16 rats). The incidence of lymphoma was increased ($P < 0.05$) in the group exposed to filtered exhaust (9 out of 24) compared with the controls (2 out of 24), but the incidence of tumours at other sites did not differ among the three groups (Iwai *et al.*, 1986). [The Working Group noted the small number of exposed animals.]

Groups of 72 male and 72 female Fischer 344 rats, aged 8–10 weeks, were exposed for 7 hours a day, 5 days a week for 24 months to: clean air (control); 2 mg/m^3 of coal dust; 2 mg/m^3 of diesel exhaust particles; or 1 mg/m^3 of coal dust plus 1 mg/m^3 of diesel exhaust particles. Additional groups of 144 male rats were also exposed to the same substances, and at least 10 animals from each group were killed at interim periods of 3, 6, 12 and 24 months. The diesel exhaust was generated by a 7.0-L displacement, four-cycle Caterpillar Model 3304 diesel engine and was diluted by a factor of 27:1 before the exposure; the nitrogen dioxide concentration in the exhaust was 1.5 ± 0.5 ppm. After 24 months of exposure, all survivors were killed. The numbers of rats necropsied and examined histologically in each of the four groups after 24 months of exposure were 120–121 males and 71–72 females. No statistically significant difference in tumour incidence was noted between the four groups (Lewis *et al.*, 1986, 1989).

Groups of 221–230 male and female Fischer 344 rats, aged 17 weeks, were exposed to one of three concentrations of diesel engine exhaust generated by a 1980 model 5.7-L V8 engine (operated according to US FTP cycles) for 7 hours a day, 5 days a week for up to 30 months. The exposure concentrations were reported as dilutions of the whole exhaust to measured soot concentrations of 0.35 (low), 3.5 (mid) or 7.0 (high dose) mg/m^3, for which the levels of nitrogen dioxide were 0.1 ± 0.1, 0.3 ± 0.2 and 0.7 ± 0.5 ppm, respectively. Sham-exposed controls received filtered air. Subgroups of animals were removed at 6, 12, 18 and 24 months for ancillary studies; all rats surviving after 30 months of exposure were killed. A total of 901 rats were necropsied and examined histologically for lung tumours. Four lung tumours types were found: bronchiolo-alveolar adenoma, adenocarcinoma, squamous cysts (mostly benign tumours) and squamous cell carcinoma. None of the tumours were found to have metastasized to other organs. The prevalence of lung tumours in males and females combined was 0.9% (adenocarcinoma and squamous cell carcinoma) in controls, 1.3% (adenocarcinoma and squamous cell carcinoma) in the low-dose, 3.6% (2.3% adenoma, 0.5% adenocarcinoma and squamous cell carcinoma, 0.9% squamous cysts) in the mid-dose and 12.8% (0.4% adenoma, 7.5% adenocarcinoma and squamous cell carcinoma, 4.9% squamous cysts) in the high-dose groups. Compared with controls, the prevalence of lung tumours at the mid-and high dose was significantly increased ($P < 0.05$) as was that of lung adenoma at the mid-dose and adenocarcinoma and squamous cell carcinoma (combined) at the high dose (Mauderly *et al.*, 1986, 1987).

Groups of 20–26 female Fischer 344 rats, aged 5 weeks, were exposed to clean air (control) or diesel exhaust from a 269-cm^3 displacement small diesel engine run at idling speed, diluted 1:2–1:4 with clean air to give a particulate matter concentration of 2–4 mg/m^3 and 2–4 ppm of nitrogen dioxide, for 4 hours a day, 4 days a week for up to 24 months. No lung tumours were observed in the 12 controls or 15 treated rats that survived for 18–24 months (Takemoto *et al.*, 1986). [The Working Group noted that the study was limited by the small number of exposed animals and the short duration of daily exposures.]

Groups of 72 male and 72 female Fischer 344 rats, aged 6–8 weeks, were exposed to one of three concentrations (0.7, 2.2 or 6.6 mg/m^3) of exhaust or particle-filtered exhaust from a 1.5-L VW Rabbit diesel engine (operated to simulate average urban driving conditions: US-72 FTP) for 16 hours a day, 5 days a week for 24 months, and survivors were maintained for a further 6 months in clean air. The exposure concentrations were reported as a dilution of the exhaust with a constant volume of 800 m^3 of air (high dose), a further 1:3 dilution of this mixture in air (mid-dose) or a further dilution of 1:9 (low dose). Levels of 0.9–2.8 ppm of nitrogen oxides and 0.7–7 ppm of nitrogen dioxide were reported in the different exposure chambers. Two control groups of 144 males and females were exposed to conditioned air. Eight animals per sex were killed at interim periods of 6, 12, 18 and 24 months. A significant increase in the incidence of lung tumours was observed in the high-dose males and high- and mid-dose females exposed to unfiltered exhaust. The highest incidence of tumours was seen in high-dose rats that died after the end of exposure (between 24 and 30 months) with a total lung tumour [mainly adenoma, squamous cell carcinoma, adenocarcinoma, and mixed adenoma/adenocarcinoma/squamous cell carcinoma, and one mesothelioma; individual incidences were not given] incidence of 12 out of 27 (44%) males (malignant lung tumours, 10 out of 27) and 24 out of 25 (96%) females (malignant lung tumours, 19 out of 25). No increase in the incidence of respiratory tract tumours was observed in rats exposed to the filtered exhaust (Brightwell *et al.*, 1989).

Groups of 64 male and 59 female Fischer 344 rats, aged 5 weeks, were exposed for 16 hours a day, 6 days a week [duration unspecified] to exhaust from a 1.8-L light-duty diesel engine or from an 11-L heavy-duty diesel engine diluted with filtered, conditioned air to give particle concentrations of 0, 0.1, 0.4, 1.1 or 2.3 mg/m^3 or 0, 0.5, 1.0, 1.8 or 3.7 mg/m^3, respectively. No significant increase in the incidence of lung tumours was observed in any of the treated animals (Takaki *et al.*, 1989). [The Working Group noted that the study was reported as a poster presentation, and some critical experimental details were absent, such as the duration of the experiment.]

To explore the importance of diesel exhaust soot-associated organic compounds in the induction of rat lung tumours, groups of approximately 100 male and female Fischer 344 rats, aged 7–9 weeks, were exposed to aerosolized carbon black or exhaust from two 1988 Model LH6 General Motors 6.2-L V8 diesel engines that was diluted with filtered conditioned air to obtain a particulate concentration of 2.5 or 6.5 mg/m^3 and levels of 0.73 or 3.78 ppm of nitrogen dioxide and 8.79 or 23.45 ppm of nitrogen oxides, respectively, for 16 hours a day, 5 days a week for 24 months, and then held for up to an additional 6 months. Both diesel exhaust soot and carbon black particles accumulated in the lungs of exposed rats, but the rate of accumulation was higher for diesel exhaust soot. Diesel exhaust and carbon black both induced concentration-related significant increases in the incidence of bronchiolo-alveolar adenoma and bronchiolo-alveolar adenocarcinoma in female rats only (Mauderly *et al.*, 1994; Nikula *et al.*, 1995).

Groups of 100–220 female Wistar rats, aged 7 weeks, were exposed for 18 hours a day, 5 days a week for 24 months to clean air (control) or diesel

exhaust from a 1.6-L diesel engine (VW; operated according to the US FTP 72-cycle or under constant load conditions) diluted 1:80, 1:27 or 1:9 with clean air to obtain average diesel soot particle concentrations of 0.8, 2.5 and 7.0 mg/m^3, respectively, and levels of 0.3–3.8 ppm of nitrogen dioxide and 4.7–33.1 ppm of nitrogen oxides in the diesel exhaust atmospheres. Surviving animals were maintained untreated for up to an additional 6 months. Exposure to diesel exhaust produced a statistically significant increase in the incidence of bronchiolo-alveolar adenoma (4 out of 100, 4%; $P < 0.01$) and adenocarcinoma (5 out of 100, 5%; $P < 0.05$) in high-dose animals and benign squamous cell tumours of the lung in the mid- (7 out of 200, 7%; $P < 0.01$) and high-dose (14 out of 100, 14%; $P < 0.001$) groups compared with controls (0 out of 217 adenoma or benign squamous cell tumours and 1 out of 217 adenocarcinoma) (Heinrich *et al.*, 1995).

Two groups of approximately 120 female Fischer 344 rats, aged 8 weeks, and another group of 24 females were exposed to clean air (control), diluted filtered diesel exhaust or diluted unfiltered diesel exhaust for 8 hours a day, 7 days a week for 24 months. Survivors were returned to clean air for up to 6 months of observation. The diesel exhaust produced by a 2.4-L diesel engine was diluted with conditioned air to give a particle concentration of 9.4 mg/m^3 and 1.8 ppm of nitrogen dioxide, and was delivered to the exposure chambers either directly or after excluding particles by passage through a high-efficiency particulate air filter. The incidence of lung tumours [mainly bronchiolo-alveolar adenoma and adenocarcinoma] in animals that survived at least 18 months was significantly higher in the group exposed to whole diesel exhaust (8 out of 19, 42%; including 5 out of 19 (26%) malignant tumours) than in the control group (5 out of 121, 4%; $P < 0.01$) or the group exposed to filtered exhaust (4 out of 108, 4%; $P < 0.01$) (Iwai *et al.*, 1997). [The Working Group noted the small size of the group exposed to whole diesel exhaust.]

In a second, concurrent experiment, a group of 48 female Fischer 344 rats, aged 8 weeks, was exposed to diluted diesel exhaust produced by the system described above (2.4-L diesel engine) for 8 hours a day, 6 days a week for 24 months. The particle concentration was 3.2 mg/m^3 and the level of nitrogen dioxide was 1.8 ppm. After treatment, survivors were maintained in clean air for up to 6 months of observation. The incidence of lung tumours (mainly bronchiolo-alveolar adenoma and adenocarcinoma) was significantly higher in the group exposed to whole diesel exhaust (5 out of 43, 12%) than in the control group (5 out of 121, 4%; $P < 0.01$) or the group exposed to filtered exhaust (4 out of 108, 4%; $P < 0.01$) from the previous experiment (Iwai *et al.*, 1997).

In a third, concurrent experiment, groups of 96 female Fischer 344 rats, aged 8 weeks, were exposed to diluted diesel exhaust for 18 hours a day, 3 days a week for 24 months. Survivors were maintained in clean air for up to 6 months of observation. The diesel exhaust was produced by a 2.4-L diesel engine and was diluted with conditioned air to give a particle concentration of 5.1 mg/m^3 and 1.8 ppm nitrogen dioxide. The incidence of lung tumours (mainly bronchiolo-alveolar adenoma and adenocarcinoma) was significantly higher in the group exposed to whole diesel exhaust (40 out of 96, 42%) than in the control group (5 out of 121, 4%; $P < 0.01$) or the group exposed to filtered exhaust (4 out of 108, 4%; $P < 0.01$) from the previous experiment (Iwai *et al.*, 1997).

Groups of 48–50 female Fischer 344 rats, aged 8 weeks, were exposed to either clean air (control) or diluted diesel exhaust for 17 hours a day, 3 days a week for 3, 6, 9 or 12 months, were moved to a clean-air room after each exposure period and were maintained in clean air until the end of the 30-month experimental period. The diesel exhaust was produced from a light-duty diesel engine (operated at 1050 rpm) and was diluted with filtered conditioned air to give a particle concentration of 3.5 ± 1.4 mg/m^3, 1.3 ± 1.0

ppm of nitrogen dioxide and 34.5 ± 10.8 ppm of nitrogen oxide. The incidence of lung tumours was significantly higher in the group exposed to whole diesel exhaust for 9 months (19 out of 47, 40%; $P < 0.001$) and 12 months (10 out of 44, 23%; $P < 0.01$) than that in the control group (1 out of 48, 2%). For all exposure periods, lung tumours were observed in treated rats only after the 18th experimental month. The histological types of lung tumour observed were bronchiolo-alveolar adenoma (14 rats) and adenocarcinoma (22 rats), which were the major types, and squamous cell carcinoma (three rats), adenosquamous carcinoma (one rat) and sarcoma (one rat) (Iwai *et al.*, 2000).

Groups of 99 male and 99 female Wistar rats, aged 40 days, were exposed by nose-only inhalation for 6 hours a day, 7 days a week for 24 months to exhaust from a 1.6-L displacement VW diesel engine (operated under US FTP conditions) that was diluted 1:5 with air and contained 3 mg/m^3 (low dose, obtained by further dilution) or 10 mg/m^3 (high dose) of particles. Levels of 7 (low dose) or 23 (high dose) ppm of nitrogen monoxide and 9 (low dose) or 28 (high dose) ppm of nitrogen oxides were found in the whole exhaust. Controls were exposed to clean air only. Surviving animals were maintained with no further treatment for an additional 6 months. A significantly increased incidence of lung tumours was observed in rats exposed to both the high dose (males, 17 out of 49; females, 29 out of 51) and low dose (males, 9 out of 50; females 14 out of 50) compared with controls (males, 2 out of 50 [adenomas only], 4%; females, 0 out of 51). A significant increase in the incidence of lung adenomas was also observed in the high-dose males (8 out of 49) and females (21 out of 51) and low-dose females (5 out of 50) (Stinn *et al.*, 2005).

(b) Intrapulmonary implantation

Groups of 35 female Osborne-Mendel rats, aged 3 months, received a single intrapulmonary implant of different fractions of organic material from a diesel exhaust condensate in beeswax:trioctanoin (1:1) and were observed for up to 140 weeks. The organic material was collected from a 3.0-L Daimler-Benz 300D diesel passenger car engine (operated under the first cycle of the European test cycle) and was separated by liquid–liquid distribution into a hydrophilic fraction (approximately 25% by weight of the total condensate) and a hydrophobic fraction (approximately 75% by weight). The latter was separated by column chromatography into several further fractions: (A) non-aromatic compounds plus polycyclic aromatic hydrocarbons (PAHs) with two and three rings (72% by weight of the total condensate), (B) PAHs with four to seven rings (0.8% by weight), (C) polar PAHs (1.1% by weight) and (D) nitro-PAHs (0.7% by weight). The animals received 6.7 mg of the hydrophilic fraction, 20 mg of the hydrophobic fraction, 19.22 mg of the hydrophobic subfraction containing non-aromatic compounds and two- and three-ring PAHs, 0.21 mg of the hydrophobic subfraction containing four to seven-ring PAHs, 0.29 mg of the hydrophobic subfraction containing polar PAHs, 0.19 mg of the hydrophobic subfraction containing nitro-PAHs or 19.9 mg of a reconstituted hydrophobic fraction (from subfractions A–D). A group of 35 females received implants of beeswax:trioctanoin only. Six lung tumours (17%; squamous cell carcinoma) were found in animals treated with the hydrophobic subfraction containing PAHs with four to seven rings. Similar carcinogenic potency was seen with the reconstituted hydrophobic subfraction (20%; seven squamous cell carcinomas) and the hydrophobic fraction (14%; five squamous cell carcinomas), whereas low carcinogenic potency was observed with the subfraction of nitro-PAHs (3%; one squamous cell carcinoma). The polar PAH subfraction induced no tumour; and one bronchiolo-alveolar adenoma (3%) was observed in animals treated with the non-aromatic subfraction with two- and three-ring PAHs. One bronchiolo-alveolar adenoma (3%)

occurred in the vehicle control group (Grimmer *et al.*, 1987). [The Working Group noted the poor reporting of the study, and that details of results, including pathology and dosing regimen, were not provided. The results were summarized in a general manner and were difficult to interpret.]

(c) Intratracheal instillation

Groups of 50–52 female Wistar Crl:(WI)BR rats, aged 7 weeks, received intratracheal instillations once a week for 16–17 weeks of 0.2–0.3 mL of suspensions of diesel particles that were collected on cellulose nitrate filters from the exhaust of a 1.6-L VW diesel engine (Golf/Passat) diluted 1:10 in a dilution tunnel and suspended in a 0.25% Tween 80 saline solution. Suspensions included: A, diesel particles (total dose, 15 mg); B and C, diesel particles extracted three times with toluene (total dose, 15 and 30 mg, respectively); D, extracted diesel particles coated with benzo[*a*]pyrene (total dose, 15 mg including 170 µg benzo[*a*]pyrene); and E and F, benzo[*a*]pyrene (total dose, 15 and 30 mg, respectively). Controls received 0.25% Tween 80 saline solution alone. Animals were then observed for up to 800 days after the first instillation. A significant increase in the incidence of lung cystic keratinizing epithelioma was observed in groups instilled with diesel particles (A: 8 out of 48, 17%; $P < 0.01$), with 30 mg of extracted diesel particles (C: 8 out of 48, 17%; $P < 0.01$), or with 30 mg benzo[*a*]pyrene (F: 22 out of 47, 47%; $P < 0.001$) compared with controls (0 out of 47) (Dasenbrock *et al.*, 1996). [The Working Group noted that early mortality (fewer than 50% survived more than 2 years) may have affected the outcome of the study.]

Groups of at least 50 female Fischer 344 rats, aged 8 weeks, received intratracheal instillations once a week for 2–10 weeks of 0.2 mL of a 5 mg/[mL] suspension (total doses, 2, 4, 8 and 10 mg) of diesel particulates collected from the exhaust of a 2.4-L diesel engine at each stage of a middle volume Andersen's sampler and suspended in 0.05% Tween 80 or DMSO phosphate buffer (pH 7.4) solution and were observed for up to 30 months after the first instillation. A dose-related increase in the incidence of lung tumours [mainly bronchiolo-alveolar adenoma and adenocarcinoma] was reported, reaching up to 74% (48% of malignant tumours) in rats instilled with 10 mg (Iwai *et al.*, 1997). [The Working Group noted that it was unclear whether a control group was used, the study was poorly described and designed, the results of the study were poorly reported, and no individual data on lung tumour incidence in exposed groups were available.]

Groups of 48 female Wistar HsdCpb:WU rats, aged 8–9 weeks, received intratracheal instillations of 2.5 mg of diesel soot suspended in 4.1 µL of a 0.5% Tween 80 phosphate buffer solution once a week for 3 weeks (A), 3 mg of diesel soot suspended in 8.1 µL of buffer solution once a week for 5 weeks (B), or 6 mg of diesel soot suspended in 16.2 µL of buffer solution once a week for 5 weeks (C) and were then observed for up to 30 months after the first instillation. A significant increase in the incidence of lung adenocarcinoma or squamous cell carcinoma (combined) in the high-dose group (C: 6 out of 45, 13%; $P = 0.03$) and significant increases in the incidence of lung adenoma or epithelioma (combined) in the mid- and high-dose groups (B: 7 out of 47, 15%; $P = 0.01$; C: 12 out of 45, 27%; $P = 0.0005$) were observed compared with the controls (0 out of 46) (Pott & Roller, 2005; Mohr *et al.*, 2006). [The Working Group noted that the control group was not treated concurrently.]

(d) Administration with known carcinogens

Groups of 20–35 female Fischer 344 rats, aged 5 weeks, were exposed to diesel exhaust from a 269-cm^3 displacement small diesel engine run at idling speed, diluted 1:2–1:4 with clean air to give concentrations of 2–4 mg/m^3 of particulate matter and 2–4 ppm of nitrogen dioxide, for 4 hours a day, 4 days a week for up to 24 months. After 1 month of exposure, two

groups were injected intraperitoneally with 1 g/kg bw of *N*-nitrosodiisopropanolamine (DiPN) once a week for 3 weeks. No lung tumours were observed in the groups treated with diesel exhaust alone or controls. In contrast, an increase in the incidence of lung tumours (adenoma and carcinoma) was observed in the groups treated with diesel exhaust plus DiPN and DiPN alone, but the difference in incidence between these groups was not significant (Takemoto *et al.*, 1986). [The Working Group noted the small group size.]

3.1.3 Hamster

See Table 3.3

(a) Inhalation

Groups of 48 female Syrian golden hamsters, aged 8 weeks, were exposed to clean air (control) or to filtered or unfiltered exhaust from a 2.4-L Daimler-Benz diesel engine, diluted 1:7 with air, for 7–8 hours a day, 5 days a week for life [up to 120 weeks]. The unfiltered exhaust contained 3.9 mg/m^3 of particles. The levels of nitrogen dioxide and nitrogen oxides were 1.2 ± 1.7 and 18.6 ± 5.8 ppm in whole exhaust and 1.0 ± 1.5 and 19.2 ± 6.1 ppm in filtered exhaust, respectively. Diesel exhaust had no effect on survival; the median lifespan was 72–74 weeks in all groups, and no lung tumours were reported in treated or control animals (Heinrich *et al.*, 1982).

Groups of 48 male and 48 female Syrian golden hamsters, aged 8–10-weeks, were exposed to clean air (control) or to filtered or unfiltered exhaust from a 1.6-L displacement diesel engine (operated to simulate average urban driving), diluted 1:17 with air, for 19 hours a day, 5 days a week for life (up to 120 weeks). The unfiltered exhaust contained 4.24 mg/m^3 of particles. The levels of nitrogen dioxide and nitrogen oxides were 1.5 ± 0.3 and 11.4 ± 2.1 ppm in whole exhaust and 1.2 ± 0.26 and 9.9 ± 1.8 ppm in filtered exhaust, respectively. The median lifespan was not significantly influenced by exposure to diesel exhaust and was 75–80 weeks for females and 80–90 weeks for males. No lung tumour was observed in treated or control animals (Heinrich *et al.*, 1986a).

Groups of 104 male and 104 female Syrian golden hamsters, aged 6–8-weeks, were exposed to filtered or unfiltered exhaust emissions generated by a VW Rabbit 1.5-L diesel engine, diluted with clean air to give particle concentrations of 0.7, 2.2 or 6.6 mg/ m^3, for 16 hours a day, 5 days a week for 24 months. The filtered exhaust was passed through a stationary filtration system that removed 99.97% of the mass of particles before delivery to the animals. Further groups of 208 male and 208 female hamsters were exposed to clean air and served as controls. In addition, groups of 52 male and 52 female exposed hamsters and 104 male and 104 female controls were pretreated with 4.5 mg/kg of *N*-nitrosodiethylamine (NDEA) 3 days before the start of exposure. No significant increase in the incidence of respiratory tract (lung, trachea, larynx or nasal cavity) tumours was observed in any treated groups compared with their respective controls (Brightwell *et al.*, 1989).

(b) Intratracheal instillation

Groups of 59–62 male Syrian golden hamsters, aged 8 weeks, received intratracheal instillations of 0.1 mL of a suspension of 0 (control), 0.1, 0.5 or 1.0 mg of tar from a heavy-duty V6 11-L diesel engine exhaust suspended in 0.1 mL of a mixed solution of Tween 60:ethanol:phosphate buffer (1.5:2.5:30 mL, respectively) solution once a week for 15 weeks and were observed for life. Survival rates were 95%, 92%, 71% and 98% in the three treated groups and vehicle controls, respectively. [The duration of survival was not reported.] No significant difference in the incidence of tumours of the lung, trachea or larynx was observed between controls and treated groups (Kunitake *et al.*, 1986).

Table 3.3 Studies of the carcinogenicity of diesel engine exhaust in hamsters

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Syrian golden (F) [Up to 120 wk] (lifetime) Heinrich *et al.* (1982)	Inhalation Clean air (control), or filtered or unfiltered exhaust from a 2.4-L Daimler-Benz diesel engine, diluted 1:7 with air (unfiltered exhaust: 3.9 mg/m^3 particles, 1.2 ± 1.7 ppm NO_2 and 18.6 ± 5.8 ppm NO_x; filtered exhaust: 1.0 ± 1.5 ppm NO_2 and 19.2 ± 6.1 ppm NO_x), 7–8 h/d, 5 d/wk Groups of 48, aged 8 wk	No lung tumours observed in treated or control animals	NS	Median lifespan was not influenced by exposure to diesel exhaust
Syrian golden (M, F) Up to 120 wk Heinrich *et al.* (1986a)	Inhalation Clean air (control), or filtered or unfiltered exhaust from a 1.6-L displacement diesel engine (operated to simulate average urban driving), diluted 1:17 with air (unfiltered exhaust: 4.24 mg/m^3 particles, 1.5 ± 0.3 ppm NO_2 and 11.4 ± 2.1 ppm NO_x; filtered exhaust: 1.2 ± 0.26 ppm NO_2 and 9.9 ± 1.8 ppm NO_x), 19 h/d, 5 d/wk Groups of 48 M and 48 F, aged 8–10 wk	No lung tumours observed in treated or control animals	NS	Median lifespan was not influenced by exposure to diesel exhaust
Syrian golden (M, F) 24 mo Brightwell *et al.* (1989)	Inhalation Clean air (control), or whole exhaust emissions generated by a VW Rabbit 1.5-L diesel engine, diluted with clean air (particle concentrations, 0.7 mg/m^3, 2.2 mg/m^3 or 6.6 mg/m^3) or exhaust passed through a stationary filtration system to remove 99.97% of the mass of particles, 16 h/d, 5 d/wk; additional groups pretreated with 4.5 mg/kg NDEA 3 days before start of exposure Treated groups of 104 M and 104 F; control groups of 208 M and 208 F; 52 M and 52 F treated and 104 M and 104 F control pretreated with NDEA; aged 6–8 wk	No significant increase in the incidence of respiratory tract (lung, trachea, larynx or nasal cavity) tumours observed in any diesel engine exhaust-treated groups compared with the respective controls	NS	Authors reported clinical evidence of 'wet-tail' disease in both NDEA-pretreated and non-pretreated hamsters in all experimental and control groups that resulted in significant mortality (~45%) between 10 and 12 mo of exposure. Oxytetracycline and dimetridazole added to the drinking-water of all hamsters at 400 mg/L and 5 g/L, respectively, from about 12 mo of exposure until 24 mo, effectively controlled the infection, and no further mortalities associated with the disease occurred up to the end of the 2-yr exposure period
Syrian golden (M) Lifetime Kunitake *et al.* (1986)	Intratracheal instillation 0 (control), 0.1, 0.5 or 1.0 mg tar from a heavy-duty V6 11-L diesel engine exhaust suspended in 0.1 mL Tween 60, ethanol and phosphate buffer solution (1.5:2.5:30), once/wk for 15 wk Groups of 59 or 62, aged 8 wk	No significant difference in the incidences of tumours of the lung, trachea or larynx observed between controls and treated groups	NS	Duration of survival was not reported

B[*a*]P, benzo[*a*]pyrene; d, day; F, female, h, hour; M, male; mo, month; NDEA, *N*-nitrosodiethylamine; NO_2, nitrogen dioxide; NO_x, nitrogen oxides; NS, not significant; VW, Volkswagen; wk, week; yr, year

Table 3.4 Study of the carcinogenicity of diesel engine exhaust in monkeys

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Comments
Cynomolgus (*Macaca fascicularis*) (M) 24 mo Lewis *et al.* (1989)	Inhalation Clean air (control), exhaust from a 7.0-L Caterpillar Model 3304 diesel engine, diluted 1:27 with clean air (particle concentration, 1.95 ± 0.25 mg/m^3; 1.5 ± 0.5 ppm NO_2 and 8.7 ± 3.6 ppm NO), 2.00 ± 0.41 mg/m^3 coal dust or 2.02 ± 0.30 mg/m^3 coal dust plus diesel exhaust, 7 h/d, 5 d/wk Groups of 15 [age unspecified]	No statistical difference in tumour incidence between the four experimental groups	Study limited by the short observation period

d, day; h, hour; M, male; mo, month; NO, nitrogen oxide; NO_2, nitrogen dioxide; wk, week

3.1.4 Monkey

See Table 3.4

Inhalation

Groups of 15 male cynomolgus (*Macaca fascicularis*) monkeys were exposed for 7 hours a day, 5 days a week for 24 months to clean air or to the exhaust from a 7.0-L Caterpillar Model 3304 diesel engine, diluted 1:27 with clean air to give concentrations of 1.95 ± 0.25 mg/m^3 of particles, 1.5 ± 0.5 ppm of nitrogen dioxide and 8.7 ± 3.6 ppm of nitrogen oxide. Additional groups were exposed to 2.00 ± 0.41 mg/m^3 of coal dust or 2.02 ± 0.30 mg/m^3 of diesel exhaust plus coal dust. At the end of the exposure period, all survivors (59 out of 60) were necropsied and examined histologically. No significant difference in tumour incidence was reported among the four groups (Lewis *et al.*, 1989). [The Working Group noted that the study was limited by the short observation period.]

3.2 Gasoline engine exhaust

The gasoline engine exhausts used in the studies evaluated here were generated from fuels and engines produced before the year 2000. These exhausts include three basic components: particles composed primarily of elemental carbon and metallic compounds (especially lead, if present in the fuel); adsorbed organic material that is readily extractable with organic solvents; and a mixture of gas and vapour phases that include volatile organic compounds. Many studies have been carried out using several animal species to evaluate the potential carcinogenicity of exposure to whole exhaust and to the components of exhaust from gasoline engines. The studies are considered under two subcategories: (i) whole gasoline engine exhaust; and (ii) condensates or extracts of gasoline engine exhaust.

Animal bioassays conducted with different gasoline engine exhausts have been reviewed previously in the *IARC Monographs* (IARC, 1989). This section provides a summary of these and a detailed review of more recent studies.

3.2.1 Mouse

See Table 3.5

(a) Inhalation

Groups of 37–38 male and female mice of 'mixed' [unspecified] strain, aged 3 months, were exposed to clean air (control) or to exhaust from either a four-cylinder, 23-hp car engine run on regular (unleaded) gasoline or a six-cylinder, 24-hp car engine run on leaded gasoline. The exhausts were initially diluted 1:145 with air for

Table 3.5 Studies of the carcinogenicity of gasoline engine exhaust in mice

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
'Mixed' [strain NR] (M, F) Up to 25 mo (lifetime) Campbell (1936)	Inhalation Clean air (control) or exhaust from (A) a 4-cylinder, 23-hp gasoline car engine run on regular (unleaded) gas or (B) a 6-cylinder, 24-hp gasoline car engine run on leaded gas (1:1800), diluted 1:145 with air for 4 h and then diluted 1:83 for 3 h (particulate content and lead concentrations NR; average CO levels, 7.5% for A and 2.1% for B), 7 h/d on 5 d/wk Groups of 37 M and 38 F, aged 3 mo	Lung (all tumours): A (M+F)–8/74 (11%), 9/75 (12%) B (M+F)–6/70 (9%), 12/75 (16%)	NS	Study poorly reported; no details on survival or pathology; information on test atmosphere generation and chamber monitoring doubtful
ICR (F) 12 mo Yoshimura (1983)	Inhalation Exhaust emission generated by a small gasoline engine, diluted 1:250 with clean air to give concentration of 0.1 mg/m^3 (300 ± 50 ppm CO, 0.21 ppm NO and 0.08 ppm NO_2), 2 h/d on 3 d/wk for 6–12 mo [Initial numbers and age NR]	Lung (all tumours): 2/15 (13% adenoma only)	-	Study poorly designed and reported; short treatment period and lack of controls
C57BL [sex NR] [duration NR, > 390 days] Kotin *et al.* (1954)	Skin application Benzene alone (control) or an oil residue of the benzene extract of filter paper used to filter exhaust from an overhauled Ford V-8 gasoline engine [doses NR] in benzene, applied at frequent but irregular intervals Control groups of 42; treated groups of 86 [age NR]	Skin (all tumours): 0/42, 38/86 (44%)* Skin (squamous cell carcinoma): 0/42, 22/86** (26%)	[*$P < 0.0001$, **$P = 0.0005$]	The study appeared not to have been completed; authors state that "This 44% figure of positive tumour production is subject to upward revision in view of the possibility of tumour demonstration in 16 remaining mice." Study poorly reported: details on sex, age, dose used and dosing administration frequency NR
Swiss (F) Up to 18 mo Wynder & Hoffmann (1962)	Skin application 0 (control), 5, 10, 25, 33 or 50% of an oil residue of a benzene extract of condensed and filtered exhaust from a V8 gasoline engine in in acetone, applied with a No. 5 camel's hair brush dipped once in the 'tar' solution, 3 × /wk for 15 mo and held for an additional 3 mo Groups of 30–50, aged 6 wk	Skin (papilloma): 0%, 4%, 50%, 60%, 60%, 70% Skin (squamous cell carcinoma): 0%, 4%, 32%, 48%, 54%, 4%	NR	All animals in the high-dose group had died by 10 mo. Study poorly designed; dose application method gave inconsistent doses and high-dose animals were tested separately

Table 3.5 (continued)

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Swiss (F) 18 mo Hoffmann *et al.* (1965)	Skin application Tar [method of extraction NR] from exhaust of a V8 gasoline engine using approximately 1 L engine oil/200 miles [0.3 L/100 km] (A) or from exhaust of an engine using approximately 1 L oil/1600 miles [0.04 L/100 km] (B) in acetone, [frequency and method of application NR] Groups of 50 [age NR]	Skin (all tumours): 60% (48% carcinoma; A), 84% (52% carcinoma; B)	-	Study poorly designed and reported; method and frequency of exposure NR; no vehicle or untreated controls
CFLP (F) Lifetime Brune *et al.* (1978)	Skin application 0 (two control groups), 0.5, 1.6 or 4.7 µg of an exhaust condensate (C1, C2, C3) produced from a VW 1.5-L Otto gasoline engine during a European test cycle, nitromethane (two groups; N1, N2) and cyclohexane (two groups; CY1, CY2) fractions of the condensate, or reconstituted condensate (two groups; R1, R2) in 0.1 mL DMSO: acetone (3:1) on the shaved intrascapular region twice/wk for life Groups of 80 studied in Hamburg and groups of 40 studied in Heidelberg, aged 12 wk	Hamburg study Skin (squamous cell tumours): 0/76, 1/76 (1%), 3/77 (4%; C1), 26/74 (35%; C2)*, 60/78 (77%; C3)*, 11/67 (16%; N1)***, 51/74 (69%; N2)*, 10/73 (14%; CY1)****, 53/77 (69%; CY2)*, 6/76 (8%; R1), 44/75 (59%; R2)* Skin (squamous cell carcinoma): 0/76, 0/76, 1/77 (1%; C1), 22/74 (30%; C2)*, 56/78 (72%; C3)*, 9/67 (13%; N1)****, 48/74 (65%; N2)*, 10/73 (14%; CY1)****, 47/77 (61%; CY2)*, 3/76 (4%; R1), 40/75 (53%; R2)*	[*$P < 0.0001$, **$P = 0.0002$, ***$P = 0.005$, ****$P = 0.009$, *****$P = 0.001$]	

Table 3.5 (continued)

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
CFLP (F) Lifetime Brune et al. (1978) (cont.)		Heidelberg study Skin (squamous cell tumours): 0/30, 0/37, 1/31 (3%; C1), 3/37 (8%; C2), 19/38 (50%; C3)**, 3/34 (9%; N1), 14/37 (38%; N2)*, 0/34 (CY1), 11/38 (29%; CY2)*, 3/34 (9%; R1), 9/30 (30%; R2)** Skin (squamous cell carcinoma): 0/30, 0/37, 1/31 (3%; C1), 2/37 (5%; C2), 18/38 (47%; C3)*, 2/34 (6%; N1), 12/37 (32%; N2)*, 0/34 (CY1), 10/38 (26%; CY2)**, 3/34 (9%; R1), 7/30 (23%; R2)***** Lung (all tumours): 3/40 (7%), 3/40 (7%), 3/40 (7%; C1), 8/40 (20%; C2)*****, 9/40 (22%; C3)*****, 7/40 (17%; N1)***, 6/40 (15%; N2)****, 6/40 (15%; CY1)****, 4/40 (10%; CY2), 5/40 (12%; R1), 3/40 (7%; R2)	[*$P < 0.0001$, **$P = 0.0002$, ***$P = 0.005$, ****$P = 0.009$, *****$P = 0.001$] [*$P < 0.0001$, **$P = 0.0002$, ***$P = 0.005$, ****$P = 0.009$, *****$P = 0.001$]	

Table 3.5 (continued)

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
CLFP (F) Lifetime Grimmer *et al.* (1983a, b)	Skin application 0 (control), 0.29, 0.87 or 2.6 mg/animal condensate (E1, E2, E3) from exhaust emissions generated by a passenger car gasoline engine (1500 cm^3, 50 hp); several fractions of condensate: 0.97 or 2.9 mg/animal PAH-free (A1, A2), 0.152 or 0.455 mg/animal PAH-containing (2 and 3 rings) (B1, B2), and 0.02 or 0.06 mg/animal PAH-containing (more than 3 rings) (C1, C2); or 0.003 or 0.009 mg/animal of a mixture of 15 PAHs (D1, D2) simulating those in automobile exhaust; dissolved in DMSO: acetone (3:1) and applied as 0.1 mL of the resultant solution, twice/wk for 104 wk Control groups of 65; treated groups of 80; aged 7 wk	Skin (mainly squamous cell carcinoma): 0/65, 6/80 (7%; E1)***, 34/80 (42%; E2)*, 65/80 (81%; E3)*, 4/80 (5%; A1), 11/80 (14%; A2)**, 3/80 (4%; B1), 1/80 (1%; B2), 7/80 (9%; C1)***, 50/80 (62%; C2)*, 1/80 (1%; D1), 29/80 (36%; D2)*	[*$P < 0.0001$, **$P = 0.003$, ***$P = 0.04$]	Clear dose–response relationships demonstrated for skin tumours in the groups treated with: condensate, the fraction containing PAHs with more than three rings, and the mixture of 15 PAHs
NMRI (F) [Duration NR] Pott et al. (1977	Subcutaneous injection 0 (control), 20 or 60 mg exhaust condensate, containing 0.163 µg/mg B[*a*]P, from a gasoline engine [specifics NR] in 0.5 mL tricaprylin, once or 60 mg, × 3 [frequency NR] Groups of 87–88 and 45 (3 injections) [age NR]	Skin (fibrosarcoma): 3/89 (3%), 10/87 (11%), 6/88 (7%), 5/45 (11%)	NR	Study poorly reported; no details of duration of study, age of animals or pathology; no statistics provided
Initiation-promotion				
SENCAR (M, F) 24–26 wk Nesnow *et al.* (1982a, 1983)	Skin application Single 0.2 mL application of 0 (control), 0.1, 0.5, 1.0, 2.0, or 3.0 mg/mouse particulates from the emissions of a 1977 Ford Mustang II-302 V-8 engine with catalyst collected on Teflon-coated fibreglass filters, extracted with dichloromethane removed by evaporation, dissolved in acetone to shaved dorsal skin followed 1 wk later by 2.0 µg TPA in 0.2 mL acetone, twice/wk for up to 25 wk Groups of 40 M and 40 F, aged 7–9 wk	Skin (papillomas/mouse): M–0.08 (8%), 0.05 (5%), 0.15 (13%), 0.18 (18%), 0.24 (22%), 0.24 (18%) F–0.05 (5%), 0.23 (13%), 0.24 (18%), 0.13 (10%), 0.23 (21%), 0.28 (23%)	NR	Authors stated that the responses at the higher doses were significantly greater than those of the TPA controls; no statistics provided; 20% of the females developed squamous cell carcinoma with the 3-mg dose

Table 3.5 (continued)

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Administration with known carcinogens				
Mouse, NMRI (F) [Duration NR] Pott *et al.* (1977)	Subcutaneous injection 10, 30, 90 or 270 µg B[*a*]P alone or together with 6.6, 20 or 60 mg of exhaust condensate (C) from a gasoline engine [specifics NR] in 0.5 mL tricaprylin, once Groups of 87–88 [age NR]	6.6 mg *C* + *10, 30 or* 90 µg *B[a]P* Skin (fibrosarcoma): 19/89 (21%), 25/88 (28%), 46/88 (52%) 20 mg *C* + *10, 30, 90 or* 270 µg *B[a]P* Skin (fibrosarcoma): 15/86 (17%), 24/88 (27%), 36/87 (41%), 45/87 (52%) 60 mg *C* + 270 µg *B[a]P* Skin (fibrosarcoma): 33/90 (37%) *3.3, 10, 30, 90 or* 270 µg *B[a]P* Skin (fibrosarcoma): 7/90 (8%), 18/90 (20%), 47/87 (54%), 67/88 (76%), 70/87 (80%)	Dose–response relationship reduced significantly by addition of both doses of the condensate.	Study poorly reported. No details on duration of study, age of animals or pathology
NMRI (F) 94 wk Heinrich *et al.* (1986c)	Inhalation/intratracheal instillation Clean air (control) or exhaust from a 1.6-L displacement gasoline engine run on leaded fuel (0.3–0.56 g/L) (operated according to US-72 FTP driving cycle), diluted 1:27 or 1:61 with air (mean concentrations: 350 ± 24 and 77.5 ± 12.5 ppm CO; 28 ± 3 and 13.7 ± 1.5 ppm NO; 1.9 ± 0.4 and 1.0 ± 0.2 ppm NO_2; 95.8 ± 16.5 and 47.9 ± 20.2 µg/m^3 particulates) for 18–19 h/d, 5 d/wk for 53 wk concomitantly with 10 instillations of 100 µg B[*a*]P, 20 instillations of 50 µg B[*a*]P or 10 instillations of 50 µg DB[*a,h*]A Groups of 60, aged 8–10 wk	Similar total numbers of lung tumour-bearing animals in clean air and exhaust-exposed groups	NS	Additional groups of 61–83 newborn NMRI mice received a single subcutaneous injection of 4 µg (M and F) or 10 µg (F only) DB[*a,h*]A followed by inhalation exposure to one of the two dilutions of gasoline exhaust for 6 mo, after which they were killed; the number of lung tumours per animal was not significantly different from that in controls exposed simultaneously to clean air

B[*a*]P, benzo[*a*]pyrene; CO, carbon monoxide; d, day; DB[*a,h*]A, diebenz[*a,h*]anthracene; DMSO, dimethyl sulfoxide; F, female; FTP, Federal Test Procedures; h, hour; M, male; mo, month; NO, nitrogen oxide; NO_2, nitrogen dioxide; NR, not reported; NS, not significant; PAH, polycyclic aromatic hydrocarbon; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; VW, Volkswagen, wk, week

4 hours and then diluted 1:83 for the final 3 hours [particulate content and lead concentrations not provided] for a total exposure of 7 hours a day, 5 days a week for up to 25 months. Levels of carbon monoxide in the chambers averaged 7.5% for the unleaded gasoline engine and 2.1% for the leaded gasoline engine. Lung tumours [type unspecified] were observed in 9 out of 75 (12%) animals exposed to the unleaded gasoline exhaust compared with 8 out of 74 (11%) controls. The lung tumour incidence in animals exposed to the leaded gasoline exhaust was 12 out of 75 (16%) compared with 6 out of 70 (9%) controls (Campbell, 1936). [The Working Group noted the poor reporting of the study, in which no details were provided on survival or pathology, and the information on test atmosphere generation and chamber monitoring was doubtful.]

A group of female ICR mice [initial number and age unspecified] was exposed for 2 hours a day, 3 days a week for 12 months to exhaust emissions generated by a small gasoline engine, diluted 1:250 with clean air to give concentrations of 0.1 mg/m^3 of engine exhaust, 300 ± 50 ppm of carbon monoxide, 0.21 ppm of nitrogen oxide and 0.08 ppm of nitrogen dioxide. No unexposed controls were available. At 12 months, the incidence of lung adenoma was 2 out of 15 (13%), and no malignant lung tumour was observed (Yoshimura, 1983). [The Working Group noted that the study was poorly designed and reported because of the short treatment period and the lack of controls.]

(b) Skin application

Groups of 42 and 86 C57BL mice [sex and age unspecified] received topical applications of benzene alone or an oil residue [doses unspecified] of a benzene extract from filter paper used to filter of exhaust from an overhauled Ford V-8 gasoline engine dissolved in benzene "at frequent but irregular intervals" [duration of exposure unspecified]. Skin tumours were observed in 38 out of 86 (44%) animals (22 out of 86 carcinomas) surviving at the appearance of the first tumour (390 days). No skin tumours were reported in 0 out of 42 controls (Kotin *et al.*, 1954). [The Working Group noted that the study did not appear to have been completed, because the authors stated that "… this 44% figure of positive tumour production is subject to upward revision in view of the possibility of tumour demonstration in 16 remaining mice."]

Groups of 30–50 female Swiss mice, aged 6 weeks, received topical applications three times a week for 15 months of acetone containing 0 (control), 5, 10, 25, 33 or 50% of an oil residue of a benzene extract of condensed and filtered exhaust from a V8 gasoline engine using with a No. 5 camel's hair brush that was dipped once into the 'tar' solution. Survivors were then maintained with no further treatment for an additional 3 months. At 18 months, the incidence of skin tumours was 0, 4, 50, 60 and 60% papillomas and 0, 4, 32, 48 and 54% carcinomas in the control, 5-, 10-, 25- and 33%-dose groups, respectively. All of the mice in the high-dose group had died by 10 months; 70% had skin papillomas and 4% had skin carcinomas (Wynder & Hoffmann, 1962). [The Working Group noted the poor design of study, in which the method of application led to inconsistent doses and high-dose animals were tested separately.]

Groups of 50 Swiss female mice [age unspecified] received topical applications of tar from the exhaust of a V8 gasoline engine that used approximately 1 L of engine oil/200 miles [0.3 L/100 km] (A) or an engine that used approximately 1 L of oil/1600 miles [0.04 L/100 km] (B) in acetone [method of extraction not reported]. Skin tumours were reported in 60% (48% carcinomas) of the animals treated with exhaust from engine A and 84% (52% carcinomas) of the animals treated with exhaust from engine B (Hoffmann *et al.*, 1965). [The Working Group noted that the study was poorly designed and reported, the method and frequency of exposure

was not reported and no vehicle or untreated controls were available.]

Groups of 80 CFLP female mice, aged 12 weeks, studied in Hamburg (Germany), and groups of 40 CFLP female mice, aged 12 weeks, studied in Heidelberg (Germany), received topical applications onto the shaved interscapular region three times a week for life of 0.1 mL of DMSO:acetone (3:1) containing 0 (control), 0.5, 1.6 or 4.7 µg of an exhaust condensate produced by a VW 1.5-L Otto gasoline engine during a European test cycle, nitromethane or cyclohexane fractions of this condensate or reconstituted condensate, or were untreated. Results of the study performed in Hamburg indicated a linear relationship between the percentage of animals with local skin tumours (squamous cell papilloma or carcinoma combined) and treatment with fractions that contained PAHs with a nitromethane phase (tumour incidence: 16.4% with 0.179 mg and 68.9% with 0.537 mg) or a cyclohexane phase (tumour incidence: 13.7% with 0.358 mg and 68.8% with 1.07 mg), the reconstituted condensate (tumour incidence: 7.9% with 1.05 mg and 54.7% with 3.16 mg) and the total condensate (tumour incidence: 3.9, 35.1 and 76.9% with 0.5, 1.6 and 4.7 µg, respectively). Local skin tumour rates in mice treated with total condensate were higher than those in the two control groups (1.3 and 0%; $P < 0.0001$). The study performed in Heidelberg gave similar results; however, the incidence of local skin tumours was significantly higher in the study performed in Hamburg, probably due to minor differences in experimental techniques. In both studies, squamous cell tumours were mainly carcinomas (Brune *et al.*, 1978).

Groups of 65 (control) or 80 female CLFP mice, aged 7 weeks, received dermal applications twice a week for 104 weeks of 0 (control), 0.29, 0.87 and 2.6 mg/animal of a condensate from the exhaust emissions generated by a passenger car gasoline engine (1500 cm^3, 50 hp). The condensate was separated into several fractions that were also applied: (A) 0.97 or 2.9 mg/animal of a PAH-free fraction, (B) 0.152 or 0.455 mg/animal of a fraction containing PAHs with two and three rings and (C) 0.02 or 0.06 mg/animal of a fraction containing PAHs with more than three rings. In addition, a mixture of 15 PAHs simulating those found in automobile exhaust was applied at 0.003 or 0.009 mg/animal. All study materials were dissolved in DMSO:acetone (3:1) and applied as 0.1 mL of the resultant solution. All survivors were observed for life. A dose–response relationship was observed for the incidence of skin tumours (mainly squamous cell carcinomas) in the groups treated with total condensate (6 out of 80, 7%; 34 out of 80, 42%; and 65 out of 80, 81%; with the low, mid and high doses, respectively), in those given fraction C that contained PAHs with more than three rings (7 out of 80, 9%; and 50 out of 80, 62%; for the low and high doses, respectively) and in those given the mixture of 15 PAHs (1 out of 80, 1%; and 29 out of 80, 36%; for the low and high doses, respectively). An increased incidence of skin tumours was observed in the group treated with the high dose of fraction A. Fraction B did not produce a significant increase in the incidence of skin tumours, and no local skin tumours were seen in controls (0 out of 65) (Grimmer *et al.*, 1983a, b).

(c) Subcutaneous administration

Three groups of 87–88 female NMRI mice [age unspecified] received a single subcutaneous injection of 0 (control), 20 or 60 mg of an exhaust condensate (containing 0.163 µg/mg of benzo[*a*]pyrene) from a gasoline engine [unspecified] in 0.5 mL tricaprylin. A fourth group of 45 animals received three injections of the 60-mg dose. The mean survival time in the low- and mid-dose groups was similar to that of the controls (80–88 weeks), but was 57 weeks in the high-dose group. The number of animals with fibrosarcomas at the injection site was 3 out of 89 (3%), 10 out of 87 (11%), 6 out of 88 (7%) and 5 out of 45 (11%), respectively (Pott *et al.*, 1977). [The Working

Group noted that no details on the duration of the study or histopathology were reported.]

(d) Initiation–promotion studies

Groups of 40 male and 40 female SENCAR mice, aged 7–9 weeks, received a single topical application on the shaved dorsal skin of 0 (control), 0.1, 0.5, 1.0, 2.0 or 3.0 mg of a dichloromethane extract of the particulates from the emissions of a 1977 Ford Mustang II-302 V-8 engine with a catalyst that were collected on Teflon-coated fibreglass filters in 0.2 mL of acetone. One week later, the mice received topical applications of 2.0 µg of TPA in 0.2 mL of acetone twice a week for up to 25 weeks. At experimental week 26, the percentages of mice with skin papillomas and the numbers of papillomas/mouse in TPA-treated controls were 8% and 0.08 in males and 5% and 0.05 in females, respectively. In the groups treated with both TPA and the gasoline extract, the percentages and numbers of papillomas/mouse were: males – 5% and 0.05 (0.1 mg), 13% and 0.15 (0.5 mg), 18% and 0.18 (1 mg), 22% and 0.24 (2 mg) and 18% and 0.24 (3 mg); females – 13% and 0.23 (0.1 mg), 18% and 0.24 (0.5 mg), 10% and 0.13 (1 mg), 21% and 0.23 (2 mg) and 23% and 0.28 (3 mg). Of the high-dose females, 20% developed squamous cell carcinomas (Nesnow *et al.*, 1982a, 1983). [The Working Group noted that the authors stated that the responses at the higher doses were significantly greater than those of the TPA controls, but no statistics were reported.]

(e) Administration with known carcinogens

Groups of 86–90 female NMRI mice [age unspecified] received a single subcutaneous injection of 3.3, 10, 30, 90 or 270 µg of benzo[*a*]pyrene alone or together with 6.6, 20 or 60 mg of an exhaust condensate from a gasoline engine [unspecified] in 0.5 mL of tricaprylin. The dose–response relationship for local fibrosarcomas produced by benzo[*a*]pyrene (20, 54 and 76%, respectively) was significantly reduced by the addition of the condensate at all doses (Pott *et al.*, 1977). [The Working Group noted that no details on the duration of the study or histopathology were reported.]

Groups of 60 female NMRI mice, aged 8–10 weeks, received 10 intratracheal instillations of 100 µg of benzo[*a*]pyrene, 20 intratracheal instillations of 50 µg of benzo[*a*]pyrene or 10 intratracheal instillations of 50 µg of DB[*a*,*h*]A and were exposed concomitantly by inhalation for 18–19 hours a day, 5 days a week for 53 weeks to clean air or exhaust from a 1.6-L displacement gasoline engine run on leaded fuel (0.3–0.56 g/L; operated according to the US-72 FTP driving cycle), diluted 1:27 or 1:61 with air. Mean concentrations of the exhaust components measured in the inhalation chambers were, respectively: carbon monoxide, 350 ± 24 and 177.5 ± 12.5 ppm; nitrogen oxide, 28 ± 3 and 13.7 ± 1.5 ppm; nitrogen dioxide, 1.9 ± 0.4 and 1.0 ± 0.2 ppm; and particulates, 95.8 ± 16.5 and 47.9 ± 20.2 µg/m^3. The animals were then followed up during a 40-week observation period with no further treatment. The mean survival time of exhaust-exposed animals was 75–85 weeks. The total numbers of lung tumour-bearing animals in the clean air- and exhaust-exposed groups did not differ significantly. Additional groups of 61–83 newborn NMRI mice received a single subcutaneous injection of 4 µg (females and males) or 10 µg (females only) of DB[*a*,*h*]A followed by inhalation exposure to one of the two dilutions of gasoline exhaust for 6 months, after which they were killed. The number of lung tumours/animal did not differ significantly from that in controls exposed simultaneously to clean air (Heinrich *et al.*, 1986c).

3.2.2 Rat

See Table 3.6

Table 3.6 Studies of the carcinogenicity of gasoline engine exhaust in rats

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
BOR,WISW (F) 30 mo Heinrich *et al.* (1986c)	Inhalation Clean air (control) or exhaust from a 1.6-L displacement gasoline engine run on leaded fuel (0.3–0.56 g/L) (operated according to US-72 FTP driving cycle), diluted 1:61 or 1:27 with air (mean concentrations, 350 ± 24 and 177.5 ± 12.5 ppm CO; 28 ± 3 and 13.7 ± 1.5 ppm NO; 1.9 ± 0.4 and 1.0 ± 0.2 ppm NO_2; 95.8 ± 16.5 and 47.9 ± 20.2 µg/m^3 particulates), 18–19 h/d, 5 d/wk for 24 mo Groups of 80–83, aged 10–12 wk	Lung (all tumours): 1/78 (1%), 1/83 (1%), 3/78 (4%)	NS	The highest levels of gasoline engine exhaust that could be tested in this study were limited by the toxicity of CO
F344 (M, F) 30 mo Brightwell *et al.* (1989)	Inhalation Clean air (control) or exhaust emission generated from unleaded fuel in a Renault R18 1.6-L gasoline engine fitted with or without a three-way catalytic converter, diluted with a constant volume of 800 m^3 of air (high dose), or further dilution 1:3 in air (low dose) (low dose: 15 ppm NO, 15 ppm NO_2, 67 ppm CO; high dose: 44 ppm NO, 49 ppm NO_2, 224 ppm CO), 16 h/d, 5 d/wk for 24 mo. Control groups of 144 M and 144 F; treated groups of 72 M and 72 F; aged 6–8 wk	With catalytic converter Lung (all tumours): M–2/134 (1%), 0/37, 0/68 F–1/126 (1%), 0/36, 1/61 (2%) Without catalytic converter Lung (all tumours): M–2/134 (1%), 1/44 (2%), 0/65 F–1/126 (1%), 0/32, 1/63 (2%)	NS	Histological types NR
Osborne-Mendel (F) Lifetime Grimmer *et al.* (1984)	Intrapulmonary implantation 0 (control), 5.0 or 10.0 mg (A1, A2) condensate from the exhaust emission generated by a 1.5-L gasoline car engine (operated on the European test cycle), or one of several fractions: 4.36, 8.73 or 17.45 mg PAH-free (B1, B2, B3), 0.50, 0.99 or 1.98 mg PAHs with 2 or 3 rings (C1, C2, C3), or 0.14, 0.28 or 0.56 mg PAHs with more than 3 rings (D1, D2, D3) in beeswax:trioctanoin (1:1) into the left lobe of the lung, once Groups of 34–35, aged 3 mo	Lung (carcinoma): 0/34, 3/35 (9%; A1), 20/35 (57%; A2)**, 0/34 (B1), 3/34 (9%; B2), 1/34 (3%; B3), 0/35 (C1), 0/35 (C2), 3/35 (9%; C3), 3/35 (9%; D1), 15/34 (44%; D2)*, 24/35 (69%; D3)** Lung (sarcoma): 0/34, 4/35 (11%; A1), 0/35 (A2), 0/34 (B1), 3/34 (9%; B2), 2/34 (6%; B3), 0/35 (C1), 0/35 (C2), 3/35 (9%; C3), 1/35 (3%; D1), 2/34 (6%; D2), 0/35 (D3)	[*P = 0.0002, **P < 0.001]	The authors reported that a lung tumour dose–response relationship was obtained with the condensate and with the fraction of PAHs with more than 3 rings

Table 3.6 (continued)

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Administration with known carcinogens				
Sprague-Dawley (F) Up to 12 mo Yoshimura (1983)	Inhalation/oral administration Exhaust emissions from a small gasoline engine, diluted 1:250 with clean air (0.1 mg/m^3 engine exhaust particulates, 300 ± 50 ppm CO, 0.21 ppm NO, 0.08 ppm NO_2), 2 h/d, 3 × / wk for 6–12 mo concomitantly with 0 or 0.01% DiPN in the drinking-water [Numbers and age NR]	Lung (all tumours): 0/23, 2/24 (8%), 11/37 (30%)*	[*P = 0.05]	Study limited by the lack of clean air controls; lung tumours observed following the combined treatment included one adenoma and 10 malignant tumours (undifferentiated carcinomas, squamous cell carcinomas, adenocarcinomas or mixed tumours)
Rat, BOR,WISW (F) 30 mo Heinrich *et al.* (1986c)	Subcutaneous injection/inhalation 0.25 or 0.5 g/kg bw NDPA, once/day for 25 days, followed by exposure to clean air (control) or exhaust from a 1.6-L displacement gasoline engine run on leaded fuel (0.3–0.56 g/L) (operated according to US-72 FTP driving cycle), diluted 1:27 (H) or 1:61 (L) with air (Mean concentrations: H– 350 ± 24 ppm CO, 28 ± 3 ppm NO, 1.9 ± 0.4 ppm NO_2, 95.8 ± 16.5 µg/m^3 particulates; L–177.5 ± 12.5 ppm CO; 13.7 ± 1.5 ppm NO; 1.0 ± 0.2 ppm NO_2, 47.9 ± 20.2 µg/m^3 particulates), 18–19 h/d, 5 d/wk for 24 mo. Groups of 60, aged 10–12 wk	0.5 g/kg NDPA Lung (all tumours): 44/49 (90%), 28/48 (58%; H)*, 33/49 (67%; L)* 0.25 g/kg NDPA Lung (all tumours): 27/48 (56%), 13/54 (24%; H)*, 15/47 (32%; L)*	*$P \leq 0.05$, decrease	

B[*a*]P, benzo[*a*]pyrene; CO, carbon monoxide; d, day; DiPN, *N*-nitrosodiisopropanolamine; F, female; FTP, Federal Test Procedures; h, hour; mo, month; NDPA, *N*-nitrosodipentylamine; NO, nitrogen oxide; NO_2, nitrogen dioxide; NR, not reported; NS, not significant; wk, week

(a) Inhalation

Groups of 80–83 female Bor:WISW rats, aged 10–12 weeks, were exposed by inhalation for 18–19 hours a day, 5 days a week for 24 months to clean air (control) or to exhaust from a 1.6-L displacement gasoline engine run on leaded fuel [0.3–0.56 g/L] (operated according to the US-72 FTP driving cycle), diluted 1:27 or 1:61 with air. Mean concentrations of exhaust components measured in the inhalation chambers were, respectively: carbon monoxide, 350 ± 24 and 177.5 ± 12.5 ppm; nitrogen oxide, 28 ± 3 and 13.7 ± 1.5 ppm; nitrogen dioxide, 1.9 ± 0.4 and 1.0 ± 0.2 ppm; and particulates, 95.8 ± 16.5 and 47.9 ± 20.2 μg/m^3. Animals surviving at the end of the 24-month exposure period were maintained in clean air for up to an additional 6 months. Neither concentration of gasoline exhaust produced a significant increase in the incidence of lung tumours: 1 out of 83 animals exposed to 1:61 had a squamous cell carcinoma; 3 out of 78 exposed to 1:27 had two squamous cell carcinomas and one adenoma; and 1 out of 78 controls had an adenoma (Heinrich *et al.* (1986c). [The Working Group noted that the highest levels of gasoline engine exhaust that could be tested in this study were limited by the toxicity of carbon monoxide.]

Groups of 72 male and 72 female Fischer 344 rats, aged 6–8 weeks, were exposed for 16 hours a day, 5 days a week for 24 months to exhaust emissions generated by a Renault R18 1.6-L gasoline engine run on unleaded fuel and fitted or not fitted with a three-way catalytic converter. The exhausts were diluted with a constant volume of 800 m^3 of air for the high dose, and further dilution of this mixture 1:3 with air produced the low dose; the levels of nitrogen oxide, nitrogen oxides and carbon monoxide were 15 and 44 ppm, 15 and 49 ppm and 67 and 224 ppm for the low and high doses, respectively. All survivors were maintained in clean air for up to an additional 6 months. No increase in the incidence of lung tumours was observed (Brightwell *et al.*, 1989).

(b) Intrapulmonary implantation

Groups of 34–35 female Osborne-Mendel rats, aged 3 months, received a single implantation into the left lobe of the lung of 0 (control), 5.0 and 10.0 mg in beeswax:trioctanoin (1:1) of a condensate from the exhaust emissions generated by a 1.5-L gasoline car engine operated on the European test cycle. The condensate was also separated into several fractions that were also implanted in beeswax:trioctanoin (1:1): 4.36, 8.73 or 17.45 mg/animal of a PAH-free fraction; 0.50, 0.99 or 1.98 mg/animal of a fraction containing PAHs with two to three rings; or 0.14, 0.28 or 0.56 mg/animal of a fraction of PAHs containing more than three rings. Animals were observed for life. Mean survival times for treated and controls ranged from 80 to 111 weeks. Only the fraction containing PAHs with more than three rings produced an increase in the incidences of lung tumours (carcinoma: 1 out of 35, 3%; 15 out of 34, 44% [$P = 0.0002$]; and 24 out of 35, 69% [$P < 0.0001$]; sarcoma: 1 out of 35, 3%; 2 out of 34, 6%; and 0 out of 35) comparable with that induced by total exhaust condensate (carcinoma: 3 out of 35, 9%; and 20 out of 35, 57% [$P < 0.0001$]; sarcoma: 4 out of 35, 11%; and 0 out of 35). No lung tumours were observed in the controls. The authors reported a dose–response relationship between the incidence of lung tumours and treatment with the total condensate and with the fraction of PAHs containing more than three rings (Grimmer *et al.*, 1984).

(c) Administration with known carcinogens

Groups of female Sprague-Dawley rats [numbers and age unspecified] were administered 0.01% DiPN in the drinking-water or were exposed by inhalation for 2 hours a day, 3 days a week to the exhaust emission generated by a small gasoline engine diluted 1:250 with clean air to give 0.1 mg/m^3 of engine exhaust particulates

(300 ± 50 ppm carbon monoxide, 0.21 ppm nitrogen oxide and 0.08 ppm nitrogen dioxide), or underwent both treatments concurrently for 6–12 months. In the animals killed between 7 and 12 months after the start of the experiment, the number of lung tumours (11 out of 37, 30%) in the combined treatment group (one adenoma and 10 malignant tumours including undifferentiated carcinomas, squamous cell carcinomas, adenocarcinomas and mixed tumours) was significantly greater than that in the group treated with DiPN alone (2 out of 24, 8% [$P < 0.05$]; two carcinomas). No lung tumours were observed in 23 animals exposed to gasoline exhaust alone (Yoshimura, 1983). [The Working Group noted that the study was limited by the lack of clean air controls.]

Groups of 60 female Bor:WISW rats, aged 10–12 weeks, received 25 daily subcutaneous injections of 0.25 or 0.5 g/kg bw of *N*-nitrosodipentylamine (NDPA) followed by exposure for 18–19 hours a day, 5 days a week for 24 months to clean air (control) or to exhaust from a 1.6-L displacement gasoline engine run on leaded fuel (0.3–0.56 g/L) (operated according to the US-72 FTP driving cycle), diluted 1:27 or 1:61 with air. Mean concentrations of exhaust components measured in the inhalation chambers were, respectively: carbon monoxide, 350 ± 24 and 177.5 ± 12.5 ppm; nitrogen oxide, 28 ± 3 and 13.7 ± 1.5 ppm; nitrogen dioxide, 1.9 ± 0.4 and 1.0 ± 0.2 ppm; and particulates, 95.8 ± 16.5 and 47.9 ± 20.2 μg/m^3. All survivors were observed for up to an additional 6 months. A decrease in the incidence of benign and malignant lung tumours combined was observed in animals exposed to gasoline exhaust and NDPA compared with those exposed to NDPA alone (Heinrich *et al.*, 1986c).

3.2.3 Hamster

See Table 3.7

(a) Inhalation

Groups of 80–83 female Syrian hamsters, aged 10–12-weeks, were exposed by inhalation for 18–19 hours a day, 5 days a week for 24 months to clean air (control) or to exhaust from a 1.6-L displacement gasoline engine run on leaded fuel (0.3–0.56 g/L) (operated according to the US-72 FTP driving cycle), diluted 1:27 or 1:61 with air. Mean concentrations of exhaust components measured in the inhalation chambers were, respectively: carbon monoxide, 350 ± 24 and 177.5 ± 12.5 ppm; nitrogen oxide, 28 ± 3 and 13.7 ± 1.5 ppm; nitrogen dioxide, 1.9 ± 0.4 and 1.0 ± 0.2 ppm; and particulates, 95.8 ± 16.5 and 47.9 ± 20.2 μg/m^3. Exposure to gasoline engine exhaust had no effect on survival; the median lifespan was 70 weeks in all groups, and only one animal out of 75 in the high-dose group and three animals out of 80 in the low-dose group developed lung tumours; no lung tumours were observed in 83 controls (Heinrich *et al.*, 1986c). [The Working Group noted that the highest levels of gasoline engine exhaust that could be tested in this study were limited by the toxicity of carbon monoxide.]

Groups of 104 male and 104 female Syrian hamsters, aged 6–8 weeks, were exposed to exhaust emissions generated by a Renault R18 1.6-L gasoline engine run on unleaded fuel and fitted or not fitted with a three-way catalytic converter. The exhausts were diluted with a constant volume of 800 m^3 of air for the high dose, and further dilution of this mixture 1:3 in air produced the low dose; levels of nitrogen oxide, nitrogen dioxide and carbon monoxide were 15 and 44 ppm, 15 and 49 ppm and 67 and 224 ppm for the low and high dose, respectively. Further groups of 208 male and 208 female hamsters served as untreated controls. In addition, groups of 52 male and 52 female exposed hamsters and 104 male and 104 female controls were pretreated with 4.5 mg/kg of NDEA 3 days before the start of inhalation exposure. The authors reported

Table 3.7 Studies of the carcinogenicity of gasoline engine exhaust in hamsters

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Syrian (F) 24 mo Heinrich *et al.* (1986c)	Inhalation Clean air (control) or exhaust from a 1.6-L displacement gasoline engine run on leaded fuel (0.3–0.56 g/L)(operated according to US-72 FTP driving cycle), diluted 1:61 or 1:27 with air (mean concentrations: 350 ± 24 and 177.5 ± 12.5 ppm CO; 28 ± 3 and 13.7 ± 1.5 ppm NO; 1.9 ± 0.4 and 1.0 ± 0.2 ppm NO_2; and 95.8 ± 16.5 and 47.9 ± 20.2 µg/m^3 particulates, respectively), 18–19 h/d, 5 d/wk for 24 mo Groups of 80–83, aged 10–12 wk	Lung (all tumours): 0/83, 3/80 (4%), 1/75 (1%)	NS	The highest levels of gasoline engine exhaust that could be tested in this study were limited by the toxicity of CO
Syrian (M, F) 24 mo Brightwell *et al.* (1989)	Inhalation Exposure to exhaust emission generated from a Renault R18 1.6-L gasoline engine run on unleaded fuel fitted with or without a three-way catalytic converter, diluted with a constant volume of 800 m^3 (high dose), or further diluted 1:3 in air (low dose) (15 and 44 ppm NO, 15 and 49 ppm NO_2 and 67 and 224 ppm CO for low and high doses, respectively), 16 h/d, 5 d/wk; additional groups were pretreated with 4.5 mg/kg NDEA 3 days before start of exposure Control groups of 208 M and 208 F; treated groups of 104 M and 104 F; pretreated control groups of 104 M and 104 F; pretreated treated groups of 52 M and 52 F; aged 6–8 wk	NDEA-pretreated groups With catalytic converter Lung (all tumours): M–4/101 (4%), 5/51 (10%), 1/51 (2%) F–3/101 (3%), 0/51, 3/51 (6%) Trachea (all tumours): M–14/101 (14%), 9/50 (18%), 5/48 (10%) F–18/103 (17%), 4/50 (8%), 10/51 (20%) Without catalytic converter Lung (all tumours): M–4/101 (4%), 5/51 (10%), 1/51 (2%) F–3/101 (3%), 2/52 (4%), 3/49 (6%) Trachea (all tumours): M–14/101 (14%), 8/52 (15%), 10/51 (20%) F–18/103 (17%), 12/50 (24%), 9/48 (19%)	[NS]	Authors reported that there was no evidence of any increase in respiratory tract tumours or tracheal tumours in hamsters (NDEA-pretreated and non-pretreated [data not shown here]) exposed to gasoline engine exhaust with or without a catalyst

Table 3.7 (continued)

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Syrian (M) Lifetime Mohr *et al.* (1976); Reznik-Schüller & Mohr (1977)	Intratracheal instillation 0 (control), 2.5 or 5.0 mg of an automobile exhaust condensate from emissions of a common German passenger car (operating according to the European test cycle), containing 0.34 µg/mg B[*a*]P, dissolved in 0.2 m Tris-saline and EDTA buffer/saline, once/2 wk Groups of 6, aged 12 wk	Lung (adenoma): 0/6, 6/6 (100%)*, 6/6 (100%)*	*[$P < 0.05$]	Study poorly described; small number of animals per group
Syrian golden (M) Lifetime Künstler (1983)	Intratracheal instillation 0 (control), 0.5, 1.0 or 2.1 mg condensate (containing 0.41 µg/mg B[*a*]P) from exhaust emission generated by a VW 1500 Otto gasoline engine; condensate also separated into several fractions including a methanol phase, a cyclohexane phase, a nitromethane phase, and a reconstituted condensate from these fractions, dissolved in 0.2 mL of Tris-saline and EDTA buffer/saline, once/2 wk Groups of 30, aged 16 wk	No lung tumours observed in any animals treated with the condensate, its fractions or the reconstituted condensate	NS	
Administration with known carcinogens				
Syrian golden (F) 24 mo Heinrich *et al.* (1986c)	Subcutaneous injection/intratracheal instillation/inhalation Single subcutaneous injection of 3 mg/kg bw NDEA or 20 intratracheal instillations of 0.25 mg B[*a*]P followed by exposure to clean air (control) or exhaust from a 1.6-L displacement gasoline engine run on leaded fuel (0.3–0.56 g/L) (operated according to US-72 FTP driving cycle), diluted 1:27 or 1:61 with air (mean concentrations: 350 ± 24 and 177.5 ± 12.5 ppm CO; 28 ± 3 and 13.7 ± 1.5 ppm NO; 1.9 ± 0.4 and 1.0 ± 0.2 ppm NO_2; 95.8 ± 16.5 and 47.9 ± 20.2 µg/m^3 particulates), 18–19 h/d, 5 d/wk for 24 mo Groups of 80–81, aged 10–12 wk	The rates of NDEA- or B[*a*]P-induced tumours not increased by exposure to either dilution of exhaust	NS	Tumour rates in NDEA- and B[*a*]P-treated animals exposed to the 1:27 dilution of exhaust were approximately 50% lower than those in treated animals exposed to the 1:61 dilution or clean air

B[*a*]P, benzo[*a*]pyrene; bw, body weight; CO, carbon monoxide; d, day; EDTA, ethylenediaminetetraacetic acid; F, female; FTP, Federal Test Procedures; h, hour; M, male; mo, month; NDEA, *N*-nitrosodiethylamine; NO, nitrogen oxide; NO_2, nitrogen dioxide; NR, not reported; NS, not significant; PAH, polycyclic aromatic hydrocarbon; VW, Volkswagen; wk, week

no evidence of any increase in the incidence of respiratory tract tumours or tracheal tumours [type unspecified] in hamsters exposed to gasoline or gasoline catalyst engine exhaust with or without pretreatment with NDEA (Brightwell *et al.*, 1989).

(b) Intratracheal instillation

Groups of six male Syrian hamsters, aged 12 weeks, received intratracheal instillations once every two weeks for life of 0 (control), 2.5, or 5.0 mg of an automobile exhaust condensate prepared from the emissions of a common German passenger car (operated according to the European test cycle; containing 0.34 µg/mg of benzo[*a*]pyrene) dissolved in 0.2 mL Tris-hydrochloric acid and ethylenediaminetetraacetic acid (EDTA) buffer/saline. Survival of the treated animals ranged from 30 to 60 weeks. Pulmonary adenomas were observed in all treated animals but not in controls (Mohr *et al.*, 1976; Reznik-Schüller & Mohr, 1977). [The Working Group noted that the study was poorly described and the number of animals per group was small.]

Groups of 30 male Syrian hamsters, aged 16 weeks, received intratracheal installations once every two weeks for life of 0.2 mL of Tris-hydrochloric acid and EDTA buffer/saline containing 0 (control), 0.5, 1.0 or 2.1 mg of a condensate (containing 0.41 µg/mg of benzo[*a*]pyrene) from the exhaust emissions generated by a VW 1500 Otto gasoline engine. The condensate was also separated into several fractions including a methanol phase, a cyclohexane phase and a nitromethane phase, and another condensate was reconstituted from these fractions. Survival ranged from 68 to 87 weeks. No lung tumours were observed in any animals treated with the condensate, its fractions or the reconstituted condensate (Künstler, 1983).

(c) Administration with known carcinogens

Groups of 80–81 female Syrian golden hamsters, aged 10–12 weeks, received either a single subcutaneous injection of 3 mg/kg bw of NDEA or 20 intratracheal instillations of 0.25 mg of benzo[*a*]pyrene followed by inhalation exposure for 18–19 hours a day, 5 days a week for 24 months to clean air (control) or to exhaust from a 1.6-L displacement gasoline engine run on leaded fuel (0.3–0.56 g/L) (operated according to the US-72 FTP driving cycle), diluted 1:27 or 1:61 with air. Mean concentrations of exhaust components measured in the inhalation chambers were, respectively: carbon monoxide, 350 ± 24 and 177.5 ± 12.5 ppm; nitrogen oxide, 28 ± 3 and 13.7 ± 1.5 ppm; nitrogen dioxide, 1.9 ± 0.4 and 1.0 ± 0.2 ppm; and particulates, 95.8 ± 16.5 and 47.9 ± 20.2 µg/m^3. Administration of NDEA or benzo[*a*]pyrene to hamsters exposed to clean air resulted in basic rates of benign respiratory tract tumours of 12.8 and 6.5% of animals, respectively. The basic tumour rate was not significantly increased by exposure to either dilution of the exhaust (Heinrich *et al.*, 1986c).

3.2.4 Dog

See Table 3.8

Inhalation

Groups of 12–20 female Beagle dogs, aged 4 months, were exposed by inhalation for 16 hours a day for 68 months to clean air (control), to exhaust emissions generated from leaded fuel in a six-cylinder 2.4-L gasoline engine (operated to simulate urban driving) and/or to specific air pollutants: non-ultraviolet (UV)-irradiated exhaust, UV-irradiated exhaust, 0.5 ppm of sulfur dioxide (SO_2) + 100 µg/m^3 of sulfuric acid (H_2SO_4) mist in control air, non-UV-irradiated exhaust + 0.5 ppm of SO_2 + 100 µg/m^3 of H_2SO_4, UV-irradiated exhaust + 0.5 ppm of SO_2 + 100 µg/m^3 of H_2SO_4, 0.2 ppm of nitrogen oxide + 0.5–1.0 ppm of nitrogen dioxide or 1.5–2.0

Table 3.8 Study of the carcinogenicity of gasoline engine exhaust in dogs

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence (%) and/or multiplicity of tumours	Significance
Beagle (F) 104 mo Stara *et al.* (1980)	Inhalation Clean air (control), UV-irradiated or non-irradiated exhaust emissions from a 6-cylinder, 2.4-L gasoline engine run on leaded fuel (operated to simulate urban driving) (non-irradiated exhaust: 100 ppm CO and 24–30 ppm hydrocarbon expressed as methane; UV-irradiated exhaust: 0.5–1.0 ppm NO_2, 0.1 ppm NO and 0.2–0.4 ppm oxygen expressed as O_3, lead concentrations, 14–26 µg/m³), 0.5 ppm SO_2 + 100 µg/m³ H_2SO_4 mist in control air, non-UV-irradiated exhaust + 0.5 ppm SO_2 + 100 µg/m³ H_2SO_4, UV-irradiated exhaust + 0.5 ppm SO_2 + 100 µg/m³ H_2SO_4, 0.2 ppm NO + 0.5–1.0 ppm NO_2, or 1.5–2.0 ppm NO + 0.2 ppm NO_2, 16 h/d for 68 mo Control group of 20; treated groups of 12; aged 4 mo	No lung tumours observed in 41 surviving dogs exposed to engine exhaust or 17 surviving controls	NS

CO, carbon monoxide; d, day; F, female; h, hour; H_2SO_4, sulfuric acid; mo, month; NO, nitrogen oxide; NO_2, nitrogen dioxide; NS, not significant; SO_2, sulfur dioxide; UV, ultraviolet

ppm of nitrogen oxide + 0.2 ppm of nitrogen dioxide. The exhaust contained 100 ppm of carbon monoxide and 24–30 ppm of hydrocarbon expressed as methane. The UV-irradiated exhaust contained 0.5–1.0 ppm of nitrogen dioxide, 0.1 ppm of nitrogen oxide and 0.2–0.4 ppm of oxygen expressed as O_3. The concentration of lead measured in the different exposure atmospheres was 14–26 µg/m³. The dogs were then maintained with no further treatment for up to an additional 36 months. No lung tumours were observed at necropsy in 41 surviving dogs from any of the groups exposed to the engine exhaust or 17 surviving controls (Stara *et al.*, 1980).

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4. MECHANISTIC AND OTHER RELEVANT DATA

4.1 Overview of the mechanisms of carcinogenesis of polycyclic aromatic hydrocarbons

The chemical exposures of greatest concern are to polycyclic aromatic hydrocarbons (PAHs) and nitro-PAHs with which diesel engine exhaust particles may be impregnated and to PAHs that are present in gasoline engine exhaust. Mechanistic and other relevant data associated with exposure to individual nitro-PAHs are reviewed elsewhere in this Volume (see Sections 4 in the individual *Monographs*). Exposure to PAHs is associated with an increased risk of cancer and was recently reviewed in the *IARC Monographs* (IARC, 2010a) and comprehensive reviews by the Agency of Toxic Substances and Disease Registry (ATSDR, 1995) and the International Programme on Chemical Safety (IPCS, 1998). A major finding of IARC (2010a) was the reclassification of the representative PAH, benzo[*a*]pyrene, as a Group 1 or known human carcinogen. Benzo[*a*]pyrene is the most extensively studied PAH, for which an exhaustive set of literature exists. Its reclassification as a known human carcinogen considered epidemiological data, data on metabolism, bioactivation, mutagenicity and tumorigenicity, and the existence of a mode of action or plausible mechanism that accounts for the end-points observed. Many of these data are relevant to the risk assessment of other PAHs found in diesel and gasoline engine exhausts, which contain many of the 16 Environmental Protection Agency (EPA) priority pollutants, e.g. naphthalene, acenaphthylene, acenaphthene, fluorene, anthracene, phenanthrene, fluoranthene, pyrene, chrysene, benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, indeno[*1,2,3-cd*]pyrene, benzo[*ghi*]perylene and dibenz[*a,h*]anthracene. An examination of the structure–activity relationships of these compounds showed that four or more of their fused benzene rings need to create an indentation known as a bay-region for the compound to be a potent carcinogen. Substitution of the bay-region by a methyl group or the introduction of another benzene ring to create a *fjord*-region often increases the carcinogenicity of the PAH. A summary of the mechanistic data that support the role of benzo[*a*]pyrene as a human carcinogen is presented below, and other PAHs present in diesel and gasoline engine exhausts are discussed in this context.

4.1.1 Mechanistic data to support the role of benzo[a]pyrene as a human carcinogen

(a) Toxicokinetics

PAHs, including benzo[*a*]pyrene, are present in airborne fine particles, and absorption occurs via the respiratory tract, as shown by studies on [^{14}C]benzo[*a*]pyrene-labelled particles (Withey

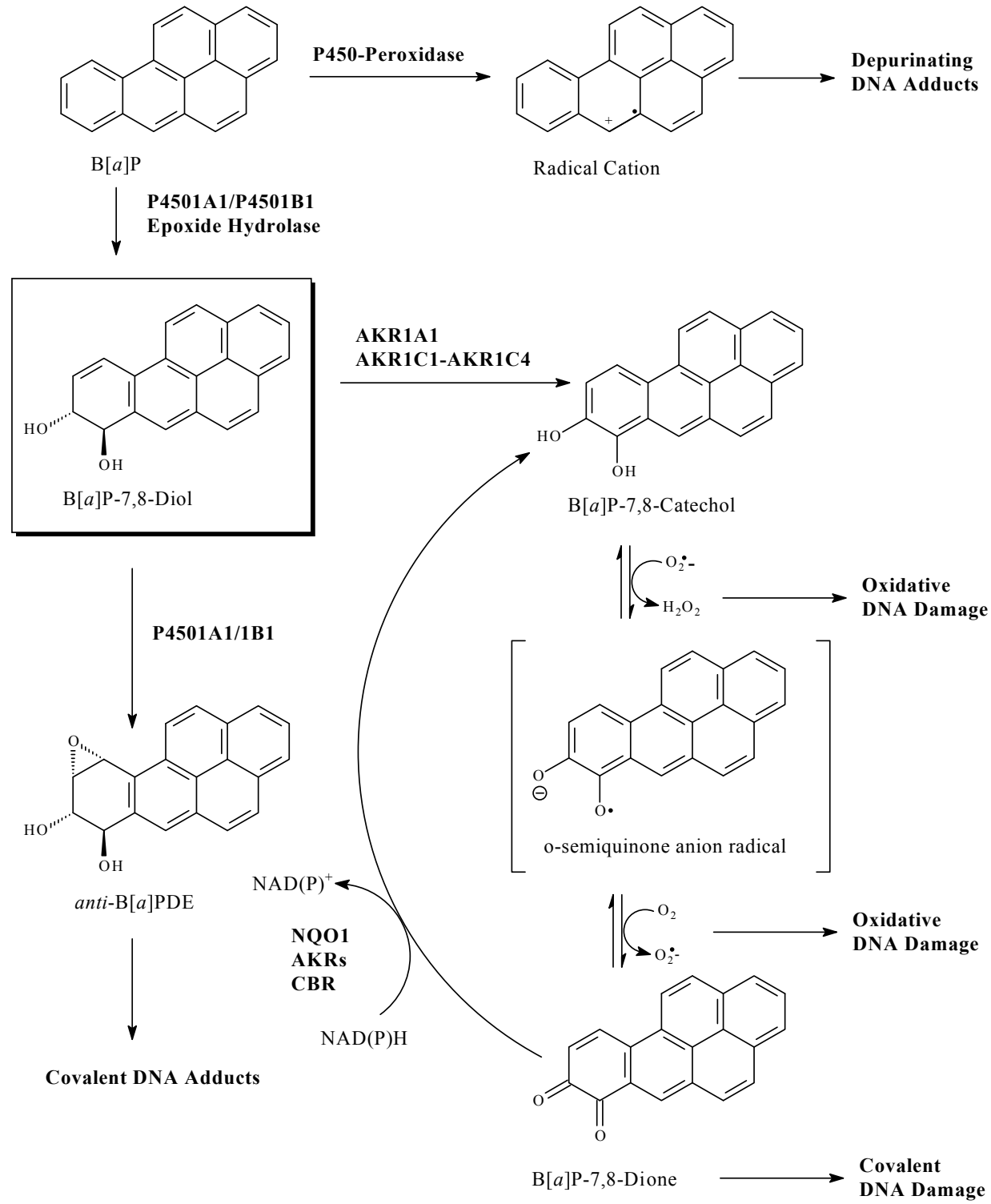
et al., 1993; ATSDR, 1995; IPCS, 1998). Because of their lipophilic character, PAHs can be cleared by the epithelial cell mucillary elevator which can lead to ingestion via the gastrointestinal tract (Withey *et al.*, 1994). Benzo[*a*]pyrene is a complete multiorgan and multispecies carcinogen and forms tumours in the lungs of newborn mice after intraperitoneal administration (Kapitulnik *et al.*, 1978), in A/J mouse lung after intraperitoneal administration (Nesnow *et al.*, 1995, 1998), in mouse skin after topical application (Levin *et al.*, 1977) and in the murine forestomach and gastrointestinal tract after oral administration (Wattenberg *et al.*, 1980; Culp & Beland, 1994). Thus, many of the sites of tumour formation in rodents correspond to sites of human exposure to diesel and gasoline engine exhausts.

PAHs are metabolically inert and must be metabolized before both their elimination and activation can occur; the balance of these events determines the carcinogenicity of PAHs in a given species and at a given organ site. For benzo[*a*]pyrene and other PAHs, a general consensus is that phase I enzymes – cytochrome P450 (CYP) and aldo-keto reductases (AKRs) – form phenols, dihydrodiols and catechols, and that each of these metabolites are then available for conjugation by phase II enzymes, leading to elimination. The phase II enzymes of interest are the sulfotransferases (SULTs) and the uridine glucuronosyl transferases (UGTs), while catechols are uniquely conjugated by catechol-*O*-methyl transferase. In each instance, the conjugate is eliminated by excretion. In addition, phase I metabolism to epoxides, diol epoxides and quinones, which are biologically reactive intermediates, may occur, and, if they are not conjugated with glutathione by glutathione *S*-transferases (GSTs), they can react with DNA, RNA and protein. These detoxication and toxication pathways of benzo[*a*]pyrene have been studied extensively *in vitro* and *in vivo* in mouse, rat and human subcellular fractions and tissues, and in cell lines. The metabolic pathways observed appear to be consistent (Gelboin, 1980; Conney, 1982). Features that can determine the balance of detoxication and activation are the induction of phase I and phase II enzymes, and the existence of polymorphic variants in enzyme isoforms that can determine individual susceptibility to exposure to PAHs. The mechanisms of phase I and phase II enzyme induction are discussed elsewhere. For a discussion of the polymorphic variants of the major enzyme families involved in the metabolism of PAHs, the reader is referred to IARC (2010a). The pathways that lead to the metabolic activation of benzo[*a*]pyrene and the production of genotoxic species are discussed below.

(b) Metabolic pathways of activation

Benzo[*a*]pyrene is a pro-carcinogen that is metabolically activated to electrophilic species, which can react with bases in DNA to form DNA adducts. When these adducts persist, they give rise to mutations and, when these mutations activate proto-oncogenes or deactivate tumour-suppressor genes, the cell is altered in a manner consistent with the hallmarks of cancer, as described by Hanahan & Weinberg (2011). The mutations of most interest are G→T transversions, which are the dominant mutations observed in the genes most commonly mutated in human lung cancer, e.g. *K-ras* and *p53*, and any molecular mechanism that explains the carcinogenicity of PAHs must provide a process that leads to these mutations. Three pathways have been proposed for the metabolic activation of benzo[*a*]pyrene that can lead to DNA adducts that can ultimately induce G→T transversions (Fig. 4.1). These include the diol epoxide pathway (mediated by CYP isozymes and epoxide hydrolase), the radical cation pathway (mediated by CYP peroxidase activity or other peroxidases) and the *ortho*-quinone pathway (mediated by AKRs following the action of CYP and epoxide hydrolase). An additional pathway involving L-region mesomethylation, e.g. the conversion of benzo[*a*]pyrene to 6-methyl-benzo[*a*]pyrene

Fig. 4.1 Three pathways of metabolic activation of benzo[*a*]pyrene



AKR, aldo-keto reductase; B[a]P, benzo[a]pyrene; CBR, carbonyl reductase; NAD(P)H/NAD(P)$^+$, nicotinamide adenine dinucleotide phosphate; NQO1, NADPH quinone oxidoreductase; P450, cytochrome P450

followed by hydroxylation and sulfation, was evaluated by IARC but no compelling evidence was found to support this mechanism (IARC, 2010a), and this pathway is not discussed further.

(i) Diol epoxides

Overwhelming evidence shows that benzo[*a*]pyrene and other carcinogenic PAHs are metabolically activated to diol epoxide ultimate carcinogens (Gelboin, 1980; Conney, 1982), which can account for the observation that bay-region PAHs are potent carcinogens. In this pathway, the principal CYP enzymes involved are CYP1A1 (ubiquitous) and CYP1B1 (extrahepatic), although others may play a minor role, e.g. CYP1A2 (Shimada *et al.*, 1996, 1999, 2001). These monooxygenases form an arene oxide on the terminal benzo-ring of benzo[*a*]pyrene to form the major 7*R*,8*S*,-benzo[*a*]pyrene-7,8-oxide and minor 7*S*,8*R*-benzo[*a*]pyrene-7,8-oxide, which, in the presence of epoxide hydrolase, are converted to (–)*R*,*R*,-7,8-dihydro-7,8-dihydroxy-benzo[*a*]pyrene [(–)benzo[*a*]pyrene-7,8-dihydrodiol] and (+)*S*,*S*,-7,8-dihydro-7,8-dihydroxy-benzo[*a*]pyrene [(+)benzo[*a*]pyrene-7,8-dihydrodiol], respectively. Each non-K-region benzo[*a*]pyrene-7,8-dihydrodiol isomer undergoes a second round of monooxygenation to form the corresponding diol epoxide (Shimada *et al.*, 1996, 1999), (+)-*anti*-7β,8α-dihydroxy-9α,10α-oxo-7,8,9,10-tetrahydro-benzo[*a*]pyrene [(+)-*anti*-B[*a*]PDE] and (+)-*syn*-7α,8β-dihydroxy-9α,10α-oxo-7,8,9,10-tetrahydro-benzo[*a*]pyrene [(+)-*syn*-B[*a*]PDE], respectively (Thakker *et al.*, 1976). These diol epoxides can undergo hydrolysis with either *cis* or *trans* ring-opening which gives rise to four stereoisomeric tetraols. Alternatively, they can undergo nucleophilic attack at the C10 position by either a guanine or adenine DNA base to form either 8-stereoisomeric N^2-deoxyguanosine adducts or 8-stereoisomeric N^6-deoxyadenosine adducts, resulting in a total of 16 possible adducts (Jennette *et al.*, 1977; Jeffrey *et al.*, 1979). The major pathway proceeds through the (–)benzo[*a*]pyrene-7,8-dihydrodiol and the major DNA adduct formed is the (+)-*anti*-*trans*-B[*a*]PDE-N^2-deoxyguanosine adduct [(+)-*anti*-B[*a*]PDE-dG] (Jennette *et al.*, 1977). This stereochemistry of metabolic activation is an important consideration when comparing other pathways that may be involved.

Compelling evidence demonstrates that (+)-*anti*-B[*a*]PDE is an ultimate carcinogen, and is produced metabolically in target tissues that develop tumours (Melikian *et al.*, 1987). (+)-*Anti*-B[*a*]PDE adducts have been detected at sites of tumour formation: in mouse skin using [^{3}H]benzo[*a*]pyrene as a precursor followed by isolation of the DNA adducts by digestion and their co-chromatography with standards of (+)-*anti*-B[*a*]PDE-dG (Koreeda *et al.*, 1978) and by ^{32}P-postlabelling (Bodell *et al.*, 1989; Suh *et al.*, 1995); in A/J mouse lung using ^{32}P-postlabelling approaches (Mass *et al.*, 1993); in the lung and forestomach of mice (Adriaenssens *et al.*, 1983); and in human bronchial and colon explants using [^{3}H]benzo[*a*]pyrene as a precursor followed by reversed phase high-performance liquid chromatography (HPLC) of the adducts obtained from DNA digests (Jeffrey *et al.*, 1977; Autrup *et al.*, 1978). (+)-*Anti*-B[*a*]PDE-dG adducts have also been detected in a variety of human cell lines by ^{32}P-postlabelling (Li *et al.*, 1996) and quantified by stable-isotope dilution liquid chromatography/mass spectrometry (SID-LC/MS) in human lung tissues (Beland *et al.*, 2005) and in human bronchoalveolar (H358) cells (Ruan *et al.*, 2006, 2007). These adducts can be repaired by nucleotide-excision repair or by transcription-coupled repair. If they persist, (+)-*anti*-B[*a*]PDE-dG adducts can undergo translesional synthesis by specific by-pass DNA polymerases to produce G→T transversions (Zhao *et al.*, 2006; Choi *et al.*, 2011). Many of these DNA polymerases show low fidelity and low processivity; thus, once the lesion is by-passed, the replication complex becomes stalled until the by-pass polymerase is substituted with replicative DNA polymerase II

(Choi *et al.*, 2011). This route to G→T transversions is considered to be less than straightforward because it requires multiple steps.

(+)-*Anti*-B[*a*]PDE is the most mutagenic of the known benzo[*a*]pyrene metabolites in the Ames test (Malaveille *et al.*, 1977) and is mutagenic in mammalian mutagenicity assays in Chinese hamster ovary cells or fibroblasts that express the *hypoxanthine-guanine phosphoribosyltransferase* (*HPRT*) gene (MacLeod *et al.*, 1988; Chen *et al.*, 1990; Wei *et al.*, 1993). (+)-*Anti*-B[*a*]PDE is also the most tumorigenic of the known benzo[*a*]pyrene metabolites in the lung of newborn mice (Buening *et al.*, 1978; Kapitulnik *et al.*, 1978) but it is less tumorigenic than benzo[*a*]pyrene in mouse skin (Slaga *et al.*, 1977). (+)-*Anti*-B[*a*]PDE can mutate *H-ras* at codons 12 and 61 which activates this proto-oncogene and leads to the transformation of NIH3T3 cells (Marshall *et al.*, 1984); it preferentially forms DNA adducts in 'hot-spots' in the *p53* tumour-suppressor gene which correspond to codons mutated in tumours from lung cancer patients (Denissenko *et al.*, 1996; Hainaut & Pfeifer, 2001).

Ample evidence indicates that this pathway of metabolic activation and hence adduct formation occurs in humans. Phenanthrene is similar to benzo[*a*]pyrene because it contains a bay-region and can be metabolically transformed to a diol epoxide that can be hydrolysed to a tetraol. Biomonitoring studies on the urine of smokers has led to the detection of phenanthrene tetraols as a surrogate biomarker of exposure to PAHs. More recently, benzo[*a*]pyrene tetraols have been detected as exposure biomarkers in the urine of smokers using SID-LC/MS (Hecht *et al.*, 2010; Hochalter *et al.*, 2011). (+)-*Anti*-B[*a*]PDE-dG-adducts have also been detected in human maternal and umbilical white blood cells following exposure to air pollution using enzyme-linked immunosorbent assay-based methods (Whyatt *et al.*, 1998; Santella, 1999). Using SID-LC/MS, it was found that (+)-*anti*-B[*a*]PDE-dG adducts in human lung tissue from 26 donors with different smoking histories contributed to only a small portion of adducts that were measured by ^{32}P-postlabelling (Beland *et al.*, 2005). These studies showed that other stable, bulky DNA adducts arise in lung tissue due to cigarette smoke, including (+)-*anti*-B[*a*]PDE-dG adducts.

Other PAHs have been shown to be activated to diol epoxides and account for either the mutagenic and/or tumorigenic properties of the parent hydrocarbon from which they are derived. A key feature of this generalized mechanism is the formation of a resonance-stabilized carbocation following epoxide ring-opening, which is stabilized further by the inductive group of a bay-region methyl group or *fjord*-region benzo group, as originally proposed and predicted by Jerina *et al.* (1976, 1986). The following PAHs are activated to diol epoxides which react with DNA to form diol epoxide–DNA adducts: benz[*a*]anthracene is converted to benz[*a*]anthracene-3,4-diol-1,2-oxide (Cooper *et al.*, 1980); benzo[*g*]chrysene is activated to benzo[*g*]chrysene-11,12-diol-13,14-oxide (Giles *et al.*, 1996; Agarwal *et al.*, 1997); benzo[*b*]fluoranthene is converted to benzo[*b*]fluoranthene-9,10-diol-11,12-oxide (Ross *et al.*, 1992; Weyand *et al.*, 1993); benzo[*j*]fluoranthene is activated to benzo[*j*]fluoranthene-4,5-diol-6,6α-oxide and the 9,10-diol-11,12-oxide (LaVoie *et al.*, 1980); benzo[*c*]phenanthrene is converted to the benzo[*c*]phenanthrene-3,4-diol-1,2-oxide (Wood *et al.*, 1980); dibenz[*a,h*]anthracene is activated to dibenz[*a,h*]anthracene-3,4-diol-1,2-oxide and can be converted to a *bis*-diol-epoxide because it has two bay-regions (Platt *et al.*, 1990); dibenz[*a,j*]anthracene is activated to the dibenz[*a,j*]anthracene-3,4-diol-1,2-oxide; dibenzo[*a,h*]pyrene is converted to dibenzo[*a,h*]pyrene–1,2-diol-3,4-oxide; dibenzo[*a,l*]pyrene is activated to dibenzo[*a,l*]pyrene-11,12-diol-13,14-oxide (Luch *et al.*, 1997, 1999); and 5-methylchrysene is activated to 5-methylchrysene-7,8-diol-9,10-oxide and 5-methylchrysene-1,2-diol-3,4-oxide (Melikian *et al.*, 1983; Koehl *et al.*, 1996). Of these

PAHs, chrysene, benz[*a*]anthracene and dibenzo[*a,l*]pyrene, as well as benzo[*a*]pyrene, belong to the 16 EPA priority PAHs found in air pollution which arise from incomplete combustion and are probably present in diesel or gasoline engine exhaust. Thus, the weight of the evidence suggests that many PAHs are activated to reactive diol epoxides that form DNA adducts which give rise to mutations, and that this mechanism is responsible for the initiation of cancer.

(ii) Radical cations

In the radical cation pathway, CYP peroxidases or other peroxidases use benzo[*a*]pyrene as a co-reductant. The pathway does not require a bay-region in the parent PAH but is governed by the ionization potential (the ease at which an already electron-deficient PAH will donate an electron) (Cavalieri & Rogan, 1985, 1995). Peroxidases require protoporphyrin IX and, when a peroxide (ROOH) is cleaved to yield an alcohol and water, the equivalent of iron(V^+) is produced (compound I) which is reduced back to iron(III^+) in the presence of a reductant. Benzo[*a*]pyrene can be a source of these electrons and generate a radical cation at C6. This reactive radical can form unstable C8-guanine [8-(benzo[*a*]pyren-6-yl)guanine], N7-guanine [7-benzo[*a*]pyren-6-yl)guanine] and N7-adenine [7-benzo[*a*]pyren-6-yl)adenine] depurinating adducts. These adducts have been detected *in vitro* following the incubation of benzo[*a*]pyrene with hydrogen peroxide horseradish peroxidase (Rogan *et al.*, 1988) or the incubation of 3-methylcholanthrene-induced rat liver microsomes with benzo[*a*]pyrene, DNA and cumene hydroperoxide (Cavalieri *et al.*, 1990), *in vivo* in mouse skin following the topical application of benzo[*a*]pyrene (Chen *et al.*, 1996) and in the urine of smokers (Casale *et al.*, 2001). These depurinating adducts give rise to abasic sites, and DNA polymerases most frequently insert an A opposite an abasic site (Sagher & Strauss, 1983; Shibutani *et al.*, 1997) providing a straightforward route to the G→T transversions found in *K-ras* and *p53* in human lung cancers. The radical cation pathway could explain the higher tumorigenicity of benzo[*a*]pyrene than that of (+)-*anti*-B[*a*]PDE in mouse skin (Slaga *et al.*, 1977) and would provide an explanation for the tumorigenicity of 1,2,3,4-tetrahydro-7,12-dimethylbenz[*a*]anthracene (DiGiovanni *et al.*, 1982), which is incapable of forming a diol epoxide. If the radical cation is not intercepted by bases in DNA, it can undergo hydroxylation and/or subsequent air oxidation to produce the remote quinones, benzo[*a*]pyrene-1,6-dione, benzo[*a*]pyrene-3,6-dione and benzo[*a*]pyrene-6,12-dione (Lesko *et al.*, 1975; Cavalieri *et al.*, 1988). These diones can undergo one-electron and two-electron redox cycling to produce reactive oxygen species (ROS) (Chesis *et al.*, 1984). The one-electron pathway leads to mutagenesis in the Ames test with a fortified exogenous metabolic activation system and is unaffected by dicoumarol but is blocked by catalase and super oxide dismutase, indicating that ROS are the culprit mutagens (Chesis *et al.*, 1984). In subsequent experiments, it was shown by ^{32}P-postlabelling that benzo[*a*]pyrene-3,6-dione produced stable covalent benzo[*a*]pyrene-3,6-dione-dG adducts provided that CYP1A1 and CYP-nicotinamide adenine dinucleotide phosphate (NADPH) oxidoreductase were present but that this was suppressed by the presence of NADPH quinone oxidoreductase 1 (NQO1), suggesting that the one-electron-reduced semiquinone radical was responsible for the adducts (Joseph & Jaiswal, 1994).

Similar pathways for the activation of PAHs that involved radical cations have been observed for 7,12-dimethylbenz[*a*]anthracene and dibenzo[*a,l*]pyrene in mouse skin, in which depurinating DNA adducts were shown to be responsible for the bulk of the covalent DNA adducts that were formed from the parent PAH (Devanesan *et al.*, 1990, 1993; Cavalieri *et al.*, 2005). Of the PAHs that are activated via this pathway, only

benzo[*a*]pyrene is found in diesel and gasoline engine exhausts.

(iii) ortho-Quinones

The third pathway is known as the *ortho*-quinone pathway (Penning *et al.*, 1999), through which (–)benzo[*a*]pyrene-7,8-*trans*-dihydrodiol and other non-K-region PAH *trans*-dihydrodiols undergo an enzymatic NAD(P)$^+$-dependent oxidation catalysed by human AKRs (AKR1A1, AKR1C1–AKR1C4) to yield a ketol (Burczynski *et al.*, 1998; Palackal *et al.*, 2001, 2002). The ketol undergoes a spontaneous rearrangement to produce benzo[*a*]pyrene-7,8-catechol. Because the transformation occurs on the terminal benzo-ring, this pathway (similarly to the diol epoxide pathway) explains why the parent PAH requires the presence of a bay-region to be tumorigenic. Benzo[*a*]pyrene-7,8-catechol is air-sensitive and undergoes primary one-electron oxidation to produce the *ortho*-semiquinone radical followed by secondary one-electron oxidation to produce the *ortho*-quinone, benzo[*a*]pyrene-7,8-dione and concomitant ROS (Smithgall *et al.*, 1988; Penning *et al.*, 1996). Benzo[*a*]pyrene-7,8-dione is a reactive Michael acceptor that can form stable and depurinating DNA adducts *in vitro* (Shou *et al.*, 1993; McCoull *et al.*, 1999; Balu *et al.*, 2004, 2006). Benzo[*a*]pyrene-7,8-dione is also redox-active and, in the presence of NQO1 and AKRs, is reduced back to the corresponding catechol establishing a futile redox cycle in which ROS are amplified until cellular reducing equivalents are depleted (Shultz *et al.*, 2011).

The catalytic efficiency of an AKR to convert the *trans*-dihydrodiol to the *ortho*-quinone is several orders of magnitude lower than that of the same AKR to reduce the quinone back to the catechol, indicating that, once the *ortho*-quinone is formed, AKRs can play an important role in exacerbating the formation of ROS (Shultz *et al.*, 2011). This formation can be attenuated if the catechol is intercepted by conjugation that is catalysed by catechol-*O*-methyl transferase, SULTs or UGTs or if the *ortho*-quinone is intercepted by conjugation that is catalysed by GST. Evidence has been obtained for the formation of 8-*O*-monomethyl-benzo[*a*]pyrene-7,8-catechol in three different human lung cell lines (H35A, A549 and HBEC-tk) (Zhang *et al.*, 2011). In addition, benzo[*a*]pyrene-7,8-dione reacts rapidly and non-enzymatically with glutathione to form 10-glutathionyl-benzo[*a*]pyrene-7,8-dione and 10-glutathionyl-benzo[*a*]pyrene-7,8-catechol (Murty & Penning, 1992).

The conversion of benzo[*a*]pyrene-7,8-*trans*-dihydrodiol to benzo[*a*]pyrene-7,8-dione, concomitant formation of intracellular ROS and the generation of 8-oxo-2'-deoxyguanosine (8-OH-dG) adducts via this pathway was recently demonstrated in human lung adenocarcinoma (A459) cells (Park *et al.*, 2008a); evidence of the entire pathway for benzo[*a*]pyrene was also found in human H358 bronchoalveolar cells (Lu *et al.*, 2011).

Benzo[*a*]pyrene-7,8-dione was found to be a *p53* mutagen at nanomolar concentrations *in vitro* only under conditions in which it can redox cycle (Yu *et al.*, 2002). In this assay, a linear correlation was observed between the frequency of *p53* mutagenicity and the formation of 8-OH-dG adducts (Park *et al.*, 2008b). The *p53* mutations observed were G→T transversions, which are consistent with the formation of 8-oxoguanine and its base mispairing with adenine. When selected for dominance, the *p53* mutations were found in 'hot-spots' in the tumours of lung cancer patients (Shen *et al.*, 2006). *In vivo*, only 8-OH-dG gives rise to G→T transversions if it is not repaired by base-excision repair. In human lung cancer, one allele of the human 8-oxoguanine glycosylase gene (*OGG1*) is deleted, suggesting that oxidative lesions are more prone to lead to mutation (Wikman *et al.*, 2000). Covalent, stable and depurinating *ortho*-quinone adducts have not yet been detected in cells or in the A/J mouse model of benzo[*a*]pyrene-induced lung cancer (Nesnow *et al.*, 2010). *In vitro*, the dominant

mutagenic lesion from this pathway is 8-OH-dG (Yu *et al.*, 2002; Park *et al.*, 2005). Furthermore, in many studies of benzo[*a*]pyrene–DNA adduct formation, the ^{32}P-postlabelling assay used would not have detected 8-OH-dG. Unlike the diol epoxide pathway, no evidence has shown that PAH-*ortho*-quinones are mutagenic in mammalian cells, have transformation potential or can cause tumours in rodent models.

Other PAH *trans*-dihydrodiols that are activated by this pathway are: chrysene-1,2-*trans*-dihydrodiol that is activated to chrysene-1,2-dione; 5-methyl-chrysene-1,2-*trans*-dihydrodiol that is converted to 5-methyl-chrsyene-1,2-dione; benz[*a*]anthracene-3,4-*trans*-dihydrodiol that is activated to benz[*a*]anthracene-3,4-dione; 7-methylbenz[*a*]anthracene-3,4-dihydrodiol that is converted to 7-methylbenz[*a*]anthracene-3,4-dione; 12-methylbenz[*a*]anthracene-3,4-dihydrodiol that is activated to 12-methylbenz[*a*]anthracene-3,4-dione; 7,12-dimethylbenz[*a*]anthracene-3,4-dihydrodiol that is converted to 7,12-dimethylbenz[*a*]anthracene-3,4-dione; benzo[*g*]chrysene-11,12-diol that is activated to benzo[*g*]chrysene-11,12-dione; and benzo[*c*]phenanthrene-3,4-*trans*-dihydrodiol that is converted to benzo[*c*]phenanthrene-3,4-dione (Palackal *et al.*, 2001, 2002). As stereochemistry is a critical determinant of the downstream metabolism of PAHs, AKR1A1 was found to have the highest catalytic efficiency for (–) benzo[*a*]pyrene-7,8-*trans*-dihydrodiol and to be stereoselective for this major isomeric metabolite (Palackal *et al.*, 2001). In contrast, AKR1C1–AKR1C4 showed no stereoselectivity and can oxidize the racemic *trans*-dihydrodiols listed. These isoforms also display a higher catalytic efficiency than AKR1A1 for *trans*-dihydrodiols that contain either a methylated bay-region or *fjord*-region (Palackal *et al.*, 2002). Of the PAHs affected by this pathway, chrysene, benz[*a*]anthracene and benzo[*a*]pyrene are found in air pollution.

(c) Induction of metabolism and/or activation

The relative contribution of these three pathways to the activation of PAHs is influenced by enzyme induction. Planar PAHs can induce their own metabolism. Compounds such as benzo[*a*]pyrene can bind to the aryl hydrocarbon receptor (AhR) (Nebert *et al.*, 1979, 1993, 2004), which is found in the cytosol and, after binding to the ligand, dissociates from heat-shock protein 90 (a chaperone protein) and translocates to the nucleus where the liganded receptor heterodimerizes with the aryl hydrocarbon nuclear translocator (Hankinson, 1995). The AhR:aryl hydrocarbon nuclear translocator heterodimer binds to the xenobiotic response element on the promoter of *CYP1A1* and *CYP1B1* genes to cause gene transcription and enzyme induction (Denison *et al.*, 1988a, b, 1989), resulting in increased monooxygenation of the parent PAH.

PAHs have been referred to as bi-functional inducers (Prochaska & Talalay, 1988), because their metabolism frequently results in the production of electrophiles that can then activate the Nrf2 (transcription factor)-Keap1 (negative regulator) system (Itoh *et al.*, 1997, 1999), which is also activated by other electrophiles, redox-active compounds, heavy metals and ROS (Dinkova-Kostova *et al.*, 2005). This activation leads to the dissociation of Nrf2 from the electrophilic/ROS sensor Keap1 followed by its translocation to the nucleus where it heterodimerizes with small c-Maf proteins (Dinkova-Kostova *et al.*, 2005). The Nrf2:small c-Maf complex then binds to the antioxidant response element in the promoter regions of responsive genes (Rushmore *et al.*, 1991; Nguyen *et al.*, 2003). The relevant genes in humans that are regulated by an antioxidant response element are: *γ-glutamyl cysteine synthase, NQO1*, *AKR1C1–AKR1C3* and *AKR1B10* (Burczynski *et al.*, 1999; Jin & Penning, 2007; Penning & Drury, 2007). In contrast, human GSTs lack an antioxidant response element in their gene promoters (Hayes

et al., 2005). Among the human genes, *AKR1C1–AKR1C3* are involved in the metabolic activation of PAH *trans*-dihydrodiols to electrophilic and redox-active *ortho*-quinones (Palackal *et al.*, 2002), when a positive feedback loop is created in which the quinone and ROS can further induce metabolism via this pathway. In addition, PAH *ortho*-quinones can also operate as bi-functional inducers by activating the Nrf2-Keap1 system and acting as ligands for the AhR (Burczynski & Penning 2000). Thus, benzo[*a*]pyrene-7,8-dione promotes nuclear translocation of the AhR and induction of the *CYP1A1* and *CYP1B1* genes. *AKR1C1*, *AKR1C2*, *AKR1B10* and *CYP1B1* have been shown to be upregulated in the bronchial epithelial cells of tobacco smokers (Woenckhaus *et al.*, 2006), downregulated in those of smokers who quit (Zhang *et al.*, 2008) and upregulated in oral dysplastic cells induced by cigarette-smoke condensate (Nagaraj *et al.*, 2006; Gümüş *et al.*, 2008), which led to the concept that they form part of a battery of genes that are induced by cigarette smoking (Penning & Lerman 2008). The same genes are probably regulated in response to particulate matter of 2.5 mm diameter ($PM_{2.5}$) and similar mixtures found in diesel and gasoline engine exhausts.

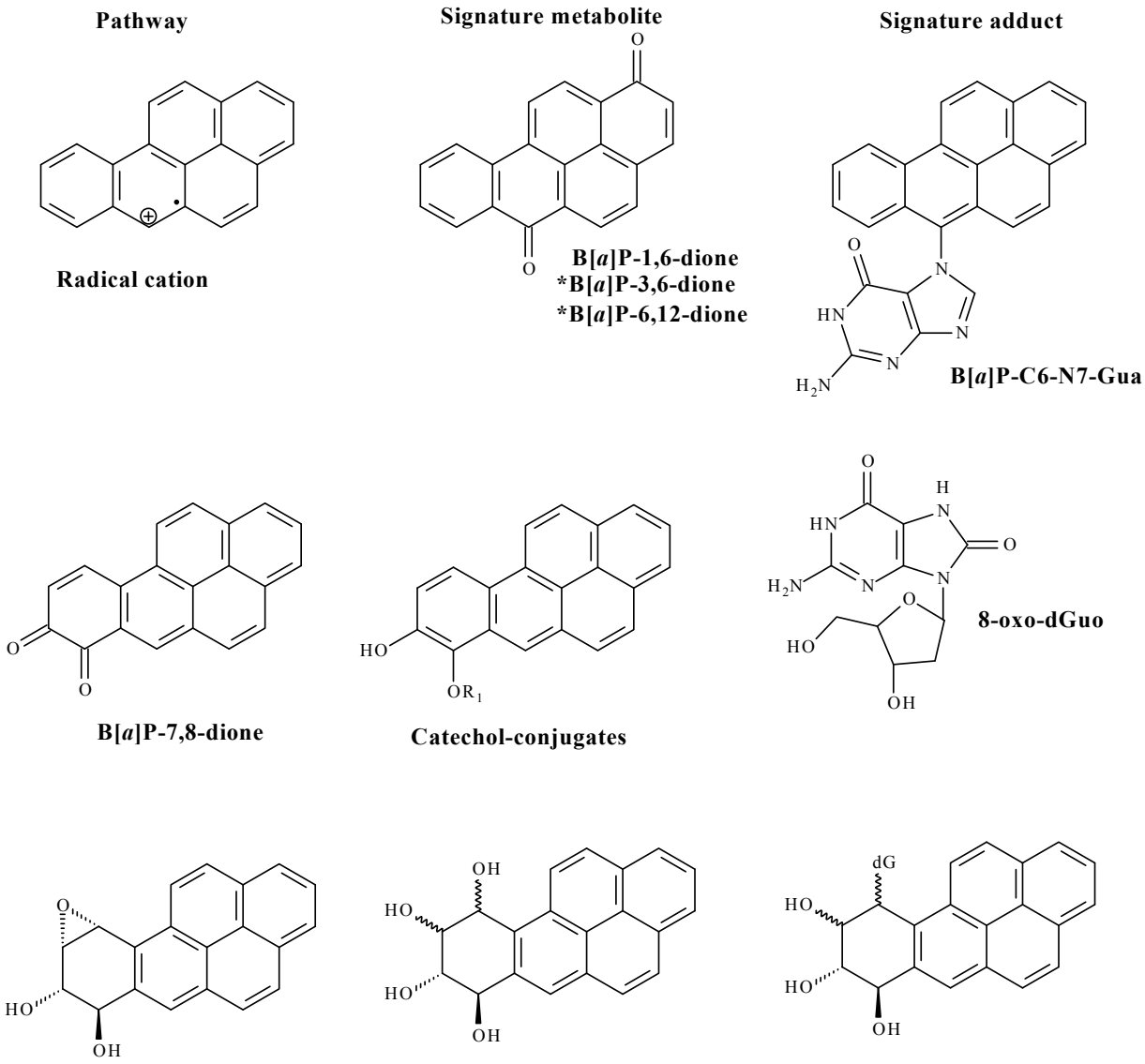
(d) Relative contributions of the three pathways of activation

While compelling evidence shows that the diol epoxide pathway of PAH activation is mechanistically important and explains how PAHs may initiate lung cancer in mice and humans, relatively few studies have been conducted to compare the contribution of all three pathways to their activation in the same cell line, tissue, organ or animal model. One approach is to compare the relative formation of signature metabolites from each benzo[*a*]pyrene pathway of activation, e.g. benzo[*a*]pyrene tetraols (diol-epoxide pathway), benzo[*a*]pyrene-1,6-dione and benzo[*a*]pyrene-3,6-dione (radical cation pathway) and benzo[*a*]pyrene-7,8-dione (*ortho*-quinone pathway). Another approach is to compare the formation of signature benzo[*a*]pyrene–DNA adducts following exposure to benzo[*a*]pyrene; these would include (+)-*anti*-B[*a*]PDE-dG adducts (diol epoxide pathway), the formation of abasic sites or the corresponding depurinating adducts (C6-C8-guanine, C6-N7-guanine and C6-N7-adenine adducts of benzo[*a*]pyrene) (radical cation pathway) and the formation of 8-OH-dG (*ortho*-quinone pathway) following treatment with benzo[*a*]pyrene (Fig. 4.2). Both approaches have been used with variable success.

Recently, SID-LC/MS/MS assays were developed to profile benzo[*a*]pyrene metabolism in human H358 bronchoalveolar cells in the absence and presence of the AhR ligand, 2,3,7,8-tetrachlorodibenzodioxin. Signature metabolites of each pathway were measured, e.g. benzo[*a*]pyrene tetraols, benzo[*a*]pyrene-1,6-dione, benzo[*a*]pyrene-3,6-dione and benzo[*a*]pyrene-7,8-dione. It was found that, in the absence and presence of 2,3,7,8-tetrachlorodibenzodioxin, all three pathways made an equal contribution to the metabolism of benzo[*a*]pyrene, and that 2,3,7,8-tetrachlorodibenzodioxin abolished its lag phase (Lu *et al.*, 2011).

Other studies compared the relative formation of dibenzo[*a,l*]pyrene-diol epoxide–DNA adducts with that of abasic sites (evidence of depurination and radical cation formation) following the treatment of HL60 cells with dibenzo[*a,l*]pyrene (Melendez-Colon *et al.*, 1997, 1999). Dibenzo[*a,l*]pyrene was chosen because it is the most carcinogenic PAH known and HL60 cells were chosen because of their high level of endogenous peroxidase activity. Only diol epoxide adducts were detected by ^{32}P-postlabelling, whereas abasic sites could not be detected with the aldehyde-reactive probe. In other studies, unstable depurinating DNA adducts of benzo[*a*]pyrene, 7,12-dimethylbenz[*a*]anthracene and dibenzo[*a,l*]pyrene were detected in mouse skin and found to exceed the formation of stable DNA adducts that would be formed from the diol epoxide

Fig. 4.2 Signature metabolites and signature DNA adducts arising from the three pathways of benzo[*a*]pyrene activation



* Structures not shown

B[*a*]P, benzo[*a*]pyrene; B[*a*]PDE, benzo[*a*]pyrene-7,8-diol-9,10-epoxide; 8-oxo-dGuo, 8-oxo-7,8-dihydro-2′-deoxyguanosine

pathway (RamaKrishna *et al.*, 1992; Chen *et al.*, 1996; Cavalieri *et al.*, 2005). In a comparison of the formation of *anti*-B[*a*]PDE-dG and 8-OH-dG adducts in human H358 bronchoalveolar cells following treatment with the proximate carcinogen (–)benzo[*a*]pyrene-7,8-*trans*-dihydrodiol using SID-LC/MS, the levels of both adducts were similar (Ruan *et al.*, 2007; Mangal *et al.*, 2009). The level of 8-OH-dG increased following treatment of the cells with a catechol-*O*-methyl transferase inhibitor, suggesting that a futile redox cycle between benzo[*a*]pyrene-7,8-catechol and benzo[*a*]pyrene-7,8-dione was responsible for the increased formation of lesions. Using a low dose to initiate DNA adducts in the A/J mouse lung model of benzo[*a*]pyrene carcinogenesis, the relative level of stable adducts formed was measured with ^{32}P-postlabelling. While (+) *anti*-B[*a*]PDE-dG adducts were detected, the corresponding stable covalent adducts that can be formed with benzo[*a*]pyrene-7,8-dione were not found (Nesnow *et al.*, 2010). These data were consistent with the concept that 8-OH-dG may be the dominant lesion formed from benzo[*a*]pyrene-7,8-dione *in vivo*.

IARC (2010a) examined four mechanisms for the activation of benzo[*a*]pyrene. Overwhelming evidence supported the formation of bay-region diol epoxides as the mechanism that generates biological reactive intermediates which form the DNA adducts that lead to mutation. Other pathways, such as the radical cation pathway or the *ortho*-quinone pathway, may also play a role but the data for each of these pathways are still incomplete, and no compelling data support L-region mesomethylation as a viable pathway.

4.2 Deposition, clearance, retention and metabolism

The general principles of inhalation, deposition, clearance and retention of poorly soluble particles have been described previously (IARC, 2010b). Modelling of inhaled particle deposition in the human lung has also been reviewed (Hofmann, 2009, 2011).

4.2.1 Humans

(a) Diesel engine exhaust

The number, concentration and size distribution of aerosol particles in the submicrometre range generated from diesel combustion that were inhaled and exhaled by 14 nonsmoking human volunteers were determined using a scanning mobility particle sizer. The subjects were in a relaxed state and inhaled nasally using a spontaneous breathing pattern. Total deposition was determined by the difference between the total measured number and concentrations of the inhaled and exhaled size distributions. The average total deposition for diesel smoke was 30 ± 9% (± standard deviation [SD]) and the count median diameter was 0.124 ± 0.025 µm (Morawska *et al.*, 2005). A Monte Carlo deposition morphometric stochastic model was applied to this data set. The theoretical prediction for total deposition was 24 ± 2.7%, which was lower than the values obtained experimentally. The apparent discrepancy between experimental data on total deposition and modelling results was reconciled by considering the non-spherical shape of the test aerosols by diameter-dependent dynamic shape factors that account for differences between mobility-equivalent and volume-equivalent or thermodynamic diameters (Hofmann *et al.*, 2009).

Other models have been developed to predict the deposition of diesel engine exhaust in humans and rats (Yu & Xu, 1986).

A group of selective studies of metabolism have been conducted on humans exposed to diesel engine exhaust, generally in the workplace. Many of these studies (described below) focused on measurements of urinary levels of hydroxylated and amino PAHs, mainly of pyrene, and demonstrated the ability of humans exposed

to diesel engine exhaust to adsorb, distribute, metabolize and excrete metabolites of PAHs. Additional studies have described haemoglobin adducts of PAHs and low-molecular-weight alkenes.

The levels of urinary 1-hydroxypyrene and haemoglobin-bound adducts (hydroxyethylvaline and hydroxypropylvaline) were measured in four groups of nonsmoking men: urban bus drivers (27), suburban bus drivers (23), taxi drivers (21) and suburban controls (22). No differences were found between the groups (Hemminki *et al.*, 1994a).

Several biomarkers were evaluated for their ability to assess differences in exposure to diesel engine exhaust and used lubricating oil in bus garage workers and mechanics, and controls by measuring the levels of both urinary 1-hydroxypyrene and hydroxyethylvaline adducts in haemoglobin. Samples were taken from 10 nonsmoking bus garage workers and mechanics and 12 nonsmoking healthy men in the administrative department. The exposed workers had significantly higher levels of the biomarkers than the controls. The level of hydroxyethylvaline haemoglobin adducts was 33.3 pmol/g haemoglobin in exposed workers versus 22.1 pmol/g haemoglobin in controls, and that of 1-hydroxypyrene in the urine was 0.11 pmol/mol creatinine in the workers compared with 0.05 pmol/mol creatinine in controls. The levels of hydroxyethylvaline adducts in the blood correlated with those of urinary 1-hydroxypyrene. The study indicated that skin absorption might be an important factor to consider when studying exposure to PAHs from air pollution sources (Nielsen *et al.*, 1996a).

Fifty Estonian underground oil-shale miners (35 smokers) who drove diesel-powered excavators and 50 surface workers (31 smokers) engaged in various production assignments above-ground that were not associated with the use of diesel-powered engines were examined for levels of urinary 1-hydroxypyrene, *S*-phenylmercapturic acid and *t,t*-muconic acid. Samples were collected before the first of a series of work shifts and after several shifts over the course of a work week. The group of underground miners had slightly but significantly increased levels of all three biomarkers at the end of the first and last shifts while no significant changes in the levels over time were observed for the surface controls (Scheepers *et al.*, 2002).

The levels of urinary 1-hydroxypyrene in groups of smoking and nonsmoking Chilean workers exposed to diesel engine exhaust emissions in the transport industry were compared with those of an unexposed rural population. The groups comprised 59 diesel-exposed workers (38 involved in diesel revision (emission compliance) and 21 in an urban area working as established street vendors) and 44 unexposed subjects living in a rural area. The levels of urinary 1-hydroxypyrene in the urban and rural populations showed significant differences between smokers and nonsmokers ($P < 0.04$), but no significant differences between smokers and nonsmokers were found among the diesel plant workers. Nonsmoking subjects from the diesel plants and the urban area showed similar levels which were significantly higher ($P < 0.05$) than those of subjects living in the rural area (Adonis *et al.*, 2003).

Levels of 1-hydroxypyrene were determined in 60 nonsmoking bus drivers in city and rural areas on a work day and on a day off and in 88 nonsmoking mail deliverers working outdoors (in the streets) and indoors (in the office). Twenty-four hour urine samples were collected on a working day and a day off. Bus drivers excreted significantly more 1-hydroxypyrene in the urine than mail deliverers ($P < 0.001$). These results indicated that bus drivers have greater exposure to PAHs than mail deliverers. Mail deliverers who worked outdoors had significantly higher urinary concentrations of 1-hydroxypyrene than those who worked indoors ($P < 0.001$) (Hansen *et al.*, 2004). [The Working Group noted that the

exposures of the study groups to specific exhaust emissions were not identified.]

In Helsinki, Finland, urinary hydroxylated metabolites of naphthalene, phenanthrene and pyrene were examined as markers of exposure to diesel engine exhaust in a group of exposed workers comprising 30 nonsmoking men and two women (20 bus garage workers and 12 waste-collection truck drivers), who were exposed daily to diesel engine exhaust. A group of 46 nonsmoking white-collar workers served as controls. Urine samples were collected from workers at three bus garages who repaired, refuelled and cleaned diesel-powered buses indoors and were exposed to diesel engine exhaust through work activities near the buses inside the garages, and from truck drivers who worked for four companies that collected household waste in a suburb. The sum of seven PAH metabolites (mean, 3.94 ± 3.40 (SD) and 5.60 ± 6.37 (SD) µmol/mol creatinine in winter and summer, respectively) was higher ($P = 0.01$) in the exposed group than in the control group (mean, 3.18 ± 3.99 (SD) and 3.03 ± 2.01 (SD) µmol/mol creatinine in winter and summer, respectively). The mean concentrations of 2-naphthol ranged from 3.34 to 4.85 µmol/mol creatinine for the exposed workers and 2.51 to 2.58 µmol/mol creatinine for the controls ($P < 0.01$ in winter, $P < 0.03$ in summer). The mean levels of hydroxyphenanthrenes were between 0.40 and 0.70 µmol/mol creatinine in the samples from exposed workers and between 0.40 and 0.60 µmol/mol creatinine in those from controls. The concentration of 1-hydroxypyrene was higher among exposed workers in both pre-shift and post-shift samples (mean, 0.10–0.15 µmol/mol creatinine) than in the control group (mean, 0.05–0.06 µmol/mol creatinine) in winter ($P = 0.002$) and in summer ($P < 0.001$). The authors concluded that the presence of urinary hydroxylated metabolites of naphthalene, phenanthrene and pyrene indicated low exposure to diesel-derived PAHs, and was higher in exposed workers than in the control group. Urinary monohydroxylated PAH metabolites measured in this study did not correlate with PAHs in the air samples as reported previously (Kuusimäki *et al.*, 2004).

Levels of 1-hydroxypyrene were measured in the urine of 47 Italian road construction workers exposed to diesel engine exhaust (17 nonsmokers and 30 smokers), who also refrained from consuming smoked and grilled food, tea and coffee the evening before and during the day of sampling. Samples were taken at several time-points: after 2 days of vacation (baseline), and before and at the end of the monitored work shift during the second part of the work week. At all sampling time-points, the levels of urinary 1-hydroxypyrene of the smoking construction workers were higher than those of the nonsmoking group ($P < 0.01$). No differences were found in the urinary levels of 1-hydroxypyrene between samples taken at baseline, before the shift and at the end of the shift in either smoking or nonsmoking workers (Campo *et al.*, 2006). The subjects served as their own controls.

Levels of 1-hydroxypyrene were determined in pre- and post-shift urine samples collected on 4 consecutive days from 17 Chinese workers (73% smokers) exposed to diesel engine exhaust at a locomotive engine inspection plant. Increased levels were observed over at least 3 consecutive sampling days. The biological kinetics of pyrene metabolism was studied using one-compartment pharmacokinetics and nonlinear mixed-effects models. The mean half-life of urinary 1-hydroxypyrene was estimated to be 29 hours (Huang *et al.*, 2007).

Urinary levels of 1-aminopyrene were compared between three diesel mechanics working in a repair shop for train engines and two office clerks. Both cumulative and average excretion of urinary 1-aminopyrene over 48 and 72 hours were significantly enhanced ($P < 0.05$) in samples from diesel mechanics compared with those in samples from the office clerks (Scheepers *et al.*, 1994).

Blood samples from 29 bus garage workers (occupationally exposed to diesel engine exhaust), 20 urban hospital workers and 14 rural council workers (controls) were analysed for sulfinic acid-type amino-haemoglobin adducts derived from 1-nitropyrene, 2-nitrofluorene, 3-nitrofluoranthene, 9-nitrophenanthrene and 6-nitrochrysene. The most abundant cleavage products were 1-aminopyrene and 2-aminofluorene at levels ranging from 0.01 to 0.68 pmol/g haemoglobin. No significant difference in the levels of 1-nitropyrene- and 2-nitrofluorene-derived haemoglobin adducts was observed between the groups. A comparison of the sum of the five nitro-PAH-derived haemoglobin adducts also indicated no differences between the three groups (Zwirner-Baier & Neumann, 1999). The same methodology was applied to a group of 30 former East German miners exposed to diesel engine exhaust emitted from heavy-duty equipment, a group of 40 Danish bus drivers and the 29 bus garage workers cited above. The sum of the five nitro-PAH-derived haemoglobin adducts was higher (no statistical comparison) in the miners and bus drivers compared with the garage workers (Neumann, 2001).

The 24-hour urine samples from 18 underground salt miners (nine smokers, nine nonsmokers) exposed to diesel engine exhaust were collected during and after their shift. Nonsmoking workers exposed to diesel engine exhaust excreted an average level of ~4 µg phenanthrene metabolites (phenol and dihydrodiols), whereas the urinary levels in smokers were up to threefold higher. 1-Aminopyrene and 3-aminobenzanthrone were detected in the urine of all miners (Seidel *et al.*, 2002).

The urinary levels of 1-hydroxypyrene of 50 Estonian underground shale-oil miners who drove diesel-powered excavators were compared with those of 50 surface workers who had no known occupational exposure to diesel engine exhaust. Personal exposure measurements of particle-associated 1-nitropyrene were collected. Urine samples were collected at the start and after the first and last shift of the same week. Levels of particle-associated 1-nitropyrene were approximately eightfold higher for miners (as determined by personal air monitoring) than for surface workers, while levels of urinary 1-hydroxypyrene showed only a small increase (Scheepers *et al.*, 2004).

Concentrations of 1-aminopyrene were measured in spot urine samples from 38 individuals collected before and during a 24-hour period following the initiation of 1-hour controlled exposures to diesel engine exhaust (target concentration, 300 µg/m^3 as PM_{10}) or clean air. Urinary samples of the *N*-acetyl-1-aminopyrene conjugate were deconjugated to 1-aminopyrene and analysed. Time-weighted average concentrations of urinary 1-aminopyrene were significantly greater following exposure to diesel engine exhaust compared with clean air (median, 138.7 versus 21.7 ng/g creatinine; $P < 0.0001$). Comparing exposures to diesel engine exhaust and clean air, significant increases in the concentration of 1-aminopyrene from pre-exposure to either first post-exposure void (24-hour timepoint) or peak spot urine concentration following exposure ($P = 0.027$ and $P = 0.0026$, respectively) were recorded. Large interindividual variability, in both the concentration of urinary 1-aminopyrene and the time course of appearance in the urine following the standardized exposure to diesel engine exhaust, was observed (Laumbach *et al.*, 2009). In a follow-up study, pharmacokinetic analyses of the data on 1-aminopyrene excretion found that two subgroups could be determined within the exposed group in terms of the timing of the excretion. Approximately 63% of the subjects had a median maximal excretion time of 5.37 hour whereas 30% of the subjects had maximal excretion times of > 24 hours (Huyck *et al.*, 2010). Genetic polymorphisms, metabolic enzymes and/or differences in respiration rates may play a role in the differences in excretion rates.

(b) Gasoline engine exhaust

The results of three studies on the deposition of gasoline engine exhaust have been reported previously (IARC, 1989). In two controlled studies, volunteers were exposed to exhaust from an engine run on gasoline containing ^{203}Pb-tetraethyl lead. Total deposition was relatively constant (30%) over a wide range of breathing patterns for sizes of typical aerosols (Chamberlain, 1985). As the size of primary particles decreased (below 0.1 μm), deposition increased sharply and the length of the respiratory cycle (start of the time between respiratory breaths) significantly affected deposition (Heyder *et al.*, 1983; Schiller *et al.*, 1986). In a separate analysis of the same data, deposition was shown to increase with the respiratory cycle (time between the start of successive breaths) in an approximately linear fashion, ranging from 10% at 3 seconds to 55% at 20 seconds; the slope of the gradient was somewhat dependent on tidal volume (Wells *et al.*, 1977). In the third study, measurements of total deposition were taken in the field by comparing inhaled and exhaled concentrations of lead; the method was found to give results comparable with those of studies on ^{203}Pb-tetraethyl lead. Total deposition was measured for inhalation at an average breathing pattern of 0.81 and a respiratory cycle of 5.2 seconds in persons seated by a motorway (69%), by a roundabout (64%), in an urban street (48%) and in a car park (48%). Median particulate sizes in the breath of persons near fast-moving traffic (0.04 μm) were found to be much smaller than those in persons in the urban environment or in a car park (0.3 μm), although the air near roundabouts also contained a large proportion by mass of adventitious particles (2 μm) (Chamberlain *et al.*, 1978).

Lung clearance was best described as a four-component exponential. The first two phases (half-times, 0.7 and 2.5 hours) were similar for exhaust particles, lead nitrate (which is soluble) and lead oxide (which is insoluble), and therefore probably represented mucociliary clearance. On average, 40% of lung deposition of the 0.35-μm aerosols was in the pulmonary region and 60% in the tracheobronchial region. The removal of lead compounds from the pulmonary region was described by a two-compartment exponential with half-times of 9 and 44 hours; one exception was the removal of lead from highly carbonaceous particles which exhibited half-times of 24 and 220 hours (Chamberlain *et al.*, 1975, 1978; Chamberlain, 1985).

4.2.2 Experimental systems

(a) Diesel engine exhaust

Engine exhaust contains material in gaseous, vapour and particulate phases, and the absorption, distribution and excretion of individual constituents are influenced by the phase in which they occur and by the properties of each compound. After inhalation, highly soluble compounds in the gaseous phase, such as sulfur dioxide, are absorbed in the upper airways and do not penetrate significantly beyond the level of the bronchioles. Compounds that interact biochemically with the body are also retained in significant quantities; thus, processes such as the binding of carbon monoxide to haemoglobin normally occur in the gas-exchange (pulmonary) region of the lung. Retention characteristics of materials not associated with the particulate phase are highly compound-specific. Factors that affect the uptake of a wide variety of vapours and gases have been summarized and modelled (Davies, 1985; Tsujino *et al.*, 2005).

A proportion of a given compound in the vapour phase condenses onto the particulate material produced in the engine exhaust. The association of a compound with the particulate phase modifies its deposition pattern and affects its lung retention; following continuous exposure, the lung burden of a compound coated on particles may be many times higher than that

after continuous exposure to the compound alone (IARC, 1984; Tsujino *et al.*, 2005).

Deposition in the respiratory tract is a function of particle size (Smith *et al.*, 2001; Hofmann *et al.*, 2009). The median particle size in a variety of long-term exposure systems has been determined to be between 0.19 and 0.54 μm (IARC, 1984; Yu & Xu, 1986; Lapuerta *et al.*, 2003; IARC, 2010a), representative of that in an urban environment. However, some of the carbonaceous mass in environmental samples results from airborne suspensions of material collected in automobile exhaust pipes and is > 5 μm in size (Lapuerta *et al.*, 2003); such particles are unlikely to be produced in a static exposure system.

At the time of the last review of diesel engine exhaust (IARC, 1989), it was known that long-term, high rates of exposure to diesel engine exhaust lead to lung tumour development in rats, but interpretation of this response for the risk of human lung cancer was in its early stages (Vostal, 1986). No evidence had been found to demonstrate that heavy doses of inhaled particles lead to the rat-specific mechanisms of lung clearance overload that trigger the release of inflammatory molecules in the lungs. The species specificity of the response of rat lungs to 'overload', and its commonality to low-solubility particles, such as those in diesel engine exhaust, are now better understood. The previous review noted that tumours were found in rats but not in other species [except for one study in mice that reported an abnormally low background incidence of tumours], and that the tumours were associated with the solid particulate and not gaseous components of the mixtures, based on the reduction in the incidence of tumours when particle traps were used. Further studies that compared the relative rates of tumour formation between similar concentrations of carbon black and diesel engine exhaust particles suggested that the tumour formation caused by diesel engine exhaust was not dependent on its organic compounds, but rather on its poorly soluble solid carbon core (Mauderly *et al.*, 1994; Heinrich *et al.*, 1995; Nikula *et al.*, 1995; Driscoll *et al.*, 1997). Recently, Stinn *et al.* (2005) reported that chronic inhalation of diesel engine exhaust at high particle concentrations resulted in tumour formation in the absence of direct genotoxicity, as measured by the levels of DNA adducts. Because of the importance of this mechanism, this section elaborates on the evidence and its potential implications for hazard identification.

This section addresses the mechanisms of carcinogenesis of particles and is based on an extensive database for poorly soluble, respirable particles of low toxicity that was reviewed previously (IARC, 2010b). The extent to which these mechanisms are fully relevant for particles generated from combustion is not known.

(i) Lung overload

The concept of 'overload' is central to the relevance of using studies in rodents to evaluate human health hazards from inhaled particles. Overload is a biological mechanism that involves the dose-dependent impairment of alveolar macrophage-mediated clearance of respirable particles. In the alveolar region of the respiratory tract, the primary mechanism for particle clearance is phagocytosis by alveolar macrophages with subsequent removal of particle-containing macrophages by mucociliary clearance.

High particle burdens in the lungs can result in overload because alveolar macrophage-mediated clearance is overwhelmed, which results in a decreased rate of clearance and an increased retention of particles. Overloading of lung clearance has been observed in rats, mice and hamsters exposed to different insoluble respirable particles (e.g. carbon black, titanium dioxide, talc, toner and diesel engine exhaust particulates) (Strom *et al.*, 1989; Muhle *et al.*, 1990a, b; Bellmann *et al.*, 1991; NTP, 1993; Warheit *et al.*, 1997; Bermudez *et al.*, 2002, 2004; Elder *et al.*, 2005) and asbestos fibres (Davis *et al.*, 1978; Bolton *et al.*, 1983).

Mechanisms that underlie lung overload

Experimentally, overloading of lung clearance has been inferred from the observation of a greater lung burden of particles or fibres than that expected on the basis of results with lower concentrations or shorter durations of exposure (Davis *et al.*, 1978). A steady-state lung burden should be achieved when the rate of deposition equals the rate of clearance, and overloading represents a deficit in that clearance. Impaired clearance attributed to overloading has been expressed as a reduction in the clearance rate coefficient (Muhle *et al.*, 1990a; Bellmann *et al.*, 1991) or an increase in the quantity of particles retained in the lungs following exposure (Strom *et al.*, 1989; Bermudez *et al.*, 2002, 2004; Elder *et al.*, 2005). Increased translocation of particles to the lung-associated lymph nodes has also been observed at doses at which overload occurs (Strom *et al.*, 1989; Bellmann *et al.*, 1991).

Morrow (1988) hypothesized that overload was a consequence of macrophages that become progressively immobilized and aggregated. When the dose of particles reaches a critical particle volume, clearance by macrophages is suppressed and particles accumulate in the lungs. Based on the lung burden of particle mass associated with increased retention in rat lungs (approximately 1 mg/g of lung tissue for unit density particles) and data on the volume and number of alveolar macrophages in rat lungs, it was hypothesized that impairment of clearance would be initiated when the particle volume exceeded an average of 6% of the macrophage volume, and would be completely impaired when particle volume exceeded an average of 60% of the macrophage volume. The upper particle volume estimate (60%) was supported by Oberdörster *et al.* (1994), who showed that clearance was no longer detectable 200 days after instillation of polystyrene particles 10 μm in diameter into rat lungs. The overload mechanism pertains specifically to poorly soluble respirable (< 10 μm) particles of low toxicity. Factors other than the volumetric overload can lead to impaired alveolar clearance. For example, particles that are toxic to macrophages (e.g. crystalline silica) can cause impaired clearance at doses lower than those of low-toxicity particles (Bellmann *et al.*, 1991). Ultrafine particles have been found to differ from fine particles with regard to overloading. Morrow (1992) noted that ultrafine particles impair clearance at lower mass or volume concentrations than those expected for larger respirable particles. Oberdörster (1996) confirmed this observation and showed that increased particle retention and inflammation were related to particle surface area.

One mechanism for the impaired clearance of ultrafine particles may be their ineffective phagocytosis (Churg *et al.*, 1998; Renwick *et al.*, 2001, 2004; Geiser *et al.*, 2005), which leaves the particles free in the alveolar region and more readily able to translocate to the lung interstitium (Ferin *et al.*, 1992; Ferin, 1994). The surface properties of particles may also influence phagocytosis. For example, Castranova *et al.* (2000) found that chronic inhalation exposure to 2 mg/m^3 of coal dust activated alveolar macrophages, while the same exposure to diesel engine exhaust depressed phagocytic activity. Wolff *et al.* (1986) noted that additional factors other than non-specific particle effects must be important, because the exposure level that resulted in overloading and lung tumours was higher for some particles than for others (e.g. 250 mg/m^3 of fine-sized titanium dioxide versus ~7 mg/m^3 of diesel engine exhaust).

Mechanisms that underlie lung response to overload

An increase in neutrophilic inflammation has been defined as the critical biological response to lung overload (ILSI Risk Science Institute Workshop Participants, 2000). An increase in polymorphonuclear leukocytes (granulocytes) in bronchoalveolar lavage (BAL) fluid in rats

has been associated with increased retention of particles in the lungs (Tran *et al.*, 1999). Mice also appear to be susceptible to overloading doses and adverse pulmonary responses, but regain normal clearance more readily after cessation of exposure. Hamsters clear particles much faster than rats or mice, experience overloading at higher doses and recover more easily. Lung responses follow the clearance kinetics for inhaled particles: rats show a more severe, sustained response to inhaled particles than mice, while hamsters demonstrate only a temporary inflammatory response (Bermudez *et al.*, 2002, 2004; Elder *et al.*, 2005). In rats, lung responses to overloading include increased lung weight, chronic inflammation, fibrosis and lung cancer (Muhle *et al.*, 1991).

The series of events that are involved in the biological process that begins with particle deposition in critical target cells or tissues within the rat lung and results in tumours include: sustained inflammation, production of ROS, depletion of antioxidants and/or impairment of other defence mechanisms, cell proliferation and gene mutations. These individual steps comprise an overall mode of action that can be used to compare the responses of rats with those of other species including humans (IARC, 2010b).

At a lung burden of particle mass at which overload is observed in rats (estimated to begin at ~0.5 mg/g of lung tissue and to be fully developed at ~10 mg/g), a sustained and widespread cellular inflammatory response occurs. The cell population is dominated by activated and probably (under these conditions) persistent neutrophil granulocytes, and secretes a collection of mediators (pro- and anti-inflammatory cytokines, proteases, cytotoxins, fibrogenic mediators and other growth factors) that act through the pulmonary milieu on surrounding cells or tissues and surrounding structures (Castranova *et al.*, 2000; IARC, 2010b).

The degree of sustained inflammation experienced by rodents (most notably rats) at high lung burdens is not observed in humans. However, humans may experience sustained inflammation during certain diseases. One such condition (which may be particle-stimulated, e.g. by silica, or cryptogenic) is late-stage interstitial pulmonary fibrosis. Patients who have interstitial pulmonary fibrosis and chronic inflammation have been reported to experience a higher incidence of lung tumours (Daniels & Jett, 2005). Rom (1991) found a statistically significant increase in the percentage of neutrophil granulocytes in the BAL fluid of workers with respiratory impairment who had been exposed to asbestos, coal or silica (4.5% in cases versus 1.5% in controls). Elevated levels (sevenfold increase over controls) of neutrophil granulocytes have been observed in the BAL fluid of miners who had simple coal workers' pneumoconiosis (Vallyathan *et al.*, 2000) and in patients with acute silicosis (a 10-fold increase over controls) (Goodman *et al.*, 1992; Lapp & Castranova, 1993).

The precise role of chronic inflammation in the development of cancer is uncertain, but considerable evidence shows that chronic inflammation may have a multifaceted role in this process. Activated cells in the lung are known to release various reactive intermediates, most notably those derived from oxygen. A sustained excess of oxidant activity is known to deplete antioxidant defences gradually. Clear differences in these lung defence mechanisms exist between humans and rats, and evidence shows that humans in general are relatively deficient in some of these mechanisms compared with rats (Hatch *et al.*, 1985). ROS within cells may directly damage DNA and potentially induce mutations. Moreover, cell damage and promitotic stimuli initiated by ROS promote cell turnover and proliferation, both of which may enhance the risk for DNA replication error and/or expand a mutated or transformed cell to initiate the tumorigenic process (see Section 4.3.1).

Dosimetric correlation between lung particle burden and response

Because particle overload is the critical determinant that underlies the adverse biological response to inhaled particles, an understanding of the appropriate dosimetric expression for overload is essential for hazard evaluation. Several studies have shown that, for particles of different sizes but with the same chemical composition, the dose expressed as particle surface area is a better predictor of adverse pulmonary inflammation than particle mass (Oberdörster *et al.*, 1992; Tran *et al.*, 1999; Bermudez *et al.*, 2002, 2004). Particle surface area is also related to pulmonary inflammation in mice (Lison *et al.*, 1997). Oberdörster & Yu (1990) and Driscoll *et al.* (1996) showed that particle surface area is also a better predictor of lung tumours than particle mass in rats exposed to various poorly soluble fine or ultrafine particles.

The particle characteristics and methods used to estimate particle surface area may influence the magnitude of the observed response. For example, carbon black that has a high specific surface area (220 m^2/g) was shown to induce a lower inflammatory response than that expected on the basis of total particle surface area dose (Driscoll *et al.*, 1996). This could be due to less disaggregation of the deposited carbon black into smaller units compared with ultrafine particles, such as titanium dioxide (Oberdörster, 1996). It may also be due to the more porous surface of carbon black (carbon black has a greater internal surface than titanium dioxide), which may increase the surface area as measured by nitrogen absorption, but does not accurately measure the effective surface area in contact with the epithelial cell surface (Tran *et al.*, 1999).

Deposition: Studies of the deposition of diesel engine exhaust, which is representative of fresh urban exhaust, showed a total deposition of inhaled particles ranging from 10 to 20% (IARC, 1984). Models for the deposition of diesel engine exhaust particles predict that, as the median size increases from 0.10 to 0.30 µm, total deposition in rats decreases from approximately 25% to ~15%, (IARC, 1984; Schroeter *et al.*, 2012), in agreement with measured deposition (Garcia & Kimbell, 2009).

Mucociliary clearance: The rapid phase of clearance of particles from the lung following a single exposure to radiolabelled diesel particles is conventionally assumed to be due to mucociliary action, and the remainder (slow phase) to pulmonary clearance. The fraction of lung deposit cleared in the rapid phase ranged from 6% to 75% whereas the slow phase ranged from 25% to 66%. The half-time for clearance in the slow phase ranged from 60 to 80 days and was occasionally dose-dependent (IARC, 1984).

Pulmonary (alveolar) clearance: The pulmonary clearance of diesel particles is much slower than mucociliary clearance, with retention half-times ranging from 60 days to more than 1000 days. In general, half-times are greater after long exposures and for larger particles. With longer half-times, the lung burden can increase linearly over the lifetime of the animal, leading to the 'overload' phenomenon. The clearance rate of insoluble particles following prolonged exposure can be seriously impaired, leading to very long-term retention of material in the lung, usually referred to as 'sequestration'. The majority of particles that are cleared by macrophages from the pulmonary region leave via the ciliated epithelium and are excreted through the gut. However, about one-third of the particles penetrate the lymphatic system, borne by macrophages, and are filtered by the lymph nodes to form aggregates (IARC, 1984). Only the gas phase appears to have no effect on pulmonary clearance in rats or hamsters (Reed *et al.*, 2004).

Retention: Organic compounds adsorbed on engine exhaust particles can be extracted by biological fluids, with a half-time for the slow phase of lung clearance of 18–25 days in rats (IARC, 1984). This phenomenon has been

reviewed (Stöber & McClellan, 1997) and additional data are available (Claxton & Woodall, 2007). In dogs, 37% and 59% of the [^{3}H]benzo[*a*]pyrene originally coated onto diesel particles did not remain on the particles after 5.6 months (Gerde *et al.*, 2001). The retention of several nitroarenes adsorbed onto diesel engine exhaust particles is described in the *Monographs* on those compounds in this Volume.

Metabolism: The metabolism of several components of engine exhausts has been reported previously: some PAHs (IARC, 2010a), formaldehyde (IARC, 1982, 2006a, 2012a), lead (IARC, 1980, 2006b), nitroarenes (IARC, 1989) and benzene (IARC, 1982, 2012a).

Three dogs were exposed to aerosolized [^{3}H]benzo[*a*]pyrene coated onto diesel particles. Blood sampling demonstrated a bi-phasic half-life, with a first half-life for benzo[*a*]pyrene in the blood of 4.3 minutes and a second half-life of 1.8 minutes. Radiolabel appeared in the blood almost immediately after exposure, indicating rapid metabolism, and persisted throughout the 1-hour sampling period (Gerde *et al.*, 2001).

Groups of eight rats were exposed to Standard Reference Material (SRM) 2975 at 0.064 or 0.64 mg/kg body weight (bw) for 6 and 24 hours. Increased levels of 8-OH-dG lesions were observed at the highest dose after 6 and 24 hours in colon, liver, and lung. Elevated levels of *OGG1* mRNA were observed after 24 hours of exposure to both doses of diesel engine exhaust particles in the lung but not in the colon or liver. Elevated levels of *haeme oxygenase 1* (*Hmox-1*) mRNA and bulky DNA were observed in the liver and lung 6 hours (bulky DNA only) and 24 hours after administration (Danielsen *et al.*, 2008).

[^{3}H]Benzo[*a*]pyrene coated onto diesel particles was instilled intratracheally into male Sprague-Dawley rats and the distribution of radioactivity was analysed at selected time-points over 3 days. About 50% of the radioactivity remained in the lungs 3 days after instillation; 30% was excreted in the faeces and the remainder was distributed throughout the organs (Bevan & Ruggio, 1991).

Interspecies comparison of particle retention in the lung

Impairment of clearance leads to an increased retention of particles, which is the hallmark of lung overload. Thus, an understanding of interspecies differences in the mechanisms of particle retention can aid hazard evaluation and risk assessment. Differences in the patterns of particle retention of coal dust or diesel engine exhaust were observed in rats and monkeys exposed by inhalation for 7 hours a day, 5 days a week to 2 mg/m^3 of coal dust and/or diesel engine exhaust particulates for 2 years. A higher volume percentage of coal dust was retained in the alveolar lumen in rats and in the interstitium in monkeys (Nikula *et al.*, 1997a, b). A greater proportion of particles was also retained in the interstitium in humans, because the pattern of retention changed as the duration of exposure and assumed concentration of coal dust increased. In contrast, the pattern of retention in rats did not vary with increasing concentrations of diesel engine exhaust particulates from 0.35 to 7.0 mg/m^3 (Nikula *et al.*, 2001).

One class of insoluble particles—carbon black—has been identified in human lungs, although no quantitative data are available on its retention in humans. However, based on studies with other poorly soluble particulate materials, it can be assumed that the normal retention half-times of particles such as carbon black in humans is longer than that measured in rats and mice. For example, Bailey *et al.* (1985) found that the retention time in humans of inhaled, monodisperse, fused aluminosilicate particles 1 and 4 µm in diameter followed a two-component exponential with phases that had half-times of the order of tens of days and several hundred days, respectively. At 350 days after inhalation, retention of the remaining material averaged 46 ± 1% for the 1-µm particles and 55 ± 11% for the 4-µm particles. In contrast, data in rats (Oberdörster,

1995) and mice (Kreyling, 1990) demonstrated retention half-times of ~70 days and ~55 days, respectively.

Heavy exposure to particles in occupational settings may lead to high particle burdens in the human lung. By analogy to the rat, if the human lung burden exceeds ~0.5–1 mg/g of lung, it is expected that the normal retention half-time would be prolonged. Indeed, there is some evidence that workers in occupations that are associated with high particle burden in the lungs (e.g. coal mining) show increased long-term retention of particles (Stöber *et al.*, 1965; Freedman & Robinson, 1988). Retention half-times of the order of years have been measured in several human studies that involved accidental exposure to radionuclides (ICRP, 1994).

Little is known about overloading in non-rodent species, including humans. The most frequently cited human data come from coal miners, who are one of the best studied occupational cohorts with regard to quantitative exposure–response relationships (Attfield & Kuempel, 2003). Coal miners have historically experienced high rates of occupational lung diseases, including increased morbidity and mortality from pneumoconiosis and chronic obstructive lung diseases (NIOSH, 1995). However, excess mortality from lung cancer has not generally been observed in coal miners (NIOSH, 1995), although, in a more recent study of German coal miners, elevated lung cancer mortality was detected in those who had developed pneumoconiosis (standardized mortality ratio, 1.57), which was independent of the effect of tobacco smoking (Morfeld & Lampert, 2002; Xiao *et al.*, 2012).

Retained lung burdens have also been reported to be relatively high; an average of ~14 mg/g of lung has been observed historically in coal miners in the USA (Kuempel *et al.*, 2001) and the United Kingdom (Tran & Buchanan, 2000). This mean lung burden is comparable with retained mass lung burdens in rats that experienced overload. Because an elevated incidence of lung cancer has not generally been observed in coal miners, and because clear differences in lung defence mechanisms exist between humans and rats (humans are deficient in some of these mechanisms compared with rats), it has been suggested that the rat may not be an appropriate model to predict lung cancer in humans. However, although the mean lung burden is relatively high in coal miners, it is actually lower than the mean lung burdens associated with the excess incidence of lung tumours in rats. For example, in rats chronically exposed to coal dust, mass lung burdens of 24 mg/g of lung tissue were associated with an 11% incidence of lung tumours (versus 0% in unexposed controls) (Martin *et al.*, 1977). In rats exposed to fine-sized titanium dioxide, lung burdens of up to ~35 mg/g were not associated with lung tumours, and increased incidences of lung tumours were observed only in rats with lung burdens greater than ~100 mg/g (approximately 16% in male and female rats, excluding keratinizing cystic squamous cell carcinomas) (Lee *et al.*, 1985a, b, 1986). In female rats that inhaled talc chronically for 2 years, 9 mg of talc/g of lung tissue were not associated with an elevated incidence of lung tumours (0/48, 0%), while an average retained burden of 29 mg/g of lung was associated with an incidence of 13 out of 50 (26%) alveolar/bronchiolar tumours (NTP, 1993).

Based on these chronic inhalation studies in rats exposed to various fine-sized, poorly soluble particles of relatively low toxicity, lung tumours were not observed in rats that had lung burdens similar to those of coal miners. Rats that developed lung tumours following chronic inhalation of these particles had retained mean mass lung burdens that were at least twice as high as those found in coal miners. Thus, the observed lung tumour response in rats and the absence of reported tumours in coal miners when both are exposed chronically to fine-sized, poorly soluble particles, such as coal dust, are somewhat consistent. The surface area of particles may be

a more appropriate dose metric for predicting response; therefore, it is useful to evaluate rat and human responses to particle surface area dose in addition to particle mass dose. In rat lungs, fine and ultrafine particles of similar composition have shown consistent dose–response relationships when the dose is expressed as particle surface area rather than as particle mass. The mean surface area dose of coal dust in the lungs of miners from studies in the USA and United Kingdom can be calculated as 0.1 m^2 of coal dust/g of lung tissue (assuming 7.4 m^2/g of coal dust; Vallyathan *et al.*, 1988; Tran & Buchanan, 2000; Kuempel *et al.*, 2001). In rats, the lowest observed surface area doses associated with elevated incidences of lung tumours (excluding keratinising cystic squamous cell tumours) following chronic inhalation were: 0.18 m^2 of coal dust/g of lung tissue in female rats (assuming 7.4 m^2/g coal dust), with an 11% tumour incidence versus 0% in controls (Martin *et al.*, 1977); 0.58 m^2 of carbon black/g of lung tissue for female rats, with a 7.5% tumour incidence versus 0% in controls (Nikula *et al.*, 1995); 6.9 m^2 of carbon black/g of lung tissue in female rats, with a 28% tumour incidence versus 0.46% in controls (Heinrich *et al.*, 1995); 1.3 m^2 of ultrafine titanium dioxide/g of lung tissue in female rats, with a 19% tumour incidence versus 0.46% in controls (Heinrich *et al.*, 1995); 1.2 m^2 of fine titanium dioxide/g of lung tissue, with a 16% and 17% tumour incidence in male and female rats versus 2% and 0% in male and female controls, respectively (Lee *et al.*, 1985a); and 0.27 m^2 of talc/g of lung tissue in female rats, with a 26% tumour incidence versus 2% in controls (NTP, 1993). These comparisons show that the retained particle surface area dose in coal miners was lower by a factor of approximately 2–70 than that associated with elevated incidences of lung tumours in rats exposed to either fine or ultrafine poorly soluble particles. Thus, using particle surface area as the dose metric, an excess incidence of lung cancer would not necessarily be expected to be observed in coal miners because of their relatively low particle surface area dose compared with that associated with lung tumours in rats.

These comparisons illustrate the importance of using normalized doses to compare responses across species. Furthermore, due to their faster clearance rate, rats do not attain lung burdens that are comparable with those observed in humans who work in dusty jobs (e.g. coal miners) unless they experience overloading of lung clearance.

(ii) Relevance of mechanistic data to assess carcinogenic hazards in humans

To evaluate the appropriateness of the rat as an experimental model to assess the carcinogenic hazards of poorly soluble particles in the lungs of humans, it is useful to evaluate the scientific evidence that allows for comparisons among species with regard to exposure, dose–response and mode of action.

Exposure–dose: Inhaled particles may present a hazard when they are deposited in sufficient quantities (dose) and interact with cells/tissues at responsive target sites along the respiratory tract. The relationship between exposure to particles and inhaled dose is described by the kinetics of particle deposition and clearance, and that retained at or within respiratory tract tissues. Inhaled and deposited particles are cleared from the normal lungs of healthy rats at a faster rate than those from humans. However, at high lung burdens, normal clearance from the rat lung can be impaired and overwhelmed, and, in time, effectively ceases. This phenomenon (termed 'overload') is observed with particles that are poorly soluble and are generally considered to be of low toxicity (Morrow, 1988).

Particle lung burdens observed in humans in some dusty jobs (e.g. coal mining) have sometimes approximated the overload dose in rats. At sufficient concentrations and durations of inhalation, the lungs of rats can accumulate higher levels of particles than the lung burdens seen in most workers. For ultrafine particles, the attained

mass doses associated with impaired clearance in rodents approximate those that could occur in workers. For any experimental model used for hazard assessment in humans or to evaluate dose–response relationships, it is important to evaluate doses in experimental animals that are comparable with those that may occur in humans.

Lung clearance can be impaired in humans and experimental animals for many reasons. In humans, toxic gases and particles have been shown to impair clearance by affecting normal cilia function, mucus rheology and phagocytosis. Ultrafine particles may be cleared less effectively than larger particles due to impaired phagocytosis (Renwick *et al.*, 2001, 2004; Geiser *et al.*, 2005).

Much more is known about overload in rats than in humans. Overwhelmed or impaired clearance in rats has been postulated to be a pivotal factor in the development of lung overload (Morrow, 1988). The same factors that can interfere with clearance in rats may contribute to mass dose accumulation in humans (e.g. the cytotoxicity of the material and/or ineffective phagocytosis). Overload was originally described in terms of mass- or volume-based dose. For fine and ultrafine carbon black and titanium dioxide, surface area dose has been shown to be a better predictor of impaired clearance (Oberdörster & Yu, 1990; Oberdörster *et al.*, 1992; Oberdörster, 1996; Tran *et al.*, 1999). Impaired clearance and overload are not unique to rats, and can also occur in other species, although to different degrees. For example, overload has not been observed in hamsters at concentrations at which it readily occurs in rats and mice (Bermudez *et al.*, 2002, 2004; Elder *et al.*, 2005). Although the behaviour of human lung clearance under similar circumstances is unclear, by analogy to coal workers, impairment of clearance occurs after chronic exposure and often persists long after the cessation of exposure (Freedman & Robinson, 1988).

Rats chronically exposed to sufficiently high concentrations of poorly soluble particles experience a steady reduction in their alveolar clearance rates and an accumulation of particles in the alveolar lumen and interstitium (Ferin *et al.*, 1992; Ferin, 1994; Warheit *et al.*, 1997; Bermudez *et al.*, 2002, 2004), and ultrafine particles translocate to the interstitium of rodents to a greater extent than fine particles (Ferin *et al.*, 1992; Oberdörster, 1996). In studies that compared the patterns of particle retention in the lungs of rats, monkeys and humans exposed to coal dust and/or diesel engine exhaust, the largest volume percentage of dust was observed in the alveolar lumen in rats and in the interstitium of monkeys and humans (Nikula *et al.*, 1997a, b, 2001). No data were available to compare the doses retained in the specific regions of the lung of each species. The biological significance of the interstitial/luminal distribution in the development of overload and toxic sequelae is not clear, either within a given species or among species.

Dose–response and mode of action: After continued inhalation of high concentrations of particles, rats that achieve overload may develop pulmonary fibrosis and both benign and malignant tumours (Lee *et al.*, 1985a, b, 1986; Warheit *et al.*, 1997). Oberdörster (1996, 2002) proposed that the high-dose effects observed in rats may be associated with two thresholds: (i) a pulmonary dose that results in a reduced macrophage-mediated clearance leading to overload; and (ii) a higher dose associated with overload, at which normal antioxidant defences within the lung are overwhelmed and pulmonary tumours may be induced.

As discussed above, the series of events that are involved in the biological process that begins with some particle deposition at critical target cells or tissues within the rat lung and results in rat lung tumours include: sustained inflammation, production of ROS, depletion of antioxidants and/or impairment of other defence mechanisms, cell proliferation and gene

mutations. These individual steps comprise an overall mode of action that can be used to compare the responses of rats with those of other species, including humans (see Fig. 4.3).

When the particle mass lung burden reaches a level at which overload is observed in rats (estimated to begin at ~0.5 mg/g of lung and to be fully developed at ~10 mg/g of lung; Muhle *et al.*, 1990b), a sustained and widespread cellular inflammatory response occurs. The degree of sustained inflammation experienced by rodents (most notably rats) with high lung burdens is not observed in humans, although humans may experience sustained inflammation during certain diseases.

Interspecies extrapolation: Several studies have shown that rats, but not mice or hamsters, develop an excess incidence of lung cancer after exposure to chronic 'overloading' doses of inhaled poorly soluble particles. Several studies have discussed this phenomenon and the challenges it poses for the extrapolation of chronic effects in rats to the human situation (Morrow, 1994; Levy, 1995; Oberdörster, 1995, 2002; Watson & Valberg, 1996; ILSI Risk Science Institute Workshop Participants, 2000; Miller, 2000; Hext *et al.*, 2005).

Uncertainty remains with regard to the detailed identification of the series of events that lead to lung cancer in rats following inhalation of poorly soluble particles (i.e. talc, carbon black and titanium dioxide). However, as shown in Fig. 4.3, several important steps can be identified that are supported by a substantial rodent database. An important question that needs to be addressed is the extent to which the steps outlined in Fig. 4.3 for rat lung cancer are also operative in other animal species, including humans. The majority of animal studies that have evaluated the effects of poorly soluble particles on the respiratory tract have been conducted in rats. It is therefore necessary to consider species differences, such as particle inhalability, breathing conditions, respiratory tract structure and pulmonary defences, when extrapolating toxicological findings from rodents to humans (Brown *et al.*, 2005).

All animal species that are routinely used in particle toxicology, as well as humans, are susceptible to the impairment of clearance of poorly soluble particles from the lungs. Impaired clearance is probably one of the first steps necessary to initiate a sequence of events that may lead to lung cancer in rats (see Fig. 4.3). Importantly, however, various animal species exhibit differences in particle-induced impairment of clearance, which can result in diverse lung burdens (expressed as mass or surface area) following exposures to the same particle concentrations. Similarly, pulmonary inflammation has been reported to be a consequence of exposures to poorly soluble particles in both experimental animals and humans. The pathophysiology of particle-induced fibrosis in humans and fibrosis and lung cancer in rats from lung overload involves chronic inflammation, hyperplasia and cell proliferation, and altered deposition and architecture of collagen.

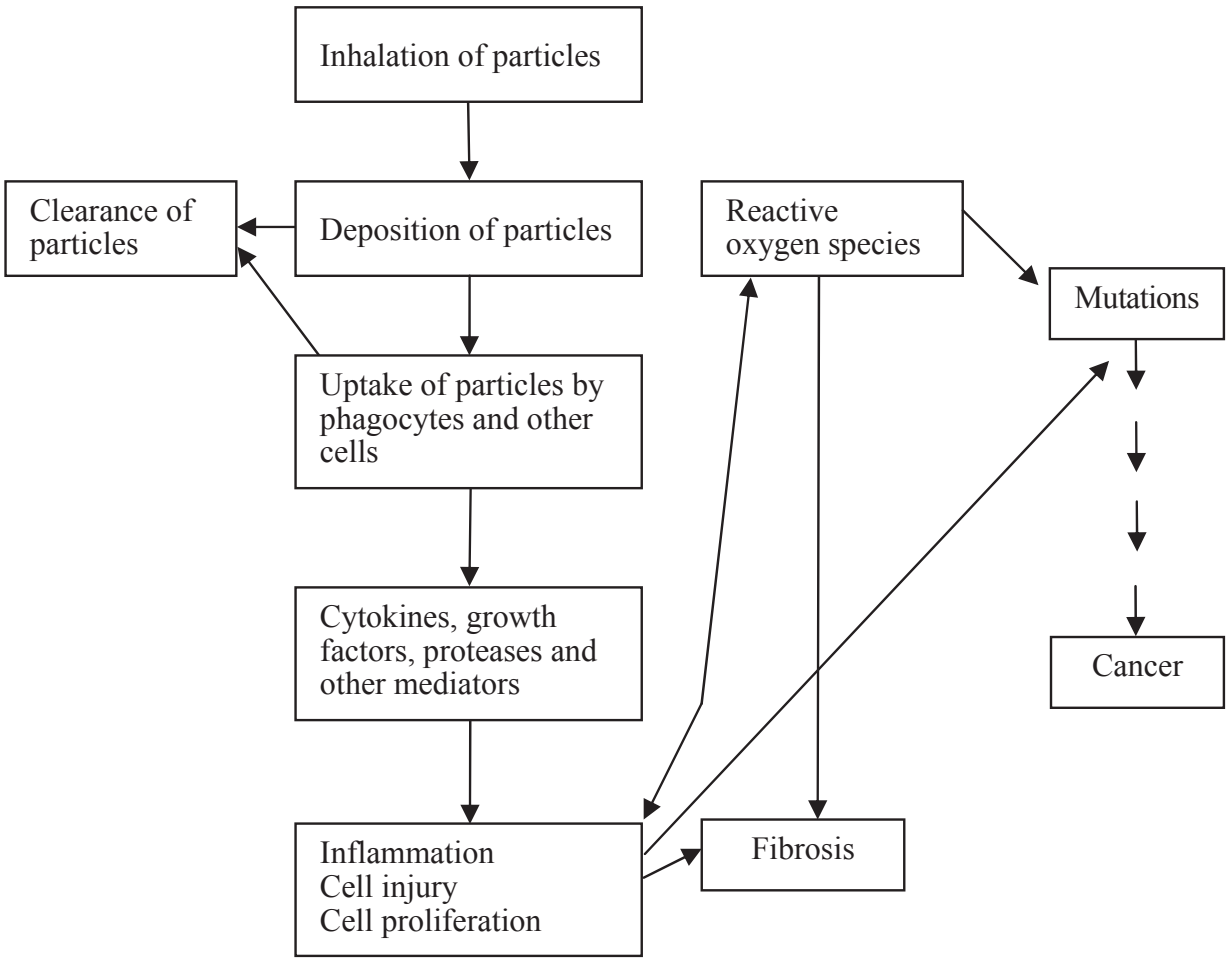
Rats and mice, in contrast to hamsters, exhibit sustained inflammation associated with particle lung burden, but lung tumours induced by poorly soluble particles have only been observed in rats. Rats have been shown to be uniquely susceptible to particle-induced lung cancer in comparison with mice and hamsters. Although some of the steps indicated in Fig. 4.3 have been demonstrated in humans exposed to poorly soluble particles, it is not known to what extent humans are susceptible to particle-induced lung cancers.

(b) Gasoline engine exhaust

(i) Deposition

Limited evidence has suggested that the deposition rate of gasoline engine exhaust particles in rat lungs is 30% (IARC, 1989).

Fig. 4.3 Conceptual framework of carcinogenesis induced by poorly soluble particles in rats



The scheme represents the sequence of events and modes of action that are considered to be involved in the formation of tumours that are observed in the lungs of rats after high exposure to poorly soluble particles (see text for further details).

(ii) Clearance

A marginal increase in the half-time for pulmonary clearance of ferric oxide by rats but not hamsters was observed following exposure to gasoline engine particles (IARC, 1989).

(iii) Metabolism

The major DNA adduct derived from benzo[*a*] pyrene-7,8-dihydrodiol-9,10-epoxide was found in male BALB/c mice following topical application of a crude extract of gasoline engine exhaust over a period of 1–2 weeks (IARC, 1989).

Blood samples from Fischer rats and Syrian golden hamsters exposed to different dilutions of gasoline engine exhaust for 6–24 months were analysed for levels of 2-hydroxyethylvaline and 2-hydroxypropylvaline in haemoglobin. A dose-dependent increase in the level of haemoglobin adducts, corresponding to the metabolic conversion of ~5–10% of inhaled ethylene and propylene to ethylene oxide and propylene oxide, respectively, was observed (IARC, 1989).

Table 4.1 Summary of genetic and related effects of atmospheres containing engine exhaust (diesel only) in humans

End-point	Exposure	Result	Association with exposure		Reference
			Urine	Air	
Oxidative stress, inflammation (gene expression)	2-h exposure to diesel, 200 μg/m³ $PM_{2.5}$, blood taken 6 h after start of exposure	↑	–	$PM_{2.5}$ +	Peretz *et al.* (2007)
Oxidative stress, protein degradation, coagulation (gene expression)	2 × 1 h exposures to diesel, 300 μg/m³ $PM_{2.5}$, blood taken 24 h after each exposure	↑	–	$PM_{2.5}$ +	Pettit *et al.* (2012)
IL-8 gene, IL-8 and GRO-α protein	1-h exposure to diesel, 300 μg/m³ PM_{10}, lavage 6 h after end of exposure	↑	–	PM_{10} +	Salvi *et al.* (2000)

GRO, growth-regulated oncogene; h, hour, IL, interleukin; PM, particulate matter; ↑, increase

4.3 Genetic and related effects

4.3.1 Humans

Various studies have identified chromosomal damage, such as micronuclei, sister chromatid exchange and chromosomal aberrations, as well as DNA adducts and oxidative damage in traffic policemen, gas station attendants and other workers exposed to traffic exhaust. Such workers are occupationally exposed to diesel and gasoline engine exhausts, but the Working Group noted that they were also exposed to ambient air that may have been especially high in engine exhaust content. Thus, the results of these studies must be viewed within that context. Only three studies involved exposure solely to engine (diesel) exhaust. Nevertheless, most of the studies found an adverse effect of exposure to engine exhaust through ambient air, and approximately half of the studies incorporated measurements of internal (urinary 1-hydroxypyrene) or external (e.g. PAHs in the air) exposure, most of which also showed an association between exposure and effect.

Fewer than half of the studies included a measure of either internal or external exposure, but the majority of these and most studies that provided exposure assessments found that individuals with chronic exposure to engine exhaust had increased frequencies of a variety of genotoxic biomarkers, and the latter also showed an association between the measured biomarkers and exposure (see Tables 4.1 and 4.2). Positive associations between biomarkers of exposure and genetic effects were also observed in the majority of the studies across all three exposure categories.

(a) Diesel only

See Table 4.1

Changes in gene expression

An experimental study in which five subjects were exposed for 2 hours to 200 μg/m³ of $PM_{2.5}$ from diesel engine exhaust (derived from a 2002 model turbocharged direct-injection 5.9-L Cummins B-series engine), blood samples were taken 6 hours after the initiation of the exposure and showed changes in the expression of genes involved in oxidative stress, inflammation, leukocyte activation and vascular homeostasis, which were associated with the levels of $PM_{2.5}$ in the air (Peretz *et al.*, 2007). In another experimental study (Pettit *et al.*, 2012), 14 healthy subjects were exposed to 300 μg/m³ of $PM_{2.5}$ from diesel engine exhaust (derived from a 5500-W Yanmar electricity generator, with a 406-ml displacement

Table 4.2 Summary of genetic and related effects of atmospheres containing engine exhaust (predominantly diesel) in humans

End-point	Exposure group	Result	Association with exposure		Reference
			Urine	Air	
DNA adducts	Bus/automobile garage workers	+			Hemminki *et al.* (1994b)
		+			Hou *et al.* (1995)
		+	OHPy –		Nielsen *et al.* (1996a)
		–			Schoket *et al.* (1999)
	Diesel vehicle drivers in a mine	–		1-NP –	Knudsen *et al.* (2005)
DNA damage	Diesel vehicle drivers in a mine	+		1-NP +	Knudsen *et al.* (2005)
	Road tunnel construction workers	–			Villarini *et al.* (2008)
Hprt mutant frequency	Bus maintenance workers	–			Hou *et al.* (1995)
Hprt spectra		+			Österholm *et al.* (1995)
Urinary mutagenicity	Railroad workers	–		PM –	Schenker *et al.* (1992)
Urinary/faecal mutagenicity	Car mechanics	–		PM –	Willems *et al.* (1989)
Sister chromatid exchange	Tunnel construction workers	–			Villarini *et al.* (2008)
MN buccal lymph		+	OHPy +	PAH +	Schoket *et al.* (1999)
MN lymph	Train mechanics	+			Lu *et al.* (1999a)
	Road tunnel construction workers	+			Villarini *et al.* (2008)

HPRT, hypoxanthine-guanine phosphoribosyltransferase; MN, micronuclei; 1-NP, 1-nitropyrene; OHPy, 1-hydroxypyrene; PAH, polycyclic aromatic hydrocarbon; PM, particulate matter; +, positive; –, negative

air-cooled engine) for 1 hour a day, during 2 days at an interval of at least 1 week (total exposure, 2 hours). Blood was drawn 24 hours after each exposure for the analysis of gene expression, which indicated that genes involved in oxidative stress, protein degradation and coagulation pathways were differentially expressed. Thus, both experimental studies found that exposure to engine exhausts caused alterations in the expression of genes involved in oxidative stress.

Salvi *et al.* (2000) exposed 15 healthy subjects to 300 μg/m^3 of PM_{10} from diesel engine exhaust (derived from an idling Volvo diesel engine) for 1 hour. Bronchial lavage was performed 6 hours after the end of the exposure, and the expression of selected gene and protein was assessed. Upregulation of the interleukin (IL)-8 gene, IL-8 protein and growth-regulated oncogene-α protein was observed and provided evidence of an inflammatory response due to exposure to diesel engine exhaust.

(b) Predominantly diesel

See Table 4.2

(i) DNA adducts

The levels of DNA adducts determined in lymphocytes by postlabelling were elevated in bus and truck terminal workers compared with hospital mechanics who were used as a control. The highest levels of adducts were found for garage workers among the bus maintenance workers and for those who drove diesel forklifts among the terminal workers (Hemminki *et al.*, 1994b). Another study also found elevated levels of DNA adducts (determined by postlabelling in lymphocytes) in bus maintenance workers exposed to diesel engine exhaust compared with controls (Hou *et al.*, 1995).

Workers and mechanics in a diesel bus garage had elevated levels of DNA adducts (determined in lymphocytes by postlabelling) compared with administrative workers in the same facility. Elevated levels of hydroxyethylvaline adducts in haemoglobin were also found among the garage workers compared with the controls and were associated with the concentrations of 1-hydroxypyrene in the urine, whereas the levels of DNA adducts were not. Postlabelling was the most sensitive assay, and the authors noted that dermal exposure to oils and grease might be an important route of exposure to PAHs and other chemical classes in addition to the inhalation of diesel engine exhaust (Nielsen *et al.*, 1996a). A study of garage mechanics in another country did not find an increase in DNA adducts (determined by postlabelling in lymphocytes; Schoket *et al.*, 1999).

The level of 1-nitropyrene–DNA adducts (determined by HPLC of postlabelled lymphocytes) was not higher in shale-oil miners who drove diesel-powered excavation machines in the mine than in surface workers (Knudsen *et al.*, 2005).

(ii) DNA damage

A study of shale-oil mine workers exposed to diesel engine exhaust found that DNA damage, as measured by the comet assay in lymphocytes, was higher among smoking miners who drove diesel-powered excavation machines compared with smoking surface workers, and the levels of DNA damage were associated with the levels of 1-nitropyrene in the air. A decrease in DNA-repair capacity (as determined by the cytogenetic challenge assay; i.e. an increase in dicentrics and deletions) was also observed, and a relationship between the increase in DNA damage, the decrease in DNA-repair capacity, the levels of 1-hydroxypyrene in the urine and the levels of PAHs in the air was established (Knudsen *et al.*, 2005). A study of road tunnel construction workers in Genoa, Italy, found no increase in DNA damage (as measured by the comet assay in lymphocytes) compared with office controls (Villarini *et al.*, 2008).

(iii) Mutagenicity

No significant difference in mutant frequency at the *Hprt* gene (assessed in lymphocytes) was found between bus maintenance workers exposed to diesel engine exhaust and controls (Hou *et al.*, 1995), whereas splice mutations were more frequent among the garage workers compared with controls (Österholm *et al.*, 1995).

No increase in urinary mutagenicity in *Salmonella typhimurium* TA98 in the presence of an exogenous metabolic activation system was found among a population of railroad workers with a wide range of exposures to diesel engine exhaust (Schenker *et al.*, 1992). Similarly, no increase in either urinary or faecal mutagenicity in *S. typhimurium* TA98 in the presence of an exogenous metabolic activation system was found among a group of car mechanics exposed to high concentrations of diesel engine exhaust compared with a group of office workers (Willems *et al.*, 1989).

(iv) Chromosomal effects

A study in Genoa, Italy, did not find an increased frequency of sister chromatid exchange in the lymphocytes of road tunnel construction workers compared with office controls (Villarini *et al.*, 2008).

Garage mechanics in Budapest, Hungary, had elevated frequencies of micronuclei in their lymphocytes, which correlated with the levels of 1-hydroxypyrene in the urine and PAHs in the air (Schoket *et al.*, 1999). Increased frequencies of micronuclei were found in diesel train attendants and wheel axle workers in Henan, China (Lu *et al.*, 1999a), as well as in road tunnel construction workers in Genoa, Italy (Villarini *et al.*, 2008).

(c) Mixed exposures

See Table 4.3

(i) DNA adducts

An early study of adducts in the DNA and haemoglobin of bus and taxi drivers in Stockholm, Sweden, found elevated levels of DNA adducts (by postlabelling in lymphocytes) in suburban bus drivers and taxi drivers (covering a mixed route) but not in city bus drivers compared with controls from a mechanical workshop in a hospital (Hemminki *et al.*, 1994a).

Taxi drivers also had elevated levels of PAH–plasma protein adducts determined by an immunoassay. Alkylvaline levels in the haemoglobin of urban bus drivers did not differ significantly from those of the controls. A study of bus drivers in Copenhagen, Denmark, found significantly elevated levels of DNA adducts (by postlabelling in lymphocytes) in drivers in central and suburban Copenhagen compared with rural controls, and drivers in central Copenhagen had more adducts than suburban drivers (Nielsen *et al.*, 1996b).

Traffic police in Genoa, Italy, had significantly higher levels of DNA adducts (by postlabelling in lymphocytes) compared with office workers in the same city; however, the levels of DNA adducts were not associated with the levels of benzo[*a*]pyrene in the air (Merlo *et al.*, 1997). A similar study of traffic police in Genoa showed that the increased levels of adducts were related to seasonal variations, with higher levels occurring in the summer, suggesting a role of atmospheric transformation products, and were associated with the levels of benzo[*a*]pyrene in the air (Peluso *et al.*, 1998). Traffic police in Prague, Czech Republic, had levels of benzo[*a*]pyrene-like DNA adducts (determined by postlabelling in lymphocytes) that correlated with the levels of carcinogenic PAHs in the air determined from personal samplers (Topinka *et al.*, 2007).

In Milan, Italy, newspaper vendors working < 50 m from streets with high traffic flow (> 1300 vehicles/hour) did not have elevated levels of DNA adducts (by postlabelling in lymphocytes) compared with those working along streets with low traffic flow (< 1300 vehicles/hour) (Yang *et al.*, 1996).

(ii) DNA damage

A study by Kim *et al.* (2004) showed that DNA damage (assessed as Olive tail moment by the comet assay in lymphocytes) was greater among automobile emission inspectors than control subjects, and the levels were associated with the urinary markers of exposure (1-hydroxypyrene and 2-naphthol). DNA damage (measured by the comet assay; both percentage of cells damaged and Olive tail moment) in lymphocytes was greater in traffic police than in resident controls in Shanghai, China, and was associated with the level of exposure to $PM_{2.5}$ determined by personal monitors (Li *et al.*, 2010).

An increase in urinary 1-hydroxypyrene and DNA damage (as measured by the comet assay in lymphocytes) was found in children at a school located near areas of high-density traffic in Bangkok, Thailand, compared with children at a school in the provincial area of Chonburi (Tuntawiroon *et al.*, 2007).

Urinary excretion of 8-OH-dG, which is a biomarker of oxidative DNA damage, was increased among bus drivers from central Copenhagen, Denmark, compared with drivers from suburban/rural areas around Copenhagen (Loft *et al.*, 1999), and was increased threefold in nonsmoking security guards who worked at an entrance gate by a road with heavy traffic following an 8-hour work shift. The latter increase in DNA damage was associated with the concentration of PAHs, $PM_{2.5}$ and metals, but not polar organics, in the air at the site (Wei *et al.*, 2009).

An analysis of lung tissue from nonsmokers with lung cancer found carbon particles that were morphologically similar to those of diesel engine exhaust-like particles; the mass of particles and the concentration of 8-OH-dG in the lung DNA

Table 4.3 Summary of genetic and related effects of atmospheres containing engine exhaust (mixed) in humans

End-point	Exposure group	Result	Association with exposure		Reference
			Urine	Air	
DNA adducts	Bus/taxi drivers	+			Hemminki *et al.* (1994a)
	Bus drivers	+			Nielsen *et al.* (1996b)
	Traffic police	+		B[*a*]P +	Peluso *et al.* (1998)
		+		PAH +	Topinka *et al.* (2007)
		+		B[*a*]P –	Merlo *et al.* (1997)
	Newspaper vendors (street)	–			Yang *et al.* (1996)
DNA damage	Auto-emission inspectors	+	OHPy + 2Naph +		Kim *et al.* (2004)
	Traffic police	+		$PM_{2.5}$ +	Li *et al.* (2010)
	Children in school (high traffic density area)	+	OHPy +		Tuntawiroon *et al.* (2007)
8-OH-dG	Bus drivers	+			Loft *et al.* (1999)
	Security guards	+		$PM_{2.5}$ + PAH + Metals +	Wei *et al.* (2009)
	Cancer patients	+			Tokiwa *et al.* (2005)
Oxidative damage	Bicycling in traffic	+		PM_{10} +	Vinzents *et al.* (2005)
	Traffic police	+			Yang *et al.* (2005)
	Airport personnel	+	OHPy +	PAH +	Cavallo *et al.* (2006)
Urinary mutagenicity	Bus drivers	+	OHPy –		Hansen *et al.* (2004)
	Cancer patients	+	1-NP +		Tokiwa *et al.* (1999)
Chromosomal aberrations	Traffic police	+			Anwar & Kamal (1988)
		+			Burgaz *et al.* (2002)
		+			Chen *et al.* (1999)
		+			Fu *et al.* (1999)
		+			Luo & Shi (1994)
		+			Sree Devi *et al.* (2009)
	Taxi drivers	+	OHPy +		Burgaz *et al.* (2002)
	Gas station attendants	+			Santos-Mello & Cavalcante (1992)

Table 4.3 (continued)

End-point	Exposure group	Result	Association with exposure		Reference
			Urine	Air	
Sister chromatid exchange	Petrochemical workers	+			Sobti & Bhardwaj (1993)
	Airport personnel	+	OHPy +	PAH +	Cavallo *et al.* (2006)
	Traffic police	+			Anwar & Kamal (1988)
		+			Chandrasekaran *et al.* (1996)
		+			Zhao *et al.* (1998)
		+			Sreedevi *et al.* (2006)
		+			Soogarun *et al.* (2006)
		+			Anbazhagan *et al.* (2010)
		–		B[*a*]P –	Bolognesi *et al.* (1997a)
	Airport personnel	+	OHPy +	PAH +	Cavallo *et al.* (2006)
MN buccal	Traffic police	+			Karahalil *et al.* (1999)
		+			Hallare *et al.*(2009)
	Gas station attendants	+			Hallare *et al.* (2009)
		+			Sellappa *et al.* (2010)
	Auto mechanics	+			Karahalil *et al.* (1999)
	Taxi drivers	+			Karahalil *et al.* (1999)
	Firebreathers	–			Torres-Bugarín *et al.* (1998)
Gene expression	Auto-emission inspection workers	↑	OHPy + 2Napth +		Kim *et al.* (2004)

B[*a*]P, benzo[*a*]pyrene; MN, micronuclei; 1-NP, 1-nitropyrene; 2Naph, 2-naphtol; 8-OH-dG, 8-hydroxy-2′-deoxyguanosine; OHPy, 1-hydroxypyrene; PAH, polycyclic aromatic hydrocarbon; PM, particulate matter; +, positive; –, negative; ↑, increase

of cancerous tissue, but not of the adjacent non-cancerous tissue, increased with the age of the patient. The lung particles were stripped of organic compounds and induced the formation of 8-OH-dG when administered to mice. The authors suggested that the 8-OH-dG found in the lung DNA of cancer patients was formed by the generation of hydroxyl radicals that were produced after inflammatory cells phagocytized non-mutagenic particles; mutation leading to lung cancer might then have been induced subsequently by unrepaired 8-OH-dG (Tokiwa *et al.*, 2005).

An evaluation of subjects who cycled in traffic in Copenhagen, Denmark, with personal sampling monitors found an association between the concentration of ultrafine particles (PM_{10}) and purine oxidation (oxidative damage) in their lymphocytes (determined by the comet assay). However, no association was found between PM_{10} and DNA strand breaks (also determined by the comet assay; Vinzents *et al.*, 2005). Increased oxidative damage was found among traffic police in Henan Province, China, as assessed by the levels of superoxide dismutase and malondialdehyde in blood serum (Yang *et al.*, 2005).

Outdoor airport workers in Rome, Italy, had higher levels of urinary 1-hydroxypyrene than airport office workers, as well as increased levels of oxidative and direct DNA damage (as measured by the comet assay) in both buccal cells and lymphocytes. An association was also found between these two biomarkers and the level of PAHs in the air (Cavallo *et al.*, 2006).

(iii) Mutagenicity

The urine of bus drivers in central Copenhagen, Denmark, showed higher levels of mutagenicity in the presence of an exogenous metabolic activation system in *S. typhimurium* YG1021 (a frameshift strain that expresses excess nitroreductase) than mail carriers, but these were not associated with levels of urinary 1-hydroxypyrene (Hansen *et al.*, 2004).

A unique *ex-vivo* study of tissues from lung cancer patients found that extracts of lung tissue were mutagenic in the *Salmonella* mutagenicity assay in a strain that responds to ROS. A simultaneous analysis suggested that the deposition of particles in the tissue correlated with the mutagenicity of the extracts. In addition, 1-nitropyrene, which may be indicative of exposure to diesel engine exhaust, was present in all of the tissues (Tokiwa *et al.*, 1999).

(iv) Chromosomal effects

Increased frequencies of chromosomal aberrations have been found in the lymphocytes of traffic policemen in Cairo, Egypt (Anwar & Kamal, 1988), Shanghai District (Luo & Shi, 1994) and Henan Province (Chen *et al.*, 1999; Fu *et al.*, 1999), China, Ankara, Turkey (Burgaz *et al.*, 2002) and Hyderabad, India (Sree Devi *et al.*, 2009) in comparison with controls. Elevated chromosomal aberrations frequencies have also been found among gas station attendants in Rio de Janeiro and São Paulo, Brazil (Santos-Mello & Cavalcante, 1992), petrochemical workers from scooter markets in Chandigarh, India (Sobti & Bhardwaj, 1993), taxi drivers in Ankara, Turkey (Burgaz *et al.*, 2002), and outdoor airport workers in Rome, Italy (Cavallo *et al.*, 2006). The increase in chromosomal aberrations in the taxi drivers in Turkey was associated with their urinary levels of 1-hydroxypyrene, and that in airport workers in Italy were associated with the levels of both urinary 1-hydroxypyrene and PAHs in the air.

Increased frequencies of sister chromatid exchange compared with controls have been found in the lymphocytes of traffic policemen in Cairo, Egypt (Anwar & Kamal, 1988), Madras, India (Chandrasekaran *et al.*, 1996), Lanzhou, China (Zhao *et al.*, 1998), Bangkok, Thailand (Soogarun *et al.*, 2006), and Hyderabad (Sreedevi *et al.*, 2006) and Chennai city (Anbazhagan *et al.*, 2010), India. An increase in sister chromatid exchange was also found among outdoor airport workers compared with office workers in Rome,

Italy, and was associated with increased levels of urinary 1-hydroxypyrene and PAHs in the air (Cavallo *et al.*, 2006). No increase in sister chromatid exchange was found among traffic policemen in Genoa, Italy (Bolognesi *et al.*, 1997a).

Increased frequencies of micronuclei have been found in the buccal cells of engine repair workers, taxi drivers and traffic police in Ankara, Turkey (Karahalil *et al.*, 1999), gasoline station attendants and traffic police in Manila, the Philippines (Hallare *et al.*, 2009), and gasoline station attendants in Coimbatore, India (Sellappa *et al.*, 2010). Although increased frequencies of micronuclei were found in the lymphocytes of traffic policemen in Lanzhou, China (Zhao *et al.*, 1998), diesel train attendants and wheel axle workers in Henan, China (Lu *et al.*, 1999a), and road tunnel construction workers in Genoa, Italy (Villarini *et al.*, 2008), no increase was found among traffic police in Genoa, Italy (Bolognesi *et al.*, 1997b).

No increase in micronuclei in either buccal cells or lymphocytes was found among outdoor airport workers compared with office workers in Rome, Italy (Cavallo *et al.*, 2006), or in buccal-cell micronuclei among 'firebreathers' who used diesel fuel (Torres-Bugarín *et al.*, 1998).

(v) Changes in gene expression

Examination of changes in global gene expression in the lymphocytes and proteomic changes in the blood plasma of automobile emission inspectors in Seoul, Republic of Korea, revealed the upregulation of genes and proteins involved in oxidative stress. The upregulated genes included *integrin-linked kinase, CYP2F1, CYP2D6, IL-1 receptor-associated kinase* and *antioxidant protein 2*. Two proteins in particular were upregulated (transthyretin and sarcolectin) and one was downregulated (haptoglobin-1). The changes in both gene and protein expression were associated with the levels of 1-hydroxypyrene and 2-naphthol in the urine (Kim *et al.*, 2004).

4.3.2 Experimental systems

(a) Introduction and brief summary of IARC Monographs Volume 46

Before the publication of Volume 46 (IARC, 1989), several comprehensive reviews had provided excellent summaries regarding the genotoxic activity of emissions released by diesel- and gasoline-driven internal combustion engines (e.g. Claxton, 1983; Lewtas *et al.*, 1981; Lewtas, 1983; Lewtas & Williams, 1986). IARC (1989) noted that the soluble organic fraction of PM from mobile-source emissions readily induced mutations in the *S. typhimurium* reverse mutation assay. *S. typhimurium* TA98 and TA100 were the most responsive strains, and the mutagenic activity was generally higher in the absence of a mammalian metabolic activation system (e.g. from induced rat liver). Chemical fractionation of PM extracts and analysis of the mutagenicity of these fractions with the nitroreductase-deficient *S. typhimurium* strain TA98 led to the recognition that nitroarenes are major contributors to the mutagenic activity of diesel engine exhaust particulates. Examination of the factors that modify the mutagenicity of mobile-source emissions revealed that fuel formulation, type and degree of combustion, ambient environmental conditions and the methods of sample collection can all influence the mutagenic activity of the soluble organic fraction of mobile-source PM. Briefly, the mutagenic activity of the soluble organic fractions from mobile-source PM samples was shown to increase with increasing aromatic content of the fuel, and to vary across engine types and between runs with the same engine. The mutagenic activity of organic extracts from gasoline-derived PM was found to be similar to that of diesel engine exhaust particles. However, when differences in PM emission levels between gasoline and diesel vehicles were taken into account, the mutagenic activity of diesel PM (i.e. per unit mass or per mile driven) was found to be much greater than that of gasoline PM.

IARC (1989) included an overview of several comprehensive studies by Lewtas and colleagues. These authors examined the mutagenic activity of exhausts from several diesel and spark-ignition (gasoline) engines in numerous *in vitro* assay systems including the *Salmonella* reverse mutation assay, the WP2 mutagenicity assay in *Escherichia coli*, the thymidine kinase ($Tk^+/-$) gene mutation assay in L5178Y mouse lymphoma cells, the ouabain-resistance mutagenicity assay in BALB/c 3T3 cells, the *Hprt* gene mutation assay in Chinese hamster ovary cells, the mitotic recombination assay in *Saccharomyces cerevisiae* strain D3, the DNA strand-break assay (assessed by means of alkaline elution) in Syrian hamster embryo cells, the test for unscheduled DNA synthesis in primary rat hepatocytes, sister chromatid exchange and chromosomal aberration assays in Chinese hamster ovary cells and the chromosomal aberration assay in cultured human lymphocytes. The *Salmonella* mutagenicity results showed that mutagenic activity per unit extractable organic matter (EOM) varied across engine types with no appreciable difference between gasoline- and diesel-derived PM samples; three out of four diesel-derived samples were more active in the presence of exogenous metabolic activation. IARC (1989) also noted that particle emission levels (per mile driven) from diesel vehicles can be 100-fold higher than those of gasoline vehicles, yielding mutagenic activities that are 45–800-fold higher. The results of the assays in mammalian cells indicated that organic PM extracts from both gasoline and diesel vehicles can induce DNA strand breaks, gene mutations and chromosomal damage. However, the test outcome and the magnitude of the response were dependent on engine type and the end-point examined. Several samples induced significant increases in $Tk^+/-$ mutations in mouse lymphoma cells; the diesel-derived material was more potent in the absence of exogenous metabolic activation and gasoline-derived samples giving a stronger response in the presence of metabolic activation. In the presence of metabolic activation, the gasoline samples were more potent than the diesel samples. A similar pattern was observed for the ouabain-resistance assay. PM extracts from two out of four diesel engines induced significant increases in *Hprt* mutations in Chinese hamster ovary cells, whereas gasoline-derived samples did not. DNA-damage assays indicated that one gasoline emission sample elicited a significant increase in the number of DNA strand breaks in Syrian hamster embryo cells. Only one diesel sample induced a significant increase in unscheduled DNA synthesis in primary rat hepatocytes. Assays for chromosomal effects indicated that both diesel- and gasoline-derived samples induced a significant increase in sister chromatid exchange; this effect diminished in the presence of exogenous metabolic activation. Only two diesel samples were examined for their ability to induce chromosomal aberrations: one induced a significant increase in aberrations in Chinese hamster ovary cells and the other had a similar effect in cultured human lymphocytes. In the absence of exogenous metabolic activation, two out of three extracts of diesel-derived PM induced a significant increase in mitotic recombination in *Saccharomyces cerevisiae*, while the third sample was weakly positive. The sole extract of gasoline-derived PM that was tested failed to induce mitotic recombination in this assay. Additional analyses showed that diesel PM extracts did not induce gene mutations or gene conversion at the *Trp-6* and *Ilv1–92* loci in *Saccharomyces cerevisiae* strain D7.

IARC (1989) also summarized the results of *in-vivo* assays to assess the mutagenicity of diesel engine emissions. Whole emissions and gaseous emission fractions (i.e. filtered exhaust) induced chromosomal damage and stamen-hair mutations in *Tradescantia*, but failed to induce sex-linked recessive lethal mutations in *Drosophila melanogaster*. Experiments with mice exposed *in vivo* indicated that whole emissions failed to induce chromosomal damage or

sister chromatid exchange in bone marrow, or specific locus mutations. In addition, the dominant lethal and heritable translocation assays in mice showed that, under the conditions of the test, whole emissions did not induce a significant increase in mutation or chromosomal damage. However, extractable organic compounds from diesel engine exhaust particles did induce significant increases in chromosomal damage in mouse bone marrow. *In-vivo* experiments with Syrian or Chinese hamsters indicated that whole emissions increase the frequency of sister chromatid exchange in lung cells, but did not induce sister chromatid exchange in fetal liver or chromosomal damage in bone marrow. In contrast, extractable organic compounds induced a weak increase in chromosomal damage in bone marrow, and significant increases in sister chromatid exchange in lung and fetal liver cells.

These studies indicated that the extractable organic fraction of particulate material released by both diesel and gasoline engine emissions can induce a variety of genetic effects (e.g. DNA damage, gene mutations, sister chromatid exchange, chromosomal aberrations and mitotic recombination) in a wide range of experimental systems *in vitro* and *in vivo* (e.g. bacteria, yeast, cultured mammalian cells and experimental animals). Although the magnitude of the induced effects varied with assay type (i.e. *in vitro* versus *in vivo*), end-point (i.e. strand breaks, mutations and chromosomal damage), engine type (i.e. gasoline or diesel), exposure conditions (i.e. whole exhaust, PM extracts or filtered exhaust) and metabolic capacity of the experimental system (i.e. in the presence or absence of exogenous activation *in vitro*), several general statements can be made.

First, the soluble organic fraction of diesel-derived PM generally induced genotoxic effects *in vitro* (e.g. mutations in *Salmonella*) in the absence of exogenous metabolic activation. Second, chemical fractionation of diesel engine exhaust particulate extracts indicated that the most potent activity was associated with the moderately polar and highly polar fractions, and, moreover, that nitroarenes such as mono- and dinitro-PAHs often accounted for a substantial proportion of the observed genotoxic responses. Third, the soluble organic fraction of gasoline-derived PM was also genotoxic, but, in this case, exogenous metabolic activation *in vitro* generally enhanced the magnitude of the response. Fourth, fractionation of extracts from gasoline-derived PM showed that the mutagenic activity was associated with the PAH-containing neutral aromatic fraction, and catalytic treatment of gasoline exhausts significantly reduced the emission of mutagenic material.

Since IARC (1989), more than 200 scientific papers and reports (e.g. from the Health Effects Institute and the Society of Automotive Engineers) have used a wide range of experimental systems to examine the genetic and related effects of exposure to diesel and gasoline engine emissions. These findings are summarized below.

Studies investigating the genotoxicity of engine emissions can be conducted on diluted exhaust, exhaust PM, filtered exhaust (i.e. the gaseous and volatile portions), PM extracts or semi-volatile organic concentrates adsorbed onto a solid matrix (e.g. XAD resin) or a chilled surface. The most sophisticated *in-vivo* studies are inhalation experiments, during which animals are exposed to diluted engine emissions. These studies require complex installations to collect, dilute and deliver emissions effectively to the experimental animals. Moreover, highly technical equipment is required to control engine speed and load (i.e. test cycle), to monitor diluted emissions for particle concentration and size distribution and to determine the concentration of selected exhaust gases. Exposures can be 'whole-body' or 'nose-only'. Doses delivered via inhalation exposure are generally expressed in terms of milligrams of PM per cubic metre of air in the exposure chamber, with additional

information on the duration and frequency of the exposure. Other *in-vivo* studies may involve the delivery of suspensions of exhaust PM or PM extracts to the pulmonary system via intratracheal instillation, or the delivery of PM or PM extracts via oral gavage, dietary intake, topical application or intraperitoneal injection. Intratracheal, intraperitoneal or dietary doses are generally expressed as total milligrams of PM delivered/consumed, or as milligrams of PM ingested per kilogram of body weight (mg/kg bw).

The majority of *in vitro* assessments of effects elicited by engine emissions involve exposures of cells suspended in liquid medium, cells attached to solid culture surfaces (e.g. polystyrene) or cells embedded in agar. More recently, it has become possible to maintain cultured cells, including primary human cells or three-dimensional constructs of pulmonary tissue (e.g. EpiAirway), on semi-permeable membranes and expose the cells at an air–liquid interface (Aufderheide & Mohr, 1999, 2000; Aufderheide *et al.*, 2003; Bakand *et al.*, 2006). Such systems (e.g. VitroCell® or Cultex®) have been employed to examine the toxicity of engine emissions, but rarely to determine genetic and related effects (Knebel *et al.*, 2002; Seagrave *et al.*, 2007; Tsukue *et al.*, 2010a). Thus, most *in vitro* assessments involve exposures to collected PM, organic extracts of PM, semi-volatile organic concentrates or, in rare instances, gaseous emissions bubbled through liquid culture medium. Collection of PM can present a substantial technical challenge, and most studies collect PM on glassfibre filters during the filtration of diluted exhaust. In some instances, bulk PM is collected by means of devices such as cascade impactors. In either case, the preparation of PM extracts generally involves Soxhlet extraction, sonication extraction or pressurized fluid extraction with solvents such as dichloromethane, acetone, hexane, ethanol, methanol or solvent mixtures. Extracts are generally exchanged with a bioassay-compatible solvent such as dimethyl sulfoxide (DMSO) before testing. Semi-volatile organic concentrates are generally collected by passing filtered exhaust over a solid adsorbent matrix (e.g. XAD resin) followed by solvent elution, concentration and exchange with DMSO. [The Working Group noted that sample collection and processing can introduce alterations in samples of engine emissions, which may modulate/affect the genetic and related effects observed in experimental systems.]

The units of concentration employed for *in vitro* assessments of genetic and related effects induced by engine emissions vary depending on the nature of the test substance and the experimental system. Exposure concentrations for PM suspensions are generally expressed as mass of PM (in micrograms or milligrams) per assay unit (e.g. per agar plate or per millilitre of culture medium). Those for organic PM extracts are often given as micrograms of EOM or microlitres of extract per assay unit. Measures of EOM per unit mass of PM can then be used to convert these concentrations into equivalent milligrams of PM per assay unit. In addition, measures of engine work, engine run-time, fuel consumption or distance travelled can be used to convert exposure concentrations to equivalent amounts of engine work in kilowatt-hours (kW-h) or horse power-hour (hp-h), equivalent volume of fuel consumed, equivalent hour of engine run-time or equivalent distance travelled. Concentrations for aqueous extracts of PM are generally expressed as milligrams of PM equivalent per assay unit, again with the possibility of converting to units of engine work, engine run-time, fuel consumed or distance travelled. Concentrations of semi-volatile organic concentrates collected by adsorption on solid resins (e.g. XAD polystyrene copolymer) are generally expressed as micrograms of EOM per assay unit.

The information in the following sections and accompanying tables is primarily organized by route of exposure (i.e. inhalation, intratracheal instillation, topical treatment, oral

administration, *in vitro* treatment of cultured cells or isolated DNA), and secondarily by end-point (i.e. gene mutation, chromosomal damage, DNA damage and DNA strand breaks).

(b) Diesel engine emissions

(i) Effects observed in vivo in experimental animals

Diesel engine exhaust particulate matter

The results of relevant studies published since 1989 are described below, and are summarized in Table 4.4.

The induction by emissions from a four-cylinder light-duty direct injection diesel engine of mutations at the *lacI* and *guanine phosphoribosyl transferase* (*gpt*) loci was assessed in transgenic BigBlue® rats and *gpt* delta mice, respectively (Sato *et al.*, 2000; Hashimoto *et al.*, 2007). *LacI* mutations, stable DNA adducts and oxidative DNA damage were studied in the lungs of rats following whole-body exposure to diesel engine exhaust at 1 or 6 mg/m^3 of PM for 4 weeks (12 hours per day, 7 days a week); a significant increase in *lacI* mutation frequency was observed with the higher dose, and significant increases in aromatic DNA adducts (measured by [^{32}P]-postlabelling) and oxidative DNA lesions (measured as 8-OH-dG/deoxyguanosine by HPLC with electrochemical detection) were found with both exposure levels. Analyses of the *lacI* mutations revealed a high frequency of AT→GC and GC→AT transitions and GC→TA transversions. *Gpt* mutations were examined in the lungs of mice following whole-body exposure to diesel engine exhaust at 1 or 3 mg/m^3 of PM for 4, 12 and 24 weeks (12 hours per day, 7 days a week); significant increases were found in *gpt* mutant frequency following all exposures, with a peak response at 12 weeks. Analyses of the *gpt* mutations showed a high frequency of GC→TA transversions, reaching 77% at 24 weeks.

Changes in gene expression in the lungs of Fischer 344 rats and the induction of stable DNA adducts in *Nrf2* knock-out mice were examined after exposure to diesel engine exhaust (Sato *et al.*, 1999; Aoki *et al.*, 2001). The rats were exposed to 6 mg/m^3 of PM for 4 weeks (12 hours per day, 7 days a week), and a significant upregulation of the proto-oncogene *Araf* was noted. The *Nrf2* knock-out mice (both *Nrf2*–/– and *Nrf2*+/–) were exposed by whole-body inhalation to 3 mg/m^3 of PM for 4 weeks (12 hours per day, 7 days a week), and a significant increase in stable DNA adducts (measured by [^{32}P]-postlabelling) was observed, with the highest levels in *Nrf2*-null (–/–) animals.

The induction of DNA damage was also assessed in rats exposed to diluted diesel engine emissions. Gallagher *et al.* (1993) examined the frequency of stable DNA adducts in the lungs of CDdl(WI)Br rats exposed to 7.5 mg/m^3 of soot for 24 months. A major nuclease-P1-sensitive adduct was detected in lung DNA, which was suspected to be derived from nitro-PAHs. Gallagher *et al.* (1994) assessed the frequency of stable adducts in the lungs of Wistar rats exposed to diesel engine exhaust (particle concentration, 7.5 mg/m^3) for 2, 6 and 24 months (18 hours per day, 5 days a week), and found no significant increase in PAH-derived adducts, but a modest increase in adducts assumed to originate from nitro-PAHs. Iwai *et al.* (2000) studied the frequency of stable DNA adducts and oxidative DNA damage (i.e. 8-OH-dG) in the lungs of Fischer 344 rats exposed to 3.5 mg/m^3 of PM for 1, 3, 6, 9 and 12 months (17 hours per day overnight, 3 days week), and observed an increase in oxidative damage that reached a plateau after 9 months. The amount of bulky DNA adducts (measured by [^{32}P]-postlabelling/HPLC) peaked after 1 month and then declined.

The induction of heritable mutations was studied in C57BL mice exposed to diluted emissions from a single-cylinder research engine (Hedenskog *et al.*, 1997). Mutations in the offspring of male mice were scored as effects on pre- and post-meiotic sperm at the hyper-variable expanded simple tandem repeat loci, *Pc1*

Table 4.4 Summary of studies in animals exposed *in vivo* to diesel emissions or diesel exhaust particulate matter

Engine type and specifications	Run conditions	Test material collection and processing	Animal model	Exposure regime	End-points examined	Results	Reference
Inhalation exposure							
Isuzu A4JB1 2.7-L, light-duty 4-cylinder, direct injection engine	Steady-state 1500 rpm, 10 kg/m; EDYC dynamometer	Constant-volume dilution tunnel	Male BigBlue® (F344) rats (5 wks)	Whole-body exposure for 12 h/d, 7 d/wk for 4 wks to 1 or 6 mg/m^3	*LacI* mutations, stable lung DNA adducts by [^{32}P]-postlabelling, oxidative DNA damage (e.g. 8-OH-dG) by HPLC	Significant increase in *LacI* mutations at 6 mg/m^3; significant increase in total DNA adducts and oxidative damage at 1 and 6 mg/m^3; major mutations: A→G and G→A transitions, G→T transversions	Sato *et al.* (2000)
Isuzu 4JG2, 3.1-L, light-duty, 4-cylinder, direct injection engine	Steady-state 1500 rpm, 10 kg/m; EDYC dynamometer	Constant-volume dilution tunnel, DEP collected on glassfibre filters; range of particle size, 10–470 nm, with mass peak at 110 nm	*Gpt* delta transgenic mice (7 wks) (C57BL/6J background)	Whole-body exposure for 12 h/d, 7 d/wk to 1 or 3 mg/m^3 for 4, 12 or 24 wks; intratracheal instillation of DEP (0.125, 0.25, 0.5 mg) or DEP extract (benzene/ethanol; 0.05, 0.1, 0.2 mg) in 50 µL PBS	*Gpt* transgene mutations in lung 3 d after exposure by inhalation or 14 days after intratracheal instillation	Significant increase in *Gpt* mutations for each of the exposure scenarios; response after inhalation peaked at 12 wks; dose-related increase following treatment with DEP or DEP extract (potency of DEP extract twice that of DEP); G→A transitions predominant, G→T transversions were induced by both DEP and DEP extract.	Hashimoto *et al.* (2007)
Hydra IDI single-cylinder diesel research engine	Steady-state 1800 rpm, 1 kg/m.	Constant-volume dilution tunnel, 1:10 dilution of exhaust	Male C57BL mice (8–10 wks)	Whole-body exposure for 8 h/d for 14 days, with a 2-days break after 5 d; exposed males then mated with unexposed females	Heritable mutations (in pre- and postmeiotic sperm) at murine mini-satellite loci *Pc1* and *Pc2*	Small sample size; no significant increase in mutation frequency	Hedenskog *et al.* (1997)

Table 4.4 (continued)

Engine type and specifications	Run conditions	Test material collection and processing	Animal model	Exposure regime	End-points examined	Results	Reference
Isuzu A4JB1 2.7-L, light-duty 4-cylinder, direct injection engine	Steady-state 1500 rpm, 10 kg/m; EDYC dynamometer	Constant-volume dilution tunnel	*Nrf2* knock-out mice (null $^{-/-}$ and heterozygotes $^{+/-}$) (7 months)	Whole-body exposure for 12 h/d, 7 d/wk for 4 wks to 3 mg/m^3	Stable lung DNA adducts by [^{32}P]-postlabelling	Significant increase in levels of DNA adducts; highest in *Nrf2* null ($^{-/-}$) mice	Aoki *et al.* (2001)
Volkswagen 1.6-L, 40-kW diesel engine	US FTP-72	Exhaust diluted 1:9 with clean air; cyclone removal of particles < 1 µm; MMAD, 0.3 µm	Female Wistar rats (7 wks)	Whole-body exposure to 7.5 mg/m^3 for 18 h/d, 5 d/wk for 2, 6 or 24 months	Stable DNA adducts in lung (by [^{32}P]-postlabelling)	No significant elevation in PAH-derived adducts; modest increase in adducts suspected to be nitro-PAH-derived	Gallagher *et al.* (1994)
Two 1988 model LH6 General Motors 6.2-L, V-8 engines	US FTP-72	Constant-volume dilution tunnel	Male and female F344//N rats (8.5 wks)	Whole-body exposure for 16 h/d, 5 d/wk for up to 24 months to 2.4–2.5 or 6.2–6.5 mg/ m^3	Stable DNA adducts in lung and isolated alveolar type-II cells by [^{32}P]-post-labelling, chromosomal aberrations in circulating lymphocytes	At 3 months, dose-related increase in total lung adducts; significant 4-fold increase in adducts in type-II cells; no increase in chromosomal aberrations	Mauderly *et al.* (1994)
Light-duty diesel engine [details not provided]	Steady-state 1050 rpm, 80% load	Constant-volume dilution tunnel	Female F344 rats (8 wks)	Whole-body exposure for 17 h/d (at night), 3 d/wk for 1, 3, 6, 9 or 12 months to 3.5 mg/m^3	Oxidative DNA damage (i.e. 8-OH-dG by HPLC) and stable lung DNA adducts by [^{32}P]-postlabelling	Increase in oxidative DNA damage, reaching plateau at 9 mo; bulky DNA adducts peaked after the first month, then declined	Iwai *et al.* (2000)
US 2007-compliant heavy-duty engine (selected in ACES Phase I)	16-h duty cycle developed for ACES study	Constant-pressure dilution tunnel, 1:5 initial dilution rate	Wistar Han rats and C57BL/6 mice (8 wks)	Whole-body exposure for 16 h/d, 5 d/wk for up to 3 mo; target NO_2 concentrations of 0.1, 0.8 or 4.2 ppm	MN in reticulocytes and normochromatic erythrocytes, DNA strand breaks in the lung, 8-OH-dG in serum	No significant increase in MN frequency, strand breaks or 8-OH-dG	Khalek *et al.* (2009), Bemis *et al.* (2012), Hallberg *et al.* (2012), McDonald *et al.* (2012)

Table 4.4 (continued)

Engine type and specifications	Run conditions	Test material collection and processing	Animal model	Exposure regime	End-points examined	Results	Reference
Heavy-duty 6-cylinder 9.2-L engine, with and without urea (32.5% in water); SCR (details not provided)	Steady-state 1320 rpm, 840 nm [84 kg/m]	Partial-dilution type dilution tunnel	Male F344 rats (7 wks)	Whole-body exposure for 6 h/d for 1, 3 or 7 days to 1:29, 1:290 or 1:580 dilutions of exhaust	Oxidative stress measured as free 8-OH-dG in serum; expression of *Il-1β*, *Hmox-1*, *Tnf-α* and *Cyp1A1* in lung	*Conventional engine*: significant dose-related increase in 8-OH-dG, significant increase in *Cyp1A1* expression (1 d) *SCR engine*: slight increase in 8-OH-dG (1 day 3 d); significant increase in *Cyp1A1* (7 d), *Hmox-1* (1 day 3 d) and *Tnf-α* (1, 3, 7d)	Tsukue *et al.* (2010b)
Isuzu A4JB1 2.7-L, light-duty 4-cylinder, direct injection engine	Steady-state 1500 rpm, 10 kg/m; EDYC dynamometer	Constant-volume dilution tunnel	Male F344 rats (5 wks)	Whole-body exposure for 12 h/d, 7 d/wk for 4 wks to 6 mg/m^3	Gene expression in lung (by cDNA micro-array and Northern blot)	Significant upregulation of the proto-oncogene *A-Raf* and of PCNA mRNA	Sato *et al.* (1999)
Intratracheal or intranasal instillation							
Isuzu A4JB1 2.7-L, light-duty 4-cylinder, direct injection engine	Steady-state 2000 rpm, 6 kg/m; EDYC dynamometer	Constant-volume dilution tunnel, DEP collected on glassfibre filters	Male ICR mice (6 wks)	Single intratracheal instillation of 0.1–0.6 mg under anaesthesia	Oxidative DNA damage (i.e. 8-OH-dG by HPLC) in the lung	Significant increase in oxidative damage 12 h after exposure to 0.1–0.4 mg; damage peaked 2 d after exposure	Sagai *et al.* (1993), Nagashima *et al.* (1995)
Isuzu A4JB1 2.7-L, light-duty 4-cylinder, direct injection engine	Steady-state 1500 rpm, 10 kg/m; EDYC dynamometer	Constant-volume dilution tunnel, DEP collected on glassfibre filters; MMAD, 0.4 µm	Male ICR mice (4 wks)	Ten weekly intratracheal instillations with 0.1 mg DEP suspended in PBS; comparison with hexane/benzene/methanol-washed DEP	Oxidative DNA damage (i.e. 8-OH-dG by HPLC) in lung 24 h after final treatment	Significant increase in oxidative damage; no significant difference between DEP and washed DEP	Sagai *et al.* (1993), Ichinose *et al.* (1997b)

Table 4.4 (continued)

Engine type and specifications	Run conditions	Test material collection and processing	Animal model	Exposure regime	End-points examined	Results	Reference
Isuzu A4JB1 2.7-L, light-duty 4-cylinder, direct injection engine	Steady-state 1500 rpm, 10 kg/m; EDYC dynamometer	Constant-volume dilution tunnel, DEP collected on glassfibre filters; MMAD, 0.4 µm	Male ICR mice (4 wks)	Ten weekly intratracheal instillations with 0.05, 0.1 or 0.2 mg DEP suspended in PBS	Oxidative DNA damage (i.e. 8-OH-dG by HPLC) in lung 24 h after final treatment	Dose-related increase in oxidative DNA damage with 0.1 and 0.2 mg DEP/wk	Sagai *et al.* (1993), Ichinose *et al.* (1997a)
Mercedes-Benz MB1620, 210-hp bus engine, with Euro-III emission profile	Not specified	PM-retention device [details not given]	Male BALB/c mice (8 wks)	Daily intranasal instillation of 30 µg DEP in saline on 5 d/wk for 30 or 60 d	Expression of *Muc5ac* mRNA in lung (by real-time RT–PCR)	Significant increase in *Muc5ac* gene expression at 60 d	Yoshizaki *et al.* (2010)
Isuzu A4JB1 2.7-L, light-duty 4-cylinder, direct injection engine	Steady-state 1500 rpm, 10 kg/m; EDYC dynamometer	Constant-volume dilution tunnel, DEP collected on glassfibre filters, suspended in saline	Female BALB/c mice (5–6 wks)	Daily intranasal instillation of 30 µg DEP for 5 d, followed by daily 1 h inhalation exposures to 6 mg/m^3 for 3 d	Expression of *Ym1* and *Ym2* in lung (by qualitative RT–PCR)	Significant increase in expression of *Ym1* and *Ym2*	Song *et al.* (2008)
2002 Cummins 5.9-L diesel engine	Steady-state operation at 75% load	DEP from outflow duct [details not given]	*Gclm* wild-type, *Gclm*–/+, *Gclm*–/– mice (8–12 wks)	Single intranasal instillation of 200 µg DEP	Expression of *Tnf-α*, and *Il-6* (by quantitative real-time RT–PCR) 6 h after exposure	Significant increase in *Tnf-α* and *Il-6* expression; *Gclm*–/– mice most sensitive to the effects of DEP	Weldy *et al.* (2011)
Topical application							
Nissan Datsun 220C engine, Volkswagen (VW) Rabbit engine (turbo-charged)	HWFET, steady-state; particle emission rate, 0.33 and 0.18 g/mile for Nissan and VW, respectively	Constant-volume dilution tunnel; DEP collected on Teflon-coated glassfibre filters; Soxhlet extraction with dichloromethane	Female C-57 mice (6–8 wks)	Topical application of 120, 50 and 20 mg/mouse DEP extract at 0, 6, 30 and 54 h	Stable DNA adducts in skin, liver and lung by [^{32}P]-postlabelling and HPLC	Significant induction of DNA adducts in all tissues 24 h after exposure; in skin, the main adduct derived from Nissan exhaust was formed by chrysene; in lung, *anti*-BPDE adducts constituted up to 67% of total adducts	Lewtas *et al.* (1981), Gallagher *et al.* (1990), Savela *et al.* (1995)

Table 4.4 (continued)

Engine type and specifications	Run conditions	Test material collection and processing	Animal model	Exposure regime	End-points examined	Results	Reference
Two 1988 model LH6 General Motors 6.2-L, V-8 engines	US FTP-72	DEP collected on glassfibre filters; sonication; extraction with dichloromethane	Male CD1 mice (4 wks)	Single topical application of 20 mg-equivalent DEP in 100 µL acetone or five daily applications of 20 mg	Stable DNA adducts in skin, lung and heart by [^{32}P]-postlabelling 1, 3, 14, 42 and 77 days after the single dose, and 1, 3, 14, 42 and 70 days after repeated doses	Significant dose-related increases in adduct frequency in skin and lung, with rapid decline after 1 and 3 d, respectively; no adducts found in heart DNA	Mauderly *et al.* (1994), Randerath *et al.* (1995)
Intraperitoneal injection							
Six diesel engines, 2 heavy-duty and 4 medium-duty	Acceleration conditions	PM collected on glassfibre filters; extraction with dichloromethane	Male Kunming mice (18–20 g)	Two daily intraperitoneal injections of 4, 20 or 100 mg-equivalent DEP extract in DMSO	MN frequency in PCEs 6 h after second treatment	Significant dose-related increase in MN	Song & Ye (1995)
Unspecified diesel engine	Idling, with periodic acceleration to maximum speed	PM collected on glassfibre filters; extract obtained by sonication in dichloromethane; fractionated on silica gel: five fractions with increasing polarity	Male Swiss mice (18–20 g)	Two intraperitoneal injections (24 h apart) of 4, 12 or 36 mg-equivalent DEP extract in DMSO	MN frequency in PCEs 6 h after second treatment	Significant dose-related increase in MN; stronger responses for base, PAH and polar fractions	Lu *et al.* (1999b)

ACES, advanced collaborative emissions study; *anti*-BPDE, (±)-*r*-7,*t*-8-dihydroxy-*t*-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene; *Cyp*, cytochrome P450 gene; d, day(s); DEP, diesel exhaust particles; DMSO, dimethyl sulfoxide; FTP, Federal Test Procedure; *Gclm*, gene encoding glutamate cysteine ligase (modifier subunit); h, hour; *Hmox*, haeme oxygenase gene; hp, horse power; HPLC, high-performance liquid chromatography; HWFET, highway fuel economy test; *Il*, interleukin gene; MMAD, mass median aerodynamic diameter; MN, micronucleus/micronuclei; mo, month; NO_2, nitrogen dioxide; 8-OH-dG, 8-oxo-2'-deoxyguanosine; PAH, polycyclic aromatic hydrocarbons; PBS, phosphate buffered solution; PCE, polychromatic erythrocytes; PCNA, proliferating cell nuclear antigen; PM, particulate matter; rpm, revolutions per minute; RT–PCR, reverse transcription-polymerase chain reaction; SCR, selective catalytic reduction; *Tnf-α*, tumour necrosis factor-α gene; wk, week

and *Pc2*. No increase in the frequency of heritable mutations was found.

Two comprehensive *in-vivo* inhalation studies of diesel engine emissions were sponsored by the Health Effects Institute. The earlier study assessed DNA damage in rats chronically exposed to diesel engine exhaust (Bond *et al.*, 1990; Belinsky *et al.*, 1995; Randerath *et al.*, 1995) as part of a larger cancer bioassay conducted by the Inhalation Toxicology Research Institute (Mauderly *et al.*, 1994). The second study, referred to as the Advanced Collaborative Emissions Study (still ongoing), examined the genetic and related effects elicited in rats and mice by chronic and subchronic inhalation of emissions from a US 2007-compliant heavy-duty diesel engine (Bemis *et al.*, 2012; Hallberg *et al.*, 2012; McDonald *et al.*, 2012). The earlier chronic inhalation study assessed the frequency of bulky DNA adducts in the lung and isolated alveolar type II cells, as well as chromosomal damage (i.e. aberrations or micronuclei) in the circulating lymphocytes of rats exposed to diluted diesel engine emissions containing 2.5 or 6.5 mg/m^3 of particles for up to 24 months (16 hours per day, 5 days a week). Analyses of adduct formation after 3 months of exposure showed a significant dose-related increase in the level of total DNA adducts in alveolar cells at the high dose and the frequency of total adducts in lung tissues. However, adduct frequencies in exposed animals examined at 6, 12, 18 and 23 months did not always exceed those in the controls. A marked increase in adduct frequency was also recorded at 18 months after exposure to the lower dose. The frequency of chromosomal aberrations in circulating lymphocytes and micronuclei in cultured bi-nucleated lymphocytes were not increased in exposed animals compared with controls.

The Advanced Collaborative Emissions Study is an ongoing *in-vivo* investigation of the toxicological effects in rats and mice chronically exposed to diluted emissions from a US 2007-compliant heavy-duty diesel engine (i.e. maximum PM emission rate, 10 mg/hp-h). The first phase of the study involved a detailed comparison of four heavy-duty engines, and the selection of an engine for biological testing. Regulated and unregulated emissions from four test engines – a Caterpillar C13, a Cummins ISX, a Detroit Diesel DDC Series 60 and a Volvo Powertrain Mack MP7 – were assessed in four test cycles (Khalek *et al.*, 2009). All engines were equipped with a water-cooled exhaust gas re-circulation or a clean gas induction system to reduce nitrogen oxides, catalysed diesel particulate filters (DPF) or a diesel-oxidation catalyst placed in front of a catalysed DPF. The engine selected for the toxicological assessments showed emission rates of 1.2 mg/hp-h of PM and 0.91 g/hp-h of nitrogen dioxide, and exposures lasted for up to 30 months.

The results of the 1- and 3-month exposures (16 hours per day overnight, 5 days a week) have now been published (Bemis *et al.*, 2012; Hallberg *et al.*, 2012; McDonald *et al.*, 2012). Chromosomal damage (i.e. micronuclei) in peripheral blood, DNA strand breaks in lung tissue and a marker of oxidative damage (free 8-OH-dG) were examined in the serum from rats and mice exposed to diluted engine emissions. Due to the low concentration of PM, the concentration of nitrogen dioxide in the exhaust was used to set the doses (i.e. 0.1, 0.8 and 4.2 ppm). The 1- and 3-month exposures to diluted emissions did not induce a significant treatment-related increase in the overall frequency of micronuclei in rats or mice. However, significant effects were observed on the frequency of micronuclei in normochromatic erythrocytes of male mice, in reticulocytes and normochromatic erythrocytes of both male and female mice with duration of exposure, and in rat reticulocytes. However, these statistically significant effects were weak, and the analyses did not show a significant interaction between the outcome of treatment and the duration of exposure (Bemis *et al.*, 2012). In contrast, the 1- and 3-month exposures to diluted diesel engine

emissions did not induce significant increases in DNA strand breaks in the lung or concentrations of 8-OH-dG in the serum, although duration of exposure had some effect on the serum levels of 8-OH-dG in both rats and mice, which was not statistically significant (Hallberg *et al.*, 2012).

The effects of emissions from a selective catalytic reduction (SCR) engine (including the use of urea to remove nitrogen oxides) were compared with those of emissions from a heavy-duty engine in male Fischer rats. Tsukue *et al.* (2010b) assessed oxidative stress (measured as 8-OH-dG in serum) and the pulmonary expression of genes involved in xenobiotic metabolism (*Cyp1A1*), oxidative stress (*Hmox-1*) and inflammation (tumour necrosis factor, *Tnf-α*) in rats exposed to diluted emissions from the SCR engine system and from a conventional engine for 6 hours per day for 1, 3 or 7 days. The results revealed a significant dose-related increase in serum concentrations of 8-OH-dG for emissions from the conventional engine, but only slight increases for those from the SCR engine. Expression of *Cyp1A1* was significantly increased at day 1 for conventional engine missions and at 7 days for SCR engine emissions; however, the expression of *Hmox-1* and *Tnf-α* was significantly increased only for emissions from the SCR engine.

Several studies investigated the induction of DNA damage after intratracheal instillation of diesel engine exhaust particulates from a four-cylinder light-duty direct injection engine. Nagashima *et al.* (1995) assessed the induction of oxidative DNA damage (i.e. 8-OH-dG) in ICR mice that received a single instillation of 0.1–0.3 mg of PM; a significant increase in oxidative damage in lung DNA was observed 12 hours after exposure, with a peak after 2 days. Ichinose *et al.* (1997a, b) determined oxidative damage in ICR mice 24 hours after the last of 10 weekly intratracheal instillations of diesel engine exhaust particles, and compared the level of damage induced by untreated with that of hexane-washed diesel engine exhaust particles suspended in phosphate-buffered solution. A significant increase in oxidative damage was observed in lung DNA, but no appreciable difference was found between the two preparations. Following instillations of 0.05, 0.1 and 0.2 mg of diesel engine exhaust particles, a dose-related increase in the level of oxidative damage in lung DNA was found.

The Working Group reviewed several studies that examined changes in the expression of inflammatory genes in the lungs of mice exposed to diesel engine exhaust particles by intranasal instillation. Yoshizaki *et al.* (2010) noted significant increases in the expression of the *Muc5ac* gene, which encodes the mucin-5ac protein and is controlled by nuclear factor-κB, in the lung of BALB/c mice that received nasal instillations of 30 μg of diesel engine exhaust particles daily on 5 days a week for 60 days. This exposure level corresponded to an average 24-hour pulmonary deposition of PM from ambient air in Sao Paulo, Brazil. Increased numbers of leukocytes were found in the BAL fluid of these mice, which is indicative of respiratory tract inflammation. In BALB/c mice, Song *et al.* (2008) noted that intranasal exposure for 5 days followed by inhalation exposure for 3 days to diesel exhaust particles from a light-duty diesel engine induced a significant increase in the pulmonary mRNA expression of *Ym1* and *Ym2*, i.e. genes involved in inflammation-related responses to air pollutants. Weldy *et al.* (2011) reported that a single intranasal exposure to diesel engine exhaust particles (200 μg) of mice heterozygous ($^{+/-}$) for or deficient ($^{-/-}$) in a glutamate–cysteine ligase modifier subunit (a factor involved in glutathione synthesis) resulted in significant increases in the inflammatory cytokines *Tnfα* and *Il6* in the BAL fluid from these mice.

The frequency of stable DNA adducts was examined in experimental animals topically exposed to organic extracts of diesel PM (Gallagher *et al.*, 1990, 1993; Savela *et al.*, 1995). Dichloromethane extracts of diesel PM collected

from several different types of vehicle were applied to the shaved dorsal skin of C57 mice. Adduct formation was measured by [^{32}P]-postlabelling in DNA isolated from the skin, lung and liver 24 hours after the last of four topical applications (200 µL each) given at 0, 6, 30 and 54 hours, or after a single application. Significant increases in adduct levels were observed in all tissues examined. The adduct profiles included both chrysene- and benzo[*a*]pyrene-diol epoxide-derived adducts, and the latter accounted for a substantial portion of the adducts observed in the lung. The Inhalation Toxicology Research Institute study also assessed the formation of stable DNA adducts after topical application of diesel PM extract, and found a significant dose-related increase in the number of adducts in both the skin and lung, which rapidly declined 5 days after exposure (Randerath *et al.*, 1985, 1995).

The induction of micronuclei was examined in bone marrow following the intraperitoneal injection of diesel engine exhaust particles into mice. Song & Ye (1995) found significant dose-related increases in the frequency of micronuclei in the polychromatic erythrocytes of Kunming mice 6 hours after administration of two daily injections of exhaust particulate extracts derived from six different diesel engines. Lu *et al.* (1999b) also found a significant dose-related increase in the frequency of micronuclei in the polychromatic erythrocytes of Swiss mice 6 hours after the second of two intraperitoneal injections (given at 24-hour intervals) of 0.1 mL of a diesel engine exhaust particulate extract containing 4, 12 or 36 mg/mL of particles. After fractionation of the extract, the strongest response in the micronucleus assay was associated with the basic, PAH-containing and polar fractions.

Standard reference materials

Two standard reference materials (SRMs) of diesel engine exhaust particulates are available from the US National Institute of Standards and Technology. SRM 1650 was formulated and issued in 1985 and contains PM collected from several direct injection diesel engines. Although not based on any particular engine, SRM 1650 is generally thought to be representative of particulate emissions from heavy-duty diesel engines from the mid-1980s. A new standard, SRM 2975, was formulated and issued in 2000 and contains PM collected from an engine equipped with a filtration system designed for use with a diesel-powered forklift. Extraction of SRM 2975 with dichloromethane produces SRM 1975. Certificates of analysis for each of these SRMs, which contain certified concentrations for numerous PAHs and nitro-PAHs, are available online at http://www.nist.gov/srm/index.cfm. Several *in-vivo* studies determined the genetic and related effects of these SRMs, and the results are summarized in Table 4.5.

Risom *et al.* (2003a) examined oxidative lesions (i.e. 8-OH-dG) and changes in the expression of the DNA-repair gene *Ogg1* in lung tissue collected from BALB/CJ mice 1, 3 or 22 hours after a single 90-minute exposure by inhalation (nose-only) to SRM 1650 at 20 or 80 mg/m^3, or after four consecutive daily exposures for 90 minutes to 5 or 20 mg/m^3 SRM 1650. Oxidative DNA damage was increased after the single 90-minute exposure, and *Ogg1* was significantly upregulated after four consecutive daily exposures. Haeme oxygenase (*Hmox-1*) mRNA was upregulated after both schedules of exposure. Additional analyses showed a significant increase in DNA strand breakage in cells from BAL fluid after the repeated exposures. Dybdahl *et al.* (2004) used the same exposure regimen to examine oxidative lesions and bulky DNA adducts in lung tissues and DNA strand breaks in BAL cells collected from exposed BALB/CJ mice. Mutations in the *cII* transgene were also measured in Muta™Mouse 28 days after exposure. The results showed increased DNA strand breakage in BAL cells and increased oxidative damage and

bulky adducts in lung tissue, but no significant increase in *cII* mutations.

Saber *et al.* (2005) found a significant increase in the induction of DNA strand breaks in BAL cells of TNF-knock-out mice exposed by inhalation (nose-only) to 20 mg/m^3 of SRM 2975 for 90 minutes a day for 4 days, suggesting that a TNF-mediated inflammatory response is not required for the induction of DNA damage in BAL cells. Additional analyses showed a significant increase in *Il-6* gene expression in exposed TNF-knock-out animals. In a follow-up study, the expression of several inflammatory cytokines was investigated in wild-type and TNF-knock-out mice exposed by inhalation (nose-only) to SRM 1650 or SRM 2975 (20 or 80 mg/m^3, 90 minutes). The results in wild-type animals showed early (< 6 hours) post-exposure increases in the expression of *Il-6*, *monocyte chemoattractant protein-1* and *neutrophil chemotactic factor Kc*, and late (1 day) increases in *Tnf* expression; in the knock-out mice, the expression of *neutrophil chemotactic factor Kc*, *monocyte chemoattractant protein-1* and *Il-6* increased in the absence of TNF (Saber *et al.*, 2006).

The effects of SRM 2975 were examined in C57/Bl/6 mice exposed *in utero* through maternal inhalation (Hougaard *et al.*, 2008; Ritz *et al.*, 2011). Pregnant mice were exposed to SRM 2975 at 19.1 mg/m^3 for 1 hour per day on gestational days 7–19. DNA strand breaks (measured in the comet assay) and the levels of mRNA expression of the *Ogg1* and *excision repair cross-complementing group 1* (*Ercc1*) genes were determined in the liver of offspring on postnatal day 2. The results showed modest, non-significant increases in DNA strand breaks and no difference in gene expression. When the offspring (F1) were reared to maturity and mated with control CBA mice, pedigree analysis of the F2 descendants was used to determine the rates of extended simple tandem repeat germ-line mutation rates at the hyper-variable alleles *Ms6-hm* and *Hm2*. A significant, twofold elevation in mutation frequency was observed in prenatally exposed F1 males. Thus, repeated daily exposures to diesel engine exhaust particles *in utero* resulted in increased rates of germ-line mutation in exposed males (Ritz *et al.*, 2011).

The induction of DNA strand breaks, DNA adduct frequency, the expression of DNA-repair genes and the frequency of *cII* transgene mutations was studied in BigBlue® rats exposed to SRM 1650 in the diet (*ad libitum*). Müller *et al.* (2004) examined lung tissue following 21 days of exposure to 0.2–80 mg/kg of diet of PM and noted significant increases in DNA strand breaks (measured in the comet assay), oxidative DNA damage (in the comet assay including endonuclease III and formamidopyrimidine DNA–glycosylase) and bulky DNA adducts (by [^{32}P]-postlabelling). No significant increase in *cII* transgene mutations or the expression of *Ogg1* (involved in the repair of 8-OH-dG) or *Ercc1* was observed. Using the same exposure protocol, Dybdahl *et al.* (2003) assessed the same end-points in the liver and colon. Significant increases in DNA strand breaks and bulky adducts were found in both organs, and an increase in *Ercc1* gene expression in the liver only. No significant increases were observed in endonuclease III-enhanced DNA strand breaks, directly measured levels of 8-OH-dG in the liver, colon cells or urine or *cII* transgene mutations. Risom *et al.* (2003b) examined effects in the liver and colon following 21 days of exposure to 0.8 mg/kg of diet of PM with or without an elevated level of sucrose. Significant increases were found in bulky DNA adducts in the colon and liver, and in DNA strand breaks in the colon but not in the liver. The elevated sucrose diet in the absence of diesel engine exhaust particles also increased the number of bulky adducts in the colon and, to a lesser extent, in the liver. Significant increases were noted in the hepatic expression of the N-*methylpurine DNA glycosylase* gene and *Ogg1*. No effect was seen on the levels of oxidative DNA damage or on mutation frequency in the *cII* transgene in either tissue. In

Table 4.5 Summary of studies in animals exposed *in vivo* to diesel particulate standard reference materials (SRMs)

SRM	Animal model	Exposure regimen	End-points examined	Results	Reference
Inhalation exposure					
SRM 1650	Female BALB/CJ mice (8 wks)	Single 1.5-h head-only exposure to 20 or 80 mg/m^3 or daily 1.5-h exposures to 5 or 20 mg/m^3 on 4 consecutive days	Oxidative DNA damage (8-OH-dG by HPLC-EC) and expression of *Ogg1* and *HO-1* genes in lung tissue collected 1, 3 or 22 h after exposure; DNA strand breaks in BAL cells (by comet assay)	Significant increase in 8-OH-dG after the single high dose; *Ogg1* significantly upregulated after repeated exposures; *HO-1* expression increased at both single doses; significant increase in DNA strand breaks in BAL cells after repeated exposures	Risom *et al.* (2003a)
SRM 1650	Female BALB/CJ mice and MutaMouse (8 wks)	Single 1.5-h head-only exposure to 20 or 80 mg/m^3 or daily 1.5-h exposures to 5 or 20 mg/m^3 on 4 consecutive days	DNA strand breaks (by comet assay) in BAL cells, oxidative DNA damage (8-OH-dG by HPLC-EC) and bulky adducts (by [^{32}P]-postlabelling) in lung collected 1, 3 or 22 h after exposure; transgene *cII* mutations in lung 4 wks after exposure	Increase in DNA strand breaks in BAL cells and in bulky adducts in lung after repeated exposures; increased oxidative DNA damage after single exposure; no increase in *cII* mutations	Dybdahl *et al.* (2004)
SRM 2975 and 1650	Female BALBcj mice (8 wks), female and male C57xCBA mice (7–8 wks), female B6 (*Tnf* –/–) and C57BL/6J (*Tnf* $^{+/+}$) mice (9–11 wks)	Single 1.5-h head-only exposure to 20 or 80 mg/m^3 SRM 1650 (BALBcj mice), 80 mg/m^3 SRM 2975 (C57xCBA mice) or 20 or 4 × 20 mg/m^3 SRM 2975 (*Tnf* –/– and *Tnf* $^{+/+}$ mice); repeat exposure was for 1.5 h on 4 consecutive days	Expression in lung tissue of *Tnf*, *Mip2*, *Kc*, *Il6* and *Mcp1* (by real-time RT–PCR)	Significant increases in *Tnf* expression late (1 day after exposure to the higher dose; significant increases in expression of *Il6*, *Mcp1* and *Kc* < 6 h after exposure; expression of *Kc*, *Mcp1* and *Il6* increased in *Tnf* –/– animals	Saber *et al.* (2006)
SRM 2975	Exposure *in utero via* maternal inhalation (pregnant C57Bl/6 mice); at 19 wks, offspring mated with unexposed CBA/J mice to generate F2	Exposure to 19.1 mg/m^3 for 1 h/d on gestational days 7–19 in 18-L inhalation chamber with a flow of 20 L/min; peak mass concentration, 292 nm; MMAD, 240 nm	F2 germ-line mutation rates at ESTR loci from full pedigrees	Significant 2-fold increase in mutation frequency for F1 males (but not females) exposed *in utero*	Hougaard *et al.* (2008), Ritz *et al.* (2011)

Table 4.5 (continued)

SRM	Animal model	Exposure regimen	End-points examined	Results	Reference
SRM 2975	Exposure *in utero via* maternal inhalation (pregnant C57Bl/6 mice)	Exposure to 19.1 mg/m^3 for 1 h/d on gestational days 7–19 in 18-L inhalation chamber with flow of 20 L/min; peak mass concentration, 292 nm; MMAD, 240 nm	In offspring at postnatal day 2, damage in liver DNA: strand breaks (by comet assay) and oxidative damage via expression of *Ogg1, HO-1* and *Ercc1*	No increase in DNA strand breaks or gene expression	Hougaard *et al.* (2008)
Intratracheal instillation					
SRM 1650	Male Sprague-Dawley rats (150–170 g); primary tracheal epithelial (RTE) cells collected 24 h after exposure	Three consecutive daily instillations of 7.5–75 mg/kg bw DEP	Frequency of transformed foci in RTE cells 5 wks after exposure and isolation	Significant dose-dependent increase in transformation efficiency at 15, 30 and 75 mg/kg bw DEP	Ensell *et al.* (1998)
Oral administration					
SRM 1650	Male BigBlue® (Fischer) rats (8 wk)	DEP fed at 0.2–80 mg/kg of diet (*ad libitum*) for 21 d	Oxidative DNA damage (8-OH-dG by HPLC-EC), DNA strand breaks and oxidative lesions (by comet assay with ENDOIII), expression of *Ogg1* and *Ercc1* genes, *cII* transgene mutation frequency, stable adducts (by [^{32}P]-postlabelling) in liver and colon	Significant increase in DNA strand breaks and bulky adducts in liver and colon; increased expression of *Ercc1* in liver, and *Ogg1* in liver and colon; no increase in ENDOIII sites, oxidative damage or *cII* transgene mutations	Dybdahl *et al.* (2003)
SRM 1650	Male BigBlue® (Fischer) rats (8 wks)	DEP fed at 0.2–80 mg/kg of diet (*ad libitum*) for 21 d	DNA strand breaks and oxidative lesions (by comet assay including ENDOIII and FPG), stable DNA adducts (by [^{32}P]-postlabelling), transgene (*cII*) mutation frequency, and expression of *Ogg1* and *Ercc1*genes in lung	Significant increase in DNA strand breaks, oxidized bases and stable DNA adducts; no increase in *cII* mutations; no change in expression of *Ogg1* or *Ercc1*	Müller *et al.* (2004)

Table 4.5 (continued)

SRM	Animal model	Exposure regimen	End-points examined	Results	Reference
SRM 1650	Male BigBlue® (Fischer) rats (8 wks)	DEP fed at 0.8 mg/kg of diet (*ad libitum*), with or without elevated sucrose, for 21 d	Oxidative DNA damage (8-OH-dG by HPLC-EC), DNA strand breaks and oxidative lesions (by comet assay including ENDOIII and FPG), expression of *Ogg1*, *Mpg* and *Ercc1* genes, *cII* transgene mutation frequency, stable adducts (by [^{32}P]-postlabelling) in liver and colon	DNA adducts and strand breaks significantly increased in colon; adducts significantly increased in liver; no increase in oxidative damage or *cII* mutations in liver or colon; significant increase in expression of *Mpg* and *Ogg1* in liver; no effect of elevated sucrose in diet	Risom *et al.* (2003b)
SRM 1650	*Ogg1*-null and wild-type C57Bl/6 mice (10–13 wks)	DEP fed at 0.8 or 8 mg/kg of diet (*ad libitum*) for 21 d	DNA strand breaks and oxidative lesions (by comet assay with ENDOIII and FPG) and oxidative DNA damage (8-OH-dG by HPLC-EC) in colon, liver and lung	Increase in FPG sites in liver and lung in *Ogg1*-null mice relative to wild-type; increased DNA strand breaks in liver; no relation with exposure to DEP for any end-point in either strain	Risom *et al.* (2007)
SRM 2975	Male F344 rats (9 wks)	Single administration (by gavage) of 0.064 or 0.64 mg/kg bw DEP in saline	Oxidative DNA damage (8-OH-dG by HPLC-EC/UV), bulky DNA adducts (by [^{32}P]-post-labelling) and *Ogg1*gene expression in colon, liver and lung 6 and 24 h after exposure	Increased oxidative damage in all tissues at the higher dose; increased levels of bulky adducts in liver and lung at both doses (colon not tested); increased expression of *Ogg1* in lung (but not in colon or liver) 24 h after exposure	Danielsen *et al.* (2008)

BAL, bronchoalveolar lavage fluid; bw, body weight; d, day; DEP, diesel exhaust particles; ENDOIII, endonuclease-III; *Ercc*, excision repair cross-complementing gene; ESTR, expanded simple tandem repeat; FPG, formamidopyrimidine-DNA glycosylase; h, hour; *HO*, haeme oxygenase gene; HPLC-EC, high-performance liquid chromatography–electrochemical detection; *Il*, interleukin gene; *Kc*, chemokine gene; min, minute; *Mcp*, monocyte chemoattractant protein-1 gene; *Mip*, major intrinsic protein gene; MMAD, mass median aerodynamic diameter; *Ogg*, 8-oxoguanine glycosylase gene; 8-OH-dG, 8-oxo-2'-deoxyguanosine; RT–PCR, reverse transcription-polymerase chain reaction; SRM, standard reference material; *Tnf*, tumour necrosis factor gene; UV, ultraviolet; wk, week

a later study, Risom *et al.* (2007) assessed DNA strand breaks and oxidative damage in Ogg1-deficient (*Ogg1*–/–) and wild-type C57Bl/6 mice following 21 days of dietary exposure to diesel engine exhaust particles (SRM 1650) at 0.8 or 8 mg/kg of diet. An increase in formamidopyrimidine DNA–glycosylase-sensitive sites was observed in the liver and lung of Ogg1-deficient mice compared with wild-type mice, but no association with exposure to diesel engine exhaust particles was found for any end-point in either strain.

Danielsen *et al.* (2008) assessed oxidative DNA damage, bulky DNA adducts and *Ogg1* expression in the colon, liver and lung of Fischer 344 rats 6 and 24 hours after a single oral administration of SRM 2975 (0.064 and 0.64 mg/kg bw) by gavage, and found increased oxidative DNA damage in all tissues, increased levels of bulky DNA adducts in the liver and lung (colon not investigated) and increased expression of *Ogg1* in the lung, but not in the colon or liver, 24 hours after exposure.

A significant increase in the frequency of transformed foci was observed in primary rat tracheal epithelial cells collected from Sprague-Dawley rats 24 hours after an intratracheal instillation of SRM 1650 (7.5, 15, 30 or 75 mg/kg bw) after 5 weeks of cell culture (Ensell *et al.*, 1998).

(ii) Effects observed in vitro

A wide range of *in vitro* systems have assessed the genetic and related effects of diesel engine emissions, diesel engine emission particulates and/or organic extracts of diesel engine emission particulates, the most common of which are the *S. typhimurium* reverse mutation test and the single-cell gel electrophoresis (comet) assay. Many studies have also investigated genotoxic effects in cultured mammalian cells (e.g. Chinese hamster ovary, Chinese hamster V79, human A549 lymphocytes, MCF-7 and BEAS-2B), and several studies have examined the induction of DNA damage in isolated calf-thymus DNA *in vitro*.

(iii) Effects in cultured mammalian cells

See Table 4.6

The single-cell gel electrophoresis (comet) assay was used to assess the ability of diesel engine emissions, diesel engine emission particulates or diesel engine emission particulate extracts to induce DNA strand breaks. Oh & Chung (2006) treated human A549 adenocarcinoma cells and Chinese hamster ovary cells with a crude dichloromethane extract or extract fractions of diesel emission particulates derived from a 1995 commercial heavy-duty diesel vehicle. The crude extract induced a significant increase in DNA strand breaks in both the absence and presence of exogenous metabolic activation. After fractionation of the crude extract, the slightly polar fraction containing nitro-PAHs, ketones and quinones induced the highest number of strand breaks. Song *et al.* (2006, 2007) exposed rat L-929 fibroblasts to dichloromethane extracts or extract fractions of diesel emission particulates from a heavy-duty diesel engine with no catalytic treatment of the exhaust and observed statistically significant concentration-dependent increases in DNA strand breaks. Each of the fractions was more potent than the crude extract itself. Song *et al.* (2007) examined diesel engine emission particulates from different diesel–ethanol blends and indicated that particulates from fuels with 0 and 20% v/v ethanol yielded higher responses in the comet assay than those obtained from blends with 5, 10 or 15% ethanol.

Totlandsdal *et al.* (2010) exposed human BEAS-2B bronchial epithelial cells to diesel emission particulates (25–400 µg/mL for 2 hours) from a light-duty engine and found a significant dose-related increase in DNA strand breaks, with a significant additional increase after treatment with formamidopyrimidine–DNA glycosylase, a DNA-repair enzyme that removes oxidized purines. Additional analyses showed induction

Table 4.6 Summary of studies of the effects of diesel emissions in cultured mammalian cells *in vitro*

Test conditions	Exposure system	End-point(s) examined	Results	Reference
Commercial 1995 diesel truck, direct injection engine; 1000 rpm, no load; dilution tunnel; DEP collected on Teflon®-coated filters; extraction with dichloromethane	Human A549 alveolar adenocarcinoma cells and Chinese hamster ovary (CHO-K1) cells exposed to crude DEP extract or extract fractions (F1–F7, obtained by acid-base partitioning) for 4 or 24 h	DNA strand breaks in A549 cells (by comet assay, with/without a CYP enzyme inhibitor) after the 24-h exposure; MN formation in CHO-K1 cells (by CBMN, with/without rat-liver metabolic activation) after the 4-h exposure	Crude extract induced significant increase in DNA strand breaks and MN; no change with metabolic activation; fraction 4 (PAHs and alkyl-PAHs) induced maximal MN frequency, higher with metabolic activation; fraction 5 (nitro-PAHs, ketones and quinones) induced maximum strand breaks, in the absence of the CYP enzyme inhibitor	Oh & Chung (2006)
Heavy-duty, 6-cylinder, 5.79-L engine; ECE R49–13 test mode; DEP collected; dichloromethane Soxhlet extraction; extract fractionated into six fractions	Rat fibrocytes exposed to DEP extract [duration of exposure not specified]	DNA strand breaks (by alkaline comet assay)	All samples induced significant concentration-related increases in DNA strand breaks; fractions were significantly more potent than crude extract	Song *et al.* (2006)
Heavy-duty, 6-cylinder, 5.79-L engine; ECE R49–13 test mode; run on diesel fuel or diesel fuel containing 5, 10, 15 or 20% ethanol by volume; PM collected on glassfibre filters; Soxhlet extract in dichloromethane	L-929 rat fibrocytes exposed to 0.125–1.0 mg/mL DEP extract for 24 h	DNA strand breaks (by alkaline comet assay)	All samples induced significant concentration-related increases in DNA strand breaks; DEP extracts from the fuels with 10 and 15% ethanol yielded a lower response; the 20% blend was as active as the unblended fuel	Song *et al.* (2007)
Two light-duty diesel engines (complying with 1996 Euro2 and 2005 Euro4 emission standards, resp.); fuels: ULSD reference, RME20, AFME20; DEP collected on quartz filters; SRM 2975 tested also	Human A549 alveolar adenocarcinoma cells exposed to 0.78–100 µg/mL DEP for 3 h	DNA strand breaks (by comet assay and FPG-assisted comet assay)	Concentration-related increase in DNA strand breaks and FPG-sensitive sites in all samples, including SRM 2975; modest increase for DEP from Euro4 engine; response with RME20 lower than that with ULSD	Hemmingsen *et al.* (2011)

Table 4.6 (continued)

Test conditions	Exposure system	End-point(s) examined	Results	Reference
DEP collected from a Deutz 4-cylinder 2.2-L diesel engine run at 500 rpm (no load)	Human BEAS-2B bronchial epithelial cells (SV40-transformed) treated with 25–400 µg/mL DEP for 2 or 4 h	DNA strand breaks (by comet assay and FPG-assisted comet assay) and level of phosphorylated p38 protein (by western blot)	Significant concentration-related increase in DNA damage and p38 phosphorylation at 2 and 4 h; at 2 h, significant elevation in FPG-sensitive sites; strand breaks were induced at concentrations above those required to elicit inflammatory responses and changes in phosphorylated p38	Totlandsdal *et al.* (2010)
Kubota 1.12-L D1105-T diesel engine, EPA Tier I[a] (EU stage II); ISO-C1 engine cycle[b]; DEP collected via a high-volume cascade impactor, polyurethane foam and Teflon®-coated membrane; ultrasonic methanol extraction; fuels: European ULSD, HVO and RME	Mouse RAW264.7 macrophage cells exposed to 5, 50, 150 or 300 µg/mL DEP extract for 24 h	DNA strand breaks (by comet assay)	Significant increase in DNA strand breaks at highest two doses; aftertreatment with DOC/POC significantly reduced response with RME fuel	Jalava *et al.* (2010)
SRM 1650 suspended in culture medium; sonication	Human A549 alveolar adenocarcinoma cells exposed to 10–500 µg/mL DEP for 2, 5 or 24 h	DNA strand breaks (by comet assay)	Significant increase in DNA strand breaks at 100 and 500 µg/mL DEP at all time-points	Dybdahl *et al.* (2004)
SRM 1650, extracted with dichloromethane; both extract and washed particles tested	Human A549 alveolar adenocarcinoma cells and THP-1 human monocytes exposed to DEP suspension, extract or washed particles for 48 h	DNA strand breaks (by comet assay)	Significant increase in DNA strand breaks by DEP suspension and extract	Don Porto Carero *et al.* (2001)
Light-duty diesel car (1988, 2-L engine; no DOC); fuels: EN97, RD1, RD2; EU91/441/EEC and 94/12/EC test runs; DEP bulk sampling via collection on Teflon®-coated glassfibre filters, Soxhlet extraction in dichloromethane	Human MCF-7 mammary carcinoma cells exposed to 17–167 µg/mL DEP extract for 3–48 h; compared with extract of SRM 1650	Frequency of stable, bulky DNA adducts (by ^{32}P-postlabelling)	Strong concentration-dependent increase in DNA adducts after 12 h of exposure; strongest response with RD2 (Swedish Class-1 fuel); PAH-derived adduct levels not explained by the sum of the adduct-forming PAHs detected	Kuljukka *et al.* (1998), Kuljukka-Rabb *et al.* (2001)

Table 4.6 (continued)

Test conditions	Exposure system	End-point(s) examined	Results	Reference
SRM 1650a, SRM 1587, DEP from light-duty diesel car (1988, 2-L engine; no DOC); fuels: EN97, RD1, RD2; modified ECE/EUDC test procedure; DEP Soxhlet-extracted with dichloromethane	Human BEAS-2B bronchial epithelial cells exposed to 37–47 µg/mL DEP extracts, 15–150 µg/mL SRM 1650a or 1–10 µL/mL SRM 1587 for up to 48 h	Frequency of stable, bulky DNA adducts (by ^{32}P-postlabelling)	Concentration-related increases in bulky adducts in all samples; agreement with concentrations of adduct-forming PAHs	Pohjola *et al.* (2003a)
SRM 1975; dichloromethane extract of SRM 1975	Human MCF-10A mammary epithelial cells exposed to 400 µg [concentration not given] DEP extract for 24 or 48 h in the presence/absence of B[*a*]P	Frequency of stable, bulky DNA adducts (by ^{33}P-postlabelling with HPLC), oxidative DNA damage (8-OH-dG by flow cytometry with fluorochrome label)	No effects of exposure to DEP alone; significant stimulation of adduct formation and 8-OH-dG induction by B[*a*]P after co-exposure	Courter *et al.* (2007)
SRM 2975	Human-hamster hybrid A_L cells (fusion of human fibroblasts and Chinese hamster ovary cells) exposed to 2.5–100 µg/mL DEP for 24 h	Frequency of mutations at the *CD59* surface-antigen locus (positive selection assay)	Concentration-related increase in *CD59* mutations, significant for treatments with ≥ 50 µg/mL DEP	Bao *et al.* (2007)
SRM 1650b	Pulmonary epithelial cells from FE1 MutaMouse exposed *in vitro* to DEP at 37.5 or 75µg/mL, for 8 exposure periods of 72 h	Mutations at the transgenic *cII* locus	Concentration-related increase in *cII* mutant frequency, significant at 75 µg/mL DEP	Jacobsen *et al.* (2008)
General Motors 5.7-L diesel engine; FTP urban driving cycle; DEP collected by high-volume sampling from dilution tunnel onto glassfibre filters; extracted with DMSO or with DPL	Chinese hamster V79 and Chinese hamster ovary (CH)) cells exposed to DEP extracts, or to dispersions of extracted DEP (34, 68 or 136 µg/mL)	MN frequency	Significant concentration-related increase in MN in V79 and CHO cells by DMSO extract, and in CHO cells by sediment after extraction with DPL	Gu *et al.* (1992)
Heavy-duty, 11.15-L, direct injection, 6-cylinder engine and light-duty, 1.82-L, 4-cylinder engine, operating with torque 35.4 and 4 kg/m, respectively; DEP collected on Teflon®-coated filters, extracted with dichloromethane	Cultured human lymphocytes from 8 nonsmoking donors exposed to 50–400 µg/mL DEP extract for 44 h	MN by cytokinesis-blocked MN assay including kinetochore labelling to assess aneuploidy	DEP extract from low-duty engine induced a significant increase in MN in cells from 6 of 8, and in kinetochore-positive MN in cells from 4 of 8 donors; DEP extract from heavy-duty engine induced significant increase in MN and kinetochore-positive MN in cells from one donor	Odagiri *et al.* (1994)

Table 4.6 (continued)

Test conditions	Exposure system	End-point(s) examined	Results	Reference
Diesel engine operating on the US FTP urban driving cycle [further details not given]; DEP collected on glassfibre filters and dispersed in DMSO or DPPC, or extracted with dichloromethane; separation into supernatant and sediment	Chinese hamster V79 cells exposed to DEP dispersions or extract (33, 67 or 133 µg/mL) for 5 h	SCE frequency	Significant increase in SCE after exposure to DEP extract and dispersion; activity contained in the supernatant of extracted samples and in the sediment of dispersed samples; all at 133 µg/mL only	Keane *et al.* (1991)
DEP from an unspecified light-duty vehicle, collected on glassfibre filters, sonication/ Soxhlet extraction in dichloromethane	Cultured lymphocytes from a single nonsmoking donor, exposed to DEP extract for 4 h	SCE frequency	Significant concentration-related increase in SCE	Barale *et al.* (1993)
SEM 1650, extracted with dichloromethane, followed by acetone:methanol (1:1)	Primary tracheal epithelial cells from male Sprague-Dawley rats exposed to DEP or DEP extract (3–200 µg/mL) for 4 h	Frequency of transformed foci 5 wks after exposure	No increase in frequency of transformed foci	Ensell *et al.* (1998)
Isuzu 2.7-L (4JB1), light-duty, 4-cylinder, direct injection engine, torque 10 kg/m, 1500 rpm; DEP collected on glassfibre filters, extraction with dichloromethane (Soxhlet)	Rat alveolar type II epithelial cells (SV40T2-transformed) exposed to DEP extract fractions (dichloromethane-soluble, hexane-soluble and hexane-insoluble) at 30 µg/mL for 6 h	Global gene expression (by the Affymetrix Rat Expression Array 230A)	Significant differential upregulation of genes involved in xenobiotic metabolism (5–98-fold), oxidative stress (7.5–19.5-fold), cell cycle/apoptosis (6–124-fold), antioxidant response (50–144-fold)	Omura *et al.* (2009)
Isuzu 2.7-L (4JB1), light-duty, 4-cylinder, direct injection engine; DEP collected by scraping surface of dilution tunnel tubing; sonication/extraction with dichloromethane	Rat alveolar type II epithelial cells (SV40T2-transformed) exposed to DEP extract at 30 µg/mL for 6 h	Global gene expression (by Motorola CodeLink Bioarray gene-expression microarrays)	Significant upregulation of genes involved xenobiotic metabolism (2.2–5.2-fold), oxidative stress (18–82-fold) and cell cycle/ apoptosis (2.1–13.2-fold)	Koike *et al.* (2004)
Heavy-duty, 6-cylinder, 9.2-L engine, torque 745 Nm [74.5 kg/m], 1000 rpm with or without continuously regenerating diesel-particulate filter; whole exhaust	Human A549 adenocarcinoma cells exposed at air–liquid interface (Vitrocell® system) to whole exhaust at 1:10 or 1:100 dilution for 60 min, 8.3 mL/min	Global gene expression (by Agilent human oligonucleotide microarray); expression of selected genes via quantitative real-time RT–PCR	Significant increase in expression of *haeme oxygenase-1* (*HMOX-1*) gene for conventional engine; significant increase in expression of *interleukin-1β* gene for engine with particulate filter	Tsukue *et al.* (2010a)
Heavy-duty 11-L, 6-cylinder diesel engine (model year 2000); steady-state: 1000–2200 rpm at 10, 50 and 100% of maximum power; DEP collected on glassfibre filters	J774A.1 murine macrophages exposed to 10, 50 or 100 µg/mL DEP suspension in PBS for 12h	Expression of *p53* (by p53-luciferase reporter assay) and *Bax* (by semiquantitative RT–PCR)	Dose-related increases in *Bax* expression and *p53* activity, both significant at 50 and 100 µg/mL	Yun *et al.* (2009)

Table 4.6 (continued)

Test conditions	Exposure system	End-point(s) examined	Results	Reference
Isuzu 2.7-L (4JB1), light-duty, 4-cylinder, direct injection engine, torque 10 kg/m; DEP collected on glassfibre filters, sonication/ extraction with methanol	Human microvascular endothelial cells exposed to 5, 15 or 25µg/mL DEP extract	Global gene-expression profiling (by Illumina BeadChips for 23 000 transcripts); expression of selected genes (by quantitative real-time RT–PCR)	Approximately 10-fold increase in *HMOX-1*; upregulated genes functionally enriched for the electrophile response element, inflammation, immune response, cell adhesion and apoptosis	Gong *et al.* (2007)
SRM 2975	Human BEAS-2 bronchial cells exposed to 10 µg/cm^2 for 1, 4, 8 or 16 h	Gene expression (by quantitative real-time RT–PCR)	Significant upregulation of *p21* mRNA and p21 protein at 1 and 4 h, and of *COX*-2 mRNA and COX-2 protein at 4 and 8 h (peak), lower at 16 h	Cao *et al.* (2010)
Deutz 4-cylinder diesel engine, partial (25%) load, bag-house collection of DEP	Human BEAS-2 bronchial cells and primary human bronchial epithelial (HBE) cells exposed to 10–100 µg/mL for 2, 6, 18 or 24 h; HBE cells exposed at air–liquid interface	Gene expression (by luciferase reporter-construct and quantitative real-time RT–PCR)	Significant time- and dose-dependent upregulation of *matrix metalloproteinase-1* gene in BEAS-2 cells, and in HBE at 24 h only	Li *et al.* (2009)
SRM 1975 (dichloromethane extract of SRM 2975)	Mouse Hepa1c1c7 hepatoma cells exposed to DEP extract (10–70 µg/mL) for 24 h	Phosphorylation of p53, p38 and JNK	No increase in phosphorylated p53; significant dose-related increases in phosphorylated MAPKs, p38 and JNK	Landvik *et al.* (2007)
Isuzu 2.7-L (4JB1), light-duty, 4-cylinder, direct injection engine [no details given on collection of DEP]	Primary human bronchial epithelial cells exposed to DEP at air–liquid interface (apical side, 10 µg/cm^2, 2 h)	MicroRNA expression via microRNA microarray	Significant change in expression of microRNAs involved in inflammatory response pathways and tumorigenesis	Jardim *et al.* (2009)

[a] The first federal standards for new off-road diesel engines over 37 kW (50 hp); adopted in 1994, phased-in from 1996 to 2000 [b] Also known as ISO 8178 (8 steady-state modes with various loads and speed)

AFME20, fuel containing 20% animal fat methyl ester; B[*a*]P, benzo[*a*]pyrene; CBMN, cytokinesis-block micronucleus assay;COX-2, cyclooxygenase-2; CYP, cytochrome P450; DEP, diesel exhaust particles; DMSO, dimethyl sulfoxide; DOC, diesel oxidation catalyst; DPL, dipalmitoyl lecithin; DPPC, dipalmitoyl phosphatidyl choline (DPL and DPPC are primary components of pulmonary surfactant); ECE/EUDC, European urban driving cycle; FPG, formamidopyrimidine DNA–glycosylase; FTP, Federal Test Procedure; h, hour; HPLC, high-performance liquid chromatography; HVO, hydro-treated vegetable oil; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; min, minute; MN, micronucleus/ micronuclei; 8-OH-dG, 8-oxo-2'-deoxyguanosine; PAH, polycyclic aromatic hydrocarbon; PBS, phosphate buffered solution; POC, particle oxidation catalyst; RME20, fuel containing 20% rapeseed methyl ester; rpm, revolutions per minute; RT–PCR, reverse transcriptase-polymerase chain reaction; SCE, sister chromatid exchange; SRM, standard reference material; ULSD, ultra-low sulfur diesel

of an inflammatory response, and increases in the level of phosphorylated p38 at concentrations below those that induced strand breaks. Jalava *et al.* (2010) treated mouse RAW264.7 macrophages with diesel emission particulates (15, 50, 150 or 300 µg/mL for 24 hours) from a light-duty engine (EPA Tier 1/EU stage II) operated with three different fuels (i.e. conventional diesel, hydro-treated fresh vegetable oil and rapeseed methyl ester), with and without a catalyst. All samples, except those derived from conventional diesel, induced a concentration-dependent increase in DNA strand breaks, which was significant at the highest two doses. Treatment of the exhaust with a combined diesel-oxidation and particle-oxidation catalyst slightly reduced the response to the samples derived from the rapeseed methyl ester-driven engine. Hemmingsen *et al.* (2011) exposed human A549 alveolar epithelial cells to diesel PM (0.78–100 µg/mL) from two light-duty engines that met Euro-2 (~1996) and Euro-4 (~2005) emission standards. All samples induced concentration-related increases in DNA strand breaks and formamidopyrimidine–DNA glycosylase-sensitive sites, which were lowest with samples from the Euro-4 engine. A comparison of different fuels showed that PM generated from a 20% v/v blend of low-sulfur diesel and biodiesel (i.e. rapeseed methyl ester or animal fat methyl ester) was less genotoxic than that from the reference low-sulfur diesel. The observed responses in the comet assay were similar to those elicited by samples of SRM 2975.

Dybdahl *et al.* (2004) exposed human A549 cells for 2, 5 or 14 hours to SRM 1650 (10, 50, 100 or 500 µg/mL) suspended in culture medium. DNA strand breaks were significantly increased at the highest two concentrations. When human A549 alveolar cells and human THP-1 monocytes were exposed for 48 hours to an SRM 1650 suspension, an SRM 1650 extract or a suspension of solvent-washed SRM 1650 (16, 160 or 1600 ng/mL), significant increases in DNA strand breaks were observed in cells exposed to high doses of the extract or the suspension of SRM 1650. Solvent-washed material did not produce an effect (Don Porto Carero *et al.*, 2001).

Kuljukka *et al.* (1998) and Kuljukka-Rabb *et al.* (2001) assessed the frequency of bulky DNA adducts in human MCF-7 mammary cells exposed for 3–48 hours to dichloromethane extracts of diesel PM produced by a 1988 light-duty diesel engine operated with three different European fuels. Strong concentration-related increases in DNA adduct levels were seen after 24 and 48 hours of exposure, and the strongest response was observed with PM from the engine run on Swedish Class 1 diesel fuel. Use of low-sulfur standard diesel fuel did not enhance the levels of adducts. The formation of bulky adducts could not be correlated with the amounts of specific strong and weak adduct-forming PAHs in the PM extracts. Using the same exposure protocol in human BEAS-2B bronchial epithelial cells, Pohjola *et al.* (2003a) found that all samples induced concentration-related increases in bulky adducts in agreement with the levels of adduct-forming PAHs. Courter *et al.* (2007) measured stable DNA adducts (by [^{32}P]-postlabelling/HPLC) and oxidative DNA lesions (i.e. 8-OH-dG) in human MCF-10A breast epithelial cells exposed for 24 or 48 hours to SRM 1975, alone or in combination with benzo[*a*]pyrene or dibenzo[*a,l*]pyrene. No effects were seen after treatment with SRM 1975 alone. The diesel particulates strongly reduced adduct formation and stimulated 8-OH-dG induction produced by benzo[*a*]pyrene, but had no effect on those produced by dibenzo[*a,l*]pyrene.

Jacobsen *et al.* (2008) assessed mutation frequency at the *cII* locus in transgenic Muta™Mouse FE1 cells exposed to SRM 1650b (37.5 or 75 µg/mL) during eight 72-hour cycles, and found a concentration-dependent increase in mutant frequency, which was statistically significant at the highest concentration. Bao *et al.* (2007, 2009) exposed human–hamster hybrid A_L cells to SRM 2975 for 24 hours or to

SRM 1975 for 30 minutes, alone or in combination with ultraviolet light. Exposure to SRM 2975 induced a concentration-related increase in CD59 mutations, with a significant response at 50 and 100 µg/mL. The short exposure to SRM 1975 alone did not elicit an increase in CD59 mutants, but caused a twofold increase in ultraviolet light-induced mutant frequency.

Oh & Chung (2006) (described above) noted that a crude extract of diesel emission particles from a heavy-duty vehicle induced a significant increase in the frequency of micronuclei in Chinese hamster ovary cells, in both the absence and presence of exogenous metabolic activation. After fractionation, the fraction containing PAHs and alkyl-PAHs induced the strongest increase in micronuclei, and the response was enhanced by exogenous metabolic activation. In the study by Bao *et al.* (2009), the short exposure to SRM 1975 alone did not have an effect on the frequency of micronuclei, but caused a twofold increase in ultraviolet light-induced micronucleus formation.

Gu *et al.* (1992) exposed Chinese hamster V79 lung fibroblasts to a suspension of diesel engine emission particles, DMSO or saline extracts of diesel engine emission particles or diesel engine emission particles washed with DMSO or saline. The DMSO extract induced a concentration-related increase in micronucleus frequency. Liu *et al.* (2005) showed that extracts of PM generated by three 1998/2000 diesel engines induced a weak increase in micronuclei in Chinese hamster V79 cells. Odagiri *et al.* (1994) assessed the frequency of micronuclei and kinetochore-positive micronuclei in cultured human lymphocytes exposed for 4 hours to organic extracts of diesel emission particles from a heavy-duty and a light-duty engine. Extracts of light-duty particles induced a significant increase in micronuclei in cells from six out of eight donors, and an increase in kinetochore-positive micronuclei in cells from four out of eight donors. Extracts of diesel emission particles from the heavy-duty engine induced a significant increase in the frequency of micronuclei and in kinetochore-positive micronuclei in cells from only one donor.

Exposure to extracts of diesel engine emission particles or diesel engine emission particles dispersed in a pulmonary surfactant (dipalmitoyl phosphatidyl choline) for 39 hours induced a significant increase in the frequency of sister chromatid exchange in Chinese hamster V79 cells (Keane *et al.*, 1991). Barale *et al.* (1993) exposed cultured human lymphocytes (from a single donor) to a dichloromethane extract of diesel emission particles from a light-duty vehicle, and found a significant concentration-related increase in sister chromatid exchange.

Ensell *et al.* (1998) treated primary rat tracheal epithelial cells with an organic extract of SRM 1650 *in vitro* and found no significant increase in transformed foci 5 weeks after exposure.

[The Working Group noted that a large number of studies reported changes in the expression of genes related to inflammation, apoptosis, cell cycle, xenobiotic metabolism, antioxidant response, invasion, migration and metastasis of transformed cells, and oxidative stress in mammalian cells exposed to diesel engine exhaust, diesel engine exhaust particles or extracts of diesel engine exhaust particles. The results of several selected publications are briefly summarized below.]

Changes in global gene expression were investigated in rat alveolar type II cells exposed to organic fractions of diesel exhaust particles from a light-duty engine. Large increases were noted in the expression of genes involved in xenobiotic metabolism, oxidative stress, antioxidant response, cell-cycle control, cell proliferation and apoptosis. Omura *et al.* (2009) exposed rat SV40T2 alveolar epithelial cells for 6 hours to various extract fractions of diesel engine exhaust particles that were soluble in dichloromethane and soluble/insoluble in *n*-hexane and found a 144-fold increase in the expression of *Hmox1*, a 98-fold increase in the expression of *Cyp1A1*, a

50-fold increase in *sulforedoxin 1 homologue*, a 13-fold increase in *Gsta3*, an 11-fold increase in *NQO1* and a five- to sixfold increase in *IL7* across the different fractions. Using the same assay system, Koike *et al.* (2004) noted a greater than twofold increase in the expression of more than 50 genes, including *Hmox1* (82-fold increase), *Gstα* (27-fold), *heat-shock protein 70–1* (18-fold) and *AkR1* (fivefold). Subsequent western blot analyses showed significant dose-related increases in the Hmox-1 protein 12 and 24 hours after exposure; this increase in protein expression was reduced by treatment with the antioxidant *N*-acetyl-L-cysteine.

Induction of apoptosis was determined in murine J774A.1 macrophages exposed for 24 hours to a suspension of diesel engine exhaust particles in saline. A significant dose-related increase was noted in the activity of p53 protein and in the expression of the *Bax* gene, a pro-apoptotic endogenous target of p53-dependent transcriptional activation (Yun *et al.*, 2009).

Changes in global gene expression were seen in human microvascular endothelial cells exposed for 4 hours to diesel engine exhaust particulate extract. Significant upregulation of genes functionally enriched for the expression of the electrophile-responsive element, immune response, cell adhesion and apoptosis was observed. Subsequent analysis of selected genes by quantitative reverse transcription–polymerase chain reaction revealed a substantial increase in oxidative stress (i.e. *Hmox1*) (Gong *et al.*, 2007; Lee *et al.*, 2012).

The transcription of *cyclin-dependent kinase inhibitor 1* (or *p21*), a negative regulator of the cell cycle, was significantly upregulated in human BEAS-2B bronchial cells exposed to SRM 2975 for 4 or 8 hours. Moreover, this increase was independent of p53 and Sp1, and involved activation by *STAT3*, a transcription factor associated with oxidative stress (Cao *et al.*, 2010). In an earlier study, the same research group reported that SRM 2975 induced increased expression of *cyclooxygenase-2*, a gene that plays an important role in inflammatory responses to external stimuli such as oxidative stress (Cao *et al.*, 2007).

Significant upregulation of the *matrix metalloproteinase-1* gene was observed following exposure to diesel eng exhaust particles oin human BEAS-2B bronchial epithelial cells and human primary bronchial epithelial cells obtained by brushing the airways during bronchoscopy. *Matrix metalloproteinase-1* is associated with inflammation, as well as invasion, migration and metastasis of transformed cells, and is known to be involved in pathological processes linked to malignant and non-malignant respiratory diseases, such as asthma, chronic obstructive pulmonary disease and bronchial carcinoma (Li *et al.*, 2009).

The Working Group reviewed two studies that use a system to expose cells to diesel engine exhaust at an air–liquid interface. Human A549 adenocarcinoma cells were exposed in such a system to diluted (1:10, 1:100) emissions from a heavy-duty engine with a normal muffler or with a continuously regenerating DPF. A significant increase in oxidative stress was observed (i.e. increased expression of *Hmox1*) for emissions from the conventional engine only. However, significant increases in pro-inflammatory processes (i.e. *IL1β* gene expression) were only seen with the continuously regenerating DPF with a high dilution of the exhaust (Tsukue *et al.*, 2010a). [The Working Group noted that the latter effect was probably the result of increased levels of nitrogen oxides with the continuously regenerating DPF.]

Jardim *et al.* (2009) exposed primary human bronchial epithelial cells obtained from cytological brushing at bronchoscopy to diesel engine exhaust particles at an air–liquid interface and noted significant, exposure-related changes in the expression of microRNAs associated with inflammatory and tumorigenic responses.

Landvik *et al.* (2007) assessed various responses of mouse Hepa1c1c7 hepatoma cells to

exposure to SRM 1975, and found an increase in the number of necrotic cells, but little or no effect on apoptosis. In addition, slightly enhanced p53 phosphorylation, and a significant concentration-related increase in the phosphorylation of mitogen-activated protein kinases, p38 and c-Jun N-terminal kinases was noted.

(iv) Effects in vitro in DNA solutions

Techniques such as [^{32}P]-postlabelling or HPLC have been used to investigate the formation of bulky DNA adducts in calf-thymus DNA exposed to diesel engine exhaust particulate extracts in the presence of exogenous metabolic activation systems, such as post-mitochondrial supernatant from Aroclor-induced rat liver, to induce the oxidative metabolism of aromatic hydrocarbons (i.e. PAHs) or to provide enzymes, such as xanthine oxidase, that catalyse the reductive metabolism of compounds such as nitro-PAHs. The results of these studies are summarized in Table 4.7.

The frequency and profile of bulky DNA adducts were studied in calf-thymus DNA after treatment with extracts of diesel engine exhaust particles in the presence of Aroclor-induced rat liver post-mitochondrial supernatant or xanthine oxidase (Jahnke *et al.*, 1990; Gallagher *et al.*, 1991, 1993; King *et al.*, 1994; Savela *et al.*, 1995; Kuljukka *et al.*, 1998; Pohjola *et al.*, 2003b). The induction of bulky adducts by dichloromethane extracts of diesel exhaust particles from a series of light- and heavy-duty vehicles was also investigated (Albert *et al.*, 1983; Gallagher *et al.*, 1991, 1993; King *et al.*, 1994). The results showed a high frequency of stable adducts that did not co-chromatograph with known PAH-diol epoxide–DNA adduct standards. Treatment in the presence of xanthine oxidase indicated that reductive metabolism contributed to the formation of unidentified diesel engine exhaust particle-derived nitro-PAH adducts. In addition, reductive metabolism yielded a nuclease P1-sensitive adduct (analysed by [^{32}P]-postlabelling) that was distinct from adducts induced by post-mitochondrial supernatant-catalysed oxidative metabolism. Savela *et al.* (1995) confirmed a high frequency of adducts after exposure to diesel exhaust particulate extracts from two light-duty vehicles; however, although benzo[*a*]pyrene- and chrysene-derived adducts were detected, the major spots did not co-chromatograph with authentic standards for these two adducts, or for adducts formed from three isomeric benzofluoranthenes.

Studies that examined extracts of diesel exhaust particles from light-duty vehicles running on three different European fuels reported higher levels of adducts in the presence of xanthine oxidase than in that of post-mitochondrial supernatant. Kuljukka *et al.* (1998) found that the potency of the extract (expressed as adduct frequency per microgram of EOM) was reduced for low-sulfur fuels and that this reduction was associated with a reduction in PM and PAH emissions per distance travelled. Pohjola *et al.* (2003b) noted that diesel engine exhaust particulate extracts yielded higher levels of adducts than gasoline PM extracts, and that the difference was greatest under reductive conditions. These results were consistent with the notion that nitro-PAHs contribute to the formation of bulky adducts induced by exposure to diesel engine exhaust particulate extracts. Topinka *et al.* (2012) examined the induction of stable adducts in calf-thymus DNA exposed, in the presence of a rat liver post-mitochondrial supernatant, to extracts of diesel exhaust particles from two engines run on diesel fuel, rapeseed oil or rapeseed oil methyl ester (B100). Depending on the test cycle used, diesel emissions contained similar or substantially higher amounts of total PAHs than rapeseed oil emissions, while the amounts of carcinogenic PAHs were comparable or significantly higher in the emissions generated from rapeseed oil. In the absence of exogenous metabolic activation, the levels of some unidentified adducts were increased under certain conditions (e.g. diesel engine test cycles), but the

Table 4.7 Summary of studies on the formation of adducts and oxidative damage in calf-thymus DNA exposed to extracts of diesel exhaust particles (DEP)

Test conditions	Exposure system	End-point(s) examined	Results	References
VW Rabbit light-duty diesel vehicle [no details given]; DEP collected and Soxhlet-extraction with dichloromethane	Incubation of calf-thymus DNA with DEP extract (100 µg/mL) for 4 h with Aroclor-induced rat-liver S9 or xanthine oxidase (to induce nitro-reduction)	Frequency of stable, bulky DNA adducts (by ^{32}P-postlabelling, TLC and HPLC)	High frequency of adducts, but those derived from DEP did not elute with any PAH or nitro-PAH adduct standards; reductive metabolism may have contributed to the formation of nitro-PAH adducts	King *et al.* (1994)
Three light-duty diesel vehicles (Mercedes, VW Rabbit, Nissan), 1 heavy-duty diesel vehicle (Caterpillar) and 1 gasoline-fuelled vehicle (Ford Van); DEP collected and Soxhlet-extraction with dichloromethane [details in Lewtas *et al.* (1981)]	Incubation of calf-thymus DNA with DEP extract (100 µg/mL) for 1.5 h with Aroclor-induced rat-liver S9 or xanthine oxidase	Frequency of stable, bulky DNA adducts (by ^{32}P-postlabelling; enrichment by nuclease P1 digestion and extraction with butanol)	All DEP samples produced adducts with S9; gasoline sample only yielded adducts without S9; reductive metabolism (with xanthine oxidase) produced a major nuclease P1-sensitive adduct distinct from S9-induced adducts; reductive metabolism may have contributed to the formation of nitro-PAH adducts	Gallagher *et al.* (1991)
Light-duty diesel engines (Datsun, Oldsmobile, VW Rabbit); HWFET driving cycle; diesel fuel No. 2; DEP collected on Teflon-coated glassfibre filters, extraction with dichloromethane	Incubation of calf-thymus DNA with DEP extract (100 µg/mL) for 1.5 h with Aroclor-induced rat-liver S9 or xanthine oxidase (under anaerobic conditions)	Frequency of stable, bulky DNA adducts (by ^{32}P-postlabelling)	A major adduct formed in DEP-treated human lymphocytes co-migrated with a benzo(*a*)pyrene-derived adduct; reductive metabolism yielded a major nuclease P1-sensitive adduct that appeared to be derived from a nitro-PAH	Albert *et al.* (1983), Gallagher *et al.* (1993)
Light-duty diesel car (1988, 2-L engine, no DOC); fuels: EN97, RD1, RD2; EU91/441/EEC and 94/12/EC test runs; DEP bulk sampling via collection on Teflon-coated glassfibre filters, extraction in dichloromethane	Calf-thymus DNA exposed to 8 or 17 µg DEP extract [no details given], without or with rat-liver S9 or xanthine oxidase	Frequency of stable, bulky DNA adducts (by ^{32}P-postlabelling)	Higher adduct levels with than without S9 or xanthine oxidase; highest levels for EN97 (high-sulfur fuel); up to 80% fewer adducts with low-sulfur RD1 and RD2 fuels; reduced PM and PAH (per km) for low-sulfur fuels	Kuljukka *et al.* (1998)

Table 4.7 (continued)

Test conditions	Exposure system	End-point(s) examined	Results	References
Light-duty diesel car (1988 Toyota, 2-L engine, no DOC); fuels: EN97, RD1, RD2; test runs: EU91/441/EEC and 94/12/EC; DEP collected on glassfibre filters, Soxhlet extraction in dichloromethane; gasoline-derived PM and SRM 1650 also tested	Incubation of calf-thymus DNA with 150 µg/mL PM extract for 4 h, with or without Aroclor-induced rat-liver S9 or xanthine oxidase	Frequency of stable, bulky DNA adducts (by ^{32}P-postlabelling)	Higher levels of adducts under reductive conditions, consistent with higher level of nitro-PAHs; yield of PAH-derived adducts (with S9) from gasoline-derived PM and DEP not significantly different (RD1/RD2 only); DEP far more potent in 'adducts per km'	Pohjola *et al.* (2003b)
Light-duty diesel vehicles (Nissan Datsun 220C engine, VW Rabbit turbocharged engine); HWFET driving cycle; diesel fuel No. 2; DEP collected on Teflon-coated glassfibre filters, Soxhlet extraction in dichloromethane	Incubation of calf-thymus DNA with 100 µg/mL DEP extract for 1.5 h with Aroclor-induced rat-liver S9	Frequency of stable, bulky DNA adducts (by ^{32}P-postlabelling and HPLC)	Both DEP extracts yielded a high frequency of adducts; the main DNA adduct did not co-migrate with B[*a*]P, B[*b*]F, B[*j*]F, B[*k*]F or chrysene adduct standards; chrysene adducts were identified	Savela *et al.* (1995)
Two direct injection, aftercooled diesel engines: Cummins ISBe4 (4.5 L; 2003; tested on WHSC and ESC) and Zetor 1505 (4.2 L; 2007; tested on NRSC); fuels: European diesel EN 590, RME (B100) and RSO; DEP collected by on high-volume sampler on Teflon-coated filters, extraction with dichloromethane	Incubation of calf-thymus DNA with DEP extract, corresponding to 0.3 or 3 m^3 Cummins engine exhaust and 0.1 or 1 m^3 Zetor engine exhaust, for 24 h, with or without rat-liver S9	Frequency of stable, bulky DNA adducts (by ^{32}P-postlabelling)	Significant concentration-related increase in adduct levels for all samples; several-fold higher response with S9; adduct-forming potency per mg PM similar for the two engines, and similar across fuel types (diesel higher for WHSC); similar potency trend per kW-h	Topinka *et al.* (2012)
Isuzu A4JB1 2.7-L, light-duty 4-cylinder, direct injection engine; steady-state 2000 rpm, 6 kg/m; constant-volume dilution tunnel; DEP collected on glassfibre filters	Incubation of DEP (5, 10 or 20 mg) with calf thymus DNA for 15–120 min	Oxidative DNA damage (8-OH-dG by HPLC)	Concentration-related increase in oxidative DNA damage reaching a maximum after approximately 60 min	Nagashima *et al.* (1995)
Light-duty diesel engine [details not provided]; steady-state, 1050 rpm, 80% load	Incubation of DEP (10 mg/mL) with calf thymus DNA (2 mg/mL) for 0.5–3 h	Oxidative DNA damage (i.e. 8-OH-dG by HPLC, after DNA isolation with pronase/ethanol)	No significant increase in oxidative DNA damage	Iwai *et al.* (2000)

B[*a*]P, benzo[*a*]pyrene; B[*b*]F, benzo[*b*]fluoranthene; B[*j*]F, benzo[*j*]fluoranthene; B[*k*]F, benzo[*k*]fluoranthene; DOC, diesel oxidation catalyst; ESC, European steady-state cycle; h, hour; HPLC, high-performance liquid chromatography; HWFET, US highway fuel economy test; kW-h, kilowatt-hour; min, minute; NRSC, non-road steady-state cycle; 8-OH-dG, 8-oxo-2'-deoxyguanosine; PAH, polycyclic aromatic hydrocarbon; PM, particulate matter; RME, rapeseed methyl ester; rpm, revolutions per minute; RSO, rapeseed oil; S9, metabolic activation system; SRM, standard reference material; TLC, thin-layer chromatography; VW, Volkswagen; WHSC, world harmonized steady-state cycle

adduct frequency per milligram of diesel exhaust particles was generally higher in the presence of such activation.

The formation of oxidative DNA lesions (i.e. 8-OH-dG) was studied in calf-thymus DNA following incubation with a suspension of diesel exhaust particles from light-duty engines. One study showed a concentration-dependent increase in oxidative damage, which reached a maximum after 60 minutes of exposure (Nagashima *et al.*, 1995), while another did not report any change in the number of oxidative DNA lesions following incubations for 0.5–3 hours (Iwai *et al.*, 2000).

(v) Effects in vitro in the Salmonella reverse mutation assay

The *Salmonella* reverse mutation test is the most common tool used to investigate the mutagenic activity of complex environmental matrices and extracts or concentrates of complex matrices (e.g., drinking-water, soil, urban air particulates and diesel engine exhaust particulates). Several *Salmonella* strains are routinely used to examine different types of mutation. The most common strains, TA98 and TA100, reveal frameshift and base-pair substitution mutations, respectively. Other common strains include TA97, used to detect frameshift mutations, and TA102 and TA104, used to assess base-pair substitution mutations containing an AT-rich allele. Detailed information on these strains is available (Maron & Ames, 1983; Mortelmans & Zeiger, 2000). Several metabolically modified versions of TA98 and TA100 are frequently used to examine extracts of PM from combustion engines. The nitroreductase-deficient strain TA98NR is resistant to the effects of nitroarenes, and the *O*-acetyltransferase-deficient strain TA98/1,8DNP$_6$ is resistant to some of the highly mutagenic dinitropyrenes (Blumer *et al.*, 1980; McCoy *et al.*, 1981, 1983; Speck *et al.*, 1981). More recently developed metabolically enhanced strains, derived from TA98 and TA100, contain higher levels of the classical bacterial nitroreductase (i.e., YG1021 and YG1026) or *O*-acetyltransferase (i.e., YG1024 and YG1029) (Watanabe *et al.*, 1989, 1990). Strains YG1041 and YG1042 are derivatives of TA98 and TA100, respectively, and contain enhanced levels of both nitroreductase and *O*-acetyltransferase (Hagiwara *et al.*, 1993).

Due to the absence of mammalian metabolism that is required to convert some mutagenic agents to DNA-reactive electrophiles, the bacterial test system is generally supplemented with a post-mitochondrial supernatant derived from rodent liver. The most common metabolic activation system included is a fraction derived from the livers of Aroclor 1254-induced Sprague-Dawley rats, but bio-activation with a combination of phenobarbital and β-naphthoflavone is also common. Most assessments use the standard plate incorporation version of the test, whereas some assays involve preincubation of the test compound with the *Salmonella* tester strain and the metabolic activation mixture before plating. Several studies have shown that a variant of the preincubation assay, known as the microsuspension assay, provides enhanced sensitivity to combustion products such as those present in coal tar, the urine of smokers, PM from urban air and diesel engine exhaust particles (Kado *et al.*, 1985, 1986; Agurell & Stensman, 1992).

Diesel emissions and extracts

More than 70 studies published since 1989 applied the *S. typhimurium* mutagenicity test to assess the mutagenic activity of diesel engine emissions. Most of these examined organic extracts of diesel PM collected on glassfibre filters. The concentration of the test compound in these studies is generally expressed as micrograms of EOM per plate, and the results are expressed as mutagenic potency – the slope of the initial, linear portion of the concentration–response curve (i.e. potency in the number of revertants per microgram of EOM). To facilitate comparisons between PM samples generated

under different engine conditions, fuel formulations and aftertreatment scenarios, these values are often converted to revertants per equivalent mass of diesel engine exhaust particles. Moreover, to facilitate comparative evaluations of the mutagenic hazard across different engines operated with different fuels and fuel blends, and/or different pollution control devices (e.g. diesel oxidation catalyst and/or different engine test cycles, potency values are often converted to revertants per unit of engine work (i.e. per kW-h or hp-h), revertants per unit of fuel consumed, revertants per distance travelled (e.g. mile or kilometre), revertants per hour of engine operation or revertants per cubic metre of exhaust. Table 4.8 summarizes the results of recent studies that used the *S. typhimurium* mutagenicity assay to assess the mutagenic activity of diesel engine emissions.

More than 40 studies employed the *S. typhimurium* mutagenicity assay with strains TA98 and/or TA100 to assess the mutagenic activity of diesel PM extracts. The results indicated overwhelmingly that their mutagenicity, when expressed in revertants per microgram of EOM, was greater in the absence than in the presence of metabolic activation, which is consistent with earlier publications (IARC, 1989), and is also consistent with the presence of nitro-PAHs in the materials tested. It should be noted that some studies showed an increase in the mutagenicity of diesel extracts, in particular the crude extract of the semi-volatile components, in the presence of metabolic activation (Westerholm *et al.*, 2001).

More than 20 studies employed a variety of metabolically altered *Salmonella* strains (e.g. TA98NR, TA98/1,8DNP$_6$, YG1024, YG1021 and YG1029) to assess the mutagenic activity of diesel engine emissions. Many reports showed substantial reductions in the mutagenic activity of extracts of diesel exhaust particulates from light-duty vehicles in *Salmonella* strains TA98NR (nitroreductase-deficient) and TA98/1,8DNP$_6$ (*O*-acetyltransferase-deficient) compared with that observed in strain TA98, thus confirming the involvement of nitroarenes in the mutagenic response (Barale *et al.*, 1993; Tahara *et al.*, 1994; Crebelli *et al.*, 1995; DeMarini *et al.*, 2004). One of these reports showed substantially reduced mutagenicity of extracts of diesel exhaust particles from a light-duty vehicle in strain TA98NR, but not in TA98/1,8DNP$_6$, which suggests a greater contribution of mono-nitroarenes to the mutagenic response (Crebelli *et al.*, 1995). Other studies reported similar reductions for extracts of diesel exhaust particles from heavy-duty engines (Hansen *et al.*, 1994; Harvey *et al.*, 1994; Bagley *et al.*, 1998; Westerholm *et al.*, 2001). For example, Bagley *et al.* (1998) noted reductions in mutagenicity of up to 69–78% with strain TA98NR and up to 73–83% with strain TA98/1,8DNP$_6$, relative to the levels found in TA98, for extracts of PM from a heavy-duty engine.

In contrast, increased mutagenicity of diesel PM from light- and heavy-duty engines was observed in assays with metabolically modified derivatives of TA98, such as YG1021 (enhanced production of nitroreductase), YG1024 (enhanced production of *O*-acetyltransferase) or YG1041 (enhanced production of both) (Mikkonen *et al.*, 1995; Kuljukka *et al.*, 1998; DeMarini *et al.*, 2004; Turrio-Baldassarri *et al.*, 2006).

Effects of engine type, test cycle and fuel formulation on Salmonella mutagenicity

Variations in engine type, test cycle, fuel formulation and pollution control technology complicate the interpretation of the published results, but several general statements can be made.

The generation of diesel engine exhaust particles requires the installation of the engine or vehicle in a test stand to monitor engine speed, and the use of a dynamometer (e.g. eddy current, water brake or chassis dynamometer) to control engine load (see Section 1.1). Numerous test protocols (cycles) have been developed to simulate a range of driving conditions. Transient test

Table 4.8 Summary of studies on the mutagenicity of extracts of diesel exhaust particles in *Salmonella typhimurium*

Test conditions	Salmonella strains[a]/test version	Results	References
SRM 1650, DEP from 1988 2-L light-duty diesel car (no DOC); three fuels (EN97, RD1, RD2); European transient test procedure (ECE15) and EUDC, run in series; DEP collected on PUF and/or Teflon®-coated glassfibre filters, dichloromethane Soxhlet extraction	TA98, standard plate incorporation assay, Aroclor-induced rat-liver S9	PM extracts more mutagenic than PUF extracts (the latter only positive with S9); without S9, DEP extracts more mutagenic (approximately 2–5-fold) than gasoline PM extracts, expressed per mg PM; when expressed per km, DEP extracts > 100-fold more mutagenic than gasoline PM extracts	Pohjola *et al.* (2003b)
DEP from a Mercedes-Benz 6.37-L, 6-cylinder and an IVECO 5.9-L, 6-cylinder diesel test engine with SCR[b]; 13-mode ESC; 4 fuel blends (DF, RME, RSO, SMDS, 5%RME in SMDS, DF/RME/GTL blend); DEP collected on Teflon®-coated glassfibre filters, dichloromethane Soxhlet extraction	TA98 and TA100, standard plate incorporation assay, PB/5,6BF-induced rat-liver S9	Mutagenic potency values uniformly greater without S9; for the Mercedes engine, no significant difference in potency (per L exhaust gas) between DF, RME, SMDS and DF/RME/GTL blend, and RSO yielded significantly elevated potency (approximately 10-fold) and highest PM output in g/kW-h; for the IVECO engine, SCR significantly reduced mutagenic potency after 1000 h, with no difference between DF and RME, although RME associated with reduced PM emissions (g/kW-h)	Krahl *et al.* (2006, 2007a)
DEP from a Mercedes-Benz 6.37-L, 6-cylinder engine; 13-mode ESC; 7 fuels (two DFs, RME, GTL, 4 FAME mixtures from soya, palm and rapeseed); DEP collected on Teflon®-coated glassfibre filters, dichloromethane Soxhlet extraction	TA98, standard plate incorporation assay, PB/5,6BF-induced rat-liver S9	Mutagenic potency (per m^3) greater without S9 and highest for DF; RME potency less than half that of DF; DEP emission rates lower (per kW-h) for all FAMEs	Krahl *et al.* (2005)
DEP from a Mercedes-Benz 4.25-L, 4-cylinder engine; 13-mode ESC; 4 fuels (DF, RME, LSDF, LSDF with high aromatics); DEP collected on Teflon®-coated glassfibre filters, dichloromethane Soxhlet extraction	TA98 and TA100, standard plate incorporation assay, PB/5,6BF-induced rat-liver S9	Mutagenic potency (per engine h) lowest for RME; DF 4–5-fold higher than RME, and LSDF 2–3-fold higher; no significant difference with or without S9; DEP emission rates (per kW-h) highest for DF	Krahl *et al.* (2003)
DEP and SVOCs from a Mercedes-Benz, 6.37-L, 6-cylinder engine; 13-mode ESC; 5 fuel blends (DF, RME, GTL, RSO, modified RSO); DEP collected on Teflon®-coated glassfibre filters, dichloromethane Soxhlet extraction and condensates from gas phase collected at 50 °C	TA98 and TA100, standard plate incorporation assay, PB/5,6BF-induced rat-liver S9	All samples yielded a positive response, and all potency values (per L exhaust gas) unchanged or reduced with addition of S9; DEP extract for RSO yielded the highest potency values (9.7–17-fold higher than DF on TA98 and 5.4–6.4-fold higher than DF in TA100); potency of modified RSO 2.8–4.4-fold higher than RSO; RSO condensate samples also yielded the highest potency values (up to 3-fold DF); few differences between DEP extracts for DF, RME and GTL, although RME significantly greater than DF in TA100 without S9	Krahl *et al.* (2007b, 2009a)

Table 4.8 (continued)

Test conditions	Salmonella strains[a]/test version	Results	References
DEP and SVOCs from a Mercedes-Benz, 5.9-L, 6-cylinder engine, with and without DOC; 13-mode ESC; 4 fuels (2 DFs, RME, RME5); DEP collected on Teflon®-coated glassfibre filters, dichloromethane Soxhlet extraction; SVOCs from condensates	TA98 and TA100, standard plate incorporation assay, PB/5,6BF-induced rat-liver S9	Mutagenic potency [unit not provided] uniformly higher without S9; response highest for DF reference fuel, and lowest for RME5 and RME; DOC further reduced activity of RME; no significant difference in potencies of SVOCs (per m^3), with complete elimination of activity by DOC	Krahl *et al.* (2009b)
DEP and SVOCs from 3 heavy-duty diesel engines: Mercedes-Benz, 6.37-L, 6-cylinder engine, MAN, 6.87-L, 6-cylinder engine, AVL single-cylinder, 1.47-L engine; 13-mode ESC, ETC, and rated power; 4 fuel types (DF, GTL RME, RME20); DEP collected on Teflon®-coated glassfibre filters, dichloromethane Soxhlet extraction; SVOCs from condensates	TA98 and TA100, standard plate incorporation assay, PB/5,6BF-induced rat-liver S9	Mutagenic potency [unit not provided] uniformly higher without S9; for Mercedes engine, GTL lowest activity followed by DF, RME similar to DF and RME20 significantly elevated; for AVL and MAN engines, RME20 significantly elevated relative to DF, but RME lower than DF; for SVOCs from the MAN engine, DF potency greater than RME blends; for the Mercedes and MAN engines, PM emission rates (g/kW-h) for RME about half those of DF	Krahl *et al.* (2008)
DEP from a Farymann single-cylinder engine, 5 load modes (0–85%), with and without DOC; 4 fuels (DF, LSDF, RME, SME); DEP collected on Teflon®-coated glassfibre filters, dichloromethane Soxhlet extraction	TA98 and TA100, standard plate incorporation assay, PB/5,6BF-induced rat-liver S9	Mutagenic potency (per h engine operation) generally lower for RME and SME, compared with DF or LSDF; under partial load, DOC generally led to reduced mutagenicity; under heavy-duty conditions (rated power), DOC frequently led to increases in mutagenic activity	Bünger *et al.* (2006)
DEP from a VW 1.9-L TDI with DOC; FTP-75, MVEG-A, and modified MVEG-A cycles; DF and RME fuels; DEP collected on Teflon®-coated glassfibre filters, dichloromethane Soxhlet extraction	TA98, TA97a, TA102 and TA100, standard plate incorporation assay, PB/5,6BF-induced rat-liver S9	Significant positive responses for DF and RME samples in TA98 and TA100, and potency (per mg DEP) generally higher without S9; potency (per mg DEP) greater for DF compared with RME, particularly in TA98 (1.9–5.1-fold); similar pattern for potency expressed per km; potency generally higher for cycles that included a cold start (modified MVEG-A)	Bünger *et al.* (1998)
DEP from a Fendt tractor; 13-mode ESC; DF and RME fuels; DEP collected on Teflon®-coated glassfibre filters, dichloromethane Soxhlet extraction	TA98 and TA100, standard plate incorporation assay, PB/5,6BF-induced rat-liver S9	All samples elicited significant positive responses; both fuels yielded more potent samples (per L exhaust) without S9; at rated power, RME potency far lower than that of DF; at idling, little difference with and without S9, and RME potency far lower than that of DF; DF 2–8-fold higher response in TA98 and 2–3-fold higher response in TA100; RME yielded higher particle emissions (g/h)	Schröder *et al.* (1999), Bünger *et al.* (2000a)
DEP and exhaust condensate from a Mercedes-Benz Euro 3 6.37-L, 6-cylinder engine; 13-mode ESC; 4 fuels (DF, RSO, RME, GTL); DEP collected on Teflon®-coated glassfibre filters, dichloromethane Soxhlet extraction	TA98 and TA100, standard plate incorporation assay, PB/5,6BF-induced rat-liver S9	All samples elicited significant positive responses; potency (per L exhaust) higher without S9 for TA100 only; DEP extracts from RSO and heated RSO fuels yielded highest potency samples (9.7–59 fold greater than DF for TA98 and 5.4–22.3-fold greater for TA100); potency of DEP extracts from RME also significant higher than DF; condensate samples from RSO and heated RSO also significantly elevated relative to DF (up to 13.5-fold).	Bünger *et al.* (2007)

Table 4.8 (continued)

Test conditions	Salmonella strains[a]/test version	Results	References
DEP from a Farymann single-cylinder engine, 5 load modes (0–85%), without DOC; 4 fuels (DF, LSDF, RME, SME); DEP collected on Teflon®-coated glassfibre filters, dichloromethane Soxhlet extraction	TA98 and TA100, standard plate incorporation assay, PB/5,6BF-induced rat-liver S9	Mutagenic potency (per mg DEP) frequently higher without S9, and DF potency far greater (up to 10-fold) than that of RME or SME; no response in TA100 for RME and SME; potency per engine h yielded similar results and indicated that DF potency was higher at increased load and speed; PAH emissions per engine h greatest for DF and SME	Bünger *et al.* (2000b)
DEP from a Cummins 14-L, 6-cylinder, heavy-duty engine; 9 fuels with sulfur content 0.04–0.3%; FTP drive cycle; DEP collected on Teflon®-coated glassfibre filters, 30% toluene in ethanol Soxhlet extraction	TA98, microsuspension preincubation version, PB/5,6BF-induced rat-liver S9	Potency without S9 generally higher, but not for all fuels; potency per µg EOM tended to increase with decreasing sulfur content; EOM decreased with decreasing sulfur and potency per unit work (hp-h) did not vary with sulfur content	Rasmussen (1990)
Pooled gasoline PM from 'normal emitters' (1982 Nissan Maxima, 1994 GMC 1500 pick-up truck, 1995 Ford Explorer, 1996 Mazda Millenia) collected at 30 °F and 72 °F, a visible white-smoke emitter (1990 Mitsubishi Montero) and a visible black-smoke emitter (1976 Ford F-150 pick-up truck), and pooled DEP from current (2000) technology (1998 Mercedes Benz E300, 1999 Dodge 2500 pick-up truck, 2000 VW Beetle TDI) collected at 30 °F and 72 °F and high-emitter diesel (1991 Dodge 2500 pick-up truck) engines; CUD cycle; PM collected on Teflon®-coated glassfibre filters, acetone sonication extraction; SVOCs collected on PUF/XAD, acetone Soxhlet extraction	TA98 and TA100, standard plate incorporation assay, Aroclor-induced rat-liver S9	All samples (i.e. combined PM and SVOC extracts) induced significant responses, with 10-fold range among samples; among the diesel samples, current diesel (30 °F) was the most potent (per µg EOM), with modest increases without S9; high-emitter diesel potency greater than current diesel (72 °F); gasoline samples generally more potent with S9; normal emitters at 30 °F and white-smoke emitter generally more potent; current gasoline and current diesel at 72 °F were generally the least potent; subsequent multivariate analyses showed associations between mutagenicity and nitro-PAH content (e.g. 6-nitroB[*a*]P, 1-NP, 7-nitroB[*a*]A) exhaust	Seagrave *et al.* (2002); McDonald *et al.* (2004a)
Two different DEP samples: 1/ scraping from exhaust pipe and cap of diesel truck idling for 30 min; 2/ collected on glassfibre filters from engine operating under US FTP; dichloromethane sonication or pulmonary surfactant extracts of DEP	TA98, preincubation version, Aroclor-induced rat-liver S9	All samples showed concentration-related responses without S9; dichloromethane extract more potent (per mg DEP) than surfactant extract	Keane *et al.* (1991)
DEP from a 1988 Cummins heavy-duty engine with CRT; 2-mode cycle representing 25 and 75% load at 1900 rpm; conventional DF and low-sulfur DF; and an Oldsmobile light-duty vehicle; HWFET cycle; DEP for both collected on Teflon®-coated glassfibre filters, dichloromethane Soxhlet extraction	TA98, standard plate incorporation assay, Aroclor-induced rat-liver S9	Mutagenic potency (per µg EOM) of DEP extracts similar for heavy-duty engine and light-duty vehicle; light-duty DEP extract more potent without S9	Lewtas *et al.* (1981), Bagley *et al.* (1996), Valberg & Watson (1999)

Table 4.8 (continued)

Test conditions	Salmonella strains[a]/test version	Results	References
PM and SVOCs from a Caterpillar heavy-duty 10-L, 4-cylinder diesel engine, with and without CDPF; transient test cycle to represent minimal conditions; PM collected on glassfibre filters, dichloromethane Soxhlet extraction; SVOCs collected on XAD	TA98, microsuspension preincubation version, unspecified S9	Dichloromethane extracts elicited significant responses with no difference with and without S9; mutagenic potency increased with CDPF, both in terms of rev/μg EOM (weighted average 14-fold increase) and rev/μg DEP (weighted average 1.5-fold increase); CDPF resulted in 90% reduction in DEP emission rate, and weighted average 70% reduction in potency per m^3 exhaust; SVOC samples more potent without S9, and CDPF reduced potency (per m^3 exhaust) by 80%; CDPF resulted in more than 98% reduction in DEP-associated PAHs (per m^3 exhaust)	Bagley *et al.* (1991)
DEP from 6 diesel engines: 2 heavy-duty and 4 medium-duty; PM collected on glassfibre filters, dichloromethane extraction	TA98 and TA100, standard plate incorporation assay, unspecified S9	Significant concentration-related increase in TA98 and TA100, with highest responses (per μg EOM) in TA98 without S9; no significant difference between engines	Song & Ye (1995)
DEP from 2 light-duty (1.93-L and 2.5-L) engines with EGR; EUCD and FTP-75 cycles; LSDF and biodiesel (unspecified), with and without DPF, DOC and EGR modifications; DEP collected on Teflon®-coated glassfibre filters, acetone sonication extraction followed by separate acetone and benzene Soxhlet extractions	TA98 and TA100, standard plate incorporation assay, unspecified S9	Mutagenic activity (per μg DEP) highest in TA98 without S9; DPF increased potency (per μg DEP or per km) for the 1.93-L engine, and decreased potency for the 2.5-L engine (per μg PM or per km), and dramatically reduced PM emissions per km; greater engine stress elicited greater mutagenic activity; biodiesel potency (per μg DEP) lower than reference LSDF, and biodiesel emissions lower in PAHs and nitro-PAHs; reduced potency (per μg DEP or per km) with EGR; DOC contributed to slight reductions for the 2.5-L engine (per μg PM) and modest reductions in potency per km	Carraro *et al.* (1997)
DEP from a 2003 low-mileage Euro 3 2003 bus, with and without DOC or CRT, chassis dynamometer; BSC and OCC cycles; low-sulfur diesel; DEP collected on filters, organic extraction [solvent/method unspecified]	TA98, standard plate incorporation assay, with S9	Mutagenic potency (per unit PM) higher with DOC (1.8-fold) or CRT (2.2–3.4-fold); potency in rev/km higher with DOC (1.4-fold), but lower (50–70%) with CRT	Nylund *et al.* (2004)
DEP and SVOCs from a heavy-duty Mercedes-Benz OM 906 LA Euro 3-compliant, 6.4-L, 6-cylinder engine; low-sulfur diesel, RME, 5% RME in diesel, with and without DOC; ESC; DEP collected on Teflon®-coated glassfibre filters, dichloromethane Soxhlet extraction; SVOC collected on chilled surface	TA98 and TA100, standard plate incorporation assay, PB/5,6BF-induced rat-liver S9	Without S9, mutagenic potency of DEP (per m^3 exhaust) modestly higher, highest for DF, and decreased for RME and 5% v/v RME; DOC contributed to modest reductions in potency of DEP extract without S9, and slight reductions with S9, and eliminated the mutagenic activity of SVOC	Westphal *et al.* (2012)

Table 4.8 (continued)

Test conditions	Salmonella strains[a]/test version	Results	References
DEP from a heavy-duty, 5.785-L, 6-cylinder engine; ECE 13-mode cycle; DF and ethanol-DF blends containing 5, 10, 15 and 20% ethanol by volume; PM collected on glassfibre filters, dichloromethane Soxhlet extraction	TA98 and TA100, standard plate incorporation assay, rat-liver S9 [inducer unspecified]	Significant positive responses in TA98 and TA100 at the highest concentration only; higher potency (per mg EOM) without S9, particularly for lower ethanol content; generally lower responses for increasing ethanol content; potency per kW-h indicated higher mutagenicity with increasing ethanol content; PAHs (µg/kW-h) increased with increasing ethanol content	Song *et al.* (2007)
DEP from a heavy-duty, 5.785-L, 6-cylinder engine; ECE 13-mode cycle; dichloromethane Soxhlet extract of PM, extract fractionated into 6 fractions	TA98 and TA100, standard plate incorporation assay, rat-liver S9 [inducer unspecified]	Crude extract elicited significant response in TA98 with S9, and in TA100 with and without S9; TA100 potency modestly higher without S9; strong direct-acting TA98 response for organic bases (e.g. amines); strong responses for fraction containing neutral aromatics	Song *et al.* (2006)
DEP and SVOCs from a 14.19-L heavy-duty diesel truck; FRG transient bus cycle; DEP collected on Teflon®-coated glassfibre filters, dichloromethane Soxhlet extraction; SVOCs collected on XAD, PUF plugs or cryogenically; DEP extract fractionated on silica into 5 fractions with increasing polarity	TA98 and TA100, standard plate incorporation assay, Aroclor-induced rat-liver S9	Crude DEP extract more potent (per km) without S9 with highest potency in TA100; highest responses in fractions containing nitro-PAHs, dinitro-PAHs and oxygenated PAHs; SVOC crude extract far less potent than DEP extract; SVOC extracts from XAD and PUF samplers more potent than cryogenic extracts; highest potency in polar fractions containing nitro-PAHs, dinitro-PAHs and oxygenated PAHs	Alsberg *et al.* (1985), Westerholm *et al.* (1991)
DEP from two heavy-duty (11-L and 9.6-L), 6-cylinder engines; THB transient bus cycle; 10 DFs; DEP collected on Teflon®-coated glassfibre filters, dichloromethane Soxhlet extraction	TA98 and TA100, standard plate incorporation assay, Aroclor-induced rat-liver S9	Range of potency values (per m^3) for different DFs; higher potency (1–2 orders of magnitude) for the 9.6-L engine, particularly without S9; multivariate analyses showed that mutagenic activity without S9 related to nitrates and 1-NP; S9-activated mutagenicity related to certain PACs; fuel density and flash- point positively correlated to mutagenic activity; fuel sulfur contributed to amount of emitted particles and genotoxicity	Westerholm *et al.* (1991), Sjögren *et al.* (1996a, b)
DEP from an Isuzu 2.369-L, 4-cylinder engine; custom 4-stage cycle; aftertreatment with DOC, DPF or DFE; DEP collected on Teflon®-coated glassfibre filters, acetone sonication extraction	TA98, microsuspension preincubation version, without S9	Without aftertreatment, mutagenic potency higher at low load and DOC eliminated the mutagenic activity; DPF and DFE samples showed equivalent or higher mutagenic activity per µg EOM; DPF and DFE reduced PM emissions by 51–71%, and mutagenic activity (per m^3) by 30–62%	Bugarski *et al.* (2007), Shi *et al.* (2010)
DEP from a light duty Isuzu (4JG2) 3.06-L, 4 cylinder engine and a Yamaha 1.0-L engine; DEP collected on glassfibre filters, sonication extraction with benzene/ethanol (3:1), fractionated on silica gel into 4 fractions with increasing polarity	TA98 without S9 and TA100 with S9 [unspecified], standard plate incorporation assay	Mutagenic potency (per mg DEP) higher for the larger engine, particularly without S9; S9-activated mutagenicity highest in less polar fraction; direct-acting mutagenicity highest in moderately polar fractions; detected PAHs accounted for less than 0.1% of the response with S9; nitro-PAHs accounted for 12% of the direct-acting response	Yang *et al.* (2010)

Table 4.8 (continued)

Test conditions	Salmonella strains[a]/test version	Results	References
Unspecified diesel engine; idling with periodic acceleration; PM collected on glassfibre filters, dichloromethane sonication extraction, fractionated on silica gel into 5 fractions with increasing polarity	TA98 and TA100, standard plate incorporation assay, Aroclor-induced rat-liver S9	Mutagenic activity (per µg DEP) highest in TA98 without S9; no significant response in TA100; strongest responses for base, PAH and polar fractions	Lu *et al.* (1999b)
Exhaust from a 2.07-L light-duty diesel engine; 4 steady-state conditions	TA98 and TA100, direct exhaust exposure method, Aroclor-induced rat-liver S9	Mutagenic potency (per m^3) without S9 higher in TA100; higher potency at low load with increasing potency at higher speeds (i.e. maximal at low load, high speed)	Courtois *et al.* (1993)
DEP from a light-duty Nissan engine; HWFET; DEP collected on Teflon®-coated glassfibre filters, dichloromethane Soxhlet extraction	TA98 and TA100, plate incorporation and spiral assays, Aroclor-induced rat-liver S9	Mutagenic potency (per µg EOM) higher without S9, especially for TA98; spiral assay, which requires less sample, yielded stronger response, possibly due to differences in relative amounts of S9	Houk *et al.* (1991)
DEP from a light-duty 2.1-L, 4-cylinder diesel engine; FTP cycle; DEP collected on Teflon®-coated glassfibre filters, Soxhlet extraction with dichloromethane, methanol, acetone or acetonitrile, single solvent or sequential extraction	TA98, plate incorporation assay, without S9	Single-solvent extraction with dichloromethane yielded highest potency (per µg EOM); sequential extraction revealed that dichloromethane extracted 97% of total mutagenic activity	Montreuil *et al.* (1992)
DEP and SVOCs from a 4.6-L, 6-cylinder Caterpillar engine; EPA heavy-duty transient test cycle; 4 fuels (DF, RME, HySEE, HySEE50 blend); DEP collected on Teflon®-coated glassfibre filters, dichloromethane Soxhlet extraction; SVOCs collected on PUF plugs, supercritical carbon dioxide extraction	TA98 and TA100, microsuspension preincubation version, Aroclor-induced rat-liver S9	Mutagenic potency of DEP extract (per hp-h) higher without S9; HySEE potency lower than 50/50 blend with DF, which was lower than DF alone; SVOC samples from DF about 2-fold more mutagenic than HySEE; HySEE associated with considerable reductions in PM and PAH emission rates (per hp-h)	Chase *et al.* (2000)
DEP and SVOCs from a heavy-duty 7.6-L, 6-cylinder engine; low idling for 150 min; DEP collected on Teflon®-coated glassfibre filters, sequential sonication extraction with dichloromethane and methanol; SVOCs collected on PUF plugs and XAD extracted with supercritical carbon dioxide	TA98 and TA100, microsuspension preincubation version, Aroclor-induced rat-liver S9	Mutagenic activity of SVOC sample highest in TA100 with S9; DEP extract potency (per µg DEP) highest in TA98 with S9; total response for sampling period showed greatest response in TA100 with S9, followed by TA98 with S9; SVOC samples accounted for about 20% of TA98 and 50% of TA100 mutagenicity	Kado *et al.* (1996b)

Table 4.8 (continued)

Test conditions	Salmonella strains[a]/test version	Results	References
DEP and SVOCs from a Cummins 10.8-L, 6-cylinder engine with EGR, with and without CPF; LSDF; 2 steady-state modes; DEP collected on Teflon®-coated glassfibre filters, and SVOCs collected on XAD, dichloromethane Soxhlet extraction	TA98, microsuspension preincubation version, without S9	No difference between mutagenic potency of DEP or SVOC extracts (per µg EOM) with and without CPF; at lower load, CPF reduced DEP potency (per m^3) by 65% and SVOC potency by 73%; CPF significantly reduced PM, EOM and PAHs (per m^3); 1-NP emissions increased at high load	Suresh *et al.* (2001)
DEP from a heavy-duty Cummins 5.9-L engine; EPA heavy-duty cycle; 4 fuels (DF, 20% REE, 50% REE, REE), with or without DOC; DEP collected on Teflon®-coated glassfibre filters, dichloromethane sonication extraction	TA98, microsuspension preincubation version, Aroclor-induced rat-liver S9	Mutagenic potency (per µg DEP) lowest for REE and highest for DF (with and without DOC); DOC contributed to increases in mutagenic potency per µg DEP; similar potency pattern when expressed per mile: higher potency with DOC; some increase in 5- and 6-ring PAH emissions (µg per mile) for REE	Kado *et al.* (1996a)
DEP from 2 heavy-duty diesel engines: 7.3-L, 6-cylinder with SCR, and 8.9-L, 5-cylinder with EGR and DOC; transient THC bus cycle; DF and HVO; DEP collected on Teflon®-coated glassfibre filters, dichloromethane Soxhlet extraction	TA98, standard plate incorporation assay, without S9	No significant response for any sample; HVO associated with lower DEP emission rate (mg/km)	Kuronen *et al.* (2007)
DEP from 3 diesel engines: light-duty 1.686-L, 4-cylinder; heavy-duty 12.8-L, 6-cylinder with DPF and SCR; heavy-duty 10.52-L, 6-cylinder with DPF; DF and 4 plant oils (peanut, rapeseed, soya, sunflower); DEP collected on glassfibre filters, dichloromethane Soxhlet extraction	TA98, TA100 and TA Mix, fluctuation assay (Xenometrics)	All samples in the range of the negative control with no evidence of differences in activity between the fuels	Dorn & Zahoransky (2009)
DEP and SVOCs from a heavy-duty Cummins 10-L, 6-cylinder engine; 2 steady-state modes of EPA heavy-duty cycle, with and without DPF; DF and LSDF; DEP collected on Teflon®-coated glassfibre filters, and SVOCs collected on XAD, dichloromethane Soxhlet extraction	TA98, microsuspension preincubation version, Aroclor-induced rat liver S9	Mutagenic potency similar with and without S9; very little variations in potency (per µg EOM, per µg DEP, per m^3) with fuel sulfur content; higher potency at low load; DPF associated with 9–67%, 30–57% and 54–94% reductions in potency per µg EOM, per µg DEP and per m^3, respectively; SVOC mutagenicity eliminated by DPF	Kantola *et al.* (1992)
DEP and SVOCs from a heavy-duty Cummins 10-L, 6-cylinder, engine; 2 steady-state modes from EPA heavy-duty cycle; LSDF and DF; DEP collected on Teflon®-coated glassfibre filters, and SVOCs collected on XAD, dichloromethane Soxhlet extraction	TA98, microsuspension preincubation version, unspecified S9	Mutagenic potency similar with and without S9; mutagenic potency (per µg DEP) for DEP extracts and SVOCs generally higher for LSDF, and higher at low load; similar pattern for potency expressed per m^3; PM emission per m^3 lower for LSDF; PAHs (per m^3) often elevated for LSDF	Opris *et al.* (1993)

Table 4.8 (continued)

Test conditions	Salmonella strains[a]/test version	Results	References
DEP and SVOCs from a heavy-duty Cummins 10-L, 6-cylinder engine, with and without DOC; 3 steady-state modes from EPA heavy-duty cycle; DEP collected on Teflon®-coated glassfibre filters, and SVOCs collected on XAD, dichloromethane Soxhlet extraction	TA98, microsuspension preincubation version, unspecified S9	Mutagenic potency similar with and without S9; DOC increased potency of DEP extract per µg EOM for 2 lower loads, and reduced potency (either per µg DEP or per m^3) for lowest load only; DOC generally reduced emission rates (per m^3) for PM, PAHs and nitro-PAHs, especially at low load	Pataky *et al.* (1994)
DEP and SVOCs from a heavy-duty Caterpillar 7-L engine with and without DOC; custom transient cycle; two DFs (cetane-adjusted fuel (CA) and LAF); DEP collected on Teflon®-coated glassfibre filters, and SVOCs collected on XAD, dichloromethane Soxhlet extraction	TA98, microsuspension preincubation version, Aroclor-induced rat-liver S9	Mutagenic potency similar with and without S9; potency of CA fuel lower than that of LAF, but no difference when potency expressed per m^3; for CA, DOC removed 41, 51 and 66% of the mutagenic activity per µg EOM, µg DEP and per m^3, respectively; similar reductions for LAF; SVOC mutagenicity (per µg EOM) increased with DOC, which contributed to substantial declines in PAH emissions rate (per m^3)	McClure *et al.* (1992)
DEP from a heavy-duty Caterpillar 6.96-L, 4-cylinder engine; 6-mode steady-state cycle; DEP collected on Teflon®-coated glassfibre filters, dichloromethane sonication extraction	TA98, preincubation version, unspecified S9	Mutagenic potency (per µg DEP) uniformly higher with S9; highest potency at 50% load and reduced at full load	Nussear *et al.* (1992)
DEP from a light-duty 2.5-L, 4-cylinder engine; 5 steady-state conditions; DEP collected on glassfibre filters, dichloromethane Soxhlet extraction	TA98, standard plate incorporation assay, Aroclor-induced rat-liver S9	Mutagenic potency (per µg EOM) higher with S9 at no or low load, and decreased with increasing load; higher direct-acting potency with increasing nitrogen oxides; PAH emission rate (per m^3) decreased with increasing load	Christensen *et al.* (1996)
DEP and SVOCs from a heavy-duty Cummins 10.8-L, 6-cylinder engine, different levels of EGR; 2 steady-state modes from EPA heavy-duty cycle; DEP collected on Teflon®-coated glassfibre filters, and SVOCs collected on XAD, dichloromethane Soxhlet extraction	TA98, standard plate incorporation assay, without S9	Substantial increases in mutagenic potency of DEP extract (per m^3) with increasing EGR; increased EGR also associated with substantial decreases in SVOC mutagenicity (per m^3), especially at high load, and some increases in PAH emission rate (per m^3), especially at low load	Kreso *et al.* (1998)
DEP from 1986 Mercedes-Benz 3.0-L, 6-cylinder and a VW Jetta 1.5-L, 4-cylinder engines with EPTO; 4 test cycles (NYCC, HFET, CFTP, HFTP); low sulfur No. 2 diesel; DEP collected on Teflon®-coated glassfibre filters, dichloromethane Soxhlet extraction	TA98 and TA100, microsuspension preincubation version, PB/5,6BF-induced rat-liver S9	All samples elicited significant positive responses; no change in TA100 with addition of S9; 3/8 samples more potent (per µg EOM) in TA98 without S9; no significant differences between samples with and without EPTO; EPTO resulted in significant (87–92%) reductions in PM for all cycles; expressed per mile, potency values with EPTO show substantial reductions (69–95%)	Rasmussen *et al.* (1989)

Table 4.8 (continued)

Test conditions	Salmonella strains[a]/test version	Results	References
DEP from a small 0.27-L diesel engine, 2000 rpm, no load; DEP collected on glassfibre filters, sonication extraction with methanol, dichloromethane or benzene/ethanol (4:1)	TA98, TA100, TA98NR and TA98/1,8DNP$_6$, standard plate incorporation assay, Aroclor-induced rat-liver S9	Significant concentration-related responses for all samples, with highest potency in TA98 without S9; benzene/ethanol extract most potent followed by dichloromethane and methanol extracts; significant reductions in potency in TA98NR and TA98/1,8DNP$_6$ indicated presence of nitroarenes; chemical analyses confirmed elevated concentrations of 1-NP and 1,6-DNP in the benzene/ethanol extract	Tahara *et al.* (1994)
DEP and SVOCs from a heavy-duty 1983 Caterpillar engine with DOC; custom 16-mode cycle representing light- and heavy-duty operations; LSDF and SME; DEP collected on Teflon®-coated glassfibre filters, and SVOCs collected on XAD, dichloromethane Soxhlet extraction	TA98, TA100, TA98NR and TA98/1,8DNP$_6$, microsuspension preincubation version, Aroclor-induced rat-liver S9	Mutagenic potency (per kW-h) greater for LSDF compared with SME; potency far greater for DEP extracts than SVOCs, and DOC resulted in > 50% reduction in DEP and SVOC mutagenicity; potency of DEP extract from LSFD dramatically reduced in TA98NR (69–78%) and TA98-DNP (73–83%); SME emissions showed lower total PM and reduced PAHs and 1-NP relative to LSFD	Bagley *et al.* (1998)
PM from Volvo 240, 4-cylinder diesel and Volvo 4-cylinder spark ignition engines; PM collected on Teflon®-coated glassfibre filters, dichloromethane Soxhlet extraction, silica fractionation into 5 fractions with increasing polarity	TA98 and TA98NR, standard plate incorporation assay, Aroclor-induced rat-liver S9	Significant concentration-related responses for all samples, with highest potency (per m^3) for crude DEP in TA98 without S9 and crude gasoline PM in TA98 with S9; potency of diesel 7-fold greater than that of gasoline (in TA98 without S9); both reduced in TA98NR; PAH-containing fraction only active with S9 and more potent for gasoline PM; most DEP extract mutagenicity in medium polarity fractions containing nitro-PAHs, dinitro-PAHs and oxygenated PAHs; active DEP fractions showed elevated 1-NP, and mutagenicity in a subfractions containing dinitro-PAHs; oxygenated PAH derivatives were a major component of mutagenic subfractions	Strandell *et al.* (1994)
DEP and SVOCs from a Volvo 12.1-L, 6-cylinder Euro 2 truck; THB transient bus cycle; 2 DFs; DEP collected on Teflon®-coated glassfibre filters, dichloromethane Soxhlet extraction; SVOCs from acetone Soxhlet extract of PUF plugs	TA98,TA98NR and TA100, standard plate incorporation assay, Aroclor-induced rat-liver S9	For both fuels, DEP extracts more potent than SVOC extracts (per km); potency higher without S9 and reduced in TA98NR; reference DF 4–9.6-fold more potent than experimental fuel (MK1). MK1 resulted in 98% and 88% reductions in PAHs and 1-NP, respectively, in DEP	Westerholm *et al.* (2001)
DEP from a 6-cylinder diesel bus; 5 modes of the 13-mode ESC, with and without DOC; DEP collected on glassfibre filters, acetone Soxhlet extraction	TA98, TA98NR and TA100, standard plate incorporation assay, Aroclor-induced rat-liver S9	Direct-acting mutagenicity (per m^3) reduced with DOC for all modes, and in TA98NR relative to TA98; lowest reduction at high load; TA98 potency with S9 enhanced with DOC for 3/5 modes; DOC reduced PM and PAH emission rates (per m^3) by 30 and 80%, respectively.	Hansen *et al.* (1994)

Table 4.8 (continued)

Test conditions	Salmonella strains[a]/test version	Results	References
DEP from 3 light-duty vehicles (2.0-L without DOC, 2.0-L with DOC, 1.6-L without DOC); FTP drive cycle; 4 fuels (DF, LSDF); DEP collected on Teflon®-coated glassfibre filters, toluene Soxhlet extraction	TA98 and TA98NR, standard plate incorporation assay, without S9	Mutagenic potency (per km) reduced with DOC; low-sulfur fuels associated with large (30–90%) reductions in potency; difference between TA98NR and TA98 significant for reference fuel only; strong positive correlations between mutagenicity (per km) and selected PAH emission rates (per km) and fuel aromatic content	Rantanen *et al.* (1996)
DEP from an unspecified light-duty vehicle; DEP collected on glassfibre filters, dichloromethane sonication and Soxhlet extraction	TA98,TA100, TA104, TA98NR and TA98/1,8DNP$_6$, standard plate incorporation assay, without S9	Significant concentration-related increase in mutagenic activity; substantial declines in TA98NR and –1,8DNP$_6$ relative to TA98	Barale *et al.* (1993)
DEP and SVOCs from a light-duty truck engine, 2000 rpm, 2 bar BMEP; 6 fuels (DF, LSDF, 4 fuels with different aromatic content); DEP collected on Teflon®-coated glassfibre filters, dichloromethane Soxhlet extract; SVOCs from exhaust condensate	TA98,TA100, TA98NR and TA98/1,8DNP$_6$, microsuspension preincubation version, Aroclor-induced rat-liver S9	Mutagenic potency (per mg EOM) of combined DEP and SVOC extracts highest without S9; fuel change related to more than 10-fold change in mutagenicity, with higher potency for fuels enriched in aromatics; comparison across strains showed higher activity in TA100 and substantial reductions in TA98NR, the latter suggesting contributions from mononitro-PAHs	Crebelli *et al.* (1995)
DEP and SVOCs from a heavy-duty Cummins 10-L engine; 2 steady-state modes of EPA heavy-duty cycle, with and without DPF; DEP collected on Teflon®-coated glassfibre filters, and SVOCs collected on XAD, dichloromethane Soxhlet extraction, Florisil fractionation into PAHs, nitro-PAHs and polar compounds	TA98, TA98NR and TA98/1,8DNP$_6$, standard plate incorporation assay, unspecified S9	Mutagenic potency uniformly higher without S9; DPF reduced the potency for low and high load by 27 and 67% (per µg EOM), 30 and 43% (per µg DEP) and 86 and 93% (per km), respectively, and eliminated SVOC mutagenicity	Gratz *et al.* (1991)
DEP and SVOCs from a heavy-duty Cummins 10-L, 6-cylinder engine; 2 steady-state modes from EPA heavy-duty cycle; LSDF with copper additive, with and without DFP; DEP collected on Teflon®-coated glassfibre filters, and SVOCs collected on XAD, dichloromethane Soxhlet extraction	TA98, TA98NR and TA98/1,8DNP$_6$, microsuspension version, Aroclor-induced rat-liver S9	Mutagenic potency similar with and without S9; mutagenic potency (per µg EOM) slightly higher for low load, and somewhat reduced with DFP, but only with elevated additive; potency (per µg DEP) substantially reduced with DPF; potency (per m^3) higher at low load, and substantially reduced with DPF; additive reduced potency, but only for high load; potency of DEP and SVOC extracts (per µg EOM) in TA98NR and TA98/1,8DNP$_6$ substantially lower than that in TA98; DPF substantially reduced PAH emission rate (per m^3) for high load only; some nitro-PAHs increase with DPF, especially at low load	Harvey *et al.* (1994)
DEP from a 12-L, 6 cylinder Euro 3 truck, no DOC, with or without DPF; 13-mode ETC; 6 fuel blends (DF, B100, B5, B10, B20, PPO); DEP collected on Teflon®-coated glassfibre filters, ethanol/dichloromethane (1:1) sonication extraction	TA98 and YG1024, standard plate incorporation assay, Aroclor-induced rat-liver S9	No significant response in the presence of S9 for any sample; for TA98, significant response for B20 and PPO only; for YG1024, significant responses for B10, B100 and PPO only; maximal responses in YG1024 for B100 and PPO; biodiesel associated with reductions in PM (g/kW-h), PAHs and oxygenated PAHs (µg/kW-h)	Kooter *et al.* (2011)

Table 4.8 (continued)

Test conditions	Salmonella strains[a]/test version	Results	References
DEP from a heavy-duty IVECO Euro 2 7.8-L, 6-cylinder engine; 13-mode ECE; DF and RME 20; DEP collected on Teflon®-coated glassfibre filters, toluene ASE extraction; SVOCs collected on PUFs, hexane/acetone (1:1) ASE extraction; fractionated on silica into 5 fractions with increasing polarity	TA98, TA100, TA98, TA98/1,8DNP$_6$ and YG1041, standard plate incorporation assay, Aroclor-induced rat-liver S9	All samples elicited significant positive responses; potency (per mg EOM) showed little difference between DF and RME20 in any strain; most samples more potent with S9; without S9, responses generally lower in 1,8DNP$_6$ than TA98; expression of potency per kW-h showed no difference between DF and RME20; fractionation showed 80–83% of the mutagenicity in fractions containing nitro-PAHs, dinitro-PAHs and oxygenated PAHs; RME20 emissions contained slightly lower levels of PAHs; subsequent study showed greater potency (per kW-h) in YG1041 without S9 relative to TA98	Turrio-Baldassarri *et al.* (2004, 2006)
SRM 2975 and DEP from a light-duty Isuzu (4JB1) 2.7-L, 4 cylinder engine, 2000 rpm; DEP collected on glassfibre filters, dichloromethane sonication extraction, fractionated on silica gel into 4 fractions with increasing polarity	TA98, TA100, TA98NR, TA98/1,8DNP$_6$, YG1021 and YG1024, plate incorporation assay, Aroclor-induced rat-liver S9	Mutagenic potency (per µg EOM) higher without S9 for SRM 2975 and with S9 for Isuzu DEP; without S9, SRM potency higher in YG1024; Isuzu extract had about 10-fold more EOM per unit DEP and much greater potency (per µg PM), particularly in YG1021; PAH-related mutagenicity over 200-fold higher and nitroarene-related mutagenicity 8–45-fold higher	DeMarini *et al.* (2004)
DEPs from 4 heavy-duty engines (1 8.5-L, 6-cylinder, 1 7.4-L, 6-cylinder and 2 9.6-L, 6-cylinder); 13-mode ESC; 6 fuels (DF, LSDF, 2 reformulated DFs, RME and RME30); DEP collected on Teflon®-coated glassfibre filters, dichloromethane Soxhlet extraction	TA98, TA98NR and YG1021, standard plate incorporation assay, Aroclor-induced rat-liver S9	Mutagenic potency uniformly higher without S9; DF showed the highest mutagenic potency (per µg EOM), followed by LSDF reformulated DFs and RME; when expressed per kW-h, RME potency lower than DF, but higher than other fuels (due to high EOM per unit mass); potency (per µg EOM) reduced in TA98NR and increased in YG1021, compared with TA98; good correlation between mutagenic potency per kW-h and PAH emissions per kW-h; RME potency higher than predicted by PAH content	Rantanen *et al.* (1993)
DEP from a heavy-duty 19-L, 6-cylinder engine; ECE steady-state cycle; DF and LSDF; DEP collected on Teflon®-coated glassfibre filters, dichloromethane Soxhlet extraction	TA98, TA98NR and YG1021, standard plate incorporation assay, unspecified S9	Mutagenic potency (per kW-h) higher without S9, reduced in TA98 NR and increased in YG1021 (relative to TA98); 51–91% reductions in potency with reformulated low-sulfur fuel; low-sulfur fuel associated with large decrease in PM and PAH emission rates (per kW-h)	Mikkonen *et al.* (1995)
DEP from a light-duty 2-L engine; ECE and EUDC cycles in series; 3 fuels (DF and LSDFs; EN97, RD1, RD2); DEP collected on Teflon®-coated glassfibre filters, dichloromethane Soxhlet extraction	TA98 and YG1021, standard plate incorporation assay, without S9	TA98 mutagenic potency for the LSDFs reduced by 44–48% per µg EOM, and 70–78% per km, relative to reference DF; large increases in potency in YG1021 relative to TA98; LSDF potency (per µg EOM) in YG1021 reduced > 50% relative to reference DF, and 72–85% reduction per km; PM and PAHs (per km) reduced for low-sulfur fuels	Kuljukka *et al.* (1998)

Table 4.8 (continued)

Test conditions	Salmonella strains[a]/test version	Results	References
DEP from a 3.5-L diesel engine; idling engine; DEP collected on glassfibre filters, sonication extraction with benzene/ethanol (3:1), fractionated on silica gel into 5 fractions with increasing polarity	YG1024, microsuspension preincubation version, without S9	Two most polar fractions accounted for almost 98% of the mutagenic activity (per mg DEP); nitro- and dinitro-PAHs accounted over 50% of mutagenic activity in fraction 4; mutagenic activity of most polar fraction from unknown compounds	Hayakawa *et al.* (1997)
DEP from a 1.997-L, single-cylinder engine; 7 steady-state conditions; 2 fuels (standard diesel, FT); total DEP collected on Teflon®-coated glassfibre filters, MOUDI size fractionated DEP, dichloromethane Soxhlet extraction	YG1024 and YG1029, standard plate incorporation assay, Aroclor-induced rat-liver S9	Mutagenic potency (per µg EOM) elevated at higher loads, no difference between fuels; effect of S9 and strain varied with load; potency per hp-h or engine h-r showed substantial reductions for FT fuel; larger particles (> 0.1µm) tended to be more mutagenic (per µg EOM) than smaller particles	McMillian *et al.* (2002)

[a] YG1021, TA98 with plasmid pYG216, nitroreductase overproducing strain; YG1024, TA98 with plasmid pYG219, *O*-acetyltransferase overproducing strain; YG1041, TA98 with plasmid pYG233, nitroreductase and *O*-acetyltransferase overproducing strain; YG1026, TA100 with plasmid pYG216, nitroreductase overproducing strain; YG1029, TA100 with plasmid pYG219, *O*-acetyltransferase overproducing strain; YG1042, TA100 with plasmid pYG233, nitroreductase and *O*-acetyltransferase overproducing strain

[b] SINOx honeycomb catalyst with reducing agent (32.5% urea solution)

ASE, accelerated solvent extraction; B[*a*]A, benz[*a*]anthracene; B[*a*]P, benzo[*a*]pyrene; BMEP, brake mean effective pressure; BSC, Braunschweig cycle; CDPF, catalysed diesel particulate filter; CFTP, cold-start Federal Test Procedure; CPF, catalysed particulate filter; CRT, continuously regenerating (particle) trap; CUD, California unified driving cycle; DEP, diesel exhaust particles; DF, diesel fuel; DFE, disposable filter element; DNP, dinitropyrene; DOC, diesel oxidation catalyst; DPF, diesel particulate filter; ECE, European driving cycle; EGR, exhaust gas recirculation; EOM, extractable organic matter; EPA, Environmental Protection Agency; EPTO, exhaust particle trap oxidizers; ESC, European stationary cycle; ETC, European transient cycle; EUDC, extra urban driving cycle; FAME, fatty acid methyl ester (biodiesel); FRG, Federal Republic of Germany; FTP, Federal Test Procedure; GTL, gas-to-liquid; h, hour; HFET, US highway fuel economy test; HFTP, hot-start Federal Test Procedure; hp, horse power; HVO, hydrotreated vegetable oil; HWFET, US highway fuel economy test; HySEE, hydrogenated soya ethyl ester; kW-h, kilowatt-hour; LAF, low-aromatics fuel; LSDF, low-sulfur diesel fuel; min, minute; MOUDI, micro-orifice uniform deposition impactor; MVEG, motor vehicles emissions group; NP, nitropyrene; NYCC, New York City drive cycle; OCC, Orange County cycle; PAC, polycyclic aromatic compound; PAH, polycyclic aromatic hydrocarbon; PB/5,6BF, phenobarbital/5,6-benzoflavone; PM, particulate matter; PPO, pure plant oil; PUF, polyurethane foam; REE, rapeseed ethyl ester; rev, revertant; RME, rapeseed oil methyl ester; rpm, revolutions per minute; RSO, rapeseed oil; S9, metabolic activation system; SCR, selective catalytic reduction; SMDS, Shell middle distillate synthesis (gas-to-liquid diesel); SME, soya bean oil methyl ester; SRM, standard reference material; SVOC, semi-volatile organic compound; TDI, turbocharged direct injection; THB, Technische Hochschule in Braunschweig; THC, total hydrocarbon; VW, Volkswagen; XAD, adsorbent resin

cycles, such as the US Federal Test Procedure 72, the European driving cycle (ECE-15), the California unified cycle and the New York City cycle of the US EPA, comprise a temporal series of load and speed changes designed to simulate representative driving conditions. Steady-state test cycles, such as the European stationary cycle, contain a series of steady-state stages with varying loads and speeds. Several studies investigated the effect of test cycle, load and/or speed on the bacterial mutagenicity of extracts of diesel engine exhaust particles.

Extracts of diesel exhaust particles emitted by a single-cylinder research engine showed higher mutagenicity (per engine–h) with a lower engine load (Bünger *et al.*, 2006). The mutagenicity of extracts (per microgram of EOM) emitted by a light-duty engine was higher under low-load conditions (Shi *et al.*, 2010), as, in general, was that of extracts (per microgram of PM) derived from two heavy-duty engines at lower load (Nussear *et al.*, 1992; Opris *et al.*, 1993). When expressed per cubic metre, the mutagenicity of extracts of exhaust particles derived from a light-duty diesel engine was generally higher at low load and increased with increasing running speed of the engine (Courtois *et al.*, 1993). The reduction in mutagenicity (per cubic metre of exhaust) of extracts by heavy-duty vehicles in strain TA98NR (compared with that in TA98) was lowest at the highest engine load, indicating that the emission rate of mutagenic nitroarenes is reduced at higher loads (Hansen *et al.*, 1994). Reductions in mutagenicity (expressed per microgram of EOM, per microgram of PM and per cubic metre of exhaust) were also noted at higher engine loads (Kantola *et al.*, 1992; Harvey *et al.*, 1994).

In an earlier study, the mutagenicity of extracts of diesel exhaust particles (per milligram of PM) released by a light-duty vehicle was reported in general to be higher for test cycles that included a cold start phase, which was probably due to incomplete combustion in a cold engine and a lower conversion rate of the catalytic converter at lower temperatures (Bünger *et al.*, 1998).

A wide range of diesel fuels are distributed commercially for use in light- and heavy-duty engines for both on- and off-road applications (see also Section 1 of this *Monograph*). Fuels vary with respect to sulfur and aromatic contents, and are generally formulated to enhance combustion and improve engine performance. Changes in formulation, and the presence of additives to enhance combustion, are applied to improve ignition quality, expressed as cetane number. In addition, formulations are adjusted to enhance engine performance in colder climates. Most diesel fuels are derived from petroleum, with a sulfur content that varies with the intended application (e.g. on-road, locomotive and marine). In many countries (e.g. the USA, Canada, Europe and Japan), numerous applications, including those in on- and off-road heavy-duty vehicles and light-duty vehicles, require the use of low or ultralow sulfur diesels. Diesel fuels can also be derived from natural gas or coal gas, and these types are often referred to as gas-to-liquid diesel. Finally, diesel fuel can be prepared from plant or animal fats. These fuels are generally referred to as bio-diesel usually comprise alkyl esters (i.e. methyl or ethyl) of fatty acids. The most common bio-diesels (i.e. fatty acid methyl esters) are derived from rapeseed and soya bean oil. Pure plant oils or hydro-treated vegetable oils can also be used as fuel for diesel engines.

Numerous studies have examined the effect of fuel formulation on the mutagenic activity of extracts of diesel engine exhaust particles. Inter-study comparisons are often hampered by variations in engine type, test cycles, fuel sources, formulations and blending, as well as by the units in which the mutagenicity is expressed. Nevertheless, some general conclusions can be drawn regarding differences between bio-diesel and conventional diesel fuel, and the effects of changes in sulfur content or aromatic content.

Mutagenicity (expressed per unit EOM) was measured to evaluate the differences between extracts of engine exhaust particles from conventional diesel and rapeseed methyl ester or ethyl ester fuels. The mutagenicity of extracts derived from rapeseed methyl ester was lower than that of diesel fuel (Rantanen *et al.*, 1993). Little difference was found between the mutagenic activities of extracts derived from a 20% v/v rapeseed methyl ester blend or from conventional diesel fuel (Turrio-Baldassarri *et al.*, 2004). Mutagenicity (expressed per microgram on PM) was measured to compare conventional diesel engine emissions with those of rapeseed methyl ester and ethyl ester, and soya bean oil methyl ester bio-diesel fuels. In general, the mutagenic activity of extracts derived from engines run on one of the three types of bio-diesel was two- to 10-fold lower than those of engines run on conventional diesel fuel (Kado *et al.*, 1996b; Bünger *et al.*, 1998, 2000b). However, expression of the mutagenicity per unit distance or per engine–hour eliminated the difference between the two fuel types. Another study reported that extracts of bio-diesel PM (unspecified source) were less mutagenic than those derived from conventional diesel fuel (Carraro *et al.*, 1997).

Numerous studies measured mutagenicity – expressed per unit distance (i.e. per mile or kilometre), per engine–h, per cubic metre of exhaust or per unit of engine work (i.e. kW-h or hp-h) – to compare extracts of engine exhaust particles from conventional diesel fuel with those of bio-diesel PM. In many cases, the mutagenicity of bio-diesel PM was significantly lower (range, 2–8-fold) than that of PM from conventional diesel (Rantanen *et al.*, 1993; Bagley *et al.*, 1998; Bünger *et al.*, 2000a, b, 2006; Chase *et al.*, 2000; Krahl *et al.*, 2003, 2005). Recently, modest reductions in the mutagenic activity of extracts of engine exhaust particles (expressed per kilometre) were reported for rapeseed methyl ester and 5% v/v rapeseed methyl ester in low-sulfur diesel fuel without metabolic activation, and slight reductions with metabolic activation, relative to low-sulfur diesel. No fuel-related differences were detected in the mutagenic activity of semi-volatile organic compounds (Westphal *et al.*, 2012). Several studies demonstrated that the use of bio-diesel was associated with considerable reductions in the emission rates (per hp-h or kW-h) of PM, PAHs, oxy-PAHs and nitro-PAHs (Rantanen *et al.*, 1993; Carraro *et al.*, 1997; Chase *et al.*, 2000; Krahl *et al.*, 2005, 2007a, b, 2008; Kooter *et al.*, 2011; Westphal *et al.*, 2012).

However, several studies failed to show a difference between the mutagenicity of PM extracts associated with bio-diesel and conventional diesel fuel. For example, in studies with diesel exhaust particles from several light- and heavy-duty vehicles, no differences were detected between the mutagenicity (per litre of exhaust) of emissions from engines run on diesel fuel, rapeseed methyl ester, gas-to-liquid, rapeseed oil or a diesel/gas-to-liquid/ rapeseed methyl ester (3:1:1) mixture (Krahl *et al.*, 2006, 2007a, b, 2009a). In these assays, samples from the engines run on rapeseed oil or preheated rapeseed oil produced a remarkably strong mutagenic response, and combustion of rapeseed oil was associated with an increase in the rate of PM emissions (per kW-h) (see also Bünger *et al.*, 2007). Similarly, the mutagenicity (per microgram of PM, tested with *Salmonella* strain YG1024) of extracts of exhaust particles derived from an engine run on bio-diesel (EN14214) or a plant oil (DIN51605) was greater than that of samples derived from a normal diesel (EN590)-driven engine (Kooter *et al.*, 2011).

Several studies investigated the effect of sulfur content of the fuel on the mutagenic activity of extracts of diesel engine exhaust particles. The mutagenicity (per microgram of PM) was generally greater for low-sulfur fuels (Opris *et al.*, 1993, Mikkonen *et al.*, 1995; Rantanen *et al.*, 1996). One of these studies reported that the mutagenicity of the emissions (expressed as revertants/kilometre) was greater at low ambient

temperature (–7 °C) than at 22 °C (Rantanen *et al.*, 1996). In an earlier study, the mutagenicity of particle- and vapour phase-associated extracts of diesel engine exhaust particles (per microgram of EOM, microgram of PM or cubic metre of exhaust) was slightly increased with a lower engine load and with lower sulfur content of the fuel (Kantola *et al.*, 1992).

The influence of other fuel properties (e.g. aromatic content, flash-point and density) on the mutagenic activity of extracts of diesel engine exhaust particles was also investigated. Multivariate analyses of the mutagenicity (per cubic metre of exhaust) using several fuel properties as variables indicated that fuel density and flash-point were positively correlated with the mutagenic activity of the extracts of diesel engine exhaust particles (Sjögren *et al.*, 1996a, b). The mutagenicity of extracts (per microgram of EOM) derived from engines run on four fuel types with different aromatic content increased with higher concentrations of aromatic compounds (Crebelli *et al.*, 1995). A later study showed that the mutagenicity of extracts of engine exhaust particles (per kilometre) from conventional diesel fuel was four to tenfold higher than that of emissions from an 'environmentally classified' Swedish diesel fuel (MK1), which produced 88% and 98% lower emissions of particle-associated PAHs and 1-nitropyrene, and 77% and 80% lower emissions of semi-volatile PAHs and 1-nitropyrene, respectively, compared with emissions from conventional fuel (Westerholm *et al.*, 2001). The mutagenic activity of diesel exhaust particles (per microgram of EOM) from an engine fuelled with diesel–ethanol blends (5–20% v/v) decreased with increasing ethanol content; this effect was clearly seen without metabolic activation in strain TA100, but to a much lesser extent in TA98 (Song *et al.*, 2007).

Many of the above studies also examined the mutagenic activity of semi-volatile organic compounds in diesel exhausts collected via adsorption onto a solid matrix (e.g. XAD resin) or through condensation onto a chilled surface, followed by solvent elution. Similarly to PM extracts, samples of semi-volatile organic compounds were generally stronger mutagens in the absence of exogenous metabolic activation, and were less mutagenic in strains TA98NR and TA98/1,8DNP$_6$ than in strain TA98 (Harvey *et al.*, 1994; Bagley *et al.*, 1998). The specific mutagenic activity of the total vapour-phase component in TA100 (with or without metabolic activation) was reported to be similar to that of the total particulate component (Kado *et al.*, 1996b). The mutagenicity of semi-volatile organic compound extracts (expressed per kilometre or kW-h) was generally much lower than that of parallel PM extracts (Westerholm *et al.*, 1991, 2001; Bagley *et al.*, 1998).

Several studies used chemical fractionation methods to determine the physico-chemical properties and the identity of mutagens in extracts of diesel engine exhaust particles and semi-volatile organic compound concentrates. More recent studies noted increased mutagenicity (per microgram of EOM or PM) in fractions containing organic bases, neutral aromatic compounds and polar compounds (Lu *et al.*, 1999b; Song *et al.*, 2006). The most strongly mutagenic chemical fractions were those that contained nitro-PAHs, dinitro-PAHs and oxygenated PAHs (Westerholm *et al.*, 1991; Strandell *et al.*, 1994; Hayakawa *et al.*, 1997). These fractions may contain up to 80% of the mutagenic activity of an organic extract from a heavy-duty engine (Turrio-Baldassarri *et al.*, 2004, 2006). Polar fractions of an extract of diesel exhaust particles from a light-duty engine accounted for 95% of the mutagenic activity; nitro-PAHs and dinitro-PAHs accounted for 53% of the activity in a moderately polar fraction, but the activity of the most polar fractions could not be attributed to any known mutagen (Hayakawa *et al.*, 1997). In a comparative study of extracts of diesel exhaust particles from two different diesel engines, the enhanced mutagenic activity

in the more potent sample obtained after fractionation was related to high levels of PAHs and nitroarenes (DeMarini *et al.*, 2004). A micro-orifice uniform deposit impactor device was used to investigate the mutagenic activity of different sized fractions of diesel engine exhaust particles: larger particles (i.e. > 0.1 μm in diameter) tended to be more mutagenic (per microgram of EOM) than smaller particles (McMillian *et al.*, 2002).

Standard reference materials

Numerous studies have used the *Salmonella* mutagenicity assay to assess the mutagenic activity of SRMs 1650, 2975 and 1975, the results of which are summarized in Table 4.9. SRM 1650 – or its later formulation SRM 1650a or SRM 1650b – was included as one of the complex test substances in the International Programme on Chemical Safety collaborative study on complex mixtures. The results were published in a series of scientific papers (summarized in Claxton *et al.*, 1992a, b). The geometric mean mutagenic activity of SRM 1650 extracts (per microgram of PM) was generally higher in TA100 than in TA98, and the response in TA100 was stronger in the presence of metabolic activation while that in TA98 was more potent in its absence (Claxton *et al.*, 1992a). However, responses were quite variable across studies, some of which noted higher mutagenic activity in TA98 in the presence of metabolic activation (Agurell & Stensman, 1992; Goto *et al.*, 1992). Two extractions with dichloromethane removed 80% of the mutagenic substances from SRM 1650, and the remainder was extracted in acetone (Nielsen, 1992); PAHs, nitro-PAHs and heterocyclic compounds were detected in the dichloromethane extracts (Savard *et al.*, 1992). A higher mutagenic response was observed in the preincubation/microsuspension *Salmonella* assay compared with the standard plate incorporation test (Agurell & Stensman, 1992; Goto *et al.*, 1992).

Several studies investigated the behaviour of the complex mixture of components in extracts of SRM 1650. By spiking with PAHs, it was shown that the mutagenic activities of the different PAH components were additive, at least at low concentrations and with sufficient metabolic activation (Bostrøm *et al.*, 1998). Most of the mutagenic activities in extracts of SRM 1650 were associated with nitro- and dinitro-PAH fractions, and those of some of the active components were additive or less than additive in the mixture (Eide & Johnsen, 1998; Rivedal *et al.*, 2003). Negative interactions were reported between nitro-PAHs and dinitro-PAHs, and between nitro-PAHs and polar fractions in the absence of metabolic activation (Ostby *et al.*, 1997).

DeMarini *et al.* (2004) noted a stronger mutagenic response to extracts of SRM 2975 in the absence than in the presence of metabolic activation for both TA100 and TA98, and substantially reduced responses with TA98NR and TA98/1,8-DNP_6. Stronger mutagenic effects with strain YG1024 were indicative of a substantial contribution from dinitro-PAHs. Higher activity in the absence of metabolic activation, and increased mutagenicity with the nitroreductase-enhanced strains YG1021 and YG1026 had been reported previously for SRM 2975 (Hughes *et al.*, 1997). The latter study also showed that the mutagenicity activity of SRM 1650 was more PAH-dependent, while that of SRM 2975 was more nitro-PAH-dependent.

Effects of aftertreatment on Salmonella mutagenicity

Numerous studies investigated the ability of aftertreatment systems – e.g., SCR, exhaust gas recirculation, diesel oxidation catalysts, DPFs, catalysed particulate filters and continuously regenerating traps – to alter the mutagenicity of diesel emissions. Four main devices are used to control the release of regulated and non-regulated emissions from diesel engines. Exhaust gas recirculation systems recirculate a portion of the exhaust back into the engine cylinders; they are used to reduce the release of nitrogen oxides,

Table 4.9 Summary of studies on the mutagenicity of diesel standard reference materials in *Salmonella typhimurium*

Test material	Salmonella strains[a]/test version	Results	References
SRM 1650, sonication or Soxhlet extraction in dichloromethane	TA98, TA100, TA98NR and TA98/1,8DNP$_6$, standard plate incorporation assay and microsuspension/ preincubation version, Aroclor-1254-induced rat-liver S9	Summary of the IPCS collaborative study; mutagenicity (geometric mean value per mg DEP) in TA100 higher with than without S9; in TA98, higher response without S9; higher response with the microsuspension version: stronger signal in both TA100 and TA98 with than without S9; mutagenicity in TA98NR and TA98/1,8DNP$_6$ about < 50% and 20–23% that in TA98; 1-nitropyrene accounted for up to 4% of direct-acting mutagenicity.	Claxton *et al.* (1992a, b)
SRM 1650, sonication extraction in dichloromethane, and sequential extractions in hexane, hexane/diethyl ether (9:1 and 1:1), diethyl ether, methanol (increasing polarity)	TA98 and TA100, standard plate incorporation assay, Aroclor-1254-induced rat-liver S9	Dichloromethane extracts much more mutagenic (per µL extract) without S9; mutagenicity of sequential extracts decreased with increasing polarity of solvent; PAHs, nitro-PAHs and heterocyclics detected in extract; no single class of compound accounted for the observed mutagenicity	Savard *et al.* (1992)
SRM 1650, sonication extraction in dichloromethane (twice) and acetone	TA98, TA98NR and TA100, standard plate incorporation assay, Aroclor-1254-induced rat-liver S9	Nearly all (89–96%) mutagenic activity (per mg DEP) present in the first dichloromethane extract; little contribution from subsequent extracts; mutagenicity higher with S9	Nielsen (1992)
SRM 1650, sonication extraction in dichloromethane	TA98 and TA100, standard plate incorporation assay and pre-incubation version, Aroclor-induced rat-liver S9	Mutagenicity (per mg DEP) higher with TA100 than with TA98; mutagenicity in TA100 higher without than with S9; mutagenicity in TA98 lower without S9; preincubation test results slightly higher than those of the standard plate incorporation assay	Goto *et al.* (1992)
SRM 1650, sonication extraction in dichloromethane	TA98,TA98NR and TA98/1,8DNP$_6$, microsuspension/ preincubation version, Aroclor-induced rat-liver S9	Mutagenicity (per µg EOM) slightly higher without S9; considerably lower mutagenicity with TA98NR and TA98/1,8DNP$_6$, compared with TA98	Bagley *et al.* (1992)
SRM 1650, sonication extraction in dichloromethane	TA98 and TA100; microsuspension/ preincubation version, Aroclor-1254-induced rat-liver S9	Mutagenicity (per µg EOM) higher with than without S9; preincubation increased mutagenicity by 3–15-fold relative to plate incorporation	Agurell & Stensman (1992)
SRM 1650, Soxhlet extraction in dichloromethane; extract spiked with nitro-PAHs, 1-nitropyrene, 2-nitrofluorene, 1,8- dinitropyrene	TA98, standard plate incorporation assay without S9	Significant mutagenicity; evidence of additive effects with no significant interactions between nitro-PAHs or between DEP and nitro-PAHs	Eide & Johnsen (1998)

Table 4.9 (continued)

Test material	Salmonella strains[a]/test version	Results	References
SRM 1650, Soxhlet extraction in dichloromethane, fractionation on silica into 5 fractions	TA100, standard plate incorporation assay without S9	Three fractions (i.e. nitro-PAH, dinitro-PAH and polar) were mutagenic; results suggested negative interactions (antagonism) between the nitro- and dinitro-PAH fractions, and between nitro-PAH and polar fractions	Ostby *et al.* (1997)
SRM 1650, Soxhlet extraction in dichloromethane, fractionation on silica with cyclohexane/ dichloromethane/ methanol (increasing polarity)	TA98, TA100, TA98NR, TA100NR and TA98/1,8DNP$_6$, S9 [assay and source of S9 unspecified]	Significant response without S9, higher in TA100 and substantially lower in TA100NR, TA98NR and TA98DNP; most of the mutagenic activity in the nitro- and dinitro-PAH fractions; results suggested that mutagenic responses of fractions are additive or less than additive	Rivedal *et al.* (2003)
SRM 1650, Soxhlet extraction in dichloromethane; extract spiked with benzo(*a*)pyrene, benz[*a*]anthracene, pyrene, fluoranthene	TA98, TA100, TA98NR and TA100NR, standard plate incorporation assay, unspecified S9	Mutagenic effects of components such as PAHs were additive at lower concentrations, provided sufficient S9 (10%) was present	Bostrøm *et al.* (1998)
SRM 1975 (dichloromethane extract of SRM 2975)	TA98, TA100, TA98NR, TA100NR, YG1021 and YG1026, standard plate incorporation assay, Aroclor-induced rat-liver S9	Comparison of mutagenicity (per µg EOM; without S9) across strains showed the rank order YG1021 > TA98 > TA98NR > YG1026 > TA100 > TA100NR; higher activity without S9 and lower activity on NR-deficient strains	Hughes *et al.* (1997)

[a] YG1021, TA98 with plasmid pYG216, nitroreductase-overproducing strain; YG1024, TA98 with plasmid pYG219, *O*-acetyltransferase-overproducing strain; YG1041, TA98 with plasmid pYG233, nitroreductase- and *O*-acetyltransferase-overproducing strain; YG1026, TA100 with plasmid pYG216, nitroreductase-overproducing strain; YG1029, TA100 with plasmid pYG219, *O*-acetyltransferase-overproducing strain; YG1042, TA100 with plasmid pYG233, nitroreductase- and *O*-acetyltransferase-overproducing strain; TA98NR and TA100NR have low nitroreductase activity compared with TA98 and TA100; TA98/1,8DNP$_6$ is deficient in a transacetylase that activates 1,8-DNP.

DEP, diesel exhaust particles; EOM, extractable organic matter; IPCS, International Programme on Chemical Safety; PAH, polycyclic aromatic hydrocarbon; S9, metabolic activation system; SRM, standard reference material

but have been associated with an increased release of diesel soot. Diesel oxidation catalysts, which comprise a range of systems often containing palladium or platinum, catalyse the conversion of carbon monoxide and unburned hydrocarbons to carbon dioxide and water. DPFs are devices that reduce the release of PM from diesel engines. Numerous types of DPF include single-use disposable devices and a range of continuously regenerating traps with ceramic filters (e.g. Cordierite) that use catalytic or high-temperature combustion to regenerate the filter medium. SCR systems use a reducing agent, such as gaseous ammonia, and active catalytic components, including oxides of metals such as vanadium, molybdenum and tungsten, to reduce nitrogen oxides to nitrogen gas and water. Details regarding pollution control technologies applied to reduce emissions of PM, semi-volatile organic compounds and nitrogen oxides are presented in Section 1.1 of this *Monograph*.

Table 4.10 summarizes the results of studies that have investigated the relative changes in response of the measured end-points associated with different exhaust aftertreatments, such as diesel oxidation catalysts, DPFs and SCR. In several studies, catalytic devices were shown to increase the mutagenic activity (expressed per unit of EOM from diesel PM). However, the use of devices to reduce the rates of PM emissions can lead to substantial reductions in mutagenic activity when expressed per unit of engine work, per cubic metre of exhaust, per engine–h or per kilometre travelled. No studies have addressed the relative changes in genetic and related effects in experimental systems associated with emissions from diesel engines fitted with emission control devices (i.e. diesel oxidation catalysts, continuously regenerating traps and SCR) that comply with current regulations in the USA and Europe.

Several studies reported that some pollution control devices caused an apparent increase in the mutagenicity of diesel engine exhaust in *S. typhimurium*, but this was strongly dependent on the units by which mutagenicity was expressed, e.g. per unit EOM, per unit PM or per cubic metre of exhaust. Bagley *et al.* (1991) noted that the mutagenicity of extracts of diesel exhaust particles from a heavy-duty engine was increased fourteenfold in the presence of a catalysed DPF, when expressed as the number of revertants per microgram of soluble organic fraction. The same study noted a 70% reduction in mutagenicity when it was expressed as the number of revertants per cubic metre of exhaust. In the Transit Bus Emission Study, Nylund *et al.* (2004) reported that the use of a continuously regenerating trap increased the mutagenicity (in TA98 without metabolic activation) of extracts of diesel exhaust particles from a 2003 Euro-3 bus approximately two- to threefold (revertants per milligram of PM). When expressed as the number of revertants per kilometre, the mutagenicity was reduced by 50–75% relative to that seen with exhaust particles from an engine without a continuously regenerating trap. Similarly, other studies of heavy-duty diesel engine emissions noted that the use of a diesel oxidation catalyst contributed to increased mutagenicity expressed per microgram of PM (Kado *et al.*, 1996a) or per microgram of EOM, respectively (Pataky *et al.*, 1994). The mutagenicity of extracts of diesel exhaust particles (per microgram of PM or per kilometre) from a 1.93-L engine increased when a DPF was used, but considerable reductions in mutagenicity were observed in material from a 2.5-L engine under the same conditions (Carraro *et al.*, 1997). A similar study of light-duty diesel engine emissions showed that the use of a particulate filter (DPF or disposable filter elements) moderately increased the mutagenic activity of extracts of diesel engine exhaust particles (expressed per unit EOM), while the use of a diesel oxidation catalyst eliminated the mutagenic activity (Shi *et al.*, 2010).

In a recent study (Westphal *et al.*, 2012), a diesel oxidation catalyst eliminated the

Table 4.10 Effect of various aftertreatment devices on the mutagenicity of diesel engine exhaust in *Salmonella typhimurium*

Engine type	Aftertreatment	End-point assessed	Exposure/mutagenicity	Effect of aftertreatment	Reference
Heavy-duty 7.3-L, 6-cylinder engine	DOC	Mutagenicity in *Salmonella*; DEP extract	Rev/mass PM, rev/mile	Increase in mutagenicity per unit PM and per mile	Kado *et al.* (1996a)
Euro 3 compliant 6.4-L, 6-cylinder engine	DOC	Mutagenicity in *Salmonella*; DEP extract, SVOCs	Rev/m^3	Modest reduction in mutagenicity of DEP extract; mutagenicity of SVOCs eliminated	Westphal *et al.* (2012)
Single-cylinder engine	DOC	Mutagenicity in *Salmonella*; DEP extract	Rev/engine–h	Decrease in mutagenicity at partial load, increase at rated power	Bünger *et al.* (2006)
Heavy-duty 10-L, 6-cylinder engine	DOC	Mutagenicity in *Salmonella*; DEP extract, SVOCs	Rev/unit EOM; rev/mass; rev/m^3	Increase in mutagenicity per µg EOM for two lower loads; reduced mutagenic effect per unit PM or per m^3 exhaust for lowest load only	Pataky *et al.* (1994)
Heavy-duty 7-L engine	DOC	Mutagenicity in *Salmonella*; DEP extract, SVOCs	Rev/unit EOM, rev/mass PM, rev/m^3	Decrease in mutagenicity of DEP extract (all units); increase in mutagenicity of SVOCs per unit EOM	McClure *et al.* (1992)
Heavy-duty 7-L engine (1983)	DOC	Mutagenicity in *Salmonella*; DEP extract, SVOCs	Rev/kW-h	Reduction in mutagenicity (50%)	Bagley *et al.* (1998)
Six-cylinder bus	DOC	Mutagenicity in *Salmonella*; DEP extract	Rev/m^3	Reduced mutagenicity	Hansen *et al.* (1994)
Light-duty 2-L vehicle	DOC	Mutagenicity in *Salmonella*; DEP extract	Rev/km	Reduced mutagenicity	Rantanen *et al.* (1996)
Light-duty 2.369-L, 4-cylinder engine	DOC, DPF or DFE	Mutagenicity in *Salmonella*; DEP extract	Rev/unit EOM, rev/m^3	DOC eliminated mutagenicity; DPF contributed to an increased mutagenic effect per unit EOM and to a 30–62% reduction in mutagenicity per m^3 exhaust	Shi *et al.* (2010)
Euro 3 bus (2003)	DOC or CRT	Mutagenicity in *Salmonella*; DEP extract	Rev/mass PM, rev/km	Increase in mutagenicity per unit PM with DOC or CRT; mutagenicity per km higher with DOC and reduced by 50–70% with CRT	Nylund *et al.* (2004)
Two light-duty (1.93-L and 2.5-L) engines	DOC or various DPFs	Mutagenicity in *Salmonella*; DEP extract	Rev/mass PM, rev/km	DPF increased mutagenicity per unit PM or per km for 1.93-L engine, and reduced the mutagenic effect for the 2.5-L engine; DOC contributed to a slight reduction in mutagenicity per unit PM for the 2.5-L engine and to a modest reduction per km	Carraro *et al.* (1997)

Table 4.10 (continued)

Light-duty 1.5-L, 4-cylinder engine	CRT	Mutagenicity in *Salmonella*; DEP extract	Rev/unit EOM, rev/mile	No effect on mutagenicity per unit EOM; mutagenicity per mile reduced by 65–95%	Rasmussen *et al.* (1989)
Heavy-duty 10-L, 4-cylinder engine	CDPF	Mutagenicity in *Salmonella*; DEP extract, SVOCs	Rev/unit EOM, rev/mass PM, rev/m^3	Increase in DEP mutagenicity per unit EOM or per unit PM; mutagenic effects per m^3 exhaust reduced by 70% and 80% for DEP and SVOCs, respectively	Bagley *et al.* (1991)
Heavy-duty 10.8-L, 6-cylinder engine	CPF	Mutagenicity in *Salmonella*; DEP extract, SVOCs	Rev/unit EOM, rev/m^3	No effect on mutagenicity per unit EOM; reduced mutagenicity per m^3 exhaust for DEP and SVOCs	Suresh *et al.* (2001)
Heavy-duty 10-L, 6-cylinder engine	DPF	Mutagenicity in *Salmonella*; DEP extract	Rev/unit EOM, rev/mass PM, rev/m^3	Reduced mutagenicity (all units)	Kantola *et al.* (1992)
Heavy-duty 12-L, 6-cylinder engine	DPF	Mutagenicity in *Salmonella*; DEP extract	[Unit not provided]	Slight increase in mutagenicity	Kooter *et al.* (2011)

CDPF, catalysed diesel particle filter; CPF, catalysed particulate filter; CRT, continuously regenerating trap; DEP, diesel exhaust particulate/particle(s); DFE, disposable filter elements; DOC, diesel oxidation catalyst; DPF, diesel-particulate filter; EOM, extractable organic material; PM, particulate matter; rev, revertant(s); SCR, selective catalytic reduction; SVOC, semi-volatile organic compound

mutagenic activity (per cubic metre) of condensate (semi-volatile organic compounds) samples of exhausts from a Euro 3-compliant heavy-duty engine operated with several types of fuel, but had only modest (without metabolic activation) or slight (with metabolic activation) effects in reducing the mutagenicity of particle extracts. A study of heavy-duty engine emissions noted that the use of a diesel oxidation catalyst reduced the mutagenicity by 41, 51 and 66% when expressed per microgram of EOM, per microgram of PM and per cubic metre of exhaust, respectively (McClure *et al.*, 1992). The use of an oxidation catalyst was shown to lead to a considerable decrease in the level of mutagenicity emitted, which was correlated with measured reductions in the emission rates of PM, PAHs and nitro-PAHs in some cases, particularly under low-load conditions (Hansen *et al.*, 1994; Pataky *et al.*, 1994).

Several studies reported that DPFs dramatically reduce the emission rate of mutagens associated with the soluble organic fraction of diesel engine exhaust particles. For example, the use of a catalysed DPF led to 90 and 70% reductions in the rate of PM emissions and mutagenicity per cubic metre, respectively (Bagley *et al.*, 1991). Similarly, the use of a DPF reduced the rates of PM emissions by 51–71% and mutagenicity per cubic metre by 30–62% (Shi *et al.*, 2010), while the use of a catalysed particle filter substantially diminished emissions of PM and PAHs, and reduced mutagenicity per cubic metre by 65% (Suresh *et al.*, 2001). Two other studies showed that the use of a DPF was associated with reductions in mutagenicity of 9–67%, 30–57% and 54–94% (Kantola *et al.*, 1992), and 27–67%, 30–43% and 86–93% (Gratz *et al.*, 1991), expressed per microgram of EOM, per microgram of PM and per cubic metre of exhaust, respectively. In a study of two light-duty engines fitted with a continuously regenerating trap, no reduction was found in the mutagenicity (expressed per microgram of EOM) of extracts of diesel exhaust particles; however, when expressed per mile travelled, the mutagenicity was reduced by 69–95% (Rasmussen *et al.*, 1989).

Only one study investigated the effect of SCR on the mutagenicity of diesel engine emissions; this process significantly reduced the number of revertants (expressed per cubic metre of exhaust) induced by extracts of diesel exhaust particles emitted by a heavy-duty engine (Krahl *et al.*, 2006).

The application of exhaust gas recirculation in a heavy-duty engine was associated with an increase in the mutagenicity (expressed as the number of revertants per cubic metre of exhaust) of extracts of diesel exhaust particles, especially at low engine-load, and with an increase in the concentration of PAHs in the exhaust in one study (Kreso *et al.*, 1998). In contrast, other studies reported that exhaust gas recirculation reduced the mutagenicity (expressed per microgram of PM or per kilometre travelled) of extracts of diesel exhaust particles from a light-duty engine (Carraro *et al.*, 1995, 1997).

Emission control devices such as exhaust gas regulation, diesel oxidation catalysts and DPFs can strongly reduce the mutagenic activity (per cubic metre) of samples of diesel engine exhaust semi-volatile organic compounds (Gratz *et al.*, 1991; Kantola *et al.*, 1992; McClure *et al.*, 1992; Kreso *et al.*, 1998). However, in line with observations for extracts of diesel engine exhaust particles, the use of a diesel oxidation catalyst increased the mutagenic activity expressed per microgram of EOM of semi-volatile organic compounds of diesel engine exhaust (McClure *et al.*, 1992).

[The Working Group acknowledged that exhaust aftertreatment devices can alter the genetic and related effects induced by diesel engine exhaust, but recognized that confounding variables such as engine design, test cycle, fuel formulation and methods of sample processing do not allow any definitive conclusions.]

(vi) Effects observed in vitro in other prokaryotic assays

The results of several studies that used other bacterial assays to assess the genotoxic activity of diesel engine emissions are summarized in Table 4.11.

The Mutatox assay is based on the use of a dark variant of the luminescent bacterium *Vibrio fischeri*, and the presence of mutagens results in the restoration of the photoluminescence. When used to examine semi-volatile organic compounds and PM extracts of diesel engine exhaust, the assay gave similar results to those observed in the *Salmonella* mutagenicity assay, i.e. that extracts of diesel engine exhaust particulates were more mutagenic in the absence than in the presence of metabolic activation, and that semi-volatile organic compounds were more potent in the presence than in the absence of metabolic activation. In addition, the mutagenicity of PM extracts was greater at low than at high engine load (Lin & Chao, 2002).

The induction of error-prone DNA repair (a so-called SOS response) was monitored in *E. coli* PQ37 (i.e. the SOS chromotest) and *S. typhimurium* TA1535/pSK1002 (i.e. the *umu* test) exposed to extracts of diesel engine exhaust particulates. The SOS chromotest was used to examine the genotoxicity of pressurized hot-water extracts, and peak activity was associated with an extract of medium polarity (Kubátová *et al.*, 2004). As part of the collaborative study of the International Programme on Chemical Safety, the SOS chromotest was also used to examine extracts of SRM 1650, for which an eightfold increase in activity was found in the absence of metabolic activation (Nylund *et al.*, 1992). The *umu* test was used in two studies of organic extracts of exhaust particulates from a diesel generator and several light-duty diesel vehicles, respectively (Wasserkort *et al.*, 1998; Yamazaki *et al.*, 2000). The former found concentration-dependent genotoxicity in the absence of metabolic activation and a greater response at higher engine loads. The second study also noted direct-acting genotoxicity (i.e. in the absence of metabolic activation), and confirmed stronger responses in the presence of human CYP1A2 and CYP1B1.

(c) Gasoline (spark ignition) engine emissions

(i) Effects observed in vivo in experimental animals

The Working Group identified four studies that examined the genotoxic effects of emissions from spark ignition gasoline engines in experimental animals *in vivo*, the results of which are summarized in Table 4.12. The first study examined changes in benzo[*a*]pyrene hydroxylation, 7-ethoxyresorufin *O*-deethylation and NADPH-cytochrome *c* reductase activity in liver, kidney and lung microsomes following whole-body inhalation exposure of rats for 1 hour twice a day, 5 days a week for 4 weeks to diluted emissions from a two-stroke motorcycle engine. The results showed significant increases in all three activities in all tissues (Ueng *et al.*, 1998). The same study also assessed enzyme induction following intratracheal administration and noted significant increases in benzo[*a*]pyrene hydroxylation and 7-ethoxyresorufin *O*-deethylation activities in all tissues. After intraperitoneal administration, all three activities were significantly increased in the three organs, except for NADPH-cytochrome *c* reductase activity in the liver. A later study by the same authors reported that 'head-only' exposures of Wistar rats to two-stroke motorcycle engine exhaust induced significant upregulation of *Cyp1A1* and *Cyp1B1*, as well as *IL-1α*, *IL-6* and *IL-11*, i.e. genes involved in xenobiotic metabolism and inflammation, respectively (Ueng *et al.*, 2005).

Groups of 10–20 male and female mice (A/J and BALBc) and rats (SHR and Fischer 344) were exposed to both unfiltered and filtered exhaust from 4.3-L 1996 General Motors engines run on national average fuel in a simulated urban

Table 4.11 Summary of results of in-vitro analyses of diesel exhausts in miscellaneous bacterial assays

Test conditions	Exposure system	End-point(s) examined	Results	Reference
Mitsubishi 6.557-L, 6-cylinder, direct injection diesel engine; HD-FTP drive cycle and steady-state tests (9.2 and 27.5 kg/m); diesel No. 2 with methanol-containing additive; DEP collected on glassfibre filters and SVOCs on PUF and XAD-4, Soxhlet extraction in dichloromethane and dichloromethane/hexane (1:1)	Dark mutants of *Vibrio fischeri* M169 (Mutatox) for 16, 20 or 24 h with and without Aroclor-induced rat-liver S9 (preincubation, 45 min)	Reverse mutation of dark mutant to luminescent wild-type	PUF/XAD and PM extracts mutagenic with and without S9; PUF/XAD extracts more mutagenic and PM extracts less mutagenic with than without S9; PUF/XAD extracts yielded higher response at lower engine load; increasing levels of methanol additive in fuel produced stronger effect for both extracts	Lin & Chao (2002)
Bulk DEP from a diesel bus [no details given]; extractions with pressurized water (25, 50, 100, 150, 200, 250, 300 °C).	Incubation (2 h) of *Escherichia coli* PQ37 with 20 µL of DEP extract	SOS chromotest: colorimetric assessment of SOS response in *E. coli* PQ37	Maximum effect observed in fraction extracted at 150 °C (mid-polarity), possibly representing nitro-aromatics	Kubátová *et al.* (2004)
Yamaha EDA 4700 TE single-cylinder diesel generator, tested at different loads; DEP collected on glassfibre filters, Soxhlet extraction in dichloromethane	*Salmonella typhimurium* TA1535/pSK1002 (umuC test) for 2 h, Aroclor-induced rat-liver S9	Induction of SOS repair	Concentration-related increase in response without S9; strongest response at high load; strong correlation between response and amount of particle-bound PAHs	Wasserkort *et al.* (1998)
DEP from 4 diesel vehicles with 2.8-L (1993 model), 2.5-L (1996), 4.1-L (1990), 7.4-L (1989) engines; idling conditions; DEP collected on glassfibre filters, sonication extraction with benzene/ ethanol (3:1)	*S. typhimurium* TA1535/pSK1002 (umuC test) for 2 h, activation with *E. coli* expressing human CYP1A1, CYP1A2 or CYP1B1, all with NPR	Induction of SOS repair	Concentration-dependent increase in response without bioactivation; stronger effect with *E. coli* expressing CYP1B1 and CYP1A2	Yamazaki *et al.* (2000)
SRM 1650, Soxhlet extraction in dichloromethane	Incubation (2 h) of *E. coli* PQ37 with 0.3–153.6 µg DEP-equivalent/mL, Aroclor-induced rat-liver S9	SOS chromotest: colorimetric assessment of SOS response in *E. coli* PQ37	Significant positive response without S9 at ≥ 10 µg DEP-equivalent/mL; response with S9 approximately 8-fold lower	Nylund *et al.* (1992)

CYP, cytochrome P450; cyp, cytochrome; DEP, diesel exhaust particles; h, hour; HD-FTP, heavy-duty Federal Test Procedure; min, minute; NPR, nicotinamide adenine dinucleotide phosphate–cytochrome P450 reductase; PAH, polycyclic aromatic hydrocarbon; PM, particulate matter; PUF, polyurethane foam; S9, metabolic activation system; SRM, standard reference material; SVOC, semi-volatile organic compounds; XAD-4, synthetic resin to remove phenols from aqueous solution

Table 4.12 Summary of studies of the exposure of animals to gasoline engine exhaust or gasoline exhaust particulate matter *in vivo*

Engine specifications	Fuel	Run conditions	Test material collection and processing	Animal model	Route of exposure/ exposure regime	End-point(s) examined	Results	Reference
Yamaha 50-mL, 2-stroke motorcycle engine	Mixture (60:1) of unleaded gasoline and low-smoke engine oil	Idle speed, no engine load	Exhaust pipe connected via mixing chamber to exposure compartment; exhaust particles collected on 0.5-µm quartz filters, Soxhlet-extracted with dichloromethane/ hexane (1:1)	Wistar rats, 100–120 g, male	Whole-body exposures to exhaust on 2 h/d, 5 d/wk for 4 wks; intratracheal (0.1 g/kg bw) or intraperitoneal (0.5 g/kg bw, daily for 4 days) administration of extract	Benzo[*a*]pyrene hydroxylation activity in liver, kidney and lung	All routes of exposure resulted in significant increases in activity in all tissues	Ueng *et al.* (1998)
Yamaha Cabin 50-ml, 2-stroke motorcycle engine	Mixture (60:1) of unleaded gasoline and low-smoke engine oil	Idle speed, no engine load	Exhaust pipe connected via mixing chamber (1:10 dilution) to exposure compartment	Wistar rats, 7 wks, female	Head-only exposure to 21.5 mg/m^3 PM from exhaust on 2 × 1 h/d, 5 d/wk for 4 wk	Gene expression (semiquantitative RT–PCR)	Significant increases in expression of *Cyp1A1* and *Il-1α*	Ueng *et al.* (2005)
Yamaha 50-ml, 2-stroke motorcycle engine	Unleaded gasoline (95% octane)	At 150 rpm, no engine load	Stainless steel collection tube, exhaust particles collected on 0.5-µm quartz filters; PM isolated by sonication of filters in methanol	ICR mice, 8–9 wks, male	Intratracheal instillation of 160, 200 or 240 mg/kg bw PM isolated from filters (suspended in corn oil)	MN formation in peripheral blood 24, 48 and 72 h after a single dose	Significant dose-related increases in MN frequency at 24 and 48 h after exposure; antioxidants reduced the response significantly	Cheng *et al.* (2004)

Table 4.12 (continued)

Engine specifications	Fuel	Run conditions	Test material collection and processing	Animal model	Route of exposure/ exposure regime	End-point(s) examined	Results	Reference
Five different gasoline-powered scooters	[Not specified]	Idling, with periodic acceleration	High-volume air sampler; PM collected on glassfibre filters	Swiss mice, 18–20 g, male	Two daily intraperitoneal injections of 0.25, 1 or 4 mg PM in DMSO	MN frequency in PCEs 6 h after second injection	Significant dose-related increases in MN for all PM samples tested	Zhou & Ye (1997)
Gasoline-powered scooters	Gasoline with 3 different lubricants [no details given]	Idling or acceleration	High-volume air sampler; PM collected on glassfibre filters; extraction by sonication in dichloromethane	Kunming mice, 18–20 g, male	Two daily intraperitoneal injections of 0.4, 2.0 or 10.0 mg-equivalent DEP extract per 20 g bw in DMSO	MN frequency in PCEs 6 h after second injection	Significant dose-related increases in MN frequency; reduction when lubricants were added to the fuel	Zhou & Ye (1998)

bw, body weight; *Cyp*, cytochrome P450 gene; d, day; DEP, diesel exhaust particle; DMSO, dimethyl sulfoxide; h, hour; *Il*, interleukin gene; MN, micronucleus; PCE, polychromatic erythrocyte; PM, particulate matter; rpm, revolutions per minute; RT-PCT, revere transcript-polymerase chain reaction; wk, week

operating cycle. Changes in lipid peroxides (SHR rats) and DNA methylation (Fischer 344 rats and A/J mice) were significantly reversed by filtration of the particles from the exhaust (Reed *et al.*, 2008).

Cheng *et al.* (2004) examined the induction of micronuclei in the peripheral blood of mice administered intraperitoneal injections of PM from a two-stroke motorcycle engine. The frequency of micronuclei was significantly increased 24 and 48 hours after exposure; simultaneous injection of antioxidants (e.g. ascorbate and α-tocopherol) significantly reduced the magnitude of the response.

Induction of micronuclei was also studied in mouse peripheral blood following intraperitoneal injections of PM extracts or suspended PM from gasoline-powered scooters. One study examined the effect of two daily doses of PM suspended in DMSO and another measured the response to two daily doses of PM extract. Both studies noted a significant dose-related increase in the frequency of micronuclei. In the second study, the magnitude of the response was dependent on the type of lubricant used in the fuel (Zhou & Ye, 1997, 1998).

(ii) Effects in cultured mammalian cells

In comparison with diesel engine emissions (see Table 4.6), relatively few studies have assessed the genotoxic effects of emissions from gasoline engines in cultured mammalian cells, the results of which are summarized in Table 4.13.

The frequency of DNA strand breaks was measured in Chinese hamster V79 cells and in human A549 adenocarcinoma cells exposed to semi-volatile organic compounds and extracts of PM from gasoline-driven engine exhaust, respectively (Liu *et al.*, 2005; Zhang *et al.*, 2007). Both studies revealed significant concentration-related increases in DNA strand breaks, and the earlier study noted stronger responses to extracts of gasoline exhaust PM compared with extracts of diesel engine exhaust particles. However, responses to extracts of gasoline and diesel engine exhaust particles were more comparable when the results were expressed per distance driven. The other study (Zhang *et al.*, 2007) compared the effects of emissions from a gasoline-driven engine with those from an engine run on pure methanol; use of the latter fuel did not show any of the effects described for gasoline. Both studies also noted significant concentration-related increases in the frequency of micronuclei after exposure to gasoline engine exhaust PM.

In a study of PM extracts derived from a Santana spark ignition engine run on leaded or unleaded gasoline, a significant concentration-related increase in the frequency of micronuclei was observed in Chinese hamster lung cells for both fuel types (Yuan *et al.*, 1999).

The induction of chromosomal alterations (e.g. structural aberrations, sister chromatid exchange and numerical alterations) was studied in rodent cells exposed to extracts or suspensions of PM from gasoline-driven engines. In Chinese hamster ovary CHO-K1 cells exposed to a suspension of PM from a two-stroke motorcycle engine, a significant concentration-related increase was noted in the frequency of aberrant cells, in both the presence and absence of exogenous metabolic activation, but the response was higher in its presence. Antioxidants, such as ascorbate, α-tocopherol and *N*-acetylcysteine, significantly reduced the frequency of aberrant cells (Cheng *et al.*, 2004). Exposure of Chinese hamster V79 and Syrian hamster kidney cells to DMSO extracts of PM from three spark ignition engines induced significant concentration-related increases in the frequency of sister chromatid exchange and *C*-metaphases, and in the proportion of hyperdiploid and polyploid cells (Hadnagy & Seemayer, 1988, 1991). In addition, these authors reported a concentration-related increase in transformed foci in Syrian hamster kidney cells (Hadnagy & Seemayer, 1989). Assessment of sister chromatid exchange and oxidative damage (i.e. 8-OH-dG and thymine

Table 4.13 Summary of studies of the effects of gasoline engine exhausts in cultured mammalian cells or isolated DNA *in vitro*

Source of exhaust	Exposure system	End-point(s) examined	Results	Reference
Cultured cells				
Spark ignition engines: 1995 Nissan Micra 1.3-L (120 km/h) and 2000 Mitsubishi Carisma 1.8-L (120 or 50 km/h); run on CEC-RF-02–99 gasoline; PM collected on Teflon-coated glassfibre filters, Soxhlet extraction in dichloromethane; DEP and SRM 1650 tested in parallel (see Table 4.6)	Human BEAS-2B bronchial epithelial cells treated with 200 µg/mL particulate extract for up to 48 h	Frequency of stable, bulky DNA adducts (by ^{32}P-postlabelling)	Samples from both engines induced concentration-related increases in bulky adducts corresponding to levels of adduct-forming PAHs; adduct-forming potency of gasoline PM stronger than that of DEP and SRM 1650 when expressed as adducts/mg PM, but 11–31-fold lower in adducts/mg PM/km	Pohjola *et al.* (2003a)
Yamaha Cabin 50-mL, 2-stroke motorcycle engine; unleaded gasoline; particulates collected on glassfibre filters, Soxhlet extraction in dichloromethane/hexane (1:1)	Chinese hamster V79 lung cells treated with particulate extract (up to 500 µg/mL) for 12 or 24 h	Sister chromatid exchange (SCE) and oxidized bases (thymine glycol, 8-OH-dG, 8-OH-dA by GC-MS)	Dose-related increase in SCE, inhibited by antioxidants; dose-related increases in thymine glycol and 8-OH-dG; no change in 8-OH-dA	Kuo *et al.* (1998)
Three spark ignition engines; gasoline with 12.4, 1.5 or 0.03 µg/mL lead; FTP-75 and ECE driving cycles; PM collected on polyvinylchloride or glassfibre filters, reflux extraction with DMSO	Chinese hamster V79 lung cells and Syrian hamster kidney cells exposed to PM extract (up to 10 µg/mL) for 16 and 18 h, respectively	SCE, structural and numerical chromosome aberrations, C-metaphases and cell transformation	All extracts induced significant concentration-related increases in SCE, C-metaphases, polyploidy, hyperdiploidy and transformed foci	Hadnagy & Seemayer (1988, 1989, 1991)
Yamaha 50-mL, 2-stroke motorcycle engine; PM collected on quartz filters and retrieved in suspension by sonication with methanol	Chinese hamster ovary K1cells exposed to PM (0.5, 5 or 50 µg/mL in DMSO) for 3 h, 3-methylcholanthrene-induced rat-liver S9	Chromosomal aberrations	Significant dose-related increase in frequency of aberrant cells; higher response with than without S9; antioxidants reduced the response significantly	Cheng *et al.* (2004)
Santana spark ignition engine; idling and medium-duty conditions; leaded and unleaded gasoline; PM collected on glassfibre filters, extracted by sonication in dichloromethane	Chinese hamster lung cells exposed to PM at 10, 30 or 90 µg/mL for 24 h	MN frequency	Significant concentration-related increase in MN frequency; no difference between leaded and unleaded gasoline	Yuan *et al.* (1999)

Table 4.13 (continued)

Source of exhaust	Exposure system	End-point(s) examined	Results	Reference
Five 1982–96 spark ignition (gasoline) engines and 3 1998–2000 diesel engines; CUD cycle; PM and SVOCs collected, acetone-extracted, sonicated in TWEEN-80; SRM 1650a was also tested	Chinese hamster V79 lung cells exposed for 20 h	MN frequency and DNA strand breaks (by comet assay)	Gasoline PM/SVOC and SRM 1650a induced significant concentration-related increases in strand breaks and MN frequency; strongest response for gasoline PM; effects of DEP and gasoline PM were comparable when expressed per mile driven.	Liu *et al.* (2005)
Spark ignition 40-passenger bus, no aftertreatment of exhaust; idling, empty-load conditions; PM collected on glassfibre filters and extracted by sonication in dichloromethane; SVOC collected in PUF and XAD-2, extracted with dichloromethane	Human A549 adenocarcinoma cells exposed to PM extract or SVOC for 2 h (comet assay) or 24 h (MN assay) at 0.025–0.4 L-equivalent/mL	MN frequency and DNA strand breaks (by comet assay)	Significant concentration-related increase in MN frequency and DNA strand breaks	Zhang *et al.* (2007)
1992 Yamaha Cabin 50-mL, 2-stroke engine; unleaded gasoline mixed (60:1) with low-smoke engine oil; idle speed, empty-load conditions; PM collected on glassfibre filters and extracted (Soxhlet) in dichloromethane/ hexane (1:1)	Human CL5 lung epithelial adenocarcinoma cells and human BEAS-2B bronchial epithelial cells treated with PM extract (CL5: 100 µg/mL; BEAS-2B: 1, 10 or 100 µg/mL) for 6 h	Changes in gene expression (by cDNA microarray and quantitative real-time RT–PCR)	Increased mRNA expression of genes active in xenobiotic metabolism (*Cyp1A1*, *Cyp1B1*), inflammation (*Il-1α*, *Il-6, Il-11*), cell growth (*Fgf-6, Fgf-9*), tumour progression (*Fra-1*) and cell cycle (*p21*)	Ueng *et al.* (1998, 2005)
1992 Yamaha Cabin 50-mL, 2-stroke engine; unleaded gasoline mixed (60:1) with low-smoke engine oil; idle speed, empty-load conditions; PM collected on glassfibre filters and extracted (Soxhlet) in dichloromethane/ hexane (1:1)	Human HepG2 hepatoma cells and human NCI-H322 lung carcinoma cells treated with 100 µg/mL for 24 h	Benzo[*a*]pyrene hydroxylase activity in microsomal fraction	Significant elevation in rate of benzo[*a*]pyrene hydroxylation	Ueng *et al.* (1998, 2000)

Table 4.13 (continued)

Source of exhaust	Exposure system	End-point(s) examined	Results	Reference
Yamaha Cabin 50-mL, 2-stroke engine; unleaded gasoline; PM collected on quartz filters, extracted in methanol, filtered (0.2 µm) to obtain extract without particles	Human A549 adenocarcinoma cells treated with 0.02–20 µg/mL PM extract with particles or 20 µg/mL PM extract without particles	Gene expression (by semiquantitative RT–PCR and nuclear factor-κB luciferase-reporter assay)	Concentration-related increase in expression of interleukin-8 and nuclear factor-κB	Lee *et al.* (2005)
DNA in solution				
Spark-ignition engines: 1995 Nissan Micra 1.3-L (120 km/h) and 2000 Mitsubishi Carisma 1.8-L (120 or 50 km/h); run on CEC-RF-02–99 gasoline; PM collected on Teflon-coated glassfibre filters, Soxhlet extraction in dichloromethane; DEP and SRM 1650 tested in parallel (see Table 4.6)	Incubation of calf-thymus DNA with 150 µg/mL PM extract for 4 h, Aroclor-induced rat-liver S9 or xanthine oxidase	Frequency of stable, bulky DNA adducts (by ^{32}P-postlabelling and HPLC)	Gasoline PM extracts formed smaller amounts of adducts than DEP, with largest difference under reductive conditions; potency of gasoline-derived PM and DEP to form PAH-derived adducts not significantly different (with S9); DEP potency much higher when expressed as adducts/km	Pohjola *et al.* (2003b)
Gasoline-fuelled vehicle (Ford Van); HWFET driving cycle; PM collected on Teflon-coated glassfibre filters, Soxhlet extraction with dichloromethane; DEP tested in parallel (see Table 4.7)	Incubation of calf-thymus DNA with gasoline-derived PM extract (100 µg/mL) for 1.5 h, Aroclor-induced rat-liver S9 or xanthine oxidase	Frequency of stable, bulky DNA adducts (by ^{32}P-postlabelling)	Gasoline samples yielded adducts only without S9; more complex and diffuse adduct pattern compared with DEP; no indication of nitroarene-derived adducts in the xanthine oxidase-treated gasoline extract	Gallagher *et al.* (1991)

CUD, California unified driving cycle; *Cyp*, cytochrome P450 gene; DEP, diesel exhaust particles; DMSO, dimethyl sulfoxide; *Fgf*, fibroblast growth factor gene; *Fra*, folate receptor α gene; GC-MS, gas chromatography-mass spectrometry; h, hour; HPLC, high-performance liquid chromatography; HWFET, US highway fuel economy test; MN, micronucleus; 8-OH-dA, 8-oxo-2'-deoxyadenosine; 8-OH-dG, 8-oxo-2'-deoxyguanosine; PAH, polycyclic aromatic hydrocarbon; PM, particulate matter; PUF, polyurethane foam; RT–PCR, reverse transcript-polymerase chain reaction; S9, metabolic activation system; SRM, standard reference material; SVOC, semi-volatile organic compound; XAD, adsorbent resin

glycol) in Chinese hamster V79 cells and in mouse BNL.C12 liver cells exposed to extracts of PM from a two-stroke spark ignition engine showed significant dose-related increases in both end-points (Kuo *et al.*, 1998).

The frequency of bulky DNA adducts was determined in human BEAS-2B bronchial epithelial cells treated with extracts of PM from two spark ignition gasoline engines and from a diesel engine. All samples induced significant concentration-dependent responses that correlated with measured concentrations of PAHs. When expressed as adducts per milligram of PM, the results indicated that gasoline-derived PM was more potent than diesel-derived PM; however, when adjusted by PM emission rate (mg/km), the results showed that PM derived from standard diesel fuel was 31-fold more potent (Pohjola *et al.*, 2003a).

A study with calf-thymus DNA showed that extracts of PM from a spark ignition gasoline engine were less potent in forming DNA adducts than extracts of diesel engine exhaust PM, particularly following incubation under reductive conditions; in particular, the diesel PM extract was far more potent when expressed per kilometre (Pohjola *et al.*, 2003b).

Several studies assessed changes in benzo[*a*]pyrene hydroxylase activity and the expression of various genes in human cells exposed to extracts of PM from a two-stroke motorcycle spark ignition engine. One study showed that benzo[*a*]pyrene hydroxylase activity in human HepG2 hepatoma and human NCI-H322 lung carcinoma cells was significantly elevated following treatment with PM extracts (Ueng *et al.*, 2000). A subsequent study with human CL5 pulmonary epithelial cells exposed to PM extracts derived from a motorcycle engine reported increased expression of genes involved in xenobiotic metabolism (e.g. *CYP1A1* and *CYP3A7*), inflammation (e.g. *IL-1α*, *IL-6* and *IL-11*), tumour progression (e.g. *folate receptor alpha-1*), angiogenesis (e.g. *vascular endothelial growth factor-D*), apoptosis (*TNFSF10*) and cell-cycle control (*P21*) (Ueng *et al.*, 2005).

A study on engine exhausts from a two-stroke motorcycle engine noted that a PM extract of these emissions induced IL-8 production by the activation of the *nuclear factor-κB* gene and increased oxidative stress (measured as dichlorofluorescein-diacetate fluorescence) in human A549 adenocarcinoma cells (Lee *et al.*, 2005).

(iii) Effects in vitro in the Salmonella reverse mutation assay

In comparison with the numerous reports on diesel engines, a relatively small number of studies used the *Salmonella* mutagenicity assay to examine emissions from spark ignition gasoline engines, the results of which are summarized in Table 4.14.

Several studies compared the bacterial mutagenicity of PM extracts and/or semi-volatile organic compound samples from diesel and gasoline engine exhausts. Although gasoline PM extracts often displayed greater mutagenic activity in the presence of metabolic activation (Crebelli *et al.*, 1991; Carroll *et al.*, 2000; Seagrave *et al.*, 2002; Zhang *et al.*, 2007), diesel and gasoline PM extracts show similar potency when expressed per microgram of EOM (Liu *et al.*, 2005). When expressed per microgram of PM, extracts of diesel engine exhaust particles were far more potent than extracts of gasoline engine exhaust PM, especially in the absence of metabolic activation. Also, when expressed per mile driven, extracts of diesel engine exhaust particles were up to more than 100-fold more potent than those of gasoline engine exhaust PM (Pohjola *et al.*, 2003b). Liu *et al.* (2005) reported that samples of semi-volatile organic compounds from gasoline-driven engine exhaust were more mutagenic than those from diesel-driven engine exhaust when mutagenicity was expressed per microgram of EOM; however, when expressed per mile, diesel semi-volatile organic compounds

Table 4.14 Summary of studies on the mutagenicity of extracts of gasoline engine exhaust particles in *Salmonella typhimurium*

Test conditions	Salmonella strains[a]/test version	Results	Reference
PM and SVOCs from 5 1982–96 spark ignition (gasoline) engines and 3 1998–2000 diesel engines; CUD cycle; acetone extracts and SRM 1650 extract	YG1024 and YG1029, microsuspension preincubation version, Aroclor-induced rat-liver S9	Highest responses in YG1024 without S9; potency of DEP and gasoline PM similar when expressed per µg extract, but much greater for diesel (4.5–17-fold) when expressed per mile; for YG1029, DEP more potent with S9; SVOC extracts yielded positive results with little difference across strains or S9 conditions; gasoline SVOC more potent per µg EOM, but diesel more potent (1.5–2.9-fold) when expressed per mile	Liu *et al.* (2005)
PM from two spark ignition engines: 1995 Nissan Micra (120 km/h) and 2000 Mitsubishi Carisma (120 or 50 km/h), SRM 1650, DEP from 1988 2-L light-duty diesel car (no DOC); three fuels (EN97, RD1, RD2); European transient test procedure (ECE15) and EUDC, run in series; PM collected on PUFs and/or Teflon®-coated glassfibre filters, dichloromethane Soxhlet extraction	TA98, standard plate incorporation assay, Aroclor-induced rat-liver S9	Gasoline PM extracts showed similar mutagenicity with and without S9; PM extracts more mutagenic than PUF extracts, the latter were only positive with S9; without S9, DEP extracts more mutagenic (approximately 2–5-fold) than gasoline PM extracts, expressed per mg PM; when expressed per km, DEP extracts more than 100-fold more mutagenic than gasoline PM extracts.	Pohjola *et al.* (2003b)
Pooled gasoline PM from 'normal emitters' (1982 Nissan Maxima, 1994 GMC 1500 pick-up truck, 1995 Ford Explorer, 1996 Mazda Millenia) collected at 30 °F and 72 °F, a visible white-smoke emitter (1990 Mitsubishi Montero), a visible black-smoke emitter (1976 Ford F-150 pick-up truck), pooled DEP from current (2000) technology (1993 Mercedes Benz E300, 1999 Dodge 2500 pick-up truck, 2000 VW Beetle TDI) collected at 30 F and 72 °F and high-emitter diesel (1991 Dodge 2500 pick-up truck); CUD cycle; PM collected on Teflon®-coated glassfibre filters, acetone sonication extraction; SVOCs collected on PUF/XAD, acetone Soxhlet extraction	TA98 and TA100, standard plate incorporation assay, Aroclor-induced rat-liver S9	Gasoline samples generally more potent (per µg EOM) with S9; normal emitters at 30 °F and white-smoke emitter generally more potent; current gasoline and diesel at 72 °F were generally the least potent; multivariate analyses showed associations between mutagenicity and nitro-PAH content (e.g. 6-nitrobenzo[*a*]pyrene, 1-nitropyrene, 7-nitrobenz[*a*]anthracene) of exhaust	Seagrave *et al.* (2002)
PM/DEP from a 1.1-L spark ignition engine and a light-duty 2.5-L diesel engine; unspecified cycle; PM collected on Teflon®-coated glassfibre filters, dichloromethane Soxhlet extraction, fractionation into acidic, neutral and basic compounds	TA98,TA97, TA102, TA100, TA104, TA98NR, TA100NR and TA98/1,8DNP$_6$, and *E. coli* WP2 *uvrA*, plate-incorporation assay, Aroclor-induced rat-liver S9	Potency of spark ignition PM extracts (per mg EOM) much higher with S9, and highest response for TA100; significant response in TA102 for spark ignition engine only; DEP and spark ignition PM extract fractions showed highest activity in TA98 in acidic and neutral fractions; mutagenicity of spark ignition PM higher with S9	Crebelli *et al.* (1991)

Table 4.14 (continued)

Test conditions	Salmonella strains[a]/test version	Results	Reference
PM from 2 light-duty spark ignition engines; FTP, ECE and EUDC cycles, with and without catalytic aftertreatment; PM collected on glassfibre filters, dichloromethane Soxhlet extraction	TA98, TA98NR and TA98/1,8DNP$_6$, standard plate incorporation assay, without S9	Mutagenic potency (per µg PM) uniformly higher without S9, with modest reductions in TA98NR and TA98/1,8DNP$_6$, relative to TA98; aftertreatment reduced mutagenicity by 61–99% (per µg) and 92–99% (per mile) and associated with reductions in PM, PAHs and 1-nitropyrene emissions rates (per mile) of 40–80%, 71–99% and 87–92%, respectively	Cooper & Shore (1989)
SVOCs and PM from a light-duty, 4-cylinder spark ignition engine; 3-stage drive cycle; fuels with various concentrations of ETBE or MTBE; PM collected on Teflon®-coated glassfibre filters, dichloromethane Soxhlet extraction; SVOCs from exhaust condensates	TA98 and TA100, standard plate incorporation assay, unspecified S9	At rated power, potency (per L exhaust) higher without S9; no response from SVOC samples; additives reduced mutagenicity of all samples; reduction most pronounced for 10% ETBE in TA98	Westphal *et al.* (2010)
PM from 6 1.3–2.0-L light-duty spark ignition engines; modified MVEG cycle; 2 reformulated gasolines; PM collected on Teflon®-coated glassfibre filters, dichloromethane Soxhlet extraction	TA98 and YG1021, plate-incorporation assay, unspecified S9	Direct-acting mutagenicity (per km) highest for older vehicle without fuel injection, lowest for lean burning and multiport injection; the engine without fuel injection showed higher activity in YG1021; S9 enhanced activity for engine tested at –7 °C; reformulated gasoline associated with reduced mutagenicity; higher mutagenicity per km associated with higher PM emissions	Kokko *et al.* (2000)
PM from a Santana spark ignition engine; idling and medium-duty cycle; PM collected on glassfibre filters, dichloromethane sonication extraction	TA98 and TA100, standard plate incorporation assay, Aroclor-induced rat-liver S9	Significant concentration-related increase in TA98; higher response without S9; no significant response in TA100; no difference between leaded and unleaded gasoline	Yuan *et al.* (1999)
PM from spark ignition passenger bus, no aftertreatment; PM collected on glassfibre filters, sonication extraction with dichloromethane; SVOCs collected on PUF and XAD, extracted with dichloromethane	TA98 and TA100, standard plate incorporation assay, Aroclor-induced rat-liver S9	Significant concentration-related increase in TA98; higher response with S9; no significant response in TA100	Zhang *et al.* (2007)
PM from 5 types of gasoline-powered scooters; idling with periodic acceleration; high-volume air sampler, PM collected on glassfibre filters, dichloromethane Soxhlet extraction	TA98 and TA100, standard plate incorporation assay, Aroclor-induced rat-liver S9	Significant concentration-related increase in TA98 without S9 for 4/5 PM extracts; significant response in TA98 with S9 for 2 samples; no significant response in TA100	Zhou & Ye (1997)

Table 4.14 (continued)

Test conditions	Salmonella strains[a]/test version	Results	Reference
PM from a gasoline-powered scooter; idling or acceleration; 3 different lubricants; high-volume air sampler, PM collected on glassfibre filters, dichloromethane sonication extraction	TA98 and TA100, standard plate incorporation assay, Aroclor-induced rat-liver S9	Significant concentration-related increase in TA98 (μg EOM/plate); no difference with and without S9; no significant response in TA100; new lubricant associated with significant decline in mutagenicity and PM emission rate	Zhou & Ye (1998)
PM from a 2-stroke spark ignition scooter engine; ISO 6460 drive cycle; 3 2-stroke engine oils, with and without exhaust treatment; PM collected on Teflon®-coated glassfibre filters, dichloromethane Soxhlet extraction	TA98 and TA100, plate incorporation assay, Aroclor-induced rat-liver S9	Highest potency (per unit PM) in TA100 with S9; potency and PM emissions reduced for synthetic engine oils, and with use of exhaust catalyst	Sakai *et al.* (1999)
PM from a Yamaha 50-ml, 2-stroke motorcycle engine; PM collected on quartz filters and isolated by sonication with methanol and suspended in DMSO	TA98, TA100 and TA102, plate incorporation, 3-methylcholanthrene-induced rat-liver S9	Significant concentration-related responses in TA98, TA100 and TA102 with S9; significant reductions with addition of antioxidants	Cheng *et al.* (2004)
PM from a 0.5-L, 2-cylinder, 2-stroke engine; 5-mode steady-state snowmobile cycle; 2 engine oils; PM collected on glassfibre filters, dichloromethane extraction	[Strains unspecified], microsuspension preincubation version, unspecified S9	Mutagenic potency (per μg PM) increased 3–5-fold with S9, and reduced for bio-synthetic engine oil; potency per hp-h also 3–5-fold higher with S9	Carroll *et al.* (2000)
PM from 1 4-stroke and 2 2-stroke marine spark ignition engines; 5-mode steady-state cycle; PM collected on Teflon®-coated glassfibre filters, dichloromethane Soxhlet extraction	TA98, microsuspension preincubation version, unspecified S9	Mutagenic potency (per μg PM) much higher for 2-stroke engine, and spark ignition engine (higher PAHs per unit PM) more than 3-fold more potent than carburetor engine; no enhancement with S9; potency per 67-min run showed highest mutagenic activity for the carburetor engine (highest PM emission rate)	Kado *et al.* (2000)
PM from 1 4-stroke and 1 2-stroke marine spark ignition engines; 5-mode steady-state cycle; PM collected on glassfibre filters, dichloromethane Soxhlet extraction	TA98, microsuspension preincubation version, without S9	Mutagenic potency (per μg DEP) for 4-stroke engine 1.5–2.5-fold higher than 2-stroke; similar pattern for potency expressed per kW-h	Wasil *et al.* (2004)

[a] YG1021, TA98 with plasmid pYG216, nitroreductase overproducing strain; YG1024, TA98 with plasmid pYG219, *O*-acetyltransferase overproducing strain; YG1041, TA98 with plasmid pYG233, nitroreductase and *O*-acetyltransferase overproducing strain; YG1026, TA100 with plasmid pYG216, nitroreductase overproducing strain; YG1029, TA100 with plasmid pYG219, *O*-acetyltransferase overproducing strain; YG1042, TA100 with plasmid pYG233, nitroreductase and *O*-acetyltransferase overproducing strain

CUD, California unified driving cycle; DEP, diesel exhaust particles; DOC, diesel oxidation catalyst; ECE, European driving cycle; EOM, extractable organic matter; ETBE, ethyl *tert*-butyl ether; EUDC, extra urban driving cycle; FTP, Federal Test Procedure; hp, horse power; ISO, International Standards Organization; kW-h, kilowatt-hour; min, minute; MTBE, methyl *tert*-butyl ether; MVEG, motor vehicles emissions group; PM, particulate matter; PUF, polyurethane foam; S9, metabolic activation system; SVOC, semi-volatile organic compound; XAD, adsorbent resin

were 1.5- to threefold more mutagenic (Liu *et al.*, 2005).

PM extracts of exhaust derived from two gasoline-driven spark ignition engines showed uniformly higher mutagenic activity in the absence of metabolic activation, and modest reductions in activity in strains TA98NR and TA98/1,8DNP$_6$. Catalytic aftertreatment reduced the mutagenicity by 61–99% when expressed per microgram of EOM and by 92–99% when calculated per mile driven; these reductions were associated with declines in the emission rates of PM, PAHs and nitro-PAHs (Cooper & Shore, 1989).

Changes in fuel formulation can reduce the mutagenic activity (expressed per kilometre driven) of PM emissions from gasoline-driven spark ignition engines (Kokko *et al.*, 2000).

Higher mutagenic activity of gasoline-derived PM emissions (expressed per litre of exhaust) in the absence rather than in the presence of metabolic activation was reported in a recent study that also showed pronounced reductions in the mutagenic response with the use of fuel additives, such as ethyl-*tert*-butyl ether (Westphal *et al.*, 2010).

Several studies examined the mutagenic activity of extracts of PM emitted by small gasoline engines. A suspension of PM from a two-stroke motorcycle engine produced a significant concentration-related mutagenic response in *S. typhimurium* TA98, TA100 and TA102, but only in the presence of exogenous metabolic activation (Cheng *et al.*, 2004). The mutagenicity decreased after pretreatment with antioxidants, such as α-tocopherol, ascorbate and *N*-acetylcysteine.

Two studies assessed the mutagenic activity (per microgram of EOM) of an extract of PM from several two-stroke scooter engines. The first study showed no difference in mutagenicity in *S. typhimurium* TA98 in the presence or absence of metabolic activation, and noted a reduction in mutagenic activity associated with a newer lubricant in the fuel (Zhou & Ye, 1998). The second study reported a stronger effect (per milligram of PM) in TA100 in the presence of metabolic activation, and showed reduced mutagenic activity when synthetic engine oil was used and catalytic exhaust aftertreatment was applied (Sakai *et al.*, 1999).

Extracts of PM from exhaust of a two-stroke snowmobile engine were three- to fivefold more mutagenic (per microgram of PM or per hp-h) in the presence than in the absence of metabolic activation, but the mutagenicity was lower when a biosynthetic lubricant was used (Carroll *et al.*, 2000).

Two studies assessed the mutagenic activity of PM extracts derived from the exhaust of two- and four-stroke outboard marine engines, with either fuel injection or a carburettor. The first study reported that the mutagenicity of PM extracts from a two-stroke engine was much higher (per microgram of PM) than that of extracts from the four-stroke engine, with no enhancement from metabolic activation (Kado *et al.*, 2000). PM emissions from the engine fitted with a fuel injection system had a higher PAH content, but the higher PM emission rate of the engine with a carburettor contributed to the higher mutagenic activity. The second study demonstrated that the mutagenic activity (per microgram of PM or per kW-h) of PM extracts from the four-stroke engine was 1.5–2.5-fold higher than that of PM extracts from the two-stroke gasoline direct injection engine (Wasil *et al.*, 2004).

4.4 Other data relevant to carcinogenicity

4.4.1 Diesel engine exhaust

Numerous human clinical and experimental animal studies have been conducted to investigate the non-cancer health effects of diesel engine exhaust. Selected studies are discussed below for a diverse range of health end-points, including lung function, lung inflammation, immunology

and infection, systemic inflammation and brain inflammation. More comprehensive reviews of the voluminous non-cancer health effects are available (EPA, 2002; Hesterberg *et al.*, 2009). Most of the findings discussed below are applicable to older types of diesel exhaust, which was emitted from diesel engines manufactured before 2007. However, a few of the studies reviewed here examined the toxicity of exhaust from modern diesel engines that has been referred to in Section 1.1 as 'new-technology diesel engine exhaust'. Because an understanding of the health consequences of new-technology diesel engine exhaust is important, a subsection regarding the limited studies that have been carried out has also been included below.

(a) Lung function

(i) Humans

The possible effects on the lung of chronic occupational exposures to low levels of diesel engine exhaust emissions were studied cross-sectionally in railroad engine house workers (Battigelli *et al.*, 1964), iron ore miners (Jörgensen & Svensson, 1970), potash miners (Attfield *et al.*, 1982), coal miners (Reger *et al.*, 1982), salt miners (Gamble *et al.*, 1983), coal miners exposed to oxides of nitrogen generated (in part) by diesel engine emissions underground (Robertson *et al.*, 1984) and bus garage workers (Gamble *et al.*, 1987). Effects of relatively high concentrations of automobile emissions have been described among bridge and road tunnel workers in two large cities (Speizer & Ferris, 1963; Ayres *et al.*, 1973). Changes in lung function over a 5-year period have also been studied longitudinally among coal miners working underground in mines with and without diesel engines (Ames *et al.*, 1984). Some, but not all, of the results from these studies showed decrements in lung function and an increased prevalence of respiratory symptoms in subgroups exposed to engine emissions.

Controlled studies of human exposure have reported mixed findings for the effects of diesel engine exhaust on lung function, including a general lack of statistically significant effects on lung volumes (e.g. forced vital capacity and forced expiratory volume in one second), but statistically significant effects on specific airway resistance (Nightingale *et al.*, 2000; Nordenhäll *et al.*, 2001; Mudway *et al.*, 2004; Stenfors *et al.*, 2004). Mudway *et al.* (2004) exposed 25 healthy adult volunteers for 2 hours to diluted whole diesel engine exhaust with a particulate concentration of approximately 100 μg/m^3. No significant effects in standard lung function tests (forced vital capacity and forced expiratory volume in one second) were observed. Consistent with these findings, Stenfors *et al.* (2004) observed a lack of statistically significant effects on forced vital capacity and forced expiratory volume in one second among groups of 25 healthy and 15 mildly asthmatic volunteers exposed for 2 hours to whole diesel engine exhaust with a particulate concentration of 108 μg/m^3. Both Mudway *et al.* (2004) and Stenfors *et al.* (2004) reported small, but significant, effects on airway resistance; Stenfors *et al.* (2004) reported similar increases in airway resistance in both healthy and asthmatic subjects (4.1 and 6.5%, respectively).

(ii) Experimental animals

Short-term exposure to diesel engine exhaust (28 days) led to a 35% increase in pulmonary air flow resistance in Hartley guinea-pigs (Wiester *et al.*, 1980), and increased vital capacity and total lung capacity in Sprague-Dawley rats exposed to raw exhaust (Pepelko, 1982a).

Prolonged exposure of rats to diluted diesel engine exhaust has led to impairment of lung function in some studies (Gross, 1981; Heinrich *et al.*, 1986a, b; McClellan, 1986; Maier *et al.*, 2008), but not in others (Green *et al.*, 1983). No significant impairment of lung function was reported in hamsters (Heinrich *et al.*, 1986b).

A classic pattern of restrictive lung disease was observed in cats after 124 weeks of exposure to diesel engine exhaust (weeks 1–61: dilution factor air:diesel, 18; particles, ~6 mg/m^3; weeks 62–124: dilution factor air:diesel, 9; particles, ~12 mg/m^3) (Moorman *et al.*, 1985). No such effect was observed during the first 61 weeks of the study (Pepelko *et al.*, 1980, 1981; Moorman *et al.*, 1985).

No impact on the function of the large airways, the elastic properties of the lung or tracheal mucous transport was observed in sheep exposed to diesel engine exhaust particulates (Abraham *et al.*, 1980).

Animal and human models have demonstrated the effects of diesel engine exhaust particulates on the enhancement of immunoglobulin (Ig) E production and the promotion of inflammatory responses (Casillas *et al.*, 1999), including asthma (Casillas & Nel, 1997; Saxon & Diaz-Sanchez, 2000; Chiaverini, 2002; Jang *et al.*, 2005; McCunney, 2005). Experimental models in which animals were exposed to diesel engine exhaust particulates then challenged with various allergens showed significantly higher responses in treated animals than in matched controls, indicating hyper-responsiveness (Muranaka *et al.*, 1986; Suzuki *et al.*, 1993, 1996; Fujimaki *et al.*, 1994, 1995; Birumachi *et al.*, 2001; Yamashita *et al.*, 2001; Farraj *et al.*, 2006).

Subchronic exposures of rats to new-technology diesel engine exhaust showed small but statistically significant trends in pulmonary function (e.g. lower values for forced vital capacity and decrements in diffusing capacity for carbon monoxide in the lung) and forced expiratory variables of mean mid-expiratory flow after 3 and 12 months of exposure (discussed in McDonald *et al.*, 2012).

(b) Alterations in the immune system, inflammation and risk of cancer

Diesel engine exhaust and, in particular, diesel engine exhaust particulates have been linked with a variety of adverse health effects that potentially have a bearing on the immune system, including pulmonary inflammation.

(i) Humans

Several human clinical studies of exposures to whole diesel engine exhaust have investigated inflammatory responses in the lungs of both healthy and asthmatic volunteers (Salvi *et al.*, 1999, 2000; Nightingale *et al.*, 2000; Nordenhäll *et al.*, 2000, 2001; Mudway *et al.*, 2004; Pourazar *et al.*, 2004, 2005; Stenfors *et al.*, 2004; Behndig *et al.*, 2006). These studies have generally reported evidence of mild pulmonary inflammatory responses to elevated short-term exposures by inhalation to diesel engine exhaust with particulate concentrations in the range of 100–300 µg/m^3.

Behndig *et al.* (2006) observed responses indicative of mild bronchial inflammation (e.g. increased numbers of neutrophils and mast cells in the bronchial mucosa, and increased numbers of neutrophils, IL-8 and myeloperoxidase concentrations in bronchial lavage), but no evidence of an inflammatory response in the alveolar compartment based on differential lavage and bronchial biopsy 18 hours after exposure to 100 µg/m^3 of airborne PM with a diameter < 10 µm for 2 hours. In addition, significant increases in urate and reduced glutathione were observed in alveolar lavage, but not in bronchial lavage. The authors suggested that the different inflammatory responses in the conducting airways and alveolar regions of the lung were related to the movement of glutathione and urate into the lung surface to protect against inflammation in the alveolar region.

Inflammatory responses in the lung are a hallmark of the lung overload response in rats caused by protracted, elevated exposures to

diesel engine exhaust. An increase in neutrophilic inflammation has been defined as the critical biological response to lung overload (see Section 4.2.2).

Human clinical studies demonstrated the detrimental effects of exposure to diesel exhaust particulates on asthma and allergy. Subjects challenged nasally with a dose of diesel engine particulates equivalent to 40 hours of ambient exposure in Los Angeles (0.3 mg) were found to have increased IgE isotype switching resulting in an increase in total IgE levels (Diaz-Sanchez *et al.*, 1994, 1997; Fujieda *et al.*, 1998). Diesel engine exhaust particulates also appear to have direct effects on mast cells and basophils (Devouassoux *et al.*, 2002; Nemmar *et al.*, 2004). BAL fluid of healthy individuals exposed to diesel engine exhaust showed increased histamine levels, indicating an acute inflammatory response (Salvi *et al.*, 1999). In addition, dust mite-sensitive subjects challenged with dust mites had a threefold greater nasal histamine response when diesel engine exhaust particulates were co-administered with the allergen and an increased sensitivity to the onset of symptoms from exposure to dust mites (Diaz-Sanchez *et al.*, 1999, 2000).

In a chamber study, human volunteers exposed to 200 μg/m^3 of diesel engine exhaust particulates for 2 hours showed an increase in sputum neutrophils and myeloperoxidase 4 hours after the exposure, indicating an inflammatory response in the airways (Nightingale *et al.*, 2000). An earlier study also showed an increase in sputum neutrophils and a migration of alveolar macrophages into the air spaces (Rudell *et al.*, 1999).

Goblet cell hyperplasia with increased metaplastic and dysplastic epithelia and an increase in leukocytes were found in 136 nonsmoking customs officers responsible for clearing diesel heavy-duty vehicles (for 8.4 hours per day, 42 hours per week) compared with a nonsmoking control group of 58 officers who worked in the office only (Glück *et al.*, 2003). The authors suggested that the significant goblet cell hyperplasia, together with a clear increase in leukocytes, could be taken as an indication of a chronic state of irritation of the nasal mucosa with an inflammatory response.

(ii) Experimental systems

McDonald *et al.* (2004a) reported evidence of diesel engine exhaust-induced lung inflammation, respiratory syncytial resistance and oxidative stress among C57BL/6 mice acutely exposed to 200 μg/m^3 of uncontrolled diesel exhaust emissions (6 hours a day for 7 days) from a single-cylinder diesel engine generator (Model YDG 5500E; Yanmar, Osaka, Japan). However, these effects were not observed among a second group of mice exposed to emissions from the same test engine operated using a catalysed ceramic trap and low-sulfur fuel (McDonald *et al.*, 2004b). The composition of the exhaust was also significantly different, as expected based on the differences in technology.

CD-1 mice exposed to diesel engine exhaust (350, 3500 or 7000 μg/m^3 for up to 24 months) showed altered IgM, IgC and IgA antibody responses to a challenge with sheep red blood cells (Bice *et al.*, 1985). The IgE antibody response of BDF_1 mice was increased after five intranasal inoculations of a suspension of diesel engine exhaust particles in ovalbumin solution (Takafuji *et al.*, 1987).

Two samples of different types of diesel exhaust particulate – automobile-derived diesel exhaust particulates and standard reference material (SRM 2975) – were administered to CD-1 mice in an involuntary aspiration test for pulmonary toxicity. Chemical analysis showed that the automobile-derived sample had more than 10 times the amount of extractable organic material and less than one-sixth of the amount of elemental carbon than SRM 2975. Both diesel engine exhaust particulates produced mild acute lung injury and an increase in IL-6. The

automobile-derived diesel exhaust particulate sample independently induced macrophage influx and activation and stimulated an increase in TNFα, macrophage inhibitory protein-2 and IL-5 (Singh *et al.*, 2004).

The airways and the alveoli of Wistar rats exposed to filtered exhaust for 24 months were infiltrated by inflammatory cells, while morphological changes in their lungs were reduced (Kato *et al.*, 2000). Similar inflammatory responses were seen in the lungs of 16 1-year-old male Wistar Kyoto rats injected with diesel engine exhaust particulates in saline; the rats also had decreased heart rates and blood pressure (Nemmar *et al.*, 2007).

Increased numbers of alveolar macrophages containing diesel engine exhaust particles and type II pneumocytes, and an accumulation of inflammatory cells within the alveoli and septal walls were observed after a 24-hour exposure of Fischer 344 rats to high concentrations of diesel engine exhaust particles (6 mg/m^3) (White & Garg, 1981). Macrophage aggregates were still present 6 weeks after a 2-week exposure to diesel engine exhaust particles (6 mg/m^3) (Garg, 1983).

Following prolonged exposure of rats to diesel engine exhaust particles (2–5 mg/m^3), particle-containing alveolar macrophages and type II cell hyperplasia were observed (Heinrich *et al.*, 1986a; Iwai *et al.*, 1986; Vallyathan *et al.*, 1986). Increases in both the number and size of macrophages and in the number of polymorphonuclear leukocytes were also observed in rats and hamsters (Chen *et al.*, 1980; Vostal *et al.*, 1982; Strom, 1984; Heinrich *et al.*, 1986a), together with elevated levels of lymphocytes (Strom, 1984; Heinrich *et al.*, 1986a).

Fischer 344 rats chronically exposed to diluted whole diesel engine exhaust showed a dose-dependent focal accumulation of soot with parallel active inflammation involving alveolar macrophages adjacent to the terminal bronchiole, progressive fibrosis, epithelial hyperplasia and squamous metaplasia (adjacent to fibrotic foci) (Mauderly *et al.*, 1987).

Significant alveolar epithelial hyperplastic, inflammatory and septal fibrotic responses were seen in lung sections from male cynomolgus monkeys and Fischer 344 rats exposed to diesel engine exhaust for 7 hours a day, 5 days a week for 24 months (Nikula *et al.*, 1997a, b).

In guinea-pigs exposed to diesel engine exhaust for up to 8 weeks, B- and T-cell counts in lymph nodes were not altered (Dziedzic, 1981). No change was observed in the immunological function of splenic B or T-cells from Fischer 344 rats exposed for up to 24 months to diesel engine exhaust (Mentnech *et al.*, 1984).

(c) Changes in lung morphology, biochemistry and cytology

(i) Humans

No data were available to the Working Group.

(ii) Experimental systems

Damage to the lungs of experimental animals has been demonstrated in numerous studies (Warheit, 1989). The lung weights of mice, rats and Syrian golden hamsters chronically exposed to diesel engine exhaust particulates were significantly greater than those of controls (Heinrich *et al.*, 1986a). An increased lung:body weight ratio was also observed in guinea-pigs following an 8-week exposure (20 hours a day, 7 days a week) to emissions from a Nissan CN6–33 engine (dilution of 1:13 in clean air) (Wiester *et al.*, 1980).

Exposure of rats for 30 months to diesel engine exhaust (particles, 1–4 mg/m^3) resulted in dose-dependent irregularity, shortening and loss of cilia in ciliated epithelia, particularly in the trachea and main bronchi (Ishinishi *et al.*, 1988).

Intratracheal instillations of three different doses of various formulations from groups of vehicles decreased potency measures for lactose dehydrogenase, lavage protein and cytotoxicity in all study groups; emissions from current-technology diesel engines elicited a similar response to

those from high-emitter diesel engines (Seagrave *et al.*, 2002). In a similar experiment in Syrian hamsters, an influx of neutrophils was observed in BAL fluid with an elevation of protein and histamine, and a rapid activation of circulating platelets (Nemmar *et al.*, 2003).

(d) Other effects

(i) Humans

No data were available to the Working Group.

(ii) Experimental systems

Exposure to diesel engine exhaust particles or their extracts has been reported to have no effect (Chen & Vostal, 1981; Rabovsky *et al.*, 1984) or only a moderate effect (Lee *et al.*, 1980; Pepelko, 1982a, b; Dehnen *et al.*, 1985; Chen, 1986) on aryl hydrocarbon hydroxylase activity in the lung and liver of mice and rats and in the lung of hamsters. Cyp1A1 expression in murine lung has been shown to increase following exposure to diesel engine exhaust particles (Takano *et al.*, 2002), which have also been shown to increase Cyp1B1 in rat brain microvessels, probably through AhR activation (Jacob *et al.*, 2011). Quinone reductase, Cyp1A1 and Cyp2B1 were increased in the lung tissue of rats exposed to diesel engine exhaust particulates whereas GST-pi protein and catalase were decreased (Rengasamy *et al.*, 2003).

Exposure of Fischer 344/Crl rats by inhalation to diesel engine exhaust doubled the rate of 1-nitropyrene metabolism in both the nasal tissue and perfused lung, and the amount of ^{14}C that bound covalently to lung macromolecules was increased fourfold (Bond *et al.*, 1985).

One week after instillation, significantly more residual benzo[*a*]pyrene was found in the lungs of A/Jax mice exposed to diesel engine exhaust for 9 months, probably because the benzo[*a*]pyrene had bound to the exhaust particles (Cantrell *et al.*, 1981; Tyrer *et al.*, 1981).

4.4.2 Gasoline engine exhaust

(a) Lung function

(i) Humans

No data were available to the Working Group.

(ii) Experimental systems

Long-lasting functional disturbances in the lung were observed in beagle dogs after exposure to raw or irradiated gasoline engine exhaust (carbon monoxide, 114–126 mg/m^3) for 68 months (Lewis *et al.*, 1974; Gillespie, 1980). In contrast, no impairment in lung function was detected in Crl:COBS CD(SD)BR rats exposed for 45 or 90 days to diluted (1:10) exhaust from a catalyst-equipped gasoline engine (particles, 11.32 ± 1.27 mg/m^3; carbon monoxide, 19.5 ± 3.5 mg/m^3; Pepelko *et al.*, 1979). No change in lung function was observed in male and female mice (A/J and BALBc) or rats (SHR and Fischer 344) exposed to both unfiltered and filtered exhaust from 4.3-L 1996 General Motors engines run on national average fuel in a simulated urban operating cycle (Reed *et al.*, 2008)

(b) Alterations in the immune system, inflammation and risk of cancer

(i) Humans

No data were available to the Working Group.

(ii) Experimental systems

The BAL fluid of mice exposed to gasoline engine exhaust showed elevated levels of total protein, alkaline phosphatase, γ-glutamyl phosphatase, lactose dehydrogenase, TNF-α and IL-6 but not of IL-1β or IL-10, indicating lung damage and an inflammatory response (Sureshkumar *et al.*, 2005). Mice exposed to gasoline engine emissions demonstrated elevated levels of plasma endothelin-1, but not systemic inflammation (Campen *et al.*, 2006).

(c) Changes in lung morphology, biochemistry and cytology

(i) Humans

No data were available to the Working Group.

(ii) Experimental systems

Several studies in beagle dogs reported atypical epithelial hyperplasia in animals exposed for 68 months to raw or irradiated gasoline engine exhaust (carbon monoxide, 114 mg/m^3). Increases in alveolar air space and cilia loss were observed after a long recovery period following exposure to the irradiated exhaust (Hyde *et al.*, 1980).

(d) Other effects

(i) Humans

No data were available to the Working Group.

(ii) Experimental systems

Lund *et al.* (2007) examined aortas and plasma to evaluate histochemical markers, gene expression and oxidative stress following exposure to gasoline engine emissions and found transcriptional upregulation of genes associated with vascular modelling and increased markers of vascular oxidative stress.

4.5 Susceptibility in humans

The factors involved in human susceptibility to exposure to diesel engine exhaust include (i) genetic polymorphisms, (ii) vulnerable populations, (iii) underlying lung and airway disease and (iv) status of the respiratory tract microbiome.

The available data do not provide clear or consistent evidence of the influence of any particular genotype on biomarkers associated with exposure to engine exhaust, a situation that is somewhat similar to the influence of genetic polymorphisms on biomarkers or the risk for lung or urinary bladder cancer associated with cigarette smoking. Although some associations have been found with increased risks for lung cancer from smoking among individuals who are *GSTM1* null or who have a particular *CYP1A1* polymorphism, the data are inconsistent (IARC, 2012b). No studies have been carried out on the influence of other susceptibility factors such as vulnerable populations, underlying disease and the microbiome in relation to exposure to diesel engine exhaust and the risk for lung cancer.

4.5.1 Genetic polymorphisms

See Table 4.15

Although at least five studies have found some influence of genotype on the biomarker response to exposure to engine exhaust in humans, the data are not consistent. A study of bus maintenance workers found that, among N-*acetyltransferase 2* (*NAT2*) slow acetylators, *GSTM1*-null subjects had higher DNA adduct levels (determined by postlabelling using thin-layer chromatography) in their lymphocytes than *GSTM*-positive subjects (Hou *et al.*, 1995). However, *GSTM1* and *NAT2* status was found to have no influence on *Hprt* mutant frequency in lymphocytes. The three major *NAT2* alleles, *M1*, *M2* and *M3*, were determined by restriction analysis.

Among a group of diesel revision workers and established street vendors in an urban area of Chile, Adonis *et al.* (2003) and Gil *et al.* (2003) found no influence of the *GSTM1* gene alone on levels of urinary 1-hydroxypyrene. However, they found that subjects who had the combined *CYP1A1*2A* and *GSTM1*-null genotype had higher levels of urinary 1-hydroxypyrene than those who did not ($P = 0.055$).

The *NAT2* slow acetylators among a group of bus drivers or mail carriers who worked outdoors in Copenhagen, Denmark, had higher concentrations of urinary 1-hydroxypyrene than fast acetylators; however, this genotype did not influence the levels of urinary mutagenicity (Hansen *et al.*, 2004). The *NAT2* phenotype was

Table 4.15 Summary of human susceptibility to exposure to atmospheres containing engine exhaust

Reference	Exposure group (exposure type)	Genotype[a]	Result	Assoc. with Exposure	
				Urine	Air
Hou *et al.* (1995)	Bus maintenance workers (predominantly diesel)	*GSTM1*-null *GSTM1*, *NAT2*	↑ DNA adducts N/E on *Hprt*		
Adonis *et al.* (2003), Gil *et al.* (2003)	Truck/bus inspectors and street vendors	*CYP1A1*2A* + *GSTM1*-null	↑ OHPy		
Hansen *et al.* (2004)	Bus drivers or mail carriers	*NAT2* slow	N/E on urinary mutagenicity	↑ OHPy	
Topinka *et al.* (2007)	Police officers (mixed)	*CYP1A1* variants *CYP1A1* +, *GSTM1*+	↑ DNA adducts ↓ DNA adducts		PAHs +
Palli *et al.* (2001)	Traffic exposure	*XP2-Lys751/Gln* variants	↑ DNA adducts		
Nielsen *et al.* (1996b)	Bus drivers (mixed)	*GSTM1*, *NAT2*	N/E DNA adducts		
Knudsen *et al.* (2005)	Miners driving heavy-duty vehicles (predominantly diesel)	*GSTM1*, *GSTT1*, *GSTP1*	N/E on DNA adducts or DNA damage		1-NP
Villarini *et al.* (2008)	Road tunnel workers (predominantly diesel)	*CYP1A1; GSTM1*	N/E on MN		

[a] All genotypes were determined from DNA extracted from blood; the *NAT2* slow phenotype was determined from urinary metabolites, as noted in the text.

CYP, cytochrome P450; GST, glutathione *S*-transferase; Hprt, hypoxanthine-guanine phosphoribosyltransferase; NAT, *N*-acetyltransferase; MN, micronuclei; N/E, no effect; OHPy, 1-hydroxypyrene; ↑, increase; ↓, decrease; +, positive

determined by analysis of the urinary metabolites of caffeine.

Among policemen in Prague, Czech Republic, those who had both *CYP1A1* and *GSTM1* polymorphic variants had the lowest levels of DNA adducts in lymphocytes determined by postlabelling/thin-layer chromatography (Topinka *et al.*, 2007). The levels of DNA adducts were also highest in subjects with variants of *CYP1A1*, independent of *GSTM1* status, and were associated with the levels of carcinogenic PAHs in the air.

Subjects in Florence, Italy, who had occupational exposure to traffic exhaust and at least one variant of the DNA nucleotide excision-repair gene, *XPD-Lys751/Gln*, had increased levels of DNA adducts in their lymphocytes (determined by postlabelling/thin-layer chromatography; Palli *et al.*, 2001).

However, *GSTM1* and *NAT2* (*M1*, *M2* and *M3* alleles) had no effect on the levels of DNA adducts (measured by postlabelling/HPLC) in the lymphocytes of Copenhagen bus drivers (Nielsen *et al.*, 1996b). Also, *GSTM1*, *GSTT1* and *GSTP1* had no effect on the levels of DNA adducts (measured by postlabelling) or DNA damage (measured by the comet assay) in the lymphocytes of shale-oil mine workers exposed to diesel engine exhaust (Knudsen *et al.*, 2005).

CYP1A1 and *GSTM1* had no influence on the observed increase in micronuclei in the lymphocytes of road tunnel construction workers relative to office controls in Genoa, Italy (Villarini *et al.*, 2008). An *in vitro* study of diesel engine exhaust extracts in the *umu* gene expression assay in *S. typhimurium* TA1535/pSK1002 found that the extract was activated by CYP1B1 and CYP1A2 but not by CYP1A1 (Yamazaki *et al.*, 2000).

4.5.2 Vulnerable populations

Children represent a population that is vulnerable to exposure to diesel engine exhaust because they spend most of their time playing outside, have higher respiratory rates than adults and have underdeveloped lungs (Suwanwaiphatthana *et al.*, 2010). Alveolar development is arrested in the young due to underlying inflammatory disease (Bäckström *et al.*, 2011). An age-dependent theoretical model was developed to predict PM dosimetry in the lungs of children. The simulation predicted that the lung deposition of 2-μm particles was 38% in adults but was as high as 73% in 7-month-old children (Musante & Martonen, 2000). However, it is uncertain how these events may affect susceptibility to lung cancer later in life.

4.5.3 Underlying lung disease

While there is evidence that exposure to diesel engine exhaust may exacerbate asthma and chronic obstructive disease and increase lung injury, it is not known how these chronic conditions may affect susceptibility to lung cancer from this exposure.

4.5.4 Respiratory tract microbiome

The respiratory tract is lined with microflora that expresses enzymes which may increase the metabolic activation of some components of diesel engine exhaust, e.g. nitroarenes. The composition of the microbiome is also affected by the use of antibiotics for upper respiratory tract infections. Thus, the microbiome represents a changing microenvironment that may affect susceptibility to the carcinogenic constituents of diesel engine exhaust.

4.6 Mechanistic considerations

4.6.1 Diesel engine exhaust

Diesel engine exhaust is a complex mixture comprised of both gaseous and particulate components. The gaseous phase comprises nitrogen oxides, sulfur, ozone and organic compounds, such as acetaldehyde, acrolein,

benzene, 1,3-butadiene, formaldehyde, naphthalene and PAHs and nitro-PAHs. Benzene, 1,3-butadiene, formaldehyde and benzo[*a*]pyrene are carcinogenic in experimental animals and have been classified as human carcinogens (IARC, 2010a, 2012a). Naphthalene (IARC, 2002) and acetaldehyde (IARC, 1999) have been classified as possibly carcinogenic to humans, and several other PAHs (IARC, 2010a) and nitro-PAHs (see the *Monographs* in this Volume) have been classified as probably or possibly carcinogenic to humans.

The particulate phase contains organic compounds including PAHs (IARC, 2010a) and nitro-PAHs (see the *Monographs* in this Volume), many of which have been classified by the IARC as possible or probable carcinogens. It also contains trace metals, including lead, manganese, arsenic and chromium, and those from the catalyst aftertreatment systems – vanadium, copper and iron. Arsenic and arsenic inorganic compounds and chromium VI have been classified as human carcinogens (IARC, 2012c), whereas lead (IARC, 1987) and inorganic lead compounds (IARC, 2006b) have been classified as probably or possibly carcinogenic to humans, respectively. These components are adsorbed onto carbon core particles that vary in size from coarse to fine to ultrafine nanoparticles.

(a) Organic solvent extracts of particulates from diesel engine exhaust

Organic solvent extracts of particulates of diesel engine exhaust contain higher-molecular-weight organic compounds, including PAHs and nitro-PAHs. Organic compounds adsorbed on particles have been evaluated for genotoxicity in *in vitro* and *in-vivo* assays, and have a broad range of activities. They are mutagenic in bacterial assays and in mammalian cells, form bulky DNA adducts, and induce unscheduled DNA synthesis, sister chromatid exchange, chromosomal aberrations and morphological cell transformation (IARC, 1989). They also induce skin papillomas in mouse skin tumour–initiation studies and adenocarcinomas in mice after dermal application in cancer bioassays (IARC, 1989). More recent studies indicate that organic solvent extracts of diesel engine exhausts induce DNA strands breaks and oxidative damage, as well as increase the expression of genes involved in xenobiotic metabolism, oxidative damage, antioxidant responses and the cell cycle in mammalian cells in culture.

There is strong mechanistic evidence that organic solvent extracts of diesel engine exhaust particulates induce cancer in experimental animals by a genotoxic mechanism.

PAHs are biotransformed by phase I metabolic enzymes to a series of dihydrodiols, phenols, quinones and polyhydroxylated metabolites. Dihydrodiols can be metabolized further to chemically reactive intermediates (diol epoxides) that bind covalently to DNA to form DNA adducts. PAHs can undergo one-electron reduction to form radical cations that can adduct to DNA forming depurinating PAH adducts. PAH quinones can undergo redox cycling, generating ROS that damage DNA. Many of these DNA modifications have been associated with the induction of mutation and, eventually, tumour formation. Further metabolism of PAH metabolites by phase II enzymes converts many of the primary metabolites to glucuronic acid and sulfate and glutathione conjugates that are excreted in the faeces and urine. Nitro-PAHs can be reduced by nitroreductases to hydroxylamino and amino metabolites; the hydroxylamino intermediates have been shown to bind to DNA to form covalent DNA adducts. Some nitro-PAHs can undergo both oxidative and reductive metabolism, forming mixtures of metabolites and DNA adducts containing nitro, dihydrodiol or amino functionalities (IARC, 1989). The detailed mechanism(s) of the metabolic activation of PAHs have been described previously (IARC, 2010a) and in this *Monograph* (see Section 4.1). Detailed mechanism(s) of the

metabolic activation of nitro-PAHs are described in the individual *Monographs* in this Volume.

(b) Bioavailability

The organic compounds adsorbed onto particles need to be bioavailable to manifest their genotoxic activities. They can be removed from diesel engine exhaust particulates extremely efficient with organic solvents, and some evidence shows that biological fluids can facilitate their bioavailability based on *in vitro* assays. Human serum and rat lung cytosol released 79–85% of the organic solvent-extractable mutagenic activity from diesel engine exhaust particles based on the results of bacterial mutation assays. Although the serum-associated mutagens were largely undetectable in a bacterial mutation bioassay, incubation of the serum with protease increased its mutagenic activity (King *et al.*, 1981). Incubation and phagocytosis of diesel engine exhaust particles by rabbit alveolar macrophages removed more than 97% of the bacterial mutagenic activity (King *et al.*, 1983). When dispersed into a simulated pulmonary surfactant, diesel engine exhaust particulates were mutagenic in bacteria and genotoxic in mammalian cells, inducing unscheduled DNA synthesis, sister chromatid exchange, micronuclei and chromosomal aberrations (Keane *et al.*, 1991; Gu *et al.*, 2005).

(c) Gaseous phase of diesel engine exhaust

Acetaldehyde, acrolein, benzene, 1,3-butadiene, naphthalene and formaldehyde have been detected in the gaseous phase of diesel engine exhaust, which was mutagenic to bacteria. Each of these individual compounds possesses a different mechanism of carcinogenic action, and it is not known whether these mechanisms contribute to the overall carcinogenicity of diesel engine exhaust in terms of their potential co-carcinogenic, cell-proliferative and/or tumour-promoting activities.

(d) Particulates from diesel engine exhaust

Diesel engine exhaust particles exhibit a variety of genotoxic effects *in vitro* and *in vivo*. After dietary exposure, they induced DNA adducts, oxidative DNA damage and DNA strand breaks in the colon, liver and lung of rats, but did not induce oxidative DNA damage in the lung or liver of mice. After intratracheal instillation, diesel engine exhaust particles caused oxidative DNA damage in the lungs of mice and transformed foci in the tracheal epithelium of rats. Exposure of mice to diesel engine exhaust particles by inhalation produced oxidative damage, adducts and strand breaks in lung DNA as well as heritable germ-cell mutations.

A major fraction of diesel engine exhaust particulates comprises nanoparticles and agglomerates (Kittelson, 1998) to which humans are exposed (Sawant *et al.*, 2008; Hesterberg *et al.*, 2010). Diesel engine exhaust particulates generate superoxide and hydroxyl radicals (Vogl & Elstner, 1989; Sagai *et al.*, 1993; Kumagai *et al.*, 1997), and increase the levels of 8-OH-dG in DNA (Møller *et al.*, 2010) *in vitro* and *in vivo*. In one seminal study, levels of 8-OH-dG were measured in the lungs of mice after intratracheal injection of particulates from diesel engine exhaust (Tokiwa *et al.*, 1999); the major contributor to the formation of 8-OH-dG was carbonaceous particles that had been stripped of organic compounds, and the smaller contributor was organic chemicals adsorbed on the particles. Diesel engine exhaust particles induce the formation of inflammatory cells (neutrophils, eosinocytes and alveolar macrophages), and it was proposed that, during phagocytosis of the particles, alveolar macrophages may contribute to oxidative damage through the formation of hydroxyl radicals that lead to the hydroxylation at the C8 position of the deoxyguanosine in DNA (Tokiwa *et al.*, 1999, 2005). After intratracheal instillation, unwashed diesel particles induced lung adenomas in mice. Furthermore,

the tumorigenic response and the formation of 8-OH-dG were directly correlated in the lungs of mice injected intratracheally with the vehicle (titanium dioxide), or washed or unwashed diesel exhaust particles (Ichinose *et al.*, 1997b).

Microarray studies in cultured rat alveolar epithelial cells exposed to fractionated organic solvent extracts of diesel engine exhaust particles indicated the upregulation of genes involved in phase I and II metabolism, oxidative stress, antioxidant response, immune/inflammatory response, cell cycle/apoptosis and response to cell damage (Omura *et al.*, 2009).

(e) Whole diesel engine exhaust

Exposure to whole diesel engine exhaust induced sister chromatid exchange in the lung cells of rodents (IARC, 1989). In more recent studies, lung tissues from mice or rats exposed to whole diesel engine exhaust showed increased levels of bulky DNA adducts, whereas increases in oxidative DNA damage and mutations were observed in exposed rats. In mice implanted with matrigel scaffolds of murine epithelial cells, exposure to whole diesel engine exhaust significantly increased mRNA expression of vascular endothelial growth factor and hypoxia-inducible factor-1, while it decreased prolyl hydroxylase 2 expression. Whole diesel engine exhaust increased inflammatory cell infiltration, enhanced the vessel volume/flow and increased capillary tube formation and sprouting, thereby inducing angiogenesis and vasculogenesis (Xu *et al.*, 2009).

Extensive evidence has shown that chronic inhalation of high concentrations of diesel engine exhaust induces lung cancer in rats, but not in hamsters or mice. Although the particulate phase of diesel engine exhaust alone also induced lung cancer in rats, the gaseous phase did not. A particle overload mechanism was proposed for the induction of cancer in rats following high particle deposition, and the ensuing overloading of the particle clearance process carried out by macrophages (phagocytosis of excessive quantities of particles) in the deep lung, that resulted in sequestration of the particles within the lung (Morrow, 1988). This engendered an influx of leukocytes that produced chronic pulmonary inflammatory effects, including the generation of ROS, which increased oxidative DNA damage in proliferating epithelial lung cells that eventually resulted in lung cancer (Mauderly, 1994, 1997; Watson & Valberg, 1996; Mauderly, 1997; Stinn *et al.*, 2005).

Additional inhalation studies in rats with titanium dioxide and carbon black revealed that, regardless of the particle type used in the studies, the rate of lung tumours increased with increasing exposure concentration of the particles (Heinrich *et al.*, 1995). This indicated that, under the conditions of the bioassay, diesel engine exhaust was carcinogenic to rats. The conclusions from these and other inhalation studies in rats indicated that lower particle loads do not produce lung cancer because they do not trigger the compensatory inflammatory responses in the lung. Moreover, rats, unlike hamsters and mice, are sensitive to the inhalation of high particle loads that elicit significant physiological responses in the lung that eventually lead to cancer (Hesterberg *et al.*, 2012). This high loading effect induced by particulates may be relevant to humans who are occupationally exposed. In addition, humans, in contrast to rodents, can mount an inflammatory response at levels corresponding to occupational exposure.

The species specificity of the rat lung response to particle overload, and its occurrence with other particle types, has been described extensively (Hesterberg *et al.*, 2012). The rat model has limitations for studying the mechanisms of carcinogenicity involved in the induction of human lung cancer following the inhalation of whole diesel engine exhaust. However, some aspects of the responses observed in rats are similar to those seen in humans exposed to diesel engine exhaust

and could help to elucidate its mechanism(s) of carcinogenic action.

Human studies have indicated that some populations exposed to diesel engine exhaust excrete 1-hydroxypyrene, an indicator of exposure to PAHs, in the urine as well as several amino-PAHs (e.g. 1-aminopyrene and 3-aminobenzanthrone), which are reduction products of 1-nitropyrene and 3-nitrobenzanthrone and are considered to be specific markers of exposure to diesel exhaust (Seidel *et al.*, 2002). Increases in the levels of bulky DNA adducts were found in the peripheral blood lymphocytes of workers exposed to diesel engine exhaust (Nielsen *et al.*, 1996b). In controlled studies of human exposure to diesel engine exhaust, healthy subjects developed airway inflammation, with airway neutrophilia and lymphocytosis, as well as increases in IL-8 protein in lavage fluid, increased *IL-8* gene transcription in the bronchial mucosa and upregulation of endothelial adhesion molecules (Salvi *et al.*, 1999, 2000; Stenfors *et al.*, 2004). Exposure to diesel engine exhaust also increased IL-6 (Nordenhäll *et al.*, 2000), B lymphocytes in airway lavage fluid (Salvi *et al.*, 1999) and growth-regulated oncogene-α protein expression in the bronchial epithelium (Salvi *et al.*, 2000). It has been proposed that diesel engine exhaust particles induce oxidative stress in humans that leads to a cascade of downstream mitogen-activated protein kinase signalling pathways, the activation of which leads to the activation of nuclear factor-κB and activator protein-1 transcription factors, which increase the levels of pro-inflammatory mediators (e.g. IL4, IL6, IL8 and, TNFα), leading to induced airway leukocyte infiltration and inflammation (Salvi *et al.*, 2000; Donaldson *et al.*, 2005; Ristovski *et al.*, 2011). Diesel engine exhaust particles induced IL-8 expression in human airway epithelial cells isolated from healthy adult human volunteers by brush biopsy of the mainstem bronchus (Tal *et al.*, 2010). Recent microarray studies of blood monocytes from young healthy subjects who underwent inhalation exposures to clean filtered air or freshly generated and diluted diesel engine exhaust reported the upregulation of the expression of genes connected with key oxidative stress, protein degradation and coagulation pathways (Pettit *et al.*, 2012).

Diesel engine exhausts and the mechanisms by which they induce cancer in humans are complex in nature, and no single mechanism appears to predominate. Organic solvent and physiological fluid extracts of diesel engine exhaust particles and several of the individual components of these exhausts are genotoxic, and some are carcinogenic, generally through a mechanism that involves DNA mutation. These modifications include the formation of bulky DNA adducts and oxidized DNA bases. Both the organic and particulate components of diesel engine exhaust emissions can generate ROS, leading to oxidative stress. ROS can be generated from washed particles, fresh particles, arene quinones formed by photochemical or enzymatic processes, metals and the phagocytosis process, and as a result of the inflammatory process. ROS can lead directly to the formation of oxidatively modified DNA and DNA adducts from by-products of lipid peroxidation (Voulgaridou *et al.*, 2011), can cause lipid peroxidation, which generates cytotoxic aldehydes (Barrera *et al.*, 2008), and can also initiate a signalling cascade that leads to inflammation, resulting in further induction of oxidative stress, which in turn leads to cell proliferation and cancer (Milara & Cortijo, 2012). In response to the inflammatory insult, cyclooxygenase-2 is upregulated and is a potent mediator of cell proliferation (Speed & Blair, 2011).

4.6.2 Gasoline engine exhaust

(a) Condensates and organic solvent extracts of particulates from gasoline engine exhaust

The particulate phase of gasoline engine exhaust contains several carcinogenic PAHs (IARC, 1989), including benzo[*a*]pyrene, which is carcinogenic in experimental animals and is classified as a human carcinogen (IARC, 2010a). The particulate phase of gasoline engine exhaust contains other PAHs, some of which have been classified as probably or possibly carcinogenic to humans (IARC, 2010a). Organic solvent extracts of gasoline engine exhaust particulates were mutagenic in bacteria and in mammalian cells, and induced oxidative DNA damage, DNA strand breaks, sister chromatid exchange, micronuclei, chromosomal abnormalities and morphological cell transformation in mammalian cells. Gasoline engine exhaust condensate and particulate extracts of gasoline engine exhaust induced cancer in rodents. These data provide strong evidence that a genotoxic mechanism is involved in the carcinogenicity of condensates and particulate extracts of gasoline engine exhausts (IARC, 1989; Liu *et al.*, 2005).

(b) Gaseous phase of gasoline engine exhaust

The gaseous phase of gasoline engine exhaust is mutagenic to bacteria (IARC, 1989) and contains nitrogen oxides, sulfur, ozone and several carcinogenic volatile organic compounds, such as benzene, 1,3-butadiene and formaldehyde (IARC, 1989), which are carcinogenic in experimental animals and have been classified as human carcinogens (IARC, 2012c). Benzene, 1,3-butadiene and formaldehyde each act through a different mechanism of carcinogenic action and it is not known whether these mechanisms are altered in the presence of the other components of gasoline engine exhaust.

(c) Particulates from gasoline engine exhaust

After intratracheal instillation or intraperitoneal injection into mice, particles from gasoline engine exhaust induced micronucleus formation in peripheral blood cells, suggesting a genotoxic mechanism (IARC, 1989).

(d) Whole gasoline engine exhaust

Whole gasoline engine exhaust is mutagenic in bacteria and induces micronuclei in mice (IARC, 1989).

Overall, the mechanistic evidence from human and experimental studies of exposures to whole gasoline engine exhaust is too weak to formulate a mechanism of action for this exposure.

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5. SUMMARY OF DATA REPORTED

5.1 Exposure data

Diesel and gasoline engines are the major sources of power used in motor vehicles. Both are internal combustion engines but differ fundamentally in terms of their fuel–air mixture preparation and ignition, and the fuels they use: diesel fuel is composed of petroleum fractions with a higher boiling range than those of gasoline.

Exhaust emissions from combustion engines comprise a complex and varied mixture of gases (e.g. carbon monoxide and nitrogen oxides), particles (including elemental and organic carbon, ash, sulfate and metals), various volatile (such as benzene) and semi-volatile organic compounds, and polycyclic aromatic hydrocarbons (PAHs), including oxygenated and nitrated PAH derivatives. The exact qualitative and quantitative composition of the exhaust depends on the fuel used, the type and age of the engine, the use of an emission control system, the tuning of the engine, its state of maintenance and its pattern of use (load and acceleration). Historically, diesel engine exhaust contained larger amounts of particulate matter, whereas gasoline engines contained higher levels of certain gases, such as carbon monoxide.

In addition to powering on-road vehicles, combustion engines are also used in a variety of off-road vehicles and equipment in different industrial sectors, such as mining, construction and transport. Diesel engines are generally used to power heavy-duty equipment (e.g. bulldozers and forklift trucks); both gasoline and diesel engines are used in lighter vehicles; and gasoline engines are used in hand-held equipment (e.g. chain saws, leaf blowers, hedge trimmers, brush cutters and clearing saws) and power generators.

Diesel-powered heavy-duty vehicles came on the market in the 1950s, and the predominant type has been sold between the 1960s and 1970s, resulting in their gradual infiltration into road traffic. Diesel engines were first used in underground mines in Germany in 1927, and their use increased considerably in the 1970s. Their use in railroad locomotives was first introduced around 1930 and they largely replaced steam engines between 1945 and the 1960s. In some countries, passenger cars may be almost exclusively powered by gasoline engines (e.g. Brazil, Switzerland and the USA), whereas cars in other countries may have either type of engine.

Increasing environmental concerns over the past two decades have resulted in regulatory actions to introduce successively stricter emission standards for both diesel and gasoline engines. Current emission standards for on-road vehicles vary across the world, and are generally introduced in North America and Europe initially, followed by other countries; however, many countries do not apply such regulations. Emission standards for off-road vehicles and industrial applications are mainly initiated after those for traffic vehicles; many off-road applications, such as for ships, trains and diesel generators, are still largely uncontrolled worldwide.

Standards and technology are strongly interlinked: standards drive improvements in technology and improved technology leads to more stringent standards. Diesel engines have progressed from 'traditional' models, for which particulate matter was not controlled, through 'transitional' models, with progressively advancing technology and lower emissions of particulates, nitrogen oxides and hydrocarbons, to 'new-technology' models that are characterized by the integration of wall-flow diesel particulate filters and diesel oxidation catalysts. Concurrently, the quality of diesel fuel has been improved in most parts of the world, especially with regard to the decrease in sulfur content (from 5000 ppm to 500 ppm, and subsequently to 15 ppm) to enable the use of advanced catalyst systems. New-technology diesel engines have only recently been introduced onto the roads in the USA and Europe. The rate of infiltration of these new technologies into on-road and off-road diesel vehicles/equipment is directly related to the level of sales of new-technology diesel engines to replace older vehicles that are currently in use.

Gasoline engine technologies have also evolved significantly. Tetraethyl lead had been banned as a fuel additive in most countries by 2000, although it is still used in a few geographical regions and in aircraft gasoline. Most gasoline automotive engines are now fitted with complex electronic feedback control systems, port fuel injection and three-way catalyst systems that have reduced emissions of particulate matter, nitrogen oxides, carbon monoxide and non-methane hydrocarbons, as well as unregulated emissions. To improve efficiency, the most recent gasoline engines have been fitted with a direct in-cylinder injection system, which may, however, increase emissions of particulate matter. Many industrial applications, especially small (below 10 kW) engines, still employ older gasoline engine technology.

The complex mixtures of gases and particulate matter in diesel and gasoline engine emissions require that suitable methods be used for their sampling and analysis. The gaseous species of interest are carbon monoxide, nitrogen oxides and various volatile and semi-volatile organic compounds. Particulate matter is a composite mixture of elemental and organic carbon, sulfate and various metals. The methods for sampling and analysing ambient air and engine exhausts have some similarities, although the concentrations tend to be much higher in exhaust. Both instruments for real-time measurement and sampling methods followed by off-line analyses are used.

Biomonitoring methods to estimate exposure to engine exhaust rely on the determination of biomarkers in body fluids and exhaled air. Analytes that have been used as biomarkers include the parent constituents of gasoline or diesel engine exhaust emissions, their metabolites and products of the covalent binding of activated biotransformation products to haemoglobin or DNA.

Exposure to diesel engine exhaust occurs in many different occupational settings, including the mining, railroad, construction and transport industries. The main determinants of exposure are the size, number and use of diesel engines indoors or outdoors, and the degree of ventilation. Several different markers of exposure have been used, such as elemental carbon, nitrogen oxides and PAHs. A generally accepted proxy for levels of exposure to diesel engine exhaust is elemental carbon, although this is not specific to diesel engine exhaust alone. Miners (in settings where diesel engines are used) and tunnel construction workers are the most highly exposed occupational groups, with average levels of exposure to elemental carbon above 100 μg/m^3. Dock workers, diesel mechanics and maintenance personnel are exposed on average to levels between 20 and 40 μg/m^3; train crews, construction workers and workers involved in loading and unloading ships are exposed to levels of elemental carbon of around 10 μg/m^3; and professional drivers

are exposed on average to lower levels of around 2 μg/m^3. Levels of exposure to elemental carbon vary largely within job titles, and these relative rankings can therefore vary in specific situations. Furthermore, the composition of diesel engine exhaust differs between occupational settings due to variations in use scenarios, operating conditions and engine technology.

Occupational exposure to gasoline engine exhaust occurs in a wide variety of professions. Exposure from on-road vehicles can occur in several occupations, with the following relative ranking: professional drivers > border inspectors > tollbooth workers > car mechanics > service station attendants > street workers > (traffic) policemen > car park attendants > shopkeepers. In addition to road traffic-related sources, exposure to gasoline engine exhaust may also occur during the use of gasoline engine-powered portable equipment, such as power chain saws. Several markers have been used to determine exposure to gasoline engine exhaust (lead, carbon monoxide, volatile organic compounds, nitrogen oxides, formaldehyde and particles), although none of these is highly specific for gasoline engine exhaust. The highest exposures, measured as carbon monoxide, are generally incurred by professional drivers, car mechanics, tollbooth workers and loggers.

Exposure of the general population to traffic emissions is dependent on proximity to such emissions, the volume and characteristics of the traffic and the presence of past traffic emissions in regional pollutants. The concentration of freshly emitted traffic-related pollutants decreases with distance from roads, reaching background levels at a distance of 100–600 m, depending on the pollutant. Source apportionment studies can help to determine the contributions of traffic to the complex mixture of air pollution. Studies conducted around the world indicated the contribution to particulate matter (< 2.5 μm in diameter) in general urban air pollution is 3–15% for diesel-powered vehicles and 8–30% for gasoline-powered vehicles. In urban areas, children may frequently be exposed when they walk or cycle near heavy traffic, or take a bus to neighbourhood schools. In addition, depending on the air exchange rate, gasoline and diesel engine exhaust emissions can represent a significant fraction of indoor air pollution.

5.2 Human carcinogenicity data

5.2.1 Diesel engine exhaust

(a) Cancer of the lung

The most informative studies were based on occupational cohorts of miners, railroad workers and workers in the transport industry with well characterized exposure, and supported a positive association between exposure to diesel engine exhaust and the risk for lung cancer.

The study of US miners included both a cohort analysis and a nested case–control study that adjusted for tobacco smoking. Both studies showed positive trends in the risk for lung cancer with increasing exposure, as estimated by elemental carbon. These trends were statistically significant in the nested case–control study, and risk increased in a near monotonic fashion. A two- to threefold increased risk was observed in the highest category for both cumulative and average exposure. The Working Group gave more weight to the nested case–control study, because it controlled for tobacco smoking, and to the combined analyses of surface and underground miners, in which the surface miners formed part of the low-exposure category. The studies of miner provided evidence of an association between exposure to diesel engine exhaust and the risk for lung cancer because few potential confounding exposures occurred in these mines, and high exposures to diesel engine exhaust had been well documented in concurrent surveys.

A large cohort study was carried out among US railroad workers. In the initial analyses, exposure was considered to have begun in 1959

after widespread conversion of the railroads to diesel engines. A significant 40% increased risk for lung cancer was found for exposed railroad workers compared with those who had low or no exposure, but no positive trends were found with duration of exposure. Indirect adjustment for tobacco smoking suggested that differences in this variate may have influenced the excess risk, but only slightly. In additional analyses, exposure assessment was refined by estimating exposure to diesel engine exhaust before 1959 on the basis of work history and the chronological conversion to diesel engines of different railroads. A significantly increased risk of 70–80% was observed for long-term and highly exposed workers, and positive trends were seen for duration of exposure, but not for an estimated index of cumulative exposure. The study of railroad workers supported an association between exposure to diesel engine exhaust and the risk for lung cancer.

A large cohort of workers in the US transport industry found an increased risk for lung cancer of 20–40% among workers with regular exposure to diesel engine exhaust (drivers and dock workers). Statistically significant positive trends of increasing risk with increasing duration of employment were found, with an approximate twofold risk after 20 years of employment. Indirect adjustment for tobacco smoking did not substantially alter these results. This study was extended using a refined exposure assessment that involved contemporary measurements and exposure reconstruction, based on elemental carbon. Exposure–response analyses showed positive trends for cumulative but not average exposure; these trends were more pronounced when an adjustment for duration of employment was included in the models in an effort to account for the healthy-worker survival effect. The studies in the transport industry supported an association between exposure to diesel engine exhaust and the risk for lung cancer.

Three further cohort studies were somewhat less informative because of limited sample size. These were carried out in Stockholm (Sweden) bus mechanics (20 cases of lung cancer), German potash miners (61 cases of lung cancer) and dock workers in Swedish ports (50 cases of lung cancer), and were supportive of an association between exposure to diesel engine exhaust and the risk for lung cancer through detailed exposure assessments. In a nested case–control study, mortality from lung cancer was assessed in persons who serviced buses in Stockholm; evidence of an increased risk with increasing exposure category was found, with borderline statistical significance. German potash miners were studied over a period of approximately 30 years. The results of the main and subgroup analyses of persons who had worked underground for 10 years or longer suggested exposure–response relationships. A nested case–control study was carried out in the cohort of dock workers in Swedish ports; tobacco smoking histories were obtained from next of kin and from retired workers. Three different indices of exposure gave similar results; the greatest odds ratio was found for workers with the highest exposure and evidence of an exposure–response relationship was observed after adjustment for tobacco smoking.

Several additional cohort studies that provided less accurate definitions of exposure (e.g. professional drivers and heavy-equipment operators) or used self-reported exposures were generally supportive of a positive association, although they showed less consistent results and were considered to be less informative.

Twelve independent case–control studies were available for evaluation after exclusion of multiple publications with overlapping study populations. A pooled analysis of studies from Europe and Canada included four of the 12 independent studies from Germany, Sweden, Turin and Montreal, but also included a new set of cases and controls that had not been reported earlier. Ten of the 12 independent studies were

population-based and two were nested within industrial populations of railroad and transport industry workers, respectively.

The two case–control studies of railroad and transport industry workers were large and provided more specific exposure assessments than general population studies. The study of railroad workers showed a tobacco smoking-adjusted increased risk of lung cancer that correlated positively with duration of employment in occupations that involved exposure to diesel engine exhaust in workers under the age of 65 years. The study of transport industry workers showed an elevated tobacco smoking-adjusted risk of lung cancer for long-term employment as a driver of heavy-duty diesel vehicles, and a positive association with cumulative exposure to diesel engine exhaust.

Seven of the 10 independent population-based case–control studies showed a positive association (statistically significant in six) between occupational exposure to diesel engine exhaust and the risk for lung cancer. All of these studies adjusted for tobacco smoking habits.

Two population-based studies showed no association with exposure to diesel engine exhaust. The study from the Turin area of Italy later showed a positive association after expansion and re-analysis with the job–exposure matrix used in a pooled European–Canadian study (see below). The other negative study from six cities in the USA was initially designed to investigate the effects of tobacco smoking and provided limited data on occupational history. A case–control study in the United Kingdom reported positive, non-statistically significant associations with some indicators of exposure to diesel engine exhaust, but did not adjust for tobacco smoking.

The European–Canadian pooled case–control study that applied a job–exposure matrix to classify exposure showed a positive association between exposure to diesel engine exhaust and the risk for lung cancer in a tobacco smoking-adjusted analysis, and a positive exposure–response relationship with duration of exposure.

Conclusions regarding cancer of the lung and diesel engine exhaust

In general, the more informative studies, many of which controlled for tobacco smoking, consistently showed a positive association between exposure to diesel engine exhaust and the risk for lung cancer. Most of the comparisons of exposed and unexposed groups indicated modest increases in risk, and analyses showed positive, statistically significant exposure–response trends.

Positive exposure–response trends were seen across different study designs and in several occupational settings. Therefore, it is improbable that the observed association between exposure to diesel engine exhaust and the risk for lung cancer was caused by chance, bias or confounding.

(b) Cancer of the urinary bladder

Eleven case–control studies reported risk estimates for exposure to diesel engine exhaust, one study reported a risk estimate for 'exhaust' and over 20 studies reported risk estimates for ever employment in an occupation associated with exposure to diesel or gasoline engine exhaust. None of the latter studies included a comprehensive exposure assessment that linked job/occupation specifically to quantitative measures of diesel or gasoline engine exhaust, which limited interpretation of the data because of potential exposure misclassification.

The most informative data resulted from a large pooled analysis of 11 European studies that observed a significantly increased risk among individuals in the highest category of exposure to diesel engine exhaust assessed from lifetime occupational histories and a job–exposure matrix. Four other studies – three (in Montreal, Canada, in Belgium and in Stockholm, Sweden) that used experts and one in British Columbia, Canada, that

used a job–exposure matrix to assess exposure to diesel engine exhaust – were also given greater weight in the evaluation. An excess risk of urinary bladder cancer was found among subjects with the highest exposure to diesel engine exhaust in the studies from British Columbia, Belgium and Stockholm. In the Montreal study, no excess risk for substantial exposure (the most comprehensive measure of exposure) was found, but elevated risks were observed among individuals in the expert-assessed categories for the highest confidence, frequency or duration of exposure to diesel engine exhaust. In all five studies, the odds ratios were generally greatest among individuals with the highest exposure, according to various metrics, but risk estimates in the different exposure categories were imprecise and most were not statistically significant. Of the two studies that reported trend tests for the level of exposure, only the study from British Columbia found a statistically significant trend in risk with increasing exposure. These studies adjusted for tobacco smoking in the analyses; however, the observed risks were small and subject to potential residual confounding from tobacco smoking or other occupational exposures.

The Working Group gave less weight to the evidence from six case–control studies that provided less accurate assessments of exposure to diesel engine exhaust and studies of occupations (heavy-duty vehicle, bus and taxi cab drivers, railroad workers and automobile mechanics) associated with potential exposure to diesel engine exhaust. Statistically non-significantly elevated risks were observed among subjects with the highest exposure to diesel engine exhaust in studies based only on job titles, but attribution of the association specifically to exposure to diesel engine exhaust was difficult.

Ten mortality risk estimates for urinary bladder cancer were based on nine occupational cohort studies. Standardized mortality ratios were 1.0 or less for six estimates and between 1.0 and 1.3 for four estimates. None of these studies included exposure–response analyses for urinary bladder cancer. The mortality studies had a limited ability to detect a positive association because they investigated mortality rather than incidence, which resulted in smaller numbers of cases, and lacked accurate exposure assessments.

Eight studies provided risk estimates for the incidence of urinary bladder cancer among workers potentially exposed to diesel engine exhaust. Four were record-linkage or population-based cohort studies and reported estimates based on a job–exposure matrix, self-reported exposure to diesel or unspecified engine exhaust or job title; only one risk estimate was significantly greater than 1.0. The studies were limited by low-quality exposure assessment, which was based on occupation at one point in time. In addition, three occupational cohort studies and a case–control study nested in one of the cohorts were available. Significantly increased standardized incidence rates for urinary bladder cancer were found in two cohorts of bus drivers. No association was found between occupation and the incidence of urinary bladder cancer in another cohort study. Most analyses in these studies did not adjust for tobacco smoking.

Overall, the epidemiological studies provide some evidence of a positive association between potential exposure to diesel engine exhaust and the risk for urinary bladder cancer.

(c) Cancer at other sites

Twenty-five case–control studies of adult cancers at sites other than the lung or urinary bladder were reviewed with regard to potential associations with exposure to diesel or gasoline engine exhausts. For most cancer sites in adults, only a small set of studies was available, the majority of which were limited with regard to exposure assessment, the number of exposed cases and other methodological problems. Occupational cohort studies showed no consistent patterns for other sites based on external comparisons and could not address potential confounders. Some

case–control studies of cancers of the larynx and colon suggested a positive association with exposure to engine exhaust (unspecified mixtures of diesel and gasoline) or proxies of exposure; however, these were not consistently supported by results from cohort studies. For pancreatic cancer, prostate cancer, multiple myeloma, leukaemia and lymphoma, the overall evidence did not support an effect of exposure to diesel and/or gasoline engine exhausts.

(d) Childhood cancer

Thirteen case–control studies assessed associations between exposure to unspecified mixtures of diesel and gasoline engine exhausts (or proxies of exposure) and childhood cancer, most of which focused on childhood leukaemia and brain tumours. The studies generally relied on the occupational titles of fathers and mothers to assess exposure, and most investigated parental occupation as a proxy for exposure to engine exhaust. Although several studies showed positive associations for acute leukaemias, the exposure assessment was generally not specific for diesel engine exhaust, which hampered their interpretation. Overall, no consistent evidence of associations between parental exposures to diesel and gasoline engine exhausts and the risk of childhood cancer was found.

5.2.2 Gasoline engine exhaust

(a) Cancer of the lung

Few studies attempted to disentangle the effects of diesel engine exhaust from those of gasoline engine exhaust. In some occupational environments, diesel engine exhaust is the only or primary source of exposure (e.g. those of railroad workers and non-metal miners), while many motor exhaust-related occupations involve exposure to a mixture of diesel and gasoline engine exhausts. The separate effects of gasoline and diesel engine exhausts have been investigated in US transport industry workers and in population-based studies in Sweden and Canada. The relative risks associated with exposure were consistently higher for diesel engine exhaust than for gasoline engine exhaust, and the modest excess risks associated with exposure to gasoline engine exhaust were possibly confounded by concomitant exposure to diesel engine exhaust. Little evidence was found for the carcinogenic effect of gasoline engine exhaust in these studies, but such an effect cannot be excluded.

(b) Cancer at other sites

The available data were too sparse and inconsistent to assess the carcinogenicity of gasoline engine exhaust at other sites.

5.3 Animal carcinogenicity data

5.3.1 Diesel engine exhaust

The whole diesel engine exhaust in these studies were generated from fuels and diesel engines produced before the year 2000, and included three basic components: elemental carbon particles in respirable clusters; organic matter adsorbed onto the surface of the carbon particles, which is readily extractable with organic solvents; and a mixture of gas and vapour phases that include volatile organic compounds. Many studies have been carried out using four animal species to evaluate the potential carcinogenicity of exposure to whole exhaust from diesel engines and its components. The studies were considered within four subgroupings: (i) whole diesel engine exhaust; (ii) gas-phase diesel engine exhaust (with particles removed); (iii) diesel engine exhaust particles or extracts of diesel engine exhaust particles; and (iv) whole or gas-phase diesel engine exhaust in combination with known carcinogens.

(a) Whole diesel engine exhaust

Whole diesel engine exhaust was tested for carcinogenicity by inhalation exposure in four studies in mice, nineteen studies in rats, three studies in hamsters and one study in monkeys. In one study in mice, the incidence of lung adenocarcinoma in high-dose animals was significantly increased compared with that in concurrent controls. Significant increases in the incidence of lung tumours were not observed in the other studies in mice. In eleven studies in two different strains of rat, an increased incidence of benign and/or malignant lung tumours was related to exposure to whole diesel engine exhaust from light-duty engines (ten studies) and a heavy-duty engine (one study). One study in rats exposed to exhaust from a heavy-duty diesel engine did not show a significant increase in the incidence of lung tumours. Three studies in rats were inadequate for an evaluation of carcinogenicity and four gave negative results. None of the three studies in Syrian hamsters showed a significant increase in the incidence of respiratory tract tumours. Monkeys exposed to whole exhaust from a heavy-duty diesel engine for 2 years did not develop lung tumours, but the short duration of exposure was inadequate for an evaluation of carcinogenicity.

(b) Gas-phase diesel engine exhaust (with particles removed)

Gas-phase diesel engine exhaust (with particles removed) was tested for carcinogenicity by inhalation exposure in three studies in mice, seven studies in rats and three studies in hamsters. In one study in mice, the incidence of lung tumours was increased in treated animals compared with concurrent controls. However, the incidence of lung tumours in the control group in this study was significantly lower than that of historical controls in this laboratory. When this study was repeated in the same strain, and in a second strain, under the same conditions of exposure and duration, and with gas-phase diesel engine exhaust generated in the same way, there was no increase in the incidence of lung tumours in either strain tested, relative to controls. Therefore, the results of the first study reported were considered to be spurious. The seven studies in rats and three studies in hamsters did not show a significant increase in the incidence of respiratory tract tumours.

(c) Diesel engine exhaust particles or their organic extracts

Diesel engine exhaust particles were tested for carcinogenicity by intratracheal instillation in one study in mice, three studies in rats and one study in hamsters. The study in mice showed a non-significant increase in the incidence of lung tumours. One of the three studies in rats was inadequate for an evaluation of carcinogenicity. The other two studies showed a significant increase in the incidence of malignant and/or benign lung tumours. The study in hamsters gave negative results.

Organic extracts of diesel engine exhaust particles were tested for carcinogenicity by subcutaneous injection in three studies, by topical application in one study and by topical application in two initiation–promotion studies in mice, and by intrapulmonary implantation in one study in rats. An increased incidence of sarcomas at the injection site was observed following subcutaneous injection into mice in one study; the other studies in two strains of newborn mice were inadequate for an evaluation of carcinogenicity. An increased incidence of skin papilloma was observed in one initiation–promotion study using extracts of particles from heavy- and light-duty diesel engines; the other study was inadequate for an evaluation of carcinogenicity. The study of topical application in mice did not show an increase in the incidence of skin tumours. The study of intrapulmonary implantation in rats showed a significant increase

in the incidence of lung carcinoma following exposure to several of the isolated fractions.

(d) Whole or gas-phase diesel engine exhausts in combination with known carcinogens

Inconclusive and inconsistent results were obtained in two studies in mice and one study in rats in which a known carcinogen was administered to animals exposed to either whole or gas-phase diesel engine exhausts.

5.3.2 Gasoline engine exhaust

The gasoline engine exhausts evaluated in these studies were generated from fuels and engines produced before the year 2000, and included three basic components: particles composed primarily of elemental carbon and metallic compounds (especially lead, if present in the fuel); adsorbed organic material that is readily extractable with organic solvents; and a mixture of gas and vapour phases that include volatile organic compounds. Many studies have been carried out on four animal species to evaluate the carcinogenicity of whole gasoline engine exhaust and its components. The studies were considered within three subgroupings: (i) whole gasoline engine exhaust; (ii) condensates or extracts of gasoline engine exhaust; and (iii) whole gasoline engine exhaust in combination with known carcinogens.

(a) Whole gasoline engine exhaust

Whole gasoline engine exhaust was tested for carcinogenicity by inhalation exposure in three studies in mice, three studies in rats, three studies in hamsters and one study in dogs. The three studies in mice were inadequate for an evaluation of carcinogenicity. None of the studies of whole gasoline engine exhaust in rats, hamsters or dogs showed a significant increase in the incidence of respiratory tract tumours.

(b) Condensates or organic extracts of gasoline engine exhaust

Condensates or organic extracts of gasoline engine exhaust were studied to evaluate the effects of carbonaceous soot particles or concentrates of the organic compounds associated with these particles.

Condensates of organic extracts of gasoline engine exhaust particles were tested for carcinogenicity: in mice, by subcutaneous injection in one study which was inadequate for an evaluation of carcinogenicity, by topical application in five studies, two of which were inadequate for an evaluation of carcinogenicity and three of which demonstrated a significant increase in the incidence of carcinomas and papillomas of the skin, and by topical application in one initiation–promotion study which indicated that the extract of gasoline engine exhaust was a skin tumour initiator; in rats, by intrapulmonary implantation in one study, in which a significant increase in the incidence of lung carcinomas was observed; and in hamsters, by intratracheal installation in two studies, one of which showed an increase the incidence of pulmonary adenomas, while the other gave negative results.

(c) Whole gasoline engine exhaust in combination with known carcinogens

One of two studies of inhalation exposure to whole gasoline engine exhaust in mice that were also exposed to a known carcinogen was inadequate for an evaluation of carcinogenicity, while the other gave negative results. Of two studies of inhalation exposure to whole gasoline engine exhaust in rats that were also exposed to a known carcinogen, one indicated that whole gasoline engine exhaust was a lung tumour promoter, while the other gave negative results. One inhalation study with whole gasoline engine exhaust in hamsters in which a known carcinogen was also given gave negative results. One subcutaneous injection study with exhaust condensate in mice

also treated with a known carcinogen was inadequate for an evaluation of carcinogenicity.

5.4 Mechanistic and other relevant data

5.4.1 Diesel engine exhaust

(a) Deposition, clearance, retention and metabolism

The general principles regarding the inhalation, deposition, clearance and retention of poorly soluble particles, and the modelling of inhaled particle deposition in the human lung have been described previously. The number, concentration and size distribution of aerosol particles in the submicron range produced by combustion of diesel fuel and inhaled and exhaled by nonsmoking human volunteers have been determined. The average fraction from diesel exhaust retained in the human lung was 30 ± 9% (± standard deviation) and the count median diameter was 0.124 ± 0.025 μm.

Studies of the metabolism have been conducted on humans exposed to diesel engine exhaust, generally in the workplace, many of which focused on measurements of urinary concentrations of hydroxylated PAHs and amino-PAHs, mainly pyrenes. These studies demonstrated that humans exposed to diesel engine exhaust can adsorb, distribute, metabolize and excrete metabolites of PAHs. Other studies have reported the presence of urinary 1-hydroxypyrene and haemoglobin adducts of nitro-PAHs and low-molecular-weight alkenes (hydroxyethylvaline and hydroxypropylvaline) in populations exposed to diesel engine exhaust.

No adequate studies on the metabolism of diesel and gasoline engine exhaust mixtures in experimental animals were available to the Working Group. The deposition and clearance of diesel engine exhaust components, especially particulates, have been studied in some detail to improve the understanding of the potential mechanisms of species differences in the formation of lung tumours (e.g. exposure to diesel engine exhaust caused tumours in rats, but not in mice or hamsters). A particle overload mechanism for the induction of cancer in rats after high particle deposition entails an overloading of the process of particle clearance, which is mediated by macrophages through the phagocytosis of excessive quantities of particles in the deep lung, and results in the sequestration of particles within the lung. This engenders an influx of leukocytes that produces chronic pulmonary inflammatory effects, including the formation of reactive oxygen species, which increase oxidative DNA damage in proliferating epithelial lung cells that eventually results in lung cancer.

Inhalation studies of titanium dioxide and carbon black in rats revealed that, regardless of the particle type used, the lung tumour rate increased with increasing exposure concentrations of the particles. The conclusions from these and other inhalation studies in rats indicated that lower particle loads do not produce lung cancer, because they do not trigger compensatory inflammatory responses in the lung. Moreover, unlike other species, rats are uniquely sensitive to the inhalation of high particle loads, and mount significant lung physiological responses that eventually lead to cancer.

(b) Genetic and related effects

Exposures of humans to diesel engine exhaust increased the expression of genes associated with oxidative stress and inflammation in blood lymphocytes and those involved in inflammation in bronchoalveolar lavage cells. Exposure of humans to air that predominantly contained diesel engine exhaust induced bulky DNA adducts, DNA damage and micronucleus formation. Positive biomarkers of genotoxicity for exposure and effect were observed among humans exposed to diesel engine exhaust or

air with a predominant diesel engine exhaust content.

Diesel engine exhaust, diesel engine exhaust particulates and diesel particulate extracts induced DNA damage (e.g. oxidative lesions and bulky adducts), gene mutations, DNA strand breaks, chromosomal alterations (e.g. chromosome breaks, sister chromatid exchange and aneuploidy) and morphological cell transformation *in vivo* and *in vitro* in a wide range of experimental systems, including rats and mice, rodent and human cell lines, and rodent and human primary cells, as well as gene mutations in bacteria. The *in-vivo* effects have been documented after multiple routes of administration, including inhalation exposure, intratracheal instillation and oral administration of whole diesel engine exhaust and/or diesel engine exhaust particulates, and topical application and intraperitoneal injection of organic extracts of diesel engine exhaust particulates.

In rodent target tissues following inhalation exposure and in mammalian cells exposed to diesel engine exhaust, diesel particulate suspensions or diesel particulate extracts, gene expression profiles showed the upregulation of genes in pathways related to oxidative stress, inflammation, DNA damage, antioxidant responses, cell cycle, cell transformation and apoptosis.

Definitive statements regarding the effect of diesel engine exhaust aftertreatment or fuel formulation on the genetic and related effects of diesel engine exhaust have been hampered by variations in the types of device and/or fuel examined and the confounding effects of engine design, sample collection and processing, and engine test cycle (i.e. speed and load). Nevertheless, evidence has shown that oxidation catalysts can increase the activity of diesel engine exhaust *in vivo* and *in vitro* and that of extracts of diesel engine particulate matter or exhaust semi-volatile organic compounds (expressed per unit of extractable organic matter or per unit of mass particulate matter) *in vitro*. However, evidence has also been found that exhaust aftertreatment can contribute to substantial reductions in the activity of extracts of diesel engine particulate matter or exhaust semi-volatile organic compounds expressed per unit of engine work or volume of emitted exhaust. No comparative data were available to the Working Group to evaluate the genetic and related effects of new-technology diesel exhaust.

(c) Other effects

Numerous human clinical studies and experimental animal studies have been conducted to investigate the non-cancer health effects of diesel engine exhaust, including recent studies on new-technology diesel engine exhaust (e.g. US 2007 compliant technology or more recent). Biological responses to this type of exposure have been reported for a diverse range of health end-points, including lung function, lung inflammation, immunology and infection, systemic inflammation and cardiovascular effects, and brain inflammation. Prolonged exposure to high concentrations has been associated with the accumulation of particles in macrophages, changes in lung cell populations, fibrotic effects and squamous metaplasia, which appeared to be associated with impaired pulmonary clearance. Other responses such as systemic inflammation, susceptibility to infection, exacerbation of allergic response and cardiovascular responses have also been reported. These systemic responses were also observed with lower exposure concentrations at which pulmonary inflammation was mild or absent. Some of these responses appeared to be absent after exposure to new-technology diesel engine emissions. However, at the present time, new-technology diesel engine emissions have not been evaluated thoroughly.

(d) Susceptibility

Some studies have investigated the role of genetic polymorphisms on the modulation of biomarker responses following exposures to

air containing primarily diesel engine exhaust or mixed exhausts. However, the data were too limited to draw any conclusions. No studies were available to the Working Group on the influence of other susceptibility factors, such as vulnerable populations, underlying disease and the microbiome, in relation to exposure to diesel engine exhaust and the incidence of lung cancer.

(e) Mechanistic considerations

Diesel engine exhaust is a complex mixture that consists of both gaseous and particulate components. The gaseous phase of diesel engine exhaust is mutagenic to bacteria and contains a series of carcinogens including acetaldehyde, acrolein, benzene, 1,3-butadiene, formaldehyde, ethylene oxide, propylene oxide and naphthalene. The particulate phase contains carcinogenic PAHs, nitro-PAHs and metals.

Organic solvent extracts of particulates of diesel engine exhaust showed a broad spectrum of genotoxic activities *in vitro* and *in vivo*, inducing bulky DNA adducts, oxidative DNA damage, DNA strand breaks, unscheduled DNA synthesis, mutations, sister chromatid exchange, chromosomal aberrations and morphological cell transformation in mammalian cells, and mutations in bacteria. They increased the expression of genes involved in xenobiotic metabolism, oxidative damage, antioxidant response and the cell cycle in mammalian cells in culture, and induced skin papillomas and adenocarcinomas in mouse skin.

PAHs are bio-transformed by phase I metabolic enzymes to a series of dihydrodiols, phenols, quinones and polyhydroxylated metabolites. Dihydrodiols can be metabolized further to chemically reactive intermediates (diol epoxides), which bind covalently to DNA to form DNA adducts. PAHs can undergo one-electron reduction to form radical cations, which can bind to DNA to form depurinating PAH adducts. PAH quinones can redox cycle, generating reactive oxygen species that modify DNA. Many of these DNA modifications have been associated with the induction of mutation and, eventually, tumour formation. Further metabolism of PAH metabolites by phase II enzymes converts many of the primary metabolites to glucuronic acid, sulfate and glutathione conjugates that are excreted in the faeces and urine. Nitro-PAHs can be reduced by nitroreductases to hydroxylamino and amino metabolites, and the hydroxylamino intermediates have been shown to bind to DNA to form covalent DNA adducts. Some nitro-PAHs can undergo both oxidative and reductive metabolism, forming mixtures of metabolites and DNA adducts that containing nitro, dihydrodiol or amino functionalities.

The genotoxic organic compounds adsorbed onto the particles need to be bioavailable to manifest their genotoxic activities. Organic solvents are extremely efficient at removing organic compounds from diesel engine exhaust particulates, and some evidence has shown that biological fluids can facilitate bioavailability of genotoxic organic compounds bound to diesel engine exhaust particulates based on *in vitro* and *in vivo* assays.

The particulate phase of diesel engine exhausts was genotoxic *in vitro* and *in vivo*, inducing bulky DNA adducts, oxidative DNA damage, DNA strand breaks, germ-line mutations and transformed foci in selected organs and/or cells. Diesel engine exhaust particulates generated superoxide and hydroxyl radicals and increased the levels of 8-oxo-2'-deoxyguanosine in DNA *in vitro* and *in vivo*. Genes involved in phase I and II metabolism, oxidative stress, antioxidant response, immune/inflammatory response and cell cycle/apoptosis, and those that respond to cell damage were upregulated in cultured rat alveolar epithelial cells exposed to fractionated organic solvent extracts of diesel engine exhaust particles.

Exposure to whole diesel engine exhaust induced sister chromatid exchange in lung cells and lung tissues, increased the levels of bulky

DNA adducts and enhanced oxidative DNA damage in rodents, caused mutations in transgenic rats and induced angiogenesis and vasculogenesis in mice.

A particle overload mechanism has been proposed that includes overloading of the process of particle clearance carried out by macrophages (phagocytosis of excessive quantities of particles) in the deep lung, which results in the sequestration of particles within the lung. This engenders an influx of leukocytes that produces chronic pulmonary inflammatory effects, including the generation of reactive oxygen species, which increase oxidative DNA damage in proliferating epithelial lung cells, and eventually results in lung cancer. Inhalation studies of titanium dioxide and carbon black in rats revealed that, regardless of the particle type used, the lung tumour rate increased with increasing particle exposure concentrations. The conclusions from these and other inhalation studies in rats indicated that lower particle loads do not trigger compensatory lung inflammatory responses, which may account for the lack of tumour response observed at these exposure levels. Moreover, rats are more sensitive to the inhalation of high particle loads than hamsters and mice because they mount significant lung physiological responses that eventually lead to cancer. The rat lung response to particle overload is species specific and its occurrence after exposure to other particle types has been described. However, some aspects of the responses observed in rats are similar to those seen in humans exposed to diesel engine exhaust, which could help to elucidate the mechanism(s) of carcinogenic action in humans. The effect induced by high-loading particulates may be relevant for occupationally exposed humans. In addition, humans – in contrast to rodents – can mount an inflammatory response at levels encountered in occupational exposures.

Human studies indicated that some populations exposed to diesel engine exhaust excrete urinary 1-hydroxypyrene, an indicator of exposure to PAHs, and several amino-PAHs (e.g. 1-aminopyrene and 3-aminobenzanthrone), which are reduction products of 1-nitropyrene and 3-nitrobenzanthrone that are considered to be specific markers of exposure to diesel engine exhaust.

Populations exposed to diesel engine exhaust showed increased levels of bulky DNA adducts, DNA strand breaks, oxidative DNA damage and micronuclei in their blood lymphocytes, as well as upregulation of the genes related to oxidative stress and inflammation.

In controlled studies of chamber exposure to diesel engine exhaust, healthy subjects developed airway inflammation, with airway neutrophilia and lymphocytosis, increases in interleukin-8 protein in lavage fluid, increased interleukin-8 gene transcription in the bronchial mucosa and upregulation of the endothelial adhesion molecules. Moreover, diesel engine exhaust induced interleukin-6 and lymphocytes in airway lavage fluids, and increased growth-regulated oncogene-α protein expression in the bronchial epithelium in humans. Exposure of humans to diesel engine exhaust particles has been purported to induce oxidative stress leading to a cascade of downstream mitogen-activated protein kinase signalling pathways, the activation of which activates nuclear factor-κB and activator protein-1 transcription factors that increase the levels of pro-inflammatory mediators (e.g. interleukin-4, −6 and −8, and tumour necrosis factor-α), producing leukocyte infiltration and inflammation in the airways. Diesel engine exhaust particles increased interleukin-8 expression in airway epithelial cells isolated from normal adult human volunteers, and upregulated the expression of genes connected with key oxidative stress, protein degradation and coagulation pathways.

Diesel engine exhaust is complex in nature, and the mechanisms by which it induces cancer in humans are also complex; no single mechanism appears to predominate. Organic solvent and physiological fluid extracts of diesel engine

exhaust particles and several of their individual components are genotoxic, and some are carcinogenic, generally through a mechanism that involves DNA mutation. These modifications include the formation of bulky DNA adducts and oxidized DNA bases. Both the organic and particulate components of diesel engine exhaust emissions can generate oxidative stress through the formation of reactive oxygen species, which can be generated from washed particles, fresh particles, arene quinones formed by photochemical or enzymatic processes, metals and the phagocytosis process, and as a result of the inflammatory process. Reactive oxygen species can lead directly to the formation of oxidatively modified DNA and DNA adducts from the by-products of lipid peroxidation. They can also cause lipid peroxidation, which generates cytotoxic aldehydes, and initiate a signalling cascade that leads to inflammation, resulting in further induction of oxidative stress, which can then cause cell proliferation and cancer. In response to the inflammatory insult, cyclooxygenase-2 is upregulated and is a potent mediator of cell proliferation.

In conclusion, there is *strong mechanistic evidence* that diesel engine exhaust, as well as many of its components, can induce lung cancer in humans through genotoxic mechanisms that include DNA damage, gene and chromosomal mutation, changes in relevant gene expression, the production of reactive oxygen species and inflammatory responses. In addition, the co-carcinogenic, cell-proliferative and/or tumour-promoting effects of other known and suspected human carcinogens present in diesel engine exhaust probably contribute to its carcinogenicity in the human lung.

5.4.2 Gasoline engine exhaust

(a) Deposition, clearance, retention and metabolism

Human studies on the deposition of gasoline engine exhaust particulates have been reported previously. Total deposition was relatively constant at 30% over a wide range of breathing patterns for sizes of typical aerosols. As the size of primary particles decreased (below 0.1 μm), deposition increased sharply and the length of the respiratory cycle significantly affected deposition. In a separate analysis of the same data, deposition was shown to increase with the respiratory cycle in an approximately linear fashion, ranging from 10% at 3 seconds to 55% at 20 seconds; the slope of the gradient was somewhat dependent on tidal volume.

Lung clearance in humans was best described by a four-component exponential. The first two phases (half-times, 0.7 and 2.5 hours) were similar for gasoline engine exhaust particles, lead nitrate (which is soluble) and lead oxide (which is insoluble), and therefore probably represent mucociliary clearance. On average, 40% of lung deposition of the 0.35-μm aerosols was in the pulmonary region and 60% in the tracheobronchial region. The removal of lead compounds from the pulmonary region was described by a two-compartment exponential with half times of 9 and 44 hours; one exception was the removal of lead from highly carbonaceous particles, which showed half times of 24 and 220 hours.

Exposure to gasoline engine exhaust has been associated with several biological responses that include lung inflammation, systemic inflammation and cardiovascular effects. The lower concentration (relative to diesel engine exhaust) of particulate matter in gasoline engine exhaust in most studies probably contributed to the reduced pulmonary responses compared with those elicited by diesel engine exhaust. However, some studies have suggested that the systemic responses to these two types of exhaust

in the cardiovascular system and the developing immune system are similar.

(b) Genetic and related effects

No studies on genetic and related effects in humans exposed to gasoline engine exhaust alone were available to the Working Group. Exposures of humans to air that contained a mixture of diesel and gasoline engine exhausts induced bulky DNA adducts, DNA damage, oxidative damage, chromosomal aberrations, sister chromatid exchange, micronuclei, mutagenic urine and altered expression of genes and proteins involved in oxidative stress. Thus, a wide array of positive genotoxicity biomarkers of exposure and effect were observed among humans exposed to mixtures of diesel and gasoline engine exhaust.

Gasoline engine exhaust induced chromosomal damage in mice, and changes in the expression of genes involved in pathways related to xenobiotic metabolism and inflammation in rat lung. Particulate matter from gasoline engine exhaust and its organic extracts induced DNA damage (e.g. strand breaks, oxidative lesions and bulky DNA adducts), chromosomal alterations (e.g. chromosome breaks and sister chromatid exchange) and morphological cell transformation in cultured rodent and human cells, and gene mutations in bacteria. The upregulation of genes involved in pathways related to inflammation, xenobiotic metabolism, tumour progression, metastasis and cell cycle has been noted in cultured human cells exposed to extracts of particulate matter from gasoline engine exhaust.

(c) Other effects

See Section 5.4.1

(d) Susceptibility

See Section 5.4.1

(e) Mechanistic considerations

The gaseous phase of gasoline engine exhaust was mutagenic to bacteria and contains several carcinogenic volatile carcinogens (benzene, 1,3-butadiene, formaldehyde and naphthalene); the particulate phase contains several carcinogenic PAHs.

Organic solvent extracts of gasoline engine exhaust particulates were mutagenic in bacterial and mammalian cells, induced DNA damage, sister chromatid exchange, micronuclei, chromosomal aberrations and morphological cell transformation in mammalian cells, and initiated tumours in mouse skin. These data provide *strong evidence* that a genotoxic mechanism is involved in the carcinogenicity of organic solvent extracts of gasoline engine exhaust particulates.

Whole gasoline engine exhaust was mutagenic to bacteria and induced micronuclei in mice. However, the mechanistic data from experimental and human studies of exposures to whole gasoline engine exhaust were insufficient to formulate a mechanism of action for this exposure.

6. EVALUATION

6.1 Cancer in humans

There is *sufficient evidence* in humans for the carcinogenicity of diesel engine exhaust. Diesel engine exhaust causes cancer of the lung. A positive association has been observed between exposure to diesel engine exhaust and cancer of the urinary bladder.

There is *inadequate evidence* in humans for the carcinogenicity of gasoline engine exhaust.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of whole diesel engine exhaust.

There is *inadequate evidence* in experimental animals for the carcinogenicity of gas-phase diesel engine exhaust.

There is *sufficient evidence* in experimental animals for the carcinogenicity of diesel engine exhaust particulate matter.

There is *sufficient evidence* in experimental animals for the carcinogenicity of extracts of diesel engine exhaust particles.

There is *inadequate evidence* in experimental animals for the carcinogenicity of whole gasoline engine exhaust.

There is *sufficient evidence* in experimental animals for the carcinogenicity of condensates of gasoline engine exhaust.

6.3 Overall evaluation

Diesel engine exhaust is *carcinogenic to humans* (Group 1).

Gasoline engine exhaust is *possibly carcinogenic to humans (Group 2B).*

ANNEX: EMISSION STANDARDS FOR LIGHT- AND HEAVY-DUTY VEHICLES

All the information presented in this Annex was retrieved in 2012 from the DieselNet web site (http://www.dieselnet.com/standards/), which is freely accessible, and where more detailed information on global emission standards can be found.

1. Europe

1.1 Light-duty vehicles

The European Union (EU) Stage 1–4 regulations on emissions for new passenger cars and light commercial vehicles that were adopted through 2004 were replaced in 2007 by Stage 5 and 6 regulations (Table 1). The standards apply to all vehicles in Categories M_1, M_2, N_1 and N_2 with a reference mass that does not exceed 2610 kg. The standards regulate both diesel and spark-ignition (fuelled with gasoline, natural gas, liquefied petroleum gas and ethanol) vehicles. The Euro Stage 5 and 6 regulations introduce particulate matter (PM) mass emission standards for spark-ignition vehicles with direct injection engines.

Emissions are tested using the New European Driving Cycle or the Motor Vehicle Emissions Group B test, and are expressed in grams per kilometre. Ultra-low sulfur gasoline and diesel fuels containing < 10 ppm sulfur became mandatory in 2009.

1.2 Heavy-duty vehicles and bus engines

The European heavy-duty vehicle and bus engine emission standards apply to all motor vehicles with a 'technically permissible maximum laden mass' greater than 3500 kg, equipped with compression-ignition engines or spark-ignition natural gas or liquefied petroleum gas engines (Table 2). The regulations were introduced in tiers from Euro I through to Euro VI, and were revised and consolidated in 2005. The most recent Euro VI standards, which become effective in 2013, were published in 2009, and are comparable in stringency to the USA 2010 standards. The European standards have undergone major revisions and changes with respect to test conditions, duty cycles and methods of measurement. Euro IV standards replaced the earlier steady-state engine test (ECE R-49) with the European stationary cycle (ESC) and the European transient cycle (ETC). Smoke opacity is evaluated using the European load response test. Euro III conventional diesel engines are evaluated using the ESC and European load response tests. Euro IV and later conventional diesel engines are evaluated under all three tests,

Table 1 European Union emission standards for passenger cars (category M1[a])

Stage	Date	CO (g/km)	HC (g/km)	HC + NO_x (g/km)	NO_x (g/km)	PM (g/km)	PN (#/km)
Compression ignition (diesel)							
Euro 1[b]	1992.07	2.72 (3.16)	–	0.97 (1.13)	–	0.14 (0.18)	–
Euro 2, IDI	1996.01	1	–	0.7	–	0.08	–
Euro 2, IDI	1996.01[c]	1	–	0.9	–	0.1	–
Euro 3	2000.01	0.64	–	0.56	0.5	0.05	–
Euro 4	2005.01	0.5	–	0.3	0.25	0.025	–
Euro 5a	2009.09[d]	0.5	–	0.23	0.18	0.005[e]	–
Euro 5b	2011.09[f]	0.5	–	0.23	0.18	0.005[e]	6.0×10^{11}
Euro 6	2014.09	0.5	–	0.17	0.08	0.005[e]	6.0×10^{11}
Positive ignition (gasoline)							
Euro 1[b]	1992.07	2.72 (3.16)	–	0.97 (1.13)	–	–	–
Euro 2	1996.01	2.2	–	0.5	–	–	–
Euro 3	2000.01	2.3	0.2	–	0.15	–	–
Euro 4	2005.01	1	0.1	–	0.08	–	–
Euro 5	2009.09[d]	1	0.10[f]	–	0.06	0.005[g,h]	–
Euro 6	2014.09	1	0.10[f]	–	0.06	0.005[g,h]	6.0×10^{11}[g,i]

[a] At the Euro 1–4 stages, passenger vehicles > 2500 kg were type approved as Category N_1 vehicles.
[b] Values in brackets are conformity of production limits
[c] Until 30 September; after that date, DI engines must meet the IDI limits
[d] 2011.01 for all models
[e] 0.0045 g/km using the Particulate Measurement Programme procedure
[f] 2013.01 for all models
[g] And NMHC = 0.068 g/km
[h] Applicable only to vehicles using DI engines
[i] 6.0×10^{12} L/km within first 3 years from Euro 6 effective dates
CO, carbon monoxide; DI, direct injection; HC, hydrocarbons; IDI, indirect injection; NMHC, non-methane hydrocarbons; NO_x, nitrogen oxides; PM, particulate matter; PN, particle number
From DieselNet (2012)

while Euro III and later natural and liquefied petroleum gas engines are evaluated only using the ETC test. Durability and on-board diagnostic requirements were introduced in 2005. Technical requirements pertaining to durability and on-board diagnostics were also established using

Table 2 European Union Euro IV-VI emission standards for conventional heavy-duty diesel engines under the ESC test and diesel and gas engines under the ETC test (in g/kWh)

Tier, year	CO		NO_x		PM[a]		HC	NMHC	CH_4[c]
	ESC	ETC	ESC	ETC	ESC	ETC	ESC	ETC	ETC
Euro IV, 2005	1.5	4.0	3.5	3.5	0.02	0.03	0.46	0.55	1.10
Euro V, 2008	1.5	4.0	2.0	2.0	0.02	0.03	0.46	0.55	1.10
Euro VI, 2013	1.5	4.0	0.4	0.46	0.01	0.01	0.13	0.16[b]	0.50

[a] Not applicable for NG fuelled engines at Euro IV stage
[b] Total HC for diesel engines
[c] For NG engines only (Euro IV-V: NG only; Euro VI: NG and LPG)
CH_4, methane; CO, carbon monoxide; ETC, European transient cycle; ESC, European stationary cycle; LPG, liquefied petroleum gas; HC, hydrocarbons; NMHC, non-methane hydrocarbons; NG, natural gas; NO_x, nitrogen oxides; PM, particulate matter
From DieselNet (2012)

Table 3 European Union Stage III A, B and Stage IV standards for off-road diesel engines (in g/kWh)

Year[a]	Category	Net power (kW)	CO	HC	NO_x + HC	PM
Stage III A standards for off-road engines						
2006	H	$130 \le P \le 560$	3.5		4.0	0.2
2007	I	$75 \le P \le 130$	5.0		4.0	0.3
2008	J	$37 \le P \le 75$	5.0		4.7	0.4
2007	K	$19 \le P \le 37$	5.5		7.5	0.6
Stage III B standards for off-road engines						
2011	L	$130 \le P \le 560$	3.5	0.19	2.0	0.025
2012	M	$75 \le P \le 130$	5.0	0.19	3.3	0.025
2012	N	$56 \le P \le 75$	5.0	0.19	3.3	0.025
2013	P	$37 \le P \le 56$	5.0	-	4.7	0.025
Stage IV standards for off-road engines						
2014	Q	$130 \le P \le 560$	3.5	0.19	0.4	0.025
2014	R	$56 \le P \le 130$	5.0	0.19	0.4	0.025

[a] Dates for constant speed engines are: 2011 for categories H, I, K; 2012 for category J
CO, carbon monoxide; HC, hydrocarbons; NO_x, nitrogen oxides; PM, particulate matter
From DieselNet (2012)

a split-level approach, in which the standards and requirements were described separately.

The Euro VI regulation established a limit for ammonia concentration of 10 ppm that applies to diesel ESC- and ETC-certified engines and gas ETC-certified engines. The Euro VI standard established the first limit on particle number to supplement the mass standard. The limit on numbers prevents the Euro VI limit on PM mass being met using technologies such as open or partial filters that enable the passage of a high number of ultrafine particles and, unlike traditional diesel particle filters, do not effectively remove them. The Euro VI standard also states that the world-harmonized steady-state and transient cycles be used for Euro VI testing. The implementation of these regulations requires that correlation factors be included to adjust measurements made under the ESC and ETC test cycles.

1.3 Off-road diesel engines

The European standards for off-road diesel engine emissions are structured in gradually more stringent tiers known as Stages I–IV. Emission standards were also adopted for small, gasoline-fuelled off-road engines. Stage I was implemented in 1999 and Stage II was implemented from 2001 to 2004, depending on the power output of the engine. Equipment covered by the standard included industrial drilling rigs, compressors, construction wheel loaders, bulldozers, off-road trucks, highway excavators, forklift trucks, road maintenance equipment, snow ploughs, ground support equipment in airports, aerial lifts and mobile cranes. Agricultural and forestry tractors were covered by the same emission standards but had different implementation dates, while ships, railway locomotives, aircraft and generating equipment were not.

In 2002, emission standards were adopted for small, gasoline-fuelled utility engines below 19 kW and Stage II standards were extended to include constant speed engines. Stages III/IV, shown in Table 3, were adopted in 2004 and were

Table 4 US EPA Tier 1 emission standards for passenger cars and light-duty trucks measured over the FTP 75 (in g/mile)

	50 000 miles/5 years						100 000 miles/10 years[a]					
Category	**THC**	**NMHC**	**CO**	**NO_x[b] diesel**	**NO_x gasoline**	**PM[c]**	**THC**	**NMHC**	**CO**	**NO_x[b] diesel**	**NO_x gasoline**	**PM[c]**
Passenger cars	0.41	0.25	3.40	1.00	0.40	0.08	–	0.31	4.20	1.25	0.60	0.10
LLDT, LVW < 3750 lb	–	0.25	3.40	1.00	0.40	0.08	0.80	0.31	4.20	1.25	0.60	0.10
LLDT, LVW < 3750 lb	–	0.32	4.40	–	0.70	0.08	0.80	0.40	5.50	0.97	0.97	0.10
HLDT, ALVW < 5750 lb	0.32	–	4.40	–	0.70	–	0.80	0.46	6.40	0.98	0.98	0.10
HLDT, ALVW < 5750 lb	0.39	–	5.00	–	1.10	–	0.80	0.56	7.30	1.53	1.53	0.12

[a] Useful life 120 000 miles/11 years for all HLDT standards and for THC standards for LLDT
[b] More relaxed NO_x limits for diesel engines applicable to vehicles through to model year 2003
[c] PM standards applicable to diesel vehicles only
ALVW, adjusted LVW (the numerical average of the curb weight and the GVWR); CO, carbon monoxide; EPA, Environmental Protection Agency; FTP, Federal Test Procedure; GVWR, gross vehicle weight rating; HLDT, heavy light-duty truck (above 6000 lb GVWR); LLDT, light light-duty truck (below 6000 lb GVWR); LVW, loaded vehicle weight (curb weight + 300 lb); NMHC, non-methane hydrocarbons; NO_x, nitrogen oxides; PM, particulate matter; THC, total hydrocarbons
From DieselNet (2012)

extended to cover agricultural and forestry tractors in 2005. Further technical details on testing methods were also adopted for Stage IIIB and Stage IV engines, and amendments were made to the rules applied to agricultural and forestry tractors. Stage III standards were divided into IIIA and IIIB and were phased in from 2006 to 2013. The Stage III/IV standards were also applied to railroad locomotive engines and marine engines used for inland waterway vessels, but apply only to new vehicles and equipment; replacement engines to be used in machinery that is already in use (except for railcars, locomotives and inland waterway vessel propulsion engines) must comply with the limit values that the engine to be replaced had to meet when originally placed on the market.

Stage I and II limits were harmonized in part with regulations in the USA, and Stage III and IV limits were harmonized with the USA Tier 3 and Tier 4 standards.

2. USA

2.1 Light-duty vehicles

The Clean Air Act Amendments of 1990 established Federal Tier 1 standards that were phased in between 1994 and 1997, and Tier 2 standards that were phased in from 2004 to 2009. The Tier 1 standards were applied to all new light-duty vehicles, including passenger cars, light-duty trucks, sport utility vehicles, minivans and pick-up trucks with a gross vehicle weight rating (GVWR) of < 8500 lb, and were further divided into light light-duty trucks with a GVWR below 6000 lb and heavy light-duty trucks with a GVWR above 6000 lb. Table 4 shows the Tier 1 federal standards for missions that were measured using the Federal Test Procedure (FTP) 75; the results are expressed in grams per mile.

The Supplemental Federal Test Procedure (SFTP) was phased in between 2000 and 2004

Table 5 US EPA Tier 1 emission standards for passenger cars and light-duty trucks, measured over the SFTP (in g/mile)

Category	Gasoline (diesel)			
	NMHC + NO_x (weighted)	CO		
		USO6	SCO3	Weighted
Passenger cars and LLDT, LVW 3750 lb	0.97/2.07 (0.65/1.48)	11.1 (9.0)	3.7 (3.0)	4.2 (3.4)
LLDT, LVW > 3750 lb	1.37 (1.02)	14.6 (11.6)	4.9 (3.9)	5.5 (4.4)
HLDT, ALVW < 5750 lb	1.44 (1.02)	16.9 (11.6)	5.6 (3.9)	6.4 (4.4)
HLDT, ALVW < 5750 lb	2.09 (1.49)	19.3 (13.2)	6.4 (4.4)	7.3 (5.0)

ALVW, adjusted LVW (the numerical average of the curb weight and the GVWR); CO, carbon monoxide; EPA, Environmental Protection Agency; GVWR, gross vehicle weight rating; HLDT, heavy light-duty truck (above 6000 lb GVWR); LLDT, light light-duty truck (below 6000 lb GVWR); LVW, loaded vehicle weight (curb weight + 300 lb); NMHC, non-methane hydrocarbons; NO_x, nitrogen oxides; SFTP, Supplementary Federal Test Procedure

From DieselNet (2012)

to measure emissions during aggressive highway driving (US06) and urban driving emissions when the air conditioning system of the vehicle was functioning (SC03). The Tier 1 SFTP standards applied to weighted non-methane hydrocarbons plus NO_x and to carbon monoxide with or without weighting are shown in Table 5.

Federal Tier 2 standards apply to all weight categories, whereby those for light-duty vehicles are extended to cover heavier vehicles (up to 10 000 lb). The same emission limits apply to all vehicles regardless of the fuel used, and emissions are expressed in grams per mile so that, to meet the standards, vehicles with larger engines are forced to use more advanced emission control technologies than those with smaller engines. Also, Tier 2 regulations included new standards for fuel quality. Since 2006, the sulfur content of gasoline fuel has been reduced to a corporate average of 30 ppm with a maximum of 80 ppm, and ultralow sulfur diesel fuel has been available since June 2006, with a maximum sulfur content of 15 ppm.

The Tier 2 standards are structured into eight permanent and three temporary certification bins (expired in 2008), and an average fleet standard for emissions of NO_x. Vehicles may be certified to any available bin. In 2009, the entire light-duty vehicle fleet sold by each manufacturer had to meet an average standard of 0.07 g/mile of NO_x (Table 6).

In addition to meeting the FTP cycle requirements, the environmental Protection Agency (EPA) introduced supplemental exhaust emission standards to be met under the US0G and SC03 driving cycles. Full useful-life Tier 2 SFTP standards for non-methane hydrocarbons plus NO_x, PM and carbon monoxide (CO) are based on both vehicle weight classification and the certification bin applicable to that vehicle. They are equal to the Tier 1 SFTP standards minus 35% of the difference between the Tier 1 and Tier 2 FTP standards:

SFTP Standard = Tier 1 SFTP – [0.35 × (Tier 1 FTP – Tier 2 FTP)]

2.2 Heavy-duty vehicles

Specific emission standards apply to new diesel engines used in heavy-duty highway vehicles. Natural gas-fuelled engines equipped with spark plugs are considered to be compression-ignition engines under this regulation. Heavy-duty refers to vehicles with a GVWR of above 8500 lb in the federal jurisdiction and above 14 000 lb in California (model year 1995 and later). Diesel engines used in heavy-duty vehicles are

Table 6 US EPA Tier 2 emission standards for light-duty vehicles, measured over the FTP 75 (in g/mile)

Intermediate life (5 years/50 000 mi)						Full useful life				
Bin No.	**NMOG**	**CO**	**NO_x**	**PM**	**HCHO**	**NMOG**	**CO**	**NO_x[a]**	**PM**	**HCHO**
Temporary bins										
11						0.28	7.3	0.9	0.12	0.032
MDPV[b]										
10[c,d,e,f]	0.125 (0.160)	3.4 (4.4)	0.4	–	0.015 (0.018)	0.156 (0.230)	4.2 (6.4)	0.6	0.08	0.018 (0.027)
9[c,d,f,g]	0.075 (0.140)	3.4	0.2	–	0.015	0.090 (0.180)	4.2	0.3	0.06	0.018
Permanent bins										
8	0.100	3.4	0.14	–	0.015	0.125	4.2	0.2	0.02	0.018
7	0.075	3.4	0.11	–	0.015	0.09	4.2	0.15	0.02	0.018
6	0.075	3.4	0.08	–	0.015	0.09	4.2	0.1	0.01	0.018
5	0.075	3.4	0.05	–	0.015	0.09	4.2	0.07	0.01	0.018
4	–	–	–	–	–	0.07	2.1	0.04	0.01	0.011
3	–	–	–	–	–	0.055	2.1	0.03	0.01	0.011
2	–	–	–	–	–	0.01	2.1	0.02	0.01	0.004
1	–	–	–	–	–	0	0	0	0	0

[a] Average manufacturer fleet NO_x standard is 0.07 g/mile for Tier 2 vehicles.
[b] An additional temporary bin restricted to MDPVs that expires after model year 2008.
[c] Bin deleted at the end of 2006 model year (2008 for HLDTs)
[d] The higher temporary NMOG, CO and HCHO values apply only to HLDTs and MDPVs and expire after 2008.
[e] Optional temporary NMOG standard of 0.195 g/mile (50 000) and 0.280 g/mile (full useful life) applies for qualifying LDT4s
[f] Optional temporary NMOG standard of 0.100 g/mile (50 000) and 0.130 g/mile (full useful life) applies for qualifying LDT2s
[g] 50 000 mile standard optional for diesel engines certified to bins 9 or 10
CO, carbon dioxide; EPA, Environmental Protection Agency; FTP, Federal Test Procedure; HCHO, formaldehyde; HLDT, heavy light-duty truck (above 6000 lb gross vehicle weight rating); MDPV, medium-duty passenger vehicles; NMOG, non-methane organic gases (hydrocarbons); PM, particulate matter
From DieselNet (2012)

further divided into service classes: light heavy-duty diesel engines, medium heavy-duty diesel engines and heavy heavy-duty diesel engines. Vehicles with a GVWR of up to 10 000 lb that are used for personal transportation have been classified as medium-duty passenger vehicles and are regulated under Tier 2 light-duty regulations. Therefore, the same model of diesel engine that is used for vehicles in the 8500–10 000-lb category may be classified as either light- or heavy-duty and certified to different standards, depending on their applications.

Federal regulations require that engines be certified when operated on a dynamometer over the transient FTP; however, a complete heavy-duty diesel vehicle with a GVWR of < 14 000 lb may be certified on a chassis dynamometer. Emissions measured on an engine dynamometer test are expressed in grams per brake horse power-hour (g/bhp-h). Chassis certification emissions are expressed in grams per mile. Additional requirements include the Supplemental Emission Test and the Not-to Exceed test.

For model year 2007 and beyond, heavy-duty highway engines are required to meet the following emission standards: PM, 0.01 g/bhp-h; NO_x, 0.20 g/bhp-h; CO, 15.5 g/bhp-h; and non-methane hydrocarbons, 0.14 g/bhp-h. The standards for non-methane hydrocarbons and NO_x were phased in for diesel engines between

Table 7 US EPA Tier 1–3 emission standards for off-road diesel engines (in g/kWh; g/bhp-h)

Engine Power	Tier	Year	CO	THC	NMHC + NO_x	NO_x	PM
Kw < 8 (hp < 11)	Tier 1	2000	8.0 (6.0)	–	10.5 (7.8)	–	1.0 (0.75)
	Tier 2	2005	8.0 (6.0)	–	7.5 (5.6)	–	0.8 (0.6)
8 ≤ kW < 19 (11 ≤ hp < 25)	Tier 1	2000	6.6 (4.9)	–	9.5 (7.1)	–	0.8 0.6)
	Tier 2	2005	6.6 (4.9)	–	7.5 (5.6)	–	0.8 (0.6)
19 ≤ kW < 37 (25 ≤ hp < 50)	Tier 1	1999	5.5 (4.1)	–	9.5 (7.1)	–	0.8 (0.6)
	Tier 2	2004	5.5 (4.1)	–	7.5 (5.6)	–	0.6 (0.45)
37 ≤ kW < 75 (50 ≤ hp < 100)	Tier 1	1998	–	–	–	9.2 (6.9)	–
	Tier 2	2004	5.0 (3.7)	–	7.5 (5.6)	–	0.4 (0.3)
	Tier 3	2008	5.0 (3.7)	–	4.7 (3.5)	–	–[a]
75 ≤ kW < 130 (100 ≤ hp < 175)	Tier 1	1997	–	–	–	9.2 (6.9)	–
	Tier 2	2003	5.0 (3.7)	–	6.6 (4.9)	–	0.3 (0.22)
	Tier 3	2007	5.0 (3.7)	–	4.0 (3.0)	–	–[a]
130 ≤ kW < 225 (175 ≤ hp < 300)	Tier 1	1996	11.4 (8.5)	1.3 (1.0)	–	9.2 (6.9)	0.54 (0.4)
	Tier 2	2003	3.5 (2.6)	–	6.6 (4.9)	–	0.2 (0.15)
	Tier 3	2006	3.5 (2.6)	–	4.0 (3.0)	–	–[a]
225 ≤ kW < 450 (300 ≤ hp < 600)	Tier 1	1996	11.4 (8.5)	1.3 (1.0)	–	9.2 (6.9)	0.54 (0.4)
	Tier 2	2001	3.5 (2.6)	–	6.4 (4.8)	–	0.2 (0.15)
	Tier 3	2006	3.5 (2.6)	–	4.0 (3.0)	–	–[a]
450 ≤ kW < 560 (600 ≤ hp < 750)	Tier 1	1996	11.4 (8.5)	1.3 (1.0)	–	9.2 (6.9)	0.54 (0.4)
	Tier 2	2002	3.5 (2.6)	–	6.4 (4.8)	–	0.2 (0.15)
	Tier 3	2006	3.5 (2.6)	–	4.0 (3.0)	–	–[a]
kW ≥ 560 (hp ≥ 750)	Tier 1	2000	11.4 (8.5)	1.3 (1.0)	–	9.2 (6.9)	0.54 (0.4)
	Tier 2	2006	3.5 (2.6)	–	6.4 (4.8)	–	0.2 (0.15)

[a] Not adopted, engines must meet Tier 2 PM standard.
bhp, brake horse power; CO, carbon monoxide; EPA, Environmental Protection Agency; hp, horse power; NMHC, non-methane hydrocarbons; NO_x, nitrogen oxides; PM, particulate matter; THC, total hydrocarbons
From DieselNet (2012)

2007 and 2010.Very few engines met the 0.20 g/bhp-h requirement for NO_x before 2010 because most manufacturers decided to meet the Family Emission Limit of between 1.2 and 1.5 g/bhp-h of nitrogen oxides for most of their engines, and a few manufacturers certified some of their engines at levels as high as 2.5 g/bhp-h NO_x plus non-methane hydrocarbons. The standards include certification under the FTP test and the Supplemental Emission Test and the Not-to-Exceed tests.

2.3 Off-road engines

The 1998 Federal off-road engine regulations are structured as a three-tiered progression (Table 7). Each tier involves a phase-in (by hp rating) over several years. Tier 1 standards were phased in from 1996 to 2000, Tier 2 from 2001 to 2006 and Tier 3 from 2006 to 2008. The Tier 3 standards apply only to engines of 37–560 kW. In 2004, the EPA established Tier 4 standards that are being phased in over the period 2008–15, and require a further 90% reduction in emissions of PM and NO_x compared with Tier 3 (Table 8). With effect from June 2012, all off-road (including marine and locomotive) diesel fuels must contain 15 ppm sulfur or less.

Table 8 US EPA Tier 4 emission standards for engines up to 560 kW (in g/kWh; g/bhp-h)

Engine power	Year	CO	NMHC	NMHC + NO_x	NO_x	PM
kW < 8 (hp < 11)	2008	8.0 (6.0)	–	7.5 (5.6)	–	0.4[a] (0.3)
8 ≤ kW < 19 (11 ≤ hp < 25)	2008	6.6 (4.9)	–	7.5 (5.6)	–	0.4 (0.3)
19 ≤ kW < 37	2008	5.5 (4.1)	–	7.5 (5.6)	–	0.3 (0.22)
(25 ≤ hp < 50)	2013	5.5 (4.1)	–	4.7 (3.5)	–	0.03 (0.022)
37 ≤ kW < 56	2008	5.0 (3.7)	–	4.7 (3.5)	–	0.3[b] (0.22)
(50 ≤ hp < 75)	2013	5.0 (3.7)	–	4.7 (3.5)	–	0.03 (0.022)
56 ≤ kW < 130 (75 ≤ hp < 175)	2012–14[c]	5.0 (3.7)	0.19 (0.14)	–	0.40 (0.30)	0.02 (0.015)
130 ≤ kW < 560 (175 ≤ hp < 750)	2011–14[d]	3.5 (2.6)	0.19 (0.14)	–	0.40 (0.30)	0.02 (0.015)

[a] Hand-startable, air-cooled, direct injection engines may be certified to Tier 2 standards through to 2009 and to an optional PM standard of 0.6 g/kWh starting in 2010.
[b] 0.4 g/kWh (Tier 2) if the manufacturer complies with the 0.03 g/kWh standard from 2012-05-17.
[c] PM/CO: full compliance from 2012; NO_x/HC: Option 1 (if banked Tier 2 credits used)–50% of engines must comply in 2012–13; Option 2 (if no Tier 2 credits claimed)–25% of engines must comply in 2012–14, with full compliance from 2014.12.31.
[d] PM/CO: full compliance from 2011; NO_x/HC: 50% of engines must comply in 2011–13.
bhp, brake horse power; CO, carbon monoxide; EPA, Environmental Protection Agency; HC, hydrocarbon; hp, horse power; NMHC, non-methane hydrocarbons; NO_x, nitrogen oxides; PM, particulate matter
From DieselNet (2012)

The standards cover mobile off-road diesel engines of all sizes that are used in a wide range of construction, agricultural and industrial equipment. In 2003, the definition of off-road engines in the State of California was changed to include all diesel-powered engines, including stationary engines used in agricultural operations. This change applies only to engines sold in

Table 9 US EPA Tier 0–2 locomotive emission standards implemented in 2000 (in g/bhp-h)

Duty-cycle	HC[a]	CO	NO_x	PM
Tier 0 (1973–2001)				
Line-haul	1	5	9.5	0.6
Switch	2.1	8	14	0.72
Tier 1 (2002–04)				
Line-haul	0.55	2.2	7.4	0.45
Switch	1.2	2.5	11	0.54
Tier 2 (2005 and later)				
Line-haul	0.3	1.5	5.5	0.2
Switch	0.6	2.4	8.1	0.24
Non-regulated locomotives (1997 estimates)				
Line-haul	0.5	1.5	13.5	0.34
Switch	1.1	2.4	19.8	0.41
Smoke standards,% opacity (normalized)				
	Steady-state	30-s peak	3-s peak	
Tier 0	30	40	50	
Tier 1	25	40	50	
Tier 2 and later	20	40	50	

[a] HC standard is in the form of THC for diesel engines.
bhp, brake horse power; CO, carbon monoxide; EPA, Environmental Protection Agency; HC, hydrocarbons; NO_x, nitrogen oxides; PM, particulate matter; THC, total hydrocarbons
From DieselNet (2012)

Table 10 US EPA Tier 0–4 locomotive emission standards implemented in year 2008 (in g/bhp-h)

Tier	Model year	Date	HC	CO	NO_x	PM
Line-haul locomotives						
Tier 0[a]	1973–92[b]	2010[c]	1	5	8	0.22
Tier 1[a]	1993[b]–2004	2010[c]	0.55	2.2	7.4	0.22
Tier 2[a]	2005–11	2010[c]	0.3	1.5	5.5	0.10[d]
Tier 3[e]	2012–14	2012	0.3	1.5	5.5	0.1
Tier 4	2015 or later	2015	0.14[f]	1.5	1.3[f]	0.03
Switch locomotives						
Tier 0	1973–2001	2010[d]	2.1	8	11.8	0.26
Tier 1[g]	2002–04	2010[d]	1.2	2.5	11	0.26
Tier 2[g]	2005–10	2010[d]	0.6	2.4	8.1	0.13[h]
Tier 3	2011–14	2011	0.6	2.4	5	0.1
Tier 4	2015 or later	2015	0.14[i]	2.4	1.3[i]	0.03

[a] Tier 0–2 line-haul locomotives must also meet switch standards of the same tier
[b] 1993–2001 locomotive that were not equipped with an intake air coolant system are subject to Tier 0 rather than Tier 1 standards.
[c] As early as 2008, if approved engine upgrade kits become available.
[d] 0.20 g/bhp-h until 1 January 2013 (with some exceptions)
[e] Tier 3 line-haul locomotives must also meet Tier 2 switch standards.
[f] Manufacturers may elect to meet a combined NO_x + HC standard of 1.4 g/bhp-h.
[g] Tier 1–2 switch locomotives must also meet line-haul standards of the same tier.
[h] 0.24 g/bhp-h until 1 January 2013 (with some exceptions)
[i] Manufacturers may elect to meet a combined NO_x + HC standard of 1.3 g/bhp-h
bhp, brake horse power; CO, carbon monoxide; EPA, Environmental Protection Agency; HC, hydrocarbons; NO_x, nitrogen oxides; PM, particulate matter
From DieselNet (2012)

California; stationary engines sold in other states are not classified as off-road. Engines that are not regulated include locomotives, marine engines, mining equipment and hobby engines below 50 cm^3 per cylinder.

2.4 Locomotives

The USA standards for railroad locomotive emissions are similar in structure to the other USA standards, in that they are tiered. Tiers 0–2 became effective in 2000 and apply to locomotives manufactured from 1973 onwards (Table 9). The standard includes engines that are manufactured or re-manufactured. Tier 0–2 standards are met though engine development without the use of exhaust gas aftertreatment. Tier 3–4 standards are more stringent. Tier 3 standards took effect in December 2011; Tier 4 standards will take effect in 2015 and are expected to require some form of exhaust aftertreatment.

Locomotive emissions are measured over two steady-state test cycles that represent two different types of service, including line-haul and switch locomotives. The duty cycles include different weighting factors for each of the eight throttle notch modes, idle and dynamic brake modes. The use of ultralow sulfur (15 ppm) diesel fuel has been required as of June 2012.

The 2008 regulation (Table 10) introduced Tier 3 and 4 standards and strengthened the Tier 0–2 standards for existing locomotives when they are re-manufactured, established more restrictive standards for newly built and re-manufactured locomotives, and established standards for newly built and re-manufactured locomotives of the future. Tier 3 standards are expected to be met by improvements in engine technology, while Tier 4 standards are expected to require

Table 11 Marine engine categories used in the USA for establishing emission standards

Category	Displacement per cylinder (D) in dm³		Basic engine technology
	Tier 1–2	Tier 3–4	
1	D < 5	D < 7	Land-based off-road diesel
2	5 ≤ D < 30	7 ≤ D < 30	Locomotive engine
3	D ≥ 30		Unique marine engine design

From DieselNet (2012)

the use of exhaust gas aftertreatment technologies. The opacity regulations remain unchanged.

2.5 Marine engines

Similarly to the other USA engine emission standards, those for marine engines are tiered and broken down by engine category. In addition, the categories consider cylinder displacement and engine technology, i.e. off-road diesel engine, locomotive or unique marine design (Table 11). Category 3 marine diesel engines are very large, typically ranging in size from 2500 to 70 000 kW (3000 to 100 000 hp). These engines are used in ocean-going vessels and emission control options are very limited. The residual fuel for these large vessels is not regulated by the EPA. The fuel has a high viscosity and density, and high ash, sulfur and nitrogen contents in comparison with other types of marine distillate fuel. The EPA estimated that residual fuel can increase engine emissions of NO_x from 20–50% and those of PM from 750% to 1250% (sulfate particulates) compared with distillate fuel. Category 1 and 2 marine diesel engines range in size from about 500 to 8000 kW (700 to 11 000 hp).

Emissions from marine diesel engines are regulated by several rules that are applied to the engine categories, and may overlap with those for mobile, land-based off-road engines described previously. For engines in Categories 1 and 2, the land-based off-road and locomotive engines serve as guidelines for the Tier 2 standards as shown in Table 12. The standards for recreational engines

Table 12 US EPA Tier 2 emission standards for marine engines

Category	Displacement per cylinder (D) dm³	Year	Standard (g/kWh)		
			CO	NO_x + THC	PM
1	Power ≥ 37 kW				
	D < 0.9	2005	5	7.5	0.4
	0. ≤ D < 1.2	2004	5	7.2	0.3
	1.2 ≤ D < 2.5	2004	5	7.2	0.2
	2.5 ≤ D < 5.0	2007[a]	5	7.2	0.2
2	5.0 ≤ D < 15	2007[a]	5	7.8	0.27
	Power < 3 300 kW				
	15 ≤ D < 20	2007[a]	5	8.7	0.5
	Power ≥ 3 300 kW				
	15 ≤ D < 20	2007[a]	5	9.8	0.5
	20 ≤ D < 25	2007[a]	5	9.8	0.5
	25 ≤ D < 30	2007[a]	5	11.0	0.5

[a] Tier 1 certification requirement started in 2004.

CO, carbon monoxide; EPA, Environmental Protection Agency; NO_x, nitrogen oxides; PM, particulate matter, THC, total hydrocarbons

From DieselNet (2012)

Table 13 US EPA Tier 2 emission standards for recreational marine engines

Displacement per cylinder (D) dm³	Standard (g/kWh)			Year
	CO	NO_x + THC	PM	
0.5 ≤ D < 0.9	5	7.5	0.4	2007
0.9 ≤ D < 1.2	5	7.2	0.3	2006
1.2 ≤ D < 2.5	5	7.2	0.2	2006
D ≥ 2.5	5	7.2	0.2	2009

CO, carbon monoxide; EPA, Environmental Protection Agency; NO_x, nitrogen oxides; PM, particulate matter, THC, total hydrocarbons
From DieselNet (2012)

are summarized in Table 13. These regulations were phased in beginning in 2006, depending on the size of the engine, and are similar to the Tier 2 standards for Category 1 commercial vessels. Recreational engines are also subject to Not-to-Exceed limits, but not to smoke requirements.

The Tier 3 standards are shown in Table 14 and Table 15. They began to be phased in beginning in 2009 and this will be completed by 2014. In addition to the NO_x plus hydrocarbons and PM standards shown in the tables, the following CO emission standards apply for all Category 1/2 engines starting with the applicable Tier 3 model year: 8.0 g/kWh for engines < 8 kW, 6.6 g/kWh for engines ≥ 8 kW and < 19 kW, 5.5 g/kWh for

Table 14 US EPA Tier 3 emission standards for marine diesel category 1 commercial, high and recreational power densities

Commercial standard power density ≤ 35 kW/dm³				
Power (P) in kW	**Displacement per cylinder (D) in dm³**	**NO_x+HC[a] (g/kWh)**	**PM (g/kWh)**	**Year**
$P < 19$	D < 0.9	7.5	0.4	2009
19 ≤ P < 75	D < 0.9[b]	7.5	0.3	2009
		4.7[c]	0.30[c]	2014
75 ≤ P < 3700	D < 0.9	5.4	0.14	2012
	0.9 ≤ D < 1.2	5.4	0.12	2013
	1.2 ≤ D < 2.5	5.6	0.11[d]	2014
	2.5 ≤ D < 3.5	5.6	0.11[d]	2013
	3.5 ≤ D < 7	5.8	0.11[d]	2012
$P < 19$	D < 0.9	7.5	0.4	2009
19 ≤ P < 75	D < 0.9[b]	7.5	0.3	2009
		4.7[c]	0.30[c]	2014
75 ≤ P < 3700	D < 0.9	5.8	0.15	2012
	0.9 ≤ D < 1.2	5.8	0.14	2013
	1.2 ≤ D < 2.5	5.8	0.12	2014
	2.5 ≤ D < 3.5	5.8	0.12	2013
	3.5 ≤ D < 7	5.8	0.11	2012

[a] Tier 3 NO_x + HC standards do not apply to 2000–3700 kW engines.
[b] < 75 kW engines ≥ 0.9 dm³/cylinder are subject to the corresponding 75–3700 kW standards.
[c] Option: 0.20 g/kWh PM and 5.8 g/kWh NO_x + HC in 2014
[d] This standard level drops to 0.10 g/kWh in 2018 for < 600 kW engines.
CO, carbon monoxide; EPA, Environmental Protection Agency; HC, hydrocarbons; NO_x, nitrogen oxides; PM, particulate matter
From DieselNet (2012)

Table 15 US EPA Tier 3 emission standards for marine diesel category 2 engines[a]

Power (P) kW	Displacement per cylinder (D) in dm³	NO_x + HC[b] (g/kWh)	PM (g/kWh)	Year
$P < 3700$	$7 \leq D < 15$	6.2	0.14	2013
	$15 \leq D < 20$	7	0.27[c]	2014
	$20 \leq D < 25$	9.8	0.27	2014
	$25 \leq D < 30$	11	0.27	2014

[a] Option: Tier 3 PM/NO_x+HC at 0.14/7.8 g/kWh in 2012, and Tier 4 in 2015
[b] Tier 3 NO_x + HC standards do not apply to 2000–3700 kW engines.
[c] 0.34 g/kWh for engines below 3300 kW
CO, carbon monoxide; EPA, Environmental Protection Agency; HC, hydrocarbons; NO_x, nitrogen oxides; PM, particulate matter
From DieselNet (2012)

engines ≥ 19 kW and < 37 kW, and 5.0 g/kWh for engines ≥ 37 kW.

The Tier 4 standards for commercial marine engines at or above 600 kW, which will require aftertreatment, will be phased in from 2014 to 2017.

2.6 State of California standards

The State of California is considered in addition to the national standards because it has the authorization to, and frequently does, set lower emission standards than those of the USA. Furthermore, other states have the option to adopt California emission standards in place of national standards, for which they often set

Table 16 US State of California LEV emission standards for light-duty vehicles, measured with FTP 75 (in g/mile)

Category	50 000 miles/5 years					100 000 miles/10 years				
	NMOG[a]	CO	NO_x	PM	HCHO	NMOG[a]	CO	NO_x	PM	HCHO
Passenger cars										
Tier 1	0.25	3.4	0.4	0.08	–	0.310	4.2	0.60	–	–
TLEV	0.125	3.4	0.4	–	0.015	0.156	4.2	0.60	0.08	0.018
LEV	0.075	3.4	0.2	–	0.015	0.090	4.2	0.30	0.08	0.018
ULEV	0.04	1.7	0.2	–	0.008	0.055	2.1	0.30	0.04	0.011
LDT1, LVW < 3750 lbs										
Tier 1	0.25	3.4	0.4	0.08	–	0.310	4.2	0.60	–	–
TLEV	0.125	3.4	0.4	–	0.015	0.156	4.2	0.60	0.08	0.018
LEV	0.075	3.4	0.2	–	0.015	0.090	4.2	0.30	0.08	0.018
ULEV	0.04	1.7	0.2	–	0.008	0.055	2.1	0.30	0.04	0.011
LDT2, LVW > 3750 lbs										
Tier 1	0.32	4.4	0.7	0.08	–	0.40	5.5	0.97	–	–
TLEV	0.16	4.4	0.7	–	0.018	0.20	5.5	0.90	0.1	0.023
LEV	0.1	4.4	0.4	–	0.018	0.13	5.5	0.50	0.1	0.023
ULEV	0.05	2.2	0.4	–	0.009	0.07	2.8	0.50	0.05	0.013

[a] NMHC for all Tier 1 standards
CO, carbon monoxide; FTP, Federal Test Procedure; HCHO, formaldehyde; LEV, low emission vehicles; LDT, light-duty truck; LVW, loaded vehicle weight (curb weight + 300 lb); NMHC, non-methane hydrocarbons; NMOG, non-methane organic gases; NO_x, nitrogen oxides; PM, particulate matter; TLEV, transitional low emission vehicles; ULEV, ultra low emission vehicles
From DieselNet (2012)

Table 17 US State of California emission standards for medium-duty vehicles, measured with FTP (in g/mile)

Category	50 000 miles/5 years					100 000 miles/10 years				
	NMOG[a]	CO	NO_x	PM	HCHO	NMOG[a]	CO	NO_x	PM	HCHO
MDV1, 0–3750 lb										
Tier 1	0.25	3.4	0.4	–	–	0.360	5.0	0.55	0.08	–
LEV	0.13	3.4	0.4	–	0.015	0.180	5.0	0.60	0.08	0.022
ULEV	0.08	1.7	0.2	–	0.008	0.107	2.5	0.30	0.04	0.012
MDV2, 3751–5750 lb										
Tier 1	0.32	4.4	0.7	–	–	0.460	6.4	0.98	0.10	–
LEV	0.16	4.4	0.4	–	0.018	0.230	6.4	0.60	0.10	0.027
ULEV	0.10	4.4	0.4	–	0.009	0.143	6.4	0.60	0.05	0.013
SULEV	0.05	2.2	0.2	–	0.004	0.072	3.2	0.30	0.05	0.006
MDV3, 5751–8500 lb										
Tier 1	0.39	5.0	1.1	–	–	0.560	7.3	1.53	0.12	–
LEV	0.20	5.0	0.6	–	0.022	0.280	7.3	0.90	0.12	0.032
ULEV	0.12	5.0	0.6	–	0.011	0.167	7.3	0.90	0.06	0.016
SULEV	0.06	2.5	0.3	–	0.006	0.084	3.7	0.45	0.06	0.008
MDV4, 8501–10 000 lb										
Tier 1	0.46	5.5	1.30	–	0.028	0.660	8.1	1.81	0.12	–
LEV	0.23	5.5	0.70	–	0.028	0.330	8.1	1.00	0.12	0.040
ULEV	0.14	5.5	0.70	–	0.014	0.197	8.1	1.00	0.06	0.021
SULEV	0.07	2.8	0.35	–	0.007	0.100	4.1	0.50	0.06	0.010
MDV5, 10 001–14 000 lb										
Tier 1	0.60	7.0	2.0	–	–	0.860	10.3	2.77	0.12	–
LEV	0.30	7.0	1.0	–	0.036	0.430	10.3	1.50	0.12	0.052
ULEV	0.18	7.0	1.0	–	0.018	0.257	10.3	1.50	0.06	0.026
SULEV	0.09	3.5	0.5	–	0.009	0.130	5.2	0.70	0.06	0.013

[a] NMHC for all Tier 1 standards

CO, carbon monoxide; FTP, Federal Rest Procedure; HCHO, formaldehyde; LEV, low emission vehicles; MDV, medium-duty vehicle (with maximum gross vehicle weight from 8500 to 14 000 lb; the MDV category is divided into five classes, MDV1–MDV5, based on vehicle test weight; the definition of 'test weight' in California is identical to the Federal adjusted loaded vehicle weight; ALVW); NMHC, non-methane hydrocarbons; NMOG, non-methane organic gases; NO_x, nitrogen oxides; PM, particulate matter; ULEV, ultra low emission vehicles; SULEV, super ultra low emission vehicles

From DieselNet (2012)

the pace. California implemented the first light-duty vehicle emission standards in 1963 with the requirement for positive crankcase ventilation that recycles previously discharged blow-by emissions. It also introduced the first emission standards in 1966 for CO, hydrocarbons and NO_x. Although these standards are on average more stringent than the EPA requirements, they are similar to the Federal standards in many respects.

The Low Emission Vehicle (LEV) Standards were applied through to model year 2003 for light- and medium-duty vehicles with the following emission categories: Tier 1, transitional LEV, LEV, ultra LEV, super ultra LEV and zero emission vehicles. The standards for gaseous pollutants applied to diesel- and gasoline-fuelled vehicles while the PM standards applied to diesel vehicles only. Emissions were measured over the FTP 75 test and were expressed in grams per mile, and the standards were tiered based upon the

Table 18 US State of California LEV II emission standards for passenger cars and light-duty vehicles < 8500 lb, measured with FTP 75 (in g/mile)

Category	50 000 miles/5 years					120 000 miles/5 years				
	NMOG	CO	NO_x	PM	HCHO	NMOG	CO	NO_x	PM	HCHO
LEV	0.075	3.4	0.05	-	0.015	0.090	4.2	0.07	0.01	0.018
ULEV	0.040	1.7	0.05	-	0.008	0.008	2.1	0.07	0.01	0.011
SULEV	-	-	-	-	-	0.010	1.0	0.02	0.01	0.004

CO, carbon monoxide; FTP, Federal Test Procedure; HCHO, formaldehyde; LEV, low emission vehicles; NMOG, non-methane organic gases; NO_x, nitrogen oxides; PM, particulate matter; SULEV, super ultra low emission vehicles; ULEV, ultra low emission vehicles
From DieselNet (2012)

vehicle weight and mileage as shown in Table 16 and Table 17. The additional SFTP procedures were phased in in California between 2001 and 2005. A percentage of vehicles was required to be certified to increasingly more stringent emission categories based on the average emissions of vehicle manufacturers' fleets. Tier 1 and transitional LEV standards were eliminated after 2003. LEV II and LEV III standards were implemented for successive time periods and are discussed below.

LEV II emission standards were applied to model years 2004–10. Light-duty and medium-duty vehicle categories below 8500 lb gross weight were reclassified and had to meet passenger car requirements, as shown in Table 18 and Table 19. This was phased in by 2007. As a result, most pick-up trucks and sport utility vehicles were required to meet the passenger car emission standards. More stringent emission standards were defined: ultra LEV and super ultra LEV. Partial zero emission vehicles have the same test emission levels as super ultra LEV, including evaporative emission control and a 150 000 mile/15 years emission durability. The standards apply to all vehicles regardless of the fuel used, and gasoline vehicles are no longer exempt from the PM standard. Light-duty LEVs and ultra LEVs are certified for a 0.05 g/mi standard for NO_x that was phased in starting with the 2004 model year. The LEV II emission standards can only be met by vehicles with advanced emission control strategies.

LEV II standards also included provisions to reduce vehicle fleet emission levels each year through to 2010. The LEV II programme is similar in structure to the Federal Tier 2 legislation, except that the Federal approach uses eight certification 'bins' (with Tier 2 Bin 5 being similar to California's LEV, and Tier 2 Bin 2

Table 19 US State of California LEV II emission standards for medium-duty vehicles, durability 120 000 miles (in g/mile)

Weight (GVW)	Category	NMOG	CO	NO_x	PM	HCHO
8500–10 000 lb	LEV	0.195	6.4	0.2	0.12	0.032
	ULEV	0.143	6.4	0.2	0.06	0.016
	SULEV	0.100	3.2	0.1	0.06	0.008
10 001–14 000 lb	LEV	0.230	7.3	0.4	0.12	0.040
	ULEV	0.167	7.3	0.4	0.06	0.021
	SULEV	0.117	3.7	0.2	0.06	0.010

CO, carbon monoxide; GVW, gross vehicle weight; HCHO, formaldehyde; LEV, low emission vehicles; NMOG, non-methane organic gases; NO_x, nitrogen oxides; PM, particulate matter; SULEV, super ultra low emission vehicles; ULEV, ultra low emission vehicles.
From DieselNet (2012)

Table 20 US State of California proposed LEV III particle standards[a]

Year	Compliance option	
	PM (g/mile)	SPN (1/mile)
2014	0.006	6×10^{12}
2017	0.003	3×10^{12}

[a] Particulate matter standards: the PM mass emission standard will be tightened and an SPN limit will be introduced. SPN emissions are to be measured over the FTP driving cycle using a sampling approach patterned after the European Particulate Measurement Programme methods. Manufacturers would demonstrate compliance using only one standard – either PM or SPN – of their choice. The more stringent standards will ensure that there is no increase in PM emissions from future engine technologies and that particulate filters are used on all diesel engines. In gasoline direct-injection engines, meeting the PM standard of 3/mile should still be possible without the need for a particulate filter. Durability: the LEV III standards would phase in a new 150 000-miles durability requirement, compared with the LEV II 50 000- and 120 000-miles standards. Evaporative emissions: all light-duty vehicles would have to meet a more stringent zero evaporative standard, while using more challenging test fuels, such as E10.

LEV, low emission vehicles; PM, particulate matter; SPN, single particle number

From DieselNet (2012)

similar to super ultra LEV) to allow averaging across greater diversification of emission levels in the fleet. The Federal fleet average non-methane organic gas emissions (around 0.090 g/mi, based on Bin 5) can be more than twice as high as those of LEV II.

The proposed LEV III emission standards, to be phased in over 2014–22, would modify the LEV II standards by: (1) combining the non-methane organic gas and NO standards, (2) introducing a more stringent fleet average requirement for non-methane organic gas plus NO_x combined, (3) adding several emission standard bins and (4) increasing the durability requirements for emission control systems. A significant change proposed in the LEV III standards is a tightening of the PM standards by the introduction of a solid particle number standard similar to that in Europe introduced by the Particle Measurement Programme. Manufacturers would select either

Table 21 People's Republic of China phase-in schedule for light-duty emission standards

Stage	Date	Region	Comments	Reference
China 1	2000.01 (2000.07[a])	Nationwide		Euro 1
China 2	2002.08	Beijing		Euro 2
	2003.03	Shanghai		
	PI: 2004.07**b** (2005.07**a**) CI: 2003.09	Nationwide		
China 3	2005.12	Beijing	European OBD from 2006.12	Euro 3
	2006.10	Guangzhou	With European OBD	
	2007.01	Shanghai	With European OBD	
	2007.07	Nationwide	European OBD: Type 1 2008.07; Type 2 2010.07	
China 4	2008.03	Beijing		Euro 4
	2009.11	Shanghai		
	PI: 2011.07 CI: 2015.07	Nationwide		
China 5	2012[c]	Beijing		Euro 5

[a] Production conformity

[b] First registration

[c] Proposed

CI, compression ignition (diesel); OBD, on-board diagnostics; PI, positive ignition (gasoline, natural gas)

From DieselNet (2012)

Table 22 People's Republic of China emission standards for heavy-duty engines (in g/kWh)

Standard	Test cycle	CO	HC	NMHC	NO_x	PM	Smoke opacity (1/m)
China III	ESC + ELR	2.1	0.66	–	5.0	0.10/0.13[a]	0.8
	ETC	5.42	–	0.78	5.0	0.16/0.21[a]	–
China IV	ESC + ELR	1.5	0.46	–	3.5	0.02	0.5
	ETC	4.0	–	0.55	3.5	0.03	–
China V	ESC + ELR	1.5	0.46	–	2.0	0.02	0.5
	ETC	4.0	–	0.55	2.0	0.03	–

[a] For engines with a per cylinder displacement of < 0.75 dm^3 and rated speed > 3000 pm.
CO, carbon monoxide; ELR, European load response; ESC, European stationary cycle; ETC, European transient cycle; HC, hydrocarbons; NMHC, non-methane hydrocarbons; NO_x, nitrogen oxides; PM, particulate matter
From DieselNet (2012)

a PM or solid particle number method to determine compliance. These proposed standards (Table 20) ensure no increase in PM emissions from future engine technologies and that particulate filters are used on all diesel engines.

3. Other countries

3.1 People's Republic of China

Chinese emission standards for new passenger cars and light-duty commercial vehicles are based on European regulations. Light-duty vehicle categories are based on the EU classification, with changes for the classifications based upon gross vehicle weight. The light-duty emission standards follow the EU standards exactly but came into force a few years later. Their strictest standards follow Euro 5 and took effect in 2012 (Table 21).

Chinese heavy-duty emission standards are based on the similar test cycles to those used by the EU but with slightly different levels. The strictest standards for NO_x and PM proposed to date are 2.0 and 0.02 g/kWh, respectively (Table 22), compared with 0.4 and 0.01 g/kWh for the corresponding Euro VI standards. The phase-in dates for Chinese heavy-duty emission standards are given in Table 23.

Table 23 People's Republic of China phase-in schedule for heavy-duty emission standards

Standard		Implementation date				Initial schedule
		Beijing	Shanghai	Guangzhou	Nationwide	
China I					2000.09	
China II					2003.09	
China III	Gasoline	2010.07			2007.07	2007.07
	Diesel	2006.01				2007.01
China IV	Gasoline	2011.01				2010.01
	Diesel	2011.01	2009.11	2010.08		2010.01
China V		2013.02 [a]				

[a] Estimate
From DieselNet (2012)

Table 24 Indian emission standards for light-duty vehicles (in g/kWh)

Year	Reference	CO	HC	HC + NO_x	NO_x	PM
Diesel						
1992	–	17.3–32.6	2.7–3.7	–	–	–
1996	–	5.0–9.0	–	2.0–4.0	–	–
2000	Euro 1	2.72–6.90	–	0.97–1.70	–	0.14–0.25
2005[b]	Euro 2	1.0–1.5	–	0.7–1.2	–	0.08–0.17
2010[b]	Euro 3	0.64	–	0.56	0.50	0.05
		0.80		0.72	0.65	0.07
		0.95		0.86	0.78	0.10
2010[c]	Euro 4	0.50	–	0.30	0.25	0.025
		0.63		0.39	0.33	0.04
		0.74		0.46	0.39	0.06
Gasoline						
1991	–	14.3–27.1	2.0–2.9	–	–	–
1996	–	8.68–12.4	–	3.00–4.36	–	–
1998[a]	–	4.34–6.20	–	1.50–2.18	–	–
2000	Euro 1	2.72–6.90	–	0.97–1.70	–	–
2005[b]	Euro 2	2.2–5.0	–	0.5–0.7	–	–
2010[b]	Euro 3	2.3	0.20	–	0.15	–
		4.17	0.25		0.18	
		5.22	0.29		0.21	
2010[c]	Euro 4	1.0	0.1	–	0.08	–
		1.81	0.13		0.10	
		2.27	0.16		0.11	

[a] For catalytic converter fitted vehicles
[b] Earlier introduction in selected regions
[c] Only in selected regions
CO, carbon monoxide; HC, hydrocarbons; NO_x, nitrogen oxides; PM, particulate matter
From DieselNet (2012)

3.2 Brazil

Brazil is noteworthy because diesel engines are banned in passenger cars; however, it has regulatory standards for diesel passenger car emissions because some neighbouring countries that do not ban diesel passenger cars use Brazilian emission standards. Brazil does allow diesel engines in heavy-duty vehicles (trucks and buses) and light commercial vehicles, and establishes its own unique emission standards that are based on Euro standards but tend to be somewhat less stringent.

3.3 India

The first light-duty standards were imposed in India in 1992 (Table 24). Since 2000, India has started adopting EU emission and fuel regulations for four-wheeled light-duty and for heavy-duty vehicles (Table 25). It has also adopted standards applying to two- and three-wheeled vehicles.

3.4 Other countries

Argentina bases its emission regulations on Euro standards.

Table 25 Indian emission standards for heavy-duty engines (in g/kWh)

Year	Reference	Test	CO	HC	NO_x	PM
1992	-	ECE R49	17.3–32.6	2.7–3.7	-	-
1996	-	ECE R49	11.20	2.40	14.4	-
2000	Euro I	ECE R49	4.5	1.1	8.0	0.36[a]
2005[b]	Euro II	ECE R49	4.0	1.1	7.0	0.15
2010[b]	Euro III	ESC	2.1	0.66	5.0	0.10
		ETC	5.45	0.78	5.0	0.16
2010[c]	Euro IV	ESC	1.5	0.46	3.5	0.02
		ETC	4.0	0.55	3.5	0.03

[a] 0.612 for engines below 85kW
[b] Earlier introduction in selected regions
[c] Only in selected regions
ECE R49, 13-mode steady-state diesel engine test cycle introduced by ECE Regulation No. 49; CO, carbon monoxide; ESC, European stationary cycle; ETC, European transient cycle; HC, hydrocarbons; NO_x, nitrogen oxides; PM, particulate matter
From DieselNet (2012)

Australian regulations follow the EU regulations.

Canadian regulations follow the USA standards.

Chile has its own regulations that generally follow USA and Euro standards but are not necessarily equivalent, and, in some cases, allows certification to either regulation.

Japan has adopted its own regulations, which generally follow the USA and EU standards.

Peru has switched from a combination of USA and Euro standards to base its regulations on Euro standards.

Russian regulations follow the EU standards for light-duty vehicles, heavy-duty engines and off-road equipment. The allowable sulfur content of fuel is higher and 50 ppm sulfur fuel will not be required until the end of 2015.

Turkish regulations also follow the EU standards, with the Euro 4 light-duty gasoline and diesel regulations being implemented in 2008. The allowable sulfur content of fuel of 50 ppm was enforced in 2008, with higher levels (1000 ppm) for agricultural equipment.

Reference

DieselNet (2012). *Emission Standards: Summary of Worldwide Emission Standards*. Available at: www.dieselnet.com/standards/. Accessed 29 July 2013.

3,7-DINITROFLUORANTHENE

3,7-Dinitrofluoranthene was evaluated by previous IARC Working Groups in 1988 and 1995 (IARC, 1989, 1996). New data have since become available, and these have been taken into consideration in the present evaluation.

1. Exposure Data

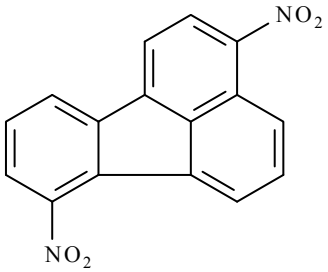
1.1 Chemical and physical data

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 105 735-71-5

Synonym: 3,7-Dinitrofluoroanthene

1.1.2 Structural and molecular formulae and relative molecular mass



$C_{16}H_8N_2O_4$

Relative molecular mass: 292.26 g/mol

1.1.3 Chemical and physical properties of the pure substance

Description: Yellow needles (Nakagawa *et al.*, 1987)

Melting-point: 203–204 °C (Nakagawa *et al.*, 1987)

Spectroscopy data: Nuclear magnetic resonance and ultraviolet spectral data have been reported (Ramdahl *et al.*, 1988)

Octanol/water partition coefficient: log K_{ow} = 4.44 (Lyman, 1985)

Vapour pressure (estimated): 9.1×10^{-10} mm Hg at 25 °C (Lyman, 1985)

Boiling-point: 526.6 °C at 760 mm Hg (Guidechem, 2012)

Flash-point: 268.2 °C (Guidechem, 2012)

Density: 1.574 g/cm^3 (Guidechem, 2012)

Solubility: Practically insoluble in water

1.2 Analysis

For a description of the analytical methods of *N*-polycyclic aromatic hydrocarbons in general, the reader is referred to Section 1.2.2(*d*) of the *Monograph* on Diesel Engine Exhausts.

Tokiwa *et al.* (1990) reported a method to separate and identify dinitrofluoranthenes in airborne particulates. The particulate matter was collected on a silica fibre filter and extracted with dichloromethane. The crude extracts were

applied to a column filled with silica gel and were eluted step by step with hexane, hexane:benzene (1:1, v/v), benzene, benzene:methanol (1:1, v/v) and methanol. The components were fractionated and identified by high-performance liquid chromatography and gas chromatography with mass spectrometry.

1.3 Production and use

3,7-Dinitrofluoranthene can be synthesized by the nitration of fluoranthene or 3-nitrofluoranthene in the presence of fuming nitric acid, followed by fractionation and purification by recrystallization (Nakagawa *et al.*, 1987; Horikawa *et al.*, 1991; Matsuoka *et al.*, 1993).

No evidence was found that either 3,7- or 3,9-dinitrofluoranthene has been produced in commercial quantities or used for any purpose other than laboratory applications.

1.4 Occurrence and exposure

1.4.1 Natural occurrence

3,7-Dinitrofluoranthene is not known to occur as a natural product.

1.4.2 Environmental occurrence and exposure

Nitrofluoranthenes can be formed during the combustion of fossil fuels that contain fluoranthene, and from atmospheric reactions of fluoranthene with nitrogen oxides. Sources of nitrofluoranthenes include diesel emissions, combustion emissions from kerosene heaters, gas fuel, liquefied petroleum, airborne particles, coal-fly ash and food (Horikawa *et al.*, 1987).

The use of 3,7-dinitrofluoranthene in laboratory applications may result in its release into the environment through various waste streams.

3,7-Dinitrofluoranthene was detected at a concentration of 0.028 mg/kg in particulates emitted from a diesel engine (Tokiwa *et al.*, 1986). In 1989, 3,7-dinitrofluoranthene was detected in airborne particulates at a level of 0.012 μg/g particulates, corresponding to 0.005 ng/m^3, and in particulate emissions from a kerosene heater at 0.14 μg/g particulates in Sapporo, Hokkaido, Japan (Tokiwa *et al.*, 1990).

Monitoring data indicated that exposure of the general population may occur through the inhalation of ambient air that contains 3,7-dinitrofluoranthene (HSDB, 2013).

2. Cancer in Humans

No data were available to the Working Group.

3. Cancer in Experimental Animals

3.1 Rat

See Table 3.1

3.1.1 Subcutaneous administration

Groups of 21 male Fischer 344/DuCrj rats (age, 6 weeks) received 3,7-dinitrofluoranthene (purity, 99.84%) by subcutaneous injection at a dose of 0 (control) or 0.05 mg dissolved in 0.2 mL of dimethyl sulfoxide twice per week for 10 weeks (total dose, 1 mg/rat). Animals were observed for 50 weeks; those with tumours at the site of injection were killed when moribund. The first subcutaneous tumour was observed in the treated group on day 155, and, within 48 weeks after the beginning of treatment, all treated rats had developed tumours at the site of injection; controls did not develop tumours. The incidence of tumours was highly statistically significant [21/21 (100%) versus 0/21; $P < 0.001$ Mann–Whitney U]. Twenty of 21 tumours were described as malignant fibrous histiocytoma and one as a rhabdomyosarcoma. Metastatic foci in the lungs were found in three rats. The mean tumour-induction time was 187 days (Tokiwa *et al.*, 1987).

Table 3.1 Studies of the carcinogenicity of 3,7-dinitrofluoranthene in rats

Strain (sex) Duration Reference	Route Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
F344/DuCrj (M) up to 50 wks Tokiwa *et al.* (1987)	Subcutaneous injection 0, 0.05 mg/rat in DMSO (0.25 mg/mL), twice/wk for 10 wks (total, 1.0 mg or 3.42 μmol) then observed up to 50 wks 21 M/group (age, 6 wk)	Injection site (subcutaneous sarcoma): 0/21, 21/21 (100%) Injection site (malignant histiocytoma): 0/21, 20/21 (95%) Injection site (rhabdomyosarcoma) : 0/21, 1/21 (5%)	[$P < 0.001$, Mann–Whitney U]	Purity, 99.84% by HPLC
F344 (M) up to 100 wks Horikawa *et al.* (1991)	Intrapulmonary implantation 0, 200 μg/rat in 0.05 mL beeswax:tricaprylin, injected directly into the left lower lung after thoracotomy then observed up until 100 wks 20 or 22 M/group (age, 11 wk)	Lung (all tumours): 0/19, 12/22 (54%) Lung (squamous-cell carcinoma): 0/19, 11/22 (50%) Lung (undifferentiated carcinoma): 0/19, 1/22 (5%)	[$P < 0.0002$, Mann–Whitney U]	Purity, > 99.90% by HPLC

DMSO, dimethyl sulfoxide; HPLC, high-performance liquid chromatography; M, male; wk, week

3.1.2 Intrapulmonary administration

Groups of 20 or 22 male Fischer 344/DuCrj rats (age, 11 weeks) received 3,7-dinitrofluoranthene (purity, > 99.9%) as a single, direct implantation at a dose of 0 (control) or 200 μg in 0.05 mL of beeswax:tricaprylin vehicle into the left lung by thoracotomy under ketamine anaesthesia, and were then observed for up to 100 weeks. The incidence of tumours of the lung was 12 out of 22 (54%; 11 squamous cell carcinomas and one undifferentiated carcinoma) in the treated group and 0 out of 19 in the controls. The incidence of tumours was highly statistically significant [$P < 0.0002$, Mann–Whitney U]. The first tumour was observed at week 51 after the start of the experiment and 12 tumours developed by day 351 after injection (Horikawa *et al.*, 1991).

4. Mechanistic and Other Relevant Data

3,7-Dinitrofluoranthene was considered by previous IARC Working Groups in 1988 and 1995 (IARC, 1989, 1996). Since that time, no new data have become available on the biological fate, metabolism, toxicity or carcinogenicity of this compound in experimental animals or in humans.

4.1 Absorption, distribution, metabolism, excretion

No data were available to the Working Group.

4.2 Genetic and related effects

4.2.1 Humans

No data were available to the Working Group.

4.2.2 Experimental systems

As reported in the previous *IARC Monograph* (IARC, 1989), 3,7-dinitrofluoranthene was highly active in the induction of reverse mutations in *Salmonella typhimurium* tester strains TA98, TA100, TA1538 and TA1537, but not in TA97, in the absence of a mammalian metabolic-activation system. Of these tester strains, TA98 was the most sensitive to 3,7-dinitrofluoranthene, indicating

that this chemical induces frameshift-type mutations (IARC, 1989; Tokiwa *et al.*, 1993). Several laboratories compared the potencies of various nitroarenes, including 3,7-dinitrofluoranthene, to induce reverse mutations in *S. typhimurium* TA98 in the absence of metabolic activation (IARC, 1989; Tokiwa *et al.*, 1993; Reifferscheid & Heil, 1996). Among several mono-, di- and trinitrofluoranthene derivatives tested, 3,7- and 3,9-dinitrofluoranthenes were highly mutagenic, to an extent that was comparable with that of dinitropyrenes.

The mutagenicity of 3,7-dinitrofluoranthene has also been determined in other strains of *S. typhimurium*, such as TA98/NR and TA98/1,8-DNP6, which are deficient in nitroreductase and *O*-acetyltransferase activities, respectively (IARC, 1989; Yamada *et al.*, 1997). The results showed that 3,7-dinitrofluoranthene was less mutagenic in these strains than in TA98 (Tokiwa *et al.*, 1987, 1993), indicating that it may be activated by nitroreductase to form mutagenic *N*-hydroxyarylamines and then by acetyltransferases to form highly reactive *N*-acetoxy esters, similarly to other potent mutagenic nitroarenes (IARC, 1989; Upadhyaya *et al.*, 1992; Chae *et al.*, 1993; Möller, 1994; Purohit & Basu, 2000).

In the Ames assay, 3,7-dinitrofluoranthene is less mutagenic in the presence than in the absence of metabolic activation, which suggests that xenobiotic-metabolizing enzymes convert this compound to a less mutagenic product. It should be noted that several other mutagenic nitroarenes, such as 1,3-, 1,6- and 1,8-dinitropyrenes, have also been reported to be inactivated by human and rat cytochrome P450 enzymes to form products that have lost their genotoxic and mutagenic activities in these bacteria (Shimada & Guengerich, 1990; Shane & Winston, 1997).

Oda *et al.* (1992) reported that 3,7-dinitrofluoranthene was genotoxic and induced *umu* gene expression (measured as β-galactosidase activity) more strongly in *S. typhimurium* NM1011, which has high nitrofurazone-reductase activity, than in the parent strain, *S. typhimurium* TA1535/pSK1002. Nitroreductase and *O*-acetyltransferase activities were both required for the full stimulation of gene expression in the *umu* tester strains by 3,7-dinitrofluoranthene (Oda *et al.*, 1993). The NM3009 strain, which has both high nitroreductase and *O*-acetylation activity, was most sensitive to the *umu* gene expression induced by 3,7-dinitrofluoranthene and 1,6-dinitropyrene, followed by strains NM2009 and NM1011, which have *O*-acetyltransferase and nitroreductase activity, respectively. As expected, NM1000 and NM2000, which are deficient in nitroreductase and *O*-acetyltransferase activity, respectively, were less sensitive to 3,7-dinitrofluoranthene and 1,6-dinitropyrene (Oda *et al.*, 1993).

3,7-Dinitrofluoranthene also induced chromosomal aberrations in a Chinese hamster cell line in the absence of a rat-liver metabolic system *in vitro* (Matsuoka *et al.*, 1993), and micronucleus formation in mouse bone marrow *in vivo* (Tokiwa *et al.*, 1993).

4.3 Mechanistic considerations

3,7-Dinitrofluoranthene was carcinogenic in experimental animals (IARC, 1989; Horikawa *et al.*, 1991). Assays for genotoxicity and mutagenicity in bacteria indicated that 3,7-dinitrofluoranthene requires metabolic activation by xenobiotic-metabolizing enzymes to form active metabolites (Nakagawa *et al.*, 1987; Oda *et al.*, 1992, 1993; Tokiwa *et al.*, 1993). Although the details of the metabolism of 3,7-dinitrofluoranthene are unknown, this nitroarene may be considered to undergo bioactivation via a similar mechanism as that described for 3,9-dinitrofluoranthene.

5. Summary of Data Reported

5.1 Exposure data

3,7-Dinitrofluoranthene is present in diesel exhaust emissions and emissions from heaters fuelled with liquefied petroleum gas. No evidence was found that it has been produced in commercial quantities or for purposes other than laboratory applications. Due to its low vapour pressure, this substance is associated with the particulate phase of these combustion emissions. Exposure of the general population can occur through inhalation of airborne particulate matter in an urban environment, and may also occur from the domestic use of burners of liquefied petroleum gas. No data on occupational exposure were available to the Working Group.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

3,7-Dinitrofluoranthene was tested for carcinogenicity in rats by subcutaneous injection in one study and by intrapulmonary implantation in another study. Subcutaneous injection of 3,7-dinitrofluoranthene caused a significant increase in the incidence of malignant fibrous histiocytoma at the injection site. Implantation of 3,7-dinitrofluoranthene into the lung caused a significant increase in the incidence of carcinoma of the lung.

5.4 Mechanistic and other relevant data

No data were available to the Working Group on the absorption, distribution, metabolism or excretion of 3,7-dinitrofluoranthene in humans, experimental animals or *in vitro*, or on the genetic and related effects of 3,7-dinitrofluoranthene in humans. The compound was strongly mutagenic in bacteria, mainly as a frameshift mutagen. The mutagenicity was weaker in bacterial strains that are deficient in nitroreductase or in *O*-acetyltransferase, indicating that 3,7-dinitrofluoranthene is activated through nitroreduction and acetyltransfer. 3,7-Dinitrofluoranthene was genotoxic in the *umu* gene-expression assay, and induced chromosomal aberrations in Chinese hamster cells and micronucleus formation in the bone marrow of mice *in vivo*. It induced tumours in rats at the sites of subcutaneous injection and intrapulmonary implantation. The evidence suggests that this chemical produces mutagenic metabolites that could play a role in its carcinogenicity.

Overall, these data provide *weak mechanistic evidence* to support the carcinogenicity of 3,7-dinitrofluoranthene.

6. Evaluation

6.1 Cancer in humans

No data were available to the Working Group.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of 3,7-dinitrofluoranthene.

6.3 Overall evaluation

3,7-Dinitrofluoranthene is *possibly carcinogenic to humans (Group 2B)*.

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3,9-DINITROFLUORANTHENE

3,9-Dinitrofluoranthene was evaluated by previous IARC Working Groups in 1988 and 1995 (IARC, 1989, 1996). New data have since become available, and these have been taken into consideration in the present evaluation.

1. Exposure Data

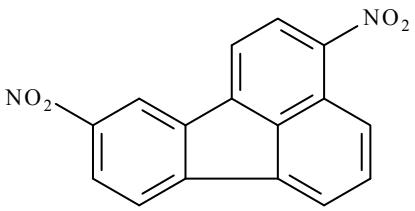
1.1 Chemical and physical data

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 22506-53-2

Synonym: 3,9-Dinitrofluoroanthene; 4,12-dinitrofluoranthene

1.1.2 Structural and molecular formulae and relative molecular mass



$C_{16}H_8N_2O_4$

Relative molecular mass: 292.26 g/mol

1.1.3 Chemical and physical properties of the pure substance

Description: Yellow needles (Nakagawa *et al.*, 1987); yellow-orange crystals (Charlesworth & Lithown, 1969)

Melting-point: 222–224 °C (Nakagawa *et al.*, 1987); 275–276 °C (Charlesworth & Lithown, 1969)

Spectroscopy data: Nuclear magnetic resonance and ultraviolet spectral data have been reported (Ramdahl *et al.*, 1988)

Octanol/water partition coefficient: log K_{ow} = 4.44 (Lyman, 1985)

1.2 Analysis

Tokiwa *et al.* (1990) reported a method to separate and identify dinitrofluoranthenes in airborne particulates. The particulate matter was collected on a silica fibre filter and extracted with dichloromethane. The crude extracts were applied to a column filled with silica gel and were eluted step by step with hexane, hexane:benzene (1:1, v/v), benzene, benzene:methanol (1:1, v/v) and methanol. The components were fractionated and identified by high-performance liquid chromatography and gas chromatography with mass spectrometry.

1.3 Production and use

3,9-Dinitrofluoranthene can be synthesized by the nitration of fluoranthene or 3-nitrofluoranthene in the presence of fuming nitric acid, with subsequent fractionation and purification by recrystallization (Nakagawa *et al.*, 1987; Horikawa *et al.*, 1991; Matsuoka *et al.*, 1993).

No evidence was found that 3,9-dinitrofluoranthene has been produced in commercial quantities or used for any purpose other than laboratory applications.

1.4 Occurrence and exposure

1.4.1 Natural occurrence

3,9-Dinitrofluoranthene is not known to occur as a natural product.

1.4.2 Environmental exposure

Nitrofluoranthenes can be formed during the combustion of fossil fuels that contain fluoranthene, and through atmospheric reactions of fluoranthene with nitrogen oxides. Sources of nitrofluoranthenes include diesel emissions, combustion emissions from kerosene heaters, gas fuel, liquefied petroleum, airborne particles, coal fly ash and food (Horikawa *et al.*, 1987).

3,9-Dinitrofluoranthene was detected at a concentration of 0.013 mg/kg in particulates emitted from a diesel engine (Tokiwa *et al.*, 1986). In 1989, 3,9-dinitrofluoranthene was detected in airborne particulates at a level of 0.009 μg/g particulates, corresponding to 0.004 ng/m^3, in Sapporo, Hokkaido, Japan (Tokiwa *et al.*, 1990).

Monitoring data indicate that exposure of the general population to 3,9-dinitrofluoranthene may occur through its inhalation in the ambient air (HSDB, 2013).

2. Cancer in Humans

No data were available to the Working Group.

3. Cancer in Experimental Animals

3.1 Rat

See Table 3.1

3.1.1 Subcutaneous administration

A group of 11 male Fischer 344/DuCrj rats (age, 6 weeks) received 3,9-dinitrofluoranthene (purity, 99.8%) by subcutaneous injection at a dose of 0.05 mg dissolved in 0.2 mL of dimethyl sulfoxide twice per week for 10 weeks (total dose, 1 mg/rat). A group of 21 males was injected similarly with the solvent alone. Animals were observed for 50 weeks; those with tumours at the site of injection were killed when moribund. The first subcutaneous tumour was observed in the treated group on day 88, and 10 out of 11 (91%) treated rats had developed tumours at the site of injection by 48 weeks after the beginning of treatment; controls did not develop tumours. The incidence of tumours was highly statistically significant [10/11 versus 0/21; $P < 0.001$]. Seven of 10 tumours were described as malignant fibrous histiocytoma and three as rhabdomyosarcoma. No metastasis was found. Mean tumour-induction time was 107 days (Tokiwa *et al.* 1987).

3.1.2 Intrapulmonary administration

Groups of 10–21 male Fischer 344/DuCrj rats (age, 11 weeks) received 3,9-dinitrofluoranthene (purity, > 99.98%) as a single implantation at a dose of 0, 50, 100 or 200 μg in 0.05 mL of beeswax:tricaprylin vehicle into the left lung by thoracotomy under ketamine anaesthesia, and were then observed for up to 100 weeks. The incidence of tumours of the lung in the groups at 50, 100 and 200 μg was 1 out of 10 (10%), 7 out of 10 (70%) and 19 out of 21 (90%), respectively. No tumours were found in the vehicle-control group (0 out of 19). In rats at 200 μg, the first tumour was observed on day 257 after injection. The incidence of tumours in the groups at 100 μg and

Table 3.1 Studies of the carcinogenicity of 3,9-dinitrofluoranthene in rats

Strain (sex) Duration Reference	Route Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
F344 (M) up to 50 wks Tokiwa *et al.* (1987)	Subcutaneous injection 0, 0.05 mg/rat in 0.25 mg/mL DMSO, twice/wk for 10 wks (total, 1.0 mg or 3.42 µmol) then observed up to 50 wk 11 or 21 M/group (age, 6 wk)	Injection site (subcutaneous sarcoma): 0/21, 10/11 (91%) Injection site (malignant histiocytoma): 0/21, 7/11 (64%) Injection site (rhabdomyosarcoma): 0/21, 3/11 (27%)	[$P < 0.001$, Mann–Whitney U]	Purity, 99.8% by HPLC
F344 (M) up to 100 wks Horikawa *et al.* (1991)	Intrapulmonary implantation 0, 50, 100, 200 µg/rat in 0.05 mL beeswax:tricaprylin, injected directly into the left lower lung after thoracotomy then observed up until 100 wk 10–21 M/group (age, 11 wk)	Lung (squamous cell and adenosquamous carcinoma combined): 0/19, 1/10 (10%), 7/10 (70%), 19/21 (90%) Lung (squamous-cell carcinoma): 0/19, 18/21 (86%) Lung (adenosquamous carcinoma): 0/19, 1/21 (5%)	[$P < 0.001$, mid- and high-dose; Mann–Whitney U]	Purity, > 99.9% by HPLC

DMSO, dimethyl sulfoxide; HPLC, high-performance liquid chromatography; M, male, wk, week

200 µg was statistically significantly increased in comparison with the control group [$P < 0.001$, Mann–Whitney U]. The tumours in the group at 100 µg were described as five squamous cell carcinomas, one adenosquamous carcinoma and one undifferentiated carcinoma, and those in the group at 200 µg as 18 squamous-cell carcinomas and one adenosquamous carcinoma (Horikawa *et al.* 1991).

4. Mechanistic and Other Relevant Data

3,9-Dinitrofluoranthene was considered by previous IARC Working Groups in 1988 and 1995 (IARC, 1989, 1996). Since that time, no new data have become available on the biological fate, metabolism or toxicity of this compound in experimental animals or humans.

4.1 Absorption, distribution, metabolism, excretion

4.1.1 Humans

No data were available to the Working Group.

4.1.2 Experimental systems

Mitchell *et al.* (1993) reported the metabolism of 3,9-dinitrofluoranthene under anaerobic conditions *in vitro* in subcellular fractions of the lung, the target organ for carcinogenicity in rats (Tokiwa *et al.*, 1987; Horikawa *et al.*, 1991). The rate of metabolism of 3,9-dinitrofluoranthene, the most carcinogenic compound of the various nitrofluoranthene derivatives, was compared with that of 2-, 3- and 8-nitrofluoranthenes, which have been reported to be relatively weak or inactive in the induction of tumours in experimental animals (Ohgaki *et al.*, 1982; Tokiwa & Ohnishi, 1986; Tokiwa *et al.*, 1986, 1993; Horikawa *et al.*, 1991). Both the cytosolic and microsomal fractions from rat lung anaerobically converted 3,9-dinitrofluoranthene and 2-, 3- or 8-monofluoranthene into their amino derivatives, i.e. 3-amino-9-nitrofluoranthene and 2-, 3- or 8-aminofluoranthene, respectively. The extent of formation of the amino derivative of 3,9-dinitrofluoranthene was found to be much greater than that of the three mononitrofluoranthenes (Mitchell *et al.*, 1993). Because the formation of amino derivatives of carcinogenic nitroarenes, such as 1,3-, 1,6- and 1,8-dinitropyrenes, has been reported to be a key step in the metabolic activation of nitroarenes to DNA-binding products (Rosenkranz & Mermelstein, 1983; Tokiwa *et al.*, 1986; Wislocki *et al.*, 1986; Möller, 1994; Purohit & Basu, 2000), 3,9-dinitrofluoranthene may also be classified as potentially carcinogenic in view of this metabolic conversion (Mitchell *et al.*, 1993; Möller, 1994).

Mitchell *et al.* (1993) also reported that 3-nitrofluoranthene was oxidized by microsomes from rat lung to form at least three metabolites, the most important of which was identified as 3-nitrofluoranthene-8-ol.

4.2 Genetic and related effects

4.2.1 Humans

No data were available to the Working Group.

4.2.2 Experimental systems

As reported in a previous *IARC Monograph* (IARC, 1989), 3,9-dinitrofluoranthene was highly active in the induction of reverse mutations in *Salmonella typhimurium* tester strains TA98, TA100, TA1538 and TA1537, but not TA97, in the absence of a mammalian metabolic-activation system. Of these tester strains, TA98 was the most sensitive to 3,9-dinitrofluoranthene, indicating that this chemical induces frameshift-type mutations (IARC, 1989; Tokiwa *et al.*, 1993). Several laboratories compared the potencies of various nitroarenes, including

3,9-dinitrofluoranthene, to induce reverse mutations in *S. typhimurium* TA98 in the absence of metabolic activation (IARC, 1989; Tokiwa *et al.*, 1993; Reifferscheid & Heil, 1996). Among several mono-, di- and trinitrofluoranthene derivatives, 3,7- and 3,9-dinitrofluoranthene were highly mutagenic, to an extent that was comparable with that of the dinitropyrenes.

The mutagenicity of 3,9-dinitrofluoranthene was also determined in other strains, such as *S. typhimurium* TA98/NR and TA98/1,8-DNP6, that are deficient in nitroreductase and *O*-acetyltransferase activities, respectively (IARC, 1989; Yamada *et al.*, 1997). The results showed that 3,9-dinitrofluoranthene was less mutagenic in these strains than in TA98 (Tokiwa *et al.*, 1987, 1993), indicating that this chemical may be activated to mutagenic products by nitroreductase (IARC, 1989).

In the Ames assay, 3,9-dinitrofluoranthene was less mutagenic in the presence than in the absence of metabolic activation, which suggests that xenobiotic-metabolizing enzymes convert this compound to a less mutagenic product. It should be noted that several other mutagenic nitroarenes, such as 1,3-, 1,6- and 1,8-dinitropyrene, have also been reported to be inactivated by human and rat cytochrome P450 enzymes to form products that have lost their genotoxic and mutagenic activity in bacteria (Shimada & Guengerich, 1990; Shane & Winston, 1997).

Oda *et al.* (1992) reported that 3,9-dinitrofluoranthene was genotoxic and induced *umu* gene expression (measured as β-galactosidase activity) more strongly in *S. typhimurium* NM1011, which has high nitrofurazone-reductase activity, than in the parent strain, *S. typhimurium* TA1535/pSK1002. The strains deficient in nitroreductase and/or *O*-acetyltransferase were less sensitive to the induction of *umu* gene expression by 3,9-dinitrofluoranthene and other nitroarenes, such as 3,7-dinitrofluoranthene, 3-nitrofluoranthene, and 1,3-, 1,6- and 1,8-dinitropyrene, which indicates that these two enzyme activities are required for the activation process (Oda *et al.*, 1993). As expected, *S. typhimurium* NM3009, which expresses both high nitroreductase and high *O*-acetyltransferase activity, showed sensitivity for *umu* gene expression induced by 3,9-dinitrofluoranthene, followed by strains NM2009 and NM1011, which have *O*-acetyltransferase and nitroreductase activity, respectively.

3,9-Dinitrofluoranthene also induced chromosomal aberrations in a Chinese hamster cell line in the absence of rat-liver metabolic activation *in vitro* (Matsuoka *et al.*, 1993) and micronucleus formation in mouse bone marrow *in vivo* (Tokiwa *et al.*, 1993).

4.3 Mechanistic considerations

(See also the corresponding Section in the *Monograph* on 3,7-Dinitrofluoranthene in this Volume.)

Assays for genotoxicity and mutagenicity in bacteria indicated that 3,9-dinitrofluoranthene requires metabolic activation by xenobiotic-metabolizing enzymes to form active metabolites (Nakagawa *et al.*, 1987; Oda *et al.*, 1992, 1993; Tokiwa *et al.*, 1993).

3,9-Dinitrofluoranthene was metabolized to its amino derivative by rat lung cytosolic and microsomal nitroreductases under anaerobic conditions *in vitro* (Mitchell *et al.*, 1993), and was hypothesized to be activated primarily to a reactive intermediate by such nitroreductases (Tokiwa *et al.*, 1986; Möller, 1994; Purohit & Basu, 2000).

Although 3,9-dinitrofluoranthene has been postulated to be activated to reactive metabolites by bacterial and mammalian enzyme systems, the detoxification pathways of this chemical by various xenobiotic-metabolizing enzymes are not fully understood. Further studies are required to understand the underlying mechanisms of the genotoxicity, mutagenicity and carcinogenicity of 3,9-dinitrofluoranthene.

5. Summary of Data Reported

5.1 Exposure data

3,9-Dinitrofluoranthene is present in diesel exhaust emissions and emissions from heaters fuelled by liquefied petroleum gas. No evidence was found that it has been produced in commercial quantities or used for purposes other than laboratory applications. Due to its low vapour pressure, this substance is associated with the particulate phase of these combustion emissions. Exposure of the general population to 3,9-dinitrofluoranthene occurs through inhalation of airborne particulate matter in urban environments. Exposure may also occur from domestic use of burners of liquefied petroleum gas. No data on occupational exposure were available to the Working Group.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

3,9-Dinitrofluoranthene was tested for carcinogenicity in rats by subcutaneous injection in one study and by direct implantation into the lung in another study. Subcutaneous injection of 3,9-dinitrofluoranthene induced a significant increase in the incidence of subcutaneous sarcomas, and intrapulmonary implantation produced a significant increase in the incidence of carcinomas of the lung.

5.4 Mechanistic and other relevant data

No data were available on the absorption, distribution, metabolism and excretion, or genetic and related effects of 3,9-dinitrofluoranthene in humans. Cytosolic and microsomal fractions from rat lung anaerobically converted 3,9-dinitrofluoranthene into 3-amino-9-nitrofluoranthene. The compound was strongly mutagenic in bacteria in the absence of metabolic activation, acting mainly as a frameshift mutagen. The mutagenicity was weaker in bacterial strains that are deficient in nitroreductase or *O*-acetyltransferase, indicating that the activation of 3,9-dinitrofluoranthene occurs through its nitroreduction and acetyltransfer. It was genotoxic in the *umu* gene-expression assay, and induced chromosomal aberrations in Chinese hamster cells *in vitro* and micronucleus formation in the bone marrow of mice *in vivo*. 3,9-Dinitrofluoranthene induced tumours in rats at the site of exposure, while its intrapulmonary implantation produced lung tumours. This evidence suggests that the mutagenic metabolites formed from this agent could play a role in its carcinogenicity.

Overall, these data provide *weak mechanistic evidence* to support the carcinogenicity of 3,9-dinitrofluoranthene.

6. Evaluation

6.1 Cancer in humans

No data were available to the Working Group.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of 3,9-dinitrofluoranthene.

6.3 Overall evaluation

3,9-Dinitrofluoranthene is *possibly carcinogenic to humans (Group 2B)*.

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1,3-DINITROPYRENE

1,3-Dinitropyrene was evaluated by a previous IARC Working Group in 1988 (IARC, 1989). New data have since become available, and these have been taken into consideration in the present evaluation.

1. Exposure Data

1.1 Chemical and physical data

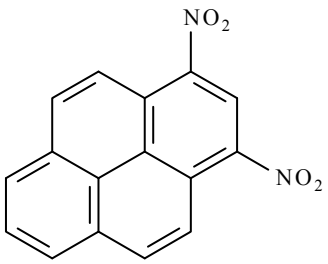
1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 75 321-20-9

Chem. Abstr. Name: Pyrene, 1,3-dinitro-

IUPAC Systematic Name: 1,3-Dinitropyrene

1.1.2 Structural and molecular formulae and relative molecular mass



$C_{16}H_8N_2O_4$

Relative molecular mass: 292.3

1.1.3 Chemical and physical properties of the pure substance

Description: Light-brown needles, recrystallized from benzene and methanol (Buckingham, 1985)

Melting-point: 274–276 °C (Buckingham, 1985); 295–297 °C (Paputa-Peck *et al.*, 1983)

Spectroscopy data: Ultraviolet (Paputa-Peck *et al.*, 1983), infrared (Hashimoto & Shudo, 1984), nuclear magnetic resonance (Kaplan, 1981; Paputa-Peck *et al.*, 1983; Hashimoto & Shudo, 1984) and mass spectral data (Schuetzle & Jensen, 1985) have been reported. The National Institute of Standards and Technology Chemistry WebBook provides extensive spectroscopic data (Linstrom & Wallard, 2011).

Solubility: Moderately soluble in toluene (Chemsyn Science Laboratories, 1988)

1.1.4 Technical products and impurities

1,3-Dinitropyrene is available for research purposes at a purity of 99% (Sigma-Aldrich, 2012). The ChemicalBook web site lists nine companies that supply 1,3-dinitropyrene (ChemicalBook, 2012).

1.2 Analysis

For analytical methods of nitro-polycyclic aromatic hydrocarbons (PAHs) in general, the reader is referred to Section 1.2.2(*d*) of the

Monograph on Diesel and Gasoline Engine Exhausts in this Volume.

A variety of analytical methods have been used to separate and quantify specific dinitropyrenes in environmental samples. Hayakawa *et al.* (1992) used the reduction of the nitro groups off-line with sodium hydrosulfide followed by separation of the mixture of amino derivatives of nitropyrenes and dinitropyrenes using high-performance liquid chromatography with chemiluminescence detection. In a later modification of this method, nitro-PAHs were separated from extraneous substances using a clean-up column and reduced to their amino derivatives on a platinum/rhodium-coated alumina reducer column. The derivatives were concentrated on a concentrator column and eluted into a separator column, and the components were measured by chemiluminescence induced by a solution of bis(2,3,6-trichlorophenyl)oxalate and hydrogen peroxide (Hayakawa *et al.*, 2001). Hutzler *et al.* (2011) reported the high sensitivity and specificity of liquid chromatography with an atmospheric-pressure photoionization source attached to an API 4000 mass spectrometer.

The measurement of dinitropyrene vapour in environmental samples is challenging because of the very low concentrations, which limited some of the earlier studies. Araki *et al.* (2009) developed an apparatus to collect nitropyrene vapours downstream of a quartz fibre filter in a high-volume sampler at 300 L/min. The specially designed cylindrical vapour collector (8 cm in diameter, 8 cm in length) contained two layers of XAD-4 resin, the first 4 cm deep and the second 2 cm deep, each followed by a 1-cm thick sheet of polyurethane foam. Dinitropyrenes were measured by gas chromatography-mass spectrometry (MS) with an electron ionizing detector.

Crimmins & Baker (2006) achieved high sensitivity using a programmed temperature vapourization method for injecting a large volume of gas into a gas chromatograph with a mass spectrometer as a detector. MS was performed using negative chemical ionization with methane ionization gas (40 mL/minute) at a temperature of 200 °C. Excellent reproducibility was obtained, with a relative standard deviation of 1.4–3.7% for three dinitropyrene compounds, but the limits of detection of the instrument were relatively high: 0.53 pg for 1,3-dinitropyrene, 2.85 pg for 1,6-dinitropyrene and 1.7 pg for 1,8-dinitropyrene, which were at least one order of magnitude higher than that for 1-nitropyrene (0.17 pg). When the method was applied to the National Institute of Standards and Technology standardized reference materials 1649 and 1650 prepared from samples of diesel engine exhaust, none of the dinitropyrenes could be detected because of matrix effects at the inlet.

1.3 Production and use

1,3-Dinitropyrene is produced by the nitration of 1-nitropyrene, and have been isolated and purified from such preparations (Yoshikura *et al.*, 1985).

No evidence was found that 1,3-dinitropyrene has been produced in commercial quantities or used for purposes other than laboratory applications.

1.4 Occurrence

1.4.1 Engine exhaust

The reader is also referred to the *Monographs* on Diesel and Gasoline Engine Exhausts and 1-Nitropyrene in this Volume.

During combustion in diesel and gasoline engines, pyrene is nitrated to form 1-nitropyrene, which is further nitrated to form small amounts of 1,3-, 1,6- and 1,8-dinitropyrene (Heeb *et al.*, 2008). A variety of tests of diesel engine emissions were performed in the 1980s, which showed a range of 1,3-dinitropyrene concentrations in the particulate matter (PM) of up to 1600 pg/mg (Table 1.1; reviewed in Fu

Table 1.1 Levels of 1,3-dinitropyrene in diesel engine exhaust particles and their extracts

Reference	Vehicle/engine	Concentration of 1,3-DNP (pg/mg particulate matter)
Nishioka *et al.* (1982)	Passenger cars (LDD)	ND–600[a]
Gibson (1983)	Diesel cars, 1978–82 (LDD)	≤ 5
Nakagawa *et al.* (1983)	Idling 6-tonne bus from 1970 (HDD), 1200 rpm	Detected
Schuetzle & Perez (1983)	*Heavy-duty vehicle*	
	Idle	< 800
	High-speed, no load	600
	High-speed, full load	400
Salmeen *et al.* (1984)	Passenger cars (LDD)	300 ± 200
Draper (1986)	Commercial mining engine (HDD), 100% load, 1–200 rpm	520
	Commercial mining engine (HDD), 75% load, 1–800 rpm	1600
Hayakawa *et al.* (1992)	Idling engine (LDD)	81.8[b]

[a] Range of three different engines
[b] Using a much more sensitive analytical method
DNP, dinitropyrene; HDD, heavy-duty diesel; LDD, light-duty diesel; ND, not detected

Table 1.2 Mass concentrations in particulate matter from diesel and gasoline engine exhausts from tailpipes in 1992

	No. of samples	Concentration (pg/mg)[a]			
		1-NP	1,3-DNP	1,6-DNP	1,8-DNP
Gasoline engine, idle	8	444 ± 210	64 ± 44	128 ± 106	102 ± 53
Diesel engine, idle	7	12 600 ± 13 100	67 ± 44	67 ± 47	61 ± 41

[a] Values are means ± standard deviations
DNP, dinitropyrene; NP, nitropyrene
From Hayakawa *et al.* (1992, 1994)

& Herreno-Saenz, 1999). The production of dinitropyrenes appears to depend on engine size and operating conditions.

Using a more sensitive method than in earlier studies, Hayakawa *et al.* (1992, 1994) examined nitroarenes in PM emissions from 15 diesel and gasoline engine vehicles (Table 1.2). Exhausts from idling diesel and gasoline engines contained approximately the same mass concentration of 1,3-dinitropyrene [64–67 pg/mg]; however, the ratio of the concentrations of 1,3-dinitropyrene to 1-nitropyrene was 14% for gasoline and 0.5% for diesel engine exhaust, which was assumed to be the result of differences in combustion conditions. The diesel engines produced many more particulates, and their total emissions of 1,3-dinitropyrene were therefore much greater. In emissions from mixed traffic, the air concentration ratio of 1,3-dinitropyrene to 1-nitropyrene decreases as the relative number of diesel vehicles increases.

In the past decade, particulate filters have been developed to filter PM from diesel engine exhausts to control emissions. The accumulated soot particles and organic carbon components, including PAHs and nitro-PAHs, that collect on the filters are removed by oxidation, aided by catalytic coatings or catalysts added to the fuel (see Section 1.1 in the *Monograph* on Diesel and Gasoline Engine Exhausts in this Volume).

In a series of laboratory tests, a range of various types of diesel particulate filter was tested

to compare their impact on PAH and nitro-PAH emissions (Heeb *et al.*, 2010). All filters tested, which removed 99% of the particles, also removed most PAH components. However, low-oxidation filters produced 63% more 1-nitropyrene than the amount present in the unfiltered exhaust; although the quantities of dinitropyrenes were not measured, they would also be expected to increase similarly.

Carrara & Niessner (2011) examined the formation of 1-nitropyrene in high- and low-oxidation filters operating at temperatures of 293–573 °K (20–300 °C). The lower temperatures produced more 1-nitropyrene on the filter, with a peak at ~100 °C that declined at higher temperatures. Measurements at 250 °C showed that < 2% of the 1-nitropyrene was on the filter and 47% ± 12% was on the vapour collector (losses of vapour were noted). Although they were not measured in the samples, dinitropyrenes would be expected to be affected similarly.

1.4.2 Environmental occurrence

(a) Air

The nitration of pyrene in atmospheric processes leads to the formation of 2- but not 1-nitropyrene, because the oxidants involved differ from those that are present during combustion, which produces 1-nitropyrene (Pitts, 1987). Thus, dinitropyrenes cannot be formed by atmospheric processes.

The earliest reports of 1,3-dinitropyrene in atmospheric air date back to the early 1980s. The presence of dinitropyrenes [not further characterized] in respirable particles from ambient atmospheric samples was inferred from mutagenicity testing of polycyclic organic matter extracts (Pitts, 1987). Tanabe *et al.* (1986) found levels of 1,3-dinitropyrene of up to 4.7 pg/m^3 in the air and up to 56.2 pg/mg in PM in the ambient atmosphere in Tokyo, Japan. Gibson (1986) found no 1,3-dinitropyrene in the ambient air at six sites in the USA, under various conditions. [The Working Group noted that the limit of detection of the analytical method used was 1 pg/mg PM and may have been too high.] Similarly, 1,3-dinitropyrene was not detected in another study in the Michigan area (Siak *et al.*, 1985).

In the early 1990s, Hayakawa and colleagues developed sensitive methods for the detection of nitro- and dinitropyrenes (see Section 1.2) and used them to perform a series of studies on PAHs and nitro-PAHs in Japan, and later in the countries surrounding the Sea of Japan. In a first study of atmospheric air, they determined that 94.3% of the 1,3-, 1,6- and 1,8-dinitropyrene, and 99.8% of the 1-nitropyrene in the air came from diesel engine emissions (Murahashi *et al.*, 1995). They later compared the atmospheric formation of nitropyrenes from highway emission sources (Hayakawa *et al.*, 2002). Samples were taken at three sites at varying distances from a highway (two urban and one suburban) in Kanazawa, Japan, using a high-volume sampler to collect total suspended particulates; 24-hour samples were collected for 6 consecutive days during each of the four seasons in 1989–96 (total, 84 samples; Table 1.3). Air concentrations of nitropyrenes rapidly declined with distance from the highway. A characteristic pattern of similar air concentrations of dinitropyrene isomers, within a factor of two, was observed; also, concentrations of dinitropyrenes were two orders of magnitude lower (approximately 0.5%) those of the parent compound, 1-nitropyrene.

A short series of five, 24-hour roadside samples were collected in Kanazawa, Japan in 2007 (Araki *et al.*, 2009). The concentration pattern was similar to that in earlier samples (Table 1.3), and the levels of dinitropyrenes in PM were less than 1% of those of 1-nitropyrene. Compared with the earlier samples, no evidence of a decline over time was found. The authors also examined the vapour-PM partitioning of the nitro-PAHs using a newly designed, high-volume vapour collector. No dinitropyrenes

Table 1.3 Airborne concentrations of 1-nitropyrene and isomers of dinitropyrene

Reference	Location, site	No. of samples	Airborne concentrations (pg/mg³)[a,b]			
			1-NP	1,3-DNP	1,6-DNP	1,8-DNP
Hayakawa *et al.* (2002)	*Kanazawa, Japan, 1989–96*					
	2 m from an urban roadside	84	170 (35–400)	0.61 (0.29–1.8)	1.1 (0.41–2.6)	1.0 (0.32–2.9)
	10 m from an urban roadside	84		0.2 (0.06–0.64)	0.24 (0.05–0.67)	0.23 (0.03–0.73)
	Suburban area not near roads	84	54 (7.4–150) 5.4 (1.0–16)	0.023 (0.006–0.06)	0.025 (0.008–0.04)	0.025 (0.003–0.06)
Araki *et al.* (2009)	*Kanazawa, Japan, 2007*					
	Urban roadside in winter	5	18.8 ± 7.41	0.12 ± 0.06	0.17 ± 0.088	[0.61][c] ± 0.17
Schauer *et al.* (2004)	*Munich area, Germany*					
	Urban site, September–23 October 2002	10	22 ± 6	16 ± 5	4.8 ± 3.4	ND
	Rural site, 28 August–15 September 2002	5	6.6 ± 3.9	4.0 ± 1.6	3.0 ± 1.8	ND
	Alpine site, 30 October–26 November 2002	9	2.2 ± 0.7	0.1 ± 0.1	0.6 ± 0.5	ND

[a] Values are means (range) or means ± standard deviations

[b] Reported values for 1,3-, 1,6- and 1,8-DNP in fmol/m³ were multiplied by 0.292 pg/fmol and those of 1-NP were multiplied by 0.247 pg/fmol for the conversion to pg/m³.

[c] The value reported in the article (21 fmol/m³) conflicts with the reported range of 1.3–2.9 fmol/m³. The Working Group therefore assumed that the correct value is 2.1 fmol/m³, or 0.61 pg/m³.

DNP, dinitropyrene; m, metres; ND, not detected; NP, nitropyrene

Table 1.4 Mass concentrations of 1-nitropyrene and three isomers of dinitropyrene in airborne particulate matter

Reference	Location, study period	No. of samples	Concentration (pg/mg)[a]			
			1-NP	1,3-DNP	1,6-DNP	1,8-DNP
Kakimoto *et al.* (2002)	Kitakyushu, Japan Industrial city centre, 1997	20	230 ± 96	4.1 ± 0.18	7.9 ± 6.5	5.9 ± 1.8
	Sapporo, Japan Roadside of highway, 1997	20	2700 ± 1600	11 ± 9.4	10 ± 8.8	16 ± 9.4
	Tokyo, Japan Roadside of highway, 1997	20	940 ± 490	4.7 ± 0.85	5.0 ± 3.2	8.2 ± 3.2
Albinet *et al.* (2006)	Sollières, France Rural site, winter 2002–03	13	10.6 (2.7–28.9)	3.7 (0.0–27.7)	1.3 (0.0–4.4)	9.5 (0.0–27.2)

[a] Values are means ± standard deviations (range)
DNP, dinitropyrene; NP, nitropyrene

were detected in the gas phase samples, whereas ~10% of the 1-nitropyrene was present in the gas phase. Nitro-PAHs were generally less volatile than their parent compounds.

1-Nitropyrene and related dinitropyrene isomers are not formed in the atmosphere, but can be removed by atmospheric processes such as photo-degradation; as a result, their concentration declines more rapidly than through dilution and dispersion alone (Morel *et al.*, 2006). Kakimoto *et al.* (2002) noted that the levels of 1-nitropyrene and dinitropyrene in airborne PM declined more rapidly with distance than the un-nitrated PAHs, which implies an active removal process. Also, the levels of dinitropyrenes in the PM collected close to the roadside in Sapporo and Tokyo, Japan, were 0.2–0.9% of those of 1-nitropyrene (Table 1.4), whereas at a distance from the roadside, in Kitakyushu, Japan, levels of dinitropyrenes were 1.7–34% of those of the 1-nitropyrene, which indicates that 1-nitropyrene is removed more rapidly than dinitropyrenes.

To link the levels to the sources, the authors also recorded the percentage of diesel-powered vehicles registered in each city and the volume of kerosene purchased per home. Levels of each dinitropyrene and 1-nitropyrene in PM increased with the percentage of diesel-powered vehicles registered in the cities. Atmospheric concentrations of 1-nitropyrene and of each dinitropyrenes were 1.3–4.3-fold higher in the winter (with the exception of 1,3-dinitropyrene in Kitakyushu), but most differences were not significant (Table 1.4). Sapporo had the highest percentage of diesel vehicles compared with Kitakyushu and Tokyo (38.5% versus 20% and 26%, respectively) and the highest volume of kerosene used per home (1343 L versus 224 L and 85 L, respectively) (Kakimoto *et al.*, 2002). This site also had the lowest dinitropyrene:1-nitropyrene ratio (0.011 versus 0.06 and 0.017, respectively). The differences in the number of vehicles and the amount of kerosene used may account in part for the variations between the three cities. Consistently, Sapporo had the highest airborne levels of each of the dinitropyrene isomers, but the differences were much smaller than those for 1-nitrropyrene.

At a rural site in France, the levels of dinitropyrenes isomers were similar to those in a previous study (Albinet *et al.*, 2006).

Schauer *et al.* (2004) collected a series of air samples in Germany during the autumn and summer of 2002 in urban (Munich), rural (Hohenpeissenberg) and high alpine (Zugspitze) locations to measure the levels of PAHs and nitro-PAHs. The method of analysis used nitro-PAH

Table 1.5 Distribution of particulate matter, dinitropyrene isomers and 1-nitropyrene by particle size

PM size (μm)	PM (μg/m³ (%))	1,3-DNP[a] (pg/m³ (%))	1,6-DNP[a] (pg/m³ (%))	1,8-DNP[a] (pg/m³ (%))	1-NP[a] (pg/m³ (%))
> 7	24 (36%)	0.02 (3%)	0.04 (5%)	0.06 (9%)	4.99 (3%)
3.3–7	7.1 (11%)	0.04 (5%)	0.05 (6%)	0.07 (10%)	8.10 (4%)
2–3.3	4.6 (7%)	0.04 (5%)	0.05 (6%)	0.06 (8%)	7.46 (4%)
1.1–2	4.4 (7%)	0.05 (6%)	0.06 (7%)	0.06 (8%)	10.20 (5%)
< 1.1	26 (39%)	0.62 (81%)	0.62 (76%)	0.46 (65%)	155 (84%)
Total	66.1	0.77	0.82	0.70	186

[a] Values were converted from fmol/m³ to pg/m³ using a conversion factor of 0.292 pg/fmol for DNPs and 0.247 pg/fmol for 1-NP.
DNP, dinitropyrene; NP, nitropyrene; PM, particulate matter
From Hayakawa *et al.* (1995)

reduction to amino-PAHs and fluorescence detection, which appears to be less sensitive than the chemiluminescence detection used by Hayakawa's group. The air concentrations for 1,3- and 1,6-dinitropyrene were among the highest observed for the urban and rural areas (Table 1.3). The concentrations of 1,3-dinitropyrene were almost of the same magnitude as those of 1-nitropyrene, which differed considerably from the Japanese data that showed a difference of at least 100-fold between the concentrations of 1-nitropyrene and the dinitropyrenes isomers. [The Working Group noted that the values for dinitropyrenes seemed to be unreasonably high.]

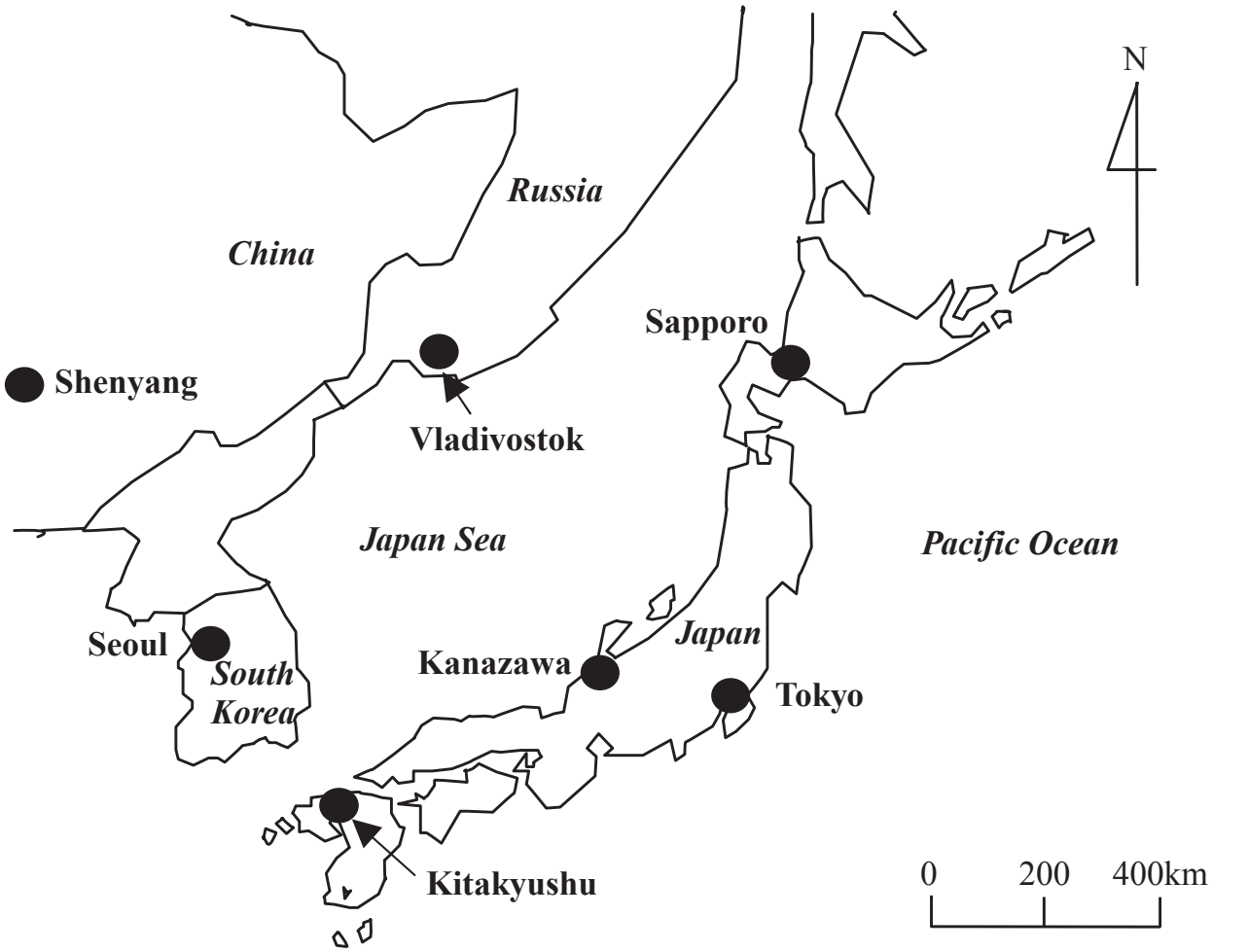
In the autumn of 1993, Hayakawa *et al.* (1995) collected size-fractionated PM with an Andersen high-volume sampler for 6 days in downtown Kanazawa, Japan (Table 1.5). Approximately one-third (36%) of the particulate mass comprised particles > 7 μm and another third (39%) comprised particles < 1.1 μm. However, 81% of 1,3-, 76% of 1,6- and 65% of 1,8-dinitropyrene, and 84% of 1-nitropyrene were found in the smallest particles (< 1.1 μm), probably due to the predominant diesel emissions in the submicron PM sizes from nearby heavy traffic.

The authors later extended these studies to the Pan-Japan Sea area (Tang *et al.*, 2005). The sampling sites are given in Fig. 1.1. The amount of 1-nitropyrene derived from pyrene associated with coal fires was much smaller than that from diesel engine emissions, which have much higher operating temperatures. Air samples were collected from six cities during the summer and winter seasons. Very clear seasonal differences were seen for all of the dinitropyrene isomers and 1-nitropyrene (Table 1.6). Lower levels in the summer were attributed to increased photochemical degradation, while higher levels in the winter were partially ascribed to stagnant weather conditions. In contrast, the patterns of the levels of nitropyrenes did not change with the seasons. [The Working Group noted that this observation indicates that coal burning was not a significant confounder.]

(b) Water

Nitropyrene and the dinitropyrene isomers were measured in rainwater, river water and sea water in Kanazawa (Murahashi *et al.*, 2001), Japan, in the autumn and winter of 1996–97. The levels of dinitropyrenes were all approximately 0.01 pmol/L in February and approximately 0.05 pmol/L in September–October.

Fig. 1.1 Sampling locations in the study by Tang *et al.* (2005)



From Tang *et al.* (2005)

1.4.3 Other sources

Small amounts of dinitropyrenes are generated by kerosene heaters, which are used extensively in Japan to heat residences and offices (Tokiwa *et al.*, 1985). Such open, oil-burning space heaters were found to emit dinitropyrenes at a rate of 0.2 ng/h after one hour of operations; 1,3-dinitropyrene was found at 0.53 ± 0.59 pg/mg in the particulate extract.

Gas and liquefied petroleum gas burners are widely used for home heating and cooking, and also produce detectable amounts of dinitropyrenes. A level of 0.6 pg/mg particulate extract of 1,3-dinitropyrene was found in emissions from one gas burner. Dinitropyrenes may result from the incomplete combustion of fuel in the presence of nitrogen dioxide (Tokiwa *et al.*, 1985).

Toners for photocopy machines have been produced commercially since the late 1950s and have been in widespread use since that time. Löfroth *et al.* (1980) and Rosenkranz *et al.* (1980) first discovered the presence of dinitropyrenes in the toner and on the copies. They traced these to impurities in the carbon black, the toner colorant. 'Long-flow' furnace black was first used in photocopy toners in 1967. Its manufacture involved an oxidation step whereby some nitration of pyrene also occurred. A carbon black

Table 1.6 Seasonal concentrations of 1-nitropyrene and dinitropyrene isomers in cities around the Sea of Japan, 1997–2002

Site, country, sampling date Season	No. of samples	Concentration (pg/m³)[a]				Comments
		1-NP	1,3-DNP	1,6-DNP	1,8-DNP	
Seoul, Republic of Korea, 2002	4					330 000 cars
Summer		-	-	-	-	
Winter		173.6 ± 38.5	1.1 ± 0.1	1.1 ± 0.2	1.6 ± 0.4	
Shenyang, China, 2001	9					330 000 cars; energy from coal
Summer		29 ± 24	0.6 ± 0.5	0.4 ± 0.1	0.3 ± 0.1	
Winter		178.6 ± 19.0	2.0 ± 0.2	1.5 ± 0.2	0.9 ± 0.3	
Vladivostok, the Russian Federation, 1999	14					200 000 cars; energy from coal
Summer		17 ± 19	0.3 ± 0.3	0.2 ± 0.2	0.3 ± 0.2	
Winter		95.1 ± 76.6	1.2 ± 0.9	0.6 ± 0.7	0.5 ± 0.4	
Kanazawa, Japan, 1999	14					2 640 000 cars
Summer		25 ± 28	0.2 ± 0.2	0.4 ± 0.4	0.4 ± 0.4	
Winter		56.3 ± 56.1	0.8 ± 0.6	0.6 ± 0.4	0.4 ± 0.3	
Sapporo, Japan, 1997	20					1 210 000 cars; 38.5% diesel
Summer		126 ± 36	0.4 ± 0.1	0.4 ± 0.1	0.8 ± 0.2	
Winter		271.7 ± 109.9	1.2 ± 0.4	1.1 ± 0.4	1.6 ± 0.5	
Tokyo, Japan, 1997	20					3 630 000 cars; 20.5% diesel
Summer		44 ± 12	0.4 ± 0.2	0.2 ± 0.1	0.4 ± 0.2	
Winter		168.0 ± 61.8	0.7 ± 0.2	0.9 ± 0.4	1.4 ± 0.4	
Kitakyushu, Japan, 1997	20					550 000 cars; 26.3% diesel
Summer		5.7 ± 2.2	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	
Winter		13.6 ± 8.6	0.1 ± 0.1	0.4 ± 0.6	0.3 ± 0.1	

[a] Values are means ± standard deviations

DNP, dinitropyrene; NP, nitropyrene

From Tang *et al.* (2005)

sample manufactured before 1979 was reported to contain 6.3 ng/mg 1,3-dinitropyrene (Sanders, 1981); using fused silica capillary gas chromatography/negative ion chemical ionization MS for analysis, a 'long-flow' furnace carbon black was also reported to contain 1,3-, 1,6- and 1,8-dinitropyrenes (Ramdahl & Urdal, 1982). Subsequent to this discovery, changes in the production process reduced the total extractable nitropyrene content from uncontrolled levels of 5–100 ng/mg to less than 0.3 ng/mg. Toners formulated from this modified carbon black (and sold since 1980) have resulted in no detectable levels of mutagenicity and, hence, of nitropyrenes (Rosenkranz *et al.*, 1980; Butler *et al.*, 1983). A sample of carbon black made in 1980 contained 0.07 ng/mg 1,3-dinitropyrene (Giammarise *et al.*, 1982), as detected by optimization of the extraction method.

2. Cancer in Humans

No data were available to the Working Group.

3. Cancer in Experimental Animals

3.1 Mouse

See Table 3.1.

3.1.1 Intraperitoneal administration

Groups of 90 or 100 male and female newborn CD-1 mice received three intraperitoneal injections of 1,3-dinitropyrene (total dose, 200 nmol [58.5 µg]; purity, > 99%) or benzo[*a*]pyrene (total dose, 560 nmol [140 µg]; purity, > 99%) in 10, 20 and 40 µL of dimethyl sulfoxide (DMSO) or three injections of DMSO alone on days 1, 8 and 15 after birth. At 25–27 days, when the mice were weaned, 30 males and 39 females in the treated group, 37 males and 27 females in the positive-control group and 28 males and 31 females in the vehicle-control group were still alive. All surviving mice were killed after 1 year. No increase in the incidence of tumours was observed in any of the organs examined in males or females (Wislocki *et al.*, 1986). [The Working Group noted the short duration of the study.]

3.1.2 Subcutaneous administration

A group of 20 male BALB/c mice, aged 6 weeks, received subcutaneous injections of 0.05 mg of 1,3-dinitropyrene (purity, > 99,9%) dissolved in 0.2 mL of DMSO (total dose, 1 mg) once a week for 20 weeks. A positive-control group of 20 males received injections of 0.05 mg benzo[*a*]pyrene, and a further 20 mice served as controls. [The Working Group could not determine whether controls were injected with DMSO.] Animals were observed for 60 weeks or until moribund. The first subcutaneous tumour in the benzo[*a*]pyrene-treated group was seen in week 21, and all 16 mice surviving beyond this time developed tumours at the injection site which were diagnosed histologically as malignant fibrous histiocytomas [a term used as a specific diagnosis for some malignant soft-tissue sarcomas]. No subcutaneous tumour was found in 1,3-dinitropyrene-treated mice or controls up to 60 weeks. Some tumours developed in the lungs, liver and spleen of 1,3-dinitropyrene-treated animals, but the incidence was not statistically different from that in the controls (Otofuji *et al.*, 1987). [The Working Group noted the small number of animals used and the relatively short observation period.]

3.2 Rat

See Table 3.2.

3.2.1 Oral administration

A group of 36 female weanling Sprague-Dawley rats received intragastric intubations of 10 µmol [3 mg]/kg body weight (bw) of 1,3-dinitropyrene (purity, > 99%) dissolved in DMSO (1.7 µmol [0.5 mg]/mL) three times per week for 4 weeks

Table 3.1 Studies of the carcinogenicity of 1,3-dinitropyrene in mice

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Newborn CD1 (M, F) 12 mo Wislocki *et al.*(1986)	Intraperitoneal administration 0 (control), 200 nmol [58.5 mg] 1,3-DNP (total dose) or 560 nmol [140 µg] B[*a*]P in 10, 20 or 40 µL DMSO once at 1, 8 and 15 d after birth Groups of 90 M, 100 F	*Liver (adenoma):* M–2/28 (7%), 6/30 (20%) F–0/31, 0/39 *Liver (carcinoma):* M–0/28, 0/30 F–0/31, 0/39 *Lung (adenoma):* M–1/28 (3%), 3/30 (10%) F–0/31, 3/39 (8%) *Lung (carcinoma):* M–0/28, 0/30 F–0/31, 0/39 *Malignant lymphoma:* M–1/28 (1%), 1/30 (3%) F–1/31 (1%), 3/39 (8%)	NS	Purity, > 99% Survival: 1,3-DNP–30 M, 39 F; controls–73 M, 65 F Short duration of the study Incidences of liver and lung tumours were increased in B[*a*]P-treated animals (positive-control group)
BALB/c (M) 60 wks or until moribund Otofuji *et al.* (1987)	Subcutaneous injection 0.05 mg 1,3-DNP or B[*a*]P in 0.2 mL DMSO (total dose, 1 mg) once/wk for 20 wks Groups of 20 aged 6 wks including a control group [unclear if injected with DMSO]	*Subcutaneous (all tumours):* 0/13 (control), 0/18 *Lung (all tumours):* 3/13 (23%), 8/18 (44%) *Liver (all tumours):* 3/13 (23%), 2/18 (11%)	NS	Purity, > 99.9% Study limited by small number of animals and the relatively short observation period Incidence of subcutaneous tumours was increased in B[*a*]P-treated animals (positive-control group)

1,3-DNP, 1,3-dinitropyrene; B[*a*]P, benzo[*a*]pyrene; d, day; DMSO, dimethyl sulfoxide; F, female; M, male; mo, month; NS, not significant; wk, week

Table 3.2 Studies of the carcinogenicity of 1,3-dinitropyrene in rats

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
CD (F) 76–78 wks King (1988); Imaida *et al.* (1991)	Oral administration (intragastric intubation) 0 or 10 µmol [3 mg]/kg bw in DMSO (total dose, 16 µmol [4.7 mg]/rat), 3 ×/wk for 4 wks Groups of 36 weanlings	Leukaemia: 0/36, 3/36 (9%) Mammary gland (adenocarcinoma): 5/35 (14%), 5/35 (14%) Mammary gland (fibroadenoma): 9/35 (26%), 7/35 (20%) Adrenal gland (pheochromocytoma): 4/36 (11%), 3/35 (9%) Adrenal gland (cortical adenoma): 6/36 (17%), 10/35 (29%) Pituitary gland (carcinoma): 2/36 (6%), 12/35 (34%)* Pituitary gland (adenoma): 9/36 (25%), 5/35 (14%)	*$P < 0.05$	Purity, > 99.9% Study limited by the short duration of both treatment and observation periods and the use of a single dose.
CD (F) 76–78 wks King (1988); Imaida *et al.* (1991)	Intraperitoneal administration 0 or 10 µmol [3 mg]/kg bw in DMSO (total dose, 16 µmol [4.7 mg]/rat), 3 ×/wk for 4 wks Groups of 36 weanlings	Peritoneal cavity (malignant fibrous histiocytoma): 0/31, 2/36 (6%) Leukaemia: 0/31, 2/36 (6%) Mammary gland (adenocarcinoma): 3/31 (10%), 9/36 (25%) Mammary gland (fibroadenoma): 5/31 (16%), 12/36 (33%) Pituitary gland (carcinoma): 3/31 (10%), 3/36 (8%) Pituitary gland (adenoma): 10/31 (32%), 13/36 (36%)	$P < 0.05$ for mammary tumour-bearing animals	Purity, > 99.9% Study limited by the short duration of both treatment and observation periods and the use of a single dose.

Table 3.2 (continued)

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
F344/DuCrj (M) 347 d Ohgaki *et al.* (1984)	Subcutaneous injection 0 or 0.2 mg in 0.2 mL DMSO (total dose, 4 mg), twice/wk for 10 wks Groups of 10 or 20 aged 6 wks	Injection site (subcutaneous sarcoma): 0/20, 10/10 (100%)	[$P < 0.0001$]	Study limited because of the small number of animals studied, the short treatment and observation periods and the possible influence of the contamination with 1,6-dinitropyrene (0.6%).
CD (F) 67 wks King (1988); Imaida *et al.* (1991)	Subcutaneous injection Suprascapular injection starting within 24 h of birth; 1st dose: 2.5 µmol/kg bw; 2nd and 3rd doses: 5 µmol/kg bw; 4th–8th doses: 10 µmol/kg bw (total dose, 6.3 µmol [1.9 mg]) in DMSO once/wk for 8 wks Treated group: 43 newborns; a group of 40 newborns served as vehicle controls	Injection site (malignant fibrous histiocytomas): 0/40, 5/43 (12%)* Mammary gland (adenocarcinoma): 1/40 (2%), 6/43 (14%) Mammary gland (fibroadenoma): 6/40 (15%), 3/43 (7%) Mammary gland (adenoma): 1/40 (2%), 1/43 (2%)	*$P < 0.05$ NS	Purity, > 99.9% Study limited by the short duration of both treatment and observation periods and the use of a single dose.

bw, body weight; d, day; DMSO, dimethyl sulfoxide; F, female; h, hour; M, male, NS, not significant; wk, week

(average total dose, 16 µmol [4.7 mg]/rat) and were observed for 76–78 weeks. A vehicle-control group of 36 animals received DMSO only. Average survival times of the treated and control animals were 527 and 517 days, respectively. Three rats (9%) administered 1,3-dinitropyrene and none of the controls developed leukaemia. Mammary adenocarcinomas were found in 5 out of 35 (14%) and fibroadenomas in 7 out of 35 (20%) treated animals, but their incidence did not differ from that observed in vehicle controls (5 out of 35 and 9 out of 35, respectively). A significant increase in the incidence of pituitary carcinomas was also observed in treated animals (12 out of 35; 34%) compared with the control group (2 out of 36; 6%) (King, 1988; Imaida *et al.*, 1991). [The Working Group noted the short duration of both treatment and observation.]

3.2.2 Intraperitoneal administration

A group of 36 female weanling Sprague-Dawley rats received intraperitoneal injections of 10 µmol [3 mg]/kg bw of 1,3-dinitropyrene (purity, > 99%) dissolved in DMSO (1.7 µmol [0.5 mg]/mL) three times a week for 4 weeks (total dose, 16 µmol [4.7 mg]/rat); 36 control animals were treated with DMSO only. Animals were killed when moribund or after 76–78 weeks. Malignant fibrous histiocytomas were found in the peritoneal cavity of two treated rats (6%), and two animals (6%) had leukaemia. Neither malignancy developed in 31 surviving controls. Mammary adenocarcinomas were observed in 9 out of 36 (25%) treated animals compared with 3 out of 31 (10%) controls, and fibroadenomas occurred in 12 out of 36 (33%) treated rats compared with 5 out of 31 (16%) controls; the difference in the number of mammary tumour-bearing animals was statistically significant ($P < 0.05$). There was also an increased incidence of pituitary adenomas ($P < 0.05$) (King, 1988; Imaida *et al.*, 1991).

3.2.3 Subcutaneous administration

Ten male Fischer 344/ DuCrj rats, aged 6 weeks, received subcutaneous injections of 0.2 mg of 1,3-dinitropyrene [purity unspecified] (impurities: 0.6% 1,6-dinitropyrene, < 0.05% other nitropyrenes) dissolved in 0.2 mL of DMSO twice a week for 10 weeks (total dose, 4 mg). A control group of 20 rats received injections of 0.2 mL DMSO alone. The animals were killed between days 169 and 347. Subcutaneous sarcomas developed at the site of the injection in all (100%) treated rats between days 119 and 320. No tumours were observed in other organs of the treated rats, and no tumours developed among control animals (Ohgaki *et al.*, 1984). [The Working Group noted that, while the study material was contaminated with 1,6-dinitropyrene, the 100% incidence of sarcomas that it caused would indicate that the small amount of the impurity had a minor effect, if any, on the outcome.]

A group of 43 female newborn Sprague-Dawley rats received subcutaneous injections into the suprascapular region of 1,3-dinitropyrene (purity, > 99%; total dose, 6.3 µmol [1.9 mg]) dissolved in DMSO (1.7 µmol [0.5 mg]/mL) starting within 24 hours after birth. A group of 40 or more animals injected with DMSO alone served as controls. The first dose was 2.5 µmol/kg bw, the second and third doses were 5 µmol/kg and doses 4–8 were 10 µmol/kg. The average length of survival was 468 days for treated animals and 495 days for the controls. The animals were killed 67 weeks after the first treatment, and 5 out of 43 (12%; $P < 0.05$) treated rats had developed malignant fibrous histiocytomas at the site of injection; no tumour of this type was found among the vehicle controls (0 out of 40). No increase in the incidence of mammary tumours was observed. Mammary tumours were observed in treated animals (6 out of 43 had adenocarcinoma (14%); 3 out of 43 had fibroadenoma (7%); and 1 out of 43 had adenoma (1%)) and in control animals

(1 out of 40 had adenocarcinoma (3%); 6 out of 40 had fibroadenoma (15%); and 1 out of 40 had adenoma (3%)) (King, 1988; Imaida *et al.*, 1995). [The Working Group noted that the study was limited by the short duration of both treatment and observation and the use of a single dose.]

4. Mechanistic and Other Relevant Data

4.1 Absorption, distribution, metabolism, excretion

4.1.1 Humans

No data were available to the Working Group.

4.1.2 Experimental systems

The metabolic activation of 1,3-dinitropyrene (reviewed previously in IARC, 1989) is caused by the reduction of one nitro group to yield the corresponding *N*-hydroxylamine derivative, which in turn can undergo acid-catalysed DNA binding or be converted into a highly reactive *O*-acetyl metabolite by bacteria and mammalian acetyltransferases; several metabolizing systems were employed and the nitroreduction of 1,3-dinitropyrene by rat liver cytosol occurred to much lesser extent than that of 1,6- or 1,8-dinitropyrene (Fu, 1990; Beland & Marques, 1994).

4.2 Genetic and related effects

4.2.1 Humans

No data were available to the Working Group.

4.2.2 Experimental systems

The genotoxicity of nitro-PAHs, including 1,3-dinitropyrene, in eukaryotic cells, including fungi, plants and mammalian cells, has been reported (IARC, 1989; IPCS, 2003). The wide differences in sensitivity to nitro-PAHs of prokaryotic and eukaryotic cells are probably due to interspecies differences in the types and concentrations of metabolizing enzyme present, as well as DNA repair or possibly other factors, including the duration of treatment (Durant *et al.*, 1996).

The activation of 1,3-dinitropyrene to a mutagen in *Salmonella typhimurium* was mainly due to the enzymes in the bacteria itself (IARC, 1989). Overall, nitroreduction is an important mutagenic activation pathway in this system. The use of a nitroreductase-deficient strain revealed a clear decrease in mutagenic activity compared with the standard tester strain, which showed the maximum mutagenic potency of 1,3-dinitropyrene (Crebelli *et al.*, 1995).

Genotoxicity studies of 1,3-dinitropyrene in bacterial systems other than the *Salmonella* microsome assay have been reported, and the results, with a few exceptions, were consistent with those in *Salmonella*. Other bacterial assays were also used, e.g. *S. typhimurium* TM677 (a quantitative bacterial forward mutation assay, based on resistance to 8-azaguanine). In general, the results were consistent with those observed in *S. typhimurium* TA98. 1,3-Dinitropyrene was a potent mutagen in *in vitro* studies in bacteria, but only marginal effects were observed *in vivo* in a host-mediated assay using the same bacterial strain (McCoy *et al.*, 1985; IARC, 1989; Mersch-Sundermann *et al.*, 1991, 1992; Shah *et al.*, 1991; Oda *et al.*, 1992, 1993; Busby *et al.*, 1994; Shimada *et al.*, 1994; Shane & Winston, 1997; Yamazaki *et al.*, 2000).

In the yeast *Saccharomyces cerevisiae* D4, 1,3-dinitropyrene at doses of up to 500 µg/mL did not induce gene conversion; in primary mouse hepatocytes at 5–20 µM, it induced marginal DNA damage (as measured by alkaline elution); and in rat, mouse and human hepatocytes, 1,3-dinitropyrene (1.1×10^{-5}–1.1×10^{-2} mg/mL) induced unscheduled DNA synthesis. At 0.5–2.0 µg/mL, it induced the synthesis of polyoma virus DNA

in polyoma virus-transformed rat fibroblasts (IARC, 1989).

1,3-Dinitropyrene (0.1–10 μg/mL) induced mutation to diphtheria toxin resistance in cultured Chinese hamster lung fibroblasts and to ouabain resistance in Chinese hamster V79 cells (1–10 μg/mL). At 0.2–2 μg/mL, it also induced mutation to 6-thioguanine resistance in Chinese hamster ovary cells in the absence of an exogenous metabolic system but was unequivocally active at 2 μg/mL and in the presence of metabolic activation. 1,3-Dinitropyrene (2 μg/mL) induced chromosomal aberrations in cultured Chinese hamster lung fibroblasts in the absence of an exogenous metabolic system but not morphological cell transformation at concentrations of up to 250 μg/mL in BALB/c3T3 cells (IARC, 1989).

The effects of nitro-PAHs, including 1,3-dinitropyrene, on cell signalling related to apoptosis have been reported (Landvik *et al.*, 2007). In Hepa1c1c7 cells, 1,3-dinitropyrene was a more effective inducer of apoptosis than 1,8-dinitropyrene, in contrast to the level of DNA damage that it causes and its carcinogenic activity. Both compounds induced cytochrome P450 1a1, and activated various intracellular signalling pathways related to apoptosis. Furthermore, 1,3-dinitropyrene was found to induce concentration-dependent lipid peroxidation, as measured using the fluoroprobe C_{11}-BODIPY$^{581/591}$. Products derived from lipid peroxidation can react with DNA leading to the formation of mutagenic etheno adducts (el Ghissassi *et al.*, 1995; Nair *et al.*, 2007).

4.3 Other relevant data

No data were available on the acute toxicity of 1,3-dinitropyrene. As previously reported (IARC, 1989), rats administered 1,3-dinitropyrene (about 3 mg/kg bw, three times a week for 4 weeks) orally showed no effects on body weight or survival. Before the appearance of tumours, local effects (ulcer and scar formation at the site of injection) were noted in rats after repeated subcutaneous injections of 0.2 mg of 1,3-dinitropyrene per animal. Three intraperitoneal injections of 1,3-dinitropyrene (at 2.5 mg/kg bw) into young male Sprague-Dawley rats resulted in a fourfold increase in 1-nitropyrene reductase activity, a carcinogen-metabolizing enzyme, in the liver microsomes compared with controls (IARC, 1989).

Oral administration of 1,3-dinitropyrene to female weanling CD rats resulted in the development of leukaemia. Subcutaneous injection of 1,3-dinitropyrene into mice and rats did not result in significant tumorigenicity. However, subcutaneous injection of 1,3-dinitropyrene into newborn CD rats resulted in the formation of malignant fibrous histiocytomas at the site of injection (IARC, 1989).

4.4 Mechanistic considerations

See the *Monograph* on 1,8-Dinitropyrene in this Volume.

5. Summary of Data Reported

5.1 Exposure data

1,3-Dinitropyrene is formed by the nitration of 1-nitropyrene. No evidence was found that it has been produced in commercial quantities or used for purposes other than laboratory applications. During the combustion of diesel and gasoline engines, pyrene is nitrated to form 1-nitropyrene, which is further nitrated to form small amounts of dinitropyrenes. This leads to a content of 1,3-dinitropyrene in the range of 0.1–10% relative to that of 1-nitropyrene in diesel and gasoline exhaust particles and ~1% in airborne particulate matter. 1,3-Dinitropyrene is present in the 0.1–10 ng/g range in airborne particulate matter collected from ambient atmospheric samples. Air concentrations clearly

declined from values in the 1–10 pg/m^3 range at urban locations to values in the 0.01–0.1 pg/m^3 range at suburban and rural locations.

1,3-Dinitropyrene is also generated by kerosene heaters. No data on occupational exposure were available to the Working Group.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

1,3-Dinitropyrene was tested for carcinogenicity in mice in one study by intraperitoneal injection and in one study by subcutaneous injection, and in rats in one study by oral administration, one study by intraperitoneal injection and two studies by subcutaneous injection. In mice, neither intraperitoneal nor subcutaneous injection of 1,3-dinitropyrene produced an increase in the incidence of tumours at any site that was significantly different from that in controls. In rats, intragastric intubation of 1,3-dinitropyrene produced a significant increase in the incidence of pituitary carcinomas; intraperitoneal injection of 1,3-dinitropyrene caused a significant increase in the incidence of mammary tumours and pituitary adenomas; and subcutaneous injection of 1,3-dinitropyrene caused significant increases in the incidence of subcutaneous sarcomas in treated animals in one study and an increase in the formation of malignant histiocytomas at the injection site in another study.

5.4 Mechanistic and other relevant data

No data were available to the Working Group on the absorption, distribution, metabolism and excretion or the genetic and related effects of 1,3-dinitropyrene in humans. Metabolic activation of 1,3-dinitropyrene occurs by reduction of one nitro group to yield the corresponding *N*-hydroxylamine, which may then be converted into the highly reactive *O*-acetyl metabolite. Mutagenicity assays in bacteria have shown that the activating enzymes are present in the bacteria themselves. 1,3-Dinitropyrene did not induce gene conversion in yeast. It was mutagenic in different mammalian systems, and induced chromosomal aberrations in Chinese hamster lung fibroblasts, but no cell transformation in BALB-c3T3 cells. 1,3-Dinitropyrene was an effective inducer of apoptosis in mammalian Hepa1c1c7 cells, and it enhanced lipid peroxidation.

Overall, these data provide *weak mechanistic evidence* to support the carcinogenicity of 1,3-dinitropyrene.

6. Evaluation

6.1 Cancer in humans

No data were available to the Working Group.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of 1,3-dinitropyrene.

6.3 Overall evaluation

1,3-Dinitropyrene is *possibly carcinogenic to humans (Group 2B).*

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1,6-DINITROPYRENE

1,6-Dinitropyrene was evaluated by a previous IARC Working Group in 1988 (IARC, 1989). New data have since become available, and these have been taken into consideration in the present evaluation.

1. Exposure Data

1.1 Chemical and physical data

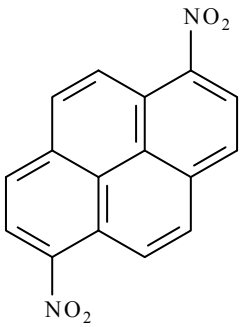
1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 42397-64-8

Chem. Abstr. Name: Pyrene, 1,6-dinitro-

IUPAC Systematic Name: 1,6-Dinitropyrene

1.1.2 Structural and molecular formulae and relative molecular mass



$C_{16}H_8N_2O_4$

Relative molecular mass: 292.3

1.1.3 Chemical and physical properties of the pure substance

Description: Light-brown needles, recrystallized from benzene and methanol (Buckingham, 1985)

Melting-point: > 300 °C (Buckingham, 1985); 309–310 °C (Paputa-Peck *et al.*, 1983)

Spectroscopy data: Ultraviolet (Paputa-Peck *et al.*, 1983), infrared (Hashimoto & Shudo, 1984), nuclear magnetic resonance (Kaplan, 1981; Paputa-Peck *et al.*, 1983; Hashimoto & Shudo, 1984) and mass spectral data (Schuetzle, 1983) have been reported. The National Institute of Standards and Technology Chemistry WebBook provides extensive spectroscopic data (Linstrom & Wallard, 2011).

Solubility: Moderately soluble in toluene (Chemsyn Science Laboratories, 1988)

1.1.4 Technical products and impurities

1,6-Dinitropyrene is available for research purposes at a purity of 98% (Sigma-Aldrich, 2012). The ChemicalBook web site lists 18 companies that supply 1,6-dinitropyrene (ChemicalBook, 2012).

Table 1.1 Levels of 1,6-dinitropyrene in diesel engine exhaust particles and their extracts

Reference	Vehicle/engine	Concentration of 1,6-DNP (pg/mg particulate matter)
Nishioka *et al.* (1982)	Passenger cars (LDD)	ND–600[a]
Gibson (1983)	Diesel cars 1978–82 (LDD)	33–34
Nakagawa *et al.* (1983)	Idling 6-tonne bus from 1970 (HDD), 1200 rpm	1.2
Schuetzle & Perez (1983)	*Heavy-duty vehicle*	
	Idle	< 800
	High-speed, no load	1200
	High-speed, full load	800
Salmeen *et al.* (1984)	Passenger cars (LDD)	400 ± 200
Manabe *et al.* (1985)	Four-cycle six-cylinder engine (HDD) at 17.7–40 km/h	810
Tokiwa *et al.* (1986)	Idling engine [not further specified]	13
Draper (1986)	Commercial mining engine (HDD), 100% load, 1200 rpm	ND (< 230)
	Commercial mining engine (HDD), 75% load, 1800 rpm	ND (< 1100)
Hayakawa *et al.* (1992)	Idling engine (LDD)	113.9[b]

[a] Range of three different engines
[b] Using a much more sensitive analytical method
HDD, heavy-duty diesel; LDD, light-duty diesel; ND, not detected

1.2 Analysis

The reader is referred to Section 1.2 of the *Monograph* on 1,3-Dinitropyrene in this Volume.

1.3 Production and use

The reader is referred to Section 1.3 of the *Monograph* on 1,3-Dinitropyrene in this Volume.

1.4 Occurrence and environmental exposure

1.4.1 Engine exhaust

The reader is referred to the *Monographs* on Diesel and Gasoline Engine Exhausts and 1-Nitropyrene in this Volume.

During the combustion of diesel and gasoline engines, pyrene is nitrated to form 1-nitropyrene, which is further nitrated to form small amounts of 1,3-, 1,6- and 1,8-dinitropyrene (Heeb *et al.*, 2008). A variety of tests of diesel engine emissions were performed in the 1980s, which showed a range of concentrations of 1,6-dinitropyrene in the particulate matter (PM) (Table 1.1). It was detected at levels of 0.81 ng/mg (Manabe *et al.*, 1985) and 1.2 ng/mg (Nakagawa *et al.*, 1983) in extracts of particles from the exhaust of heavy-duty diesel engines; and at 0.4 ± 0.2 ng/mg extract (Salmeen *et al.*, 1984), 0.6 ng/mg extract (Nishioka *et al.*, 1982) and 0.033–0.034 ng/mg particles (Gibson, 1983) from the exhaust of light-duty diesel engines (reviewed in Fu & Herreno-Saenz, 1999). The production of dinitropyrene therefore appears to depend on engine size and operating conditions.

Hayakawa *et al.* (1992, 1994) examined nitro-polycyclic aromatic hydrocarbons (PAHs) in PM emissions from 15 diesel and gasoline engine vehicles. Compared with diesel engine exhaust, those of gasoline engines contained approximately twice as much 1,6-dinitropyrene ([128 pg/mg] versus [67 pg/mg]; Table 1.2); however, the ratio of concentrations of 1,6-dinitropyrene to 1-nitropyrene was 29% for gasoline and 0.5% for diesel engines, which was assumed to be the result of differences in combustion conditions. Diesel engines produced much more PM, and their total emissions of dinitropyrene isomers were much greater. In air

Table 1.2 Mass concentrations of particulate matter from diesel and gasoline engine exhausts from tailpipes in 1992

	No. of samples	Concentration (pg/mg)[a]			
		1-NP	1,3-DNP	1,6-DNP	1,8-DNP
Gasoline engine, idle	8	444 ± 210	64 ± 44	128 ± 106	102 ± 53
Diesel engine, idle	7	12 600 ± 13 100	67 ± 44	67 ± 47	61 ± 41

[a] Values are the means ± standard deviations
DNP, dinitropyrene; NP, nitropyrene
From Hayakawa *et al.* (1992, 1994)

concentrations of emissions from mixed traffic, the ratio of 1,6-dinitropyrene to 1-nitropyrene decreased as the relative number of diesel vehicles increased.

In the past decade, particulate filters have been developed to filter PM from diesel engine exhaust to control emissions. The accumulated soot particles and organic carbon components, including PAHs and nitro-PAHs, that collect on the filters are removed by oxidation, aided by catalytic coatings or catalysts added to the fuel (see Section 1.1 of the *Monograph* on Diesel and Gasoline Engine Exhausts in this Volume).

In a series of laboratory tests, a range of diesel particulate filter types were tested to compare their impact on PAH and nitro-PAH emissions (Heeb *et al.*, 2010). All filters tested, which removed 99% of the particles, also removed most PAH components. However, low-oxidation filters produced 63% more 1-nitropyrene than the amount present in the unfiltered exhaust; although they were not measured, the quantities of dinitropyrenes would also be expected to increase similarly.

Carrara & Niessner (2011) examined the formation of 1-nitropyrene in high- and low-oxidation filters operating at temperatures of 293–573 °K (20–300 °C). Lower temperatures produced more 1-nitropyrene on the filter, the level of which peaked at ~100 °C and declined at higher temperatures. Measurements at 250 °C showed that < 2% of the 1-nitropyrene was on the filter and 47% ± 12% was on the vapour collector (losses of vapour were noted). Although they were not measured in the samples, dinitropyrenes would be expected to be similarly affected.

1.4.2 Environmental occurrence in air and water

The nitration of pyrene during atmospheric processes leads to the formation of 2- but not 1-nitropyrene, because the oxidants that are present differ from those that occur during combustion, which produces 1-nitropyrene (Pitts, 1987). Thus, dinitropyrenes are not produced by atmospheric processes.

The presence of dinitropyrenes [not characterized further] in respirable particles from ambient atmospheric samples was inferred from the mutagenicity testing of extracts of polycyclic organic matter (Pitts, 1987). Early sampling data collected in several locations showed a wide range of concentrations of 1,6-dinitropyrene (Table 1.3). In remote, rural or unindustrialized areas, the content of 1,6-dinitropyrene in airborne PM was in the range of 4.6–8.3 pg/mg and the corresponding levels in air were 0.12–0.30 pg/m^3. In contrast, the PM from the heavily industrialized areas had a content of 43–46 pg/mg, with air concentrations of 4.44–7.50 pg/m^3 (Gibson, 1986). The large urban cities of Tokyo, Japan, and Santiago, Chile, had levels of 1,6-dinitropyrene ranging up to 200 pg/mg (Tokiwa *et al.*, 1983; Tanabe *et al.*, 1986). One study in Michigan, USA, found much lower levels of 1,6-dinitropyrene than other investigators (Siak *et al.*, 1985).

Table 1.3 Concentrations of 1,6-dinitropyrene in air samples and collected particulate matter

Reference	Site/country Season	Concentration	
		Particulate matter (pg/mg)	Atmosphere (pg/m^3)
Tokiwa *et al.* (1983)	Santiago, Chile (urban)	200	–
Siak *et al.* (1985)	*South-eastern MI, USA*	0.26–0.35	0.020–0.032
	Summer		
Gibson (1986)	*Bermuda (remote)*		
	Summer	8.1	0.15[a]
	Winter	8.3	0.12[a]
	Delaware, USA (rural)		
	Summer	4.9	0.12[a]
	Warren, MI, USA (suburban)		
	Winter	< 6	0.15[a]
	Summer	4.6	0.30[a]
	Detroit, MI, USA (urban)		
	Summer	3.6	0.48[a]
	River Rouge, MI, USA (industrial)		
	Summer	46	4.44[a]
	Dearborn, MI, USA (industrial)		
	Summer	41	7.50[a]
Tanabe *et al.* (1986)	Tokyo, Japan	4.7–105	0.33–8.74

[a] Calculated by the IARC Working Group (IARC, 1989)

More recent studies have assessed 1-nitropyrene and 1,3-, 1,6- and 1,8-dinitropyrene in ambient air simultaneously. These studies are presented in Section 1.4.2 of the *Monograph* on 1,3-Dinitropyrene in this Volume.

1.4.3 Other sources

Small amounts of dinitropyrenes are generated by kerosene heaters, which are used extensively in Japan to heat residences and offices (Tokiwa *et al.*, 1985). Such open, oil-burning space heaters were found to emit dinitropyrenes at a rate of 0.2 ng/h after one hour of operations; a mixture of 1,6- and 1,8-dinitropyrenes was found at 3.25 ± 0.63 mg/kg particulate extract.

Gas and liquefied petroleum gas burners are widely used for home heating and cooking. Levels of 1,6-dinitropyrene of 1.88 mg/kg extract were reported from one gas burner (Tokiwa *et al.*, 1985). Dinitropyrenes may result from the incomplete combustion of fuel in the presence of nitrogen dioxide.

Toners for photocopy machines have been produced commercially since the late 1950s and have been in widespread use since that time. 'Long-flow' furnace black was first used in photocopy toners in 1967; its manufacture involved an oxidation process, during which some nitration of pyrene also occurred. A carbon black sample manufactured before 1979 was reported to contain 21 mg/kg 1,6-dinitropyrene (Sanders, 1981); another 'long-flow' furnace carbon black sample was also found to contain this compound (Ramdahl & Urdal, 1982). Toners produced from a new type of carbon black since 1980 had no detectable levels of mutagenicity, and hence of nitropyrenes (Rosenkranz *et al.*, 1980; Butler *et al.*, 1983). A sample of carbon black made in 1980 contained 0.13 mg/kg 1,6-dinitropyrene

after optimization of the extraction method (Giammarise *et al.*, 1982).

2. Cancer in Humans

No data were available to the Working Group

3. Cancer in Experimental Animals

3.1 Mouse

See Table 3.1.

3.1.1 Intraperitoneal administration

Groups of 90 or 100 male and female newborn CD-1 mice received three intraperitoneal injections of 1,6-dinitropyrene (purity, > 99%; total dose, 200 nmol [58.7 µg]) or benzo[*a*]pyrene (purity, > 99%; total dose, 560 nmol [140 µg]) in 10, 20 and 40 µL of dimethyl sulfoxide (DMSO) on days 1, 8 and 15 after birth or DMSO alone. At 25–27 days, when the mice were weaned, 25 males and 29 females in the treated group, 37 males and 37 females in the positive-control group, and 28 males and 31 females in the control group were still alive. All surviving mice were killed after 1 year. In the group injected with 1,6-dinitropyrene, 8 out of 25 (32%) male mice developed liver tumours (three adenomas, five carcinomas); this incidence was significantly greater than that in the vehicle controls ($P < 0.025$). No increase in the incidence of lung tumours or malignant lymphomas was observed in males or females compared with DMSO-treated animals (Wislocki *et al.*, 1986).

3.1.2 Subcutaneous administration

A group of 20 male BALB/c mice, aged 6 weeks, received subcutaneous injections of 0 (vehicle control) or 0.1 mg of 1,6-dinitropyrene (purity, > 99,9%) dissolved in 0.2 mL of DMSO once a week for 20 weeks (total dose, 2 mg). Animals were observed for 60 weeks or, for mice that developed tumours at the site of injection, until moribund. The first tumour in the 1,6-dinitropyrene-treated group was seen on day 112; 45 weeks after the first treatment, 10 out of 20 mice ($P < 0.002$) had developed tumours at the injection site that were diagnosed histologically as malignant fibrous histiocytomas [a term used as a specific diagnosis for some subcutaneous and intraperitoneal sarcomas]. No subcutaneous tumour was detected in the vehicle controls (Tokiwa *et al.*, 1984).

3.2 Rat

See Table 3.2.

3.2.1 Oral administration

A group of 36 female weanling Sprague-Dawley rats received intragastric intubations of 0 (vehicle control) or 10 µmol [3 mg]/kg body weight (bw) of 1,6-dinitropyrene (purity, > 99%) dissolved in DMSO (1.7 µmol [0.5 mg]/mL), three times a week for 4 weeks (average total dose, 16 µmol [4.7 mg]/rat) and were observed for 76–78 weeks. Two rats (6%) treated with 1,6-dinitropyrene and none of the controls developed leukaemia. Mammary adenocarcinomas and fibroadenomas were found in 11 out of 36 (31%) and 10 out of 36 (28%) treated animals, respectively, which was not statistically different from the incidence in controls (5 out of 35 (14%) adenocarcinomas and 9 out of 35 (26%) fibroadenomas). Adrenal and pituitary tumours were also observed in treated animals at an elevated but non-significant level compared with controls (King, 1988; Imaida *et al.*, 1991). [The Working Group noted the short duration of both treatment and observation periods and the use of a single dose.]

Table 3.1 Studies of the carcinogenicity of 1,6-dinitropyrene in mice

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Newborn CD1 (M, F) 12 mo Wislocki *et al.* (1986)	Intraperitoneal administration 0 (control), 200 nmol [58.7 mg] 1,6-DNP or 560 nmol [140 µg] B[*a*]P in 10, 20 or 40 µL DMSO (total dose), at 1, 8 and 15 days after birth Groups of 90 M, 100 F newborn mice	Liver (adenoma): M–2/28 (7%), 3/25 (12%), 11/37 (30%) F–0/31, 0/29, 0/27 Liver (carcinoma): M–0/73, 5/25 (20%), 7/37 (19%) F–0/65, 0/29, 0/27 Lung (adenoma): M–1/28 (4%), 1/25 (4%), 13/37 (35%) F–0/31, 2/29 (7%), 13/27 (48%) Lung (carcinoma): M–0/28 (3%), 0/25, 0/37 F–0/31, 0/29, 0/27 Malignant lymphoma: M–1/28 (4%), 0/25, 2/37 (5%) F–1/31 (1%), 2/29 (15%), 4/27 (15%)	$P < 0.05$ (liver carcinoma in M versus control)	Purity, > 99% Study limited by a small number of animals per group and short observation period.
BALB/c (M) 60 wks or until moribund Tokiwa *et al.*, (1984)	Subcutaneous injection 0 (control) or 0.1 mg 1,6-DNP in 0.2 mL DMSO (total dose, 2.0 mg), once/wk for 20 wks Groups of 20 aged 6 wks	Site of injection (malignant fibrous histiocytoma): 0/20, 10/20 (50%)	$P < 0.002$	Purity, > 99%

B[*a*]P, benzo[*a*]pyrene; d, day; DMSO, dimethyl sulfoxide; DNP, dinitropyrene; F, female, M, male; mo, month; wk, week

Table 3.2 Studies of the carcinogenicity of 1,6-dinitropyrene (1,6-DNP) in rats

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
CD (F) 76–78 wks King (1988); Imaida *et al.* (1991)	Oral administration (intragastric intubation) 0 (control) or 10 µmol [3 mg]/kg bw 1,6-DNP in DMSO (total dose, 16 µmol [4.7 mg]/rat), 3 × /wk for 4 wks Groups of 35 or 36 weanlings	Leukaemia: 0/36, 2/36 (6%) Mammary (adenocarcinoma): 5/35 (14%), 11/36 (31%) Mammary (fibroadenoma): 9/35 (26%), 10/36 (28%) Adrenal (pheochromocytoma): 4/36 (11%), 7/36 (19%) Adrenal (cortical adenoma): 6/36 (17%), 7/36 (19%) Pituitary (carcinoma): 2/36 (6%), 12/36 (33%) Pituitary (adenoma): 9/36 (25%), 13/36 (36%)	NS	Purity > 99% Study limited by the short duration of both treatment and observation periods and the use of a single dose
CD (F) 76–78 wks King (1988); Imaida *et al.* (1991)	Intraperitoneal administration 0 (control) or 10 µmol [3 mg] 1,6-DNP/kg bw in DMSO (total dose, 16 µmol [4.7 mg]/rat), 3 × /wk for 4 wks Groups of 36 weanlings	Peritoneal cavity (malignant fibrous histiocytoma): 0/31, 23/23 (100%)	$P < 0.0001$	Purity, > 90% Some early deaths
F344/DuCrj (M) 72 wks Maeda *et al.* (1986)	Implantation into the lung 0 (control), 0.15 mg 1,6-DNP or 0.5 mg 3-methylcholanthrene in 0.05 mL beeswax:tricaprylin, single injection Groups of 19–31, aged 10–11 wks	Lung (squamous cell carcinoma): 0/31, 21/28 (75%) 19/19 (100%) Lung (undifferentiated carcinoma): 0/31, 0/19, 2/28 (7%)	$P < 0.005$ NS	Purity, > 99.9% Single-dose study
F344 (M) 104 wks Iwagawa *et al.* 1989,	Implantation into the lung 0 (control), 0.003, 0.01, 0.03, 0.1 or 0.15 mg 1,6-DNP or 0.03, 0.1, 0.3 or 1.0 mg B[*a*]P in beeswax Groups: 40, control; 39, 0.003 mg 1,6-DNP; 30, 0.011 mg 6-DNP; 31, 0.03 mg 1,6-DNP; 26, 0.1 mg 1,6-DNP; 9, 0.15 mg 1,6-DNP; 29, 0.03 mg B[*a*]P; 30, 0.1 mg B[*a*]P; 29, 0.3 mg B[*a*]P; 13, 1.0 mg B[*a*]P, aged11 wks	Lung (all tumours): 0–0/40 0.003 1,6-DNP–0/39 0.01 1,6-DNP–4/30 (13%) 0.03 1,6-DNP–13/31 (42%) 0.1 1,6-DNP–22/26 (85%) 0.15 1,6-DNP–6/9 (67%) 0.03 B[*a*]P–1/29 (3%) 0.1 B[*a*]P–7/30 (23%) 0.3 B[*a*]P–22/29 (76%) 1.0 B[*a*]P–9/13 (69%)	 [$P < 0.0299$] [$P < 0.0001$] [$P < 0.0001$] [$P < 0.0001$] NS [$P < 0.0017$] [$P < 0.0001$] [$P < 0.0001$]	Purity, > 99.8%

Table 3.2 (continued)

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
F344/DuCrj (M) 320 d for treated, 650 d for controls Ohgaki *et al.* (1985)	Subcutaneous injection 0 (control) or 0.2 mg 1,6-DNP in 0.2 mL DMSO (total dose, 4 mg), twice/wk for 10 wks Groups of 10 or 20, aged 6 wks	Site of injection (subcutaneous sarcoma): 0/20, 10/10 (100%)	[$P < 0.0001$]	Purity not reported Study limited by the small number of treated animals and the use of only one dose
CD (F) 149 d for treated, 495 d (control group) King (1988); Imaida *et al.* (1991)	Subcutaneous injection Suprascapular injection starting within 24 h of birth; 1st dose: 2.5 µmol 1,6-DNP/kg bw; 2nd and 3rd doses: 5 µmol/kg bw; 4th–8th doses: 10 µmol/kg bw (total dose in DMSO, 6.3 µmol [1.9 mg]); once/wk for 8 wks Treated groups of 46 newborns; vehicle-control group of 40 newborns	Site of injection (malignant fibrous histiocytoma): 0/40, 46/46 (100%) Leukaemia: 0/40, 9/46 (20%) Mammary (adenocarcinoma): 1/40 (3%), 3/46 (7%) Mammary (fibroadenoma): 6/40 (15%), 0/46 Mammary (adenoma): 1/40 (3%), 2/46 (4%)	$P < 0.0001$ $P < 0.005$	Purity, > 99%

B[*a*]P, benzo[*a*]pyrene; bw, body weight; d, day; DMSO, dimethyl sulfoxide; DNP, dinitropyrene; F, female; h, hour; M, male; NS, not significant; wk, week

3.2.2 Intraperitoneal administration

A group of 36 female weanling CD rats received intraperitoneal injections of 0 (vehicle control) or 10 µmol [3 mg]/kg bw of 1,6-dinitropyrene (purity, > 99%) dissolved in DMSO (1.7 µmol [0.5 mg]/mL) three times a week for 4 weeks (average total dose, 16 µmol [4.7 mg]/rat) and were then maintained for 76–78 weeks. Treatment with 1,6-dinitropyrene resulted in some early deaths 12–15 weeks after the initial treatment. Tumours were first identified in a rat autopsied 17 weeks after the first injection. All 23 1,6-dinitropyrene-treated animals (100%) that survived longer than 21 weeks had developed malignant fibrous histiocytomas in the peritoneal cavity, whereas none of the vehicle controls that were observed for 76–78 weeks developed these tumours ($P < 0.0001$). Mammary tumours developed in both groups with approximately the same incidence (King, 1988; Imaida *et al.*, 1991). [The Working Group noted the short duration of both treatment and observation periods.]

3.2.3 Intrapulmonary administration

A group of 28 male Fischer 344/DuCrj rats, aged 10–11 weeks, received a single injection of 0.05 mL of beeswax:tricaprylin containing 0.15 mg of 1,6-dinitropyrene (purity, > 99.9%) directly into the lower third of the left lung after left lateral thoracotomy. One group of 19 males received a single injection of 0.05 mL of beeswax:tricaprylin containing 0.5 mg of 3-methylcholanthrene [purity unspecified], and another group of 31 males received an injection of beeswax:tricaprylin alone. Animals were observed for 72 weeks after treatment, at which time the experiment was terminated. In the 1,6-dinitropyrene-treated rats, 21 out of 28 (75%) developed squamous cell carcinomas ($P < 0.005$) and 2 out of 28 developed undifferentiated carcinomas of the lung. Squamous cell carcinomas were induced earlier in all 19 rats treated with 3-methylcholanthrene than in those treated with 1,6-dinitropyrene. No squamous cell carcinoma was observed in the control group. Distant metastases of induced tumours were observed in four 1,6-dinitropyrene-treated and one 3-methylcholanthrene-treated rats. The incidence of Leydig-cell tumours of the testis was significantly lower in 1,6-dinitropyrene- and 3-methylcholanthrene-treated rats than in the controls ($P < 0.005$). The incidence of other tumours did not differ among the groups (Maeda *et al.*, 1986).

Groups of male Fischer 344/NSIc rats, aged 11 weeks, received injections of 0 (vehicle control), 0.003, 0.01, 0.03, 0.1 or 0.15 mg of 1,6-dinitropyrene (purity, > 99,8%), or 0.03, 0.1, 0.3 or 1.0 mg of benzo[*a*]pyrene as suspensions in beeswax:tricaprylin (1:1) into the lung after anaesthesia with ketamine hydrochloride. The animals were then observed for up to 104 weeks. The incidence of lung cancers (mainly undifferentiated neoplasms) was 0 out of 39 (0%), 4 out of 30 (13%), 13 out of 31 (42%), 22 out of 26 (85%) and 6 out of 9 (67%) rats in the groups treated with 0.003, 0.01, 0.03, 0.1 and 0.15 mg of 1,6-dinitropyrene, respectively. Benzo[*a*]pyrene induced lung cancers (well differentiated squamous cell carcinomas) in 1 out of 29 (3%), 7 out of 30 (23%), 22 out of 29 (76%) and 9 out of 13 (69%) rats treated with 0.03, 0.1, 0.3 and 1.0 mg, respectively. No lung cancer was found in control rats. Thus, the incidence of lung cancer induced by 1,6-dinitropyrene and benzo[*a*]pyrene showed significant dose dependence. At equal doses, the incidence of lung cancer was much higher with 1,6-dinitropyrene than with benzo[*a*]pyrene, and was still higher with a dose equivalent to one-third of the dose of benzo[*a*]pyrene (Iwagawa *et al.*, 1989).

3.2.4 Subcutaneous administration

Ten male Fischer 344/DuCrj rats, aged 6 weeks, received subcutaneous injections of 0.2 mg of 1,6-dinitropyrene ([purity unspecified];

Table 3.3 Studies of the carcinogenicity of 1,6-dinitropyrene in hamsters

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Syrian golden (M, F); 11 mo Takayama *et al.* (1985)	Intratracheal instillation 0 (control) or 0.5 mg in 0.2 ml saline, once/wk for 26 wks Groups of 10 M, 10 F aged 10 wks	*Lung (adenocarcinoma):* M–0/10 10/10* (100%) F–0/10, 9/10* (90%) *Myeloid leukaemia:* M–0/10, 6/10 (60%)* F–0/10, 6/10* (60%)	*[$P < 0.05$]	Purity > 99.9% Study limited by the small number of animals, short duration of both treatment and observation periods and use of a single dose

F, female, M, male; mo, month; wk, week.

impurities: < 0.05% each of 1,3-dinitropyrene, 1,8-dinitropyrene, 1,3,6-trinitropyrene and 1,3,6,8-tetranitropyrene) dissolved in 0.2 mL of DMSO twice a week for 10 weeks (total dose, 4 mg). A control group of 20 rats received injections of 0.2 mL of DMSO alone. Treated animals were killed on day 320 and control rats on day 650. Sarcomas developed at the site of injection in all dinitropyrene-treated rats between days 103 and 123. No tumour developed at the injection site among control animals (Ohgaki *et al.*, 1985). [The Working Group noted that, while it recognized the contamination of the study material by other nitropyrenes, the tumour response was so strong that it can be attributed to the exposure to 1,6-dinitropyrene.]

In a lifetime study, a group of 46 female newborn Sprague-Dawley rats received subcutaneous injections of 1,6-dinitropyrene (purity, > 99%) dissolved in DMSO (1.7 µmol [0.5 mg]/mL) into the suprascapular region once a week for 8 weeks (total dose, 6.3 µmol [1.8 mg]). A group of 40 animals injected with DMSO alone served as controls. The average survival time was 149 days for treated rats and 495 days for controls. Malignant fibrous histiocytomas developed rapidly at the site of injection among treated rats; the first tumour was seen 15 weeks after the initial treatment, and by 18 weeks all rats had developed this tumour ($P < 0.0001$). In addition, nine rats had leukaemia ($P < 0.005$). Vehicle controls developed no such malignancies. Mammary tumours (mainly adenocarcinomas) were observed in 5 out of 46 (11%) treated rats, and 8 out of 40 (20%) controls had mammary tumours (mainly fibroadenomas) (King, 1988; Imaida *et al.*, 1991).

3.3 Hamster

See Table 3.3.

3.3.1 Intratracheal administration

Groups of 10 male and 10 female Syrian hamsters, aged 10 weeks, received intratracheal instillations of 0 (control) or 0.5 mg of 1,6-dinitropyrene (purity, > 99.9%) suspended in 0.2 mL of saline once a week for 26 weeks (total dose, 13 mg). The experiment was terminated at 11 months. Lung adenocarcinomas developed in 10 out of 10 (100%) males and 9 out of 10 (90%) females treated with 1,6-nitropyrene during weeks 20–48; 65% had multiple tumours. In addition, myeloid leukaemia developed in 6 out of 10 (60%) males and 6 out of 10 (60%) females. No tumours were detected in the controls (Takayama *et al.*, 1985).

4. Mechanistic and Other Relevant Data

4.1 Absorption, distribution, metabolism and excretion

4.1.1 Humans

No data were available to the Working Group.

4.1.2 Experimental systems

The metabolic activation of 1,6-dinitropyrene has been reviewed previously (IARC, 1989) and is caused by the reduction of one of the nitro groups to yield the corresponding hydroxylamine derivative, which in turn can undergo acid-catalysed DNA binding or be converted into a highly reactive *O*-acetylated metabolite by bacteria and mammalian acetyltransferases (Beland & Marques, 1994). The nitroreduction is catalysed by intestinal bacteria, and this metabolic activation pathway has been shown to be responsible for the mutagenicity of 1,6-dinitropyrene in *Salmonella* (Fu, 1990). In mammalian systems, the nitroreduction is catalysed by a variety of enzymes, including cytosolic aldehyde oxidase, xanthine/xanthine oxidase, nicotinamide adenine dinucleotide phosphate (NADPH) quinone oxidoreductase and microsomal NADPH cytochrome P450 reductase (Bauer & Howard, 1990, 1991). These enzymes are found in a variety of tissues: xanthine oxidase occurs in the liver, intestinal mucosa and mammary glands, and in the milk and colostrum of most mammals (Howard *et al.*, 1995).

4.2 Genetic and related effects

4.2.1 Humans

No data were available to the Working Group.

4.2.2 Experimental systems

Within 24 hours after the administration of a single intraperitoneal dose of 1,6-dinitropyrene ([33.6 µmol]) to pre-weanling male CD mice, one major adduct, *N*-(deoxyguanosin-8-yl)-1-amino-6-nitropyrene, was identified in the lung and liver; this adduct was also detected in the mammary epithelium, liver, lung, kidney and urinary bladder of rats (IARC, 1989; Smith *et al.*, 1993).

Oral administration of 1,6-dinitropyrene to rats resulted in the formation of measurable DNA adducts in the intestinal mucosa and urinary bladder; after intraperitoneal injection, higher levels of DNA adducts were found, mostly in the bladder, white blood cells and lung, but only a lower level was found in the liver (Wolff *et al.*, 1993). Following direct pulmonary instillation of 1,6-dinitropyrene into male Fischer 344 rats, *N*-(deoxyguanosin-8-yl)-1-amino-6-nitropyrene was identified in the target (lung) and surrogate tissues (liver, white blood cells and splenic lymphocytes). The extent of the DNA binding by 1,6-dinitropyrene *in vivo* depends on its nitroreduction and *O*-acetylation (Beland *et al.*, 1985).

The effect of co-administration of 1-nitropyrene on the levels of DNA adducts derived from 1,6-dinitropyrene was investigated (IPCS, 2003). Following intraperitoneal administration of 100 nmol of 1,6-dinitropyrene to B6C3F1 mice, 0.46 ± 0.05 fmol of DNA adducts/µg DNA were detected. Co-administration of a 25-fold molar excess of 1-nitropyrene (but not a 2.5-fold excess) increased the level of DNA adducts (derived from 1,6-dinitropyrene) to 0.59 ± 0.07 fmol/µg DNA. In contrast, co-administration of 25-fold molar excess of pyrene resulted in a significant decrease in 1,6-dinitropyrene–DNA adducts to 0.34 ± 0.04 fmol/µg DNA. Collectively, these results suggest that the metabolic activation of 1,6-dinitropyrene can be greatly altered by other agents (e.g. other nitro-PAHs or PAHs) found in

complex mixtures, such as diesel exhaust and air pollutants (IPCS, 2003).

In *Salmonella typhimurium*, 1,6-dinitropyrene (0.005 μg/mL) induced DNA damage (IARC, 1989). Its genotoxicity in bacterial systems other than the *Salmonella* microsome system have been reported, and the results, with a few exceptions, are consistent with those in *Salmonella* (IARC, 1989; Mersch-Sundermann *et al.*, 1991, 1992; Oda *et al.*, 1992, 1993; Busby *et al.*, 1994; Shimada *et al.*, 1994; Shane & Winston, 1997; Yamazaki *et al.*, 2000). Several mammalian systems were used to demonstrate the genotoxicity of 1,6-dinitropyrene reported previously (IARC, 1989). 1,6-Dinitropyrene induced DNA damage and mutations in several strains of bacteria, and unscheduled DNA synthesis in mouse, rat and human hepatocytes, in rat and human cultured tracheal or bronchial epithelial cells and in rabbit lung cells. It was mutagenic in several mammalian cell lines, induced sister chromatid exchange and chromosomal aberrations in mammalian cells in culture and caused chromatid-type chromosomal aberrations in cultured rat and hamster liver epithelial cells and human fibroblasts. In rats, 1,6-dinitropyrene produced fibrosarcomas that contained activated H-*ras* and N-*ras* oncogenes (IARC, 1989).

A dose-dependent increase in DNA adducts was found in splenic lymphocytes, but not in the lung, following the direct implantation of 1,6-dinitropyrene into the lung of Fischer 344 rats. In parallel, a significant increase of gene mutation frequency (at the hypoxanthine-guanine phosphoribosyltransferase locus) was detected in splenic T lymphocytes (Smith *et al.*, 1993). Beland and his team (IARC, 1989; Beland & Marques, 1994; Beland, 1995) compared adducts in the lung, liver and lymphocytes and gene mutations in splenic T lymphocytes. The outcome of these studies indicated that the levels of 1,6-dinitropyrene that produce lung tumours in a dose-dependent fashion also induced DNA adducts and mutations in T lymphocytes dose-dependently; however, the dose–response curves for DNA binding differ from those of mutations. Taken together, these authors suggested that mutation in T lymphocytes may be a more sensitive and longer-lasting biomarker than DNA adducts to assess previous exposure to nitro-PAHs.

Gene mutations were analysed in 20 rat lung tumours induced by 1,6-dinitropyrene; five mutations were detected in *k-Ras* codon 12 (four GGT to TGT transversions and one GGT to GAT transition) but not in *k-Ras* codons 13 or 61. Mutations in *p*53 exons 5–8 (eight substitutions at G:C base-pairs and one deletion) were identified in 9 out of 20 tumour samples (Smith *et al.*, 1997).

4.3 Other relevant data

Studies of the carcinogenicity of 1,6-dinitropyrene in various species were reported previously (IARC, 1989) and are briefly summarized below.

No data were available to the Working Group on the acute toxicity of 1,6-dinitropyrene. Administration of 10 μmol [3 mg]/kg bw of 1,6-dinitropyrene to rats by gavage, three times a week for 4 weeks had no effects on body weight or survival (Imaida *et al.*, 1991). A single injection of 0.15 mg directly into the lower third of the left lung after left lateral thoracotomy in a group of 28 male Fischer 344 rats resulted in the formation of squamous metaplasia of the lung in two rats and granulomatous lesions containing foreign-body giant cells in three rats. Intraperitoneal administration of 1,6-dinitropyrene to young male Sprague-Dawley rats (three times at 2.5 mg/kg bw) resulted in a 2.5-fold increase in the activity of 1-nitropyrene reductase, a carcinogen metabolizing enzyme, in the liver microsomes compared with controls (IARC, 1989).

4.4 Mechanistic considerations

See the *Monograph* on 1,8-Dinitropyrene.

5. Summary of Data Reported

5.1 Exposure data

1,6-Dinitropyrene is produced by the nitration of 1-nitropyrene. No evidence was found that it has been produced in commercial quantities or used for purposes other than laboratory applications. During the combustion of diesel and gasoline engines, pyrene is nitrated to form 1-nitropyrene, which is further nitrated to form small amounts of dinitropyrenes. This leads to a content of 1,6-dinitropyrene in the range of 0.1–10% relative to the 1-nitropyrene content in diesel and gasoline exhaust particles and of ~1% in airborne particulate matter. 1,6-Dinitropyrene was present at a range of 1–10 ng/g in airborne particulate matter collected from ambient atmospheric samples. Air concentrations clearly declined from values in the 0.1–10 pg/m^3 range at urban locations to values in the 0.01–0.1 pg/m^3 range at suburban and rural locations.

1,6-Dinitropyrene is also generated by kerosene heaters. No data on occupational exposure were available to the Working Group.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

1,6-Dinitropyrene was tested for carcinogenicity in mice in one study by intraperitoneal injection and one study by subcutaneous injection, in rats in one study by oral administration, one study by intraperitoneal injection, two studies by implantation into the lung and two studies by subcutaneous injection, and in one study in hamsters by intratracheal instillation. Intraperitoneal injection of 1,6-dinitropyrene into newborn mice caused a significant increase in the incidence of liver carcinomas in males and its subcutaneous injection caused a significant increase in the incidence of malignant subcutaneous histiocytomas in males. In rats, oral intubation with 1,6-dinitropyrene caused a significant increase in the incidence of pituitary carcinomas in females; its intraperitoneal injection caused a significant increase in the incidence of malignant histiocytomas of the peritoneal cavity in females; its implantation into the lung (two studies) caused a significant increase in the incidence of squamous cell carcinomas of the lung in males; and its subcutaneous injection caused injection site sarcomas in males in one study and malignant histiocytomas and leukaemia in females in another study. In hamsters, intratracheal instillation of 1,6-dinitropyrene caused a significant increase in the incidence of lung adenocarcinomas and myeloid leukaemia in males and females.

5.4 Mechanistic and other relevant data

No data were available to the Working Group on the absorption, distribution, metabolism and excretion or genetic and related effects of 1,6-dinitropyrene in humans. Activation of this compound in bacteria or in mammalian cells occurs via the reduction of one nitro group initially to form a nitroso intermediate, that undergoes further reduction to the *N*-hydroxylamino derivative. 1,6-Dinitropyrene was strongly mutagenic in bacteria. It induced DNA-adduct formation and caused mutation in the splenic T lymphocytes of rats, and induced chromosomal aberrations in human fibroblasts. The mutagenicity of 1,6-dinitropyrene is related to the ability of its corresponding hydroxylamino derivative to bind to DNA. *O*-Acetylation of the *N*-hydroxylamino group by acetyltransferases followed by removal of the acetoxy group yields the active electrophilic nitrenium ion, which reacts with deoxyguanosine at the C8 position

to form *N*-(deoxyguanosin-8-yl)-1-amino-6-nitropyrene. Mutations in the K-*Ras* oncogene and the *Tp53* tumour-suppressor gene were observed in 1,6-dinitropyrene-induced lung tumours in rats. 1,6-Dinitropyrene-induced rat fibrosarcomas contained activated *H-ras* and *N-ras* oncogenes.

Overall, these data provide *moderate mechanistic evidence* to support the carcinogenicity of 1,6-dinitropyrene.

6. Evaluation

6.1 Cancer in humans

No data were available to the Working Group.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of 1,6-dinitropyrene.

6.3 Overall evaluation

1,6-Dinitropyrene is *possibly carcinogenic to humans (Group 2B)*.

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1,8-DINITROPYRENE

1,8-Dinitropyrene was evaluated by a previous IARC Working Group in 1988 (IARC, 1989). New data have since become available, and these have been taken into consideration in the present evaluation.

1. Exposure Data

1.1 Chemical and physical data

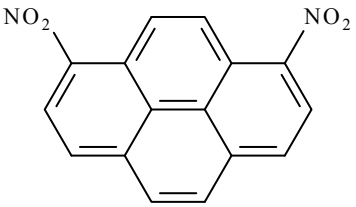
1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 42397-65-9

Chem. Abstr. Name: Pyrene, 1,8-dinitro-

IUPAC Systematic Name: 1,8-Dinitropyrene

1.1.2 Structural and molecular formulae and relative molecular mass



$C_{16}H_8N_2O_4$

Relative molecular mass: 292.3

1.1.3 Chemical and physical properties of the pure substance

Description: Light-brown needles, recrystallized from benzene and methanol (Buckingham, 1985)

Melting-point: > 300 °C (Buckingham, 1985); 299–300 °C (Paputa-Peck *et al.*, 1983)

Spectroscopy data: Ultraviolet, infrared, nuclear magnetic resonance (Kaplan, 1981; Paputa-Peck *et al.*, 1983; Hashimoto & Shudo, 1984) and mass spectral data (Schuetzle, 1983) have been reported. The National Institute of Standards and Technology Chemistry WebBook provides extensive spectroscopic data (Linstrom & Wallard, 2011).

Solubility: Moderately soluble in toluene (Chemsyn Science Laboratories, 1988)

1.1.4 Technical products and impurities

1,8-Dinitropyrene is available for research purposes at 98% purity (Sigma-Aldrich, 2012). The ChemicalBook web site lists five companies that supply 1,8-dinitropyrene (ChemicalBook, 2012). Radiolabelled (^{14}C and ^{3}H) 1,8-dinitropyrene can be prepared in commercial laboratories.

Table 1.1 Levels of 1,8-dinitropyrene in diesel engine exhaust particles and their extracts

Reference	Vehicle/engine	Concentration of 1,8-DNP (pg/mg particulate matter)
Nishioka *et al.* (1982)	Passenger cars (LDD)	ND–400[a]
Gibson (1983)	Diesel cars 1978–82 (LDD)	13–25
Nakagawa *et al.* (1983)	Idling 6-tonne bus from 1970 (HDD), 1200 rpm	3400
Schuetzle & Perez (1983)	Heavy-duty vehicle	
	Idle	< 800
	High-speed, no load	1200
	High-speed, full load	800
Salmeen *et al.* (1984)	Passenger cars (LDD)	500–700
Draper (1986)	Commercial mining engine (HDD), 100% load, 1200 rpm	ND (< 290)
	Commercial mining engine (HDD), 75% load, 1800 rpm	ND (< 1300)
Tokiwa *et al.* (1986)	Idling engine [not further specified]	13
Hayakawa *et al.* (1992)	Idling engine (LDD)	128.5[b]

[a] Range of three different engines
[b] Using a much more sensitive analytical method
DNP, dinitropyrene; HDD, heavy-duty diesel; LDD, light-duty diesel; ND, not detected

1.2 Analysis

The reader is referred to Section 1.2 of the *Monograph* on 1,3-Dinitropyrene in this Volume.

1.3 Production and use

The reader is referred to Section 1.3 of the *Monograph* on 1,3-Dinitropyrene in this Volume.

1,8-Dinitropyrene has been reported to be a photosensitizer, and to increase the spectral activity of bis-azide compounds with light (Tsunoda *et al.*, 1973).

1.4 Occurrence and environmental exposure

1.4.1 Engine exhaust

The reader is also referred to the *Monographs* on Diesel and Gasoline Engine Exhausts and 1-Nitropyrene in this Volume.

During the combustion of diesel and gasoline engines, pyrene is nitrated to form 1-nitropyrene, which is further nitrated to form small amounts of 1,3-, 1,6- and 1,8-dinitropyrene (Heeb *et al.*, 2008). A variety of tests on diesel engine emissions were performed in the 1980s, and showed a range of concentrations of 1,8-dinitropyrene in the particulate matter (PM) (Table 1.1).

1,8-Dinitropyrene was detected at a level of 3.4 ng/mg in an extract of particles from the exhaust of a heavy-duty diesel engine (Nakagawa *et al.*, 1983). Other investigators have found concentrations of between 0.5 ± 0.3 and 0.7 ± 0.2 ng/mg in extracts (Salmeen *et al.*, 1984), not detected and 0.4 ng/mg in extracts from three different diesel engines (Nishioka *et al.*, 1982), and 0.013 and 0.025 ng/mg in particles (Gibson, 1983) from the exhausts of light-duty diesel engines (reviewed in Fu & Herreno-Saenz, 1999). The production of dinitropyrenes therefore appears to be dependent on engine size and operating conditions.

Hayakawa *et al.* (1994) examined nitroarenes in PM emissions from 15 diesel and gasoline engine vehicles. Compared with diesel engine exhausts, gasoline engine exhausts contained

Table 1.2 Mass concentrations in particulate matter from diesel and gasoline engine exhausts from tailpipes in 1992

	No. of samples	Concentration (pg/mg)[a]			
		1-NP	1,3-DNP	1,6-DNP	1,8-DNP
Gasoline engine, idle	8	444 ± 210	64 ± 44	128 ± 106	102 ± 53
Diesel engine, idle	7	12 600 ± 13 100	67 ± 44	67 ± 47	61 ± 41

[a] Values are the means ± standard deviations
DNP, dinitropyrene; NP, nitropyrene
From Hayakawa *et al.* (1992, 1994)

approximately twice the mass concentration of 1,8-dinitropyrene ([102 pg/mg] versus [61 pg/mg]; Table 1.2); however, the ratio of the concentrations of 1,8-dinitropyrene to 1-nitropyrene was 29% for gasoline and 0.5% for diesel engine exhaust, which was assumed to be the result of differences in combustion conditions. The diesel engines produced many more particulates, and their total emissions of dinitropyrene isomers were therefore much greater. In air concentrations of emissions from mixed traffic, the ratio of 1,8-dinitropyrene to 1-nitropyrene decreased as the relative number of diesel vehicles increased.

In the past decade, several types of particulate filters have been developed to filter PM from diesel engine exhaust to control emissions (Heeb *et al.*, 2010). The accumulated soot particles and organic carbon components, including polycyclic aromatic hydrocarbons (PAHs) and nitro-PAHs, that collect on the filters are removed by oxidation, aided by catalytic coatings or catalysts added to the fuel (see Section 1.1 in the *Monograph* on Diesel and Gasoline Engine Exhausts in this Volume).

In a series of laboratory tests, a range of diesel particulate filters were tested to compare their impact on PAH and nitro-PAH emissions (Heeb *et al.*, 2010). All filters tested, which removed up to 99% of the particles, also removed most PAH components. However, low-oxidation filters produced 63% more 1-nitropyrene than the amount present in the unfiltered exhaust; although they were not measured, the quantities of dinitropyrenes would also be expected to increase similarly.

Carrara & Niessner (2011) examined the formation of 1-nitropyrene in high- and low-oxidation filters at temperatures of 293–573 °K (20–300 °C). The lower temperatures produced more 1-nitropyrene on the filter, which peaked at ~100 °C and declined at higher temperatures. Measurements at 250 °C showed that < 2% of the 1-nitropyrene was on the filter and 47% ± 12% was on the vapour collector (losses of vapour were noted). Although they were not measured in the samples, dinitropyrenes would be expected to be affected similarly.

1.4.2 Atmospheric particulate matter

Nitration of pyrene during atmospheric processes leads to the formation of 2- but not 1-nitropyrene, because the oxidants present differ from those involved in combustion, which produces 1-nitropyrene (Pitts, 1987). Thus, dinitropyrenes are not produced by atmospheric processes.

The presence of dinitropyrenes [not further characterized] in respirable particles from ambient atmospheric samples was inferred from mutagenicity testing of polycyclic organic matter extracts (Pitts, 1987). Early sampling data collected at several locations showed a wide range of 1,8-dinitropyrene concentrations (Table 1.3). In remote, rural or unindustrialized areas, the content of 1,8-dinitropyrene in airborne PM was

Table 1.3 Concentrations of 1,8-dinitropyrene in air samples and collected particulate matter

Reference	Sample location Season	Concentration	
		Particulate matter (pg/mg)	Atmosphere (pg/m³)
Tokiwa *et al.* (1983)	Santiago, Chile (urban)	200	–
Siak *et al.* (1985)	*South-eastern MI, USA*		
	Summer	0.29–0.61	0.023–0.061
Tanabe *et al.* (1986)	Tokyo, Japan	ND–79.3	ND–6.6
Gibson (1986)	*Bermuda (remote)*		
	Summer	3.5	0.07[a]
	Winter	4.4	0.06[a]
	Delaware, USA (rural)		
	Summer	2.4	0.06[a]
	Warren, MI, USA (suburban)		
	Winter	< 4	< 0.10[a]
	Summer	2.1	0.13[a]
	Detroit, MI, USA (urban)		
	Summer	2.5	0.34[a]
	River Rouge, MI, USA (industrial)		
	Summer	13.1	1.26[a]
	Dearborn, MI, USA (industrial)		
	Summer	20	3.80[a]

[a] Calculated by the IARC Working Group (IARC, 1989)
ND, not detected

in the range of 4.6–8.3 pg/mg and the corresponding air levels were 0.12–0.30 pg/m³. In contrast, in heavily industrialized areas, the PM contained 43–46 pg/mg 1,8-dinitropyrene with air concentrations of 4.44–7.50 pg/m³ (Gibson, 1986). The large urban cities of Tokyo, Japan, and Santiago, Chile, had levels of 1,8-dinitropyrene that ranged up to 200 pg/mg (Tokiwa *et al.*, 1983; Tanabe *et al.*, 1986). One study in Michigan, USA, found much lower levels of 1,8-dinitropyrene in total suspended particles than other investigators (Siak *et al.*, 1985).

More recent studies have assessed the concentrations in ambient air of 1-nitropyrene and the 1,3-, 1,6- and 1,8-dinitropyrene simultaneously, and are presented in Section 1.4.2 of the *Monograph* on 1,3-Dinitropyrene in this Volume.

1.4.3 Other sources

Small amounts of dinitropyrenes are generated by kerosene heaters, which are used extensively in Japan to heat residences and offices (Tokiwa *et al.*, 1985). Such open, oil-burning space heaters were found to emit dinitropyrenes at a rate of 0.2 ng/h after one hour of operations; a mixture of 1,6- and 1,8-dinitropyrenes was found at a level of 3.25 ± 0.63 mg/kg of particulate extract.

Gas and liquefied petroleum gas burners are widely used for home heating and cooking. Levels of 1,8-dinitropyrene of 0.88 mg/kg extract were reported from one gas burner (Tokiwa *et al.*, 1985). Dinitropyrenes may result from the incomplete combustion of fuel in the presence of nitrogen dioxide.

Toners for photocopy machines have been produced commercially since the late 1950s and have been in widespread use since that time.

'Long-flow' furnace black was first used in photocopy toners in 1967; its manufacture involved an oxidation process whereby some nitration of pyrene also occurred. A carbon black sample manufactured before 1979 was reported to contain 23.4 mg/kg 1,8-dinitropyrene (Sanders, 1981); another 'long-flow' furnace carbon black sample was also found to contain this compound (Ramdahl & Urdal, 1982). Subsequent changes in the production technique reduced the total extractable nitropyrene content from uncontrolled levels of 5–100 ng/mg to below 0.3 ng/mg (Rosenkranz *et al.*, 1980; Sanders, 1981; Butler *et al.*, 1983). Toners produced from a new type of carbon black since 1980 had no detectable levels of mutagenicity, and hence of nitropyrenes (Rosenkranz *et al.*, 1980; Butler *et al.*, 1983). A sample of carbon black made in 1980 contained 0.16 mg/kg 1,8-dinitropyrene after optimization of the extraction method (Giammarise *et al.*, 1982).

2. Cancer in Humans

No data were available to the Working Group

3. Cancer in Experimental Animals

3.1 Mouse

See Table 3.1

3.1.1 Intraperitoneal administration

Groups of 90 or 100 male and female newborn CD-1 mice received three intraperitoneal injections of 1,8-dinitropyrene (total dose, 200 nmol [58.7 µg]; purity, > 99%) or benzo[*a*]pyrene (total dose, 560 nmol [140 µg]; purity, > 99%) in 10, 20 and 40 µL of dimethyl sulfoxide (DMSO) on days 1, 8 and 15 after birth or DMSO alone. At 25–27 days, when the mice were weaned, 31 males and 33 females in the treated group, 37 males and 27 females in the positive-control group and 28 males and 31 females in the vehicle-control group were still alive. All surviving mice were killed after 1 year. In the group injected with 1,8-dinitropyrene, 5 out of 31 (16%) males developed liver tumours compared with 2 out of 28 (7%) controls. No increase in the incidence of lung tumours or malignant lymphomas was observed in males or females compared with DMSO-treated animals (Wislocki *et al.*, 1986). [The Working Group noted the short observation period.]

3.1.2 Subcutaneous administration

A group of 20 male BALB/c mice, aged 6 weeks, received subcutaneous injections of 0.05 mg of 1,8-dinitropyrene (purity, > 99.9%) dissolved in 0.2 mL DMSO (total dose, 1 mg) once a week for 20 weeks. A positive-control group of 20 males received injections of 0.05 mg of benzo[*a*]pyrene, and a further group of 20 mice served as controls. [It was unclear whether the animals were untreated or injected with DMSO.] Animals were observed for 60 weeks or until moribund. After 60 weeks, 6 out of 15 (40%) mice injected with 1,8-dinitropyrene had developed subcutaneous tumours; no such tumours were found in controls ($P < 0.05$). All of the subcutaneous tumours were diagnosed histologically as malignant fibrous histiocytomas [a term used as a specific diagnosis for subcutaneous sarcomas]. Some animals in the 1,8-dinitropyrene-treated group developed tumours of the lung or liver (Otofuji *et al.*, 1987). [The Working Group noted the small number of animals used.]

3.2 Rat

See Table 3.2

Table 3.1 Studies of the carcinogenicity of 1,8-dinitropyrene in mice

Strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
CD1 (M, F) 12 mo Wislocki *et al.* (1986)	Intraperitoneal administration 0 (control), 200 nmol [58.5 mg] 1,8-DNP or 560 nmol [140 µg] B[*a*]P in 10, 20 or 40 µL DMSO at 1, 8 and 15 d after birth Groups of 90 M, 100 F newborn	Liver (adenoma): M–2/28 (7%), 4/31 (13%), 11/37 (30%) F–0/31, 0/33, 0/27 Liver (carcinoma): M–0/28, 1/31 (3%), 7/37 (19%) F–0/31, 0/33, 0/27 Lung (adenoma): M–1/28 (4%), 1/31 (3%), 13/37 (35%) F–0/31, 2/33 (6%), 13/27 (48%) Lung (carcinoma): M–0/28, 0/31, 0/37 F–0/31, 0/33, 0/27 Malignant lymphoma: M–1/28 (4%), 1/31 (3%), 2/37 (5%) F–1/31 (3%), 1/33 (3%), 4/27 (15%)	NS (1,8-DNP)	Purity, > 99% Small number of animals per group and short observation period.
BALB (M) 60 wks or until moribund Otofuji *et al.* (1987)	Subcutaneous injection 0.05 mg 1,8-DNP or 0.05 mg B[*a*]p in 0.2 mL DMSO (total dose, 1 mg), once/wk for 20 wks Groups of 20 aged 6 wks old; 20 controls (unclear if injected with DMSO)	Subcutaneous (all tumours): 0/13 (control), 6/15 (40%)*, 16/16 (100%)* Lung (all tumours): 3/13 (23%), 6/15 (40%), 1/16 (6%) Liver (all tumours): 3/13 (23%), 2/15 (13%), 0/16	*$P < 0.05$ compared with controls	Purity, > 99.9%

B[*a*]p, benzo[*a*]pyrene; d, day; DMSO, dimethyl sulfoxide; DNP, dinitropyrene; F, female; M, male; mo, month; NS, not significant; wk, week

Table 3.2 Studies of the carcinogenicity of 1,8-dinitropyrene in rats

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
CD (F) 76–78 wks King (1988); Imaida *et al.* (1991)	Oral administration (intragastric intubation) 0 or 10 µmol [3 mg]/kg bw in DMSO; (total dose, 16 µmol [4.7 mg]/rat), 3 ×/wk for 4 wks Groups of 36 weanlings	Leukaemia: 0/36, 1/36 (3%) Mammary (all tumours): 12/35 (34%), 22/36 (61%)* Mammary (adenocarcinoma): 5/35 (14%), 12/36 (33%)* Mammary (fibroadenoma): 9/35 (26%), 12/36 (33%) Adrenal gland (pheochromocytoma): 4/36 (11%), 12/36 (33%) Adrenal gland (cortical adenoma): 6/36 (17%), 14/36 (39%) Pituitary gland (carcinoma): 2/36 (6%), 13/36 (36%)** Pituitary gland (adenoma): 9/36 (25%), 12/36 (33%)	[*$P = 0.05$] [**$P < 0.005$]	Purity > 99% Study limited by the short duration of both treatment and observation periods and the use of a single dose
CD (F) Death 12–15 wks after initial treatment; control group: 76–78 wks King (1988); Imaida *et al.* (1991)	Intraperitoneal administration 0 or 10 µmol [3 mg] in DMSO (total dose, 16 µmol [4,7 mg]/rat), 3 × /wk for 4 wks Groups of 33–36 weanlings	Peritoneal cavity (malignant fibrous histiocytoma): 0/31, 29/33 (88%) Leukaemia: 0/31, 7/33 (21%) Mammary (adenocarcinoma): 3/31 (10%), 14/33 (42%) Mammary (fibroadenoma): 5/31 (16%), 4/33 (12%)	$P < 0.0001$ $P < 0.01$ $P < 0.01$ NS	Purity, > 90% Study limited by short duration of both treatment and observation periods
F344/DuCrj (M) 169 d Ohgaki *et al.* (1984)	Subcutaneous injection 0 or 0.2 mg in 0.2 mL DMSO (total dose, 4 mg), twice/wk for 10 wks Groups of 10 or 20 aged 6 wks	Injection site (subcutaneous sarcoma): 0/20, 10/10 (100%)	[$P < 0.0001$]	Impurities: 0.4% 1,3-dinitropyrene, 0,6% 1,6-dinitropyrene; < 0.05% other nitropyrenes Study limited because of the small number of animals studied, the short treatment and observation periods and the possible influence of the contamination with 1,3-dinitropyrene and 1,6-dinitropyrene

Table 3.2 (continued)

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
F344/DuCrj (M) 320 d Ohgaki *et al.*, (1985)	Subcutaneous injection 0, 0.02 or 0.002 mg in 0.2 mL DMSO (total dose, 0.04 or 0.4 mg), twice/wk for 10 wks Groups of 10 or 20 aged 6 wks	Injection site (subcutaneous sarcoma): 0/20, 9/10 (90%)*, 10/10 (100%)*	*[$P < 0.0001$]	Impurities: 0.4% 1,3-dinitropyrene Study limited because of the small number of animals studied, the short treatment and observation periods and the possible influence of the contamination with 1,3-dinitropyrene.
CD (F) Exposure group: 164 d; control group: 495 d King (1988); Imaida *et al.* (1995)	Subcutaneous injection Suprascapular injection starting within 24 h of birth; 1st dose: 2.5 mol/kg bw; 2nd–3rd doses: 5 mol/kg bw; 4th–8th doses: 10 mol/kg bw (total dose, 6.3 mmol [1.9 mg] in DMSO), once/wk for 8 wks 37, treated group; 40 controls (DMSO)	Injection site (malignant fibrous histiocytoma): 0/40, 37/37 (100%)* Leukaemia: 0/40, 8/37 (22%)** Mammary (adenocarcinoma): 1/40 (3%), 5/37 (14%) Mammary (fibroadenoma): 6/40 (15%), 0/46 Mammary (adenoma): 1/40 (3%), 0/37	*$P < 0.0001$ **[$P < 0.005$]	Purity > 99% Study limited by the short duration of both the treatment and observation periods and the use of a single dose

bw, body weight; d, day; DMSO, dimethyl sulfoxide; F, female; h, hour; M, male; NS, not significant; wk, week

3.2.1 Oral administration

Groups of 36 female weanling Sprague-Dawley rats received intragastric intubations of 0 (control) or 10 µmol [3 mg]/kg body weight (bw) of 1,8-dinitropyrene (purity, > 99%) dissolved in DMSO (1.7 µmol [0.5 mg]/mL) three times a week for 4 weeks (average total dose, 16 µmol [4.7 mg]/rat) and were observed for 76–78 weeks. The animals were observed for 76–78 weeks and then killed. One rat (3%) treated with 1,8-dinitropyrene and none of the controls developed leukaemia. The total number of mammary-tumour bearing animals in the 1,8-dinitropyrene-treated group was significantly increased. The incidence of mammary gland adenocarcinoma (12 out of 36; 33%, $P < 0.05$) was significantly higher than that observed in the control rats (5 out of 35; 14%), but that of fibroadenoma in treated rats (12 out of 36; 33%) was not significantly different compared with controls (9 out of 35; 26%). Adrenal gland and pituitary tumours were also observed in treated animals at an elevated but non-significant level compared with controls (King, 1988; Imaida *et al.*, 1991). [The Working Group noted the short duration of both treatment and observation periods and the use of a single dose.]

3.2.2 Intraperitoneal administration

Groups of 36 female weanling CD rats received intraperitoneal injections of 0 (control) or 10 µmol [3 mg]/kg bw of 1,8-dinitropyrene (purity, > 99%) dissolved in DMSO (1.7 µmol [0.5 mg]/mL) three times a week for 4 weeks (total dose, 16 µmol [4.7 mg]/rat). Treatment with 1,8-dinitropyrene resulted in early deaths 12–15 weeks after the initial treatment. The first intraperitoneal tumour was detected at week 17; 29 out of 33 (88%) of the treated rats developed malignant fibrous histiocytomas of the peritoneal cavity ($P < 0.0001$), and a significantly increased incidence of myelocytic leukaemia (7 out of 33; 21%) was observed in this group ($P < 0.01$). No such malignancies developed among 31 vehicle controls after an observation period of 76–78 weeks. Mammary gland adenocarcinomas were observed in 14 out of 33 (42%) treated animals compared with 3 out of 31 (10%) controls; the difference in the incidence was statistically significant ($P < 0.001$) (King, 1988; Imaida *et al.*, 1991).

3.2.3 Subcutaneous administration

Ten male Fischer 344/ DuCrj rats, aged 6 weeks, received subcutaneous injections of 0.2 mg of 1,8-dinitropyrene ([purity unspecified]; impurities: 0.4% 1,3-dinitropyrene, 0.6% 1,6-dinitropyrene and < 0.05% other nitropyrenes) dissolved in 0.2 mL DMSO (total dose, 4 mg) twice a week for 10 weeks. A control group of 20 rats received injections of 0.2 mL DMSO alone. The animals were killed between days 140 and 169. Sarcomas developed at the site of injection in all treated rats between days 113 and 127. No tumours were observed in other organs of treated rats, and no local tumours developed among the control animals (Ohgaki *et al.*, 1984). [The Working Group noted that, while it recognized the contamination of the study material with 1,3- and 1,6-dinitropyrene, the tumour response was so strong that it can be attributed to the exposure to 1,8-dinitropyrene.]

Two groups of 10 male Fischer 344/ DuCrj rats, aged 6 weeks, received subcutaneous injections of 0.002 or 0.02 mg of 1,8-dinitropyrene ([purity unspecified]; impurities: 0.4% 1,3-dinitropyrene; 0.6% 1,6-dinitropyrene and < 0.05% other nitropyrenes) dissolved in 0.2 mL of DMSO (total doses, 0.04 or 0.4 mg) twice a week for 10 weeks. A control group of 20 rats received injections of 0.2 mL of DMSO alone. All treated animals were killed on day 320 and control rats on day 650. Sarcomas developed at the site of injection between days 123 and 156 in all 10 rats treated with 0.4 mg of 1,8-dinitropyrene and between days 213 and 320 in 9 out of 10 (90%) rats treated

with 0.04 mg of 1,8-dinitropyrene. No tumours were observed in other organs of treated rats or at the injection site in control animals (Ohgaki *et al.*, 1985). [The Working Group noted that, while it recognized the contamination of the study material by 1,3- and 1,6-dinitropyrene, the tumour response was so strong that it can be attributed to the exposure to 1,8-dinitropyrene.]

In a lifetime study, a group of 37 female newborn Sprague-Dawley rats received subcutaneous injections into the suprascapular region of 1,8-dinitropyrene (purity, > 99%; total dose, 6.3 µmol [1.8 mg]) dissolved in DMSO (1.7 µmol [0.5 mg]/mL) at weekly intervals starting within 24 hours of birth (first dose, 2.5 mmol/kg bw; second the third doses, 5 mmol/kg bw; doses 4–8, 10 mmol/kg bw). A group of 40 animals injected with DMSO alone served as controls. Average survival was 164 days for treated animals and 495 days for controls. Malignant fibrous histiocytomas developed rapidly at the injection site in treated rats; the first tumour was seen 122 days after the initial injection and, by 20 weeks, all treated rats had developed this tumour (37 out of 37; $P < 0.0001$). In addition, 8 out of 37 treated rats (22%; $P < 0.005$) had leukaemia. Controls developed no such malignancies. Mammary gland adenocarcinomas were also observed in 5 out of 37 (14%) treated rats, although this incidence did not differ significantly from that in the control group (1 out of 40; 3%) (King, 1988; Imaida *et al.*, 1995).

4. Mechanistic and Other Relevant Data

4.1 Absorption, distribution, metabolism and excretion

4.1.1 Humans

No data were available to the Working Group.

4.1.2 Experimental systems

The metabolism of 1,8-dinitropyrene was studied *in vitro* using rat liver and mammary gland cytosols, as well as dog liver cytosols, and in in-vivo systems (IARC, 1989). In conventional CD rats, several metabolites were detected following the oral administration of 1,8-dinitropyrene (1.0 µmol, 0.3 mg): *N*,*N*-diacetyl-1,8-diaminopyrene, 1,8-diaminopyrene, 1-acetylamino-8-nitropyrene and unidentified polar metabolites in the faeces. In germ-free animals, only 1-amino-8-nitropyrene and the polar metabolites were detected.

4.2 Genetic and related effects

4.2.1 Humans

No data were available to the Working Group.

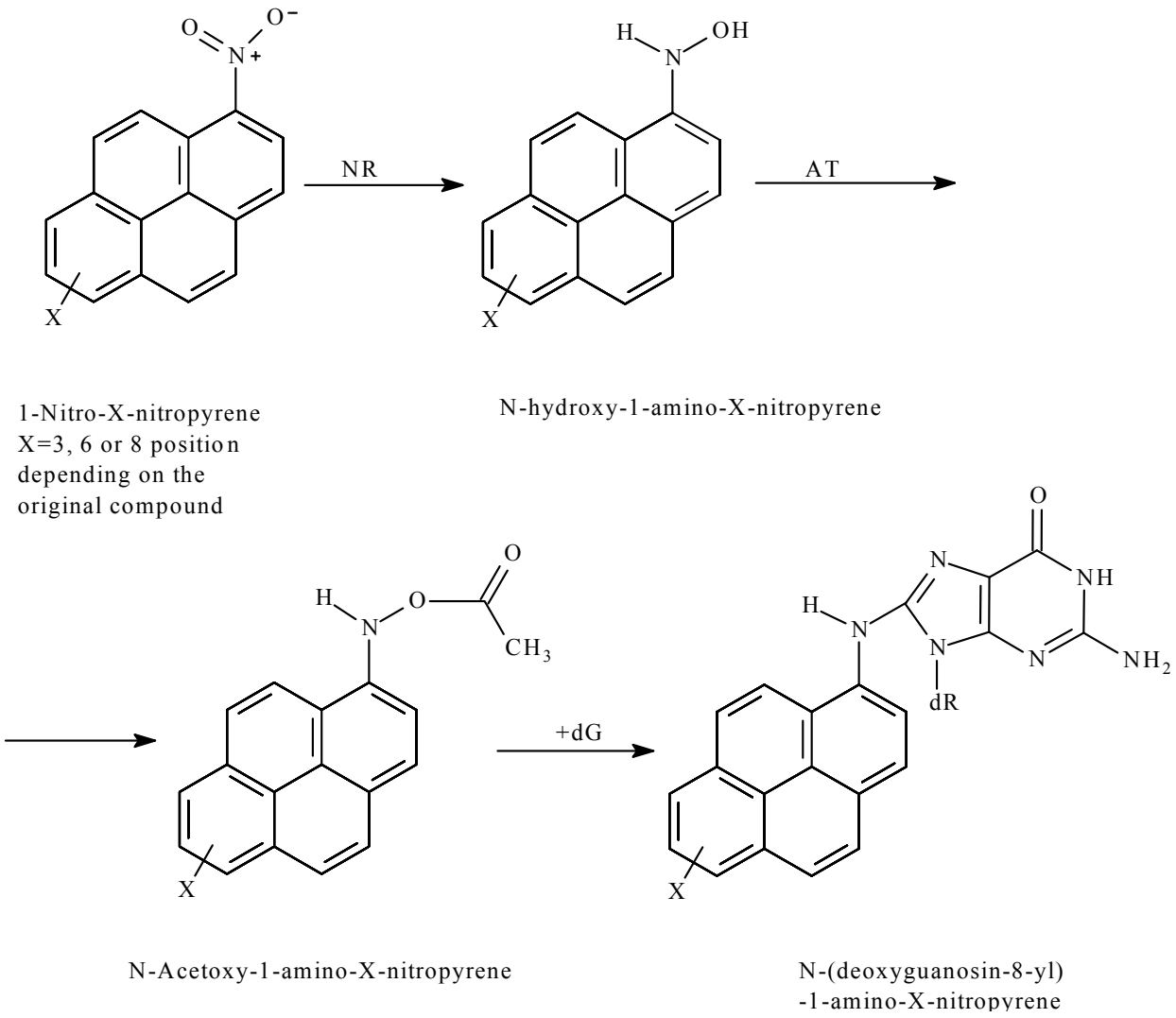
4.2.2 Experimental systems

See Fig. 4.1.

In both conventional and germ-free male CD rats, *N*-(deoxyguanosin-8-yl)-1-amino-8-nitropyrene was detected as the major DNA adduct in the liver and mammary gland, but the levels of binding were considerably lower in germ-free animals (IARC, 1989).

The majority of the studies on the genotoxicity of nitro-PAHs have used the *Salmonella typhimurium* microsome assay with a standardized test protocol using several strains (TA98, TA100, TA1535, TA1537 or TA1538). Overall, nitroreductases and *O*-acetyltransferases have been shown to be important enzymes in the mutagenic activation of 1,8-dinitropyrene in bacteria (IPCS, 2003). No significant difference in mutagenicity was seen between nitroreductase-deficient strains, standard strains and nitroreductase-overexpressing strains (IPCS, 2003). 1,8-Dinitropyrene showed stronger mutagenicity in this system in the absence of metabolic activation. The mutagenic activity of 1,8-dinitropyrene

Fig. 4.1 Metabolic activation pathway and DNA adduct formation



NR, nitroreductase; AT, acetyltransferase; dG, deoxyguanosin
Compiled by the Working Group

was clearly decreased with *O*-acetyltransferase-deficient strains (AT^-) and increased with *O*-acetyltransferase-overproducing strains (AT^+) compared with that observed in standard tester strains (IARC, 1989; Tokiwa *et al.*, 1994). These results indicate that *O*-acetylation appears to be a critical mutagenic activation pathway in *S. typhimurium*. Nitrated pyrenes induced primarily frameshift mutations.

The genotoxicity of 1,8-dintropyrene has been reported in *S. typhimurium* and in bacterial systems other than the *Salmonella* microsome assay (IARC, 1989; Mersch-Sundermann *et al.*, 1991, 1992; Oda *et al.*, 1992, 1993; Jurado *et al.*, 1993; Busby *et al.*, 1994; Jurado *et al.*, 1994; Nohmi *et al.*, 1995; Shane & Winston, 1997; Yamazaki *et al.*, 2000); in general, the results, with a few exceptions, were consistent. 1,8-Dinitropyrene induced DNA damage and mutations in several strains of bacteria, and DNA single-strand breaks in mouse hepatocytes, cultured Chinese hamster V79 cells and cultured rat hepatoma

cells, and activated the synthesis of viral DNA in polyoma virus-transformed rat fibroblasts. It induced unscheduled DNA synthesis in mouse and rat hepatocytes, and in rabbit lung Clara and alveolar type II cells. 1,8-Dintropyrene induced mutations in cultured mouse lymphoma and Chinese hamster ovary cells, chromosomal aberrations in Chinese hamster ovary cells and human fibroblasts, chromatid-type chromosomal aberrations in rat epithelial cells, and morphological transformation in Syrian hamster embryo cells. Sarcomas produced in 1,8-dinitropyrene-treated rats contained activated C-K_i-*ras* oncogenes (IARC, 1989).

Landvik *et al.* (2007) showed that 1-nitropyrene and dinitropyrenes induced apoptosis in Hepa1c1c7 cells with the following potency: 1,3-dinitropyrene > 1-nitropyrene > 1,8-dinitropyrene. These compounds induced cytochrome P450 (CYP) 1a1 and activated various intracellular signalling pathways related to apoptosis. The most important finding was that the most mutagenic and carcinogenic compound tested in this study, 1,8-dinitropyrene, induced little (if any) cell death, despite the fact that this compound seemed to cause the greatest DNA damage as determined by DNA adduct formation, increased phosphorylation of p53 and accumulation of cells in the S-phase. Immunohistochemical studies revealed that the p53 protein did not accumulate in the nucleus, suggesting that 1,8-dinitropyrene inactivated the pro-apoptotic function of the p53 protein by a non-mutagenic event. Taken together, Landvik *et al.* (2007) suggested that, after exposure to 1,8-dinitropyrene, more cells may survive with DNA damage and thereby increase the mutagenic and carcinogenic potential of the compound.

4.3 Other relevant data

No data were available to the Working Group on the acute toxicity of 1,8-dinitropyrene. As previously reported (IARC, 1989), ulcer and scar formation at the site of injection were observed in rats after repeated subcutaneous injection of 0.2 mg per animal. 1,8-Ditropyrene administered intraperitoneally to young male Sprague-Dawley rats (three times at 2.5 mg/kg bw) resulted in increases in the activities of aryl hydrocarbon hydroxylase, 7-ethoxycoumarin-*O*-deethylase, aminopyrine-*N*-demethylase and 1-nitropyrene reductase in the liver microsomes compared with untreated controls.

4.4 Mechanistic considerations

To gain insights into the mechanisms by which 1,6-dinitropyrene induced mutations in human cells, Boldt *et al.* (1991) investigated the mutagenic effects of *N*-hydroxy-1-amino-6-nitropyrene, which is derived from the nitroreduction of 1,6-dinitropyrene. The shuttle vector plasmids, pS189, in human 293 cells were exposed to [^{3}H]1-nitro-6-nitrosopyrene for one hour in the presence of ascorbic acid to generate the corresponding hydroxylamine. A linear increase was observed in the number of DNA adducts per plasmid (as a function of applied concentration) and also in the frequency of *supF* mutants (as a function of adducts per plasmid). 1,6-Ditropyrene induced base substitutions, primarily GC→TA transversions, but produced a significant fraction of –1 frameshifts, most of which were located in a unique run of the five Gs in the gene. The 'hot spots' for adduct formation were not perfectly correlated with those for the induction of mutation. Thus, the ultimate biological effect of 1,6-dinitropyrne depends not only on the number of adducts (measured by the ^{32}P-postlabelling method) originally formed, but also on other processes such as cellular DNA repair, which may remove these adducts from the plasmids before DNA replication occurs, as well as on the structure of the neighbouring bases at the site of adduction. *In vivo*, mutagenesis was reported in the lungs of gpt-delta transgenic mice that received intratracheal instillations of

1,6-dinitropyrene (Hashimoto *et al.*, 2006), and the major mutations induced included G:C→T:A transversions and 1-base deletions.

To understand further the mechanisms that can account for mutagenesis, a commercially available mixture of dinitropyrenes (1,3-, 1,6- and 1,8-dinitropryene and unidentified isomer(s) with contents of 20.2%, 30.4%, 35.2% and 14.2%, respectively) was administered by intragastric intubation in the Muta Mouse model at doses of 200 and 400 mg/kg bw once a week for 4 weeks. Several organs (the liver, lung, colon, stomach and bone marrow) were collected 7 days after the final treatment, and the mutation frequencies of *lacZ* and *cII* genes were analysed. Spontaneous mutation frequencies were in the range of 3.1×10^{-5} to 7.6×10^{-5} and 1.6×10^{-5} to 5.9×10^{-5} for the *lacZ* and *cII* genes, respectively. These increases above spontaneous levels were most apparent in the colon, where six- and eightfold increases were observed in the *lacZ* and *cII* genes, respectively. The increase was also evident in the stomach for both genes, although this was not statistically significant at the higher dose for the *cII* gene. A statistically significant increase was observed in the liver and lung for the *lacZ* gene, but was not evident in the liver for the *cII* gene. A fourfold increase was observed in bone marrow for both genes, but was statistically significant only for the *cII* gene. Base-substitution mutations in the colon predominated in both untreated and dinitropyrene-treated mice. The treatment with dinitropyrenes increased the incidence of G:C→T:A transversions and decreased that of G:C→A:T transitions. The G:C→T:A transversions were probably caused by the guanine-C8 adduct (Kohara *et al.*, 2002).

Female Sprague-Dawley rats were given a single intraperitoneal injection of 1,6-dinitropyrene and both covalent DNA adduct formation (*N*-deoxyguanoisin-8-yl)-1-amino-6-nitropyrene) and oxidative DNA damage (5-hydroxymethyl-2′-deoxyuridine and 8-hydroxy-2′-deoxyguanosine) were assessed in the liver, mammary gland, urinary bladder and nucleated blood cells at 3, 12, 24 and 48 hours after treatment (Djurić *et al.*, 1993). The covalent adduct was detected in all tissues and the bladder had the highest levels. The levels of 5-hydroxymethyl-2′-deoxyuridine were highest in the liver and mammary glands. 1,6-Dinitropyrene did not affect the levels of 8-hydroxy-2′-deoxyguanosine. These results suggest that 1,6-dinitropyrene can induce both covalent DNA binding and certain DNA oxidative damage and that both types of DNA damage may contribute to its carcinogenicity in the rat mammary gland.

Tokiwa *et al.* (1999) showed that 8-hydroxy-2′-deoxyguanosine was detected in all 22 cases of carcinoma in human lung tissues. Intratracheal administration of diesel exhaust particles to rodents (without analysis of the organic components) increased the levels of this oxidative lesion. These results suggest that carbonaceous particles, but not mutagens and carcinogens, promote the formation of this lesion and that, as a mechanism, alveolar macrophages may be involved in diesel particle-induced oxidative damage. Murata *et al.* (2004) investigated the extent of oxidative DNA damage induced by 1,3-, 1,6- and 1,8-dinitropyrene in the presence of nicotinamide adenine dinucleotide phosphate (NADPH)-CYP reductase using the ^{32}P-5′-end-labelled DNA method. The intensity of DNA damage caused by 1,6- or 1,8-dinitropyrene was stronger than that caused by 1,3-dinitropyrene. Further experiments suggest that dinitropyrenes are enzymatically reduced to the corresponding 1-nitro-X(3,6,8)-nitrosopyrene via the nitro-radical anion, and that these nitroso intermediates are further reduced non-enzymatically by NADPH. Subsequently, auto-oxidation of the nitro radical anion resulted in $O^{-}\cdot$ generation leading to DNA damage. These results indicate that both covalent DNA adducts and DNA oxidative damage may contribute to the mutagenic and carcinogenic effects of dinitropyrenes.

SnrA and cnr bacterial nitroreductases have been previously identified in *Salmonella enterica* serovar Typhimurium (Salamanca-Pinzón *et al.*, 2010). Both SnrA and cnr have been purified, and their capacity to activate dinitropyrenes in the Ames test and their kinetic parameters (*Km* and *Vmax*) were examined. 1,3-Dinitropyrene was efficiently activated by cnr, whereas 1,6- and 1,8-dinitropyrene were scarcely activated by either nitroreductase. A good correlation was obtained between the catalytic efficiency (*Vmax*/*Km*) of the purified cnr (but not of SnrA) and the redox potential of the dinitropyrene. These results suggest that factors other than redox potential are involved in the catalytic activity of SnrA.

The activity of hepatic microsomal enzymes in rats pretreated with a series of nitro-PAHs was examined by Chou *et al.* (1987), who found that some of these compounds, including dinitropyrenes, increased the activities of aryl-hydrocarbon hydroxylase, 7-ethoxycoumarin *O*-deethylase and 1-nitropyrene reductase. None of the compounds caused significant increases in epoxide hydrolase or NADP-cytochrome C reductase. Because nitro reduction appears to be important in the metabolic activation of dinitro-pyrenes, the results of this study suggest that chronic exposure to these agents may result in an increase in DNA adduct formation, which in turn could result in increased tumorigenicity.

The treatment of fish hepatoma PLHC-1 cells with 1,6-dinitropyrene resulted in the induction of CYP1A (Jung *et al.*, 2001). The mRNA levels of CYP1A1, -1A2 and -1B1 were determined using the reverse transcription-polymerase chain reaction in various human cell lines treated with several nitro-PAHs, amino PAH derivatives and PAHs (Iwanari *et al.*, 2002). In inducible cell lines, such as human breast cancer MCF-7 cells, the induction profile of chemical specificity was similar for CYP1A1, -1A2 and -1B1, although the extent of induction differed among cell lines and for the CYP isoforms. 1,3-, 1,6- and 1,8-Dinitropyrene slightly induced CYP1 mRNA, but the 1,3- isomer induced a sixfold induction of CYP1A1 mRNA in MCF-7 cells. The cell-specific induction of the CYP1 family was not related to the expression levels of the arylhydrocarbon receptor, aryl hydrocarbon nuclear translocator or estrogen receptors α and β.

Landvik *et al.* (2007) demonstrated that nitro-PAHs induced apoptosis in Hepa1C1C7 cells with the following order: 1,3-dinitro-pyrene > 1-nitropyrene > 1,8-dinitropyrene. These compounds induced CYP1A1, and activated various intracellular signalling pathways related to apoptosis. 1,3-Dinitropyrene and 1-nitropyrene induced concentration-dependent lipid peroxidation. 1,3-Dinitropyrene caused pro-apoptotic events (increased phosphorylation and accumulation of p53 in the nucleus, cleavage of bid and of caspases 8 and 3, down-regulation of bcl-X_L and phosphorylation of p38 and c-Jun N-terminal kinase/mitogen-activated protein kinase). It also increased the activation of survival signals (phosphorylation of AKt and inactivation [phosphorylation] of pro-apoptotic bad). 1,8-Dinitropyrene induced little (if any) cell death, despite the fact that this compound seemed to induce the greatest DNA damage (as determined by DNA adduct formation, increased phosphorylation of p53 and accumulation of cells in the S-phase). Immunohistochemical analysis revealed that the p53 protein did not accumulate in the nucleus, suggesting that 1,8-dinitro-pyrene inactivated the pro-apoptotic function of p53 by a non-mutagenic event. On the basis of these results, these investigators suggested that, following exposure to 1,8-dinitropyrene, more cells may survive with DNA damage and thereby increase its mutagenic and carcinogenic potential.

Mutagenic activity was evaluated using the umuC test in the presence and absence of metabolic activation (Bonnefoy *et al.*, 2012). The umuC test is based on the induction of the umuC gene as part of the *Salmonella typhimurium*

TA1535 [pSK1002] SOS response to genotoxic lesions induced by xenobiotics. To evaluate the genotoxic effects further, these authors used both the cytokinesis-blocked micronucleus assay and fluorescent *in situ* hybridization of human pan-centromeric DNA probes on human lymphocytes. 1,3-, 1,6-, and 1,8-Dinitropyrene were mutagenic in the umuC test and the effect was dose-dependent in the presence and absence of metabolic activation; however, some exceptions were evident. 1,3- and 1,6-Dinitropyrene induced a clear but statistically non-significant increase in micronucleated cells. However, a significant induction of micronucleated cells was observed for 1,8-dinitropyrene at 0.5 μg/mL. The percentages of observed centromere-negative micronuclei were 63.2% and 81.8% for 1-nitropyrene and 1,8-dinitropyrene, respectively; the corresponding values for 1,3- and 1,6-dinitropyrene were 56.3% and 58.3%, respectively. 1,3- and 1,6-Dinitropyrene exhibited both clastogenic and aneugenic activities but 1,8-dinitropyrene exhibited a dominant clastogenic mechanism.

In summary, 1,6- and 1,8-dinitropyrene are more powerful mutagens in bacterial systems and mammalian systems than 1,3-dinitropyrene. Moreover, their carcinogenic activities exceed those of 1,3-dinitropyrene and 1-nitropyrene. The activation of dinitropyrenes occurs by nitroreduction of one nitro group initially to form nitroso intermediates that are then converted to the corresponding hydroxylamino derivatives. 1,3-Dinitropyrene and 1-nitropyrene were reduced to a much lesser extent than 1,6- and 1,8-dinitropyrene. The mutagenicity of dinitropyrene is related to the ability of the corresponding hydroxylamino derivative to bind to DNA. *O*-Acetylation of the *N*-hydroxylamino group is followed by removal of the acetyl group to yield the active electrophilic nitronium ion that reacts with deoxyguanosine at the C8 position to form *N*-(deoxyguanosine-8-yl)-1-amino-X(3,6,8) nitropyrene. Mutations in the *K-ras* oncogene and *p53* tumour-suppressor gene were observed in 1,6-dinitropyrene-induced lung tumours in rats. 1,8-Dinitropyrene induced greater DNA damage than 1,3-dinitropyrene and 1-nitropyrene but less, if any, cell death in Hepa1c1c cells; this result suggested that, following exposure to 1,8-dinitropyrene, more cells may survive with DNA damage, and thereby increase its mutagenic and carcinogenic activities.

5. Summary of Data Reported

5.1 Exposure data

1,8-Dinitropyrene is produced by the nitration of 1-nitropyrene. No evidence was found that it has been produced in commercial quantities or used for purposes other than laboratory applications. During the combustion of diesel and gasoline engines, pyrene is nitrated to form 1-nitropyrene, which is further nitrated to form small amounts of dinitropyrenes. This leads to a content of 1,8-dinitropyrene in the range of 0.1–10% relative to the 1-nitropyrene content in diesel and gasoline exhaust particles and ~1% in airborne particulate matter. 1,8-Dinitropyrene is present in the 1–10 ng/g range in airborne particulate matter collected from ambient atmospheric samples. Air concentrations clearly decline from values in the 0.1–10 pg/m^3 range at urban locations to values in the 0.01–0.1 pg/m^3 range at suburban locations.

1,8-Dinitropyrene is also generated by kerosene heaters. No data on occupational exposure were available to the Working Group.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

1,8-Dinitropyrene was tested for carcinogenicity in mice in one study by intraperitoneal injection and one study by subcutaneous injection, and in rats in one study by oral administration, one study by intraperitoneal injection and three studies by subcutaneous injection. In mice, intraperitoneal injection of 1,8-dinitropyrene into newborns did not produce an increase in the incidence of tumours at any site that was significantly different from that in controls; the study of the subcutaneous injection of 1,8-dinitropyrene was inadequate to evaluate carcinogenicity. In rats, intragastric administration of 1,8-dinitropyrene caused a significant increase in the incidence of mammary tumours and pituitary carcinomas in females; intraperitoneal injection caused a significant increase in the incidence of malignant histiocytomas of the peritoneal cavity, mammary adenocarcinoma and myelocytic leukaemia in females; and subcutaneous injection of 1,8-dinitropyrene induced injection-site sarcomas in males in two studies and a significant increase in the incidence of malignant histiocytomas and leukaemia in females in one study.

5.4 Mechanistic and other relevant data

No data were available to the Working Group on the absorption, distribution, metabolism and excretion or genetic and related effects of 1,8-dinitropyrene in humans. The metabolism of 1,8-dinitropyrene was investigated in rat liver and mammary gland cytosols *in vitro*. After oral administration to rats, 1,8-dinitropyrene produced the metabolites *N*,*N*-diacetyl-1,8-diaminopyrene, 1,8-diaminopyrene and 1-acetylamino-8-nitropyrene. Studies with nitroreductase/acetyltransferase-deficient or -overproducing strains of bacteria revealed that *O*-acetylation is probably the critical mutagenic activation pathway in *Salmonella*. 1,8-Dinitropyrene induced little, if any, cell death, despite the induction of extensive DNA damage, DNA adduct formation, phosphorylation of *Tp53* and enhanced cell proliferation. A greater number of cells may thus survive with DNA damage, and thereby increase the mutagenic and carcinogenic potential of 1,8-dinitropyrene. Sarcomas in rats treated with 1,8-dinitropyrene contained activated K-*ras* oncogenes.

Overall, these data provide *moderate mechanistic evidence* to support the carcinogenicity of 1,8-dinitropyrene.

6. Evaluation

6.1 Cancer in humans

No data were available to the Working Group.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of 1,8-dinitropyrene.

6.3 Overall evaluation

1,8-Dinitropyrene is *possibly carcinogenic to humans (Group 2B).*

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Yamazaki H, Hatanaka N, Kizu R *et al.* (2000). Bioactivation of diesel exhaust particle extracts and their major nitrated polycyclic aromatic hydrocarbon components, 1-nitropyrene and dinitropyrenes, by human cytochromes P450 1A1, 1A2, and 1B1. *Mutat Res*, 472: 129–138. PMID:11113705

3-NITROBENZANTHRONE

3-Nitrobenzanthrone has not previously been evaluated by an IARC Working Group.

1. Exposure Data

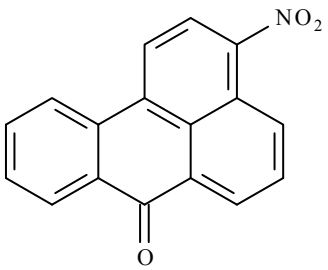
1.1 Chemical and physical data

1.1.1 Nomenclature

Chem. Abst. Serv. Reg. No.: 17117-34-9

Synonym:
3-Nitro-7*H*-benzo[*d*,*e*]anthracen-7-one

1.1.2 Structural and molecular formulae and relative molecular mass



$C_{17}H_9NO_3$
Relative molecular mass: 275.27 g/mol

1.1.3 Chemical and physical properties of the pure substance

Description: Yellow powder (Enya *et al.*, 1997)

Melting-point: 256–257 °C (Enya *et al.*, 1997); 252 °C (Suzuki *et al.*, 1997)

Boiling-point: 506.2 °C at 760 mm Hg

Flash-point: 256.6 °C

Spectrometry data: Infrared and nuclear magnetic resonance data have been reported (Enya *et al.*, 1997; Suzuki *et al.*, 1997).

1.2 Analysis

For the analytical methods of nitro-polycyclic aromatic hydrocarbons (PAHs) in general, the reader is referred to Section 1.2.2(*d*) of the *Monograph* on Diesel and Gasoline Engine Exhausts in this Volume.

3-Nitrobenzanthrone is a semi-volatile compound with a high molecular weight, and can therefore be collected on a filter.

Phousongphouang & Arey (2003) measured low concentrations of 3-nitrobenzanthrone in ambient air by mass spectrometry, and Tang *et al.* (2004) developed a method using high-performance liquid chromatography (HPLC) with chemiluminescence detection for the analysis of nitrobenzanthrones in airborne particulates.

1.3 Production and use

1.3.1 Production

3-Nitrobenzanthrone can be produced by the direct nitration of benzanthrone with nitric acid in an organic solvent (Suzuki *et al.*, 1997), or with gaseous nitrogen dioxide and ozone (Enya

et al., 1998). Suzuki *et al.* (1997) synthesized the compound using the modified Ullmann cross-coupling reaction between 4-nitro-substituted 1-iodonaphthalene and methyl-iodo-benzoate, followed by ring closure of the resulting 2-(1-naphthyl)benzoic acid derivative.

1.3.2 Use

No evidence was found that 3-nitrobenzanthrone has been used in commercial applications.

1.4 Occurrence and exposure

1.4.1 Environmental occurrence

3-Nitrobenzanthrone was first discovered by Suzuki *et al.* (1997) in organic extracts of both diesel exhaust and airborne particles. It is formed by the combustion of organic material and from reactions of complex PAHs with nitrogen oxides. It has been suggested that 2-nitrobenzanthrone might be formed more specifically by atmospheric processes (Inazu *et al.*, 2008), while 3-nitrobenzanthrone seems to be formed preferentially by combustion processes, such as in a diesel engine (Feilberg *et al.*, 2002; Phousongphouang & Arey, 2003; Tang *et al.*, 2004).

Table 1.1 summarizes the available data on environmental exposure from point sources and on environmental samples of air, soil and rainwater.

(a) Point sources

3-Nitrobenzanthrone has been detected in diesel exhaust particles at concentrations of up to 6.6 μg/g particles (Enya *et al.*, 1997; Murahashi, 2003). Furthermore, it was detected in extracts of particles collected from the chimney of a domestic coal-burning stove at a concentration of 0.23 μg/g particles, suggesting that particles emitted from industrial and domestic coal-burning sources should be considered as possibly minor sources of 3-nitrobenzanthrone in urban air pollution (Taga *et al.*, 2005).

(b) Environmental levels

Airborne concentrations of 3-nitrobenzanthrone in ambient particles have been reported to be in the order of several picgrams per cubic metre (up to > 11.5 pg/m^3) in urban and semi-rural areas, and at sites affected by traffic or industrial emissions. Higher levels (up to > 80 pg/m^3) have been recorded in ambient air at workplaces exposed to high levels of diesel emissions (Seidel *et al.*, 2002).

More recently, Inazu *et al.* (2008) measured airborne levels of 3-nitrobenzanthrone in central Tokyo, Japan, in the range of 0.5–3.5 fmol/m^3 (0.2–1.0 pg/m^3); the highest levels were observed in the winter (average, 0.6 pg/m^3) and the lowest levels were found in the spring (average, 0.2 pg/m^3).

Murahashi *et al.* (2003a) detected 3-nitrobenzanthrone in rainwater samples collected at a residential area in Kyoto, Japan, at levels ranging from 0.07 to 2.6 ng/L. It was also detected in surface soil at levels of up to 1200 pg/g soil (Murahashi *et al.*, 2003b; Watanabe *et al.*, 2003).

Substantially higher concentrations of 2-nitrobenzanthrone than of 3-nitrobenzanthrone have been found in ambient air, with a ratio of 37.5–70:1 (Phousongphouang & Arey, 2003; Inazu *et al.*, 2008).

1.4.2 Exposure of the general population

Exposure to 3-nitrobenzanthrone occurs mainly by inhalation and secondarily by oral intake. Inhalation occurs through contamination in air that is formed by combustion. Oral exposure can occur either by mucociliary clearance and subsequent swallowing of the material that has been inhaled or by the consumption of foods that have been affected by dry or wet deposition of airborne 3-nitrobenzanthrone. Overall, inhalation is considered to be the greatest and perhaps most important source of exposure. On the basis of the reported levels of 3-nitrobenzanthrone in diesel exhaust particles and a daily

Table 1.1 Sources of environmental exposure to 3-nitrobenzanthrone

Reference	Country	Sampling information	Concentration of 3-NBA
Diesel exhaust particles			
Enya *et al.* (1997)	Japan	Isuzu engine Model 6HEL 7127 c (maximum power, 250 sp/2700 rpm) working under various loading conditions	< 0.001–6.61 µg/g particulate
Murahashi (2003)	Japan	Diesel engine vehicles, including heavy- and light-duty vehicles, driven at 80 km/h (n = 3)	0.027–0.056 µg/g particulate
Phousongphouang & Arey (2003)	USA	Standard reference material (SRM) 1975 obtained from the National Institute for Standards and Technology (NIST), Gaithersburg, MD	Identified by GC–MS SIM
Airborne particles			
Enya *et al.* (1997)	Japan	Sampling point was in central Tokyo; sampling time, winter 1994; same day and night time collection	5.2–11.5 pg/m^3
Zhu *et al.* (2001)	USA	2.5 µm particles ($PM_{2.5}$) were collected in Salt Lake City, UT; sampling time, 5 October 1999 (n = 1)	Tentatively identified by MS
Feilberg *et al.* (2002)	Denmark	Sampling site was located at Riso in a semi-rural area about 35 km west of Copenhagen; sampling time, from February 1998 to February 1999 (3-NBA was present ~25% of collected samples; n = 31)	ND–68.4 pg/m^3
Seidel *et al.* (2002)	Germany	Samples were collected at five typical workplaces in an underground salt mine (n = 5)	ND–80 pg/m^3
Phousongphouang & Arey (2003)	USA	Sampling point was at an industrial site affected by emissions in Concord, CA (n = 1); sampling time, January 1987	0.4 pg/m^3
Tang *et al.* (2004)	Japan	Sampling point was a heavy-traffic road in Kanazawa (n = 1)	6.79 pg/m^3
Inazu *et al.* (2008)	Japan	Samples collected in central Tokyo: n = 3 n = 4 n = 10 n = 8	0.13–0.96 pg/m^3 spring: 0.19 pg/m^3 summer: 0.38 pg/m^3 autumn: 0.44 pg/m^3 winter: 0.57 pg/m^3
Rainwater			
Murahashi *et al.* (2003a)	Japan	Sampling point was a building roof in a residential area of Kyoto (n = 6); sampling time, from May to July and from October to December 2001	0.07–2.6 ng/L

Table 1.1 (continued)

Reference	Country	Sampling information	Concentration of 3-NBA
Surface soil			
Murahashi *et al.* (2003b)	Japan	Samples were collected in the Chuba area (n = 6)	1.2–1020 pg/g
Watanabe *et al.* (2003)	Japan	Samples were collected in the Kinki region, particularly in Osaka and neighbouring cities (n = 8); sampling time, between February and December 1999	144–1158 pg/g
Lübcke-von Varel *et al.* (2012)	Germany	Polar fraction of a sediment extract of chemical industrial area of Bitterfeld (n = 1)	60 pg/g SEQ
Coal-burning-derived particles			
Taga *et al.* (2005)	China	Sampling point was the chimney of a domestic coal stove in Shenyang (n = 1)	0.234 µg/g particulate

3-NBA, 3-nitrobenzanthrone; GC–MS SIM, gas chromatography–mass spectroscopy selected ion monitoring; MS, mass spectroscopy; ND, not detected; SEQ, sediment equivalents
Adapted from Arlt (2005)

intake by inhalation of 1 μg of particles per cubic metre of air, Arlt (2005) estimated a human lung dose of approximately 90 pg per day, based on a breath intake of about 15 m^3 air per day, although the value may be exceeded in highly exposed populations.

1.5 Regulations and guidelines

There are currently no regulations or guidelines regarding exposure to 3-nitrobenzanthrone.

2. Cancer in Humans

No data were available to the Working Group

3. Cancer in Experimental Animals

See Table 3.1

3.1 Mouse

Initiation-promotion

A study was conducted in NMRI mice to evaluate the initiating and carcinogenic potential of 3-nitrobenzanthrone and one of its putative metabolites. Groups of 20 female mice [age unspecified] received a single topical application of 0, 25, 100 or 400 nmol of 3-nitrobenzanthrone or its primary metabolite, *N*-hydroxy-3-aminobenzanthrone, in acetone followed by weekly applications of 0 or 5 nmol of the tumour promoter, 12-*O*-tetradecanoylphorbol-13-acetate, in acetone for up to 40 weeks. Another group received topical applications of 3-nitrobenzanthrone twice a week. No tumours were observed in any of the treatment groups except for a positive control group treated with 7,12-dimethylbenz[*a*]anthracene (Schmeiser *et al.*, 2009).

3.2 Rat

Intratracheal administration

A long-term study of the intratracheal instillation of 3-nitrobenzanthrone was conducted to determine the formation of DNA adducts at short-term time-points and to evaluate tumour formation at 24 months after the initiation of treatment. Groups of 25, 21 and 33 female Fischer 344 rats, aged 4 weeks, received intratracheal instillations of 0 (vehicle control) or 0.5 mg of 3-nitrobenzanthrone in 10% propylene glycol/saline three or five times a week (total doses, 1.5 or 2.5 mg/rat [15 or 22 mg/kg body weight (bw)] for the low- and high-dose groups, respectively) for up to 18 months. Animals were killed at interim time-points to evaluate DNA adduct formation, and after 18 months or when the animals were moribund for the study of carcinogenesis, at which time the respiratory tracts were collected and analysed histologically. Animals in the high-dose group started to become moribund after 7 months of treatment. Squamous cell carcinomas were found in the lungs of animals in the high-dose group after 7–9 months (3 out of 33; 9%), and in the low-dose group after 10–12 months (2 out of 33; 6%). The study was terminated at 18 months due to excess mortality in the high-dose group. The incidence of squamous cell carcinoma at 18 months was 3 out of 15 and 11 out of 19 [$P < 0.0001$] low- and high-dose animals, respectively. The sum at all time-points yielded an incidence of 3 out of 21 and 11 out of 33 [$P < 0.0013$] squamous cell carcinomas in the low- and high-dose groups, respectively. A single lung adenocarcinoma developed in each of the low- and high-dose groups, but not dose-dependently. None of the above lesions was observed in controls (0 out of 25) and no other tumour types were observed in the lungs of the treated groups (Nagy *et al.*, 2005a).

Table 3.1 Studies of the carcinogenicity of 3-nitrobenzanthrone in experimental animals

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, NMRI (F) 40 wks Schmeiser *et al.* (2009)	Topical application 0 (control), 25, 100 or 400 nmol/mouse in 0.1 mL acetone, followed 1 wk later by 0 or 5 nmol TPA; DMBA was used as a positive control Groups of 20 mice	No tumours observed except in positive control	No initiation or promotion observed	No tumour initiation or promotion observed; a second part of the study investigated a putative metabolite that also showed no initiation or promotion activity.
Rat, F344 (F) 18 mo or when moribund Nagy *et al.* (2005a)	Intratracheal instillation 0 (control), 15 or 22 mg/kg bw in 0.1 mL propylene glycol/saline (9:1), once/wk for 3 or 5 wks Groups of 21–33 aged 4 wks	Lung (squamous cell carcinoma): 0/25, 3/21 (14%), 11/33 (33%)* Lung (adenocarcinoma): 0/25, 1/21 (5%), 1/33 (3%)	*[$P < 0.0013$]	High dose expected to simulate environmental exposure; tumours were first observed at 7–9 mo in the high-dose group and 10–12 mo in the low-dose group. Study terminated at 18 mo due to excess mortality in the high-dose group. No statistics reported for tumour formation

bw, body weight; DMBA, 7,12-dimethylbenz[*a*]anthracene; F, female; M, male; mo, month; TPA, 12-O-tetradecanoylphorbol-13-acetate; wk, week

4. Mechanistic and Other Relevant Data

4.1 Absorption, distribution, metabolism, and excretion

4.1.1 Humans

Seidel *et al.* (2002) examined the urinary levels of PAHs and nitrated PAHs (nitroarenes) in the 24-hour urine of 18 (nine smokers and nine non-smokers) underground salt-mine workers occupationally exposed to diesel engine exhaust. 3-Aminobenzanthrone, the major metabolite of 3-nitrobenzanthrone (Borlak *et al.*, 2000; Arlt *et al.*, 2003a; Hansen *et al.*, 2007), and 1-aminopyrene, a reductive metabolite of 1-nitropyrene (El-Bayoumy *et al.*, 1983; Howard *et al.*, 1985; van Bekkum *et al.*, 1998; Chae *et al.*, 1999), were identified in both groups of workers at similar levels of 1–143 ng/24-hour urine and 2–200 ng/24-hour urine, respectively. These results suggest that 3-nitrobenzanthrone and 1-nitropyrene bind to diesel engine exhaust particles and are ingested/inhaled by humans (Seidel *et al.*, 2002), distributed in different organs, metabolized by various enzymes to more polar products (such as 3-aminobenzanthrone and 1-aminopyrene) and excreted in the urine. Thus, 3-aminobenzanthrone and 1-aminopyrene could be used as urinary biomarkers in humans exposed to diesel engine exhaust (Arlt, 2005; Stiborová *et al.*, 2005).

4.1.2 Experimental systems

Several *in vivo* studies have shown that 3-nitrobenzanthrone is metabolically activated to DNA-binding products in the tissues of animals administered the compound by various routes (Arlt *et al.*, 2001, 2003a, 2004a, 2006a, 2007; Bieler *et al.*, 2005, 2007; Nagy *et al.*, 2005a, 2006, 2007; Schmeiser *et al.*, 2009). These studies have been confirmed by several in-vitro studies which showed that 3-nitrobenzanthrone first requires metabolic activation through nitroreduction by various xenobiotic-metabolizing enzymes,

including nicotinamide adenine dinucleotide phosphate (NADPH)-quinone oxidoreductase (NQO1), xanthine/xanthine oxidase, NADPH-P450 oxidoreductase (POR) or cytochrome P450 (CYP) enzymes, leading to the formation of the DNA-binding reactive intermediate, *N*-hydroxy-3-aminobenzanthrone (Borlak *et al.*, 2000; Arlt *et al.*, 2003a, b, 2004b; Bieler *et al.*, 2003) (Fig. 4.1). *N*-Hydroxy-3-aminobenzanthrone can be further activated by acetylation and sulfation, which are catalysed by *N*-acetyltransferases (NATs) and sulfotransferases (SULTs), to form highly reactive *N*-acetoxy or *S*-sulfoxy esters, respectively, leading to increased DNA binding (Arlt *et al.*, 2002, 2003c). Although 3-aminobenzanthrone has been found to be the reductive metabolite of 3-nitrobenzanthrone in mammalian cells in culture, including 3-nitrobenzanthrone-treated human lung adenocarcinoma A549 cells (Borlak *et al.*, 2000; Hansen *et al.*, 2007), detailed chemical analyses to identify 3-nitrobenzanthrone and its metabolites *in vivo* in experimental animals have been scarce.

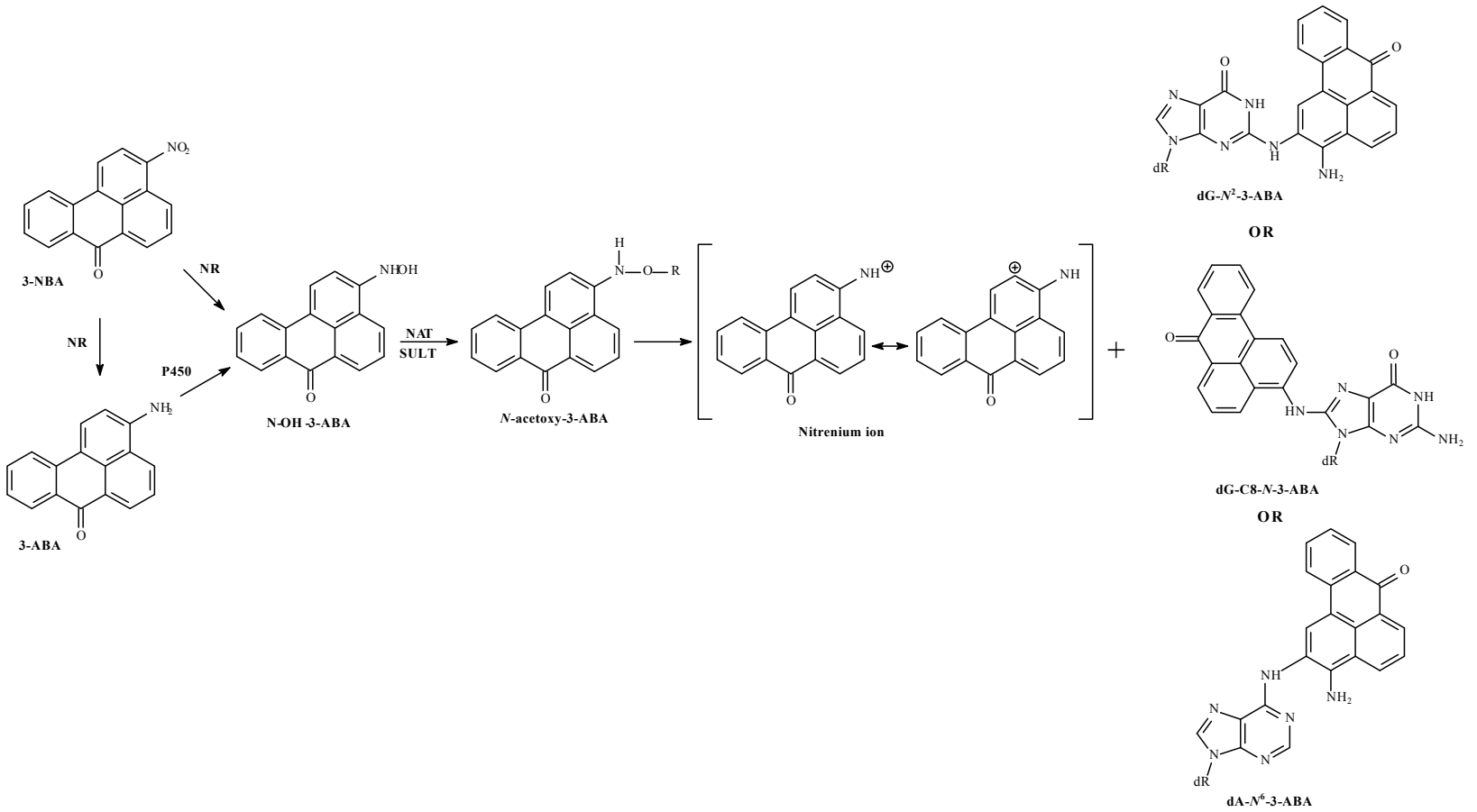
Recently, a mercapturic acid metabolite of 3-aminobenzanthrone, *N*-acetyl-*S*-(3-aminobenzanthrone-2-yl)cysteine, as well as 3-aminobenzanthrone and 3-acetylaminobenzanthrone, have been identified in the urine of rats administered 3-nitrobenzanthrone (2 mg/kg bw) by gavage, and it has been suggested that this metabolite could be used as a biomarker in exposed humans (Linhart *et al.*, 2012). The same authors also showed that 2-nitrobenzanthrone, which is reported to be less active in forming DNA adducts (Arlt *et al.*, 2007; Stiborová *et al.*, 2010), does not produce a mercapturic acid metabolite, suggesting that reactive metabolites of 3-, but not those of 2-nitrobenzanthrone, can be trapped by glutathione *S*-transferase.

4.1.3 Role of xenobiotic-metabolizing enzymes in the metabolic activation of 3-nitrobenzanthrone

Several *in vitro* and *in vivo* studies have indicated that the metabolic conversion of 3-nitrobenzanthrone is catalysed by xenobiotic-metabolizing enzymes to form DNA-binding metabolites in humans and experimental animals (Fig. 4.1) (reviewed by Arlt, 2005). 3-Nitrobenzanthrone is first bio-activated in humans and experimental animals to *N*-hydroxy-3-aminobenzanthrone by various nitroreductases, such as NQO1, xanthine/xanthine oxidase and POR (Arlt *et al.*, 2003b, 2004c, 2005; Bieler *et al.*, 2003). Cytosolic NQO1 is the major enzyme involved in the bio-activation of 3-nitrobenzanthrone, based on experiments with an NQO1 inhibitor (dicoumarol) and human recombinant NQO1 (Arlt *et al.*, 2005; Stiborová *et al.*, 2006, 2010). The role of POR in the nitroreduction of 3-nitrobenzanthrone *in vivo* may be minor, because no difference in the levels of DNA adducts in the liver was observed between 3-nitrobenzanthrone-treated wild-type C57BL/6 and hepatic POR-null mice lacking hepatic CYP enzyme activity (Arlt *et al.*, 2005; Stiborová *et al.*, 2006, 2008). In contrast, the DNA binding induced by 3-aminobenzanthrone in the liver was reduced in POR-null compared with wild-type mice, indicating that the bio-activation of 3-aminobenzanthrone depends on hepatic CYP enzymes (Stiborová *et al.*, 2006).

3-Aminobenzanthrone, the reductive metabolite of 3-nitrobenzanthrone (Borlak *et al.*, 2000; Arlt, 2005; Hansen *et al.*, 2007), can be oxidized by rat CYP enzymes (mainly CYP1A) to *N*-hydroxy-3-aminobenzanthrone, as detected by HPLC analysis (Mizerovská *et al.*, 2008). Human CYP1A1 and -1A2 (Arlt *et al.*, 2004c, 2006b; Stiborová *et al.*, 2008) and other human CYPs, including CYP2A6, -3A4 and -2B6 (Arlt *et al.*, 2003c; Bieler *et al.*, 2003), have also been shown to be involved in this reaction step. Several

Fig. 4.1 Metabolic activation of 3-nitrobenzanthrone and DNA-adduct formation



3-ABA, 3-aminobenzanthrone; 3-NBA, 3-nitrobenzanthrone; dG-N^2-3-ABA, 2-(2′-deoxyguanosin-N^2-yl)-3-aminobenzanthrone; dG-C8-*N*-3-ABA, *N*-(2′-deoxyguanosin-8-yl)-3-aminobenzanthrone; dA-N^6-3-ABA, 2-(2′-deoxyadenosin-N^6-yl)-3-aminobenzanthrone; dR, 2′-deoxyribose; NAT, *N*-acetyltransferase; NR, nitroreductase; P450, cytochrome P450; R, $-COCH_3$ or SO_3H; SULT, sulfotransferase

peroxidases, such as lactoperoxidase, myeloperoxidase and prostaglandin H synthase, were also able to catalyse the conversion of 3-aminobenzanthrone to *N*-hydroxy-3-aminobenzanthrone (Stiborová *et al.*, 2005; Arlt *et al.*, 2006b). The reactivity of *N*-hydroxy-3-aminobenzanthrone in biological systems has been reported in human cell lines, *in vivo* in rats after intraperitoneal injection (Arlt *et al.*, 2007) and in cultured embryonic fibroblasts from human *TP53* knock-in mice (vom Brocke *et al.*, 2009). Arlt *et al.* (2003b) found that purified POR isolated from rabbit liver microsomes catalysed the activation of 3-nitrobenzanthrone to DNA-binding products in a reconstituted system, indicating that *N*-hydroxy-3-aminobenzanthrone is the DNA-reactive intermediate.

N-Hydroxy-3-aminobenzanthrone has been shown to be further activated by NATs (NAT1 and NAT2) or SULTs (SULT1A1 and SULT1A2) (Arlt *et al.*, 2002, 2003a, c, 2004b, 2005; Stiborová *et al.*, 2006) (Fig. 4.1). Reactive *N*-acetoxy or *N*-sulfoxy esters can bind covalently to DNA (Snyderwine *et al.*, 1988; Arlt, 2005), and evidence has been presented that an aryl nitrenium ion, which is formed through heterolytic cleavage of these ester metabolites, is the ultimate DNA-binding species of 3-nitrobenzanthrone (Arlt, 2005). NATs (i.e. NAT2) may be more important than SULTs in the bio-activation of 3-nitrobenzanthrone (Arlt *et al.*, 2002, 2003c, 2005). By incorporating human SULT1A1, -1A2 and -1A3 into a *Salmonella typhimurium* strain deficient in *O*-acetyltransferase activity, Oda *et al.* (2012) found that the strain that expressed human SULT1A1 was more effective in activating 3-nitrobenzanthrone to genotoxic metabolites than those that harboured SULT1A2 or -1A3, as determined in the *umu* gene expression assay.

NATs have also been reported to be involved in the acetylation of *N*-hydroxy-3-aminobenzanthrone to form *N*-acetyl-*N*-hydroxy-3-aminobenzanthrone, and the resultant metabolite is also activated by NATs to form highly reactive *N*-acetyl-*N*-acetoxy-3-aminobenzanthrone (Enya *et al.*, 1997; Kawanishi *et al.*, 1998, 2000; Arlt *et al.*, 2002, 2003a). However, this activation step has no relevance to the metabolic activation of 3-nitrobenzanthrone in experimental animals, because all of its major DNA adducts identified *in vivo* lack an *N*-acetyl group in the adduct molecules (see Section 4.2.1) (Arlt *et al.*, 2002, 2003a; Kanno *et al.*, 2007; Takamura-Enya *et al.*, 2007); moreover, *N*-acetyl-*N*-hydroxy-3-aminobenzanthrone was weakly mutagenic in the *Salmonella* strain DJ450, which expresses human recombinant NAT2 (Arlt *et al.*, 2002). It has also been reported that *N*-acetyl-*N*-hydroxy-3-aminobenzanthrone is readily deacetylated by microsomal enzymes (Arlt *et al.*, 2002, 2003b, c).

4.1.4 *Induction of xenobiotic-metabolizing enzymes by 3-nitrobenzanthrone and 3-aminobenzanthrone*

3-Nitrobenzanthrone and 3-aminobenzanthrone induce several forms of xenobiotic-metabolizing enzyme in experimental animals *in vivo* (Stiborová *et al.*, 2006, 2009; Mizerovská *et al.*, 2011). Intraperitoneal administration of 3-nitrobenzanthrone or 3-aminobenzanthrone to rats (at a single dose of 0.4, 4 or 40 mg/kg bw) caused increases in cytosolic menadione reduction and 7-ethoxyresorufin *O*-deethylation activities with increased levels of DNA adducts in the liver, indicating that these compounds induced NQO1 and CYP1A; the induction of NQO1, and CYP1A1 and -1A2 protein was confirmed by western blot analysis (Stiborová *et al.*, 2006). Intraperitoneal administration of 3-nitrobenzanthrone and 3-aminobenzanthrone to rats also induced CYP1A1 and NQO1 in the lung and kidney (Stiborová *et al.*, 2008, 2009). Because these enzymes play important roles in both the activation and detoxification of PAHs and nitro-PAHs, it is possible that 3-nitrobenzanthrone and 3-aminobenzanthrone not only

alter their own toxic responses by inducing xenobiotic-metabolizing enzymes but also modulate the metabolism of related PAHs and nitro-PAHs found in diesel-engine exhaust.

4.2 Genetic and related effects

4.2.1 Humans

No data were available to the Working Group.

4.2.2 Experimental systems

(a) DNA adduct formation

In vivo results in experimental animals show that the levels of 3-nitrobenzanthrone–DNA adducts in different tissues are largely dependent on the route of administration (Arlt *et al.*, 2001, 2003a, 2004a, 2006a, 2007; Bieler *et al.*, 2005, 2007; Nagy *et al.*, 2005a, 2006, 2007; Schmeiser *et al.*, 2009) (Table 4.1). DNA-adduct formation by 3-nitrobenzanthrone has been mainly investigated by ^{32}P-postlabelling using thin-layer chromatography (TLC) or HPLC. Oral administration of 3-nitrobenzanthrone to rats induced DNA adducts in the small intestine, stomach, liver, kidney and urinary bladder, but relatively fewer in the lung (Arlt *et al.*, 2001; Nagy *et al.*, 2006). A single intraperitoneal dose of 3-nitrobenzanthrone resulted in the distribution of DNA adducts in several organs, such as the pancreas, kidney, liver, lung, urinary bladder, heart and colon (Arlt *et al.*, 2003a, 2005, 2006a, 2007; Bieler *et al.*, 2005, 2007; Nagy *et al.*, 2005a). Intratracheal instillation of 3-nitrobenzanthrone to rats, which induced squamous cell carcinomas in the lung of Fischer 344 rats (Nagy *et al.*, 2005a), resulted in DNA-adduct formation in the lung and other tissues, such as the kidney, pancreas, urinary bladder, heart, small intestine and liver (Bieler *et al.*, 2005, 2007; Nagy *et al.*, 2006). The highest levels of DNA adducts in female Fischer 344 rats that received intratracheal instillations of 3-nitrobenzanthrone (10 mg/kg bw) were observed in the lungs (~250 adducts/10^8 nucleotides); adduct levels were also high in the kidney (~200 adducts/108 nucleotides), but low in the liver (~30 adducts/10^8 nucleotides). Although the kidney showed high levels of 3-nitrobenzanthrone–DNA adducts, tumour formation was not reported in this organ (Nagy *et al.*, 2005a). Topical application of 3-nitrobenzanthrone and *N*-hydroxy-3-nitrobenzanthrone to the skin of NMRI mice was found to produce DNA adducts in the epidermis, but adducts in other organs were only found after treatment with 3-nitrobenzanthrone (Schmeiser *et al.*, 2009). These results suggest that 3-nitrobenzanthrone can largely be metabolized by xenobiotic-metabolizing enzymes near the sites of application to produce DNA adducts, and that 3-nitrobenzanthrone and its metabolites may be distributed via the blood to other organs in which further metabolism may occur (i.e. activation or detoxification).

TLC- and HPLC-^{32}P-postlabelling analyses have been used to characterize 3-nitrobenzanthrone-derived DNA adducts *in vitro* and *in vivo* (Kawanishi *et al.*, 1998; Arlt *et al.*, 2001; Bieler *et al.*, 2003; Nagy *et al.*, 2005a; Osborne *et al.*, 2005) (Table 4.1). In TLC, the pattern of DNA adducts induced by 3-nitrobenzanthrone consisted of a characteristic cluster of four major DNA adducts (spots 1, 2, 3 and 4); initial studies showed that all four adducts formed were derived from reductive metabolites bound either to deoxyadenosine (spots 1 and 2) or deoxyguanosine (spots 3 and 4) (Arlt *et al.*, 2001, 2003a, c). Among a series of deoxyguanosine and deoxyadenosine adducts characterized to date, the following have been identified as the major lesions *in vivo*: 2-(2′-deoxyguanosin-*N2*-yl)-3-aminobenzanthrone (spot 3), *N*-(deoxyguanosin-8-yl)-3-aminobenzanthrone (spot 4) and 2-(2'-deoxyadenosin-N^6-yl)-3-aminobenzanthrone (spot 1) (Nagy *et al.*, 2005a, 2006; Osborne *et al.*, 2005; Arlt *et al.*, 2006a, 2008, 2011; Kanno *et al.*, 2007; Takamura-Enya *et al.*, 2007;) (Fig. 4.1). The formation of 2-(2′-deoxyguanosin-*N2*-yl)-3-aminobenzanthrone and

Table 4.1 Detection of DNA adducts in tissues of experimental animals treated with 3-nitrobenzanthrone and *N*-hydroxy-3-aminobenzanthrone by different routes of administration *in vivo*

Species, strain (sex) Reference	*In vivo* conditions for experiments; time of sacrifice of animals for analysis	DNA adducts in various organs (relative adduct levels/10^8 nucleotides)
Rat, SD (F) Arlt *et al.* (2001)	Single oral dose (2 mg/kg bw) of 3-NBA in tricaprylin (1 mg/mL); 4 h after the dose	Small intestine (38), forestomach (33), kidney (13), liver (10), lung (9.5)
Rat, F344 (F) Nagy *et al.* (2006)	Single oral dose (9 mg/kg bw) of 3-NBA in trioctanoin (1 mg/mL); 48 h after the dose	Fore stomach (50), glandular stomach (47), kidney (45), caecum (43), liver (37), small intestine (33), colon (31), lung (30)
Mouse, C57BL/6 (M) Arlt *et al.* (2005)	Single ip dose (2 mg/kg bw) of 3-NBA in tricaprylin (0.5 mg/mL); 24 h after the dose	Liver (200), urinary bladder (50), kidney (27), colon (24), lung (4)
Rat, Wistar (F) Arlt *et al.* (2003a)	Single ip dose (2 mg/kg bw) of 3-NBA in tricaprylin (0.5 mg/mL); 24 h after the dose	Pancreas (300), colon (84), lung (52), kidney (47), heart (45), liver (13)
Rat, Wistar (F) Arlt *et al.* (2007)	Single ip dose (2 mg/kg bw) of 3-NBA in tricaprylin (0.5 mg/mL); 24 h after the dose	Kidney(134), lung (89), colon (87), pancreas (84), liver (28)
	Single ip dose (10 mg/kg bw) of N-OH-3-ABA in DMSO; 24 h after the dose	Kidney (262), lung (312), colon (191), pancreas (430), liver (55)
Rat, SD (F) Bieler *et al.* (2005)	Single it dose (0.2 mg/kg bw) of 3-NBA in tricaprylin (0.4 mg/mL); 48 h after the dose	Pancreas (55). lung (39), kidney (33), urinary bladder (28), heart (26), small intestine (19), liver (12), blood (10)
	Single it dose (2 mg/kg bw) of 3-NBA in tricaprylin (0.4 mg/mL); 48 h after the dose	Pancreas (620), lung (350), kidney (330), heart (220), urinary bladder (215), small intestine (98), liver (59), blood (41)
Rat, SD (F) Bieler *et al.* (2007)	Single it dose (0.2 mg/kg bw) of 3-NBA in tricaprylin (0.4 mg/mL); 2 d after the dose	Pancreas (55), lung (39), kidney (33), urinary bladder (28), heart (26), small intestine (19), liver (12)
	Single it dose (0.2 mg/kg bw) of 3-NBA in tricaprylin (0.4 mg/mL); 36 wks after the dose	Pancreas (11), lung (11), kidney (12), urinary bladder (5), heart (not detected), small intestine (5), liver (4)
Rat, F344 (F) Nagy *et al.* (2005a)	Single it dose (10 mg/kg bw) of 3-NBAin propylene glycol:saline (9:1; 0.1 mL); 24 h after the dose	Lung (250), kidney (170), liver (15)
Rat, F344 (F) Nagy *et al.* (2007)	Single it dose (5 mg/kg bw) of 3-NBA in trioctanoin (5 mg/mL); 48 h after the dose	Lung (55), kidney (45), liver (31), glandular stomach (27), forestomach (24), spleen (20), caecum (17), colon (16), small intestine (15)
Mouse, NMRI (F) Schmeiser *et al.* (2009)	Single or repeated (once daily for 4 d) topical dose (100 nmol) of 3-NBA in acetone; 24 h after the dose	Skin (17 for single dose; 40 for four daily doses)
	Single or repeated (once daily for 4 d) topical dose (100 nmol) of N-OH-3-ABA in acetone; 24 h after the dose	Skin (140 for single dose) or skin (170 for 4 daily doses)

Some of the values in this table were taken from figures in the references.
bw, body weight; d, day; DMSO, dimethyl sulfoxide; F, female; h, hour; ip, intraperitoneal; it, intratracheal; M, male; 3-NBA, 3-nitrobenzanthrone; *N*-OH-3-ABA, *N*-hydroxy-3-aminobenzanthrone; wk, week

N-(deoxyguanosin-8-yl)-3-aminobenzanthrone in 3-nitrobenzanthrone-treated rodents has been confirmed by mass spectrometry (Gamboa da Costa *et al.*, 2009). None of the DNA adducts detected *in vivo* contained an *N*-acetyl group, suggesting that there is no important role of *N*-acetyl-*N*-acetoxy-3-aminobenzanthrone in the reaction with DNA (Arlt, 2005; Kanno *et al.*, 2007). Overall, the pattern of 3-nitrobenzanthrone-derived DNA adducts in experimental animals is the same *in vivo* and *in vitro* (Arlt, 2005; Stiborová *et al.*, 2010).

3-Nitrobenzanthrone–DNA adducts have been detected in various organs of rats within 36 weeks after intratracheal instillation of a single dose (0.2 mg/kg bw), including in the target organ, the lung; the most abundant and persistent DNA adduct was 2-(2′-deoxyguanosin-*N2*-yl)-3-aminobenzanthrone (Bieler *et al.*, 2007). Lukin *et al.* (2011) showed that this lesion increased the stability of the damaged DNA duplex, which may provide an explanation for its long persistence *in vivo*.

TP53-dependent adduct formation by 3-nitrobenzanthrone was investigated in two isogenic human colorectal HCT116 cell lines, one that expressed *TP53* (p53-wild-type) and another that had this gene knocked out (p53-null) (Hockley *et al.*, 2008; Simoes *et al.*, 2008). The authors showed that the levels of DNA adducts after treatment with benzo[*a*]pyrene and nitroarene aristolochic acid I was lower in p53-null cells compared with p53-wild-type cells, whereas DNA binding by 3-nitrobenzanthrone was *TP53*-independent. These results suggest that the cellular *TP53* status is linked to the CYP-mediated metabolism of benzo[*a*]pyrene and nitroarene aristolochic acid I, whereas the nitroreduction of 3-nitrobenzanthrone is not *TP53*-dependent. The possible lack of protective functions mediated by p53 after the treatment of p53-null cells with 3-nitrobenzanthrone needs to be explored further.

(b) Mutagenicity of 3-nitrobenzanthrone and its metabolites

(i) In-vivo studies

Arlt *et al.* (2004a) reported that intraperitoneal administration of 3-nitrobenzanthrone to the MutaMouse increased the mutant frequency in the *cII* gene in the colon, liver and urinary bladder, but not in the lung, kidney, spleen or testis. In addition, the percentage of G:C to T:A transversions in the liver was found to be higher in 3-nitrobenzanthrone-treated mice than in untreated mice (49% versus 6%). The authors also showed preferential DNA binding at deoxyguanosine (70–80%), which correlated with the preferential occurrence of G:C to T:A transversions; 2-(2′-deoxyguanosin-*N2*-yl)-3-aminobenzanthrone and *N*-(deoxyguanosin-8-yl)-3-aminobenzanthrone appeared to be the pre-mutagenic lesions. *LacZ* mutant frequencies were also investigated in the MutaMouse strain treated with 3-nitrobenzanthrone and 3-aminobenzanthrone (0, 2 and 5 mg/kg bw per day for 28 days) (Arlt *et al.*, 2008). Dose-related increases in mutant frequency were seen in the liver and bone marrow, but not in the lung; the mutagenic activity of 3-aminobenzanthrone was approximately twofold lower than that of 3-nitrobenzanthrone. Chen *et al.* (2008) showed that 3-nitrobenzanthrone induced *lacZ* mutations in the bone marrow and liver, but not in the lung or intestinal epithelium, in the MutaMouse after single and repeated oral administration. High 3-nitrobenzanthrone nitroreductase activity was found in all tissues examined, whereas no NAT activity was observed in bone marrow.

(ii) In-vitro studies

3-Nitrobenzanthrone was highly mutagenic in *S. typhimurium* strain TA98, only in the absence of metabolic activation. It was less mutagenic in *S. typhimurium* TA100 than in TA98, suggesting that it causes frameshift-type mutations in the bacteria. In strain TA98, the mutagenic potency of

3-nitrosobenzanthrone was comparable with that of 1,8-dinitropyrene, and both compounds were found to be highly mutagenic in *S. typhimurium* YG1024, an *O*-acetyltransferase-overexpressing strain (Enya *et al.*, 1997; Watanabe *et al.*, 2005). It should be noted that 3-nitrosobenzanthrone induced 6.3 million revertants/nmol in strain YG1024, and is thus one of the most potent bacterial mutagens known to date. Takamura-Enya *et al.* (2006) compared the mutagenic activities of several derivatives of mono-, di- and trinitrobenzanthrone in *S. typhimurium* TA98, and found that 3-nitrobenzanthrone was more mutagenic than 1-, 2-, 9- and 11-nitrobenzanthrone, 1,9-, 3,9- and 3,11-dinitrobenzanthrone, and 3,9,11-trinitrobenzanthrone. In comparison, 3-nitrobenzanthrone showed high mutagenic potency, similar to that of 1,3-, 1,6- and 1,8-dinitropyrene, and 3,7- and 3,9-dinitrofluoranthene, in *S. typhimurium* TA98 in the absence of metabolic activation. In general, these nitroarenes have been less active in inducing reverse mutations in *S. typhimurium* TA100. It should be mentioned that the nitrenium ion of 3-nitrobenzanthrone is more stable than comparable ions derived from other nitrated benzanthrone derivatives, which could provide a possible explanation for its potent mutagenic and DNA adduct-forming activities (Arlt *et al.*, 2007, 2011; Reynisson *et al.*, 2008).

Watanabe *et al.* (2005) found that 3-aminobenzanthrone and 3-acetyl-3-aminobenzanthrone, two metabolites of 3-nitrobenzanthrone, were activated by a rat-liver metabolic activation system to reactive metabolites that caused the induction of reverse mutations in *S. typhimurium* TA98, TA100, YG1024 and YG1029; the highest activity was seen in the YG1024 strain, which overexpresses *O*-acetyltransferase. They also showed that 3-nitrobenzanthrone itself was deactivated by the metabolic activation system in this assay, suggesting that enzymes present in this fraction may detoxify 3-nitrobenzanthrone or its metabolites.

Oda *et al.* (2007) reported that a very low concentration (~2 nM) of 3-nitrobenzanthrone induced *umu* gene expression in *S. typhimurium* NM2009 and NM3009, which express *O*-acetyltransferase and exhibit both nitroreductase and *O*-acetyltransferase activities, respectively. They also showed that 3-nitrobenzanthrone was cytotoxic in these tester strains, suggesting the formation of active metabolites. More recently, Oda *et al.* (2012) reported that 3-nitrobenzanthrone induced *umu* gene expression more actively in *S. typhimurium* NM7001, which expresses SULT1A1, than in strains NM7002 and 7003, which express human SULT1A2 and 1A3, respectively.

3-Nitrobenzanthrone induced base-substitution mutations in mammalian cell systems (Phousongphouang *et al.*, 2000; Arlt *et al.*, 2008; Nishida *et al.*, 2008). The induction of base-substitution mutations in mammalian cells compared with frameshift mutations in bacteria is probably due to differences in the target genes or in the mechanisms of mutagenesis between bacterial and mammalian systems. In human B-lymphoblastoid cell lines, 3-nitrobenzanthrone induced mutations at the *thymidine kinase+/–* and *hypoxanthine phosphoribosyltransferase* loci (Phousongphouang *et al.*, 2000). 3-Nitro- and 3-aminobenzanthrone produced a dose-dependent increase in mutant frequency in lung epithelial FE1 cells derived from the MutaMouse (in the presence and absence of metabolic activation), which correlated with an increase in DNA-adduct formation (Arlt *et al.*, 2008).

Nishida *et al.* (2008) determined the mutagenic specificity of *N*-acetoxy-3-aminobenzanthrone, probably the most active metabolite of 3-nitrobenzanthrone, in the *supF* system using human fibroblast cell lines. They found that *N*-acetoxy-3-aminobenzanthrone bound to guanine rather than adenine and preferentially induced G:C to T:A transversions; deoxyadenosine adducts may be repaired more efficiently by

nucleotide excision repair than deoxyguanosine adducts.

3-Nitrobenzanthrone also induced G:C to T:A transversions in the *TP53* gene in immortalized human *TP53* knock-in murine embryonic fibroblasts (vom Brocke *et al.*, 2009; Kucab *et al.*, 2012), suggesting that G:C to T:A transversions in *TP53* could be used as a signature mutation for exposure to 3-nitrobenzanthrone in human lung tumours, when exposure to this carcinogen has been documented (Kucab *et al.*, 2010).

(c) *Other genetic effects of 3-nitrobenzanthrone* in vivo *and* in vitro

3-Nitrobenzanthrone induced micronuclei in mouse peripheral blood reticulocytes after intraperitoneal injection (25 or 50 mg/kg bw) into male ICR mice (Enya *et al.*, 1997). Similar results have been reported by Arlt *et al.* (2004a). Intraperitoneal administration of 3-nitrobenzanthrone (25 mg/kg bw once a week for 4 weeks) increased the frequency of micronuclei in the peripheral reticulocytes of the male MutaMouse.

In vitro induction of micronucleus formation and DNA damage following treatment with 3-nitrobenzanthrone has also been reported in human hepatoma HepG2 cells (Lamy *et al.*, 2004), human B-lymphoblastoid MCL-5 cells (Arlt *et al.*, 2004b) and human A549 lung cells (Nagy *et al.*, 2005b). Both 3-nitrobenzanthrone and 3-aminobenzanthrone increased the tail moment in the alkaline comet assay in human lung epithelial A549 cells and in the liver, kidney, spleen, lung and bone marrow of male ICR mice (Watanabe *et al.*, 2005; Hansen *et al.*, 2007).

N-Hydroxy-3-aminobenzanthrone induced oxidative DNA damage through the formation of 8-hydroxydeoxyguanosine in the presence of divalent copper and nicotinamide adenine dinucleotide *in vitro* (Murata *et al.* 2006; Hansen *et al.*, 2007). Using ^{32}P-labelled DNA fragments from human *TP53*, Murata *et al.* (2006) showed that the DNA damage caused by *N*-hydroxy-3-aminobenzanthrone could be inhibited by catalase and bathocuproline, suggesting the involvement of hydrogen peroxide and monovalent copper in the induction of DNA damage; DNA damage occurred at the cytosine and guanine residues of an ACG sequence complementary to codon 273, which is a well known hotspot for *TP53* mutations (Murata *et al.*, 2006). Shimohara *et al.* (2008) reported that 3-nitrobenzanthrone caused double-strand DNA breaks by analysing the phosphorylation of histone H2AX in human Hela cells.

3-Nitrobenzanthrone induced apoptosis, DNA-adduct formation and DNA damage in human bronchial epithelial BEAS-2B cells (Ovrevik *et al.*, 2010; Oya *et al.*, 2011). In mouse hepatoma Hepa1c1c7 cells, Landvik *et al.* (2010) reported that 3-nitrobenzanthrone caused cell death with increasing levels of DNA adducts, single-strand DNA breaks and oxidative DNA damage (measured in the comet assay).

4.3 Mechanistic considerations

The main metabolite of 3-nitrobenzanthrone, the reductive amino metabolite 3-aminobenzanthrone (Borlak *et al.*, 2000; Hansen *et al.*, 2007; Linhart *et al.*, 2012), has been found in individuals occupationally exposed to diesel engine exhaust, clearly indicating that human exposure to 3-nitrobenzanthrone can be significant and is detectable (Seidel *et al.*, 2002). 3-Nitrobenzanthrone is bio-activated to DNA-binding products by various xenobiotic-metabolizing enzymes *in vivo* in humans and experimental animals as well as *in vitro* (Arlt, 2005). It is first activated by nitroreduction, which is catalysed most efficiently by NQO1, to *N*-hydroxy-3-aminobenzanthrone (Arlt, 2005; Arlt *et al.*, 2005; Stiborová *et al.*, 2010). 3-Aminobenzanthrone is oxidized by CYPs (mainly CYP1A1 and CYP1A2) or by several peroxidases to the same reactive intermediate, *N*-hydroxy-3-aminobenzanthrone (Arlt *et al.*, 2004c, 2006b; Stiborová *et al.*, 2005), which can

be further activated by NATs (NAT1 and NAT2) or SULTS (SULT1A1 and SULT1A2) to form highly reactive *N*-acetoxy or sulfoxy esters (Arlt *et al.*, 2002, 2003a, c, 2004b, 2005; Stiborová *et al.*, 2006). The aryl nitrenium ions formed through the heterolytic cleavage of these ester metabolites are the most reactive with DNA, and lead to the formation of covalent DNA adducts (Arlt, 2005). NATs may be more important in the bio-activation of 3-nitrobenzanthrone than SULTs (Arlt *et al.*, 2002, 2003c, 2005). Collectively, comparable metabolic activation pathways have been found in rodents and humans.

^{32}P-Postlabelling analyses have detected multiple DNA adducts bound to activated 3-nitrobenzanthrone metabolites *in vivo* and *in vitro* (Arlt, 2005). The major purine-based DNA adducts have been structurally identified *in vivo* as 2-(2′-deoxyguanosin-*N2*-yl)-3-aminobenzanthrone, *N*-(deoxyguanosin-8-yl)-3-aminobenzanthrone and 2-(2′-deoxyadenosin-N^6-yl)-3-aminobenzanthrone (Arlt, 2005; Osborne *et al.*, 2005; Arlt *et al.*, 2006a; Nagy *et al.*, 2006; Kanno *et al.*, 2007; Takamura-Enya *et al.*, 2007; Gamboa da Costa *et al.*, 2009). The deoxyguanosine adducts are found most persistently in the DNA of the target tissue – the lung (Bieler at al., 2007) – and induce G:C to T:A transversions in mammalian cells and in transgenic mouse mutation assays (Arlt *et al.*, 2004b, 2008; Nishida *et al.*, 2008). Such increases in G:C to T:A transversions have also been found in the DNA-binding domain sequence of *TP53* in human *TP53* knock-in murine embryonic fibroblasts exposed to 3-nitrobenzanthrone (vom Brocke *et al.*, 2009). 3-Nitrobenzanthrone was a strong direct-acting mutagen in *S. typhimurium* strain TA98, in which its high mutagenic potency is comparable with that of 1,3-, 1,6- and 1,8-dinitropyrene. It was also a powerful mutagen in *S. typhimurium* YG1024, an *O*-acetyltransferase-overexpressing strain, and induced more than 6 million revertants/nmol (Enya *et al.*, 1997). 3-Nitrobenzanthrone induced *umu* gene expression in *S. typhimurium* tester strains that overexpress nitroreductase and *O*-acetyltransferase activties (Oda *et al.*, 2007), the development of micronuclei in mouse peripheral blood reticulocytes *in vivo* (Enya *et al.*, 1997) and increases in micronuclei in male MutaMouse peripheral reticulocytes, as well as increased mutant frequencies in the *cII* gene in the colon, liver and urinary bladder or in *lacZ* in the liver and bone marrow (Arlt *et al.*, 2004a; 2008; Chen *et al.*, 2008). Many *in vitro* bioassays in human cells showed that 3-nitrobenzanthrone can induce micronuclei (Arlt *et al.*, 2004c; Lamy *et al.*, 2004), DNA strand breaks (Nagy *et al.*, 2005b) and gene mutations (Arlt *et al.*2008; Nishida *et al.*, 2008, Phousongphouang *et al.*, 2000. It induced apoptosis with increasing levels of DNA adducts in human bronchial epithelial BEAS-2B cells and the expression of cytokine/chemokine (Landvik *et al.*, 2010; Ovrevik *et al.*, 2010).

Nagy *et al.* (2005a) showed that intratracheal instillation of 3-nitrobenzanthrone induced lung squamous cell carcinomas in rats after 18 months. Tumour formation was also associated with a high level of DNA-adduct formation in the lungs Nagy *et al.* (2005a); 2-(2′-deoxyguanosin-*N2*-yl)-3-aminobenzanthrone was the most abundant and persistent DNA adduct in the lung (Bieler *et al.*, 2007). No skin tumours were found after topical administration of 3-nitrobenzanthrone or *N*-hydroxy-3-aminobenzanthrone to NMRI mice (Schmeiser *et al.*, 2009). In addition to differences in the various routes of administration, the authors concluded that the bioactivation of 3-nitrobenzanthrone in mouse skin, which has also been shown for some other nitro-PAHs (Nesnow *et al.*, 1984; Möller *et al.*, 1993), may not be sufficient to initiate tumour formation in this model.

Collectively, these data indicate that 3-nitrobenzanthrone, which has been identified in diesel engine exhaust, is a powerful mutagen and rodent carcinogen.

5. Summary of Data Reported

5.1 Exposure data

3-Nitrobenzanthrone is primarily formed as a product of the combustion of fossil fuels. It is a constituent of diesel exhaust emissions and was also detected in particulate matter collected from the chimney of a domestic coal-burning stove. No evidence was found that it has been produced in commercial quantities or used for purposes other than laboratory applications. 3-Nitrobenzanthrone was detected in the air, soil and water. Concentrations of 3-nitrobenzanthrone in airborne particulate matter collected at urban locations were reported to be in the subpicogram per cubic metre range (up to 70 pg/m^3), and similar values were reported in confined workplaces. Occupational exposure to this substance was reported after urine analysis.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

3-Nitrobenzanthrone was evaluated in one initiation–promotion study in mice and in one study in rats by intratracheal instillation. Intratracheal instillation resulted in an increase in the incidence of squamous cell carcinoma of the lung in rats. The initiation–promotion study in mice was negative.

5.4 Mechanistic and other relevant data

3-Nitrobenzanthrone is a powerful mutagen in bacterial and mammalian assays, and produces lung tumours in rats after intratracheal administration. It induced 6.3 million revertants/nmol in a bacterial strain that overexpresses *O*-acetyltransferase, and is therefore one of the most potent bacterial mutagens known to date. The main reductive metabolite of 3-nitrobenzanthrone, 3-aminobenzanthrone, was found in the urine of salt-mine workers exposed to diesel engine exhaust, indicating that human exposure to 3-nitrobenzanthrone from diesel emissions can be significant and is detectable. 3-Nitrobenzanthrone is first metabolically activated by nitroreduction, leading to the formation of *N*-hydroxy-3-aminobenzanthrone, which can also be formed by the *N*-oxidation of 3-aminobenzanthrone and can be further activated by acetylation or sulfation to form highly reactive *N*-acetoxy- or *S*-sulfoxy esters that can bind to DNA and form adducts. Comparable metabolic activation pathways have been found in rodents and humans. The major DNA adducts identified *in vivo* are deoxyguanosin-N^2-yl-3-aminobenzanthrone, deoxyguanosin-C8-yl-*N*-3-aminobenzanthrone and deoxyadenosin-N^6-yl-3-aminobenzanthrone; the deoxyguanosin-N^2-yl-3-aminobenzanthrone adduct is the most abundant and persistent DNA lesion in the lung – the target organ for carcinogenesis. 3-Nitrobenzanthrone induces predominantly G:C to T:A transversions in mammalian and transgenic rodent mutation assays; the same mutation pattern has been found in the human DNA-binding domain of *TP53*. Both 3-nitrobenzanthrone and 3-aminobenzanthrone can induce oxidative damage to DNA, and 3-nitrobenzanthrone has also been shown to induce micronuclei and DNA strand breaks.

These data provide *strong mechanistic evidence* to support the carcinogenic properties of 3-nitrobenzanthrone in animals and *moderate mechanistic evidence* to support its carcinogenic properties in humans.

6. Evaluation

6.1 Cancer in humans

No data were available to the Working Group.

6.2 Cancer in experimental animals

There is *limited evidence* in experimental animals for the carcinogenicity of 3-nitrobenzanthrone.

6.3 Overall evaluation

3-Nitrobenzanthrone is *possibly carcinogenic to humans (Group 2B).*

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6-NITROCHRYSENE

6-Nitrochrysene was evaluated by a previous IARC Working Group in 1988 (IARC, 1989). New data have since become available, and these have been taken into consideration in the present evaluation.

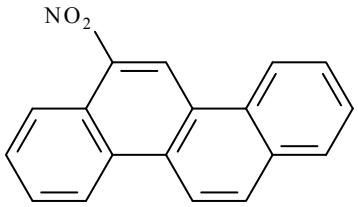
1. Exposure Data

1.1 Chemical and physical data

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 7496-02-8

1.1.2 Structural and molecular formulae, and relative molecular mass



$C_{18}H_{11}NO_2$

Relative molecular mass: 273.3

1.1.3 Chemical and physical properties of the pure substance

Description: Chrome-red, thick prismatic crystals (Prager & Jacobson, 1922); orange-yellow needles (Boit, 1965); light-yellow needles (Chemsyn Science Laboratories, 1988)

Boiling-point: Sublimes without decomposition (Prager & Jacobson, 1922)

Melting-point: 209 °C (Boit, 1965); 211.5–212.5 °C (Chemsyn Science Laboratories, 1988)

Spectroscopy data: Mass spectral data have been reported (Schuetzle & Jensen, 1985).

Solubility: Insoluble in many organic solvents; slightly soluble in cold ethanol, diethyl ether and carbon disulfide; somewhat more soluble in benzene, toluene and acetic acid; soluble in hot nitrobenzene (Prager & Jacobson, 1922; Chemsyn Science Laboratories, 1988).

Reactivity: Forms 6-aminochrysene on heating with tin and concentrated hydrochloric acid in acetic acid at 100 °C; reacts with bromine to form 12-bromo-6-nitrochrysene; reacts with fuming nitric acid to form 6,12-dinitrochrysene (Boit, 1965).

1.1.4 Technical products and impurities

6-Nitrochrysene (99% pure) is sold in small quantities for analytical purposes by one company each in Germany, China and the USA (Chemexper, 2012; Chemical Buyers, 2012). It is also available as a reference material with a certified purity of 98.91% (Belliardo *et al.*, 1988)`

Table 1.1 Levels of 6-nitrochrysene in gasoline and diesel engine emissions

Engine	Year of manufacture	Carburant	T[a] (°C)	No.[b]	Emission (µg/km)[c]
Mazda Millenia, Ford Explorer, Nissan Maxima, GMC 1500 Pickup, Mercury Sable	1982–96	Normal gasoline	22	18	0.11
Ford F-150 pick-up	1976	Gasoline black smoker	22	5	0.60
Mitsubishi Montero	1990	Gasoline white smoker	22	2	0.13
Dodge Ram 2500 Pickup, Mercedes Benz E300, Volkswagen Beetle TDI	1998–2000	Current technology diesel vehicles	22	9	0.24
Dodge Ram 2500 Pickup	1991	Diesel high articulate matter emitter	22	6	1.19
Mazda Millenia, Ford Explorer, Nissan Maxima, GMC 1500 Pickup, Mercury Sable	1982–96	Normal gasoline	–1	12	0.03
Dodge Ram 2500 Pickup, Mercedes Benz E300, Volkswagen Beetle TD	1998–2000	Current technology diesel vehicles	–1	6	2.08

[a] Temperature of the engine when tested, calculated from Fahrenheit by the Working Group
[b] Number of test cycles
[c] Calculated from µg/mile by the Working Group
From Zielinska *et al.* (2004)

1.2 Analysis

For the analytical methods of nitro-polycyclic aromatic hydrocarbons (PAHs) in general, the reader is referred to Section 1.2.2(*a*) of the *Monograph* on Diesel and Gasoline Engine Exhausts in this Volume.

6-Nitrochrysene has been detected in particles that were collected on glass and quartz fibre or Teflon-coated silica fibre membrane filters (Tokiwa *et al.*, 1990; Schauer *et al.*, 2004). It can be extracted from particulate matter using dichloromethane for extraction by sonification (Tokiwa *et al.*, 1990). To analyse 6-nitrochrysene, gas chromatography-negative ion chemical ionization-mass spectrometry was used, following pressurized-fluid extraction (Bamford *et al.*, 2003; Albinet *et al.*, 2006). This substance was also analysed after reduction to 6-aminochrysene with titanium(III) citrate and subsequent detection by fluorescence spectrometry (Zielinska & Samy, 2006), or, alternatively, by chemiluminescence detection to increase the sensitivity for 6-aminochrysene in airborne particulates (Murahashi & Hayakawa, 1997).

1.3 Production and use

1.3.1 Production

6-Nitrochrysene was first synthesized in 1890 by heating chrysene with aqueous nitric acid in acetic acid at 100 °C (Prager & Jacobson, 1922). It can also be synthesized by briefly heating chrysene with nitric acid and concentrated sulfuric acid in acetic acid at 40 °C (Boit, 1965).

1.3.2 Use

No evidence was found that 6-nitrochrysene has been used in commercial applications.

1.4 Occurrence and exposure

1.4.1 Diesel and gasoline exhaust emissions

Different types of in-use passenger cars were tested on a dynamometer using the California Unified Driving Cycle with commercial fuel and used crankcase oil 'as received' (Zielinska *et al.*, 2004). The model year and testing conditions strongly influenced emission levels (Table 1.1). The 6-nitrochrysene emissions were one order of magnitude higher in cold diesel-powered cars

Table 1.2 Mean air concentrations of 6-nitrochrysene associated with particulate matter

Reference	Source	No. of samples	Concentration (pg/m³) [mean ± SD (range)]
Garner *et al.* (1986)	Urban (Bayreuth, Germany)	2	800; 1500[a]
Murahashi & Hayakawa (1997)	Urban (Kanazawa, Japan), October 1994	1	1.1[b]
	Suburban (Kanazawa, Japan), October 1994	1	0.13[b]
Schauer *et al.* (2004)	Urban (Münich, Germany), 2002	10	54 ± 8
	Rural (Münich, Germany)	5	18 ± 13
	Rural (alpine site)	9	2.1 ± 1.7
Albinet *et al.* (2006)	Rural (Maurienne Valley, France), 2002–03	13	0.6 (0.2–2.4)
Albinet *et al.* (2007)	Urban (Marseille, France)	12	33.1 (0.1–147.5)
	Suburban (La Penne sur Huveaune, France)	14	0.7 (ND-4.4)
	Remote (Plan d'Aups, France)	14	0.3 (ND-2.5)
Araki *et al.* (2009)	Urban road side (Kanazawa, Japan), winter 2007	5	13.4 ± 5.2 (8.0–21.8)

[a] Estimated from a graph (no numbers presented)
[b] Calculated from fmol/m³ by the Working Group
ND, not detected; SD, standard deviation

relative to warm engines. Such a change was not observed in the gasoline-powered cars.

In three types of heavy-duty diesel engine, different fuels were tested in the heavy-duty transient Federal Test Procedure: neat biodiesel fuel (B100), a blend of 20% biodiesel with 80% normal diesel fuel by volume (B20) and neat diesel fuel (2D). Following the use of B100, only traces of 6-nitrochrysene were detected in the exhaust. When fuelled with B20 and 2D, the amounts of 6-nitrochrysene detected in the exhaust of two of the three engines (both 1997 models) were in the range of 0.6–0.9 ng/horse power (hp)–h. After the addition of an oxidation catalyst, 6-nitrochrysene was detected at 1.8 ng/hp-h in the exhaust of one of the two engines that used B100 fuel. In the exhaust from the third engine (a 1995 model), levels of 6-nitrochrysene without a catalyst were 1.9 and 11 ng/hp-h for B20 and 2D, respectively. After the addition of an oxidation catalyst, the production of 6-nitrochrysene increased to 58 and 56 ng/hp-h for B20 and 2D, respectively (Sharp *et al.*, 2000).

1.4.2 Ambient air and dust

Garner *et al.* (1986) first identified 6-nitrochrysene in the nanogram per cubic metre range in airborne particulate matter (Table 1.2). More recent reports reported values in the subnanogram per cubic metre range in urban areas, and in the lower and subpicogram per cubic metre range (or lower) at rural or remote locations (Murahashi & Hayakawa, 1997; Schauer *et al.*, 2004; Albinet *et al.*, 2006, 2007).

1.4.3 Biomonitoring of the general population

Blood samples were analysed for the presence of haemoglobin adducts of 6-aminochrysene, a metabolite of 6-nitrochrysene (Zwirner-Baier & Neumann, 1999). The study comprised groups of garage workers, inhabitants of Southampton and inhabitants of small villages in the region (for details, see Scheepers *et al.*, 1999). The proportion of blood samples that contained haemoglobin adducts of 6-aminochrysene was 2/29, 3/20 and 0/20 in the three groups, respectively. Adduct

levels were all below 0.27 pmol/g haemoglobin, with a median of 0.03 pmol/g haemoglobin in all three groups. The method of analysis was based on gas chromatography-negative ion chemical ionization-mass spectrometry (see Section 1.2 of the *Monograph* on Diesel and Gasoline Engine Exhaust in this Volume).

2. Cancer in Humans

No data were available to the Working Group.

3. Cancer in Experimental Animals

No lifetime bioassays on the carcinogenesis of 6-nitrochrysene have been carried out, but a few selected studies have shown tumour formation in rodents.

3.1 Mouse

See Table 3.1

3.1.1 Intraperitoneal administration

Groups of 21–29 male and female newborn Swiss-Webster BLU-Ha mice were administered intraperitoneal injections of 0 (control), 38 or 189 µg/mouse of 6-nitrochrysene in dimethyl sulfoxide (DMSO) on days 1, 8 and 15 after birth. Mice were necropsied at 26 weeks of age and were analysed histologically. A 100% incidence of tumours was observed in the lung for all groups treated with 6-nitrochrysene compared with 3 out of 22 (14%) and 1 out of 15 (7%) male and female controls. The increased number of tumours (compared with controls) was highly significant ($P < 0.001$) (Busby *et al.*, 1985).

Groups of 45 or 33 male and 34 or 40 female newborn CD-1 mice received intraperitoneal injections of 0 (control) or 2800 (total dose) nmol of 6-nitrochrysene in DMSO on days 1, 8 and 15 after birth. A further group of 33 males and 40 females received a single injection of 700 nmol of 6-nitrochrysene 10 weeks after birth. Mice were observed for up to 1 year, and those that were moribund and died in the interim were evaluated histologically. In the single 700 nmol-dose group, 25 out of 33 males (76%; one adenoma, 24 carcinomas; $P < 0.05$) and 9 out of 40 females (23%; five adenomas, four carcinomas; $P < 0.005$) developed liver tumours. The 2800-nmol dose produced liver tumours in 3 out of 9 males (33%; carcinomas; $P < 0.05$) and 3 out of 11 females (27%; two adenomas, one carcinoma; $P < 0.05$); hepatic tumours were observed in 5 out of 45 (11%) male and 0 out of 34 female vehicle controls. In the 700-nmol group, lung tumours were observed in 28 out of 33 males (85%; 11 adenomas, 17 carcinomas; $P < 0.05$) and 36 out of 40 females (90%; 19 adenomas, 17 carcinomas; $P < 0.05$). At 2800 nmol, 7 out 9 (78%) males and 9 out of 11 (82%) females developed lung tumours, while vehicle controls had an incidence of 4 out of 45 males (9%; two adenomas, two carcinomas) and 2 out of 34 females (6%; one adenoma, one carcinoma). The incidence of malignant lymphoma was also increased in treated males (at 700 nmol: 6 out of 33, 18%; at 2800 nmol: 3 out of 9, 33%) and females (at 700 nmol: 9 out of 40, 23%; at 2800 nmol: 4 out of 11, 36%) compared with their respective controls (Wislocki *et al.*, 1986).

Groups of 91 and 26 male and 101 and 22 female newborn Swiss-Webster BLU-Ha mice received intraperitoneal injections of 0 (control) and 7 µg/mouse 6-nitrochrysene, respectively, in DMSO on days 1, 8 and 15 days after birth, and were necropsied at 26 weeks of age, when their lungs were analysed histologically. Male mice had an incidence of 11 out of 26 (42%) lung adenomas and 8 out of 26 (31%) lung adenocarcinomas. The incidence for females was 8 out of 22 (36%) and 5 out of 22 (28%), respectively, and that in the vehicle-treated groups was 12 out of 91 (13%) and 1 out of 91 (1%) for males and 7 out of 101

Table 3.1 Studies of the carcinogenicity of 6-nitrochrysene in mice

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Swiss-Webster BLU:ha (ICR) (M, F) 26 wks Busby *et al.* (1985)	Intraperitoneal injection 0 (control), 38.5 or 189 µg in DMSO 1, 8 and 15 d after birth; animals killed and analysed 23 wks later Newborn; 26 M, 22 F	Lung (all tumours): M–3/22 (14%), 26/26 (100%), 26/26 (100%) F–1/15 (7%), 22/22 (100%), 22/22 (100%)	$P < 0.001$	70% of tumours were adenocarcinomas
CD-1 (M, F) 1 yr Wislocki *et al.* (1986)	Intraperitoneal injection 0 (control) or 2800 nmol (total dose) in DMSO on 1, 8 and 15 d after birth, or 700 nmol (once) at 10 wks Newborn; Vehicle control: 45 M, 34 F; 2800 nmol: 9 M, 11 F 700 nmol: 33 M, 40 F	Liver (adenoma and carcinoma): M–5/45 (11%), 3/9 (33%), 25/33 (76%) F–0/34, 3/11 (27%), 9/40 (23%) Lung (adenomas and carcinomas): M–4/45 (9%), 28/33 (85%), 7/9 (78%) F–2/34 (6%), 9/11 (82%), 36/40 (90%) Malignant lymphoma: M–0/45, 3/9 (33%), 6/33 (18%) F–0/34, 4/11 (36%), 9/40 (23%)	$P < 0.05$ for all treated groups	Significant mortality led study to be stopped at 1 year.
Swiss-Webster BLU:ha (ICR) (M, F) 26 wks Busby *et al.* (1989)	Intraperitoneal injection 0 (control) or 7.7 µg in DMSO 1, 8 and 15 d after birth; animals killed and analysed 23 wks later Newborn; 26 M, 22 F	Lung (adenoma): M–12/91 (13%), 11/26 (42%) F–7/101 (7%), 8/22 (36%) Lung (adenocarcinoma): M–1/91 (1%), 8/26 (31%) F–0/26, 5/22 (23%)	$P < 0.001$ for total lung tumours when genders combined	Compared with other nitroarenes in this study, 6-nitrochrysene was the most potent at producing tumours.
Swiss-Webster BLU:ha (M, F) 30 wks El-Bayoumy *et al.* (1989a)	Intraperitoneal injection 0 (control), 100 or 700 nmol (total doses) in DMSO on 1, 8 and 15 d after birth or 100 nmol (once) on day 1 Newborn; Vehicle control: 38 M, 28 F 100 nmol: 23 M, 24 F 700 nmol: 37 M, 46 F; 100 nmol (one injection): 25 M, 21 F	Lung (all tumours): M–18%, 100%, 100%, 100% F–11%, 100%, 100%, 100% Liver (all tumours): M–0%, 84%*, 84%*, 65%* F–0%, 10%, 3%, 4%	*$P < 0.01$	Metabolites also evaluated, and the putative metabolite 1,2-dihydro-1,2-dihydroxy-6-aminochrysene showed similar or greater tumour production, suggesting 6-nitrochrysene is metabolically activated

Table 3.1 (continued)

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
HSD:ICR (M, F) 30 wks El-Bayoumy *et al.* (1992)	Intraperitoneal injection 0 (control) or 100 nmol (total dose) in DMSO on 1, 8 and 15 d after birth; animals killed and analysed 23 wks later Newborn; 38 M, 24 F	Lung (all tumours): M–12%, 100% F–3%, 96% Liver (all tumours): M–8%, 64% F– 0%, 0%	$P < 0.001$ for lung tumours, and liver tumours in males	Study aimed at comparing isomers; 6-nitrochrysene was the most potent and 1,2- and 3-nitrochrysene did not differ from controls.
Crj:CD-1(ICR) (M, F) 24 wks Imaida *et al.* (1992)	Intraperitoneal injection 0 (control) or 1.4 µmol (total dose) in DMSO on 1, 8 and 15 d after birth; animals killed analysed 24 wks later Newborn; Control: 22 M, 26 F 1.4 µmol: 9 M, 11 F	Lung (adenoma): M–0/22, 9/9 (100%) F–0/26, 11/11 (100%)	$P < 0.001$	Study to determine potential for colon tumours observed in rats; no colon tumours observed in mice
B6C3F1 (M) 1 yr Fu *et al.* (1994)	Intraperitoneal injection 0 or 400 nmol in DMSO on 1, 8 and 15 d after birth (treatment (T) 1: 1/7, 2/7 and 4/7; or treatment (T) 2: 0/7, 3/7 and 4/7 of the dose); fed ad-libitum diet or calorie-restricted diet at 14 wks of age; killed and analysed at 1 yr Newborn: ~20/group	Liver (adenoma): Ad-libitum–2/18 (11%), 19/19 (100%)* T1, 21/21 (100%)* T2 Calorie-restricted–0/21, 4/23 (17%) T1, 0/21 T2 Liver (carcinoma): Ad-libitum–0/18, 14/19 (74%)* T1, 21/21 (100%)* T2 Calorie-restricted: 0/21, 1/23 (4%) T1, 0/21 T2 Ad libitum Lung (adenoma): Ad-libitum–1/18 (6%), 11/19 (58%)* T1, 3/21 (14%) T2 Calorie-restricted– 1/19 (5%), 0/23 T1, 0/21 T2	*$P < 0.01$ versus control and versus ad-libitum diet	Purity > 99%; diet had a significant impact on tumour formation; liver tumours more prevalent than lung tumours

Table 3.1 (continued)

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
CD-1 (M) 11 mo Li *et al.* (1994)	Intraperitoneal injection 0 (control) or 250 nmol in DMSO on 1, 8 and 15 d after birth; animals killed at 11 mo and analysed Newborn; 21 or 27/group	Lung (adenoma): 3/27 (11%), 21/21 (100%)* Lung (carcinoma) 0/27, 5/21 (24%)	*$P < 0.01$	Comparative treatment with trans 1,2 dihydro-1,2-dihydroxy-6-aminochrysene, a putative metabolite, showed similar tumour and DNA-adduct formation as 6-nitrochrysene; other metabolites were less potent.
Crl/CD-1(ICR) BR (F) 25 wks El-Bayoumy *et al.* (1982)	Skin application 0 (control) or 0.1 mg in acetone on alternate d for 20 d; 10 d later, 2.5 µg TPA 3 ×/wk for 25 wks 20/group	Skin (predominantly squamous cell papillomas): 1/20 (5%), 12/20 (60%)	$P < 0.01$	Purity > 99%

d, day; DMSO, dimethyl sulfoxide; F, female; M, male; mo, month; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; wk, week; yr, year

(7%) and 0 out of 101 for females, respectively. The increased number of tumours (compared with controls) in males and females combined was highly significant when analysed for total tumours (32 out of 48; $P < 0.001$), but not when analysed by gender (Busby *et al.*, 1989).

Groups of newborn Swiss-Webster BLU-Ha mice received intraperitoneal injections of 0 (control; 38 males and 28 females), 100 (total dose; 23 males and 24 females) or 700 (total dose; 37 males and 46 females) nmol/mouse of 6-nitrochrysene in DMSO on days 1, 8 and 15 days after birth, or a single injection on day 1 of 100 nmol/mouse (25 males and 21 females) of 6-nitrochrysene in DMSO. Mice were necropsied at 30 weeks of age and their liver and lungs were analysed histologically. All male mice treated with 6-nitrochrysene had a 100% incidence of lung tumours. The incidence of liver tumours was 65%, 84% and 84% in males and 4%, 3% and 10% in females treated with 100 (three doses), 700 (three doses) and 100 (single dose) nmol of 6-nitrochrysene, respectively. The incidence of lung and liver tumours in all treated males was statistically significant compared with that in vehicle controls [type of tumours unspecified]. These results confirm the effects in the lungs, and show a gender-related sensitivity. Metabolites of 6-nitrochrysene were also evaluated in the study, and the putative metabolite, 1,2-dihydro-1,2-dihydroxy-6-amino chrysene, showed carcinogenicity similar to or greater than that of 6-nitrochrysene (El-Bayoumy *et al.*, 1989a; see also Table 3.2).

An assay was conducted in male and female newborn HSD:ICR mice to compare the potency of 6-nitrochrysene with that of isomers of nitrochrysene in the induction of tumour formation. A total dose of 100 nmol/mouse of 6-nitrochrysene in DMSO was administered by intraperitoneal injection to 38 males and 24 females on days 1, 8 and 15 after birth. A group that received DMSO alone served as a vehicle control. Animals were killed and analysed histologically for lung and liver tumours 30 weeks after the last dose. The lung tumour incidence was 100% in males and 96% in females, and was statistically significantly increased compared with that in controls (12% and 3%, respectively; $P < 0.001$). Liver tumours were observed in 64% ($P < 0.001$) of the treated males, none of the treated females, 8% of the control males and none of the control females. The types of tumour were not specified. 6-Nitrochrysene was significantly more potent than the isomers of nitrochrysene, which did not show a significant induction of tumours (El-Bayoumy *et al.*, 1992).

Groups of 22 or 9 male and 26 or 11 female newborn ICR mice received intraperitoneal injections of 0 (control) or 1.4 µmol/mouse (total dose) of 6-nitrochrysene in DMSO on days 1, 8 and 15 days after birth, were killed 24 weeks after the last dose and were analysed histologically. The incidence of lung tumours (adenoma) was 9 out of 9 (100%) treated males and 11 out of 11 (100%) treated females lung tumours versus none in the vehicle controls. No tumours were observed in the colon or the liver (Imaida *et al.*, 1992).

The effects of diet and modality of treatment on the formation of liver and lung tumours were investigated in groups of ~20 male newborn B6C3F1 mice that received intraperitoneal injections of a total dose of 0 (control) or 400 nmol/mouse of 6-nitrochrysene (purity, > 99%) in DMSO on days 1, 8 and 15 after birth; the three injections of 6-nitrochrysene contained 1/7th, 2/7th and 4/7th (treatment 1) or 0/7th, 3/7th and 4/7th (treatment 2) of the total dose, respectively. The two treatment groups were then separated at 14 weeks of age and either received the standard diet *ad libitum* or received a 70% calorie-restricted diet. The incidence of liver tumours in animals that received the standard diet *ad libitum* was 19 out of 19 (100%; adenomas) 14 out of 19 (74%; carcinomas) in treatment 1 group and 21 out of 21 (100%; adenomas) and 21 out of 21 (100%; carcinomas) in treatment 2 group. The calorie-restricted diet decreased the tumour

Table 3.2 Induction of lung and liver tumours in newborn mice treated with 6-nitrochrysene and its metabolites

Group Compound	Total dose (nmol)	Mice with lung tumours (%)	No. of lung tumours per mouse	Mice with liver tumours (%)	No. of liver tumours per mouse
1 DMSO	–	M – 18.4 F – 10.7	M – 0.2 ± 0.5 F – 0.1 ± 0.3	M – 0 F – 0	M – 0 F – 0
2 6-NC	700	M – 100[a] F – 100[a]	M – 50.6 ± 29.6[a,b] F – 47.8 ± 26.3[a,b]	M – 84[a] F – 3	M – 15.4 ± 15.1[a,b] F – 0.1 ± 0.5
3 6-NC	100	M – 100[a] F – 100[a]	M – 11.4 ± 6.9[a] F – 13.2 ± 9.2[a]	M – 65[a] F – 4	M – 5.4 ± 8.6[a] F – 0.1 ± 0.19
4 1,2-DHD-6-NC	100	M – 100[a] F – 100[a]	M – 14.1 ± 9.9[a] F – 15.8 ± 12.3	M – 81[a] F – 10.0	M – 17.6 ± 18.9[a,b,c] F – 0.1 ± 0.3
5 1,2-DHD-6-AC	100	M – 89[a] F – 89[a]	M – 12.2 ± 12.1[a] F – 13.4 ± 11.8[a]	M – 83[a] F – 5	M – 7.7 ± 7.5[a] F – 0.3 ± 1.2

[a] Significantly different from group 1, $P < 0.01$
[b] Significantly different from group 3, $P < 0.01$
[c] Significantly different from group 6, $P < 0.05$
1,2-DHD-6-AC, 1,2-dihydroxy-6-aminochrysene; 1,2-DHD-6-NC, 1,2-dihydroxy-6-nitrochrysene; 6-NC, 6-nitrochrysene; DMSO, dimethyl sulfoxide; F, female; M, male
From El-Bayoumy *et al.* (1989a)

incidence to 4 out of 23 (17%; adenomas) and 1 out of 23 (4%; carcinomas) in treatment 1 mice, but did not decrease that in treatment 2 group. The ad-libitum diet had a statistically significant effect ($P < 0.01$) on tumour incidence in comparison with both controls and the calorie-restricted diet. The incidence of lung tumour was 11 out of 19 (58%; adenomas) in the treatment 1 group and 3 out of 21 (14%; adenomas) in the treatment 2 group fed the ad-libitum diet, whereas no lung tumours were observed in mice fed the calorie-restricted diet (Fu *et al.*, 1994).

In a study to compare the relative DNA adducts, mutagenicity and tumour formation induced by 6-nitrochrysene and several of its major metabolites in the lungs, groups of 21–27 newborn male CD-1 mice were administered intraperitoneal injections of a total dose of 0 (control) or 250 nmol/mouse of 6-nitrochrysene on days 1, 8 and 15 after birth (1/7th, 2/7th and 4/7th of the total dose, respectively). In parallel, several metabolites of 6-nitrochrysene were evaluated at under similar experimental conditions. Tumour formation was investigated 11 months after the last dose of 6-nitrochrysene. A statistically significant increase in the incidence of lung adenoma (21 out of 21; 100%) and adenocarcinoma (5 out of 21; 24%) was observed in the 6-nitrochrysene-treated animals compared with controls (adenomas only; 3 out of 27; 11%) (Li *et al.*, 1994).

3.1.2 Initiation–promotion

Groups of 20 female CD-1 mice, aged 50–55 days, received 10 topical applications of 0 (control) or 0.1 mg/mouse of 6-nitrochrysene (purity, > 99%) in 0.1 mL acetone onto the shaved back every other day for 20 days (total dose, 1 mg/mouse). Ten days later, 2.5 µg of the tumour promoter 12-*O*-tetradecanoylphorbol 13-acetate were applied three times a week for an additional 25 weeks, after which the animals were killed and analysed histologically. An increase in the incidence of squamous cell papilloma was observed in the treated group; 12 out of 20 (60%) treated mice developed skin tumours (primarily papillomas; $P < 0.01$) compared with 1 out of 20 (5%) controls. No systemic tumours were observed (El-Bayoumy *et al.*, 1982).

3.2 Rat

See Table 3.3

3.2.1 Oral administration

In a study to evaluate the formation of mammary tumours that had previously been demonstrated in female CD1 rats and to compare the effects of several metabolites of 6-nitrochrysene that had also been reported to cause tumours, groups of 20 female Sprague-Dawley rats, aged 30 days, received intragastric intubations of 0 (control), 100 (low dose), 200 (mid-dose) or 400 (high dose) µmol/rat of 6-nitrochrysene [purity unspecified] in trioctanoin once a week for 8 weeks, and were killed 23 weeks after the last dose, when mammary tissue were analysed by histopathology. An increase in the incidence of mammary adenocarcinomas was observed: 3 out of 30 (10%) vehicle-control, 25 out of 30 (83%) low-dose ($P < 0.0001$), 25 out of 30 (83%) mid-dose ($P < 0.0001$) and 27 out of 30 (90%) high-dose ($P < 0.0001$) animals. A statistically significant increase in the incidence of fibroadenomas occurred in the low-dose group (5 out of 30 (17%) versus 0 out of 30; $P = 0.02$) (El-Bayoumy *et al.*, 2002).

Groups of transgenic (BBR × CD)F1 rats (developed by mating Big Blue (BBR) and Sprague-Dawley (CD) strains), aged 30 days, received intragastric intubations of 0 (control), 100 or 200 µmol (total doses) of 6-nitrochrysene in trioctanoin once a week for 8 weeks. The rats were killed 32 weeks after the last dose, and mammary tissue were analysed histologically. An increase in the incidence of mammary tumours was observed: fibroadenomas – 0 out of 33 control, 29 out of 31 (29%) low-dose ($P < 0.01$) and 2 out of 14 (15%) high-dose ($P < 0.05$) animals; adenomas – 0 out of 33 control, 1 out of 31 (3%) low-dose and 1/14 (7%) high-dose animals; adenocarcinomas – 1 out of 33 (3%) control, 5 out of 31 (16%) low-dose and 5 out of 31 (36%) high-dose ($P < 0.01$) animals; and fibromas – 0 out of 33 control, 1 out of 31 (3%) low-dose and 0 out of 14 high-dose females (Boyiri *et al.*, 2004).

3.2.2 Intraperitoneal administration

Groups of 31 male and 32 female newborn Crj:CG rats received intraperitoneal injection of 0 (control) or 14.8 µmol/rat (total dose) of 6-nitrochrysene in DMSO on days 1, 8 and 15 after birth. The animals were killed 32 weeks after the last administration and analysed histologically. The incidence of colon adenomas/dysplasias and adenocarcinomas was 23% and 16% in males ($P < 0.01$) and 44% and 6% in females ($P < 0.01$ for adenomas/dysplasias only), respectively. No colon tumours were observed in the controls, and no lung or liver tumours were observed in the treated or control rats (Imaida *et al.*, 1992).

3.2.3 Intramammary administration

Groups of 30 female CD1 rats, aged 30 days, received intramammary injection of 0 (control) or 12.3 µmol/rat (total dose) of 6-nitrochrysene in DMSO over 2 days. Animals were killed and analysed grossly and histologically for tumours 43 weeks after the last injection. Fibroadenomas, but no other tumours, developed in 5 out of 30 (16%) vehicle controls. The incidence of tumours in the 6-nitrochrysene group was 24 out of 30 (80%; $P < 0.0001$) fibroadenomas, 15 out of 30 (50%; $P < 0.0001$) adenocarcinomas and 10 out of 30 (33%; $P < 0.001$) spindle cell carcinomas (El-Bayoumy *et al.*, 1993).

Table 3.3 Studies of the carcinogenicity of 6-nitrochrysene in rats

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
CD (F) 23 wks El-Bayoumy *et al.* (2002)	Oral administration 0 (control), 100, 200 or 400 µmol in trioctanoin once/wk for 8 wks; animals killed and analysed 23 wks later Newborn; 20/group	Mammary (adenoma): 0/30, 0/30, 3/30 (10%), 1/30 (3%) Mammary (fibroadenoma): 0/30, 5/30 (17%)**, 3/30 (10%), 3/30 (3%) Mammary (adenocarcinoma): 3/30 (10%), 25/30 (83%)*, 25/30 (83%)*, 27/30 (90%)*	*$P < 0.0001$ **$P = 0.02$	High doses; not exposed by expected route in the environment; also evaluated tumour-forming potential of putative metabolites, and found that several metabolites also cause mammary tumours, none as potent as 6-nitrochrysene
Transgenic (Big Blue F344 × Sprague-Dawley) (F) 32 wks Boyiri *et al.* 2004	Oral administration 0 (control), 100 µmol or 200 µmol (total dose) in trioctanoin once/wk for 8 wk Aged 30 d; 33 (0), 31 (100 µmol) and 14 (400 µmol)	Mammary (fibroadenoma): 0/33, 9/31 (29%)**, 2/14 (14%)* Mammary (adenoma): 0/33, 1/31 (3%), 5/14 (7%) Mammary (fibroma): 0/33, 1/31 (3%), 0/14 Mammary (adenocarcinoma): 1/33 (3%), 5/31 (16%), 5/14 (36%)**	*$P < 0.05$ **$P < 0.01$	Oral administration induced mammary tumours but intraperitoneal administration in other studies produced colon tumours
Crj:CD (CD) (M, F) 32 wks Imaida *et al.* (1992)	Intraperitoneal injection 0 (control) or 14.8 µmol (total dose) in DMSO on 1, 8, 15, 22 and 29 d after birth; animals killed and analysed 32 wks later 31 M, 31 F (0); 31 M, 32 F (14.8 µmol)	Colon (dysplasia/adenoma): M–0/31, 7/31 (23%)* F–0/31, 14/32 (44%)* Colon (adenocarcinoma): M–0/31, 5/31 (16%) F–0/31, 2/32 (6%)	*$P < 0.01$	No lung or liver tumours observed
CD (F) 43 wks El-Bayoumy *et al.* (1993)	Intramammary injection 0 (control) or 12.3 µmol/rat (total dose) in DMSO on 2 d; animals palpated every 2 wks 3 mo after treatment, and killed and analysed after 43 wks Aged 30 d; 30/group	Mammary (fibroadenoma): 5/30 (17%), 24/30 (80%)** Mammary (spindle cell carcinoma): 0/30, 10/30 (33%)* Mammary (adenocarcinoma): 0/30, 15/30 (50%)**	*$P < 0.001$ **$P < 0.0001$	Not exposed by expected route in the environment

d, day; DMSO, dimethyl sulfoxide; F, female; M, male; mo, month; wk, week

4. Mechanistic and Other Relevant Data

4.1 Absorption, distribution, metabolism, excretion

4.1.1 Humans

No data were available to the Working Group.

4.1.2 Experimental systems

(a) In-vivo studies

In CD rats treated intraperitoneally with a single dose of [3,4,9,10-^{3}H] 6-nitrochrysene ([^{3}H]6-nitrochrysene; 9 µmol/rat), only 1.3% of the dose was excreted in the urine and 23% in the faeces after 24 hours (Chae *et al.*, 1996). The extent of metabolism was extremely limited; 6-nitrochrysene was the major component found in the faeces after 24 hours, accounting for 98% of the radioactivity. In the faeces, *trans*-1,2-dihydro-1,2-dihydroxy-6-nitrochrysene (1,2-DHD-6-NC), chrysene-5,6-quinone and 6-aminochrysene were identified as 6-nitrochrysene metabolites; the major free products (unconjugated) identified in urine were 6-aminochrysene, 1,2-DHD-6-NC and *trans*-9,10-dihydro-9,10-dihydroxy-6-nitrochrysene (9,10-DHD-6-NC) (Chae *et al.*, 1996). The same metabolites were detected in extracts from whole Blu:Ha (ICR) mice after intraperitoneal injection of [^{3}H]6-nitrochrysene (Delclos *et al.*, 1988). The role of intestinal microflora on the metabolism of 6-nitrochrysene was investigated in Balb/c mice after treatment with a single intraperitoneal injection of 0.03 µmol/5 µL/g body weight (bw) [^{3}H]6-nitrochrysene (Delclos *et al.*, 1990). The amount of 6-aminochrysene excreted in the faeces of germ-free mice within 48 hours after treatment was approximately 25% of that excreted in identically treated conventional mice.

6-Nitrochrysene induced hepatic cytochrome P450 (CYP) expression after oral treatment of Sprague-Dawley rats with 2.5, 5 or 25 mg/kg bw for 3 days (Chou *et al.*, 1987). 6-Nitrochrysene was significantly more active than its parent compound, chrysene. In Sprague-Dawley rats treated with 5 mg/kg bw for 3 days, 6-nitrochrysene significantly increased the enzyme activities of uridine diphosphate-glucuronosyl-transferase and glutathione *S*-transferase whereas chrysene did not (Pegram & Chou, 1989).

(b) In-vitro studies

The metabolism of 6-nitrochrysene ([12-^{3}H]6-nitrochrysene) was studied in primary cultures of human breast cells prepared from tissues obtained from reduction mammoplasty (Boyiri *et al.*, 2002). 1,2-DHD-6-NC and 6-aminochrysene were identified as the major metabolites; chrysene-5,6-quinone was also detected. Similar results were found in cultured, immortalized human mammary epithelial MCF-10A cells, as well as estrogen-dependent (MCF-7) and estrogen-independent (MDA-MB-435) human breast cancer cell lines, in which *trans*-1,2-dihydro-1,2-dihydroxy-6-aminochrysene (1,2-DHD-6-AC) was also found (Boyiri *et al.*, 2002).

In microsomes prepared from human liver and lung tissue and incubated with [^{3}H]6-nitrochrysene, 1,2-DHD-6-NC, 9,10-DHD-6-NC, 6-aminochrysene and chrysene-5,6-quinone were identified as metabolites but with quantitative differences (Chae *et al.*, 1993). The levels of the major metabolite, 1,2-DHD-6-NC, ranged from 33 to 132 pmol/mg protein/hour using numerous human hepatic microsomal samples; the corresponding levels using human pulmonary microsomes ranged from non-detectable to 33 pmol/mg protein/hour. The remaining metabolites were detected at much lower levels.

The oxidation of 1,2-DHD-6-NC was catalysed by *CYP1A2* in human liver and *CYP1A1*

in human lung; CYP3A4 catalysed the reduction of 6-nitrochrysene to 6-aminochrysene. In human hepatoma HepG2 cells, 6-nitrochrysene induced the expression of *CYP1A1* protein and mRNA levels that may be an important factor to assess the metabolism of 6-nitrochrysene in the liver (Chen *et al.*, 2000). 6-Nitrochrysene also induced *CYP1A1* mRNA in human lung carcinoma NCI-H322 cells. The induction of *CYP1A1*, *CYP1A2* and *CYP1B1* mRNAs by 6-nitrochrysene was investigated in various human-derived cell lines (Iwanari *et al.*, 2002). The induction of all three *CYP* genes by 6-nitrochrysene was observed in HepG2 (hepatocellular carcinoma), MCF-7 (breast carcinoma), LS-180 (colon carcinoma), OMC-3 (ovarian carcinoma) and NEC14 (testis embryonal carcinoma) cells; a strong induction of *CYP1B1* was observed in A549 (lung carcinoma) cells.

Incubation of 6-nitrochrysene with an exogenous metabolic activation system from rat liver resulted in the formation of 1,2-DHD-6-NC and 6-aminochrysene as the major metabolites (El-Bayoumy & Hecht, 1984). Similar results were obtained in incubations containing [^{3}H]6-nitrochrysene and an exogenous metabolic activation system from the liver of 1- or 8-day-old BLU:Ha (ICR) mice; the formation of 1,2-DHD-6-NC (approximately twofold), 9,10-DHD-6-NC (~1.5-fold) and 6-aminochrysene (approximately fourfold) was greater in younger animals (Delclos *et al.*, 1988). Further metabolism of [^{3}H]1,2-DHD-6-NC by rat liver metabolic activation yielded 1,2-DHD-6-AC as the major product.

Mixed cultures of rat and mouse intestinal bacteria and pure cultures of anaerobic bacteria reduced 6-nitrochrysene to 6-aminochrysene. In the fungus, *Cunninghamella elegans*, 74% of the [^{3}H]6-nitrochrysene added was metabolized within 6 days to form two isomeric sulfate conjugates (Pothuluri *et al.*, 1998). These conjugates were identified as 6-nitrochrysene-1-sulfate and 6-nitrochrysene-2-sulfate.

4.2 Genetic and related effects

The genetic and related effects of 6-nitrochrysene and some of its metabolites have been reviewed (Rosenkranz & Mermelstein, 1983; Tokiwa & Ohnishi, 1986; Purohit & Basu, 2000; IPCS, 2003)

4.2.1 Humans

No data were available to the Working Group.

4.2.2 Experimental systems

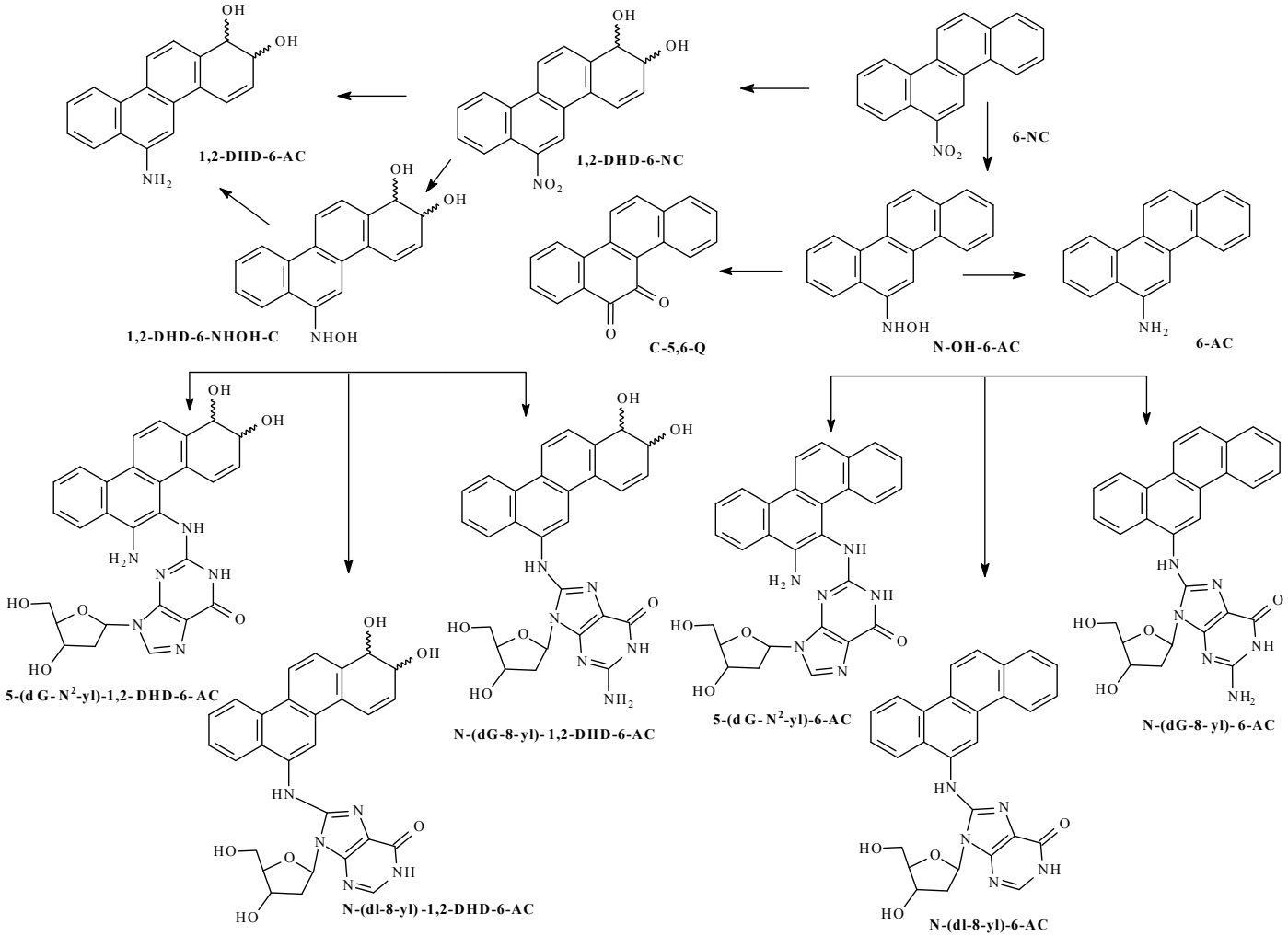
(a) DNA adduct formation

See Fig. 4.1.

(i) In-vivo studies

The formation of DNA adduct by 6-nitrochrysene was predominantly measured using the ^{32}P-postlabelling method and studied in rats. The intraperitoneal injection of CD rats with 14.8 µmol/rat of 6-nitrochrysene on days 1, 8, 15, 22 and 29 after birth resulted in the formation of DNA adducts, 24 hours after the last administration, in the colon (the target organ for carcinogenesis) as well as in the liver and lung (non-target organs for carcinogenesis) (Chae *et al.*, 1996). Two major DNA adducts were detected (adducts 3 and 4). DNA adduct 3 was chromatographically indistinguishable from 5-(deoxyguanosin-N^2-yl)-6-aminochrysene [5-(dG-N^2-yl)-6-AC]. Adduct 4 was proposed by the authors to be derived from 1,2-DHD-6-AC and to be a deoxyguanosine adduct modified by 1,2-DHD-6-AC-3,4-epoxide (Chae *et al.*, 1996), but was later shown to be derived from 1,2-DHD-6-hydroxylamino-chrysene and was structurally identified as 5-(deoxyguanosin-N^2-yl)-1,2-dihydroxy-1,2-dihydro-6-aminochrysene [5-(dG-N^2-yl)-1,2-DHD-6-AC] (El-Bayoumy *et al.*, 2004). *N*-(Deoxyguanosin-8-yl)-6-aminochrysene [*N*-(dG-8-yl)-6-AC; adduct 1] and *N*-(deoxyinosin-8-yl)-6-aminochrysene

Fig. 4.1 Metabolism and DNA-adduct formation of 6-nitrochrysene



6-AC, 6-aminochrysene; C-5,6-Q, chrysene-5,6-quinone; 5-(dG-N^2-yl)-6-AC, 5-(deoxyguanosin-*N*^{2}-yl)-6-aminochrysene; 5-(dG-N^2-yl)-1,2-DHD-6-AC, 5-(deoxyguanosin-*N*^{2}-yl)-1,2-dihydroxy-1,2-dihydro-6-aminochrysene; 1,2-DHD-6-AC, 1,2-dihydroxy-1,2-dihydro-6-aminochrysene; 1,2-DHD-6-NC, 1,2-dihydroxy-1,2-dihydro-6-nitrochrysene; 1,2-DHD-6-NHOH-C, *trans*-1,2-dihydroxy-1,2-dihydro-*N*-hydroxy-6-aminochrysene; 6-NC, 6-nitrochrysene; N-(dG-8-yl)-6-AC, *N*-(deoxyguanosin-8-yl)-6-aminochrysene; N-(dG-8-yl)-1,2-DHD-6-AC, *N*-(deoxyguanosin-8-yl)-1,2-dihydroxy-1,2-dihydro-6-aminochrysene; N-(dI-8-yl)-6-AC, *N*-(deoxyinosin-8-yl)-6-aminochrysene; N-(dI-8-yl)-1,2-DHD-6-AC, *N*-(deoxyinosin-8-yl)-1,2-dihydroxy-1,2-dihydro-6-aminochrysene; N-OH-6-AC, *N*-hydroxy-6-aminochrysene

[*N*-(dI-8-yl)-6-AC; adduct 2] were not detectable *in vivo*.

The role of intestinal microflora on the formation of 6-nitrochrysene-induced DNA adducts was investigated in Balb/c mice after a single intraperitoneal injection of [^{3}H]6-nitrochrysene (0.03 µmol/5 µL/g bw) (Delclos *et al.*, 1990). The levels of DNA adducts were similar in conventional and germ-free mice; however, the adducts in the liver were derived from both *N*-hydroxy-6-aminochrysene and 1,2-DHD-6-AC, whereas only adducts derived from 1,2-DHD-6-AC were detected in the lung. These findings are in contrast to other findings in preweanling mice (Delclos *et al.*, 1987a), in which the single major adduct detected in both the liver and the lung was derived from 1,2-DHD-6-AC.

As part of the newborn mouse lung adenoma bioassay, newborn CD-1 mice received intraperitoneal injections of total doses of 250 nmol of 6-nitrochrysene, 6-aminochrysene, *N*-hydroxy-6-aminochrysene or 6-nitrosochrysene or 80 nmol of 1,2-DHD-6-AC within 24 hours, and on days 8 and 15 after birth (Li *et al.*, 1994). In the lung, 6-nitrochrysene, 6-aminochrysene and 1,2-DHD-6-AC produced predominantly a single major DNA adduct, which was subsequently identified as 5-(dG-N^2-yl)-1,2-DHD-6-AC (El-Bayoumy *et al.*, 2004). 6-Nitrosochrysene induced a single major adduct that was most probably derived from reaction at the C8 position of deoxyadenosine (Li *et al.*, 1994).

(ii) In-vitro studies

N-Hydroxy-6-aminochrysene was reacted *in vitro* with DNA. The three major DNA adducts identified, *N*-(dG-8-yl)-6-AC, *N*-(dI-8-yl)-6-AC and 5-(dG-N^2-yl)-6-AC, accounted for 22%, 32% and 28%, respectively, of the total DNA adducts formed (Delclos *et al.*, 1987b). The deoxyinosine adduct probably results from the oxidative deamination of the corresponding deoxyadenosine adduct. *Anti*-1,2-dihydroxy-3,4-epoxy-1,2,3,4-tetrahydro-6-NC was shown to react with DNA, as well as with deoxyguanosine or deoxyadenosine, *in vitro* but these DNA adducts did not correspond to adducts found in 6-nitrochrysene-treated rats *in vivo* (Krzeminski *et al.*, 2000). The reaction of *trans*-1,2-dihydroxy-1,2-dihydro-6-hydroxyaminochrysene (1,2-DHD-6-NHOH-C) with DNA *in vitro* resulted in the formation of three major DNA adducts, namely 5-(dG-N^2-yl)-1,2-DHD-6-AC, *N*-(deoxyguanosin-8-yl)-1,2-dihydroxy-1,2-dihydro-6-aminochrysene and *N*-(deoxyinosin-8-yl)-1,2-dihydroxy-1,2-dihydro-6-aminochrysene (El-Bayoumy *et al.*, 2004).

N-(dG-8-yl)-6-AC and 5-(dG-N^2-yl)-6-AC were repaired to similar extents by nucleotide excision repair, because the N^2-deoxyguanosine adduct formed by benzo[*a*]pyrene-diol-epoxide [i.e. (+)-*trans*-benzo[*a*]pyrene-diol-epoxide-N^2-deoxyguanosine] in DNA duplexes constructs incubated with nucleotide excision repair-competent nuclear extracts from human HeLa cells (Krzeminski *et al.*, 2011). As (+)-*trans*-benzo[*a*]pyrene-diol-epoxide-N^2-deoxyguanosine is considered to be a poorer substrate for nucleotide excision repair, *N*-(dG-8-yl)-6-AC and 5-(dG-N^2-yl)-6-AC lesions may be repaired slowly and may thus persist in mammalian tissues.

In rat hepatocytes treated with [^{3}H]6-nitrochrysene or [^{3}H]6-aminochrysene, a high degree of DNA binding was observed (0.2–4 adducts per 10^6 nucleotides); the two major DNA adducts formed were identified as *N*-(dG-8-yl)6-AC and *N*-(dI-8-yl)-6-AC (Delclos *et al.*, 1987a). Similarly, 6-nitrochrysene formed one major adduct by ^{32}P-postlabelling in primary rat hepatocytes, NCI-H322 derived human lung cells and Chinese hamster lung V79NH cells with endogenous acetyltransferase activity (Topinka *et al.*, 1998). In human MCF-7 cells treated with [12-^{3}H]6-nitrochrysene and [^{3}H]1,2-DHD-6-NC, one major DNA adduct was detected which was chromatographically indistinguishable from a major DNA adduct found in the [12-^{3}H]6-nitrochrysene-treated mammary

glands of rats and a deoxyguanosine adduct derived from the nitroreduction of [^{3}H]1,2-DHD-6-NC in the presence of xanthine oxidase (Boyiri *et al.*, 2002).

(b) *Mutagenicity of 6-nitrochrysene*

(i) *Mutagenesis and chromosomal instability* in vivo

K-ras mutations in codon 12, 13 or 61 were found in 64 (88%) of 73 lung adenomas and all 15 adenocarcinomas analysed from newborn CD-1 mice treated intraperitoneally with 6-nitrochrysene (Li *et al.*, 1994). All of the mutations in codon 12 and 13 involved a G:C base pair. Liver tumours were induced by the intraperitoneal administration of 6-nitrochrysene to preweanling B6C3F1 (total dose, 300 nmol/mouse) and CD-1 (total dose 250 nmol/mouse) mice and analysed for *ras* gene mutations (Manjanatha *et al.*, 1996). The frequency of H- and K-*ras* mutations in 6-nitrochrysene-induced tumours was only 4% in B6C3F1 mice, while 19 liver tumours (90%) from CD-1 mice had the identical CAA to AAA mutation in H-*ras* codon 61. Transgenic mice have been used as powerful toxicological models to study the role of specific genetic alterations as predisposing factors for chemical carcinogenesis. In a previous study, transgenic mice carrying a prototype human c-Ha-*ras* gene was susceptible to lung carcinogens such as 6-nitrochrysene; the authors stated that this susceptibility may be due to somatic mutations in the *ras* transgene (Ogawa *et al.*, 1996).

The mutagenicity of 6-nitrochrysene in the mammary glands was examined in the *cII* gene of transgenic (Big Blue F344 × Sprague-Dawley) F1 rats treated once a week for 8 weeks with 100 or 200 µmol/rat 6-nitrochrysene (Boyiri *et al.*, 2004). The mutant frequency measured 32 weeks after the last administration was comparable in normal, non-involved and tumour tissue from the mammary glands of 6-nitrochrysene-treated rats but was significantly higher than that observed in control rats. 6-Nitrochrysene induced G:C to T:A, G:C to C:G, A:T to G:C and A:T to T:A mutations, whereas G:C to A:T transitions were primarily found in control rats.

Injection of Balb/c mice with 0–500 mg/kg bw 6-nitrochrysene led to a significant increase in micronuclei in the bone marrow (Sakitani & Suzuki, 1986).

(ii) *Mutagenesis and DNA damage in bacteria*

Since the previous review of 6-nitrochrysene (IARC, 1989), additional studies have shown that 6-nitrochrysene was mutagenic to *Salmonella typhimurium* TA98, TA100 and TA1538 in the presence of absence of an exogenous metabolic activation system (El-Bayoumy *et al.*, 1989b; Jung *et al.*, 1991; Chen *et al.*, 2000). The mutagenicity of 6-nitrochrysene, 1,2-DHD-6-NC and 6-aminochrysene was compared in *S. typhimurium* TA100 and 1,2-DHD-6-NC was identified as the major proximate mutagen of 6-nitrochrysene. Bay region methyl substitution has been shown to either inhibit (5-position) or enhance (11-position) the mutagenic activity of 6-nitrochrysene in *S. typhimurium* (El-Bayoumy *et al.*, 1989b).

6-Nitrochrysene induced DNA damage in *S. typhimurium* (SOS umu test) (Shimada *et al.*, 1989; Rafii *et al.*, 1994; Shimada *et al.*, 1996; Yamazaki *et al.*, 2000); human CYP1B1 was required to activate 6-nitrochrysene in this assay, whereas human *CYP1A1* and *CYP1A2* did not activate the compound (Shimada *et al.*, 1996). 6-Nitrochrysene inhibited the growth of DNA repair-deficient *Bacillus subtilis* (Tokiwa *et al.*, 1987).

(iii) *Mutagenesis and cellular transformation in mammalian cells*

6-Nitrochrysene induced mutations in the hypoxanthine-guanine phosphoribosyltransferase (*Hprt*) forward mutation assay in Chinese hamster ovary cells in the presence of a liver homogenate from Aroclor 1254-pretreated rats (Delclos & Heflich, 1992). The mutation

frequency was higher in DNA repair-deficient Chinese hamster ovary cells than in repair-proficient cells. In contrast, exposure to 6-nitrochrysene and 6-aminochrysene did not result in a concentration-dependent increase in mutation frequency at the *HPRT* locus in human lymphoblastoid AHH-1 cells (Morris *et al.*, 1994). Minimal metabolic activation of 6-nitro- or 6-aminochrysene by AHH-1 cells may account for the lack of a positive mutagenic response.

6-Nitrosochrysene also induced mutations at the *Hprt* locus in Chinese hamster ovary cells, predominantly base-pair substitution at A:T base pairs (Manjanatha *et al.*, 1993). However, it mainly formed DNA adducts at deoxyguanosine (80%) while only 20% were probably formed through binding at deoxyadenosine, indicating that 6-nitrosochrysene-derived deoxyadenosine adducts are much more effective at producing mutations than their deoxyguanosine counterparts. In contrast, 1,2-DHD-6-AC-induced *Hprt* mutations in Chinese hamster ovary cells involved primarily G:C base-pair substitutions, which was consistent with the formation of a single major deoxyguanosine adduct formed by the microsome-catalysed reaction of 1,2-DHD-6-AC with DNA (Li *et al.*, 1993).

The mutational profiles of 6-nitrochrysene and its metabolites, *N*-hydroxy-6-aminochrysene, 6-aminochrysene, 1,2-DHD-6-NC, 1,2-DHD-6-AC and 1,2-DHD-6-NHOH-C, were compared in the *cII* gene of *lacI* rat mammary epithelial cells *in vitro* (Guttenplan *et al.*, 2007). 1,2-DHD-6-NHOH-C induced a mutational profile that was most similar to that of 6-nitrochrysene *in vivo* (Boyiri *et al.*, 2004). When the mutation profiles of (±)-1,2-DHD-6-NC were examined in these cells, the [*R*,*R*]-isomer was a more potent mutagen than the [*S*,*S*]-isomer (Sun *et al.*, 2009). The major types of mutation induced by the [*R*,*R*]-isomer were G:C to T:A, A:T to G:C and A:T to T:A mutations, which were similar to those obtained in the mammary gland of rats treated with 6-nitrochrysene *in vivo* (Boyiri *et al.*, 2004).

6-Nitrochrysene induced morphological transformation in cultured Syrian hamster embryo cells (DiPaolo *et al.*, 1983; Sala *et al.*, 1987), Syrian hamster kidney BHK-21/cl13 cells (Purchase *et al.*, 1978), rat tracheal epithelial cells (Mitchell & Thomassen, 1990; Gray *et al.*, 1994; West & Rowland, 1994) and human lung fibroblast WI38 or human liver cells (Purchase *et al.*, 1978) but not in murine BALB/c 3T3 or C3H 10T1/2 cells (Sala *et al.*, 1987; Sheu *et al.*, 1994).

(c) *Other biological effects of 6-nitrochrysene*

The effect of 6-nitrochrysene on the expression of p53 and $p21^{Cip1}$ proteins and cell-cycle regulation was examined in MCF-7 and MCF-10A cells (Sun *et al.*, 2007). Although treatment with of 6-nitrochrysene did not increase the level of total p53 protein in either cell line, $p21^{Cip1}$ protein and a concomitant increase in cells in the G1 phase were observed in MCF-10A, but not MCF-7 cells. Treatment with 1,2-DHD-6-NHOH-C resulted in a significant induction of p53 protein in MCF-7 cells. The authors concluded that the lack of a p53 response to 6-nitrochrysene may imply a lack of the protective functions that are mediated by p53 (i.e. DNA repair mechanisms).

6-Nitrochrysene inhibited the growth of cultured rat but not human epidermal cells (Chun *et al.*, 2000).

4.3 Other relevant data

No data were available to the Working Group.

4.4 Mechanistic considerations

6-Nitrochrysene can be activated metabolically via ring oxidation, nitroreduction or a combination of both pathways to yield reactive electrophilic species that can react with DNA and result in the formation of DNA adducts (Boyiri *et al.*, 2002; El-Bayoumy *et al.*, 2004). 6-Nitrochrysene induces hepatic and pulmonary

CYP1A1 and can thereby enhance its own CYP-mediated bioactivation (Chen *et al.*, 1998). Several DNA adducts have been detected in rats and mice treated with 6-nitrochrysene (IPCS, 2003). Such DNA damage has been suggested to contribute to its potent mutagenic and carcinogenic activities, including the induction of rat mammary adenocarcinomas (Delclos *et al.*, 1987b; Li *et al.*, 1994; Chae *et al.*, 1996; Krzeminski *et al.*, 2000). These DNA lesions are liable to cause mutations if they are not removed by cellular defence mechanisms before DNA replication occurs. A recent study in human cell extracts demonstrated the inefficient nucleotide excision repair of two DNA adducts – *N*-(dG-8-yl)-6-AC and 5-(dG-N^2-yl)-6-AC – derived from the nitroreduction pathway of 6-nitrochrysene (Krzeminski *et al.*, 2011). However, the efficiency of nucleotide excision repair of DNA adducts of 6-nitrochrysene derived from a combination of ring oxidation and nitroreduction pathways has not yet been determined.

Nitroreduction and ring oxidation leads to the formation of 1,2-DHD-6-NHOH-C. The mutation profile of 6-nitrochrysene in the rat mammary gland *in vivo* was compared with that of its known metabolites in the *cII* gene of *lacI* mammary epithelial cells *in vitro* and showed that the profile of 1,2-DHD-6-NHOH-C was the most similar (Guttenplan *et al.*, 2007). These results, in conjunction with the known structure of the major DNA adduct detected in the rat mammary gland (i.e. 5-(dG-N^2-yl)-1,2-DHD-6-AC), support the hypothesis that 1,2-DHD-6-NHOH-C is the ultimate mutagen/carcinogen. The mutation profile and spectra of the proximate mutagens [*R,R*]- and [*S,S*]-1,2-DHD-6-NC were compared in the *cII* gene of *lacI* mammary epithelial cells *in vitro* and, although the [*R,R*]-enantiomer was a significantly more potent mutagen than the [*S,S*]-enantiomer, the mutation spectra were similar (Sun *et al.*, 2009). While the *in vitro* results suggest that the [*R,R*]-enantiomer is the proximate carcinogen in the rat mammary gland, the comparative carcinogenic activity of both enantiomers in this organ *in vivo* has not yet been determined.

In contrast to numerous other DNA-damaging agents, such as benzo[*a*]pyrene, that are known to stimulate the expression of p53 protein, the lack of a p53 response to 6-nitrochrysene implies the lack of protective functions mediated by this protein (e.g. DNA repair mechanisms) after exposure to 6-nitrochrysene, which may, in part, account for the carcinogenicity of 6-nitrochrysene in rat mammary glands (Sun *et al.*, 2007).

5. Summary of Data Reported

5.1 Exposure data

6-Nitrochrysene was reported to be a constituent in the particulate phase of both diesel and gasoline engine exhaust emissions. No evidence was found that it has been produced in commercial quantities or used for purposes other than laboratory applications. No evidence was found of any source of exposure to 6-nitrochrysene other than traffic exhaust emissions. In ambient air, 6-nitrochrysene was found at concentrations in the subnanogram and low to subpicagram per cubic metre range in airborne particulate matter collected at urban, and rural and remote locations, respectively. Blood analysis confirmed exposure to 6-nitrochrysene of inhabitants in a rural area and several occupational groups.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

Several studies have investigated tumour formation in rodents that were administered 6-nitrochrysene shortly after birth and then observed for up to 1 year after treatment. Eight studies of intraperitoneal injection were conducted in newborn mice and one in newborn rats.

In mice, increases in the incidence of malignant lymphoma in one study, and of benign or malignant tumours of the lung (adenoma or adenocarcinoma) and liver (hepatocellular adenoma or carcinoma) in both sexes were observed. The study in rats showed an increased incidence of colon adenocarcinoma in both sexes, but no lung or liver tumours. Thus, intraperitoneal injection caused a species-specific increase in the incidence of lung and liver tumours in mice and of colon tumours in rats. In two studies of oral administration and one of intramammary injection in rats, an increased incidence of mammary adenocarcinomas was observed. In addition, 6-nitrochrysene showed initiating activity in one skin tumour initiation–promotion study in mice.

5.4 Mechanistic and other relevant data

6-Nitrochrysene is metabolically activated via nitroreduction, ring-oxidation or a combination of both pathways, leading to the formation of DNA adducts. When administered orally or by intramammary injection to rats, 6-nitrochrysene induced mammary adenocarcinomas; its carcinogenic activity in the rat mammary gland exceeds that of benzo[*a*]pyrene – classified as a Group 1 human carcinogen by the IARC. Furthermore, 6-nitrochrysene was found to induce lung, liver and skin tumours in mice. Human hepatic and pulmonary microsomes and human mammary epithelial cells metabolized 6-nitrochrysene to reactive metabolites that caused DNA damage. 6-Nitrochrysene induced the same DNA adducts in human mammary epithelial cells as those detected in the mammary gland – the target organ – of rats. Haemoglobin adducts derived from 6-nitrochrysene have been identified in humans exposed to diesel engine exhaust. The mutation spectrum induced by 6-nitrochrysene in mammary tissue in *lacZ*-transgenic mice was dominated by mutations at G and A residues and could be linked to the mutation profile of 1,2-dihydro-dihydroxy-6-hydroxyamino-chrysene, a metabolite that is formed by a combination of nitroreduction and ring-oxidation. The formation of its major adduct, 5-(deoxyguanosin-N^2-yl)-1,2-dihydro-dihydroxy-6-amino-chrysene, in the rat mammary gland supports the hypothesis that 1,2-dihydro-dihydroxy-6-hydroxyamino-chrysene is the ultimate carcinogen. Mutations in the H-*Ras* and K-*Ras* oncogenes were observed in lung tumours from mice exposed to 6-nitrochrysene.

Overall, the Working Group considered that there is *strong mechanistic evidence* to support the carcinogenic properties of 6-nitrochrysene.

6. Evaluation

6.1 Cancer in humans

No data were available to the Working Group.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of 6-nitrochrysene.

6.3 Overall evaluation

6-Nitrochrysene is *probably carcinogenic to humans (Group 2A).*

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2-NITROFLUORENE

2-Nitrofluorene was evaluated by a previous IARC Working Group in 1988 (IARC, 1989). New data have since become available, and these have been taken into consideration in the present evaluation.

1. Exposure Data

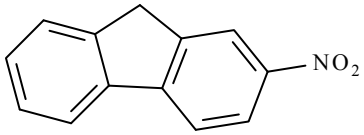
1.1 Chemical and physical data

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 607-57-8

IUPAC Systematic Name: 2-Nitrofluorene

1.1.2 Structural and molecular formulae and relative molecular mass



$C_{13}H_9NO_2$

Relative molecular mass: 211.2 g/mol

1.1.3 Chemical and physical properties of the pure substance

Description: Needles, recrystallized from 50% acetic acid or acetone (Weast, 1985); light-yellow, fluffy solid (Chemsyn Science Laboratories, 1988)

Melting-point: 156 °C (Buckingham, 1982); 158 °C (Weast, 1985)

Spectroscopy data: Ultraviolet, nuclear magnetic resonance, infrared and mass spectral data have been reported (Schuetzle & Jensen, 1985; Chemsyn Science Laboratories, 1988).

Solubility: Sparingly soluble in water (Beije & Möller, 1988a); soluble in acetone, benzene, tetrahydrofluorenone and toluene (Weast, 1985; Chemsyn Science Laboratories, 1988)

1.1.4 Technical products and impurities

2-Nitrofluorene is available for research purposes at purities of 95%, 98% or > 99%, and in radiolabelled form at purities of ≥ 98% (^{14}C) or ≥ 99% (^{3}H) (IARC, 1989).

Currently, 2-nitrofluerene (98% pure) is sold in small quantities for research purposes (Sigma-Aldrich, 2012).

1.2 Analysis

For the analytical methods of nitro-polycyclic aromatic hydrocarbons (PAHs) in general, the reader is referred to Section 1.2.2(d) of the

Monograph on Diesel and Gasoline Engine Exhausts in this Volume.

2-Nitrofluorene is present in the gas phase and in the particle phase, and polyurethane foam can be used for its collection in the gas phase (Albinet *et al.*, 2006). 2-Nitrofluorene has been detected in particles that were collected on glass and quartz fibre or Teflon-coated silica fibre membrane filters (Tokiwa *et al.*, 1990; Schauer *et al.*, 2004).

2-Nitrofluorene can be extracted from particulate matter using dichloromethane for extraction by sonification (Tokiwa *et al.*, 1990). Gas chromatography-negative ion chemical ionization-mass spectrometry was used for its analysis following pressurized-fluid extraction (Bamford *et al.*, 2003; Albinet *et al.*, 2006). This substance was also analysed after its reduction to 2-aminofluorene with titanium(III)citrate and subsequent detection by fluorescence spectrometry (Zielinska & Samy, 2006). Chemiluminescence was also used as an alternative and sensitive method of detection in place of fluorescence (Murahashi & Hayakawa, 1997).

1.3 Production and use

1.3.1 Production

No evidence was found that 2-nitrofluorene is currently produced for purposes other than laboratory use.

1.3.2 Use

2-Nitrofluorene was not reported to be used in commercial applications.

1.4 Occurrence and exposure

1.4.1 Diesel engine exhaust

2-Nitrofluorene has been identified in diesel engine exhaust emissions in several studies (Table 1.1). Two unspecified isomers of nitrofluorene were reported in exhaust emissions from three light-duty diesel passenger cars at concentrations in the range of 50–200 µg/g of particulates (Schuetzle, 1983). Emission levels from 1980–85 model light-duty diesel engines running on an urban Federal Test Procedure cycle were 90 µg/mile [56 µg/km] for the gas phase and 97 µg/mile [61 µg/km] for the particulate phase (Schuetzle & Frazier, 1986). Concentrations in particulates from a heavy-duty mining diesel engine were reported to be 0.63 µg/g when running at a 100% load and 1200 rpm and 8.8 µg/g when running at a 75% load and 1800 rpm (Draper, 1986).

The use of biodiesel and an oxidation catalyst was studied in the heavy duty transient Federal Test Procedure. In three types of heavy-duty diesel engine, different fuels were tested: neat biodiesel fuel (B100), a blend of biodiesel with normal diesel fuel (20:80 by volume; B20) and neat diesel fuel (2D). The formation of 2-nitrofluorene in the exhaust from B100 ranged widely between 6.5 and 142 ng/horse power (hp)–h. When an oxidation catalyst was added, emissions of 2-nitrofluorene were reduced in two of the engines to values of 14 and 73 ng/hp-h. When fuelled with B20 and 2D, the amounts of 2-nitrofluorene ranged from 70 to 144 ng/hp-h in two of the three engines (two 1997 models). In the third engine (a 1995 model), the level of 2-nitrofluorene was 257 ng/hp-h for 2D. After the addition of an oxidation catalyst, the production of 2-nitrofluorene increased to 478 ng/hp-h with (Sharp *et al.*, 2000). [This comparison could not be made for the blended fuel B20 due to the absence of data.]

1.4.2 Ambient air and dust

Low levels of 2-nitrofluorene were detected in most, but not all, samples of airborne particulate matter or gas-phase samples collected at urban locations in Japan, Germany and France (Tanabe *et al.*, 1986; Tokiwa *et al.*, 1990; Schauer *et al.*, 2004; Albinet *et al.*, 2006, 2007; Table 1.2), whereas no detectable quantities of 2-nitrofluorene were

Table 1.1 Levels of 2-nitrofluorene in particulate matter from diesel exhaust emissions and other sources

Reference	Source	Type of sampling	No. of samples	Concentration		
				(µg/km)	(µg/g)	(ng/bhp-h)
Nishioka *et al.* (1982)	Light-duty diesel passenger car	PM	4	–	ND-0.4	–
Schuetzle & Perez (1983)	Heavy-duty vehicle	PM				
	Idle		1	–	84	–
	High speed; zero load (2100 rpm)		1	–	62	–
	High speed; full load (2100 rpm)		1	–	1.9	–
Schuetzle (1983)	Light-duty diesel passenger car	PM	3	–	71, 78, 186[a]	–
Schuetzle & Frazier (1986)	Light-duty diesel (1980–1985 models)	GP	7	56	–	–
		PM	7	61	–	–
Draper (1986)	Heavy-duty diesel					
	100% load (1200 rpm)	PM	1	–	0.63	–
	75% load (1800 rpm)	PM	1	–	8.8	–
	Diesel exhaust particles (SRM 1650)	PM	3	–	15.1	–
Tokiwa *et al.* (1990)	City gas (methane)	PM	1	–	0.021	–
	Heavy oil	PM	1	–	0.013	–
Khalek *et al.* (2011)	Heavy-duty engines	PM				
	12 repeats of 16-h cycles over FTP transient cycle					
	2007 technology		4	–	–	3.6 ± 4.1
	2000-technology		1	–	–	65

[a] Concentrations are accurate to within ± 30–40%

bhp, break horse power; FTP, Federal Test Procedure; GP, gas phase; h, hour; ND, not detected; PM, particulate matter; rpm, revolutions per minute; SRM, Standard Reference Material

found in similar samples collected from rural locations (Schauer *et al.*, 2004; Albinet *et al.*, 2006, 2007).

1.4.3 Other sources

2-Nitrofluorene has been detected in particulate extracts of emissions from kerosene heaters, gas burners and liquefied petroleum gas burners, but no attempt was made to quantify the contents (Tokiwa *et al.*, 1985). In another study, Tokiwa *et al.* (1990) reported low values of 2-nitrofluorene in city gas and in heavy oil (see Table 1.1).

1.4.4 Exposure of the general population and in occupational settings

Zwirner-Baier & Neumann (1999) analysed blood samples for the presence of haemoglobin adducts of 2-aminofluorene, a metabolite of 2-nitrofluorene. The study comprised groups of garage workers, inhabitants of Southampton and inhabitants of small villages in the region (for details, see Scheepers *et al.*, 1999). The proportion of blood samples that contained haemoglobin adducts of 6-nitrofluorene in the three groups was 22/29, 14/20 and 10/14, respectively. The levels of adducts were all below than 0.17 pmol/g haemoglobin. The method of analysis was based on gas chromatography-negative ion chemical ionization-mass spectrometry (see Section 1.2 of

Table 1.2 Mean air concentrations of 2-nitrofluorene associated with particulate matter

Reference	Source	Type of sampling	No. of samples	Mean ± standard deviation (range) (in pg/m³)
Tanabe *et al.* (1986)	Urban (Tokyo, Japan)	PM	8	1.8; 27.2[a]
Tokiwa *et al.* (1990)	Urban (Sapporo, Japan)	PM	1	210
Schauer *et al.* (2004)	Urban (Munich, Germany)	GP	10	3.6 ± 3.0
	Rural (Munich, Germany)	GP	5	< 0.01
	Rural (alpine site)	GP	9	< 0.02
Albinet *et al.* (2006)	Rural (Maurienne Valley, France)	PM GP	13 13	0.2 (0.0–2.0) ND
Albinet *et al.* (2007)	Urban (Marseille, France)	GP + PM	12	21.4 (0.1–92.9)
	Suburban (La Penne sur Huveaune, France)	GP + PM	14	5.1 (ND–24.4)
	Rural (Plan d'Aups, France)	GP + PM	14	1.4 (ND–8.4)

[a] Not detected in six samples
GP, gas phase; ND, not detected; PM, particulate matter

the *Monograph* on Diesel and Gasoline Engine Exhausts in this Volume).

2. Cancer in Humans

No data were available to the Working Group.

3. Cancer in Experimental Animals

See Table 3.1

3.1 Mouse

Initiation/promotion studies

Groups of 20 or 25 female SENCAR mice [age unspecified] received a single topical application of 0 or 50–1500 µg of 2-nitrofluorene or 5–10 µg of 7,12-dimethylbenz[*a*]anthracene (positive control) in acetone followed by promotion with topical applications of 2 or 5 µg of 12-*O*-tetradecanoylphorbol-13-acetate twice a week for 13 or 19 weeks. Administration of 2-nitrofluorene did not result in the formation of skin papillomas, even at the highest dose, whereas the positive control induced the formation of many papillomas (Möller *et al.*, 1993a).

3.2 Rat

3.2.1 Oral administration

Six male and three female Minnesota rats [age unspecified] were fed a diet containing 2.37 mmol [500 mg]/kg of 2-nitrofluorene [purity unspecified] for 23 weeks, and were then placed on a basal diet until they developed tumours or became moribund. Three males and three females fed a basal diet served as controls. At necropsy (300 days), animals were analysed grossly and histologically for tumours. Two females (67%) in the treated group had one adenocarcinoma of the mammary gland and one squamous cell carcinoma in the ear duct. No tumours were observed in the controls (Morris *et al.*, 1950). [The Working Group noted that the small number of animals hampered an evaluation of the study.]

Nine male and nine female Holtzman rats [age unspecified] were fed a diet containing 1.62 mmol [342 mg]/kg of 2-nitrofluorene [purity unspecified] for 8 months, after which they were placed on a basal diet for 2 additional months and

Table 3.1 Studies of the carcinogenicity of 2-nitrofluorene in mice and rats

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, SENCAR (F) 13 or 19 wks Möller *et al.* (1993a)	Topical application Study 1: 0 (control), 10 µg DMBA (positive control), 50 or 150 µg 2-NF in acetone followed by TPA for 13 wks (2 µg) and an additional 7 wks (5 µg) Study 2: 0 (control), 5 µg DMBA (positive control), 500 or 1500 µg 2-NF in acetone followed by TPA for 19 wks 20 or 25 mice/group	The positive control had the expected incidence of skin tumours. No skin papilloma was observed in any 2-NF -treated group.	NS	The absence of initiation may have been due to a lack of metabolism to a putative metabolite.
Rat, Minnesota albino (F) 310 d Morris *et al.* (1950)	Oral administration (diet) 0 (control) or 500 mg/kg of diet (total estimated dose, 756 mg) for 23 wks then normal diet until moribund death or tumour development Control: 3 M, 3 F; 2-NF: 6 M, 3 F	Mammary gland (adenocarcinoma): F–0/3, 2/3 (67%) Ear duct (squamous cell carcinoma): M–0/3, 1/6 (17%)	NS	The number of animals was too small to evaluate the study effectively.
Rat, Holtzman (M, F) 10 mo Miller *et al.* (1955)	Oral administration 0 (control) or 1.62 mmol/kg of diet for 8 mo then normal diet for 2 mo Control: 18 M, 18 F; 2-NF, 9 M, 9 F	Mammary gland (all tumours): F–1/18 (6%), 4/9 (44%)* Liver (all tumours): M–0/18, 1/9 (11%) Small intestine (all tumours): M–0/18, 1/9 (11%)	*$P < 0.05$	
Rat, Holtzman (M) 12 mo Miller *et al.* (1955)	Oral administration 0 (control) or 1.62 mmol/kg of diet for 12 mo Control, 10; treated, 20	Forestomach (squamous cell carcinoma): 0/10, 17/18 (94%)* Liver (all tumours): 0/10, 13/18 (72%)* Ear duct (all tumours): 0/10, 4/18 (22%) Small intestine (epithelial): 0/10, 2/18 (11%) Mammary gland (all tumours): 0/10, 1/18 (11%)	*$P < 0.05$	Some tumour types not specified

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Rat, Wistar (M) 24 mo Cui *et al.* (1995)	Oral administration 0, 0.24, 0.95 or 2.37 mmol/kg of diet for 11 mo; surviving animals held for an additional 13 mo before necropsy, histological analysis and DNA adduct determination 18–20/group	Liver (hepatocellular carcinoma): 0/20, 2/18 (11%), 15/19 (79%)*, 20/20 (100%)** Forestomach (squamous cell carcinoma): 0/20, 10/18 (55%), 16/19 (84%), 10/20 (50%) Cortical kidney (renal cell carcinoma): 0/20, 1/18 (5%), 15/19 (79%)*, 11/20 (55%)	*$P < 0.01$ **$P < 0.001$	Purity, > 98%; high- and mid-dose animals died before end of experiment (high-dose animals within 10–13 months); body weight was reduced in the high-dose group. DNA adducts correlated with tumour locations.
Rat, Minnesota Albino (M, F) 310 d Morris *et al.* (1950)	Topical application 0 (control) or 3 drops in acetone, 3 ×/wk for 6 mo then 6 drops, 3 ×/wk (total dose, 69 mg) Control: 3 M, 3 F; treated: 7 M, 7 F	Mammary gland (carcinoma): M–0/3, 1/7 (14%) Adrenal gland (carcinoma): M–0/3, 3/7 (43%) Lung (lymphosarcoma): 0/3, 2/7 (28%) Skin (subcutaneous fibroma): M–0/3, 1/7 (14%) Salivary gland (anaplastic carcinoma): M–0/3, 1/7 (14%)	NS	The number of animals was too small to evaluate the study effectively.

d, day; DMBA, 7,12-dimethylbenz[*a*]anthracene; F, female; M, male; mo, month; 2-NF, 2-nitrofluorene; NS, not significant; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; wk, week

evaluated grossly and histologically for tumours. A group of 18 males and 18 females were fed the basal diet alone and served as controls. Four females in the treated group developed mammary gland tumours (44%) and one fibroadenoma was observed in a control female (1 out of 18). Most 2-nitrofluorene-treated rats developed multiple papillomas or squamous cell carcinomas in the forestomach (5 out of 7 males and 2 out of 2 females examined) (Miller *et al.*, 1955).

To confirm these findings, groups of 10 and 20 male Holtzman rats [age unspecified] were fed a basal diet containing 0 and 1.62 mmol/kg of 2-nitrofluorene [purity unspecified] for 12 months. In the treated group, 17 out of 18 (94%; $P < 0.05$) survivors had squamous cell carcinomas of the forestomach, 13 out of 18 (72%) had liver tumours ($P < 0.05$) [type not specified], 4 out of 18 (22%) had tumours of the ear duct, 2 out of 18 (11%) had tumours in the epithelium of the small intestine and 1 out of 18 (5%) had a tumour of the mammary gland. No tumours were observed in the control group (Miller *et al.*, 1955).

Groups of 18–20 male Wistar rats [age unspecified] were fed 0, 0.24, 0.95 or 2.37 mmol/kg of 2-nitrofluorene (purity, > 98%) in the diet for 11 months, and were then placed on a basal diet for an additional 13 months before gross or histological evaluation. The incidence of tumours was: hepatocellular carcinoma – 2 out of 18 (11%) low-dose, 15 out of 19 (79%; $P < 0.01$) mid-dose and 20 out of 20 (100%; $P < 0.01$) high-dose rats; forestomach squamous cell carcinoma – 10 out of 18 (5%) low-dose, 16 out of 19 (84%) mid-dose and 11 out of 20 (55%) high-dose rats; and cortical kidney [renal cell] carcinoma – 1 out of 18 (5%) low-dose, 15 out of 19 (79%; $P < 0.01$) mid-dose and 10 out of 20 (50%; $P < 0.05$) high-dose rats. No tumours were observed in the control animals (Cui *et al.*, 1995). [The Working Group noted that the high-dose animals died within 10–13 months.]

3.2.2 Skin application

Seven male and three female Minnesota rats [age unspecified] received a single topical application of 69 mg of 2-nitrofluorene [purity unspecified] and were then maintained on a basal diet and analysed grossly and histologically for tumours at approximately 300 days (approximate average life-span of the group). An untreated group of three males and three females served as controls. No tumours were observed in females or in the controls. In the males, 1 out of 7 (14%) had mammary gland carcinoma, 3 out of 7 (43%) had adrenal gland carcinoma, 2 out of 7 (28%) had lung lymphosarcoma, 1 out of 7 (14%) had subcutaneous fibroma and 1 out of 7 (14%) had anaplastic carcinoma of the salivary glands. The incidence of these tumours was not statistically significant compared with controls (Morris *et al.*, 1950). [The Working Group noted that the small number of animals hampered an evaluation of the study.]

4. Mechanistic and Other Relevant Data

4.1 Absorption, distribution, metabolism, excretion

The metabolism of 2-nitrofluorene *in vivo* has been reviewed extensively (Möller, 1988, 1994).

4.1.1 Humans

No data were available to the Working Group.

4.1.2 Experimental systems

(a) In-vivo studies

In Sprague-Dawley rats treated with a single oral dose of 1 mg/kg body weight (bw) of 2-nitro[9-^{14}C]fluorene, 2-nitrofluorene was excreted rapidly in the urine and faeces; 60%

was excreted in the urine and 30% in the faeces (Möller *et al.*, 1985, 1987a). The major free products (unconjugated) were 5-hydroxy (OH)- and 7-OH-2-acetylaminofluorene (5- and 7-OH-2-AAF), but also *N*-, 1, 3-, 8- and 9-OH-2-AAF were identified as metabolites (Möller *et al.*, 1985, 1987a). No difference in the excretion profiles of 5- and 7-OH-2-AAF was observed in rats pretreated intraperitoneally with β-naphthoflavone, an inducer of cytochrome P450 (CYP) 1A1/2; however, the relative proportion of excreted hydroxylated 2-nitrofluorenes increased substantially after treatment with β-naphthoflavone (Möller *et al.*, 1987a).

Similar experiments were performed in conventional and germ-free AGUS rats (single oral dose of 5 mg of [^{14}C]2-nitrofluorene per rat) (Möller *et al.*, 1988). The metabolism of 2-nitrofluorene was similar in AGUS and Sprague-Dawley rats (Möller *et al.*, 1987a, 1988). However, the excretion profiles of metabolites in germ-free animals differed considerably from those in conventional animals. Whereas 7- and 5-OH-2-AAF were the major metabolites of 2-nitrofluorene in conventional animals (3- and 1-OH-2-AAF were excreted as minor metabolites), five different hydroxylated 2-nitrofluorenes were observed in the excreta from germ-free animals (Möller *et al.*, 1988). Hydroxylated 2-nitrofluorenes were also observed in another study of germ-free Wistar rats that received 1 mmol/kg bw of 2-nitrofluorene, whereas very little OH-2-AAF was detected in the urine (Scheepers *et al.*, 1994a).

The metabolism of 2-nitrofluorene was studied in isolated perfused rat lung and liver (Möller *et al.*, 1987b). The compound was metabolized in the lung to hydroxylated 2-nitrofluorenes, mainly 9-hydroxy-2-nitrofluorene. After intratracheal administration, 2-nitrofluorene was rapidly excreted into the perfusate, indicating that other organs might be exposed to the unmetabolized compound. In the liver, hydroxylated 2-nitrofluorenes were further conjugated to glucuronides and were excreted in the bile. Mutagenicity was only observed in the bile after treatment with β-glucuronidase, suggesting that biliary 2-nitrofluorene glucuronides could be hydrolysed by the β-glucuronidase expressed in the colon (Möller *et al.*, 1987b). Törnquist *et al.* (1990) compared the absorption of 2-nitrofluorene in isolated, perfused and ventilated lungs of treated rats with that in control animals and animals pretreated with β-naphthoflavone. The clearance of intratracheally instilled 2-nitrofluorene increased approximately fourfold after treatment with β-naphthoflavone.

The pharmacokinetics of 2-nitrofluorene was studied in Wistar rats with cannulated bile ducts after intravenous administration of a single oral dose of 6 μmol/kg bw of ^{3}H-labelled 2-nitrofluorene (Mulder *et al.*, 1990). 2-Nitrofluorene was removed from the blood in a biphasic manner: the initial removal was very rapid, with a half-time of 2.5 minutes, which was followed by a much slower phase (half-time > 2.5 hours). Excretion *via* the bile was also biphasic, with half-time values of 9 minutes and 1 hour, respectively. After 2 hours, approximately 40% of the 2-nitrofluorene dose had been excreted in the bile. In contrast, the excretion of 2-nitrofluorene and its metabolites in urine was low (20% within 24 hours), possibly due to reabsorption of the parent compound or its metabolites from the gut and subsequent enterohepatic recirculation (Mulder *et al.*, 1990). The glucuronide conjugate of 9-hydroxy-2-nirofluorene was identified as the major metabolite.

The time-dependent metabolism of 2-nitrofluorene was studied after a single intraperitoneal injection of 1 mmol/kg bw of 2-nitrofluorene in Sprague-Dawley rats. In contrast to previous studies of the metabolism of 2-nitrofluorene following its oral administration, OH-AAFs (*N*-, 1-, 3-, 5-, 7- or 9-OH-2-AAF), 2-AAF and 2-aminofluorene were not detected in the urine by high-performance liquid chromatography or nuclear magnetic resonance analysis. After hydrolysis with β-glucuronidase/arylsulfatase,

five hydroxylated 2-nitrofluorenes were isolated and structurally identified as *trans*-6,9-dihydroxy-2-nitrofluorene, and 6- , 7-, 8- and 9-hydroxy-2-nitrofluorene. Furthermore, two conjugated metabolites were identified as 6- and 7-[(hydroxysulfonyl)oxy]-2-nitrofluorene (Castañeda-Acosta *et al.*, 1997).

The acylated derivative, 2-formylaminofluorene (2-FAF), was identified in rats and albino rabbits treated with 2-nitrofluorene (Tatsumi & Amano, 1987). 2-FAF was formed by the *N*-formylation of 2-aminofluorene catalysed by liver formamidase in the presence of *N*-formyl-L-kynurenine. The metabolism of 2-nitrofluorene, 2-aminofluorene, 2-FAF and 2-AAF was compared in rats and dogs (Ueda *et al.*, 2001a). 2-AAF and its hydroxylated derivatives, 5- and 7-OH-2-AAF, were the major metabolites identified in the urine and faeces of rats, but FAF and its hydroxylated derivatives, 5- and 7-hydroxy-2-FAF, were mainly excreted in dogs.

The metabolism of 2-nitrofluorene *in vivo* was also studied in goldfish after exposure to 5 mg/L for 2 days (Ueda *et al.*, 2001b); the metabolites identified were 7-OH-2-AAF, 7-hydroxy-2-aminofluorene, 2-FAF, 2-AAF and 2-aminofluorene.

(b) In-vitro studies

The metabolism of 2-nitrofluorene in human lung tissue was studied after its incubation with surgical resectate containing adenocarcinoma and normal surrounding tissue (Gøtze *et al.*, 1994). 9-Hydroxy-2-nitrofluorene was detected as the main metabolite.

After the incubation of 2-nitrofluorene with rat pulmonary microsomes, 9-hydroxy-2-nitrofluorene was the predominant metabolite formed (Törnquist *et al.*, 1990). In microsomes isolated from rats pretreated with β-naphthoflavone, the formation of 9- and X-hydroxy-2-nitrofluorene increased significantly. In lung microsomes, the formation of X-hydroxy-2-nitrofluorene was inhibited *in vitro* by the addition of α-naphthoflavone, an inhibitor of CYP1A1/2. Anti-P4502B1-immunoglobulin G inhibited the formation of 9-hydroxy-2-nitrofluorene, whereas that of X-hydroxy-2-nitrofluorene was unaffected (Törnquist *et al.*, 1990), indicating that CYP2B1 catalyses the hydroxylation of 2-nitrofluorene at the 9-position.

The metabolism of 2-nitrofluorene was studied *in vitro* in isolated rat lung cells (Gøtze *et al.*, 1994). The main metabolites in rat lung Clara cells and rat alveolar type II cells were 9- and X-hydroxy-2-nitrofluorene. The formation of 9-hydroxy-2-nitrofluorene was efficiently catalysed by CYP2B1 in Clara cells and increased with the age-dependent up-regulation of CYP2B1 in the lung (Törnquist *et al.*, 1988). Alveolar type II cells showed a preferentially formed X-hydroxy-2-nitrofluorene and metabolism increased after pretreatment of the rats with β-naphthoflavone.

The reduction of 2-nitrofluorene and 9-hydroxy-2-nitrofluorene in the rat mammary gland was investigated *in vitro* (Ritter *et al.*, 2000). Cytosolic fractions catalysed the nicotinamide adenine dinucleotide- and nicotinamide adenine dinucleotide phosphate (NADPH)-dependent formation of 2-aminofluorene and 9-hydroxy-2-aminofluorene, respectively. Nitroreduction was primarily catalysed by a xanthine oxidase and partially by a diaphorase (Ritter *et al.*, 2000). Similar results were obtained in rat skin, in which the reduction of 2-nitrofluorene was mainly catalysed by xanthine oxidase (Ueda *et al.*, 2003). Skin cytosols from various mammals including rabbits, hamsters, guinea-pigs, mice and rats all exhibited significant nitroreductase activity towards 2-nitrofluorene (Ueda *et al.*, 2005). The species differences reflected differences of relative aldehyde oxidase and xanthine oxidase activities.

In liver preparations of sea bream (*Pagrus major*), 2-nitrofluorene was effectively reduced to 2-aminofluorene by CYP enzymes or aldehyde oxidase (Ueda *et al.*, 2002). 2-Aminofluorene,

2-AAF and 2-FAF were oxidized to their 7- or 5-hydroxy derivatives by CYPs.

4.2 Genetic and related effects

The genetic and related effects of 2-NF and some of its metabolites have been reviewed (Beije & Möller, 1988a; Purohit & Basu, 2000;. IPCS, 2003).

4.2.1 Humans

No data were available to the Working Group.

4.2.2 Experimental systems

(a) Formation of DNA and protein adducts

(i) In-vivo studies

See Fig 4.1

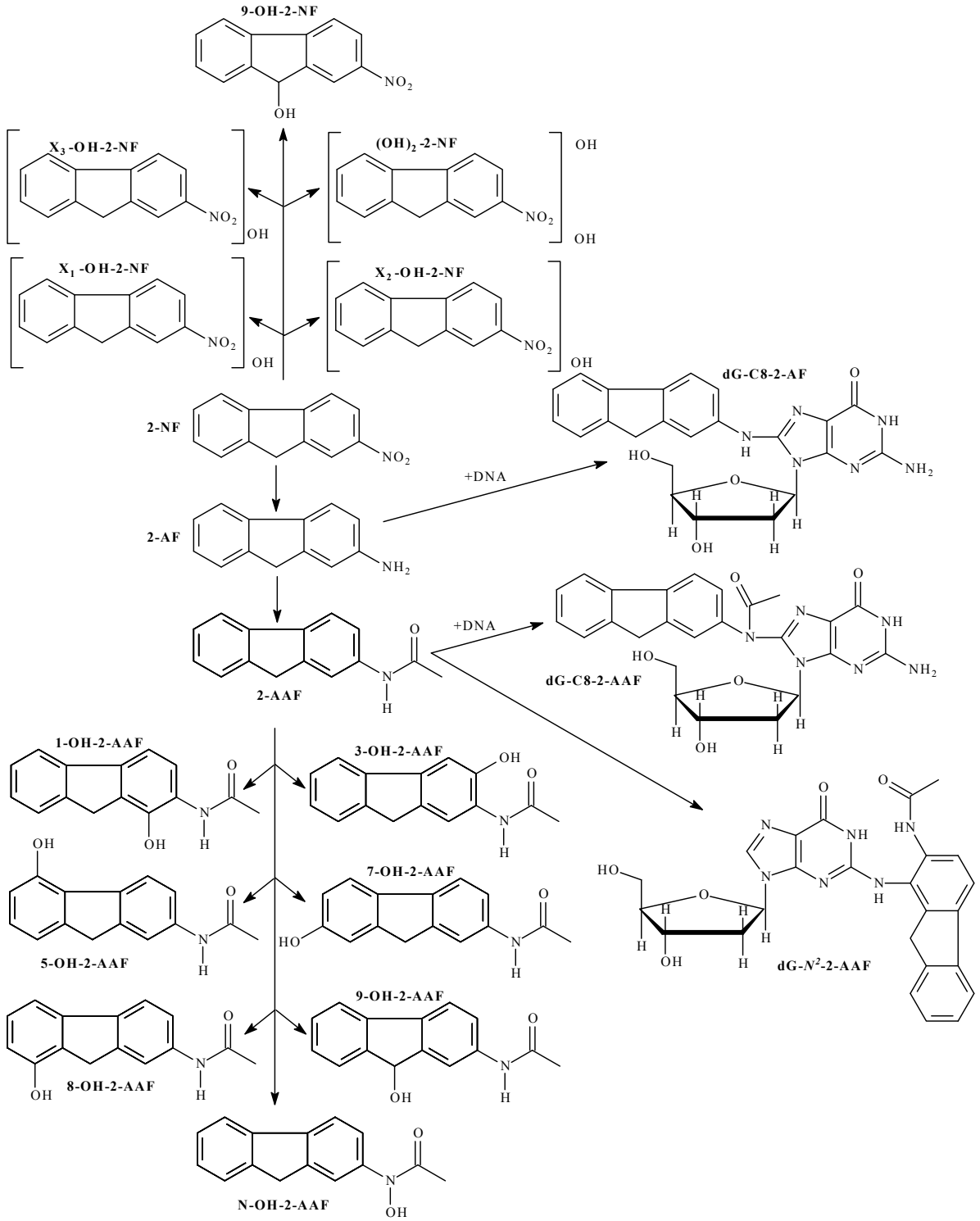
[^{3}H]-Labelled 2-nitrofluorene (a single oral dose of 6 µmol/kg bw) was used to demonstrate DNA binding in the liver, kidney, forestomach and gut mucosa of Wistar rats (Wierckx *et al.*, 1990). The formation of DNA adducts by 2-nitrofluorene (measured using the ^{32}P-postlabelling method) was predominantly studied in rats (Möller *et al.*, 1993b; Möller & Zeisig, 1993; Cui *et al.*, 1995, 1999). Four major DNA adducts were detectable (adducts A–D). DNA adduct D was chromatographically indistinguishable from *N*-(deoxyguanosin-8-yl)-2-aminofluorene (dG-C8-2-AF) and DNA adduct C co-migrated with C3-(deoxyguanosin-N^2-yl)-2-acetylaminofluorene (dG-N^2-2-AAF), whereas adducts A and B could not be identified.

In Wistar rats treated with 2-nitrofluorene, the formation of DNA adducts was dose- and time-dependent, with higher levels in tumour target tissues (liver, kidney and forestomach) compared with non-tumour target organs (heart, spleen and glandular stomach) (Cui *et al.*, 1995, 1999). Animals were treated with 0.24–2.4 mmol/kg of 2-nitrofluorene in the diet and were killed after 1, 2, 6 and 10 days or 11 months after continuous feeding. The four 2-nitrofluorene-induced DNA adducts showed different kinetics of formation and persistence. A and B were the major DNA adducts detected after 11 months of feeding. All of the adducts persisted after the cessation of treatment, and adducts A and C (i.e., dG-N^2-2-AAF) were only repaired to a minor extent (Cui *et al.*, 1999). Another study in which Wistar rats were treated with 200 mg/kg bw of 2-nitrofluorene for 48 hours showed that oral administration was more potent in the formation of DNA adducts than intraperitoneal administration (Möller *et al.*, 1993b). Nuclear magnetic resonance spectroscopy and restrained molecular dynamics showed that the presence of dG-N^2-2-AAF in DNA increased the thermal and thermodynamic stability of duplex DNA (Zaliznyak *et al.*, 2006), which has important implications for the recognition of this adduct by the DNA-repair machinery and may explain its persistence in rat tissue DNA *in vivo*.

The role of the intestinal microflora on the formation of 2-nitrofluorene-induced DNA adducts was investigated in several studies. Conventional and germ-free AGUS rats were treated with a single oral dose of 35 mg/kg bw of 2-nitrofluorene for 48 hours, and DNA adduct formation was analysed in the liver, kidney, lung and heart by ^{32}P-postlabelling. The presence of intestinal microflora enhanced the formation of DNA adducts in all tissues examined, and dG-C8-2-AF was the major adduct detected (Möller *et al.*, 1994). Similar results were obtained in rats and mice with human microflora (Scheepers *et al.*, 1994a; Hirayama *et al.*, 2000).

Wistar rats received an intraperitoneal injection of 100 mg/kg bw of 2-nitrofluorene, or 5-, 7- or 9-hydroxy-2-nitrofluorene and were killed 72 hours later (Cui *et al.*, 1996). 2-Nitrofluorene and 9-hydroxy-2-nitrofluorene had similar potency for the formation of DNA adducts, while 5- and 7-hydroxy-2-nitrofluorene generated approximately fourfold less DNA adducts. The ^{32}P-thin-layer chromatography autoradiograms revealed

Fig. 4.1 Metabolism of and formation of DNA adducts by 2-nitrofluorene



2-AAF, 2-acetylaminofluorene; 2-AF, 2-aminofluorene; 2-NF, 2-nitrofluorene; 9-OH-2-NF, 9-hydroxy-2-NF; dG-C8-2-AAF, *N*-(deoxyguanosin-8-yl)-2-AAF; dG-C8-2-AF, *N*-(deoxyguanosin-8-yl)-2-aminofluorene; dG-N^2-2-AAF, C3-(deoxyguanosin-N^2-yl)-2-AAF; X-OH-2-AAF, X-hydroxy-2-AAF (X = 1, 3, 5, 7, 8, or 9); X-OH-2-NF, X-hydroxy-2-NF (X = 1,2,3)

that 2-nitrofluorene and 9-hydroxy-2-nitrofluorene showed three adduct spots with similar positions on the chromatogram. 7-Hydroxy-2-nitrofluoene showed four weak DNA adduct spots while 5-hydroxy-2-nitrofluorene did not induce a distinct adduct spot pattern.

^{3}H-Labelled 2-nitrofluorene (a single oral dose of 6 μmol/kg bw) was used to demonstrate protein binding *in vivo* in Wistar rats (Wierckx *et al.*, 1990). After oral administration of 1 mmol/kg bw of 2-nitrofluorene, the number of haemoglobin adducts in the blood was low compared with administration of 2-aminofluorene (5.1 ± 0.6 versus 0.04 ± 0.01 μmol/g haemoglobin) and no haemoglobin adducts were detected in the blood in the absence of intestinal microflora (Scheepers *et al.*, 1994a, b).

(ii) In-vitro studies

DNA binding by ^{3}H-labelled 2-nitrofluorene was catalysed by rat hepatic microsomes and was CYP-dependent (Wierckx *et al.*, 1990). 2-Nitrofluorene binding was also observed in rat hepatocytes and a higher degree of binding was found in those pretreated with Aroclor 1254, an inducer of various CYP isoenzymes, indicating that DNA-reactive intermediates of 2-nitrofluorene are formed by CYP enzymes (Wierckx *et al.*, 1990).

(b) Mutagenesis of 2-nitrofluorene

In-vitro studies

Urinary mutagenicity in 2-nitrofluorene-treated Sprague-Dawley rats was observed in *Salmonella typhimurium* strain TA98 (in the presence or absence of an exogenous metabolic activation system) and was associated with a range of OH-2-AAFs in addition to several hydroxy-2-nitrofluorenes (Möller *et al.*, 1987a, 1989). The mutagenicity (measured in TA98 in the presence or absence of an exogenous metabolic activation system), in particular the direct-acting mutagenicity, detected in excreta from 2-nitrofluorene-treated germ-free rats was considerably higher than that in the excreta from conventional 2-nitrofluorene-treated animals (Möller *et al.*, 1988). 9-Hydroxy-2-nitrofluorene was mainly liable for the total urinary mutagenicity, whereas 2-nitrofluorene was only responsible for a small part (~2%). Thus, the 2-nitrofluorene metabolite that mainly contributes to its direct-acting mutagenicity is formed in germ-free animals or by metabolic routes in which microflora are not involved (Möller *et al.*, 1987a, 1988).

When evaluated previously (IARC, 1989), more than 200 independent reports were already available on the mutagenicity of 2-nitrofluorene in *S. typhimurium*, because the compound was often used as positive reference compound. These studies gave generally positive results in the standard *S. typhimurium* tester strains TA97, TA98, TA100, TA1538 or TA1978, and these results have been confirmed in more recent studies (Jurado *et al.*, 1994; Nohmi *et al.*, 1995; Cui *et al.*, 1996; Hughes *et al.*, 1997; Suzuki *et al.*, 1997; Yamada *et al.*, 1997). When TA98 was compared with the corresponding nitroreductase-deficient strain, TA98NR, the mutagenicity of 2-nitrofluorene decreased, while stronger mutagenicity (~12-fold) was observed in TA98 with high nitroreductase activity (TA98NR+), compared with the standard tester strain (Hagiwara *et al.*, 1993). In strain TA98AT+, which overexpresses *O*-acetyltransferase, 2-nitrofluorene showed a ~20-fold increase in mutagenicity compared with TA98. Thus, nitroreduction followed by *O*-acetylation appears to be the mutagenic activation pathway of 2-nitrofluorene in *S. typhimurium* (Hagiwara *et al.*, 1993). The importance of nitroreduction followed by *O*-acetylation in 2-nitrofluorene-induced mutagenicity has also been observed in other studies (Watanabe *et al.*, 1989, 1990). It has also been suggested that part of the mutagenicity of 2-nitrofluorene in *Salmonella* is related to oxidative stress (i.e., formation of 8-hydroxydeoxyguanosine), because *Salmonella* strains that are deficient in 8-oxo-guanine DNA glycosylase were shown to

exhibit enhanced mutagenicity when incubated with 2-nitrofluorene (Suzuki *et al.*, 1997).

The bacterial mutagenicity of 2-nitrofluorene, and 5-, 7- and 9-hydroxy-2-nitrofluorene was compared in strain TA98 (in the absence of an exogenous metabolic activation system) (Cui *et al.*, 1996). 5- and 9-Hydroxy-2-nitrofluorene were less mutagenic (two- to four-fold) compared with 2-nitrofluorene (absence of an exogenous metabolic activation system), while 7-hydroxy-2-nitrofluorene was twice as mutagenic as 2-nitrofluorene. 2-Nitrofluorene, and 5- and 7-hydroxy-2-nitrofluorene were stronger mutagens in the absence of an exogenous metabolic activation system, while 9-hydroxy-2-nitrofluorene had a reverse pattern and displayed stronger mutagenicity in the presence of metabolic activation.

Additional studies also confirmed the mutagenicity of 2-nitrofluorene in *Escherichia coli* (Kranendonk *et al.*, 1996; Hoffmann *et al.*, 2001).

2-Nitrofluorene induced mutations in mouse lymphoma L5178Y $Tk^{+/-}$ cells (Amacher *et al.*, 1979; Oberly *et al.*, 1984, 1996). In contrast, no mutagenicity by 2-nitrofluorene was found at the thymidine kinase locus in human B-lymphoblastoid cells that constitutively express CYP1A1 (designated h1A1v2) (Durant *et al.*, 1996). 2-Nitrofluorene gave positive results in the hypoxanthine-guanine phosphoribosyltransferase (*Hprt*) forward mutation assay in Chinese hamster ovary cells (Oberly *et al.*, 1990), but did not induce mutations at the *Hprt* locus in Chinese hamster V79 cells with (V79NH) or without (V79-MZ) endogenous acetyltransferase activity that stably express human *CYP1A2* (Kappers *et al.*, 2000).

Chromosomal aberrations were observed in Chinese hamster lung cells treated with 2-nitrofluorene, which was more clastogenic in the presence than in the absence of an exogenous metabolic activation system (Matsuoka *et al.*, 1991). 2-Nitrofluorene induced micronuclei in Chinese hamster V79 cells, rat epithelial intestinal IEC-17 and IEC-18 cells, mouse BALB/c 3T3 cells and embryonic human liver HuFoe-15 cells (Glatt *et al.*, 1990; Gu *et al.*, 1992). 2-Nitrofluorene showed higher clastogenicity in Chinese hamster lung cells that express bacterial *O*-acetyltransferase or human *N,O*-acetyltransferase (1 or 2) (Watanabe *et al.*, 1994).

(c) Other genetic effects of 2-nitrofluorene

(i) In-vivo studies

Studies reviewed in the previous *Monograph* (IARC, 1989) are summarized below. Oral administration of 2-nitrofluorene (125–500 mg/kg bw) to Chinese hamsters induced sister chromatid exchange in bone-marrow cells, while intraperitoneal injection of 50–200 mg/kg bw had no effect. 2-Nitrofluorene did not induce micronuclei in the bone marrow of treated mice, or unscheduled DNA synthesis in the liver of rats treated orally with up to 50 mg/kg bw. In the mouse host-mediated recombination assay, 2-nitrofluorene (at 125–1600 mg/kg bw) induced mutation in *S. typhimurium*, but not recombination in *Saccharomyces cerevisiae* D3. Treatment of *E. coli* with 2-nitrofluorene induced binding of cellular DNA to the bacterial envelope, while conflicting results were reported regarding its ability to induce prophages in *E. coli*. 2-Nitrofluorene preferentially inhibited the growth of DNA repair-deficient *E. coli* and *Bacillus subtilis* (rec assay) (IARC, 1989).

In more recent studies, oral treatment of Fischer 344 rats with 2-nitrofluorene (125 mg/kg bw) caused significant micronucleus formation in the liver, but not in the bone marrow (Parton & Garriott, 1997), while micronuclei were not detected in the peripheral reticulocytes isolated from ddY mice treated intraperitoneally (80 mg/kg bw) (Murakami *et al.*, 1996).

The expression profile induced by 2-nitrofluorene in the liver was examined using Affimetrix GeneChip technology after the treatment of Wistar rats with 44 mg/kg per day

for 1, 3, 7 and 14 days, a dose known to induce liver tumours in a 2-year rat bioassay (Ellinger-Ziegelbauer *et al.*, 2005). Cellular pathways affected were related to oxidative stress/DNA-damage response, and the activation of proliferative and survival signalling. Interestingly, 2-nitrofluorene deregulated genes that comprised cellular responses to various forms of oxidative stress, including the target genes of the reactive oxygen species-response transcription factor Nrf-2, NADPH:quinone oxidoreductase, haeme oxygenase 1, glutathione-synthase (light chain)) and other genes known to be induced under oxidative stress conditions (e.g., epoxide hydrolase 1) (Ellinger-Ziegelbauer *et al.*, 2005), indicating that oxidative stress might be a tumour promoter in 2-nitrofluorene-induced carcinogenesis.

(ii) In-vitro studies

2-Nitrofluorene induced DNA damage in *S. typhimurium* (SOS *umu* test) (Oda *et al.*, 1992, 1993, 1996) and *E. coli* (SOS chromotest) (IARC, 1989; Mersch-Sundermann *et al.*, 1991, 1992), and inhibited DNA synthesis in HeLa cells. Conflicting results were obtained in the unscheduled DNA synthesis assay: one study reported that 2-nitrofluorene gave positive results in mouse and rat hepatocytes, whereas another showed a negative response in rat hepatocytes. 2-Nitrofluorene induced sister chromatid exchange in cultured Chinese hamster ovary and V79 cells in the presence of an exogenous metabolic activation system. It induced morphological transformation in Syrian hamster embryo cells co-cultured with hamster hepatocytes (IARC, 1989).

In human lymphoblastoid MCL-5 cells that express high levels of native *CYP1A1* and that carry *CYP1A2*, *CYP2A6*, *CYP3A4* and *CYP2E1* as cDNA in plasmids, 2-nitrofluorene showed a positive response in the alkaline single-cell gel electrophoresis (comet) assay, in the presence or absence of the DNA-repair inhibitors hydroxyurea or cytosine arabinoside (Martin *et al.*, 1999). In contrast, it did not cause any marked DNA damage (measured by the alkaline elution technique) in isolated rabbit lung cells (Becher *et al.*, 1993).

4.3 Other relevant data

2-Nitrofluorene showed estrogenic activity (measured in an estrogen reporter assay using estrogen-responsive yeast and human breast cancer MCF-7 cells) after incubation with liver microsomes from of 3-methylcholanthrene-treated rats in the presence of NADPH, whereas no estrogenic activity was observed in liver microsomes from untreated or phenobarbital-treated rats (Fujimoto *et al.*, 2003). 7-Hydroxy-2-nitrofluorene was the main metabolite identified in liver microsomes isolated from 3-methylcholanthrene-treated rats, whereas little of the metabolite was formed in those of untreated or phenobarbital-treated rats. Rat recombinant *CYP1A1* catalysed the formation of 7-hydroxy-2-nitrofluorene which appears to be responsible for the estrogenic activity of 2-nitrofluorene.

4.4 Mechanistic considerations

2-Nitrofluorene can enter an oxidative or a reductive pathway (Möller *et al.*, 1987a, b, 1988). 2-Aminofluorene and 2-AAF, which are both carcinogenic in rodents (Heflich & Neft, 1994), are the main reductive metabolites that are formed after oral administration of 2-nitrofluorene and are mediated by the intestinal microflora; 2-nitrofluorene is mainly excreted as OH-2-AAFs in urine and faeces (Möller *et al.*, 1988). A minor metabolic pathway results in the formation of hydroxylated nitrofluorenes after oral administration of 2-nitrofluorene to conventional rats; hydroxylated nitrofluorenes are major metabolites in germ-free rats after oral administration and in perfusates from isolated

rat lung and liver (Möller *et al.*, 1987b, 1988). While OH-2-AAFs show low mutagenic activity, hydroxylated nitrofluorenes, although not detected individually, appear to be the mutagenic metabolites of 2-nitrofluorene (Beije & Möller, 1988b; Möller *et al.*, 1988).

2-Nitrofluorene induces the formation of DNA adducts *in vivo*, one of which has been characterized as dG-C8-2-AF (Wierckx *et al.*, 1990; Möller *et al.*, 1993b; Möller & Zeisig, 1993). Together with other known 2-aminofluorene- and 2-AAF-related DNA adducts (i.e., dG-C8-2-AF and dG-N^2-2-AAF), these lesions are suspected to be pre-mutagenic (Heflich & Neft, 1994). The formation of these DNA adducts suggests that nitroreduction, the first step of which is its conversion to 2-aminofluorene, is a major route for the metabolic activation of 2-nitrofluorene, and appears to be catalysed mainly by the cytosolic nitroreductase xanthine oxidase and partially by a diaphorase (Ritter *et al.*, 2000). After oral administration of 2-nitrofluorene to rats, dG-C8-AF was the major adduct detected in liver while dG-C8-AAF was found as a minor adduct (Möller & Zeisig, 1993).

2-Nitrofluorene is both an initiator and a promoter of preneoplastic liver lesions in rats (Möller *et al.*, 1989), and resulted in tumour formation in different organs when administered to rats orally or by topical application (Miller *et al.*, 1955; Cui *et al.*, 1995). In addition to some common target organs, such as the liver and kidney, the forestomach appears to be a prime target organ for 2-nitrofluorene when compared with 2-aminofluorene and 2-AAF (Miller *et al.*, 1955). This difference could possibly be related to genotoxic hydroxylated derivatives of 2-nitrofluorene, such as 7- and 9-hydroxy-2-nitrofluorene, which are not formed after exposure to 2-aminofluorene or 2-AAF (Cui *et al.*, 1995). Tumour development and DNA adduct formation were dose- and time-dependent, and the levels of DNA adducts within the first days during a 2-year bioassay correlated with the localization of tumours, indicating that the formation of DNA adducts is an important factor for tumour development (Cui *et al.*, 1995).

5. Summary of Data Reported

5.1 Exposure data

Diesel exhaust was identified as the primary source of exposure for 2-nitrofluorene. No evidence was found that it has been produced in commercial quantities or used for purposes other than laboratory applications. 2-Nitrofluorene was detected in both the gas phase and particulate phase of air samples collected at urban locations in the low picagram per cubic metre range, but was not detected in air sampled at rural and remote locations. Analyses of the blood and urine indicated that workers in some occupations and the general population are exposed.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

The carcinogenesis of 2-nitrofluorene was evaluated in one initiation–promotion study by topical application in mice, and after oral administration in the diet in three studies and topical application in one study in rats. It caused squamous cell carcinomas of the forestomach and hepatocellular carcinoma in two separate feeding studies and cortical kidney (renal cell) carcinoma in one study in rats. Two studies of dietary exposure and topical application in rats were not included in the evaluation due to the small numbers of animals tested and insufficient methodological details. The results of the initiation–promotion study were negative.

5.4 Mechanistic and other relevant data

No data on the absorption, distribution, metabolism and excretion or genetic and related effects of 2-nitrofluorene in humans were available to the Working Group. When given to rats, the compound was rapidly excreted as a mixture of hydroxylated metabolites of aminofluorene and acetylaminofluorene produced by nitroreduction and *O*-acetylation. These metabolites were mutagenic in bacteria. 2-Nitrofluorene induced mutations in mouse lymphoma cells and had cytogenetic effects in experimental animals. The nitroreduction of 2-nitrofluorene, which is catalysed by cytosolic nitroreductases, is the major activation pathway that leads to the formation of DNA-binding metabolites and DNA adducts. The major adduct detected in the liver, i.e., the target organ for carcinogenesis, after oral administration was deoxyguanosin-8-yl-2-aminofluorene, although 2-acetylaminofluorene-related DNA adducts may also contribute to the mutagenic potency of 2-nitrofluorene. When administered to experimental animals orally or by topical application, 2-nitrofluorene caused tumour formation in different organs, including the liver, kidney and forestomach, in a time- and dose-dependent manner, which was linked with the increased formation of DNA adducts. The initial levels of DNA adducts correlated well with the location of tumours.

Collectively, these data provide *strong evidence* in animals and *weak evidence* in humans that the formation of DNA adducts plays an important role in the tumour development induced by 2-nitrofluorene.

6. Evaluation

6.1 Cancer in humans

There is *inadequate evidence* in humans for the carcinogenicity of 2-nitrofluorene.

6.2 Cancer in experimental animals

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

6.3 Overall evaluation

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

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1-NITROPYRENE

1-Nitropyrene was evaluated by a previous IARC Working Group in 1988 (IARC, 1989). New data have since become available, and these have been taken into consideration in the present evaluation.

1. Exposure Data

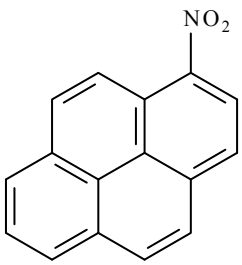
1.1 Chemical and physical data

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 5522-43-0

IUPAC Name: 1-Nitropyrene

1.1.2 Structural and molecular formulae and relative molecular mass



$C_{16}H_9NO_2$

Relative molecular mass: 247.25

1.1.3 Chemical and physical properties of the pure substance

Description: Yellow needles or prisms when crystallized from ethanol at room temperature (Luckenbach, 1980)

Melting-point: 155 °C (Luckenbach, 1980); 153 °C (Yaffe *et al.*, 2001)

Boiling-point: 472 °C at 101.3 kPa (Yaffe *et al.*, 2001)

Vapour pressure: 4.4×10^{-6} Pa at 20 °C (Yaffe *et al.*, 2001)

Octanol/water partition coefficient: Log $P_{o/w}$ 4.69 (Yaffe *et al.*, 2001)

Sorption coefficient: Log K_{oc} 4.48 (Yaffe *et al.*, 2001)

Henry's law constant: 6.4×10^{-2} kPa/m^3/g/mol at 25 °C (Yaffe *et al.*, 2001)

Spectroscopy data: Electron impact mass spectral data on ninefold deuterated 1-nitropyrene (1-nitro[$^2H_9$]pyrene) have been reported (Fatiadi & Hilpert, 1989).

Solubility: Soluble in water (0.017 mg/L; Yaffe *et al.*, 2001), ethanol, benzol and acetic acid (Luckenbach, 1980)

Reactivity: Reacts with ethanolic potassium hydroxide to form 1,1′-azoxypyrene; also reacts with zinc powder in ethanol in the presence of catalytic amounts of ammonium chloride or ammonia to form 1,1′-azoxypyrene or, in the absence of air, 1-aminopyrene and 1-hydroxylaminopyrene (Boit *et al.*, 1965).

Stability: Readily decomposes on exposure to ultraviolet/visible light (Stärk *et al.*, 1985; Holloway *et al.*, 1987). Fan *et al.* (1996) reported half-lives of 0.8 hour for native and 0.5 hour for 1-nitro[$^{2}H_{9}$]pyrene adsorbed on diesel exhaust or wood smoke particles when exposed to sunlight in a smog chamber under ambient conditions.

1.1.4 Technical products and impurities

1-Nitropyrene is produced by the nitration of pyrene, the impurities of which are presumed to be dinitropyrenes. High-purity 1-nitropyrene is produced as a reference material for analytical determinations (see Section 1.3). 1-Nitro[$^{2}H_{9}$]pyrene of high isotopic purity was synthesized by Fatiadi & Hilpert (1989) and is commercially available at a purity of 98% from two companies in Germany and the USA. Native 1-nitropyrene and 1-nitro[$^{2}H_{9}$]pyrene are produced by several companies for use as an internal standard. Radiolabelled isotopes such as [^{14}C]- or [^{3}H]1-nitropyrene have been synthesized by research laboratories (el-Bayoumy & Hecht, 1984) or were acquired from commercial suppliers for use in animal studies.

1.2 Determination in air and analysis

1.2.1 Air sampling

1-Nitropyrene may be present at very low concentrations in the gas phase (Albinet *et al.*, 2006). To determine 1-nitropyrene adsorbed onto particles, a filter is used to collect the particles from diluted exhaust fumes or ambient air. During sampling, two types of artefact may occur: (a) conversion of the pyrene trapped on the filter surface to 1-nitropyrene and (b) conversion of the pyrene adsorbed onto particles to 1-nitropyrene (Grosjean *et al.*, 1983). This conversion was first observed on glass fibre membrane filters but not on polytetrafluoroethylene. To prevent the conversion of pyrene to 1-nitropyrene, pure polytetrafluorene (Teflon) or Teflon-coated filters can be used. Alternatively, 1-nitropyrene from diesel engine exhaust can be analysed in tailpipe deposits (Paschke *et al.*, 1992).

The amount of 1-nitropyrene adsorbed to the surface of diesel exhaust particles did not change after exposure to pure air, 100 ppb of ozone and 100 ppb of sulfur dioxide or 100 ppb of nitrogen dioxide (Grosjean *et al.*, 1983), suggesting that the influence of atmospheric conditions on the formation and stability of 1-nitropyrene is limited. However, when diesel exhaust particles are captured on a filter, the pyrene adsorbed on soot particles may still be nitrated (Butler & Crossley, 1981). The extent of the artificial formation of 1-nitropyrene was estimated to be below 20% when the duration of sampling engine exhaust in a dilution tunnel of a chassis dynamometer was less than 1 hour (Schuetzle, 1983; Hartung *et al.*, 1986; Lies *et al.*, 1986); however, due to the low temperatures and low concentrations of nitrating species, sampling of ambient air may last more than 4–8 hours (Dolan & Kittelson, 1979).

1.2.2 Analysis

More details on air sampling and the analysis of nitroarenes in general can be found in Section 1.2.2(d) of the *Monograph* on Diesel and Gasoline Engine Exhaust in this Volume.

To analyse 1-nitropyrene, three approaches have generally been applied: high-performance liquid chromatography (HPLC) with fluorescence detection, HPLC with chemiluminescence detection and gas chromatography (GC) or liquid chromatography with different types of mass spectrometry (MS)-based detection. Immunoaffinity methods have also been used with some success (Zühlke *et al.*, 1998).

An important step in the quantitative determination of 1-nitropyrene is extraction from the particles deposited on a filter. Acetone and

dichloromethane have been used to extract 1-nitropyrene from diesel exhaust particles, and sonication with acetone yielded quantitative extracts from standard reference material (SRM) 1650 (Jäger, 1978; Scheepers *et al.*, 1993), but good results were also obtained using other techniques such as supercritical fluid or Soxhlet extraction (Paschke *et al.*, 1992).

A small number of methods use the direct analysis of 1-nitropyrene by GC with electron capture (Jinhui & Lee, 2001), MS (Gorse *et al.*, 1983; Albinet *et al.*, 2006) or chemiluminescence detection (Hayakawa *et al.*, 1995). The National Institute of Occupational Safety and Health method 2560 is based on GC with chemiluminescence detection, with a working range of 0.020–50 µg and a sensitivity of 0.021–104 µg/m^3 for a 500-L sample, but is only suitable for samples with extremely high levels that are rarely encountered, even in indoor workplaces. More sensitive methods involve the reduction of the nitro group by bacterial nitroreductases or chemical nitroreduction. This step can be performed off-line using sodium hydrosulfide hydrate (Hisamatsu *et al.*, 1986) or online as part of the HPLC analysis using a zinc and glass granulate column (Scheepers *et al.*, 1993; Hayakawa *et al.*, 1996) or platinum/rhodium-coated aluminium (Murahashi *et al.*, 2003; Ohno *et al.*, 2009). The detection of 1-aminopyrene by fluorescence detection is highly sensitive, but that with chemiluminescence is more sensitive by approximately one order of magnitude (Murahashi *et al.*, 2003) and approaches the sensitivity accomplished by the use of MS. To enhance the sensitivity of detection using GC-MS, 1-aminopyrene is derivatized with heptafluoro butyric anhydride (Scheepers *et al.*, 1993). A small amount of deuterated 1-nitro[$^2H_9$]pyrene with high isotopic purity can be applied to the raw sample extract as an internal standard to adjust for the recovery in the reduction and derivatization steps. This analysis uses both retention time in capillary GC and the MS/MS spectrum to verify the identity of and was able to determine the lowest concentrations of 1-nitropyrene detected in airborne particulate matter (PM) to date (> 1 pg/m^3) (Scheepers *et al.*, 1993; Albinet *et al.*, 2006; Miller-Schulze *et al.*, 2007). Recently, a deuterated internal standard was also used in an HPLC analysis with fluorescence detection (Ohno *et al.*, 2009).

Certified SRMs for 1-nitropyrene are available from the National Institute of Standards and Technology (NIST) in Gaithersburg, USA: SRM 1648 (atmospheric PM collected in St. Louis, MO, in 1978; NIST, 1998), SRM 1649a (atmospheric PM collected in an urban area in Washington DC in the late 1970s; NIST, 2001), SRM 1650b (diesel exhaust PM collected from a heat exchanger; NIST, 2006) and SRM 2975 (industrial forklift; NIST, 2000). Albinet *et al.* (2006) reported values of 1-nitropyrene for each of these SRMs based on liquid chromatography-MS/MS analysis.

1.3 Production and use

The synthesis of 1-nitropyrene by heating pyrene with nitric acid and water was first reported by Graebe in 1871 (IARC, 1984). 1-Nitropyrene was also produced in a mixture with dinitropyrenes following the addition of potassium nitrite to pyrene in diethyl ether (Prager & Jacobson, 1922). More recently, Boit *et al.* (1965) described its synthesis by heating pyrene in nitric acid and glacial acetic acid. At present, 1-nitropyrene of up to 99% purity is produced in small amounts for use as a reference standard in chemical analysis, and is also available as a certified reference material (BCR305; 99% pure). Deuterated 1-nitro[$^2H_9$]pyrene is produced for use as an internal standard, and tritium-labelled 1-nitropyrene is produced for use in animal studies.

1.4 Occurrence

1.4.1 Diesel and gasoline exhaust emissions

1-Nitropyrene is the most abundant nitroarene in diesel engine emissions (Bamford *et al.*, 2003), and its formation is facilitated by the high temperatures and excess air supply in the combustion chamber of diesel engines, where it is generated by the addition of nitrogen oxide or nitrogen dioxide to free pyrene radicals (carbonium ions) (Schuetzle & Perez, 1983). Du & Kittelson (1984) observed a fourfold increase in 1-nitropyrene from the cylinder to the exhaust manifold, and Olah *et al.* (1981) also suggested that polycyclic aromatic hydrocarbons (PAHs) reacted with nitric acid in the exhaust manifold. When a diesel engine was run on nitrogen-free air, the amount of 1-nitropyrene produced was much lower (Herr *et al.*, 1982). The addition of pyrene to a pure *n*-hexadecane fuel resulted in increased emissions of 1-nitropyrene and other nitroarenes (Henderson *et al.*, 1984). Concentrations of 1-nitropyrene measured in emissions from vehicles that were placed on a chassis dynamometer are presented in Table 1.1, and showed wide variation, depending on the type of engine, the make of car and the driving cycle used. Passenger cars tended to have higher emissions of 1-nitropyrene during driving cycles when the engine was hot compared with those when the engine was cold (at the start of a driving pattern) (Scheepers *et al.*, 2001).

It has been suggested that the formation of 1-nitropyrene is dependent on the production of nitrogen dioxide in the combustion chamber, which is normally generated at much higher levels under elevated combustion temperatures. However, the formation of soot and PAHs tends to decrease at higher speeds (Steenlage & Rijkeboer, 1985), and the formation of 1-nitropyrene is therefore difficult to predict. Moreover, engines that have an indirect fuel injection system, which are used in light-duty vehicles such as passenger cars, small power generators and forklifts, yield somewhat higher levels of 1-nitropyrene in diesel exhaust particles by weight than direct-injection engines with which heavy-duty vehicles are usually equipped (Table 1.1; Yamaki *et al.*, 1986). 1-Nitropyrene was found in the vicinity of heavy-duty road traffic (heavy goods vehicles [HGVs] and buses), the platforms of airports (push-back tractors, tanker HGVs and luggage transport trains), railway tracks (diesel trains used for surface operations and underground mining), military or agricultural vehicles and excavation in the building and construction industry (surface and underground operations) (Scheepers *et al.*, 1993, 1994a, b, 1995a). Diesel-powered engines are also used in shipping, but few data were available on the emissions of ships' engines (Scheepers *et al.*, 1995a).

Several studies have measured the levels of 1-nitropyrene in emissions from diesel-powered road and off-road vehicles, such as forklifts, electric power supplies and lawn mowers (Table 1.2). Some earlier studies (Salmeen *et al.*, 1982; Schuetzle *et al.*, 1982) [not presented in Table 1.2] reported levels of 1-nitropyrene in emissions from light-duty passenger cars (mean per vehicle tested) in the range of 3.9–14.2 μg/g of total suspended fraction. Gibson (1982) reported levels of 55–2280 μg/g of soluble organic extract of 1-nitropyrene in emissions from light-duty passenger car models from 1978 to 1982.

Different types of current passenger car that were run on commercial fuel under conditions of the California unified driving cycle were tested on a dynamometer, together with used crankcase oil 'as received' (Zielinska *et al.*, 2004); some influence of the year of the model of car and testing conditions was observed.

The use of biodiesel and an oxidation catalyst was studied (Sharp *et al.*, 2000) using the heavy-duty transient Federal Test Procedure (FTP). In three separate heavy-duty diesel engines (one 1995 and two 1997 models), different fuels were used: neat biodiesel fuel (B100), a blend of biodiesel and normal diesel fuel 20:80 by

Table 1.1 1-Nitropyrene in exhaust emissions generated by simulated driving cycles on a chassis dynamometer

Reference	Type of car	Driving cycle or pattern	No. of samples or of driving cycles	Year	1-NP (AM ± SD)	
					µg/g	µg/km
Gasoline-powered engines						
Gibson (1982)	4.3-L 8-cylinder car with its catalyst removed using unleaded gasoline	23-min hot-start portion of the FTP	3	1981	4.3 ± 3.2	0.06 ± 0.06[a]
	2.5-L 4-cylinder catalyst car	23-min hot-start portion of the FTP	2	1980	0.63 ± 052	0.029[a]
	5.7 -L 8-cylinder precatalyst car using leaded gasoline	23-minute hot-start portion of the Federal Test Procedure	2	1974	3.9 ± 1.3	0.11 ± 0.033
Zielinska *et al.* (2004)	Mazda Millenia, Ford Explorer, Nissan Maxima, GMC 1500 Pickup, Mercury Sable	*Normal PM emitters*				
		California United Driving Cycle at 72 F	18	1982–96		0.016[a]
		California United Driving Cycle at 30 F	12	1992–96		0.03[a]
	Ford F-150 pick-up	Gasoline black smoker; California United Driving Cycle at 72 F	5	1976		0.19[a]
	Mitsubishi Montero	Gasoline white smoker; California United Driving Cycle at 72 F	2	1990		0.13[a]
Light-duty diesel-powered engines						
Gibson *et al.* (1981)	Oldsmobile	FTP	1	1978	3.9	–
			1	1978	8.2	–
Gibson (1982)	General motors 5.7-L 8-cylinder production model	23-min hot-start portion of the FTP	4	1980	8.0 ± 2.4	2.0 ± 0.75[a]
	General motors 5.7-L diesel car equipped with an experimental tube-type trap coated with ceramic fibres	23-min hot-start portion of the FTP	2	1980	14.2	0.75[a]
Gibson (1983)	Oldsmobile	FTP	1	1982	24.5	–
			1	1982	7.6	–
	Opel	FTP	1	1978	3.9	
Gorse *et al.* (1983)	Not specified	FTP	2	–		4.6
		Highway Fuel Economy Test	2	–		4.2
Scheepers *et al.* (1994a)	Not specified	European Driving Cycle (cold start)	1	1990		0.32
		European Driving Cycle (hot start)	1	1990		0.22
		US'75 Driving Cycle	1			0.36

Table 1.1 (continued)

Reference	Type of car	Driving cycle or pattern	No. of samples or of driving cycles	Year	1-NP (AM ± SD)	
					µg/g	µg/km
Scheepers *et al.* (2001)	French make (Citroen, Peugeot, Renault)	Urban Driving Cycle	7	1996	2.2 ± 0.6	–
		Urban Driving Cycle (hot start)	7	1996	5.1 ± 1.5	
		Extra-urban driving cycle	7	1996	10.4 ± 4.9	–
	German make (Opel)	Urban Driving Cycle	4	1996	2.5 ± 0.6	–
		Urban Driving Cycle (hot start)	4	1996	3.2 ± 0.8	–
		Extra-urban driving cycle	4	2001	7.4 ± 4.9	–
	Japanese make (Mazda)	Urban Driving Cycle	5	2001	21.2 ± 1.2	–
		Urban Driving Cycle (hot start)	5	2001	21.4 ± 6.9	–
		Extra-urban driving cycle	5	2001	91.4 ± 36.8	–
	American make (Chrysler)	Urban Driving Cycle	1	2001	6.7	
		Urban Driving Cycle (hot start)	1	2001	8.1	–
		Extra-urban driving cycle	1	2001	33.5	–
	German make (Mercedes)	FTP – cold transition period (0–505 s)	3	2001	6.0 ± 3.1	–
		FTP – stabilized period (505–1372 s)	3	2001	16.7 ± 9.1	–
		FTP – hot transition period (1372–1877 s)	3	2001	28.2 ± 4.7	–
	Japanese make (Nissan)	FTP – cold transition period (0–505 s)	5	2001	28.4 ± 15.8	–
		FTP – stabilized period (505–1372 s)	5	2001	18.5 ± 10.1	–
		FTP – hot transition period (1372–1877 s)	5	2001	44.9 ± 22.9	–
Bamford *et al.*(2003)	Diesel engine exhaust from industrial forklift (SRM2975)	Not specified	3	–	39.64 ± 1.7	–
	Diesel engine exhaust PM (SRM1975)	Not specified	3		16.07 ± 0.59	–
	Diesel engine exhaust PM (SRM1650a)	Collected from a heat exchanger	3	-	18.33 ± 0.34	–
Zielinska *et al.* (2004)	Dodge Ram 2500 Pickup, Mercedes Benz E300, Volkswagen Beetle TDI	Current technology diesel engine; California United Driving Cycle at 72 F	9	1998–2000		3.13[a]
	Dodge Ram 2500 Pickup	High PM emitter; California United Driving Cycle at 72 F	6	1991		1.77[a]
	Dodge Ram 2500 Pickup, Mercedes Benz E300, Volkswagen Beetle TDI	Current technology diesel engine; California United Driving Cycle at 30 F	6	1998–2000		6.21[a]

Table 1.1 (continued)

Reference	Type of car	Driving cycle or pattern	No. of samples or of driving cycles	Year	1-NP (AM ± SD)	
					µg/g	µg/km
Heavy-duty diesel-powered engines						
Draper (1986)	Mining engine	100% load, 1200 rpm	1	–	< 12	–
		75% load, 1800 rpm	1	–	5.0	–
Scheepers *et al.* (1994a)	Not reported	Suburban	1	1990	1.92	2.13
			1	1990	1.94	3.28
		Urban	1	1990	3.39	6.48
		Motorway	1	1990	1.94	0.87
			1	1990	2.73	1.37
Westerholm *et al.* (2001)	Volvo FH12 truck with D12A 420 diesel engine equipped with turbo, intercooler and electronic fuel-injection system (complies with WHO Regional Office for Europe 2 requirements)	Transient driving cycle for buses; engine fuelled with EPEFE reference fuel CEC-RF-73-A93	4	–	-	0.026 ± 0.04
		Transient driving cycle for buses; engine fuelled with a Swedish Environmental Classified diesel fuel (MK1)	4	–	–	< 0.005

[a] Converted from µg/mile by the Working Group

AM, arithmetic mean; EPEFE, European Programme on Emissions, Fuels and Engine Technologies; FTP, Test Procedure; 1-NP, 1-nitropyrene; PM, particulate matter; rpm, revolutions per minute; SD, standard deviation; SRM, standard reference material; TDI, Turbocharged Direct Injection; WHO, World Health Organization

Table 1.2 Concentrations of 1-nitropyrene in particulate matter emitted from diesel-powered engines at fixed locations

Reference	Description of source	Location and conditions	Year	No. of samples	1-NP (μg/g)		
					Respirable dust (μg/g)	Total suspended particles (μg/g)	Soluble organic fraction (μg/g)
Scheepers *et al.* (1994a)	Forklift truck in concrete production plant	Indoor; Netherlands	1992	2	1.9; 4.2	3.2 ± 2.8 (n = 12)	–
	Forklift truck in chemical plant	Indoor; Netherlands	1992	2	5.6; 7.0		–
	Forklift truck in aluminium rolling	Indoor; Netherlands	1992	2	5.4; 7.7		–
	Ship's aggregate in river vessel	Outdoo; Netherlands; wind speed 8–9 m/s	1992	1	9.1	7.6	11.9
	Lawn mowers in gardening	Outdoor; Netherlands; wind speed 5 m/s	1992	1	–	0.099	0.33
Rappaport *et al.* (1982)	Long distance road truck	4-stroke; 6-cylinder engine	1979–80	6	–	–	< 2 – 44
Nakagawa *et al.* (1983)	Bus	Isuzu BY30 at 1200 rpm (idle)	1970	1	70.5	30[a]	–
Scheepers *et al.* (1994a)	Train engine	Repair shop for diesel-powered trains; engines entered the workshop with a cold engine in the morning and exhaust was emitted during engine test runs; exhaust scavenging system did not have sufficient capacity.	1992	3	6.6 ± 1.6	1.3 ± 0.8	6.0 ± 2.6
			1994	3	–	7.4 ± 1.9[a]	–
	Ship's engine	Outdoor; Netherlands; wind speed 8–9 m/s	1992	3	–	0.97 ± 0.57	2.0 ± 0.94
	Airport platform vehicle	Outdoor; Netherlands; wind speed 6–13 m/s	1992	3	–	0.60 ± 0.23	1.4 ± 0.45
	Armoured cars	Military driving lessons facility			0.11 ± 0.0014	0.40 ± 0.025	
Yamazaki *et al.* (2000)	2.8-L diesel engine	Sample collected from the tailpipe of an idling engine	1993				4.3 pmol/mg
	2.5-L diesel engine		1996				29 pmol/mg
	7.4-L diesel engine		1989				63 pmol/mg

[a] Calculated by the Working Group for comparative purposes from data in the reference

1-NP, 1-nitropyrene; rpm, revolutions per minute

Table 1.3 Emissions from heavy-duty vehicles with alternative fuels, particle filter and catalyst

Vehicle (engine)	Substance	B100 (100% biofuel[a]) (in ng/hp-h)			B20 (20% biofuel[a] in conventional diesel)		2D (100% conventional diesel fuel)	
Oxidation catalyst		–	–	+	–	+	–	+
Urban transit bus (DDC Series 50, 205 kW, n = 1)[b]	2-Nitrofluorene	48	40	14	70	67	88	90
	1-Nitropyrene	8.5	5.2	37	19	249	83	76
	6-Nitrochrysene	< 0.5	ND	1.8	0.8	4.0	0.8	5.8
Full-size pick-up truck (Cummins B5.9, 119 kW, n = 1)[c]	2-Nitrofluorene	142	122	73	–	365	257	478
	1-Nitropyrene	34	20	325	265	1644	210	2171
	6-Nitrochrysene	< 0.5	< 0.5	6.2	1.9	58	11	56

[a] Methyl ester from virgin soya bean oil (AG Environment Products)
[b] Low sulfur #2 diesel fuel (Chevron Phillips) and engine equipped with exhaust gas recirculation system for reduction of nitrogen oxides
[c] Ultra-low sulfur certified diesel fuel (Chevron Phillips) and engine equipped with exhaust gas recirculation system for reduction of nitrogen oxides, a crankcase emissions coalescer for nitrogen oxides and reduction of particulate matter and a two-stage particle filter consisting of a ceramic flow-through monolith diesel oxidation catalyst and a wall-flow monolithic catalysed soot filter
hp, horse power; ND, not detected
From Sharp *et al.* (2000)

volume (B20) and neat diesel fuel (2D). In the exhaust from B100, the formation of 1-nitropyrene was between 4.7 and 34 ng/horse power (hp)–h in the three engines (see Table 1.3). The addition of an oxidation catalyst increased the emission of 1-nitropyrene from one of the three engines to 325 ng/hp-h. One of the 1997 model engines run on B20 and 2D produced 19 and 83 ng/hp-h of 1-nitropyrene, respectively. The addition of a catalyst increased the values to 249 and 76 ng/hp-h for B20 and 2D, respectively. The 1995 model engine produced levels of 265 and 210 ng/hp-h of 1-nitropyrene with B20 and 2D, respectively, which increased to 1644 and 2171 ng/hp-h, respectively, after the addition of an oxidation catalyst.

Liu *et al.* (2010) compared two similar 15-L heavy-duty engines [not further specified] in an FTP cycle: a 2004 model equipped with a system to reduce nitrogen oxides and a 2007 model fitted with a reduction system for nitrogen oxides, a crankcase emission reducer of nitrogen oxides and a two-stage particle filter (diesel exhaust catalyst and catalysed soot filter). The level of 1-nitropyrene emitted from the 2004 model engine was 55 ± 6.44 ng/hp-h but was below the limit of quantification (0.25 ng/hp-h) in the 2007 model engine.

1.4.2 Exhaust fumes from gasoline-powered cars

Gasoline-powered spark ignition engines are less liable to emit nitroarenes than diesel engines. Alsberg *et al.* (1985) analysed different fractions of PAHs, including the polar fractions, but did not report 1-nitropyrene as a constituent in the extracts of particulate fractions of exhausts from gasoline-powered cars. Murahashi *et al.* (2003) reported a single measurement of 1-nitropyrene in the exhaust from a gasoline-powered vehicle [type of vehicle and driving cycle unspecified]. The emission of particles was low (0.01 g/km) and the amount of 1-nitropyrene was reported to be 0.02 µg/km compared with an [unspecified] diesel-powered vehicle that emitted 3.0 µg/km of 1-nitropyrene (also a single observation with a particle emission of 0.36 g/km). Gorse *et al.* (1983) determined that the on-road emission of 1-nitropyrene was < 0.03 µg/km in the Allegheny Mountain Tunnel, PA, USA, for predominantly light-duty gasoline passenger cars (of which 74% were estimated to be equipped with catalysts).

1.4.3 Aircraft exhaust

McCartney *et al.* (1986) observed elevated mutagenic activity in extracts from PM collected close to the runway of an airport, suggesting that nitrated mutagens are emitted by airplanes. [The Working Group noted that no data of chemical analysis were available to support this statement, and it was also not clear to what extent road traffic at the airport, remote sources of traffic or nitroarenes derived from atmospheric photochemistry may have contributed to the observed mutagenicity.]

1.4.4 Liquefied petroleum gas, gas burners and kerosene heaters

1-Nitropyrene, and 1,6- and 1,8-dinitropyrene in fumes produced by burning propane (or a mixture of gases including methane), using a Bunsen burner, co-eluted with standards in GC frame thermionic detection (Tokiwa *et al.*, 1985). A strong mutagenic response in the *Salmonella typhimurium* TA97 assay in the absence of an exogenous metabolic activation system was observed in the fraction that containing the reported nitroarenes. However, 1-nitropyrene was not detected in these samples [limit of detection not reported], or observed in kerosene heaters. In contrast, Kinouchi *et al.* (1988) detected 1-nitropyrene in the emissions from kerosene heaters using HPLC with fluorescence detection (see Table 1.4).

1.4.5 Emissions from industrial processes

Following the observation of elevated mutagenicity of extracts of carbon black-based photocopy toners (Löfroth *et al.*, 1980), 1-nitropyrene was found as a trace contaminant in toners, copiers and furnace carbon black produced before 1980 (Rosenkranz *et al.*, 1980; Ramdahl & Urdal, 1982). This was apparently the result of the production process used in 1967, which involved an oxidation step that resulted in the nitration of pyrene. After this discovery, the content of 1-nitropyrene was reduced from 5–100 µg/g to > 0.3 µg/g (Rosenkranz *et al.*, 1980; Sanders, 1981).

1-Nitropyrene was purported to be formed by waste incinerators (Gibson, 1982) and has been identified in coal fly ash (Harris *et al.*, 1984). Indirect evidence for the presence of nitroarenes, including 1-nitropyrene, was provided by the positive results of extracts from the emissions of a municipal-waste incinerator in the *Salmonella* mutagenicity assay (DeMarini *et al.*, 1996). Williams *et al.* (1986) did not detect 1-nitropyrene in roofing tar or remains from a coke oven, while another study reported levels of 27 µg/g of 1-nitropyrene in extracts of coke oven emissions (Topinka *et al.*, 1998).

1.4.6 Crankcase oils and wastewater

1-Nitropyrene was detected in crankcase oil at extremely high levels (138 µg/L of oil) and in water from oil–water separating tanks in gasoline stations at a range of < 0.00025–25.6 µg/L (Manabe *et al.*, 1984).

1.4.7 Surface water

1-Nitropyrene was detected at very low levels in river and seawater (Murahashi *et al.*, 2001). The highest values were observed in wet precipitations and were attributed to airborne particulates (see Table 1.4).

1.4.8 Food contamination and preparation

Table 1.5 summarizes levels of 1-nitropyrene in a variety of dietary products. The highest values were reported for products that may be contaminated by the deposition of particulates in outdoor air pollution (spices and different types of tea), through food preparation, such as the grilling of fish or meat, or during growing and further treatment (e.g. roasted tea) (Kinouchi *et al.*, 1986; Ohnishi *et al.*, 1986; Schlemitz & Pfannhauser, 1996a, b). High concentrations of 1-nitropyrene were found in fumes from different cooking oils at a range of 0.9–3.4 µg/m^3 (Table 1.4; Wu *et al.*,

Table 1.4 Concentrations of 1-nitropyrene in air/particulate matter and water from non-diesel sources

Reference	Source	Method of sampling and conditions	Method of analysis	No. of samples	1-NP (µg/g extracted PM)	1-NP (ng/m³)
Gibson (1982)	Wood fire smoke (burning of red oak in a fire place)	25-fold diluted flue gas sampled with a high volume sampler, collecting an unspecified fraction of PM	HPLC-fluorescence according to Gibson *et al.* (1981)	2	0.09 0.012	–
Thrane & Stray (1986)	Aluminium reduction plant	Unspecified fraction of particles collected on glassfibre filters by high-volume sampling in the potroom of a Söderberg electrode aluminium reduction plant	GC-MS analysis using a negative ion chemical ionization MS	1	–	64
Topinka *et al.* (1998)	Coke oven emissions	Total suspended particles collected on the top-side of the coke oven battery by high volume sampling on PTFE coated glassfibre filters	GC-MS analysis according to Scheepers *et al.* (1994a)	1	27	–
Taga *et al.* (2005)	Emissions from coal burning	Unspecified fraction of particle collected from the chimney of a domestic coal stove on a glassfibre filter	HPLC analysis with chemiluminescence detection	1	[240]	–
Kinouchi *et al.* (1988)	Indoor use of kerosene heater	Continuous sampling for 8 h	HPLC analysis with nitroreduction using enzymatic nitroreductase and fluorescence detection according to Manabe *et al.* (1984)	1	–	0.147
		21 samples of 20 min each at the beginning of burning with intermittent intervals of 20 min during which the room was ventilated		1	–	1.62
		Continuous sampling for 7 h with a latency of 1 h after lighting the heater during which the room was ventilated		1	–	0.044
Tokiwa *et al.* (1985)	Kerosene heater	Unspecified fraction of particles collected on XAD-2 resin for 2 h at an air flow rate of 20 L/min	Co-elution with standards in a GC analysis with frame thermionic detection according to Møller & Alfheim (1983)	2	ND	
	Gas[a] burned in a Bunsen burner			2	20.6	
	LPG (almost entirely propane) burned in a Bunsen burner			1	1.88	

Table 1.4 (continued)

Reference	Source	Method of sampling and conditions	Method of analysis	No. of samples	1-NP (µg/g extracted PM)	1-NP (ng/m³)
Murahashi *et al.* (2001)	River water	Midstream from Asano River, Suzumi, Kanazawa, Japan	HPLC with chemiluminescence detection according to Murahashi & Hayakawa (1997)	5		*pg/L* 1 2 3 5 27
	Seawater	Collected at seashore of the Sea of Japan at Kanaiwa, Kanazawa, Japan		2		0.2 0.5
	Precipitation (rain)	Collected on roof top in residential area of Kanazawa, Japan		2		370 3200

[a] 4–20% methane, 40–45% hydrogen, 10% carbon monoxide, 3–4% oxygen, 5–25% nitrogen, 3–4% butane and traces of pentane, isopentane and propane

GC, gas chromatography; HPLC, high-performance liquid chromatography; LPG, liquefied petroleum gas; min, minute; MS, mass spectrometry; 1-NP, 1-nitropyrene; ND, not detected; PM, particulate matter; PTFE, polytetrafluoroethylene; XAD, polymeric resin

Table 1.5 Occurrence of 1-nitropyrene in food stuffs and beverages

Reference	Category	Food stuff or beverage	No. of samples	1-NP (µg/g)
Schlemitz & Pfannhauser (1996a)	Vegetables and nuts	Lettuce	–	< 0.2
		Parsley	–	1.7
		Carrot	–	0.4
		Peanuts	–	< 0.5
	Spices and herbs	Paprika	–	9.3
		Marjoram	–	14.1
		Caraway	–	10.9
Spitzer *et al.* (2000)	Herbs	Basil	1	0.0014
		Chervil	1	0.0001
		Marjoram	2	0.0002; 0.0004
		Oregano	1	0.0014
		Sage	1	0.0001
Wu *et al.* (1998)	Cooking oils	Lard oil	3	1.1 ± 0.1[a]
		Soya bean oil	3	2.9 ± 0.3[a]
		Peanut oil	3	1.5 ± 0.1[a]
Schlemitz & Pfannhauser (1996b)	Milk products	Alp-cheese I	3	ND
		Alp-cheese II	3	ND
		Smoked cheese	3	ND
Ohnishi *et al.* (1986)	Fish	Grilled fish	3	< 0.00003–0.00035
		Grilled mackerel	1	0.45
Ohnishi *et al.* (1986)	Meat	Bacon		0.012
		Beef with sauce	1	0.00050
Ohnishi *et al.* (1986)	Chicken meat	Chicken (Yakitori)	1	0.00009
		Chicken white with sauce	1	0.00151
		Chicken (Yakitori), grilled for 3 min	1	0.0038
		Chicken (Yakitori), grilled for 5 min	1	0.019
		Chicken (Yakitori), grilled for 7 minute	1	0.043
Ohnishi *et al.* (1986)	Pork meat	Pork	1	0.00066
		Pork with sauce	1	0.00313

Table 1.5 (continued)

Reference	Category	Food stuff or beverage	No. of samples	1-NP (µg/g)
Schlemitz & Pfannhauser (1996a, b)	Pork meat	Grilled meat (pork)	–	1.0
		Grilled sausages	3	1.4
		Smoked meat (pork)	–	2.2
		Smoked sausages	–	4.2
		Roasted meat (pork)	3	0.3
Schlemitz & Pfannhauser (1996a, b)	Turkey meat	Roasted turkey		ND
Schlemitz & Pfannhauser (1997)	Tea	Assam	3	2.32
		Earl grey	3	7.75
		Ceylon	3	1.54
		Darjeeling	3	4.00
		Mate (roasted)	3	37.89
		Mate (green)	3	0.80
		Formaosa Sencha (green)	3	3.10
		Nettle leaf	3	1.96
		Peppermint	3	3.79
		Fennel (instant)	3	ND
		Fruit (instant)	3	0.55

[a] Fumes from cooking oils in µg/m^3

1-NP, 1-nitropyrene; ND, not detected

1998), and lower levels were detected in grilled food (< 0.03–11.90 ng/g).

1.4.9 Tobacco smoke

The presence of nitroarenes in cigarette-smoke condensate is improbable because the combustion of tobacco during smoking is reductive. 1-Nitropyrene was not found in cigarette smoke using analytical methods with a limit of detection of 1 ng/cigarette (el-Bayoumy *et al.*, 1985) or 10 pg on-column (Williams *et al.*, 1986), or in five commercial brands of cigarette with a limit of detection of 30 pg/cigarette (Scheepers *et al.*, 2001).

1.5 Exposure

1.5.1 Exposure of the general population

(a) Ambient air

After 2-nitrofluoranthene, 1-nitropyrene is the most abundant of 28 mononitro- and dinitro-PAHs identified in airborne PM (Bamford *et al.*, 2003). Its occurrence in the ambient air originates primarily from combustion sources (Atkinson *et al.*, 1991), and in particular from diesel engines (Arey *et al.*, 1986; Zielinska *et al.*, 1986). Photochemical formation in the atmosphere was reported for 2-nitropyrene, but not for 1-nitropyrene (Arey *et al.*, 1987).

An extensive overview of ambient air concentrations was made available (IPCS, 2003), the most recent reports from which are presented below (Table 1.6). Air concentrations of 1-nitropyrene appeared to be associated with sources from urban areas, and more specifically with traffic sources. No 1-nitropyrene was detected in Antarctica or Nepal (Ciccioli *et al.*, 1995). The lowest concentrations detected (< 2 pg/m^3) were observed at 'remote' locations, such as nature reserves in Brazil, the Netherlands, the United Kingdom and the USA (Arey *et al.*, 1988; Ciccioli *et al.*, 1995). Concentrations in suburban and urban areas varied over several orders of magnitude. The highest outdoor concentrations were reported in cities with heavy traffic such as Algiers, Damascus, Milan, Santiago and Tokyo (Tokiwa *et al.*, 1983; Tanabe *et al.*, 1986; Cecinato *et al.*, 1998; Yassaa *et al.*, 2001). Only a few studies reported indoor levels of 1-nitropyrene in homes. Geometric mean indoor concentrations were reported to be 0.67 pg/m^3 in urban residences in Southampton (United Kingdom) and 0.28 pg/m^3 in rural residences in small villages (Scheepers *et al.*, 1999).

(b) Human tissues

Tokiwa *et al.* (1993) retrieved tissues from 137 Japanese nonsmokers (97 men and 40 women) whose cause of death had been registered as lung cancer (squamous cell, small cell and adenocarcinoma), and compared them with 21 specimens from lung cancers with similar histology recovered from Chinese women (aged 28–64 years) who had lived in Wuyuan County, an area with known higher mortality from lung cancer than other regions, and had been farmers and cooks, primarily exposed to soot derived from indoor heating and cooking using coal. The mean 1-nitropyrene content (± standard deviation) per gram of lung tissue of these women (5.9 ± 2.4 pg/g) was much lower than the levels observed in the Japanese subjects (21.3 ± 12.4 pg/g). In contrast, the concentration of benzo[*a*]pyrene was higher in the Chinese women (608.7 ± 447.1 pg/g) than in the Japanese subjects (180 ± 103.7 pg/g).

Toriba *et al.* (2007) developed a liquid chromatography-MS/MS method to determine the urinary metabolites of 1-nitropyrene that was used on urine samples from 17 men and five women living in the city of Kanazawa, Japan (no occupations associated with diesel exhaust emissions or smoking status were reported). Five metabolites of 1-nitropyrene were found (see Section 4.1.1), mostly as glucuronide or sulfate conjugates, including 117, 109, 203, 137 and ≤ 0.54 pmol/mol of creatinine of 6- and 8-hydroxy-*N*-acetyl-1-aminopyrene, 6- and 8-hydroxy-1-nitropyrene and 3-hydroxy-1-nitropyrene, respectively.

Table 1.6 Concentrations of 1-nitropyrene in ambient air

Reference	Location	Description	Season and/or year	No. of samples	1-NP (μg/g) (AM ± SD)	1-NP (ng/m³)
Industrial						
Morita *et al.* (1982)	Japan	Industrial area	–	1		0.0208
Gibson (1986)	River Rouge, MI, USA	Heavy industrial site	Summer 1982	5	0.59 ± 0.56	0.057
	Dearborn, MI, USA	Heavy industrial site	Summer 1980	8	0.15 ± 0.13	0.029
Atkinson *et al.* (1988)	Yuba City, USA	Biomass burning	Autumn 1986	3	–	0.009
	Concord, USA	-	Winter 1986–87	5	–	0.029
	Mammoth Lakes, USA	Wood smoke	Winter 1987	1	–	0.008
	Oildale, USA	Oil production	Summer 1987	3	–	0.007
Yassaa *et al.* (2001)	Oued Smar, Algeria	Landfill	Winter 1999	1	–	0.080
			Summer 1998	1	–	< 0.01
Urban–suburban						
Gibson (1982)	Detroit, MI, USA	Suburban	Spring and summer 1981	2	0.27 0.18	0.016 0.030
	Warren, MI, USA	Suburban	Spring and summer 1981	2	0.56 0.42	–
Tokiwa *et al.* (1983)	Santiago, Chile	Downtown	Winter 1981	4	0.06–0.15	0.028–0.11
Siak *et al.* (1985)	Michigan, USA	–	Summer	4	0.037–0.11	0.0024–0.012
Garner *et al.* (1986)	Bayreuth, Germany	Suburban	November 1983	2	–	1.5; 1.7
Tanabe *et al.* (1986)	Tokyo, Japan	Downtown	February	2	1.6; 0.579	0.041–0.13
			April	2	0.30–0.83	0.032–0.062
			August	2	0.19–0.90	0.015–0.038
			October	2	0.61–0.75	0.051–0.080
Ramdahl *et al.* (1986)	Claremont, CA, USA	Suburban	Summer 1985	1	0.36	–
	St Louis, MO, USA	Urban	Sampled over 1 year	1	0.16	–
	Washington DC, USA	Urban	Sampled over 1 year	1	0.20	-
Gibson (1986)	Warren, MI, USA	Suburban	Winter 1982	7	0.36 ± 0.15	0.015
			Summer 1984	5	0.35 ± 0.12	0.022
	Detroit, MI, USA	Urban	Summer, 1981	15	0.22 ± 0.20	0.030
Arey *et al.* (1987)	Torrance, CA, USA	Suburban	Winter daytime	1	–	0.04
			Winter night-time	1	–	0.03

Table 1.6 (continued)

Reference	Location	Description	Season and/or year	No. of samples	1-NP (μg/g) (AM ± SD)	1-NP (ng/m³)
Atkinson *et al.* (1988)	Glendora, USA	–	Summer 1986	6	–	0.020
	Reseda, USA	–	Summer 1987	2	–	0.008
Zielinska *et al.* (1989)	Claremont, USA	Suburban	Autumn	1	–	0.016
Bayona *et al.* (1994)	Barcelona, Spain	–	1989–90	1	–	0.026
Scheepers *et al.* (1994a)	Nijmegen, Netherlands	Busy street crossing	February, 1992	1	–	0.036
Legzdins *et al.* (1995)	Hamilton, Canada	–	Spring/summer 1990–91	1	–	0.012
Ciccioli *et al.* (1995)	Madrid, Spain	Suburban	Autumn 1991–93	1	–	0.010
	Montelibretti, Italy	Suburban	1991–93	1	–	0.012
	Milan, Italy	–	Winter 1991–93	1	–	0.220
	Rome, Italy	–	Winter 1991–93	1	–	0.070
	Sao Paulo, Brazil	–	Winter 1991–93	1	–	0.016
Dimashki *et al.* (1996)	Damascus, Syrian Arab Republic	–	Winter 1994	1	–	0.120
Murahashi & Hayakawa (1997)	Kananazawa, Japan	Suburban	Summer 1994	1	–	[0.010]
		Downtown	Summer 1994	1	–	[0.032]
Cecinato *et al.* (1998)	Milan, Italy	Viale Marche	Winter 1991	1	–	0.14
	Rome, Italy	Brera Tower	Winter 1993	1	–	0.59
		via Urbana	Summer 1991–1993	1	–	0.08
Scheepers *et al.* (1999)	Southampton, United Kingdom	Daytime	Weekday in May 1998	13	–	[0.0086 ± 0.0021[a]]
		Night-time		9	–	[0.0011 ± 0.0024[a]]
		Urban residence, indoor 1st floor	Night-time	4	–	[0.00067 ± 0.0053[a]] (< 0.001–0.0038)
		Urban	May 1998	3	–	0.0012–0.0035
	Kananazawa, Japan	Urban	Summer 1994	1	–	0.032

Table 1.6 (continued)

Reference	Location	Description	Season and/or year	No. of samples	1-NP (μg/g) (AM ± SD)	1-NP (ng/m³)
Marino *et al.* (2000)	Athens, Greece	–	1996	1	–	0.040
Yassaa *et al.* (2001)	Algiers, Algeria	Urban	Winter 1999	1		0.14
Bamford *et al.* (2003)	Washington, DC, USA (SRM1649a)	Urban	1970s	3	0.0715 ± 0.0051	–
	St. Louis, MO, USA (SRM1648)	Urban	1978	3	0.155 ± 0.029	–
	Baltimore, MD, USA	Urban	1998–99	3	0.196 ± 0.003	–
Ari *et al.* (2010)	Payas, Turkey	Urban industrial				
		PM-10 fraction	May 2008	7	0.212 (0.144–0.323)[b]	0.0059 (0.0037–0.0096)[b]
		PM-2.5 fraction	May 2008	7	0.191 (0.103–0.246)[b]	0.0024 (0.0014–0.0137)[b]
	Iskenderun, Turkey	Urban background; PM-10 fraction	May 2008	8	0.273 (0.108–0.621)[b]	0.0041 (0.0007–0.0123)[b]
	Eskişehir, Turkey	Urban road side; PM-10 fraction	May–June 2008	5	0.930 (0.168–1,270) [b]	0.0192 (0.0066–0.0229)[b]
Rural						
Nielsen (1983)	Copenhagen, Denmark	Rural	February–April 1982			0.02 (< 0.001–0.04)
Gibson (1986)	Delaware, USA	Rural	Summer 1982		0.54 ± 0.24	0.013
	Bermuda	Remote	Summer 1982	1	0.52 ± 0.29	0.0096
			Winter 1983	1	0.72 ± 0.43	0.0103
Ramdahl *et al.* (1986)	Aurskog, Norway	Rural residential	Winter, 1984	1	0.15	–
Atkinson *et al.* (1988)	Pt. Arguello, USA	Remote	Summer, 1987	2	–	0.0005
	San Nicolas Island, USA	Remote	Summer 1987	1	–	0.0003
Saitoh *et al.* (1990)	Morioka, Japan	Rural	November–April		–	0.038–0.245
Ciccioli *et al.* (1995)	Castelporziano, Italy	Forest area	Winter 1992	1	–	0.002
	Alta Floresta, Brazil	Not specified	Winter/spring 1993	1	–	0.002
Scheepers *et al.* (1994a)	Hoenderloo, Netherlands	Nature reserve area	July 1992	1	0.034	0.0017

Table 1.6 (continued)

Reference	Location	Description	Season and/or year	No. of samples	1-NP (μg/g) (AM ± SD)	1-NP (ng/m³)
Scheepers *et al.* (1999)	Southampton, United Kingdom	Rural	Night-time, weekend May 1998	3	–	0.0005 0.0006 0.0008
	New Milton, United Kingdom	Rural residence, indoor	Night-time	6	–	[0.0028 ± 0.0088][a] (< 0.0001–0.0086)
Cecinato *et al.* (2000)	Svalbard Island, Norway	Remote	1998–99	1	–	0.016
Albinet *et al.* (2006)	Sollieres, Maurienne Valley, France, 1373 m altitude	Rural	Winter 2002–03	13	–	0.0106 (0.0027–0.0289)
Ari *et al.* (2010)	Eskişehir, Turkey	PM-10 fraction	May–June 2008	4	0.30 (0.18–0.50)[b]	0.0048 (0.0028–0.0062)[b]
		PM-2.5 fraction	May–June 2008	4	0.21 (0.11–0.49)[b]	0.0029 (0.0012–0.0060)[b]

[a] Geometric mean and geometric standard deviation calculated by the Working Group; for results reported as 'not detected', half of the limit of quantification was used; the classification by the Working Group was based on Google Maps.

[b] Median and range

1-NP, 1-nitropyrene; PM, particulate matter; SRM, standard reference material

3-Hydroxy-*N*-acetyl-1-aminopyrene, 1-aminopyrene and *N*-acetyl-1-aminopyrene were not detected with this method.

1.5.2 Occupational exposure to engine exhaust

(a) Personal air sampling

Workers who operate or maintain diesel-powered engines or vehicles are liable to be exposed to their exhaust. Most measurements were obtained by high-volume sampling because of the low levels observed in air, specifically at outdoor locations. Table 1.7 summarizes air concentrations of 1-nitropyrene determined at fixed outdoor sampling locations, where different types of diesel engine were used. Workers often try to avoid remaining downwind from sources of exhausts, which, together with dilution by ambient air, maintains their exposure at relatively low levels, compared with those found indoors, and within the range of those observed in the general ambient air of most urban locations (usually < 1 ng/m^3; see Table 1.6).

At indoor workplaces, exposures tend to be higher (occasionally > 1 ng/m^3; (Table 1.7), with the exception of engine repair shops, where exhaust is removed by connecting the tailpipe to an effective local exhaust ventilation system. When the capacity of this system is insufficient, levels of 1-nitropyrene may reach > 5 ng/m^3 (Scheepers *et al.*, 1994a). Overall, train repair shops and mining operations represented indoor workplaces with the highest exposures (average exposure levels, > 1 ng/m^3; see Fig. 1.1).

A few studies that attempted to determine exposure to 1-nitropyrene by taking personal air samples from the breathing zone of workers (Table 1.8) were only feasible when the analysis used was sensitive, because the airflow that can be used in compact air suction pumps is limited. The exposure of mechanics at a bus garage in Southampton (United Kingdom) was relatively low, but sampling periods were long and included the time spent commuting from home to work and vice versa [which may have underestimated the contribution of exposure in the workplace, where levels were probably higher than during commuting]. Mechanics had higher exposures than office clerks, and, on average, more than 75% of the exposure resulted from working in the repair shop. The mean contribution of local outdoor concentrations of 1-nitropyrene to the average exposure of workers was 16.1% (Scheepers *et al.*, 1999).

The highest exposures to 1-nitropyrene were observed in oil-shale mining (Estonia) and black coal mining (Czech Republic). Underground oil-shale miners were exposed to levels of 1-nitropyrene one to two orders of magnitude higher than those of workers employed in surface operations, including indoors. The personal air samples of workers exposed to exhausts from loaders that were continuously moving back and forth in narrow confined spaces contained concentrations of up to 40 ng/m^3 of 1-nitropyrene. In coal mines, the workers were exposed to exhausts from trains used for the transportation of coal and personnel that contained concentrations of 1-nitropyrene ranging up to 2.5 ng/m^3, which on average were much lower than those detected in oil-shale mining; no difference was found in average exposures to 1-nitropyrene between underground miners and workers in surface operations (Scheepers *et al.*, 1993, 1994a).

(b) Biomonitoring

Levels of 2–200 ng of 1-aminopyrene, a urinary metabolite of 1-nitropyrene, were observed in 24-hour urine samples of nine smoking and nine nonsmoking salt miners exposed to diesel exhaust in Germany (Seidel *et al.*, 2002). Seidel *et al.* (2002) and Scheepers *et al.* (1995b) developed an immunoassay to detect the urinary excretion of 1-aminopyrene, which was used in a study of three nonsmoking mechanics and two nonsmoking office clerks in a repair workshop for trains (Scheepers *et al.*, 1994b), from whom 24-hour urine samples were collected over 3 days. Combined urine aliquots

Table 1.7 Concentrations of 1-nitropyrene in extracts of total suspended particulate matter associated with the use of diesel engines collected at workplaces

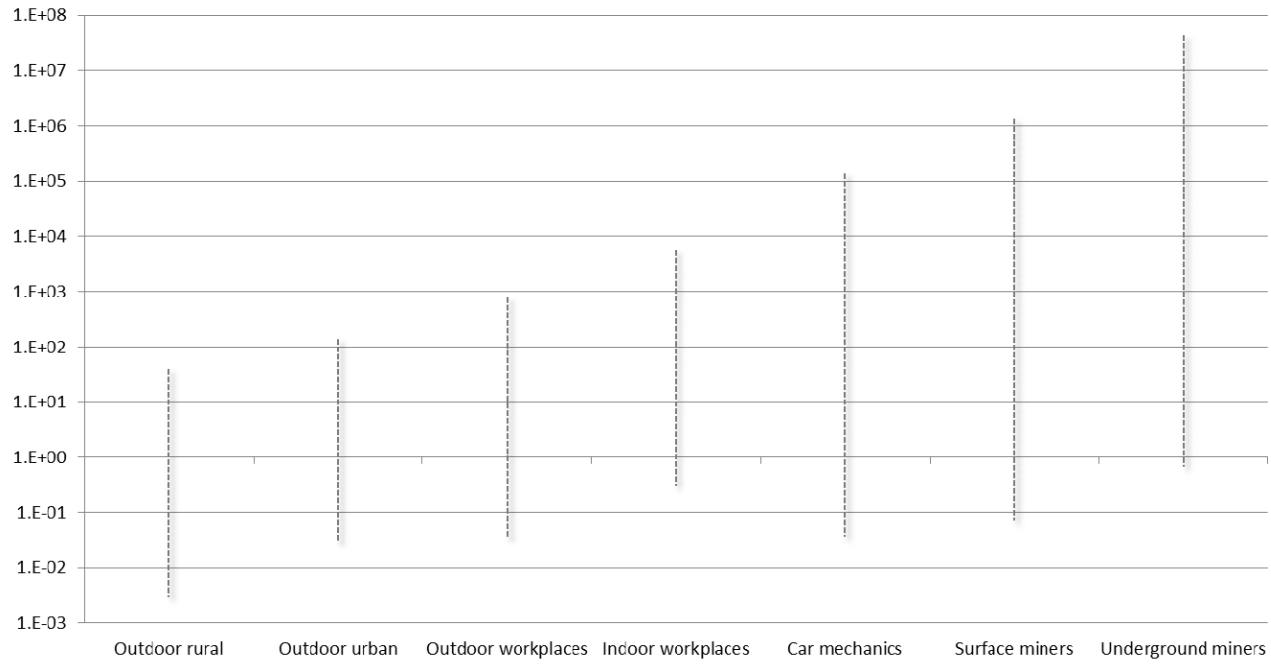
Reference	Job title	Source	Working conditions	Location and conditions	Year	No. of samples	1-NP (ng/m³)[a] (range)
Outdoor							
Scheepers *et al.* (1994a)	Professional driver	Armoured cars	Training in field operation of armed vehicle driving	Netherlands; wind speed 4–6 m/s	1992	2	0.012; 0.015
	Ship's crew	Ship's engine	River vessel	Netherlands and Belgium; wind speed 8–9 m/s	1992	3	0.034 ± 0.0019
		Power supply			1992	1	0.79
	Gardener	Passing traffic	City park	Netherlands	1992	1	0.036
		Lawn mowers	Grass verge maintenance	Netherlands	1992	1	0.0066
	Platform personnel	Lift platforms, power supplies, trucks, air craft push back tractors	Platform of Amsterdam international airport	Schiphol, Netherlands	1992	3	0.034 ± 0.0014 (0.037–0.045)
Indoor							
Scheepers *et al.* (1999)	Mechanics	Buses	Garage	United Kingdom	1998	14	0.054 ± 2.4 (0.010–0.227)
			Fitting shop			13	0.053 ± 2.0 (0.015–0.120)
Scheepers *et al.* (1994a)	Mechanics	Train engines	Repair workshop	Netherlands	1992	4	0.31 ± 1.2 (0.26–0.39)
					1994	7	1.8 ± 2.4[a] (0.5–5.6)
	Logistic personnel	Trucks	Flower auction building	Netherlands	1992	1	0.08
	Driver	Forklift truck	Concrete manufacturing			2	0.61; 0.71
			Aluminium rolling			4	1.1 ± 1.1 (0.87–1.2)
			Galvanization workshop			4	0.09 ± 1.7 (0.044–0.15)
			Chemical plant			4	0.22 ± 0.21 (0.11–0.56)
Seidel *et al.* (2002)	Miner	Not specified	Underground salt mine	Germany		3	0.70; 1.3; 1.00

Table 1.7 (continued)

Reference	Job title	Source	Working conditions	Location and conditions	Year	No. of samples	1-NP (ng/m³)[a] (range)
Scheepers *et al.* (2002, 2003)		Train	Coal mining (surface operations)	Czech Republic	1999	8	0.0028 ± 0.0037 (0.007–0.185)
			Coal mining (underground)			8	0.078 ± 0.0033 (0.028–0.697)
		Loader	Oil-shale mine (surface operations)	Estonia		6	0.070 ± 0.0021 (0.025–0.193)
			Oil-shale mine (underground)			5	0.216 ± 0.002 (0.111–0.506)

[a] Geometric mean and geometric standard deviation summarizing observations covering three workweeks, calculated by the Working Group; for results reported as 'not detected', half of the limit of quantification was used.
1-NP, 1-nitropyrene

Fig. 1.1 Range of air concentrations of 1-nitropyrene (in ng/m³)



The values for car mechanics, surface miners and underground miners were measured in the breathing zone by personal air sampling. Other values were the results of air sampling at fixed locations (stationary high-volume sampling). The results presented in this figure are also summarized in Tables 1.4–1.7.
Compiled by the Working Group.

of mechanics showed an almost twofold increase in the level of excreted 1-aminopyrene (expressed as nanomolar equivalents; $P < 0.05$) after 48 and 72 hours, and also after adjustment for creatinine ($P < 0.05$), compared with the levels excreted by office clerks.

Only one study described the determination of 1-aminopyrene in blood samples (Zwirner-Baier & Neumann, 1999) following the hydrolysis of haemoglobin adducts. Before hydrolysis, the erythrocytes were washed with water and 0.9% saline, and proteins were then precipitated and rinsed with aliquots of ethanol:water (80:20), ethanol and ethanol:ether (25:75) after lysis of the cells. [It is not clear to what extent free metabolites (from plasma and erythrocytes) were removed by this pretreatment, or may have contributed to the 1-aminopyrene content.] The blood samples were from 63 male nonsmokers, divided into three subgroups: 29 bus garage workers, 20 hospital workers and 14 men living in rural areas around Southampton, United Kingdom. 1-Aminopyrene was detected in 86–100% of the subjects, with levels ranging from < 0.03 to 0.68 pmol/g of haemoglobin. 1-Aminopyrene was the most abundant nitroarene metabolites (other cleavage products detected were 2-aminofluorene, 3-aminofluoranthene, 9-aminophenanthrene and 6-aminochrysene). Interestingly, the highest median levels of 1-aminopyrene were observed in hospital workers (0.16 pmol/g), followed by bus garage workers (0.13 pmol/g) and inhabitants of a rural environment (0.10 pmol/g).

1.6 Regulations and guidelines

No known air quality guidelines or reference values have been established by national or international authorities for 1-nitropyrene.

Table 1.8 Concentrations of 1-nitropyrene determined by personal air sampling in the breathing zone of workers

Reference	Source	Working conditions	Location and conditions	Year	No. of workers	Concentration of 1-NP (pg/m³)	
						AM ± SD	GM ± GSD (range)
Car mechanics							
Scheepers *et al.* (1999)	Buses	Work shift in bus garage including the time commuting from home to work and vice versa	Southampton, United Kingdom	1998	5	52 ± 21[a]	48 ± 1.6[a]
						19 ± 16[a]	4.0 ± 8.1[a]
						8.0 ± 9.2[a]	2.2 ± 6.0[a]
						36 ± 32[a]	8.5 ± 10.2[a]
						12.9 ± 6.9[a]	7.8 ± 4.0[a]
Miners							
Seidel *et al.* (2002)	Not specified	Salt mine	Germany		4	[1171 ± 867][b]	(972 ± 740][b]
Scheepers *et al.* (2002, 2003)	Loaders	Oil shale mine, surface operations	Estonia				
			Pilot study (1st shift)	1999	9	–	8 ± 2.3 (3–50)
			Pilot study (2nd shift)	1999	9	–	45 ± 3.8 (12–686)
			Main study (3 shifts)	2000	42	–	85 ± 2.7 (13–1332)
		Oil shale miners, underground	Estonia				
			Pilot study (1st shift)	1999	10	–	2483 ± 3.4 (602–42 190)
			Pilot study (2nd shift)	1999	10	–	984 ± 2.7 (134–3455)
			Main study (3 shifts)	2000	50	–	637 ± 3.3 (29–5031)
	Trains	Coal mine, surface operations	Czech Republic				
			Pilot study (1st shift)	1999	10	–	110 ± 2.8 (19–666)
			Pilot study (2nd shift)	1999	9	–	71 ± 7.6 (7–2167)
		Coal mine, underground	Czech Republic				
			Pilot study (1st shift)	1999	9	–	197 ± 1.5 (123–437)
			Pilot study (2nd shift)	1999	9	–	209 ± 3.6 (28–2495)

[a] Mean over one workweek (4–5 days) calculated by the Working Group; for results reported as not detected, half of the limit of quantification was used.

[b] Calculated by the Working Group; combination of stationary and personal air samples.

AM, arithmetic mean; GM, geometric mean; GSD geometric standard deviation; 1-NP, 1-nitropyrene; SD, standard deviation

2. Cancer in Humans

No data were available to the Working Group.

3. Cancer in Experimental Animals

3.1 Mouse

See Table 3.1

3.1.1 Subcutaneous administration

A group of 20 male BALB/C mice, aged 6 weeks, received subcutaneous injections of 0 (control) or 0.1 mg of 1-nitropyrene (purity, > 99.9%) dissolved in dimethyl sulfoxide (DMSO) once a week for 20 weeks (total dose, 2 mg), and were observed for up to 60 weeks after the initial treatment or until moribund. No subcutaneous tumours developed at the injection site in any animals. Lung tumours developed in 6 out of 20 (30%) 1-nitropyrene-treated and 7 out of 20 (35%) control mice (Tokiwa *et al.*, 1984). [The Working Group noted the small number of animals used in this study.]

3.1.2 Intraperitoneal administration

Three groups of 12–16 male and 12–16 female A/J mice, aged 6–8 weeks, received a total of 17 intraperitoneal injections (three per week) of 0 (control), 0.71, 2.14 of 6.14 mmol/kg body weight (bw) of 1-nitropyrene (purity, > 99%) in 0.1 mL of trioctanoin over a period of 6 weeks (total doses, 0, 175, 525 and 1575 mg/kg bw, respectively), and were killed 18 weeks after termination of the treatment (total, 24 weeks). The number of high-dose males with lung tumours (14 out of 16) was significantly greater [$P < 0.001$] than that of male controls (3 out of 16). The mean number of lung tumours per mouse was also significantly increased in the low- (0.6 ± 0.5) and high- (1.6 ± 1.1) dose males compared with male controls (0.3 ± 0.6; $P < 0.001$). The mean number of lung tumours per mouse was also significantly increased in high-dose females (0.8 ± 0.8) compared with female controls (0.3 ± 0.6; $P < 0.05$) (el-Bayoumy *et al.*, 1984).

Male and female newborn CD-1 mice [initial numbers unspecified] received three intraperitoneal injections of 1-nitropyrene (purity, > 99%) on day 1 (100 or 400 nmol), day 8 (200 or 800 nmol) and day 15 (400 or 1600 nmol) in DMSO (total doses, 700 or 2800 nmol [173 or 692 µg] in 20 or 40 µL of DMSO/mouse) after birth or DMSO alone. A positive control group received a total dose of 560 nmol [140 µg] of benzo[*a*]pyrene (purity, > 99%) in DMSO. All surviving mice were killed after 1 year. Hepatocellular adenomas or carcinomas (combined) developed in 5 out of 34 (15%) low-dose (two adenomas and three carcinomas) and 8 out of 29 (27%) high-dose 1-nitropyrene-treated males; the latter incidence was significantly greater than that in DMSO controls (8 out of 29 versus 2 out of 28; $P < 0.05$). No liver tumours developed in 1-nitropyrene-treated females. The incidence of lung tumours did not differ significantly in 1-nitropyrene-treated males (6 out of 34 low-dose and 1 out of 29 high-dose) or females (3 out of 50 low-dose and 2 out of 26 high-dose) compared with controls (1 out of 28 males and 0 out of 31 females), nor did that of malignant lymphomas (males: 4 out of 28 control, 0 out of 34 low-dose and 7 out of 29 high-dose; females: 3 out of 31 control, 6 out of 50 low-dose and 4 out of 26 high-dose) (Wislocki *et al.*, 1986).

Groups of newborn male and female Swiss-Webster BLU:Ha [ICR] mice [initial numbers unspecified] received three intraperitoneal injections of 1-nitropyrene (purity, > 99.9%) in DMSO or DMSO alone on day 1 (1/7th of the dose), day 8 (2/7th of the dose) and day 15 (4/7th of the dose) after birth (total doses: 21 µg (0.08 µmol) in 35 µL of DMSO/mouse or 105 µg (0.43 µmol) in 35 µL of DMSO/mouse). The mice were then maintained untreated for 24 weeks and killed at

Table 3.1 Studies of the carcinogenicity of 1-nitropyrene in mice

Strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
BALB/c (M) 60 wks Tokiwa *et al.* (1984)	Subcutaneous injection 0 (control) or 0.1 mg/mouse in DMSO once/wk for 20 wks (total dose, 2.0 mg/mouse) then observed up to 60 wks Groups of 20	Injection site (subcutaneous tumour): 0/20, 0/20 Lung (adenoma or carcinoma combined): 7/20 (35%), 6/20 (30%)	NS	Purity, > 99.9% by HPLC
A/J derived (M, F) 24 wks El Bayoumy *et al.* (1984)	Intraperitoneal injection 0 (control), 0.71, 2.14 or 6.44 mmol/kg bw in 0.1 mL trioctanoin, 3 ×/wk for 6 wks (total of 17 injections; total doses: 175, 525 or 1575 mg/kg bw) then held untreated for an additional 18 wks Groups of 12–16	Lung (all tumours): M–3/16 (19%), 4/15 (27%), 6/15 (40%), 14/16 (86%) F–4/16 (25%), 3/14 (21%), 5/14 (36%), 8/12 (67%) Multiplicity (lung tumours): M–0.3 ± 0.6, 0.6 ± 0.5, 0.6 ± 0.8, 1.6 ± 1.1 F–0.3 ± 0.6, 0.2 ± 0.4, 0.4 ± 0.7, 0.8 ± 0.8	M–[high dose, $P < 0.001$] F–[NS] M–[low dose, $P < 0.05$; high dose, $P < 0.001$] F–[high dose, $P < 0.05$]	Purity, > 99% by TLC; small number of animals, short durations of treatment and observation
CD-1 (F, M) 52 wks Wislocki *et al.* (1986)	Intraperitoneal injection 0 (control), 700 or 2800 nmol 1-NP or 560 nmol B[*a*]P in 10, 20 or 40 µL DMSO (total doses) on d 1, 8 and 15 after birth Groups of 28–50 newborn	Liver (adenoma): M–7/73 (10%), 2/34 (6%), 3/29 (10%)*, 11/37 (30% ; B[*a*]P) F–0/65, 0/50, 0/26, 0/27 (B[*a*]P) Lung (carcinoma): M–0/73, 3/34 (9%), 5/29 (17%)*, 7/37* (19%; B[*a*]P) F–0/65, 0/50, 0/26, 0/27 (B[*a*]P) Lung (adenoma): M–3/73 (4%), 6/34 (18%), 1/29 (3%), 13/37 (35%; B[*a*]P) F–1/65 (1%), 3/50 (6%), 2/26 (8%), 13/27 (48%; B[*a*]P) Lung (carcinoma): M–3/73 (4%), 0/34, 0/29, 0/37 (B[*a*]P) F–1/65 (1%), 0/50, 0/26, 0/27 (B[*a*]P)	*$P < 0.05$	Purity, > 99% by HPLC

Table 3.1 (continued)

Strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Swiss Webster (M, F) 26 wks Busby *et al.* (1989)	Intraperitoneal injection 0 (control), 21 µg (0.08 µmol) or 105 µg (0.43 µmol) in 35 µL DMSO/mouse (total doses); 1/7 dose at d 1 after birth, 2/7 dose on d 8, 4/7 dose on d 15 Groups of newborn (NR)	Lung (adenoma or adenocarcinoma combined): M–13/91 (14%), 2/29 (7%), 1/23 (4%) F–7/101 (7%), 3/23 (13%), 0/30	NS	Purity > 99.9%
B6C3F$_1$ (M, F) 72 wks Mori *et al.* (1992)	Intraperitoneal injection 0 (control), 100 nmol within 24 h of birth, 200 nmol on d 8, 400 nmol d 15 (total dose, 700 nmol/mouse); untreated and control also available Groups of (NR) newborn	Liver (hepatocellular adenoma): M–1/39 (3%; untreated), 4/28 (14%), 4/21 (19%) F–0/38 (untreated), 0/27, 0/20	NS	Purity > 99.9% by HPLC
CD-1 (F) 28 wks el-Bayoumy *et al.* (1982)	Initiation–promotion: skin application 0 (control) or 0.1 mg in 0.1 mL acetone onto shaved back skin, once/2 d for 20 d (10 doses; total dose, 1 mg); 10 d later 2.5 µg TPA in 0.1 mL acetone 3 ×/wk for 25 wks Groups of 20	Skin (mainly squamous-cell papillomas): [3/19] (16%), 1/20 (5%) Multiplicity (skin tumours): 0.2, 0.1	NS	Purity > 99%
SENCAR (M, F) 31 wks Nesnow *et al.* (1984)	Initiation–promotion: skin application/ intraperitoneal injection 0 (control), 0.03, 0.10, 0.30 or 1.00 mg (once) or 3.00 mg (1.50 mg twice) in 0.2 mL acetone (skin) or 0.2 mL corn oil (injection); 1 wk later 2 µg TPA, twice/wk (skin) for 30 wks Groups of 37–40 M, 37–40 F	Skin (papillomas per effective mouse): M–0.06 (31), 0.11 (38), 0.18 (40), 0.08 (39), 0.11 (38), 0.10 (39) F–0 (39), 0.06 (36), 0.28 (40), 0 (38), 0.10 (39), 0.21 (38)	NS NS	Purity > 99.9% (HPLC)

B[*a*]P, benzo[*a*]pyrene; bw, body weight; d, day; DMSO, dimethyl sulfoxide; F, female; HPLC, high-performance liquid chromatography; M, male; 1-NP, 1-nitropyrene; NR, not reported; NS, not significant; TLC, thin-layer chromatography; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; wk, week

26 weeks of age. The incidence of lung adenomas or adenocarcinomas (combined) was 13 out of 91 (14%), 2 out of 29 (7%) and 1 out of 23 (4%) males, and 7 out of 101 (7%), 3 out of 23 (13%, all adenocarcinomas) and 0 out of 30 females in the control, 21-µg and 105-µg groups, respectively, showing no difference between controls and treated animal (Busby *et al.*, 1989).

Male and female newborn B6C3F$_1$ mice [initial numbers unspecified] received intraperitoneal injections of 1-nitropyrene dissolved in DMSO or DMSO alone on day 1 (100 nmol), day 8 (200 nmol) and day 15 (400 nmol) after birth. An untreated control group was also available. Surviving animals were killed after 72 weeks. The incidence of hepatocellular adenomas in treated males (4 out of 21, 19%) and females (0 out of 20) was not statistically different from that in DMSO-treated controls (4 out of 28, 14% and 0 out of 27, respectively) (Mori *et al.*, 1992).

3.1.3 Initiation–promotion

Group of 20 female CD-1 Charles River mice, aged 50–55 days, received applications of 0 (control) or 0.1 mg of 1-nitropyrene (purity > 99%) in 0.1 mL of acetone onto the shaved back skin by pipette every other day for 20 days (total dose, 1 mg). Ten days after the completion of initiation, the animals received applications of 2.5 of µg 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in 0.1 mL of acetone three times a week for 25 weeks. The mice were killed 28 weeks after the first treatment with 1-nitropyrene. Neither the incidence of skin tumours (mainly squamous cell papillomas; 16% [3 out of 19] versus 5% [1 out of 20]) nor the multiplicity of skin tumours per mouse (0.2 versus 0.1) in 1-nitropyrene-treated mice differed from those in controls (el-Bayoumy *et al.*, 1982).

Groups of 37–40 male and 37–40 female SENCAR mice, aged 7 weeks, initially received treated a single dermal application of 0 (control), 0.03, 0.10, 0.30 or 1.00 mg/mouse of 1-nitropyrene (> 99.9% pure) dissolved in 0.2 mL of acetone onto the back. The highest-dose group received 3.0 mg of 1-nitropyrene as two applications of 1.5 mg each. One week later, the mice received applications of 2 µg of TPA in 0.2 mL of acetone twice a week for 30 weeks. For comparison of tumour yield, a positive-control group was pretreated with 0.051 mg/mouse of benzo[*a*]pyrene. The number of papillomas/mouse for each dose group was 0.06, 0.11, 0.18, 0.08, 0.11 and 0.10 in males and 0, 0.06, 0.28, 0, 0.10 and 0.21 in females, respectively. Thus, dermal application of 1-nitropyrene did not act as a skin tumour initiator (Nesnow *et al.*, 1984).

3.2 Rat

See Table 3.2

3.2.1 Oral administration

Groups of 22–36 male and 24–33 female newborn Sprague-Dawley rats were administered 0 (control), 100 or 250 µmol/kg bw of 1-nitropyrene (320 µmol/rat in 16.2 µmol of trioctanoin or 800 µmol/rat in 40.5 µmol of trioctanoin) by gavage within 24 hours of birth and then once a week for 16 weeks. Animals were then maintained without treatment and were killed at week 96. The incidence of mammary gland adenocarcinomas was increased in low-dose (14 out of 33, 42%) and high-dose (15 out of 24, 62%) females compared with controls (1 out of 31, 3%; $P < 0.0001$). The incidence of lung adenomas or adenocarcinomas (combined) in males was increased in the high-dose group only (0 out of 22 versus 4 out of 36, 11%; $P < 0.05$) (el-Bayoumy *et al.*, 1988).

Groups of 35 female weanling Sprague-Dawley rats were administered 0 (control) or 10 µmol/kg bw of 1-nitropyrene in DMSO by intragastric intubation three times a week for 4 weeks (total dose, 16 µmol/rat in 1.7 µmol/mL of DMSO) and were then observed with no further treatment until they were killed at 78 weeks.

Table 3.2 Studies of the carcinogenicity of 1-nitropyrene in rats

Strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
SD (F, M) 96 wks el-Bayoumy *et al.* (1988)	Oral administration 0 (control), 100 (320 µmol/rat in 16.2 µmol/mL trioctanoin) or 250 µmol/kg bw (800 µmol/rat in 40.5 µmol/mL trioctanoin) by gavage within 24 h of birth, once/wk for 16 wks, then observed up to 96 wks Groups of 22–36 M, 22–36 F	Mammary gland (adenoma): M–1/22 (4%), 2/25 (8%), 1/36 (3%) F–14/31 (45%), 10/33 (30%), 7/24 (29%) Mammary gland (adenocarcinoma): M–0/22, 2/25 (8%), 2/36 (6%) F–1/31 (3%), 14/33 (42%), 15/24 (62%)* Lung (adenoma or adenocarcinoma combined): M–0/22, 1/25 (4%), 4/36 (11%) F–0/31, 0/33, 3/24 (12%)** Pancreas (islet cell adenoma): M–0/22, 4/25 (16%), 3/36 (8%) F–0/31, 1/33 (3%), 1/24 (4%)	*$P < 0.01$ (high, low) **$P < 0.05$ otherwise, NS	Purity, > 99.9%; other mammary tumours included fibromas, sarcomas and myxomas
CD (F) 78 wks King (1988), Imaida *et al.* (1991a)	Intragastric administration 0 (control) or 10 µmol/kg bw, 3 ×/wk for 4 wks (total dose, 16 µmol/rat) in DMSO then observed to 78 wks Groups of 36 weanling	Mammary gland (all tumours): 12/35 (34%), 16/35 (46%) Mammary gland (fibroadenoma): 9/35 (26%), 9/35 (26%) Mammary gland (adenocarcinoma): 5/35 (9%), 5/35 (9%)	NS	Purity, > 99.9%
SD (F) 49 wks el-Bayoumy *et al.* (1995)	Intragastric administration 0 (control) or 50 µmol in 0.5 mL trioctanoin, once/wk for 8 wks (total dose, 400 µmol/rat), then observed for 41 wks Groups of 30	Mammary gland (fibroadenoma): 0/30, 10/30 (33%)* Mammary gland (desmoplastic adenoma [adenoma criss-crossed by thick bands of fibrous connective tissue]): 8/30 (27%), 15/30 (50%) Mammary gland (adenoma): 2/30 (7%), 7/30 (23%) Mammary gland (adenocarcinoma): 1/30 (33%), 2/30 (67%) Multiplicity (mammary tumours): 0, 15** (fibroadenoma); 14, 29 (desmoplastic adenoma); 2, 10 (adenoma); 1, 3 (adenocarcinoma)	*$P < 0.01$ **$P < 0.05$	Purity, > 99.9%; study limited by use of a single dose and short duration of exposure

Table 3.2 (continued)

Strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
F344/DuCrj (M) Duration (NR) Ohgaki *et al.* (1982)	Subcutaneous injection 0 (control) or 20 mg/rat in 0.2 mL DMSO, twice/wk for 10 wks (total dose, 40 mg) Groups of 20	Injection site (subcutaneous tumour[a]): 0/20, 8/17 (47%)	P < 0.003	Purity, > 99%; later, Ohgaki *et al.* (1985) reported that the 1-NP used contained impurities: 1,3-dinitropyrene (DNP) (0.2%); 1,6-DNP (0.3%); 1,8-DNP (0.3%)
CD (SD-derived) (F, M) 62 wks Hirose *et al.* (1984)	Subcutaneous injection 0 (control), 50 or 100 µmol/kg bw in DMSO into the suprascapular area, starting within 24 h of birth, once/week for 8 wks and observed until aged 62 wks Groups of 28–35 newborn	Tumour-bearing animals (all tumours[b]): M–6/28 (21%), 10/29 (34%), 15/31 (48%)* F–8/31 (26%), 19/31 (61%)**, 24/32 (75%)*** Injection site (subcutaneous fibrous histiocytomas): M–0/28, 2/29 (7%), 10/31 (32%)* F–0/31, 3/31 (10%), 9/32 (28%)** Mammary gland (all tumours): F–2/31 (6%), 7/31 (23%), 15/32 (47%)* Mammary gland (fibroadenoma): F–1/31 (3%), 5/31 (16%), 7/32 (22%)* Mammary gland (adenocarcinoma): F–0/31, 3/31 (10%), 10/32 (31%)*	*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ *$P < 0.001$; **$P < 0.01$ *$P < 0.001$ (high) *$P < 0.05$ *$P < 0.01$	Purity, 99.9%; other mammary tumours included adenomas and carcinosarcomas
F344/DuCrj (M) Up to 650 d Ohgaki *et al.* (1985)	Subcutaneous injection 0 (control), 0.2 or 2 mg/rat in 0.2 mL DMSO, twice/wk for 10 wks (total dose, 4 and 40 mg), then observed untreated up to 650 days Groups of 10 or 20	Injection site (subcutaneous tumour[a]): 0/20, 0/10, 0/10	NS	Purity checked by HPLC; 1,3-, 1,6-, 1,8-DNP, were minimized to 0.05% each; study limited by the small numbr of treated animals and use of one dose only; authors concluded that tumour incidence in the previous study (Ohgaki *et al.* (1982) was due to contaminants (1,8- and 1,6-DNP) and that 1-NP was not a skin carcinogen following subcutaneous injection.

Table 3.2 (continued)

Strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
CD (F) 90 wks King (1988), Imaida *et al.* (1991b)	Subcutaneous injection 0 (control) or 100 µmol/kg bw in DMSO (70 µmol/mL) once/wk for 4 wk (total dose, 74.3 µmol), then observed for up to 90 wks Groups of 30	All tumours [b]: 27/30 (90%), 23/29 (79%) Mammary gland (all tumours): 11/30 (37%), 17/29 (57%) Mammary gland (fibroadenoma): 8/30 (27%), 15/29 (48%)* Mammary gland (adenocarcinoma): 4/30 (13%), 6/29 (21%)	*$P < 0.05$	Purity (NR; purchased from the Midwest Research Institute, MO)
CD (F) 67 or 86 wks King (1988), Imaida *et al.* (1995)	Subcutaneous injection 0 (control) or 2.5 (within 24 h of birth), 5 (2 injections) and 10 µmol/kg bw in DMSO into suprascapular region, once/wk for 8 wks (total dose, 6.3 µmol [18 mg]), and observed untreated for up to 78 wk Groups of 37–49 newborn	Mammary gland (all tumours): 8/40 (20%), 16/49 (33%) Mammary gland (adenocarcinoma): 1/40 (2%), 10/49 (20%) Mammary gland (fibroadenoma): 6/40 (15%), 9/49 (18%)	$P < 0.05$ $P < 0.005$ NS	Purity, > 99.9%; no tumours at the injection site; other mammary tumours included adenomas and carcinosarcomas
CD or F344 (F) 67 or 86 wks King (1988), Imaida *et al.* (1995)	Subcutaneous injection 0 (control) or 100 µmol/kg bw in DMSO into suprascapular region, starting within 24 h of birth once/wk for 8 wks (total dose, 800 µmol/kg bw) then observed untreated for up to 78 wks Groups of 48–49 newborn CD; 55 newborn F344	**CD rats** Mammary gland (all tumours): 17/47 (36%), 26/48 (54%) Mammary gland (adenocarcinoma): 3/47 (6%), 10/48 (21%) Mammary gland (fibroadenoma): 16/47 (34%), 19/48 (40%) **F344 rats** Mammary gland (all tumours): 1/55 (2%), 5/55 (9%) Mammary gland (adenocarcinoma): 0/55, 0/55 Mammary gland (fibroadenoma): 1/55 (2%), 3/55 (6%)	$P < 0.005$ $P < 0.05$ NS NS NS NS	

Table 3.2 (continued)

Strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
CD (F) 61–90 wks King (1988), Imaida *et al.* (1991b)	Intraperitoneal injection *Experiment 1* 0 (control) or 67 µmol/kg bw as 25 µmol/mL in DMSO, 3 ×/wk for 4 wks (total dose, 119 µmol), then observed up to 61 wks Groups of 30, aged 30 d *Experiment 2* 0 (control) or 100 µmol/kg bw in 70 µmol/mL DMSO, once/wk for 4 wks (total dose, 77.3 µmol), then observed up to 87 wks Groups of 30, aged 30 d	*Experiment 1* All tumours: 15/29 (52%), 12/29 (41%) Mammary gland (all tumours): 4/29 (14%), 4/29 (14%) *Experiment 2* All tumours: 26/30 (87%), 25/29 (86%) Mammary gland (all tumours): 11/30 (37%), 17/29 (59%) Mammary gland (fibroadenoma): 9/30 (30%), 14/29 (48%) Mammary gland (adenocarcinoma): 2/30 (7%), 8/29 (28%)*	 NS NS *$P < 0.05$	Purity (NR); mammary tumours were fibroadenomas, adenomas and adenocarcinomas; study limited by short durations of treatment and observation, and use of single doses
CD (F) 76–78 wks King (1988), Imaida *et al.* (1991a)	Intraperitoneal injection 0 (control) or 10 µmol/kg bw as1.7 µmol/mL in DMSO, 3 ×/wk for 4 wks (total dose, 40 µmol/rat) then observed until 76–78 wks Groups of 36 weanling	Mammary gland (all tumours): 7/31 (23%), 25/36 (69%) Mammary gland (fibroadenoma): 5/31 (16%), 19/36 (53%) Mammary gland (adenocarcinoma): 3/31 (10%), 14/36 (39%)	 $P < 0.0001$ $P < 0.001$ $P < 0.01$	Purity, > 99.9%; study limited by short durations of treatment and observation and use of a single dose
F344/DuCrj (M) 72 wks Maeda *et al.* (1986)	Intrapulmonary implantation 0 (control) or 1.50 mg/rat 1-NP or 0.5 mg/rat 3-MC in 0.05 mL beeswax:tricaprylin (1:1) into the lower third of the left lung (× 1) and observed up to 72 wks Groups of 19–32, aged 10–11 wks	Injection site tumours Lung (squamous cell carcinoma): 0/31, 0/32, 19/19 (100%; 3-MC)	 NS for 1-NP	Purity, > 99.9%; study limited by short durations of treatment and observation and use of a single dose; other tumours observed: adrenal gland (4 pheochromo-cytomas, 1 adenoma), pituitary gland (2 adenomas), pancreas (1 islet cell tumour), skin (1 fibroma), leukaemia (1 granulocytic), thymus (1 thymoma)

Table 3.2 (continued)

Strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
CD (F) 77 wks Imaida *et al.* (1991b)	Direct injection into the mammary gland 0 (control) or 2.03 µmol 1-NP in 0.1 mL DMSO/rat, directly into the 3 left thoracic nipples (d 1) then 3 inguinal nipples (d 2) (total dose 12.3 µmol/rat); inner control: 0.1 mL DMSO injected into the right side of the same rats; then observed until up to 77 wks Groups of 30 aged 30 d	All tumours: 15/28 (53%), 16/27 (59%) Mammary gland (all tumours): 7/28 (25%), 7/27 (26%) Mammary gland (fibroadenoma): 6/28 (21%), 5/27 (18%) Mammary gland (adenocarcinoma): 1/28 (4%), 2/27 (7%)	NS	Purity (NR); tumours other than mammary gland were: 7 pituitary adenomas, 2 pituitary adenocar-cinomas, 2 adrenal cortical adenomas, 1 haemangioma in small intestine

[a] One extraskeletal osteosarcoma and 7 malignant fibrous histiocytomas

[b] Mammary gland tumours, injection site tumours (malignant fibrous histiocytomas) and pituitary, adrenal, thyroid, lung and other tumours at low incidence

bw, body weight; d, day; DMSO, dimethyl sulfoxide; F, female; h, hour; 3-MC, 3-methylcholanthrene; M, male; 1-NP, 1-nitropyrene; NR, not reported; NS, not significant; wk, week

The incidence of mammary tumours in treated animals (total, 16 out of 35, 46%; fibroadenoma, 9 out of 35, 26%; and adenocarcinoma, 5 out of 35, 14%) did not differ from that in the control group (12 out of 35, 34%; 9 out of 35, 26%; and 5 out of 35, 14%, respectively) (King, 1988; Imaida *et al.*, 1991a).

Groups of 30 female Sprague-Dawley rats, aged 30 days, were administered 0 or 50 µmol of 1-nitropyrene in 0.5 mL of trioctanoin by gavage once a week for 8 weeks (total dose, 400 µmol/rat) and then observed for 41 weeks at which time they were killed. 1-Nitropyrene induced an increased incidence of primarily benign mammary tumours (fibroadenomas) in treated females (10 out of 30, 33%) compared with the controls (0 out of 30; $P = 0.01$) (el-Bayoumy *et al.*, 1995). [The Working Group noted the short durations of treatment and observation.]

3.2.2 Subcutaneous administration

Groups of 20 male Fischer 344/DuCrj rats [age unspecified] received subcutaneous injections of 0 (control) or 20 mg/rat of 1-nitropyrene in 0.2 mL of DMSO twice a week for 10 weeks (total dose, 40 mg). Animals were then observed untreated for an unspecified time until they were killed. The incidence of subcutaneous tumours at the injection site (one extraskeletal osteosarcoma and seven malignant fibrous histiocytomas) was significantly increased compared with controls (0 out of 20 versus 8 out of 17; $P < 0.003$) (Ohgaki *et al.*, 1982). [Ohgaki *et al.* (1985) later reported that the 1-nitropyrene used in this study contained 1,3-dinitropyrene (0.2%), 1,6-dinitropyrene (0.3%) and 1,8-dinitropyrene (0.3%).]

Groups of 28–35 male and 28–35 female newborn Sprague-Dawley-derived CD rats received subcutaneous injections into the suprascapular region of 0 (control), 50 or 100 µmol/kg bw of 1-nitropyrene dissolved in DMSO within 24 hours of birth, then once a week for 7 weeks (total of eight injections), and were observed until 62 weeks of age. Rats that survived longer than 113 days (when the first mammary tumour was detected) were included in the effective numbers. The numbers of tumour-bearing rats (all sites) in the control, low-dose and high-dose groups were 6 out of 28 (21%), 10 out of 29 (34%) and 15 out of 31 (48%) males and 8 out of 31 (28%), 19 out of 31 (61%) and 24 out of 32 (75%) females, respectively, with significant increases compared with controls in high-dose males ($P < 0.05$) and low- and high-dose females ($P < 0.01$ and 0.001, respectively). The incidence of malignant fibrous histiocytomas at the injection site was 0 out of 28, 2 out of 29 (7%) and 10 out of 31 (32%) males and 0 out of 31, 3 out of 31 (10%) and 9 out of 32 (28%) females in the control, low-dose and high-dose groups, with significant increases in both high-dose groups ($P < 0.001$). The number of rats with mammary tumours was 2 out of 31 (6%) control, 7 out of 31 (23%) low-dose and 15 out of 32 (47%) high-dose females with a significant increase in the high-dose group ($P < 0.001$). Similarly, the incidence of mammary fibroadenomas (1 out of 31, 3%; 5 out of 31, 16%; and 7 out of 32, 22%) and adenocarcinomas (0 out of 31; 3 out of 31, 10%; and 10 out of 32, 31%) was significantly increased in the high-dose females (7 out of 32 versus 1 out of 31, $P < 0.05$; and 10 out of 32 versus 0 out of 31, $P < 0.01$; respectively) (Hirose *et al.*, 1984).

In a study similar to that of Ohgaki *et al.* (1982), groups of 10 or 20 male Fischer 344/DuCrj rats [age unspecified] received subcutaneous injections of 0 (control), 0.2 or 2.0 mg/rat of 1-nitropyrene (purified by HPLC, with no detectable dinitropyrene impurities: the concentrations of 1,3-dinitropyrene, 1,6- dinitropyrene and 1,8-dinitropyrene were less than 0.05% each, the lowest detectable concentration), dissolved in 0.2 mL of DMSO twice a week for 10 weeks and were then observed for up to 650 days (total doses per rat: 1-nitropyrene, 4 or 40 mg). No subcutaneous sarcomas were observed in the 1-nitropyrene-treated or control rats. The authors concluded that the carcinogenicity of

1-nitropyrene at the injection site observed in the previous study (Ohgaki *et al.*, 1982) was due to contamination from dinitropyrenes and not to 1-nitropyrene itself (Ohgaki *et al.*, 1985).

Groups of 30 female Sprague-Dawley CD rats, aged 30 days, received subcutaneous injections of 0 (control) or 100 µmol/kg bw of 1-nitropyrene dissolved in DMSO once a week for 4 weeks (total dose, 74.3 µmol), and surviving rats were killed 90 weeks after the first injection. No difference was found in the incidence of all tumours (27 out of 30 versus 23 out of 29) or all mammary tumours (fibroadenomas and adenocarcinomas combined; 11 out of 30 versus 17 out of 29). However, the incidence of mammary fibroadenomas was significantly increased compared with controls (8 out of 30 versus 15 out of 29; $P < 0.05$) (King, 1988; Imaida *et al.*, 1991b).

Groups of 37–49 female newborn CD 344 rats received subcutaneous injections into the suprascapular region of 0 (control) or 2.5 µmol/kg bw of 1-nitropyrene in DMSO 24 hours after birth, followed by two weekly injections of 0 or 5 µmol/kg bw and five weekly injections of 0 or 10 µmol/kg bw (total dose, 6.3 µmol [18 mg] of 1-nitropyrene in 10 µmol/5.9 mL of DMSO), and were killed at week 67. The incidence of mammary tumours (adenomas, fibroadenomas and adenocarcinomas combined) and adenocarcinomas alone was significantly increased compared with the DMSO controls (8 out of 40 versus 16 out of 49 and 1 out or 40 versus 10 out of 49, respectively) (King, 1988; Imaida *et al.*, 1995).

Groups of 48 or 49 female newborn Sprague-Dawley CD and 55 female newborn Fischer 344 rats received subcutaneous injections into the suprascapular region of 0 (control) or 100 µmol/kg bw of 1-nitropyrene dissolved in DMSO (10 µmol in 5.9 mL) once a week for 8 weeks starting 24 hours after birth and were then observed for up to 86 weeks. In CD rats, the incidence of mammary tumours (adenomas, fibroadenomas and adenocarcinomas combined) and adenocarcinomas was significantly increased compared with the DMSO controls (17 out of 47 versus 26 out of 48 and 3 out of 47 versus 10 out of 48, respectively). In Fischer 344 rats, the incidence of mammary tumours did not different significantly between treated rats and controls (1 out of 55 versus 5 out of 55) (King, 1988; Imaida *et al.*, 1995).

3.2.3 Intraperitoneal injection

Groups of 30 female Sprague-Dawley CD rats, aged 30 days, received intraperitoneal injections of 0 or 67 µmol/kg bw of 1-nitropyrene in 25 µmol/mL of DMSO three times a week for 4 weeks (total dose, 119 µmol), and were then observed up to 61 weeks after the initial injection. The incidence of tumours at all sites and mammary tumours did not differ significantly between treated animals and controls (12 out of 29 versus 15 out of 29 and 4 out of 29 versus 4 out of 29, respectively) (King, 1988; Imaida *et al.*, 1991b).

Groups of 30 female Sprague-Dawley CD rats aged 30 days, received intraperitoneal injections of 0 or 100 µmol/kg bw of 1-nitropyrene in 70 µmol/mL of DMSO once a week for 4 weeks (total dose, 77.3 µmol), and were observed until moribund or up to 87–90 weeks after the initiation of treatment. A significant increase in the incidence of mammary adenocarcinomas was observed (8 out of 29 treated animals versus 2 out of 30 controls; $P < 0.05$) and a non-significant increase in that of fibroadenomas (14 out of 29 treated rats versus 9 out of 30 controls) (King, 1988; Imaida *et al.*, 1991b).

Groups of 36 female weanling Sprague-Dawley CD rats received intraperitoneal injections of 0 or 10 µmol [2.5 mg]/kg bw of 1-nitropyrene (purity, > 99.9%) in 1.7 µmol [0.4 mg]/mL of DMSO three times a week for 4 weeks (total dose, 16 µmol [4 mg]/rat), and were killed when moribund or after 76–78 weeks. The incidence of mammary tumours was significantly increased in treated animals (25 out of 36; 14 adenocarcinomas and 19 fibroadenomas)

Table 3.3 Study of the carcinogenicity of 1-nitropyrene in hamsters

Strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Syrian golden (M) Yamamoto *et al*. (1987) 663–684 d	Intratracheal instillation 0 (control), 2.0 mg or 2.0 mg benzo[*a*]pyrene (B[*a*]P)/hamster in 0.2 mL phosphate buffer directly into the lung, once/wk for 15 wks (total dose, 30 mg) Groups of 19–35, aged 8 wks	Lung (benign tumours): 0/15, 2/21 (9%), 19/35 (54%; B[*a*]P)	NS for 1-NP	Purity, 98%; impurities (2%): 1,6-DNP, 0.6%; 1,8-DNP, 0.008%; 1,3-DNP, 1.3%; study limited by short duration of treatment, use of a single dose and impurities in the study material

d, day; DNP, dinitropyrene; M, male; 1-NP, 1-nitropyrene; NS, not significant; wk, week

compared with controls (7 out of 31; 3 adenocarcinomas and 5 fibroadenomas; $P < 0.0001$) (King, 1988; Imaida *et al*., 1991a).

3.2.4 Intrapulmonary implantation

Groups of 31 or 32 male Fischer 344/DuCrj rats, aged 10–11 weeks, received a single injection of 0.05 mL beeswax:tricaprylin (1:1) containing 0 or 1.5 mg of 1-nitropyrene (purity, > 99.9%) or 0.5 mg of methylcholanthrene directly into the lower third of the left lung after left lateral thoracotomy. Animals were observed for 72 weeks after treatment, at which time the experiment was terminated. No lung tumours were found in the 1-nitropyrene-treated or control rats, and no difference in the incidence of tumours in other organs was observed among the three groups (Maeda *et al*., 1986). [The Working Group noted that the study was limited by the short durations of both treatment and observation and the use of a single dose.]

3.2.5 Direct injection into the mammary gland

Groups of 30 female CD rats, aged 30 days, received injections of 0 or 2.03 µmol/rat of 1-nitropyrene in 0.1 mL of DMSO directly into the three left thoracic nipple areas (day 1) and then into the three left inguinal nipple areas (day 2) (total dose, 12.3 µmol/rat). As an internal control, 0.1 mL of DMSO was injected into the right-side of the nipple areas of the same rats. Thereafter, the rats were observed for up to 77 weeks. The incidence of all tumours (16 out of 27), total mammary tumours (5 out of 27), fibroadenoma (5 out of 27) and adenocarcinoma (2 out of 27) in the treated group did not differ from that in the controls (15 out of 28, 7 out of 28, 5 out of 28 and 1 out of 28, respectively) (Imaida *et al*., 1991b).

3.3 Hamster

See Table 3.3

3.3.1 Intratracheal instillation

A group of 34 male Syrian hamsters, aged 8 weeks, received intratracheal instillations of 2 mg of 1-nitropyrene (purity, 98%; impurities: 1,3-dinitropyrene, 0.008%; 1,6-dinitropyrene plus 1,8-dinitropyrene, 0.6%; pyrene, 1.3%) suspended in 0.2 mL of phosphate buffer solution once a week for 15 weeks. A further group of 35 hamsters received instillations of 2 mg of benzo[*a*]pyrene and a control group of 19 animals received phosphate buffer solution alone. All hamsters in the 1-nitropyrene-treated and control groups died within 663 and 684 days, respectively, after the initial instillation; after the 15 instillations, 24 and 16 animals in these groups, respectively,

were still alive (some died from pneumonia). Lung adenomas were detected in 2 out of 21 (9%) 1-nitropyrene-treated animals, one of which also had a squamous cell papilloma of the trachea. Tumours of the respiratory organs occurred in 19 out of 22 (54%) benzo[*a*]pyrene-treated hamsters, but not in controls (Yamamoto *et al.*, 1987). [The Working Group noted that the study was limited by the short duration of treatment, the use of a single dose level and dinitropyrene impurities in the material studied.]

4. Mechanistic and Other Relevant Data

1-Nitropyrene is a constituent of many combustion emissions, especially diesel exhaust, and has been used as a marker in air for occupational exposure to diesel exhaust. 1-Nitropyrene has been reviewed previously (IARC, 1989; IPCS, 2003); additional studies were reviewed by the Working Group.

4.1 Absorption, distribution, metabolism and excretion

4.1.1 Humans

No studies were available to the Working Group on 1-nitropyrene alone, which forms part of complex mixtures – typically diesel exhaust and ambient air contaminated with diesel exhaust – to which humans are exposed. Nevertheless, several studies have shown greater concentrations of 1-nitropyrene in diesel exhaust-contaminated air or increased urinary metabolites of 1-nitropyrene in people working in such atmospheres, suggesting that this compound may be a suitable marker for exposure to diesel exhaust in the atmosphere (Scheepers *et al.*, 1995a).

Five metabolites of 1-nitropyrene were found in the urine samples from 17 men and five women living in the city of Kanazawa, Japan (for whom no occupations associated with diesel exhaust emissions or smoking status were reported) (see Section 1.5.1(*b*)), using liquid chromatography-MS/MS, were mostly glucuronides or sulfate conjugates: 117, 109, 203, 137 and ≤ 0.54 pmol/mol of creatinine of 6- and 8-hydroxy-*N*-acetyl-1-aminopyrene, 6- and 8-hydroxy-1-nitropyrene and 3-hydroxy-1-nitropyrene, respectively (Toriba *et al.*, 2007; see Fig. 4.1).

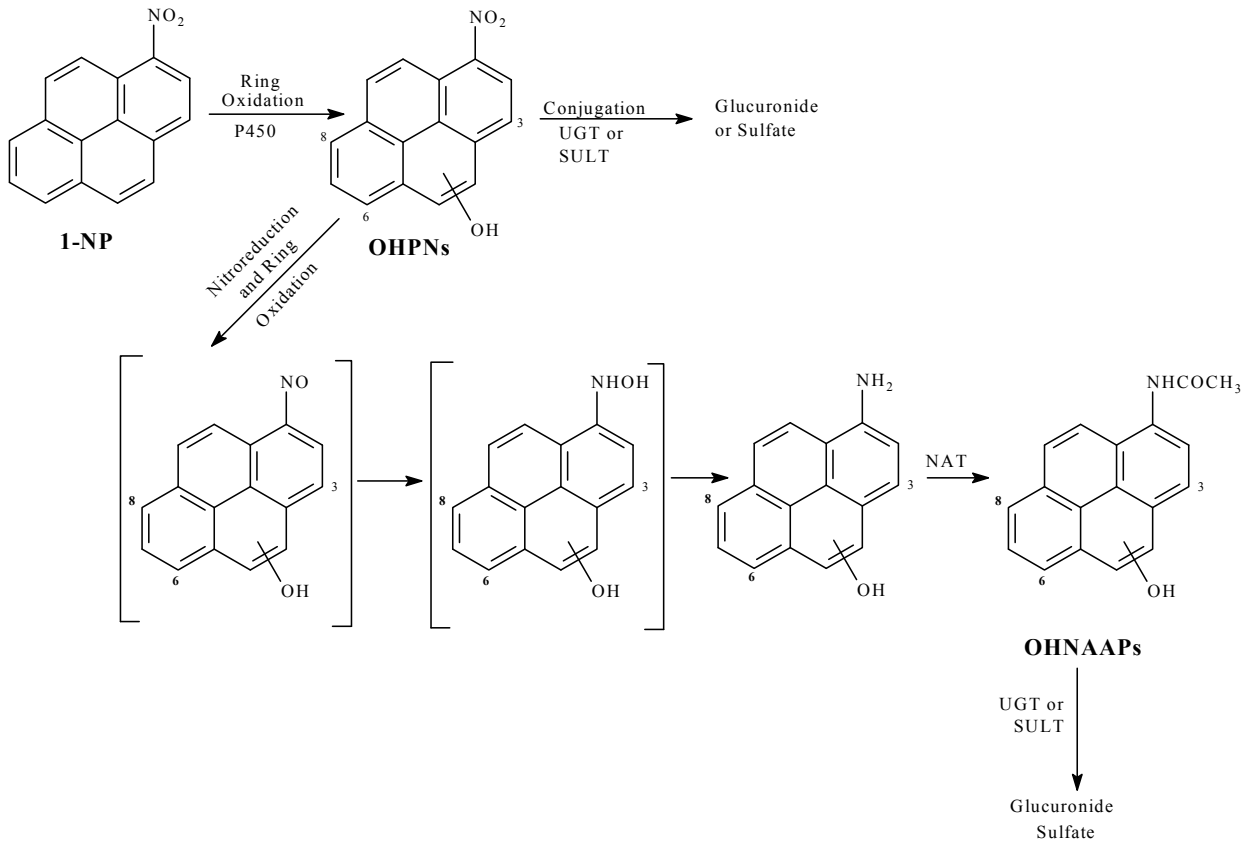
Scheepers *et al.* (1995a) found higher concentrations of 1-nitropyrene in the air of a workshop of diesel bus mechanics than in office air, and showed that the concentration was one order of magnitude greater in the shop during work hours than during off-work hours. Similarly, concentrations of 1-nitropyrene were higher in the air of a repair shop for diesel train engines than in office air, and a similar correlation was found for urinary metabolites of 1-nitropyrene in the workers (Scheepers *et al.*, 1994b). High levels of urinary metabolites of 1-nitropyrene have also been found in salt miners using diesel equipment underground (Seidel *et al.*, 2002).

4.1.2 Experimental systems

Since the previous review of 1-nitropyrene (IARC, 1989), additional studies have clarified its kinetics and metabolism, especially with regard to the role of microflora in and the organ specificity of its metabolism. A large number of studies have combined aspects of metabolism, particularly the role of nitroreductase, with genotoxicity end-points, and these are reviewed in Section 4.2.2.

Howard *et al.* (1995) found that administration of 1.03 mg/kg of [4,5,9,10-^{3}H]1-nitropyrene to C57B1/6N mice by gavage resulted in biphasic elimination kinetics from the blood with half-lives of 0.3 and 1.8 days; intraperitoneal administration resulted in similar biphasic elimination but with half-lives of 0.5 and 3 days. They also showed that, after administration of 1 mg/kg of

Fig. 4.1 Major metabolites of 1-nitropyrene in human urine



NAT, *N*-acetyltransferase; 1-NP, 1-nitropyrene; OHNAAPs, hydroxy-*N*-acetyl-1-aminopyrenes (6- and 8-OHNAAP); OHNPs, hydroxy-1-nitropyrenes (3-, 6-, and 8-OHNP); P450, cytochrome P450; SULT, sulfotransferase; UGT, UDP-glucuronyltransferase
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[4,5,9,10-^{3}H]1-nitropyrene by gavage to pregnant mice, 1-nitropyrene crossed the placenta and accumulated in the fetus and amniotic fluid; both carbon oxidized and nitroreduced metabolites were formed. The neonates received ~0.1% of the administered dose and harboured both types of metabolite.

Wolff *et al.* (1989) noted that 1-nitropyrene is typically associated with carbonaceous particles in the environment rather than as an aerosol of the pure compound, and showed that, when [^{14}C]1-nitropyrene adsorbed to carbon black particles was inhaled by Fischer 344 rats, the interaction of the reactive metabolites of [^{14}C]1-nitropyrene with target macromolecules was greater than that caused by inhalation of [^{14}C]1-nitropyrene as a pure compound.

Using a DNA repair-deficient Chinese hamster ovary cell line, Thornton-Manning *et al.* (1991a) tested the mutagenicity of 1-nitropyrene under aerobic and anaerobic metabolic conditions and analysed the metabolites. The results suggested that the exogenous metabolic activation-mediated metabolites produced under anaerobic conditions resulted from nitroreductive metabolism (1-aminopyrene), whereas those produced under aerobic conditions of exogenous metabolic activation-mediated metabolism resulted from ring-oxidized metabolites (1-nitropyrene phenols and dihydrodiols).

The treatment of HepG2 cells with 1-nitropyrene, followed by administration of 3-methylcholanthrene, increased the ratio of ring-oxidation to nitroreduction, which was accompanied by a decrease in the C-8-guanyl adduct of 1-nitropyrene (*N*-(deoxyguanosine-8-yl)-1-aminopyrene; dG-C8-AP) via nitroreduction with no further increase in other 1-nitropyrene adducts (Silvers *et al.*, 1994). The authors suggested that the cytochrome P450 (CYP)-mediated metabolism of 1-nitropyrene to epoxides, phenols and dihydrodiols is not an activation pathway in HepG2 cells, and indicated that this might explain the weak carcinogenicity 1-nitropyrene *in vivo*, in which CYP-mediated ring-oxidation predominates.

Van Bekkum *et al.* (1999) administered [^{14}C]-1-nitropyrene intragastrically to rats and found that it was absorbed rapidly from the gastrointestinal tract based on early peak concentrations of radioactivity in blood and other tissues. These data indicated an important role of intestinal microflora in the enterohepatic recirculation but not in the nitroreduction of 1-nitropyrene before adsorption from the gastrointestinal tract. The levels of radioactivity associated with plasma proteins were approximately four times higher than those associated with haemoglobin, and those associated with DNA were highest initially in the liver but decreased rapidly to levels lower than those observed in the kidney. The radioactivity associated with DNA in the lungs was 8–50 times lower than that in the liver and kidneys.

Nitroreductase activity, measured by the production of 1-aminopyrene from 1-nitropyrene, following treatment with various organ homogenates from Sprague-Dawley rats was found to be high in the liver and small intestine but low in the lung and alveolar macrophages (Kinouchi & Ohnishi, 1986). The intestinal contents also had high nitroreductase activity that was proportional to the number of bacteria (especially anaerobic bacteria) in the intestine, which provided further evidence of the potential importance of the gut flora in the metabolism of 1-nitropyrene via nitroreduction.

To examine this further, Kinouchi *et al.* (1992, 1993) pretreated rats with antibiotics to kill the gut flora *in vivo* followed by oral administration of K-region epoxides of 1-nitropyrene [1-nitropyrene-4,5-oxide and 1-nitropyrene-9,10-oxide] and found no DNA adducts in the lower intestinal mucosa, whereas adducts were found in unpretreated rats exposed to these conjugates. The authors showed that the intestinal microflora played an important role in the adsorption of the metabolites of glutathione conjugates of 1-nitropyrene oxides from the intestinal tract and the activation of the metabolites in the intestine. Kinouchi *et al.* (1993) also showed that DNA adducts were formed in the intestinal mucosa after administration of glutathione conjugates of 1-nitropyrene oxides (1-nitropyrene-4,5-oxide *S*-glutathione and 1-nitropyrene-9,10-oxide *S*-glutathione) to control mice, which indicated the metabolic activation of cysteine conjugates of 1-nitropyrene oxides (1-nitropyrene oxide-cysteine) by microfloral β-lyase. Kataoka *et al.* (1995) also showed that 1-nitropyrene oxide-cysteine is converted to a more genotoxic form by β-lyase-mediated deconjugation and nitroreduction.

Ball *et al.* (1991) administered [^{14}C]1-nitropyrene intraperitoneally to germ-free and conventional rats and showed that formation of the metabolite, 6-hydroxy-1-acetylaminopyrene, in the urine involved the gut flora; both nitroreduction and the hydrolysis of glucuronides released for enterohepatic recirculation were essential for the generation of the mutagenic urinary metabolites of 1-nitropyrene.

However, Ayres *et al.* (1985) demonstrated that gut microfloral metabolism was not the only pathway for the bioactivation of 1-nitropyrene in rats, because the level of macromolecular binding of radiolabelled 1-nitropyrene administered orally to rats was initially reduced

by almost half in the lungs of animals pretreated with antibiotics to kill the gut flora, compared with unpretreated rats, although the levels were similar 1 week later.

In an in-vitro study, King *et al.* (1990) found that 1-nitropyrene was metabolized by human, rat or mouse intestinal microflora to 1-aminopyrene, *N*-acetyl-1-aminopyrene, *N*-formyl-1-aminopyrene and two unknown metabolites. 1-Aminopyrene was the predominant metabolite generated by all three microflora, accounting for 98, 79 and 88% of the total radioactivity, respectively. Thus, microflora from all of the species that were tested metabolized 1-nitropyrene in a similar manner.

Rafii & Cerniglia (1995) identified and isolated various species of *Clostridium* and *Eubacterium* from human intestinal microbial flora that metabolized 1-nitropyrene *in vitro* to produce aromatic amines. The nitroreductase activities of the bacteria were found to be constitutive and extracellular and, although each bacterial species had one isozyme, each species had different isozymes.

Studies in rodents involving intragastric, intraperitoneal or inhalation routes of exposure to 1-nitropyrene or 1-nitropyrene coated onto diesel exhaust particles found that the majority (50–60%) of the administered dose was excreted in the faeces and ~15–20% appeared in the urine (Bond *et al.*, 1986; IARC, 1989; Howard *et al.*, 1990; Kataoka *et al.*, 1991; Silvers *et al.*, 1992). As reviewed by van Bekkum *et al.* (1999), the major pathways in the biotransformation of 1-nitropyrene administered to rats, on the basis of the metabolites found in the urine and faeces, are nitroreduction with subsequent acetylation or *N*-oxidation, ring-hydroxylation and conjugation. After intraperitoneal administration of 1-nitropyrene to rats, Chae *et al.* (1997) identified metabolites in the urine and faeces that had been formed via nitroreduction and ring-oxidation pathways.

A comparison of the macromolecular binding of radiolabelled 1-nitropyrene following its oral administration to or inhalation by rats showed that the greatest amount of binding occurred in the kidney, followed by the liver and then the lung, regardless of the route of administration (Medinsky *et al.*, 1988).

Coal fly ash particles (≤ 3 μm) were coated with 1-nitropyrene by exposure to vapour in a nitrogen atmosphere. Extraction with organic solvents and chemical analysis showed that the concentration of 1-nitropyrene was 160–220 ppm. 1-Nitropyrene was shown to be bioavailable in rabbit alveolar macrophages exposed to this coated fly ash in either agar or aqueous culture medium. The coated fly ash showed mutagenic activity when the particles were tested directly, whereas the uncoated fly ash did not (Mumford *et al.*, 1986).

Rosser *et al.* (1996) proposed a generalized scheme for the metabolism of 1-nitropyrene in rodents that involves nitroreduction, epoxidation, enhancement of the genotoxicity of a hydroxylamine intermediate by *O*-acetylation, and further activation of an arylacetamide. They suggested that *N*-hydroxylation followed by *O*-esterification, as opposed to further exogenous metabolic activation-catalysed ring-oxidation, is a major route of activation for the urinary metabolites of 1-nitropyrene in rodents.

Using human lung and liver microsomes, Chae *et al.* (1999) identified some differences between the metabolism of 1-nitropyrene in humans and rodents. They suggested that human liver does not metabolize 1-nitropyrene to 1-aminopyrene, whereas rodent liver does. They also showed that CYP3A4, and to a lesser extent CYP1A2, produced 3-hydroxy-1-nitropyrene as the major hydroxylated metabolite in humans, whereas 6-hydroxy- and 8-hydroxy-1-nitropyrene were produced at lower levels. In contrast, the 6- and 8-hydroxy metabolites were produced to a greater extent than 3-hydroxy-1-nitropyrene in rodents. The authors also showed

that *trans*-4,5-dihydrodiol-1-nitropyrene was produced preferentially to the glutathione conjugate in humans, whereas the reverse occurred in rodents. The authors concluded that, due these metabolic differences, rodents may not accurately predict the susceptibility of humans to 1-nitropyrene-induced carcinogenicity. Sun *et al.* (2004) found that primary cultures of human breast cells and cultured human breast cell lines activated 1-nitropyrene to genotoxic metabolites via ring-oxidation.

Using sensitive analytical techniques, Toriba *et al.* (2007) analysed the urine from healthy, non-occupationally exposed subjects and identified various 1-nitropyrene metabolites, including 6- and 8-hydroxy-*N*-acetyl-1-aminopyrenes and 3-, 6- and 8-hydroxy-1-nitropyrenes. These metabolites had been predicted to occur in human urine on the basis of in-vitro and rodent studies of the metabolism of 1-nitropyrene.

Ueda *et al.* (2005) also showed that molybdenum hydroxylases from the skin of mice, rats and guinea-pigs were involved in the nitroreduction of 1-nitropyrene.

Probably due to differences in metabolism, 1-nitropyrene produces tumours at different organ sites in different species: lung and liver cancer in mice, tracheal cancer in hamsters and mammary cancer in rats (see Chae *et al.*, 1999).

4.2 Genetic and related effects

See Table 4.1

4.2.1 Humans

No studies were available to the Working Group in which humans were exposed solely to 1-nitropyrene. However, studies have been carried out in humans exposed to complex combustion emissions (especially diesel exhaust) containing 1-nitropyrene, and these are reviewed in Section 4.2.1 of the *Monograph* on Diesel and Gasoline Engine Exhausts in this Volume.

4.2.2 Experimental systems

(a) Formation of DNA adducts

1-Nitropyrene induced DNA adducts in a variety of systems, and additional studies reviewed below have confirmed and extended the initial observations described in the previous *Monograph* (IARC, 1989).

(i) In-vivo studies

See Fig. 4.2

Administration of 1-nitropyrene (100 mg/kg bw) by gavage to rats produced DNA adducts detected by ^{32}P-postlabelling in the liver and mammary fat pads (Roy *et al.*, 1989). In the same study, the authors exposed calf-thymus DNA to 1-nitropyrene in the presence of xanthine and xanthine oxidase and confirmed the formation of the adduct dG-C8-AP, which co-chromatographed with a minor adduct found in the liver and mammary fat pads. The authors concluded that the major adduct formed *in vivo* did not seem to originate from the simple nitroreduction of 1-nitropyrene and that other pathways, such as ring-oxidation or ring-oxidation followed by nitroreduction, may account for the major adduct found *in vivo*. However, Chae *et al.* (1997) found no DNA adducts in the liver but did find adducts in mammary DNA in rats after intraperitoneal injection of 1-nitropyrene.

Herreno-Saenz *et al.* (1995) also found the primary dG-C8-AP DNA adduct in the mammary tissue of rats exposed to 1-nitropyrene [dose not reported], and also identified two minor adducts: 6-(deoxyguanosin-N2-yl)-1-aminopyrene and 8-(deoxyguanosin-N2-yl)-1-aminopyrene. Smith *et al.* (1990) identified dG-C8-AP and *N*-(deoxyguanosin-8-yl)-1-amino-3-, 6- and/or 8-nitropyrene DNA adducts in Sprague-Dawley rats, and CD-1 and A/J mice.

Haemoglobin and albumin adducts were identified in rats administered 1-nitropyrene (dose range, 0.1–1000 µg/kg bw) by gavage (el-Bayoumy *et al.*, 1994a, b), and another study

Table 4.1 Genetic and related effects of 1-nitropyrene

Reference	System	Result
DNA adducts *in vivo*		
Roy *et al.* (1989)	Rat, gavage; mammary fat pads and liver	+
Chae *et al.* (1997)	Rat, intraperitoneal; mammary tissue	+
Chae *et al.* (1997)	Rat, intraperitoneal; liver	–
Herreno-Saenz *et al.* (1995)	Rat, gavage; mammary tissue	+
Smith *et al.* (1990)	Rat and mouse, subcutaneous or intraperitoneal; mammary gland and site of injection (rat), liver and lung (mouse)	+
el-Bayoumy *et al.* (1994b)	Rat, gavage	+
DNA adducts *in vitro*		
Arimochi *et al.* (1998)	*Salmonella typhimurium*	+
Herreno-Saenz *et al.* (1995)	*Salmonella typhimurium*	+
Herreno-Saenz *et al.* (1995)	Calf-thymus DNA plus xanthine oxidase or rat liver microsomes and cytosol	+
Roy *et al.* (1989)	Calf-thymus DNA plus xanthine oxidase	+
King *et al.* (1994)	Calf-thymus DNA	+
Mitchell & Akkaraju (1989)	Rat lung-cell nuclei	+
Kucab *et al.* (2012)	Hupki (human *TP53* knock-in) mouse embryo fibroblasts (J201)	+
Silvers *et al.* (1997)	HepG2 cells	+
Qu & Stacey (1996)	Rat hepatocytes	+
Haemoglobin adducts *in vivo*		
van Bekkum *et al.* (1997)	Rat, gavage	+
el-Bayoumy *et al.* (1994b)	Rat, gavage	+
Suzuki *et al.* (1989)	Rat, gavage	+
Albumin adducts *in vivo*		
el-Bayoumy *et al.* (1994a)	Rat, gavage	+
DNA damage *in vivo*		
el-Bayoumy *et al.* (2000)	Rat, gavage; 8-OH-dG in mammary fat pad	–
Igarashi *et al.* (2010)	Mouse, intraperitoneal; comet assay, liver	+
DNA damage *in vitro*		
Becher *et al.* (1993)	Rabbit lung cells (Clara and type II) and alveolar macrophages; alkaline elution	–
Andersson *et al.* (2009)	Human umbilical vein endothelial cells; comet assay	+
Martin *et al.* (1999)	Human breast cancer cell line (MCL-5); comet assay	–
Martin *et al.* (2000)	Exfoliated human breast milk cells; comet assay	+
Kucab *et al.* (2012)	Hupki (human *TP53* knock-in) mouse embryo fibroblasts (J201); comet assay	+

Table 4.1 (continued)

Reference	System	Result
Mitchelmore *et al.* (1998)	Digestive gland cells from mussels; comet assay	+
Mori *et al.* (1991)	Rat primary hepatocytes; tritium	+
Kim *et al.* (2005b)	Human lung cell line A549; 8-OH-dG	+
Asare *et al.* (2009)	Mouse Hepa1c1c7 cells; comet assay, formamidopyrimidine-DNA glycosylase, 8-oxoguanine	+
Li *et al.* (2009)	Human lymphocytes; comet assay	+
Schehrer *et al.* (2000)	Rat and human hepatocytes; unscheduled DNA synthesis	+
Gene mutation in bacteria		
Hakura *et al.* (1999)	*Salmonella typhimurium* TA98 with human or rat S9	+
Hatanaka *et al.* (2001)	*Salmonella umu* assay with rat lung, liver, kidney S9	+
DeMarini *et al.* (1989)	*Salmonella typhimurium* TA98 micro-suspension assay	+
Lewtas *et al.* (1990)	*Salmonella typhimurium* TM677, TA98 coupled to HPLC	+
Yu *et al.* (1991)	*Salmonella typhimurium* TA98, TA97, TA98, TA98NR, TA98/1,8-DNP6, TA100, TA100NR, TA102, TA104, TA1538	+
Salamanca-Pinzón *et al.* (2006, 2010)	*Salmonella typhimurium*, enzyme extracts of nitroreductases	+
Watanabe *et al.* (1989)	*Salmonella typhimurium* YG1021	+
Oda *et al.* (1992)	*Salmonella typhimurium* NM1011	+
Oda *et al.* (1993)	*Salmonella typhimurium* NM3009	+
Carroll *et al.* (2002)	*Salmonella typhimurium* TA1535, TA 1538, TA100, TA98	+
Østergaard *et al.* (2007)	*Salmonella typhimurium* TGO1, TGO2	+
Consolo *et al.* (1989)	*Salmonella typhimurium* TA98, TA98NR, TA98/1,8DNP6	+
Arimochi *et al.* (1998)	*Salmonella typhimurium* TA98, TA98NR, YG1021	+
Hagiwara *et al.* (1993)	*Salmonella typhimurium* YG1021, YG1024, YG1041	+
Watanabe *et al.* (1993)	*Salmonella typhimurium* YG1024, YG1012	+
Watanabe *et al.* (1990)	*Salmonella typhimurium* YG1024, YG1029	+
Oda *et al.* (1999)	*Salmonella typhimurium* NM6001, NM6002	+
Mersch-Sundermann *et al.* (1991)	*Escherichia coli* PQ37, SOS Chromotest; –S9	+
Rosser *et al.* (1996)	*Salmonella typhimurium* YG1024, TA98, TA98NR, TA98/1,8-DNP6	+
Yamazaki *et al.* (2000)	*Escherichia coli* expressing human CYP1B1	+
Oda *et al.* (1996)	*Salmonella typhimurium* NM5004 expressing rat GST T1	w+
Oda *et al.* (2012)	*Salmonella typhimurium* expressing human CYP1A2/sulfotransferase	+
Bonnefoy *et al.* (2012)	*Salmonella umu* assay ± S9	+/+

Table 4.1 (continued)

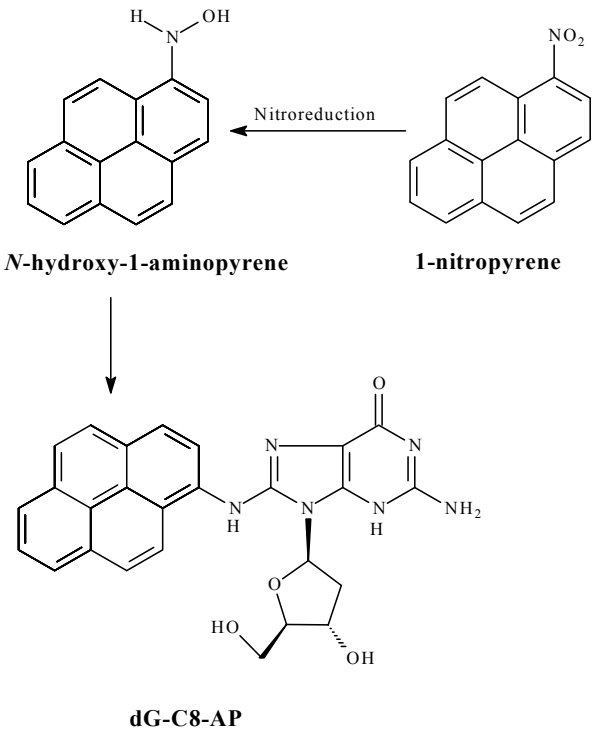
Reference	System	Result
Watanabe *et al.* (1997)	*Salmonella typhimurium* TA7005, TA7006, related strains; –S9	+
DeMarini *et al.* (1996)	*Salmonella typhimurium* UTH8413, TA1538, TA98, TA100 ; –S9	+
DeMarini *et al.* (1996)	*Salmonella typhimurium* TA1978, TA1975, UTH8414, TA1535 ; –S9	–
Watanabe-Akanuma & Ohta (1994)	*Escherichia coli lacZ* ZA1607, 08, 10, 11; ZA8108, 10, 11; –S9	+
Nohmi *et al.* (1995)	*Salmonella umuDCST* required for –GC mutagenesis,–-S9	+
Gene mutation in mammalian cells *in vivo*		
Ball *et al.* (1991)	Rat, germ-free, intraperitoneal; urinary mutagenicity in *Salmonella typhimurium* TA98 ± S9	w+
Ball *et al.* (1991)	Rat, conventional, intraperitoneal; urinary mutagenicity in *Salmonella typhimurium* TA98 ± S9	+
Varga *et al.* (2006)	Rat, intraperitoneal; urinary mutagenicity *Salmonella typhimurium* TA100	+
Varga *et al.* (2006)	Rat, intraperitoneal; urinary mutagenicity *Salmonella typhimurium* TA98	–
Varga *et al.* (2006)	Rat, gavage; urinary mutagenicity *Salmonella typhimurium* TA98, TA100	+
Scheepers *et al.* (1991)	Rat, intraperitoneal; urinary mutagenicity *Salmonella typhimurium* TA98, TA1538, YG102, YG1024	+
Gene mutation in mammalian cells *in vitro*		
Rodent cells		
Heflich *et al.* (1990)	CHO/*Hprt* –S9	–
Heflich *et al.* (1990)	CHO/*Hprt* +S9	+
Thornton-Manning *et al.* (1991a, b)	CHO-UV5/*Hprt* (repair-deficient) –S9	+
Kappers *et al.* (2000)	Chinese hamster V79/*Hprt* expressing CYP1A2 or CYP3A4 –S9	–
Kappers *et al.* (2000)	NIH/3T3/*lacZ* reporter gene, expressing CYP1A2 –S9	+
	Human cells	
Silvers *et al.* (1994)	HepG2/*HPRT* –S9	+
Durant *et al.* (1996)	Human B-lymphoblastoid h1A1vs/*HPRT* expressing CYP1A1 –S9	+
Gene mutation in yeast		
Rhenimi *et al.* (2008)	Gene mutation, gene conversion, crossing-over	+
Gene mutation in plants		
Katoh *et al.* (1994)	Soya bean, gene mutation	+
Cytogenetic effects		
Chromosomal aberrations		
Lafi & Parry (1987)	Chinese hamster DON:Wg3H cells, –S9	+
Matsuoka *et al.* (1991)	Chinese hamster lung cells, –S9	–
Matsuoka *et al.* (1991)	Chinese hamster lung cells, +S9	+

Table 4.1 (continued)

Reference	System	Result
Pusztai *et al.* (1998)	Mouse, intraperitoneal; bone marrow *in vivo*	+
Micronuclei		
Bonnefoy *et al.* (2012)	Human lymphocytes, 63% no centromere (chromatid breakage)	+
Igarashi *et al.* (2010)	Mouse, intraperitoneal; liver *in vivo*	+
Transformation		
West & Rowland (1994)	Rat tracheal epithelial cells *in vitro*	-
Ensell *et al.* (1998)	Rat tracheal epithelial cells *in vitro*	-
Mitchell & Thomassen (1990)	Rat tracheal epithelial cells *in vitro*	-
Ensell *et al.* (1998, 1999)	Rat tracheal epithelial cells *in vivo*	+
Sheu *et al.* (1994)	BALB/3T3 A31-1-1 cells *in vitro*	+
Denda *et al.* (1989)	F344 rat liver, γ-glutamyl transpeptidase-positive foci *in vivo*	+

CHO, Chinese hamster ovary cells; CYP, cytochrome P450; GST, glutathione *S*-transferase; Hprt/HPRT, hypoxanthine-guanine phosphoribosyltransferase; 8-OH-dG, 8-hydroxydeoxyguanosine; S9, exogenous metabolic activation system

Fig. 4.2 The major DNA adduct identified *in vivo* in animals treated with 1-nitropyrene



dG-C8-AP, *N*-(deoxyguanosin-8-yl)-1-aminopyrene

found that the levels of haemoglobin adducts were twofold higher than those of albumin adducts in rats after oral administration of 1-nitropyrene (van Bekkum *et al.*, 1997). Suzuki *et al.* (1989) also identified haemoglobin adducts in rats exposed to 1-nitropyrene *in vivo*.

(ii) In-vitro studies

Arimochi *et al.* (1998) exposed *S. typhimurium* strains that had three levels of nitroreductase activity (low, wild-type and excessive) to 1-nitropyrene and showed that the levels of DNA adducts and mutagenicity correlated with those of nitroreductase.

The dG-C8-AP adduct was found in 1-nitropyrene-treated calf-thymus DNA in the presence of xanthine and xanthine oxidase, as well as two minor adducts: 6-(deoxyguanosin-N2-yl)-1-aminopyrene and 8-(deoxyguanosin-N2-yl)-1-aminopyrene. All three adducts were also found in 1-nitropyrene-treated calf-thymus DNA in the presence of rat liver microsomes and cytosols and in 1-nitropyrene-treated DNA from *Salmonella* (Herreno-Saenz *et al.*, 1995). The dG-C8-AP adduct was also formed after exposure of calf-thymus DNA to 1-nitropyrene in the presence of xanthine oxidase (Roy *et al.*, 1989).

Another study of 1-nitropyrene-exposed calf-thymus DNA found a variety of DNA adducts determined by HPLC-^{32}P postlabelling; however, none of these peaks co-eluted with those produced by the incubation of calf-thymus DNA with an organic solvent extract of diesel exhaust particles (King *et al.*, 1994).

Mitchell & Akkaraju (1989) exposed rat lung cell nuclei *in vitro* to radiolabelled 1-nitropyrene and found that more of the label was bound to active chromatin and the nuclear matrix than to bulk chromatin fractions. They concluded that the selective binding of 1-nitropyrene to specific regions was related to the open state of the chromatin structure.

1-Nitropyrene induced DNA adducts (detected by ^{32}P-postlabelling) to a greater extent in an immortal Hupki (human *TP53* knock-in) mouse embryo fibroblast (HUF) cell line (J201) than in primary Hupki (human *TP53* knock-in) mouse embryo fibroblasts (Kucab *et al.*, 2012). 1-Nitropyrene also induced DNA adducts (detected by postlabelling) in freshly isolated rat hepatocytes (Qu & Stacey, 1996).

Exposure of a HepG2 human hepatoblastoma cell line to 1-nitropyrene resulted in the formation of the dG-C8-AP adduct, and the induction of ring-oxidative metabolism by treatment with 2,3,7,8-tetrachlorodibenzodioxin resulted in a decrease in the levels of this adduct (Silvers *et al.*, 1997). The authors concluded that CYP-mediated ring-oxidative pathways were detoxification pathways in HepG2 cells because no DNA adducts of oxidized 1-nitropyrene metabolites were detected in the 2,3,7,8-tetrachlorodibenzodioxin-treated cells exposed to

1-nitropyrene. The authors also exposed HepG2 cells to 1-nitropyrene and 3-methylcholanthrene to increase the ratio of ring-oxidation to nitroreduction and found a decrease in the dG-C8-AP adduct (via nitroreduction) with no increase in any other 1-nitropyrene DNA adducts.

(b) DNA damage

8-Hydroxy-2′-deoxyguanosine, a marker of oxidative damage, was not found in mammary fat pad tissue isolated from rats administered 1-nitropyrene by gavage (el-Bayoumy *et al.*, 2000). Intraperitoneal administration of 1-nitropyrene to mice induced DNA damage in the liver detected by the comet assay (Igarashi *et al.*, 2010).

Using alkaline elution, Becher *et al.* (1993) did not find any DNA damage in isolated rabbit lung cells (Clara and type II) or lung alveolar macrophages exposed to 1-nitropyrene *in vitro*. No DNA damage was found in the comet assay in 1-niropyrene-treated human breast cancer cells (MCL-5) *in vitro*, even in the presence of DNA-repair inhibitors (Martin *et al.*, 1999).

The induction of DNA damage was demonstrated in the comet assay in other cell types exposed to 1-nitropyrene *in vitro*, including human umbilical vein endothelial cells (Andersson *et al.*, 2009), exfoliated human breast milk cells in the presence of DNA-repair inhibitors (Martin *et al.*, 2000), Hupki (human *TP53* knock-in) mouse embryo fibroblasts (Kucab *et al.*, 2012), mouse Hepa1c1c cells (Asare *et al.*, 2009), human lymphocytes (Li *et al.*, 2009) and digestive gland cells isolated from mussels (Mitchelmore *et al.*, 1998). The use of tritium to monitor unscheduled DNA synthesis also showed that 1-nitropyrene induced DNA damage in primary rat hepatocytes *in vitro* (Mori *et al.*, 1991).

1-Nitropyrene also has been shown to induce oxidative damage, as demonstrated by the presence of 8-hydroxy-2′-deoxyguanosine, after in-vitro treatment of the A549 human lung adenocarcinoma cell line (Kim *et al.*, 2005a). It induced DNA damage, as detected in the unscheduled DNA synthesis assay, in human and rat hepatocytes (Schehrer *et al.*, 2000).

(c) Mutagenicity

1-Nitropyrene was mutagenic in a variety of systems, as reviewed in the previous *Monograph* (IARC, 1989). Additional studies that have explored the metabolism required to produce mutagenic metabolites of 1-nitropyrene, its mutation spectrum and the molecular mechanisms by which it induces mutations are reviewed below.

(i) Bacterial mutagenesis

Hayakawa *et al.* (1997) fractionated organic extracts of diesel engine exhaust particles and showed that 1-nitropyrene, together with several other nitro-PAHs, accounted for half of the mutagenic activity of the semi-polar fraction of diesel exhaust particles. Thus, 1-nitropyrene is a highly relevant component of diesel exhaust in terms of the mutagenicity of this complex combustion emission. Although the mechanisms of the interactions of 1-nitropyrene with other components of diesel exhaust emissions have not been elucidated, Lee *et al.* (1994) showed that, when benzo[*a*]pyrene in combination with 1-nitropyrene was tested for mutagenicity in *Salmonella* in the absence of an exogenous metabolic activation system, the mutagenic potency of 1-nitropyrene was reduced, and presumed that this was due to alterations in the nitroreductase activity of the bacterium.

Hakura *et al.* (1999) demonstrated that the mutagenic potency of 1-nitropyrene in *S. typhimurium* TA98 in the presence of five exogenous metabolic activation systems produced from the livers of different human subjects was similar to or slightly greater than that in the presence of a system produced from non-induced Sprague-Dawley rat liver. Because 1-nitropyrene is one of the primary mutagens in diesel exhaust, Hatanaka *et al.* (2001) exposed rats to 0.3 mg/m^3 of diesel exhaust particles and found that the level of induction of CYP1B1 was sufficient for

the exogenous metabolic activation systems produced from their lungs, liver or kidneys to metabolize 1-nitropyrene to a genotoxic agent in the *Salmonella umu* gene-expression assay in a strain that overexpressed *O*-acetyltransferase.

1-Nitropyrene was mutagenic in a micro-suspension assay in *Salmonella* (DeMarini *et al.*, 1989), which was then used to detect 1-nitropyrene metabolites generated by exogenous metabolic activation systems (Lewtas *et al.*, 1990), by incubating 1-nitropyrene with a system from rabbit lung and characterizing the mutational profile of the metabolites that were separated by bioassay-directed fractionation using HPLC and then tested in this assay. The HPLC fractions were tested in forward mutation assays in *S. typhimurium* strains TM677 and TA98. Approximately 12 peaks of mutagenic activity were detectable by TA98, suggesting that at least the same number of mutagenic metabolites were formed from the metabolism of 1-nitropyrene by the rabbit lung activation system. Six of the peaks were identified using standards that co-chromatographed with the peaks of mutagenic activity in the 'mutagram': 3- or 8-hydroxy-*N*-acetyl-l-aminopyrene, [*trans*]4,5-dihydro-4,5-dihydroxy-l-nitropyrene, *N*-acetyl-1-aminopyrene, 1-aminopyrene and 3-, 6- or 8-hydroxy-nitropyrene.

1-Nitropyrene was found to be a direct-acting mutagen in a wide variety of *S. typhimurium* strains, including TA98, TA97, TA98, TA98NR, TA98/1,8-DNP_6, TA100, TA100NR, TA102, TA104, TA1035 and TA1538 (Yu *et al.*, 1991). It was most potent in TA97, which contains a series of six cytosines and reverts by the frameshift deletion of a C in this sequence. This result was consistent with the formation of 1-nitropyrene adducts at GC sites and with the known frameshift activity of 1-nitropyrene in TA98, which contains repeated series of GCs. This study confirmed that the lower levels of nitroreductase present in TA98NR reduced the mutagenic potency of 1-nitropyrene relative to that in TA98. It also showed that 1-nitropyrene was a potent mutagen in strain TA104, which reverts by mutation at AT sites, indicating that the compound also forms adenine adducts. Several studies have indicated that the *cnr* gene in *Salmonella* codes for the major nitroreductase responsible for activating 1-nitropyrene to a mutagen (Salamanca-Pinzón *et al.*, 2006, 2010).

Whereas *S. typhimurium* strains TA98NR or TA100NR, which are deficient in nitroreductase, reduced the mutagenic potency of 1-nitropyrene compared with that in the parent strains, its mutagenic potency was increased in strains that overexpress nitroreductase (Watanabe *et al.*, 1989; Oda *et al.*, 1992, 1993; Carroll *et al.*, 2002; Østergaard *et al.* 2007). Consolo *et al.* (1989) also confirmed that the mutagenicity of 1-nitropyrene in *Salmonella* was dependent on nitroreductase but not on *O*-esterificase on the basis of results obtained in TA98/1,8DNP_6. Arimochi *et al.* (1998) demonstrated that the mutagenic potency and induction of DNA adducts of 1-nitropyrene were highest in a strain of *Salmonella* that overexpresses nitroreductase (YG1021), were lower in a strain with normal levels of nitroreductase (TA98) and lowest in a strain that had reduced levels of the enzyme (TA98NR).

Further insight into the metabolism of 1-nitropyrene to mutagenic forms in *Salmonella* was obtained by the introduction of derivatives of strain TA98 that overexpress acetyltransferase (YG1024) and overexpress both nitroreductase and acetyltransferase (YG1041) (Hagiwara *et al.*, 1993). The mutagenic potency of 1-nitropyrene relative to that in TA98 was 37.1-fold in YG1021 (overexpressing nitroreductase), 8.2-fold in YG1024 (overexpressing acetyltransferase) and 356.4-fold in YG1041 (overexpressing both enzymes). This study clearly demonstrated the role of *O*-acetyltransferase as well as nitroreductase in the metabolic activation of 1-nitropyrene to a frameshift mutagen. Watanabe *et al.* (1993) noted that the pKM101 plasmid, which is found in strain YG1024 that overexpresses acetyltransferase, was also essential for

1-nitropyrene-enhanced mutagenesis in this strain because the compound was far less mutagenic in an isogenic strain (YG1012) that does not carry the plasmid. Watanabe *et al.* (1990) introduced the *Salmonella* strain YG1029, which is a base-substitution strain derived from TA100 that overexpresses acetyltransferase, that also enhanced the mutagenicity of 1-nitropyrene, which was, however, much less mutagenic at the base-substitution allele than at the frameshift allele.

Some studies have indicated that the metabolic activation of 1-nitropyrene to a mutagen in bacteria may be simpler than and different from that in mammalian systems. Oda *et al.* (1999) showed that 1-nitropyrene was more mutagenic in the *Salmonella umu* test in which human *N*-acetyltransferase 1 was expressed than in a test in which the human *N*-acetyltransferase 2 was expressed, and was least mutagenic in a strain that overexpressed *O*-acetyltransferase. It was also mutagenic in the absence of an exogenous metabolic activation system in the standard *Escherichia coli* PQ37 chromotest for SOS induction (Mersch-Sundermann *et al.*, 1991).

Rosser *et al.* (1996) characterized the mutagenicity of the primary oxidized metabolites of 1-nitropyrene that were identified following mammalian metabolism in strains of *Salmonella* with low, standard or elevated levels of nitroreductase or acetyltransferase. The results indicated that *N*-hydroxylation followed by *O*-esterification, as opposed to further exogenous metabolic activation-catalysed ring-oxidation, was a major route of activation for the urinary metabolites of 1-nitropyrene in rodents.

The roles of other enzymes in the activation of 1-nitropyrene to mutagenic metabolites were investigated in an *umu* assay in *E. coli* into which human CYP1A1, 1A2 and 1B1 had been cloned (Yamazaki *et al.*, 2000); human CYP1B1 was found to activate 1-nitropyrene to a mutagen in this system. Using a strain of *Salmonella* that expressed rat glutathione *S*-transferase 5–5 (NM5004), Oda *et al.* (1996) showed that this enzyme reduced the mutagenic potency of 1-nitropyrene compared with strains in which it was not expressed. The mutagenic potency of 1-nitropyrene was also enhanced by human sulfotransferase 1A1 in a *Salmonella umu* assay expressing this enzyme (Oda *et al.*, 2012).

(ii) Mammalian cell mutagenesis

Since the previous *Monograph* (IARC, 1989), additional studies have confirmed and extended the finding that 1-nitropyrene is mutagenic in mammalian cells *in vivo* and *in vitro*. Germ-free and conventional rats were injected intraperitoneally with radiolabelled 1-nitropyrene and the urinary mutagenicity was evaluated in *S. typhimurium* TA98 in the presence of an exogenous metabolic activation system. The mutagenicity of urine from conventional rats was 10-fold greater than that from germ-free rats, showing that the gut flora was critical to the formation of the mutagenic urinary metabolites (Ball *et al.*, 1991). Conventional rats were administered 1-nitropyrene by gavage or intraperitoneal injection and the urinary mutagenicity was evaluated in *S. typhimurium* TA98 and TA100. Base-substitution urinary mutagenicity (detected by TA100) was produced only by intraperitoneal injection of 1-nitropyrene, whereas frameshift urinary mutagenicity (detected by TA98) was found only after deconjugation and was produced by both routes of exposure (Varga *et al.*, 2006). After intraperitoneal administration of 1-nitropyrene to rats, a considerable amount of the urinary mutagenicity was excreted as glucuronide conjugates (Scheepers *et al.*, 1991). Thus, different types of metabolite are produced depending on the route of exposure. Heflich *et al.* (1990) showed that 1-nitropyrene was mutagenic at the hypoxanthine-guanine phosphoribosyltransferase (*Hprt*) gene in Chinese hamster ovary cells only in the presence of exogenous metabolic activation, and that most of the mutagenicity was due to two metabolites – the

4,5- and 9,10-oxides of 1-nitropyrene. The lack of mutagenicity of 1-nitropyrene in the absence of exogenous metabolic activation in the Chinese hamster ovary cell/*Hprt* assay was confirmed by Thornton-Manning *et al.* (1991b), who showed that the compound was also mutagenic in the absence of exogenous metabolic activation at the *Hprt* locus in a nucleotide repair-deficient Chinese hamster ovary cell line (CHO-UV5). Using this repair-deficient cell line, Thornton-Manning *et al.* (1991a) tested the mutagenicity of 1-nitropyrene under aerobic and anaerobic metabolic conditions and analysed the metabolites. The results suggested that the exogenous metabolic activation-mediated mutagenicity under anaerobic conditions produced mutations and DNA adducts that resulted from nitroreductive metabolism, whereas those produced under aerobic conditions resulted from ring-oxidized metabolism.

Kappers *et al.* (2000) demonstrated that 1-nitropyrene was also not mutagenic at the *Hprt* gene in Chinese hamster V79 cells that express either CYP1A2 or CYP3A4 in the absence of exogenous metabolic activation; however, it was mutagenic at a *lacZ* reporter gene in NIH/3T3 cells that express CYP1A2. [The Working Group noted that this result implied the requirement of sulfotransferase for metabolic activation.]

1-Nitropyrene was also mutagenic in human HepG2 cells at the *Hprt* gene in the absence of exogenous metabolic activation, indicating that this cell line has sufficient nitroreductase and other metabolic enzymes to convert the compound to a mutagen (Silvers *et al.*, 1994). Similarly, 1-nitropyrene was mutagenic at the *Hprt* gene in a human B-lymphoblastoid cell line (h1A1vs) that constitutively expresses CYP1A1 in the absence of exogenous metabolic activation (Durant *et al.*, 1996).

(iii) Other systems

1-Nitropyrene induced gene mutation, gene conversion and crossing-over, but not chromosome loss, in the yeast *Saccharomyces cerevisiae* in the absence of exogenous metabolic activation (Rhenimi *et al.*, 2008). When soya bean seeds were exposed to 1-nitropyrene, an increased frequency of yellow and dark green spots on the leaves was found in the adult plant, which indicated the induction of gene mutation; however, no increase in twin spots was observed, indicating that no induction of somatic crossing-over had occurred (Katoh *et al.*, 1994).

(iv) Mutational mechanisms

The mechanisms by which 1-nitropyrene or its metabolites induce gene mutation (base substitutions, small deletions, insertions or frameshifts within a gene) are reviewed below; however, no data were available on the mechanisms by which 1-nitropyrene induces chromosomal mutations (chromosomal aberrations or micronuclei).

In cell-free studies, dG-C8-AP was part of a fragment of DNA or a plasmid to which nucleotide excision-repair enzymes from bacteria or human cell extracts were added. The results showed that the repair complex recognized a large (six-base) bulge in which the adduct was located and that repair was efficient when a C was opposite, but inefficient when no base was opposite, the adducted G. The aminopyrene moiety intercalated, and the adducted G and paired C were displaced into the major groove of the DNA (Mao *et al.*, 1996; Gu *et al.*, 1999; Nolan *et al.*, 1999; Hoare *et al.*, 2000; Sherrer *et al.*, 2009). An in-vitro study showed that the dG-C8-AP lesion is a strong block of DNA replication but, when translesion synthesis occurs, it is largely accurate (Vyas & Basu, 1995).

In bacteria, analysis by probe hybridization and polymerase chain reaction/DNA sequencing of revertants of *Salmonella* exposed to 1-nitropyrene (in the absence of exogenous metabolic activation) showed that 67% of the base

substitutions in TA100 were G→T and 28% were G→A (DeMarini *et al.*, 1996; DeMarini, 2000). The enhanced mutagenic potency of 1-nitropyrene in constructed homologues of *S. typhimurium* TA100 (SN13497, SN15939 and SN15942) compared with that in the parent TA100 was due to the deletion of not only *moeA* and *uvrB* but also that of other genes in TA100 (Swartz *et al.*, 2007). Molecular analysis of 1-nitropyrene-induced revertants of TA98 showed that 100% of the frameshift mutations were a two-base GC deletion within a repeating GC site (Bell *et al.*, 1991; DeMarini *et al.*, 1996; DeMarini, 2000), and that the induction of this mutation required the presence of *umuDCST*, but not *samAB* (Nohmi *et al.*, 1995).

Molybdenum hydrolase is involved in the nitroreduction of 1-nitropyrene; the deletion of both *uvrB* and *moeA* (a gene involved in molybdenum cofactor biosynthesis) from *S. typhimurium* TA100 resulted in an increase in the base-pair mutagenic potency of 1-nitropyrene compared with that in TA98; thus, the absence of other genes that were also deleted in TA98 caused a reduction in the mutagenic potency of 1-nitropyrene compared with that in strains in which no genes other than *uvrB* and *moeA* were deleted (Swartz *et al.*, 2007). 1-Nitropyrene was not mutagenic at either the base-substitution (*hisG46*) or frameshift (*hisD3052*) allele of *Salmonella* when only *moeA* was deleted; the deletion of *uvrB* also was necessary for the mutagenicity of 1-nitropyrene at these alleles (Swartz *et al.*, 2007). Moreover, 1-nitropryene required the pKM101 plasmid, which provides SOS repair, to induce frameshift mutations (TA98), but required both pKM101 and deletion of *uvrB* to induce base-substitution mutations (TA100) in *Salmonella* (DeMarini *et al.*, 1996).

The important influence of the DNA sequence context on the mutation spectra induced was illustrated by the finding that treatment of *Salmonella* strains of the 7001–7006 series (each of which reverts by a specific base substitution) resulted in G→T being the primary mutation followed by G→C (Watanabe *et al.*, 1997). This contrasts with the studies reviewed above, in which the secondary class of base substitution (in TA100) was G→A. Nevertheless, in both *Salmonella* systems, the primary base substitution induced by 1-nitropyrene was G→T. Moreover, 1-nitropyrene was seven times more mutagenic in TA98 (frameshift) than in TA100 (base substitution) (DeMarini, 2000).

In *E. coli*, the nucleotide excision-repair system recognized distortions (a bulge) in the DNA helix when 1-nitropyrene was adducted to double-stranded DNA (Watanabe-Akanuma & Ohta, 1994), as was noted *in vitro* (Zou *et al.*, 2003). However, the nucleotide excision-repair system also recognized the actual chemical modification (i.e. the aminopurine adduct) in single-stranded DNA (Watanabe-Akanuma & Ohta, 1994). As in *Salmonella*, SOS repair in *E. coli* resulted in the induction of –1 or +1 frameshift mutations by 1-nitropyrene, as well as G→T base substitutions; however, C→T base substitutions and two-base GC deletions tended to be induced in the absence of SOS (Stanton *et al.*, 1988; Malia & Basu, 1994; Melchior *et al.*, 1994; Malia & Basu, 1995; Malia *et al.*, 1996; Bacolod *et al.*, 2000; Luo *et al.*, 2000; Bacolod & Basu, 2001; Hilario *et al.*, 2002).

In mammalian cells, 1-nitrosopyrene or dG-C8-AP induced primarily G→T or G→A base substitutions (Yang *et al.*, 1988; McGregor *et al.*, 1994; Watt *et al.*, 2007). A direct comparison of the mutation spectra induced by dG-C8-AP at the underlined G in the sequence CGCGCG resulted in a G→T base substitution when this construct was replicated in mammalian cells (Watt *et al.*, 2007) but in a two-base CG deletion when it was replicated in bacterial cells (Hilario *et al.*, 2002). This comparison illustrated variations in the way in which the same adduct within the same DNA sequence context can be processed by different cells into a different spectrum of mutations.

Similarly, the influence of sequence context on the resulting mutation spectrum was illustrated

in mammalian cells, in which the 9,10-oxide of 1-nitropyrene was two- to threefold more mutagenic than the 4,5-oxide in either the Chinese hamster ovary cell/*Hprt* (Kim *et al.*, 2005b) or at the *SupF* gene in a shuttle vector in human XP-A fibroblasts (Kim *et al.*, 2008). However, these compounds induced primarily G→A, followed by G→T, base substitutions at *Hprt*, whereas they induced primarily G→T base substitutions at *SupF*.

The potential mutation spectrum of 1-nitropyrene is highly dependent on the type, the DNA sequence context (neighbouring DNA sequence), the methylation status of the DNA and the DNA repair status of the cell. Few generalizations emerged from the studies reviewed here; however, base substitutions targeted primarily at G and, to a certain extent, at A, as well as small frameshifts, were the primary classes of mutation induced by the active metabolites of 1-nitropyrene. The frameshifts could generally be explained by a standard slippage model (DeMarini *et al.*, 1998; DeMarini, 2000) and the base substitutions by polymerase misincorporation (Sherrer *et al.*, 2009).

(d) Cytogenetic effects

1-Nitropyrene induced chromosomal aberrations in Chinese hamster Don:Wg3H cells in the absence of exogenous metabolic activation, producing primarily chromatid exchanges and chromosomal and chromatid aberrations (Lafi & Parry, 1987). However, in the Chinese hamster lung cell line, 1-nitropyrene induced chromosomal aberrations in the presence but not in the absence of exogenous metabolic activation (Matsuoka *et al.*, 1991). One study reported the induction of chromosomal aberrations in the bone marrow of mice injected intraperitoneally with 1-nitropyrene (Pusztai *et al.*, 1998).

1-Nitropyrene induced micronuclei in human lymphocytes *in vitro*; the majority (62%) of the micronuclei did not hybridize with a centromeric probe, indicating that they were caused by chromosome breakage as opposed to aneuploidy (Bonnefoy *et al.*, 2012). It also induced micronuclei in mouse liver *in vivo* (Igarashi *et al.*, 2010).

1-Nitropyrene did not induce cellular transformation in rat tracheal epithelial cells *in vitro* (Mitchell & Thomassen, 1990; West & Rowland, 1994; Ensell *et al.*, 1998), but did induce transformation in cells that had been removed from the tracheas of 1-nitropyrene-treated rats and cultured *in vitro* (Ensell *et al.*, 1998). Approximately 50% of the transformed foci produced *in vivo* were able to be passaged > 20 times to become immortal cell lines (Ensell *et al.*, 1998). Furthermore, all five of the 1-nitropyrene-induced cell lines displayed anchorage-independent growth and grew in nude mice, indicating that 1-nitropyrene-transformed cells had a high probability of developing into tumours (Ensell *et al.*, 1999). Sheu *et al.* (1994) found that 1-nitropyrene induced a significant increase in the frequency of BALB/3T3 A31-1-1 cell transformation *in vitro* at the highest level tested.

(e) Oncogenes and tumour-suppressor genes

A/J mice received an intraperitoneal injection of 1-nitropyrene and were examined for lung tumours 24 weeks later. K_i-*Ras* mutations were found in 75% of adenocarcinomas, 26% of adenomas and 12% of hyperplasias; no mutations were found in normal tissues adjacent to the tumour (Bai *et al.*, 1998). The most frequent *Ki-ras* mutation was the arginine (CGA) AT→GC transition at codon 61 in exon 2. In addition, the frequencies of tumours that expressed proliferating-cell nuclear antigen and silver-staining nucleolar organizer regions were higher among those with K_i-*Ras* mutations than in those with no such mutations.

CAA→CGA mutations in codon 61 or GGT→GAT mutations in codon 12 of the K_i-*Ras* gene were found in the combined lung adenomas and adenocarcinomas from A/J mice 18 weeks after intraperitoneal administration of 1-nitropyrene (Nakanishi *et al.*, 2001).

Intraperitoneal administration of 1-nitropyrene to CBA/Ca mice followed by examination 48 hours later revealed elevated expression of Ha-*Ras* in the liver, lung, kidney, spleen and thymus, with the largest increase in the lung; the increases were greater in males than in females in all organs (Pusztai *et al.*, 1998). 1-Nitropyrene has been shown to increase the expression of c-*Myc* in the spleen, lymph and bone marrow, but to decrease its expression in the thymus of CBA/Ca mice (Ember *et al.*, 2000). The authors also found showed that 1-nitropyrene increased the expression of *p53* in the spleen and bone marrow but not in the lymph of these mice. In rats, 1-nitropyrene increased the expression of Ha-*Ras* and *p53* in leukocytes and the spleen but not in the lung, liver or kidney (Ember *et al.*, 2000).

(f) Gene expression

1-Nitropyrene induced apoptosis in mouse Hepa1c1c7 cells (Landvik *et al.*, 2007) via a caspase- and AMP-activated protein kinase-dependent pathway involving CYP1A1 that is also associated with a decrease in the expression of stearoyl-coenzyme A desaturase 1, resulting in an alteration in lipid homeostasis (Podechard *et al.*, 2011). In this regard, 1-nitropyrene has also been shown to stabilize the mRNA of CYP1A1 via the Akt pathway (Chu *et al.*, 2009), and the expression of p53 protein that it induced was mediated by CYP1A1 (Su *et al.*, 2011). The induction of apoptosis in mouse Hepa1c1c7 cells by 1-nitropyrene also involved ionic imbalance, as indicated by an intracellular accumulation of Ca^{2+}, as well as oxidative damage (Asare *et al.*, 2009). 1-Nitropyrene also increased the ubiquitination of p21 protein after the stabilization of p53 and expression of p21 (Nakanishi *et al.*, 2000). A study in human HepG2 cells found that aldo-keto reductase 1C2 was essential for the induction of p53 phosphorylation and apoptosis by 1-nitropyrene (Su *et al.*, 2008).

1-Nitropyrene induced reactive oxygen species and the expression of the endoplasmic reticulum stress chaperone protein GRP78 in human umbilical vein endothelial cells *in vitro* (Andersson *et al.*, 2009). It induced interleukin (IL)-8, IL-6, tumour necrosis factor-α and CXC chemokine ligand 8 in BEAS-2B cells (Øvrevik *et al.*, 2009, 2010, 2011), as well as inflammation-related genes such as pentaxin, IL-1β, IL-6, IL-8, 2(CXC chemokine ligand 2) and tumour necrosis factor-α in human bronchial epithelial BEAS-2B cells (Park & Park, 2009). 1-Nitropyrene also induced CYP1 mRNA and protein in various human cell lines (Iwanari *et al.*, 2002; Cherng *et al.*, 2006; Hirano *et al.*, 2011).

4.3 Other data relevant

Administration of 1-nitropyrene by gavage to Fischer 344 rats produced γ-glutamyl transpeptidase-positive foci in the liver after partial hepatectomy; such foci are thought to be an early lesion of hepatocarcinogenesis (Denda *et al.*, 1989).

4.4 Mechanistic considerations

The carcinogenicity of 1-nitropyrene in rodents has been reviewed previously (IARC, 1989). Those and more recent studies have demonstrated that 1-nitropyrene was generally carcinogenic in newborn rodents but not in adults, and was not carcinogenic in the following studies in young or adult rodents: by injection in male Fischer 344 rats (Ohgaki *et al.*, 1985), topical application in Crl/Cd-1(ICR) BR mice aged 4–5 weeks (el-Bayoumy *et al.*, 1982), topical application or intraperitoneal injection in SENCAR mice aged 7 weeks (Nesnow *et al.*, 1984), subcutaneous injection in BALB/c mice (Tokiwa *et al.*, 1984), intrapulmonary implantation in Fischer 344/DuCrj rats (Maeda *et al.*, 1986) or gavage in CD rats (el-Bayoumy *et al.*, 1995). In contrast, it was carcinogenic in the following studies in newborns: by suprascapular

injection in Sprague-Dawley rats, producing histocytomas at the injection site (Hirose *et al.*, 1984); gavage in Sprague-Dawley rats, producing mammary adenocarcinomas in females (el-Bayoumy *et al.*, 1988); intraperitoneal and subcutaneous injection in weanling CD rats, producing mammary fibroadenomas in females (Imaida *et al.*, 1991b); intraperitoneal injection in weanling CD rats, producing mammary tumours in females (Imaida *et al.*, 1991a); intraperitoneal and subcutaneous injection in B6C3F$_1$ mice, producing liver carcinomas (Wislocki *et al.*, 1986); and intraperitoneal injection in female CD and Fischer 344 rats, producing mammary tumours in the CD rats and leukaemia in the Fischer 344 rats (Imaida *et al.*, 1995). However, intraperitoneal injection of newborn B6C3F$_1$ mice with either the 4,-5-oxide or 9,10-oxide of 1-nitropyrene was not carcinogenic (Mori *et al.*, 1992).

An explanation for the carcinogenicity of 1-nitropyrene in newborns but not in adults has been proposed by Silvers *et al.* (1997), who noted that newborn rodents have a low ratio of ring-oxidation to nitroreduction compared with adults, and therefore also have a relatively low ratio of CYP levels to nitroreductase compared with adults. Thus, newborns would favour the nitroreductase (activation) pathway more than the inactivation (oxidation) pathway, whereas the converse would occur in adults. Consequently, newborns are more liable than adults to reduce 1-nitropyrene to hydroxylamine and to the mutagenic dG-C8-AP adduct, which could lead to cancer. Conversely, adult rodents would be more liable to oxidize 1-nitropyrene to phenols, which do not form adducts, are not mutagenic and would not cause cancer.

Silvers *et al.* (1997) also considered the comparison between rodents and humans, and noted that human liver microsomes converted 1-nitropyrene by CYP3A4 to 1-nitropyrene-3-ol, whereas other CYPs were involved in rodents, the liver microsomes of which produced only the 6-ol and 8-ol of 1-nitropyrene. They also noted that the ratio of ring-oxidation to nitroreduction was sufficiently low in human HepG2 liver cells for 1-nitropyrene to be metabolized preferentially via nitroreductase (an activation pathway) in human liver, resulting in the formation of the dG-C8-AP adduct and mutation in human HepG2 cells *in vitro*. Thus, adult rodents may not be a suitable model for the human metabolism of 1-nitropyrene, which may favour activation via nitroreductase rather than inactivation via CYP pathways.

An inverse association between 1-nitropyrene in the lung and 5-year survival from lung cancer in humans has been identified (Tokiwa *et al.*, 1998). The concentration of 1-nitropyrene was measured in 256 human lung specimens with carcinoma and did not differ significantly from that in a set of lung samples from patients with tuberculosis. However, the 5-year survival was markedly lower among lung cancer patients with 1-nitropyrene concentrations > 18 pg/g of lung tissue compared with those who had concentrations < 18 pg/g.

5. Summary of Data Reported

5.1 Exposure data

1-Nitropyrene is an incomplete combustion product and does not appear to be formed in the atmosphere. No evidence was found that it has been produced in commercial quantities or used for purposes other than laboratory applications. In exhaust emissions and in ambient air, 1-nitropyrene is associated with particles and is not observed in the gas phase. The most important source of 1-nitropyrene is from traffic emissions. In diesel and gasoline engine exhaust emissions, 1-nitropyrene is the most abundant nitroarene. It has also been found in emissions from industrial sources, such as waste incinerators and coke ovens. Other measurements of 1-nitropyrene

in the outdoor environment (surface water, water sediments, snow and rain) can probably be explained by the aforementioned primary combustion sources. During sampling for 1-nitropyrene from emissions with high concentrations of nitrogen oxides, pyrene could possibly be converted to 1-nitropyrene on the medium used. This artificial source of 1-nitropyrene is thought to be negligible when particles are collected from ambient air. Workers who operate, repair or work close to idling or moving diesel-powered vehicles or stationary equipment are probably exposed to 1-nitropyrene at concentrations in the range of 0.1–2.5 ng/m^3. Three overlapping subcategories can be distinguished: occupations with high exposures associated with the use or repair of diesel engines in confined spaces, such as mining and repair shops, with concentrations in the 1.0–2.5 ng/m^3 range; other indoor use of diesel-powered engines, such as indoor use of forklifts, leading to exposures ranging from 0.1 to 1.5 ng/m^3; and outdoor use of diesel engines, in occupations such as a driver, resulting in exposures below 0.5 ng/m^3. Potential sources of exposure for the general population are indoor and outdoor air, and indoor and domestic use of liquefied petroleum gas, kerosene heaters and oil fumes from cooking. The presence of 1-nitropyrene as a food contaminant can probably be explained by its ubiquitous occurrence as an air pollutant, although it may be produced by some food processing, such as smoking, grilling and roasting. Foodstuffs with the highest observed levels of contamination include tea, spices and some cooking oils. In addition, dietary intake can originate from the preparation of foods, such as grilled or smoked meat or fish. 1-Nitropyrene has not been detected in tobacco smoke.

In the general environment, 1-nitropyrene is the most frequently used chemical marker to characterize nitroarenes in the particulate matter of ambient air. 1-Nitropyrene has been used to characterize these compounds in many urban, rural and remote locations worldwide. In outdoor air, the highest concentrations were observed in urban areas, and were presumably related to vehicle exhaust emissions. Ambient air concentrations ranged from 10 to 1000 pg/m^3 in urban areas and from 1 to 100 pg/m^3 in rural and remote areas with low traffic intensity. The concentrations of 1-nitropyrene tend to be higher during the cold seasons (winter) than during the warm seasons (summer).

1-Nitropyrene is metabolized to 1-aminopyrene, which can be measured in human tissues, and was detected in the urine of workers exposed to exhaust from operating diesel-powered equipment. 1-Aminopyrene was also found in the blood of health-care workers employed in an urban environment and in the blood of office workers who lived in a rural environment.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

1-Nitropyrene was tested for carcinogenicity by subcutaneous injection in one study, by intraperitoneal injection in four studies and in two initiation–promotion studies in mice; by oral administration in three studies, by subcutaneous injection in five studies, by intraperitoneal injection in three studies, by implantation into the lung in one study and by direct injection into the mammary area in one study in rats; and by intratracheal instillation in one study in hamsters.

In mice, subcutaneous injection of 1-nitropyrene did not cause an increase in the incidence of tumours at any site. Intraperitoneal injection caused a significant increase in the incidence of liver tumours in males in one study and of lung tumours in males and females in another study. The other two studies by intraperitoneal injection

gave negative results, as did the two skin tumour initiation–promotion studies.

Oral administration of 1-nitropyrene to rats caused a significant increase in the incidence of mammary tumours (mostly benign) in females in two studies and of lung tumours in males in one study. The third study of oral administration gave negative results. Subcutaneous injection to rats caused a significant increase in the incidence of malignant tumours at the injection site in one study and of mammary tumours in two studies. Two other studies of subcutaneous injection gave negative results and another was inadequate for an evaluation of carcinogenicity. Intraperitoneal injection to rats caused a significant increase in the incidence of mammary tumours in one study but gave negative results in two other studies. Studies of the implantation of 1-nitropyrene into the lung or injection into the mammary area of rats also gave negative results.

In hamsters, intratracheal instillation did not cause a significant increase in the incidence of tumours at any site.

5.4 Mechanistic and other relevant data

1-Nitropyrene is a constituent of diesel exhaust, and its concentration in air has been used as a marker for exposure to diesel exhaust. In addition, urinary metabolites of 1-nitropyrene have been detected in people who worked in diesel-contaminated atmospheres, suggesting that such metabolites may be a suitable marker for exposure to diesel exhaust in humans. 1-Nitropyrene is metabolized by a combination of ring-oxidation and nitroreduction, and the gut flora also plays a role. Bacterial studies have implied that molybdenum hydrolase is also involved in the metabolism and base-substitution mutagenicity of 1-nitropyrene. The compound is metabolized to hydroxyl amino metabolites that are electrophilic and form DNA adducts, the primary form of which is *N*-(deoxyguanosin-8-yl)-1-aminopyrene.

The DNA damage induced by the metabolites of 1-nitropyrene produced mutations, primarily frameshifts in bacterial systems and base substitutions (mostly GC→TA) in mammalian cells. Standard slippage models explained the frameshifts, and polymerase misincorporation models explained the base substitutions. In addition to gene mutation, 1-nitropyrene also caused chromosomal mutation, such as micronucleus formation, and morphological cell transformation. In contrast to the mutation spectrum of 1-nitropyrene in mammalian cells *in vitro* or in bacterial cells, oncogenes in 1-nitropyrene-induced tumours in rodents have primarily AT→GC mutations, as well as altered patterns of expression.

1-Nitropyrene was carcinogenic in newborn but not in adult rodents. This was probably due to the increased ratio of nitroreduction to ring-oxidation in newborn compared with adult animals; the nitroreduction pathway leads to the formation of mutagenic and carcinogenic metabolites, whereas ring-oxidation is a detoxification route. In human liver cells *in vitro*, the ratio of nitroreduction to ring-oxidation was sufficiently high to result in the preferential activation of 1-nitropyrene via nitroreductase. Thus, adult rodents may not be a suitable model for the human metabolism of 1-nitropyrene. Similarly, the formation of urinary metabolites of 1-nitropyrene in humans favours the nitroreduction pathway. 1-Nitropyrene also induced oxidative stress and the formation of reactive oxygen species, inflammatory proteins and apoptosis in mammalian cell systems and rodents. These mechanisms, together with its direct genotoxicity, could contribute to the carcinogenicity of 1-nitropyrene.

Overall, these data provide *strong mechanistic evidence* to support the carcinogenicity of 1-nitropyrene.

6. Evaluation

6.1 Cancer in humans

There is *inadequate evidence* in humans for the carcinogenicity of 1-nitropyrene.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of 1-nitropyrene.

6.3 Overall evaluation

1-Nitropyrene is *probably carcinogenic to humans (Group 2A).*

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4-NITROPYRENE

4-Nitropyrene was evaluated by a previous IARC Working Group in 1988 (IARC, 1989). New data have since become available, and these have been taken into consideration in the present evaluation.

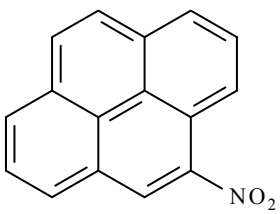
1. Exposure Data

1.1 Chemical and physical data

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 57835-92-4

1.1.2 Structural and molecular formulae and relative molecular mass



$C_{16}H_9NO_2$

Relative molecular mass: 247.25

1.1.3 Chemical and physical properties of the pure substance

Description: Slender orange needles (Bavin, 1959)

Melting-point: 190–192 °C (Paputa-Peck *et al.*, 1983); 196–197.5 °C (Bavin, 1959)

Boiling-point: 472 °C at 101.3 kPa (Yaffe *et al.*, 2001)

Vapour pressure: 4.4×10^{-6} Pa at 25 °C (Yaffe *et al.*, 2001)

Octanol/water partition coefficient: Log $P_{o/w}$ 4.69 (Yaffe *et al.*, 2001)

Sorption coefficient: Log K_{oc} 4.48 (Yaffe *et al.*, 2001)

Henry's law constant: 6.4×10^{-2} kPa.m^3/g/mol at 25 °C (Yaffe *et al.*, 2001)

Spectroscopy data: Ultraviolet and nuclear magnetic resonance data have been reported (Paputa-Peck *et al.*, 1983).

Solubility: Soluble in water (0.017 mg/L) (Yaffe *et al.*, 2001)

1.1.4 Technical products and impurities

One company in Japan produces 4-nitropyrene for analytical or reference purposes, with a purity of > 97%.

Table 1.1 Concentrations of 4-nitropyrene in airborne particulate matter[a]

SRM	Characterization	Year of collection	Location	Content (in ng/g ± 95% CI)		
				4-NP	1-NP	2-NP
1648	Urban area	1978	St. Louis, MO, USA	9.1 ± 0.7	155 ± 29	48.9 ± 2.4
1649a	Urban area	1970s	Washington, DC, USA	5.5 ± 0.6	71.5 ± 5.1	24.4 ± 4.0
BALT. PM-2.5	Airborne particulate matter (PM-2.5 fraction)	1998–90	Baltimore, MD, USA	8.8 ± 1.2	196 ± 3	35.5 ± 5.8
2975	Diesel exhaust particulate matter collected from industrial forklift	NR	NR	173 ± 11	39640 ± 590	< 4[b]
1975	Diesel exhaust particulate extract	NR	NR	68.2 ± 2.4	16070 ± 3	< 4[b]
1650a	Diesel exhaust particulate matter collected from a heat exchanger	NR	NR	135 ± 8	18330 ± 340	< 4[b]

[a] Based on gas chromatography-negative ion chemical ionization-mass spectrometry analysis
[b] Below the limit of detection
CI, confidence interval; NP, nitropyrene; NR, not reported; PM, particulate matter; SRM, standard reference material
From Bamford *et al.* (2003)

1.2 Analysis

Further details on the air sampling and analysis of nitroarenes can be found in Section 1.2 of the *Monographs* on Diesel and Gasoline Engine Exhaust or 1-Nitropyrene in this Volume.

4-Nitropyrene can be extracted from particulate matter with dichloromethane. A method for the determination of 4-nitropyrene by gas chromatography (GC) and chemiluminescence detection has been described (Yu *et al.*, 1984). Bamford *et al.* (2003) and Albinet *et al.* (2006) separated 1-, 2- and 4-nitropyrene by purification with liquid chromatography and solid-phase extraction on alumina and silica columns, followed by GC with negative ion chemical ionization-mass spectrometry (GC-NICI-MS) on a 5% or 50% phenyl-substituted methylpolysiloxane capillary column. [$^2H_9$]-Nitrofluoranthene was used as an internal standard.

1.3 Production and use

4-Nitropyrene of unspecified purity is manufactured by one company each in Germany, Norway and the People's Republic of China.

4-Nitropyrene is not produced for purposes other than use in chemical analysis and scientific research.

1.4 Occurrence and exposure

4-Nitropyrene is assumed to be formed by photochemical conversion in the atmosphere (Atkinson *et al.*, 1991), although this would occur through a very slow two-step reaction mechanism (Murahashi *et al.*, 1999).

Murahashi *et al.* (2001) identified 4-nitropyrene (together with seven other nitroarenes) in precipitation water samples collected on the roof of a four-storey building in a residential area and in airborne particulates in Kanazawa, Japan, in September–October 1996, October–November 1996 and in February 1997. [The authors included a chromatogram that showed a peak corresponding to 0.030 pmol/L [7.42 pg/L] in precipitation water samples, but no chemiluminescence spectrum.] In air samples, the nitroarenes were present at a concentration range of 2–1400 ng/g of particulate matter. The abundance of the compounds was 1-nitropyrene

> 2-nitrofluoranthene > 6-nitrochrysene > 2-nitropyrene > 1,3-, 1,6- and 1,8-dinitropyrenes in all three samples.

Albinet *et al.* (2007) reported air concentrations of 4-nitropyrene in Marseille, France. In urban locations, a median of 1.4 pg/m^3 and a range of 0.7–2.6 pg/m^3 of 4-nitropyrene were observed. In a suburban location, the median and range were 0.6 pg/m^3 and 0.1–1.2 pg/m^3, respectively. In rural locations, the concentrations were below 0.1 pg/m^3.

The presence of 4-nitropyrene was confirmed in the certified standard reference material (SRM) 1649a, an update of SRM1649 produced from airborne particulate matter collected using a baghouse collector in an urban area in Washington, DC, USA, in the late 1970s (NIST, 2001).

The contents of 1-, 2- and 4-nitropyrene in several SRMs are presented in Table 1.1.

In 2003, Bamford *et al.* (2003) reported a value of 5.5 ± 0.6 ng/g of particulate matter when SRM1649a was analysed in triplicate by GC-NICI-MS. In this analysis, 4-nitropyrene was not available as a reference standard and the calibration was based on the response factor of 3-nitrofluoranthene. Using the same technique, Albinet *et al.* (2006) reported a similar value of 6.0 ± 0.9 ng/g in SRM1648a. In these samples, the concentrations of 4-nitropyrene were much higher in diesel exhaust particles than in airborne particulate matter.

Newly developed catalytic diesel particulate filters have been shown to reduce the concentration of 4-nitropyrene in diesel emissions by 40–60% (Heeb *et al.*, 2008, 2010).

1.5 Regulations and guidelines

No regulations or guidelines for 4-nitropyrene were found by the Working Group.

2. Cancer in Humans

No data were available to the Working Group

3. Cancer in Experimental Animals

3.1 Mouse

See Table 3.1

Intraperitoneal administration

Male and female newborn CD-1 mice received three intraperitoneal injections of 4-nitropyrene (purity, > 99%) in dimethyl sulfoxide (DMSO) on day 1 (400 nmol), day 8 (800 nmol) and day 15 (1600 nmol) after birth (total dose, 2800 nmol [692 µg]/mouse). Control mice received either three injections of DMSO with the same dose schedule or a single injection of 560 nmol [140 µg] of benzo[*a*]pyrene (purity, > 99%; vehicle controls). At 25–27 days, mice were weaned and separated according to sex. All surviving mice were killed after 1 year. In males, hepatocellular adenoma or carcinoma (combined) developed in 24 out of 29 (83%; four adenomas, 20 carcinomas) treated mice and 7 out of 28 (25%) controls [$P < 0.005$]. In females, no difference in the incidence of liver tumours was found. The incidence of hepatocellular carcinoma was 20 out of 29 (69%) treated males and 0 out of 30 controls [$P < 0.005$]. Lung tumours occurred in 11 out of 29 (38%; 10 adenomas, one carcinoma) treated males and in 1 out of 28 (4%) male controls [$P < 0.002$], and in 9 out of 29 (31%) treated females and 0 out of 31 female controls [$P < 0.001$] (Wislocki *et al.*, 1986).

3.2 Rat

See Table 3.2

Table 3.1 Study of the carcinogenicity of 4-nitropyrene in mice

Strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
CD-1 (M, F) up to 52 wk Wislocki *et al.* (1986)	Intraperitoneal injection 0 (control) or 2800 nmol/ mouse in DMSO (400 nmol at 24 h after birth, 800 nmol on d 8 and 1600 nmol at d 15) 28–31 animals/group	Liver (hepatocellular adenoma or carcinoma combined): M–7/28 (25%), 24/29 (83%)* F–0/31, 2/29 (7%) Liver (hepatocellular adenoma): M–2/28 (7%), 4/29 (14%) F–0/31, 1/29 (3%) Liver (hepatocellular carcinoma): M–0/28, 20/29 (69%)* F–0/31, 1/29 (3%) Lung (adenoma or carcinoma combined): M–1/28 (4%), 11/29 (38%)** F–0/31, 9/29 (31%)***	*[$P < 0.005$] **[$P < 0.002$] ***[$P < 0.001$]	Purity, > 99%

d, day; DMSO, dimethyl sulfoxide; h, hour; F, female; M, male; wk, week

3.2.1 Subcutaneous administration

A group of female newborn Sprague-Dawley rats [initial number unspecified] received an initial suprascapular injection of 2.5 µmol/kg body weight (bw) of 4-nitropyrene (purity, > 99.9%) in DMSO within 24 hours of birth, followed by two weekly injections of 5 µmol/kg bw and eight weekly injections of 10 µmol/kg bw (total dose, 75 µmol). Another group of rats received DMSO alone. Animals were killed when moribund or at 86 weeks. A statistically significant increase ($P < 0.005$) in the number of total mammary tumours was observed in the treated group (20 out of 27, 74%; 18 adenocarcinomas, 14 fibroadenomas; induction period, 262 days) compared with controls (17 out of 47, 36%). The incidence of mammary gland adenocarcinoma in treated animals was significantly increased (18 out of 27, 67%, versus 3 out of 47, 6%). The incidence of malignant fibrous histiocytoma (10 out of 27, 37%; $P < 0.001$), leukaemia (5 out of 27, 18%; $P < 0.005$) and Zymbal gland carcinoma (4 out of 27, 14%; $P < 0.05$) was also significantly increased compared with controls (0 out of 47). In the same study, similarly treated Fischer 344 rats showed no differences in the incidence of mammary or other tumours (King, 1988; Imaida *et al.*, 1995).

3.2.2 Intraperitoneal administration

Groups of 30 weanling Sprague-Dawley rats, aged 30 days, received intraperitoneal injections of 0 or 67 µmol/kg bw of 4-nitropyrene (solution of 25 µmol) dissolved in 1 mL DMSO three times a week for 4 weeks (total dose, 119 µmol). The surviving rats were killed 61 weeks after the first injection. The incidence of total mammary tumours (fibroadenoma, adenoma or adenocarcinoma) in treated rats was significantly increased (17 out of 29, 59%; $P < 0.005$) compared with vehicle controls (4 out of 29, 14%). The incidence of adenocarcinoma was also significantly increased (13 out of 29, 45%; $P < 0.005$) compared with controls (1 out of 29, 3%) (King, 1988; Imaida *et al.*, 1991).

Table 3.2 Studies of the carcinogenicity of 4-nitropyrene in rats

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
CD (F) 86 wk King (1988), Imaida et. al. (1995)	Subcutaneous (suprascapular) injection 0 (control) or 75 µmol/kg bw (total dose) in DMSO (2.5 µmol/kg bw at 24 h after birth, 5 µmol/kg bw once/wk for 2 wk, and 10 µmol/kg bw once/wk for 5 wk) Numbers at start unspecified	Mammary gland (total tumours[a]): 17/47 (36%), 20/27 (74%)	$P < 0.005$	Purity, > 99.9% by HPLC
		Mammary gland (adenocarcinoma): 3/47 (6%), 18/27 (67%)	$P < 0.05$	
		Mammary gland (fibroadenoma): 16/47 (34%), 14/27 (52%)	NS	
		Soft tissue (malignant fibrous histiocytoma): 0/47, 10/27 (37%)	$P < 0.001$	
		Leukaemia: 0/47, 5/27 (18%)	$P < 0.005$	
		Zymbal gland (carcinoma): 0/47, 4/27 (14%)	$P < 0.05$	
CD (F) 61 wk King (1988), Imaida *et al.* (1991)	Intraperitoneal injection 0 or 67 µmol/kg bw in 25 µmol/mL DMSO, 3 ×/wk for 4 wk (total dose, 119 µmol) 30 animals/group	Mammary gland (total tumours[a]): 4/29 (14%), 17/29 (59%)	$P < 0.005$	Purity NR, synthesized
		Mammary gland (adenocarcinoma): 1/29 (3%), 13/29 (45%)	$P < 0.005$	
CD (F) up to 77 wk King (1988), Imaida *et al.* (1991)	Direct injection into the mammary gland 0 (control) or 2.03 µmol in 0.1 mL DMSO/rat, directly into 3 left thoracic gland nipple area (d 1) then 3 left inguinal nipple area (d 2) (total dose, 12.3 µmol/rat); inner control, 0.1 mL DMSO into the right side of the same rats 30 animals/group	All tumours[b]: 15/28 (53%), 24/28 (86%)	$P < 0.025$	Purity NR
		Mammary gland (total tumours[a]): 7/28 (25%), 23/28 (82%)	$P < 0.001$	
		Mammary gland (fibroadenoma): 5/28 (18%), 15/28 (54%)	$P < 0.01$	
		Mammary gland (adenocarcinoma): 1/28 (4%), 19/28 (68%)	$P < 0.001$	

[a] Fibroadenoma, adenoma or adenocarcinoma combined

[b] One pituitary adenoma, five pituitary adenocarcinomas, one thyroid adenocarcinomas, one Zymbal gland carcinomas, one adenocortical adenoma, four malignant fibrous histiocytomas, two cutaneous fibromas, one pituitary adenoma, 11 pituitary adenocarcinomas

bw, body weight; d, day; DMSO, dimethyl sulfoxide; F, female; h, hour; HPLC, high-performance liquid chromatography; M, male; NR, not reported; NS, not significant; wk, week

3.2.3 Direct injection into the mammary gland

Groups of 30 female Sprague-Dawley rats, aged 30 days, received injections of 2.03 µmol of 4-nitropyrene in 0.1 mL DMSO each directly into three left thoracic gland nipple areas (on day 1) and then into three inguinal gland nipple areas (day 2) (total dose, 12.3 µmol/rat). As an internal control, 0.1 mL DMSO alone was injected into the right side of the nipple area of the same rats. A separate control group received injections of 0.1 mL DMSO alone into both the left and right side nipple areas (12 injections per rat). Thereafter, rats were observed for up to 77 weeks. The incidence of all tumours, total mammary tumours, mammary fibroadenoma and mammary adenocarcinoma (24 out of 28, 86%; 23 out of 28, 82%; 15 out of 28, 54%; and 19 out of 28, 68%; respectively) in the treated rats was significantly greater than that in their respective controls (15 out of 28, 53%; $P < 0.025$; 6 out of 28 [7 out of 28, 25%; personal communication of the author]; $P < 0.001$; 5 out of 28, 18%; $P < 0.01$; and 1 out of 28, 4%; $P < 0.001$; respectively) (King, 1988; Imaida *et al.*, 1991).

4. Mechanistic and Other Relevant Data

4-Nitropyrene is a constituent of diesel exhaust and is not an atmospheric transformation product; however, because of its concentration and mutagenic potency, it can contribute significantly to the mutagenicity of urban air (Murahashi *et al.*, 1999). As reported in the previous *Monograph* (IARC, 1989), subcutaneous exposure of newborn female CD rats to 4-nitropyrene produced mammary tumours, and intraperitoneal exposure of newborn mice produced liver tumours in males and lung tumours in males and females. Studies published that have been studied since that time are reviewed here.

4.1 Absorption, distribution, metabolism and excretion

4.1.1 Humans

No data were available to the Working Group.

4.1.2 Experimental systems

Upadhyaya *et al.* (1994) showed that 4-nitropyrene was metabolized by 3-methylcholanthrene-induced Sprague-Dawley rat liver microsomes or by a rat-liver metabolic activation system to two primary metabolites, one of which was identified as 4-nitropyrene-9,10-dione. This differs considerably from the in-vitro metabolism of 1-nitropyrene, which yields several phenolic derivatives and *trans*-dihydrodiols, together with other major metabolites. The major metabolite of 4-nitropyrene in the presence of the epoxide hydrolase inhibitor, 3,3,3-trichloropropylene-1,2-oxide, was 9,10-epoxy-9,10-dihydro-4-nitropyrene. This K-region epoxide may play a role in the genotoxicity of 4-nitropyrene, because such epoxides contribute to the mutagenicity of other nitropyrenes, such as 1-nitropyrene.

Upadhyaya *et al.* (1994) also showed that, 48 hours after oral administration of [^{3}H]4-nitropyrene to female Sprague-Dawley rats, 32% of the dose was excreted in the urine and 30.6% in the faeces. Compounds in the faeces were identified as the metabolites 4-aminopyrene and 9(10)-hydroxy-4-(acetylamino) pyrene, and 4-nitropyrene itself. The urinary metabolites comprised sulfates and glucuronides of 9(10)-hydroxy-4-(acetylamino)pyrene. Thus, similarly to 1-nitropyrene, 4-nitropyrene is metabolized *in vivo* via nitroreduction, ring oxidation and a combination of both pathways; however, the pattern of excretion of these two nitropyrenes differs. Approximately 40% of the dose of 4-nitropyrene and its metabolites was found in the urine 168 hours after administration, whereas this value was only 20% for

1-nitropyrene. Thus, the position of the nitro group on the pyrene affects the excretion pattern, and this may play some role in the differences in carcinogenicity between the two compounds. A higher level of 4-nitropyrene metabolites in the urine indicates a higher level in the blood and, thus, more are delivered to the target organ (mammary tissue). Consistent with this is the observation that 4-nitropyrene is a more potent mammary carcinogen than 1-nitropyrene.

A comparative study of the metabolism of [^{3}H]1- and 4-nitropyrene after intraperitoneal injection into female CD rats (Chae *et al.*, 1997) showed that neither the excretion patterns nor the metabolite profiles readily explained why 4-nitropyrene is a more potent mammary carcinogen than 1-nitropyrene (see Section 3 of this *Monograph*). However, high-performance liquid chromatographic analysis of hydrolysates of liver DNA found that only 4-nitropyrene yielded putative multiple DNA adducts, whereas 1-nitropyrene produced none. In addition, 4-nitropyrene bound to mammary DNA at a rate 3.5 times higher than 1-nitropyrene, which might account for the greater potency of 4-nitropyrene as a mammary carcinogen in relation to 1-nitropyrene.

Chae *et al.* (1999a) examined the ability of microsomes from 15 human livers and 8 human lungs to metabolize 1-, 2- and 4-nitropyrene. All of the liver microsomes produced qualitatively similar metabolites, which were also similar to those produced by all of the lung microsomes; however, the levels of metabolites produced by the lung were lower than those produced by the liver. Ring-oxidized metabolites (phenols and *trans*-dihydrodiols) were produced by all three nitropyrenes, but the nitroreductive metabolism that leads to the formation of aminopyrene was observed only with 4-nitropyrene. The authors concluded that most of the liver microsomal metabolism of 1- and 4-nitropyrene was due to cytochrome P450 (CYP) 3A4, but they could not rule out a minor role for CYP1A2. CYP3A4 metabolized 4-nitropyrene to *trans*-9,10-dihydro-9,10-dihydroxy-4-nitropyrene, 9(10)-hydroxy-4-nitropyrene and 4-aminopyrene.

4.2 Genetic and related effects

4.2.1 Humans

No data were available to the Working Group.

4.2.2 Experimental systems

(a) DNA adduct formation

DNA adducts (determined by ^{32}P-postlabeling/thin-layer chromatography) were induced in three cell lines treated with 4-nitropyrene: NCI-H322 cells that are derived from a human lung tumour, V79NH cells that are derived from Chinese hamster lung and isolated hepatocytes from Wistar rats (Topinka *et al.*, 1998). Pretreatment of either the NCI-H322 cells or hepatocytes with an inducer of CYP1A1, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, reduced the levels of 4-nitropyrene-induced DNA adducts by nearly 100-fold or to non-detectable levels, respectively. This inactivation of 4-nitropyrene by increased CYP1A1 was probably due to an oxidative attack on the aromatic ring leading to the formation of nitrophenols and conjugates, which are unable to form DNA-binding metabolites.

High-performance liquid chromatographic analysis of hydrolysates of liver and mammary tissue DNA obtained from female CD rats 24 hours after intraperitoneal exposure to [^{3}H]4-nitropyrene showed that the DNA adducts from these tissues co-eluted with those derived from in-vitro incubations of 4-nitropyrene that used a nitroreductive pathway (Chae *et al.*, 1999b).

Zhou & Cho (1998) synthesized *N*-(deoxyguanosin-8-yl)-4-aminopyrene, which is a predicted *N*-(deoxyguanosin-8-yl) adduct of 4-nitropyrene, and found that it had an *anti*-glycosyl conformation with C2'-*endo*(S) sugar

puckering and a nearly planar conformation at the central amine nitrogen; it also had a substitution adjacent to a fused aromatic ring. The authors considered that this adduct was probably responsible for much of the mutagenicity and carcinogenicity of 4-nitropyrene.

(b) DNA damage

4-Nitropyrene induced DNA adducts in rats (Chae *et al.*,1997) and in rat hepatocytes *in vitro* (Topinka *et al.*, 1998), and nitroreduction was shown to be responsible for the formation of adducts in the rat mammary gland (Chae *et al.*, 1999b).

(c) Mutagenicity

4-Nitropyrene induced DNA damage in the *Bacillus subtilus* rec assay (Horikawa *et al.*, 1986), and was mutagenic in a forward mutation assay for resistance to 8-azaguanine in *Salmonella typhimurium* TM677; it was ~10 times more potent in the absence than in the presence of an exogenous metabolic activation system (Busby *et al.*, 1994a). 4-Nitropyrene was also mutagenic in a forward mutation assay at the thymidine kinase +/– locus in human B-lymphoblastoid (MCL-5) cells that express CYP1A1, 1A2, 2A6, 2E1 and 3A4, as well as microsomal epoxide hydrolase (Busby *et al.*, 1994b). A structure–activity study based on the mutagenicity of 4-nitropyrene in *S. typhimurium* TA98 in the presence of an exogenous metabolic activation system found that its mutagenic potency could not be explained fully by the orientation of the nitro group relative to the aromatic plane (Onchoke *et al.*, 2004).

4.3 Other relevant data

The ability of 4-nitropyrene to induce cytogenetic effects, alterations in oncogenes or tumour-suppressor genes, or alterations in gene expression has not been reported.

4-Nitropyrene did not induce cell transformation in isolated rat tracheal cells exposed *in vitro* (West & Rowland, 1994).

4.4 Mechanistic considerations

In addition to the two studies reviewed in the previous *Monograph* (IARC, 1989), which showed that 4-nitropyrene induced tumours in newborn rats and mice, an additional study has confirmed that subcutaneous exposure of female CD rats to 4-nitropyrene induces mammary adenocarcinomas, as well as malignant fibrous histiocytomas (Imaida *et al.*, 1995).

The age-specific carcinogenicity of 1-nitropyrene has been ascribed to the enhanced ratio of nitroreductase to CYP activity in the newborn compared with adults. As noted above, the reductive metabolic pathway for 4-nitropyrene has been shown to produce the 4-nitropyrene-associated DNA adducts found *in vivo* in rodents. Moreover, the *N*-(deoxyguanosin-8-yl)-aminopyrene adducts of 4- and 1- nitropyrene are structurally similar (Onchoke *et al.*, 2004). Thus, 1- and 4-nitropyrene may undergo similar metabolism, which results in mutagenic DNA adducts. In comparison with 1-nitropyrene, the greater mutagenic potency of 4-nitropyrene in bacterial and mammalian cells, as well as its greater carcinogenic potency in rodents, may be due to the higher levels of its metabolites in the blood (Upadhyaya *et al.*, 1994), which may result in the delivery of more mutagenic 4-nitropyrene metabolites than 1-nitropyrene metabolites to the target tissue (the mammary gland).

Similarly to 1-nitropyrene (see Section 3 of the *Monograph* on 1-Nitropyrene in this Volume), 4-nitropyrene may not be carcinogenic in adult rodents; however, no studies in adult rodents have been reported.

5. Summary of Data Reported

5.1 Exposure data

4-Nitropyrene has been detected in the particulate phase of diesel exhaust emissions. No evidence was found that it has been produced in commercial quantities or used for purposes other than laboratory applications. 4-Nitropyrene has not been detected in gasoline engine exhaust or emissions from other products or processes. Recently, it was identified as a constituent of airborne particulate matter. Concentrations of 4-nitropyrene in outdoor air were in the picogram per cubic metre range and were higher in urban and suburban locations than in rural locations.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

4-Nitropyrene was tested for carcinogenicity in mice in one study by intraperitoneal injection and in rats, in one study by subcutaneous injection, in one study by intraperitoneal injection and in one study by direct injection to the mammary gland area. In mice, intraperitoneal injection of 4-nitropyrene into newborns caused a significant increase in the incidence of liver carcinomas in males and of lung tumours in males and females. In rats, subcutaneous injection of 4-nitropyrene into newborns caused a significant increase in the incidence of mammary tumours, malignant fibrous histiocytomas, leukaemia and Zymbal gland carcinomas; intraperitoneal injection resulted in a significant increase in the incidence of mammary tumours; and direct injection into the mammary gland area produced a significant increase in the incidence of mammary tumours.

5.4 Mechanistic and other relevant data

No data were available to the Working Group on the absorption, distribution, metabolism and excretion or genetic and related effects of 4-nitropyrene in humans. When administered orally to rats, 4-nitropyrene was converted to 4-aminopyrene and 9(10)-hydroxy-4-(acetylamino)pyrene. Metabolic conversion to 4-nitropyrene-9,10-dione has also been shown *in vitro* in the presence of induced rat-liver microsomes. In the presence of an epoxide hydrolase inhibitor, a major metabolite of 4-nitropyrene was 9,10-epoxy-9,10-dihydro-4-nitropyrene, a K-region epoxide. Most of the metabolism observed in incubations with human liver microsomes was due to the cytochrome P450 3A4 enzyme, which produced the metabolites *trans*-9,10-dihydro-9,10-dihydroxy-4-nitropyrene, 9(10)-hydroxy-4-nitropyrene and 4-aminopyrene. An adduct that is probably responsible for the mutagenicity and carcinogenicity of 4-nitropyrene is *N*-(deoxyguanosin-8-yl)-4-aminopyrene. 4-Nitropyrene was mutagenic in bacteria, and at the thymidine kinase +/– locus in human lymphoblastoid cells. It did not cause cell transformation in cultured rat tracheal cells. No data were available on the cytogenetic effects, induction of mutation in oncogenes or tumour-suppressor genes, or on the effects on gene expression of 4-nitropyrene.

Overall, these results provide *moderate mechanistic evidence* to support the carcinogenicity of 4-nitropyrene.

6. Evaluation

6.1 Cancer in humans

There is *inadequate evidence* in humans for the carcinogenicity of 4-nitropyrene.

6.2 Cancer in experimental animals

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

6.3 Overall evaluation

4-Nitropyrene is *possibly carcinogenic to humans (Group 2B).*

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LIST OF ABBREVIATIONS

AAF	2-acetylaminofluorene
AhR	aryl hydrocarbon receptor
AKR	aldo-keto reductase
AM	arithmetic mean
ATSDR	Agency of Toxic Substances and Disease Registry
B[*a*]P	benzo[*a*]pyrene
B[*a*]PDE	benzo[*a*]pyrene-7,8-diol-9,10-epoxide
BAL	bronchoalveolar lavage
BG	gasoline, high-emitting black smoker
bhp	brake horse power
bw	body weight
CI	confidence interval
CO	carbon monoxide
CYP	cytochrome P450
DB[*a*,*h*]A	dibenz[*a*,*h*]anthracene
dG	deoxyguanosine
dG-C8-2-AF	*N*-(deoxyguanosin-8-yl)-2-aminofluorene
dG-C8-AP	*N*-(deoxyguanosin-8-yl)-1-aminopyrene
dG-N^2-2-AAF	C3-(deoxyguanosin-N^2-yl)-2-acetylaminofluorene
5-(dG-N^2-yl)-6-AC	5-(deoxyguanosin-N^2-yl)-6-aminochrysene
5-(dG-N^2-yl)-1,2-DHD-6-AC	5-(deoxyguanosin-N^2-yl)-1,2-dihydroxy-1,2-dihydro-6-aminochrysene
N-(dG-8-yl)-6-AC	*N*-(deoxyguanosin-8-yl)-6-aminochrysene
1,2-DHD-6-AC	*trans*-1,2-dihydro-1,2-dihydroxy-6-aminochrysene
1,2-DHD-6-NC	*trans*-1,2-dihydro-1,2-dihydroxy-6-nitrochrysene
9,10-DHD-6-NC	*trans*-9,10-dihydro-9,10-dihydroxy-6-nitrochrysene
1,2-DHD-6-NHOH-C	*trans*-1,2-dihydroxy-1,2-dihydro-6-hydroxyaminochrysene
N-(dI-8-yl)-6-AC	*N*-(deoxyinosin-8-yl)-6-aminochrysene
DiPN	*N*-nitrosodiisopropanolamine
DMSO	dimethyl sulfoxide
DPF	diesel particle filter
EC	elemental carbon
EDTA	ethylenediaminetetraacetic acid
EOM	extractable organic matter
EPA	Environmental Protection Agency
ERCC	excision repair cross-complementing

ESC	European stationary cycle
ETC	European transient cycle
EU	European Union
F_1	offspring
2-FAF	2-formylaminofluorene
FINJEM	Finnish job-exposure matrix
FTP	Federal Test Procedure
G	gasoline, normal emitter at 72 °F
G30	gasoline, normal emitter at 30 °F
GC	gas chromatography
GC-ECD	gas chromatography-electron capture detector
GC-MS	gas chromatography-mass spectrometry
GC-NICI-MS	gas chromatography-negative ion chemical ionization-mass spectrometry
GDI	gasoline direct injection
g/bhp-h	grams per brake horsepower-hour
g/kg_{fuel}	mass emissions per mass of fuel
g/kWh	grams per kilowatt-hour
g/mi	grams per mile
g/km	grams per kilometre
GPT	guanine phosphoribosyl transferase
GST	glutathione *S*-transferase
GVWR	gross vehicle weight rating
HGV	heavy goods vehicle
Hmox	haeme oxygenase
hp	horse power
HPLC	high-performance liquid chromatography
HPRT	hypoxanthine-guanine phosphoribosyltransferase
HR	hazard ratio
H_2SO_4	sulfuric acid
IARC	International Agency for Research on Cancer
Ig	immunoglobulin
IL	interleukin
IPCS	International Programme on Chemical Safety
JEM	job-exposure matrix
kW	kilowatt
kW-h	kilowatt-hour
LEV	low emission vehicle
LPG	liquefied petroleum gas
MAPK	mitogen-activated protein kinase
MS	mass spectrometry
NADPH	nicotinamide adenine dinucleotide phosphate
NAT	*N*-acetyltransferase
NCI	National Cancer Institute
NDEA	*N*-nitrosodiethylamine
NDPA	*N*-nitrosodipentylamine
NDPHA	*N*-nitroso-bis(2-hydroxypropyl)amine
NG	new technology gasoline
NICI	negative ion chemical ionization

NIOSH	National Institute of Occupational Safety and Health
NIST	National Institute of Standard and Technology
NQO1	nicotinamide adenine dinucleotide phosphate-quinone oxidoreductase
NTDE	new technology diesel engines
OGG	8-oxoguanine glycosylase
8-OH-dG	8-oxo-2'-deoxyguanosine
OR	odds ratio
PAH	polycyclic aromatic hydrocarbon
PFI	port fuel injection
PM	particulate matter
POR	P450 oxidoreductase
R^2	explanatory strength
ROS	reactive oxygen species
rpm	revolutions per minute
RR	relative risk
SCR	selective catalytic reduction
SD	standard deviation
SFTP	Supplemental Federal Test Procedure
SID-LC/MS	stable-isotope dilution liquid chromatography/mass spectrometry
SIR	standardized incidence ratio
SMR	standardized mortality ratio
SO_2	sulfur dioxide
SRM	standard reference material
SULT	sulfotransferase
TDE	traditional technology diesel engines
TK	thymidine kinase
TLC	thin-layer chromatography
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
UGT	uridine glucuronosyl transferase
UV	ultraviolet
WG	gasoline, high-emitting white smoker

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M IARC MONOGRAPHS

This volume of the *IARC Monographs* provides evaluations of the carcinogenicity of diesel and gasoline engine exhausts, and of 10 nitroarenes found in diesel engine exhaust: 3,7-dinitrofluoranthene, 3,9-dinitrofluoranthene, 1,3-dinitropyrene, 1,6-dinitropyrene, 1,8-dinitropyrene, 6-nitrochrysene, 2-nitrofluorene, 1-nitropyrene, 4-nitropyrene, and 3-nitrobenzanthrone.

Diesel engines are used for transport on and off roads (e.g. passenger cars, buses, trucks, trains, ships), for machinery in various industrial sectors (e.g. mining, construction), and for electricity generators, particularly in developing countries. Gasoline engines are used in cars and hand-held equipment (e.g. chainsaws). The emissions from such combustion engines comprise a complex and varying mixture of gases (e.g. carbon monoxide, nitrogen oxides), particles (e.g. PM_{10}, $PM_{2.5}$, ultrafine particles, elemental carbon, organic carbon, ash, sulfate, and metals), volatile organic compounds (e.g. benzene, formaldehyde) and semi-volatile organic compounds (e.g. polycyclic aromatic hydrocarbons) including oxygenated and nitrated derivatives of polycyclic aromatic hydrocarbons. Diesel and gasoline engines thus make a significant contribution to a broad range of air pollutants to which people are exposed in the general population as well as in different occupational settings.

An *IARC Monographs* Working Group reviewed epidemiological evidence, animal bioassays, and mechanistic and other relevant data to reach conclusions as to the carcinogenic hazard to humans of environmental or occupational exposure to diesel and gasoline engine exhausts (including those associated with the mining, railroad, construction, and transportation industries) and to 10 selected nitroarenes.