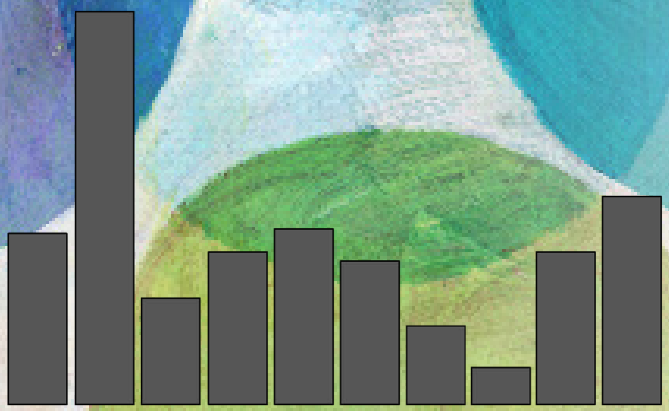
IARC SCIENTIFIC PUBLICATIONS

TUMOUR SITE CONCORDANCE AND MECHANISMS OF CARCINOGENESIS

EDITED BY ROBERT A. BAAN,
BERNARD W. STEWART, AND KURT STRAIF



IARC SCIENTIFIC
PUBLICATION NO. 165

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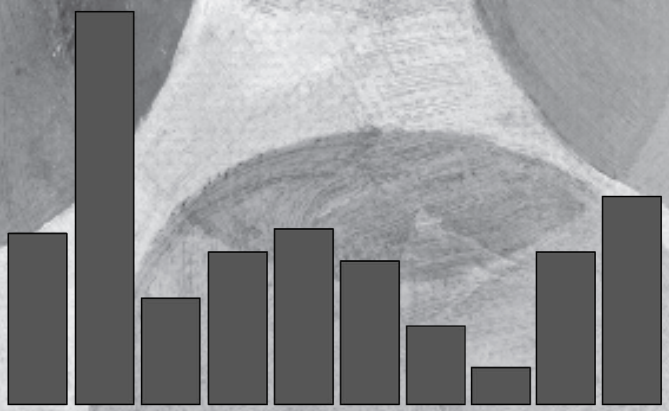

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Published by the International Agency for Research on Cancer,
150 cours Albert Thomas, 69372 Lyon Cedex 08, France

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

About the cover: The background image (credit: Qweek/E+/Getty Images) reflects the concept of "overlap" discussed in this volume, where agents cause tumours in the same target organs in humans and in experimental animals (see Chapter 21 and Annex 1). The bar graph (credit: Daniel Krewski) shows a "mechanistic profile" of the 86 carcinogens included in the analysis described in Chapter 22. The 10 bars represent the key characteristics (see Chapter 10); the height of a bar indicates the number of agents that display that particular characteristic. Genotoxicity (the second bar from the left) is the most prominent characteristic.

This book is available in electronic format from
http://publications.iarc.fr.

IARC Library Cataloguing in Publication Data

Tumour site concordance and mechanisms of carcinogenesis / edited by Robert A. Baan, Bernard W. Stewart, Kurt Straif

(IARC Scientific Publications; 165)

1. Neoplasms – etiology 2. Neoplasms – epidemiology 3. Neoplasms, Experimental 4. Carcinogenicity Tests 5. Risk Assessment 6. Public Health 7. Carcinogenesis
I. Baan, R. A. II. Title III. Series

ISBN 978-92-832-2215-6 (NLM Classification: W1)
ISSN 0300-5085

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Contributors

Editors

Robert A. Baan
Senior Visiting Scientist, Section of IARC Monographs
International Agency for Research on Cancer
Lyon, France

Bernard W. Stewart
Cancer Control Program
South Eastern Sydney Public Health Unit
Randwick, NSW, Australia

Kurt Straif
Head, Section of IARC Monographs
International Agency for Research on Cancer
Lyon, France

Workshop Participants

Lawrence Banks (April meeting only)
Tumour Virology Laboratory
International Centre for Genetic Engineering and Biotechnology
Trieste, Italy

Frederick A. Beland (April meeting only)
Division of Biochemical Toxicology
National Center for Toxicological Research
Jefferson, AR, USA

James A. Bond
Santa Fe, NM, USA

Maarten C. Bosland
Department of Pathology
College of Medicine
University of Illinois at Chicago
Chicago, IL, USA

John R. Bucher
National Institute of Environmental Health Sciences
Research Triangle Park, NC, USA

Jane C. Caldwell
National Center for Environmental Assessment
U.S. Environmental Protection Agency
Research Triangle Park, NC, USA

Vincent J. Cogliano
Integrated Risk Information System (IRIS)
National Center for Environmental Assessment
U.S. Environmental Protection Agency
Washington, DC, USA

David M. DeMarini
Integrated Systems Toxicology Division
U.S. Environmental Protection Agency
Research Triangle Park, NC, USA

Bice Fubini
Department of Chemistry
Interdepartmental Center "G. Scansetti" for Studies on Asbestos and other Toxic Particulates
Faculty of Pharmacy
University of Turin
Turin, Italy

Bernard D. Goldstein
Department of Environmental and Occupational Health
University of Pittsburgh
Pittsburgh, PA, USA

Stephen S. Hecht
Masonic Cancer Center
University of Minnesota
Minneapolis, MN, USA

Uwe Heinrich (unable to attend)
Fraunhofer Institute of Toxicology and Experimental Medicine
Hanover, Germany

Kari Hemminki
German Cancer Research Center (DKFZ)
Heidelberg, Germany

Mark A. Hill
Radiation Biophysics Group
Gray Institute for Radiation Oncology and Biology
University of Oxford
Oxford, United Kingdom

Charles William Jameson
CWJ Consulting, LLC
Cape Coral, FL, USA

Agnes B. Kane
Department of Pathology and Laboratory Medicine
Brown University
Providence, RI, USA

Robert J. Kavlock
National Center for Computational Toxicology
U.S. Environmental Protection Agency
Research Triangle Park, NC, USA

Daniel Krewski
R. Samuel McLaughlin Centre for Population Health Risk Assessment
School of Epidemiology, Public Health and Preventive Medicine
University of Ottawa
Risk Sciences International
Ottawa, Ontario, Canada

Paul F. Lambert
McArdle Laboratory for Cancer Research
University of Wisconsin School of Medicine and Public Health
Madison, WI, USA

Ronald L. Melnick
Ron Melnick Consulting, LLC
Chapel Hill, NC, USA

Christopher J. Portier (April meeting only)
National Center for Environmental Health
Agency for Toxic Substances and Disease Registry
Centers for Disease Control and Prevention
Atlanta, GA, USA

Jerry M. Rice
Georgetown University Medical Center
Department of Oncology
Lombardi Comprehensive Cancer Center
Washington, DC, USA

Ivan I. Rusyn
Environmental Sciences and Engineering
Gillings School of Global Public Health
University of North Carolina
Chapel Hill, NC, USA

Martyn T. Smith (November meeting only)
Division of Environmental Health Sciences
School of Public Health
University of California Berkeley
Berkeley, CA, USA

Leslie Stayner
Division of Epidemiology and Biostatistics
School of Public Health
University of Illinois at Chicago
Chicago, IL, USA

Bernard W. Stewart (Observer at April meeting)
Cancer Control Program
South Eastern Sydney Public Health Unit
Randwick, NSW, Australia

Robert L. Ullrich
UTMB Cancer Center
University of Texas Medical Branch
Galveston, TX, USA

Harri Vainio
Finnish Institute of Occupational Health
Helsinki, Finland

Paolo Vineis (April meeting only)
Department of Environmental Epidemiology
Imperial College London
St Mary's Campus
London, United Kingdom

Michael P. Waalkes
Inorganic Toxicology Group
National Toxicology Program Laboratory
Division of the National Toxicology Program
National Institute of Environmental Health Sciences
Research Triangle Park, NC, USA

Lauren Zeise
California Environmental Protection Agency
Reproductive and Cancer Hazard Assessment
Oakland, CA, USA

Representatives

Representative of the French Agency for Food, Environment and Occupational Health Safety
Cécile Michel (November meeting only)
French Agency for Food, Environment and Occupational Health Safety (ANSES)
Maisons-Alfort, France

Representative of the World Cancer Research Fund International
Martin Wiseman (April meeting only)
World Cancer Research Fund International
London, United Kingdom

Observers

Observer for the R. Samuel McLaughlin Centre for Population Health Risk Assessment
Mustafa Al-Zoughool
R. Samuel McLaughlin Centre for Population Health Risk Assessment
University of Ottawa
Ottawa, Ontario, Canada
Department of Community and Environmental Health
King Saud bin Abdulaziz University for Health Sciences
Riyadh, Saudi Arabia

Observer for the R. Samuel McLaughlin Centre for Population Health Risk Assessment
Michael Bird
R. Samuel McLaughlin Centre for Population Health Risk Assessment
University of Ottawa
Risk Sciences International
Ottawa, Ontario, Canada

Observer for the Centre Léon Bérard
Béatrice Fervers
Department of Cancer and the Environment
Centre Léon Bérard
University of Lyon
Lyon, France

Observer for the R. Samuel McLaughlin Centre for Population Health Risk Assessment
Pascale Lajoie (November meeting only)
R. Samuel McLaughlin Centre for Population Health Risk Assessment
University of Ottawa
Ottawa, Ontario, Canada
Department of Epidemiology
Queens University
Kingston, Ontario, Canada

IARC Secretariat

Robert Baan, Senior Visiting Scientist (Co-Responsible Officer)
Lamia Benbrahim-Tallaa
Véronique Bouvard
Graham Byrnes
Fatiha El Ghissassi
Yann Grosse (Co-Responsible Officer)
Neela Guha
Zdenko Herceg (April meeting only)
Marie-Pierre Lambert (April meeting only)
Béatrice Lauby-Secretan
Dana Loomis
Heidi Mattock
Magali Olivier (April meeting only)
Ghislaine Scélo (April meeting only)
Chiara Scoccianti (April meeting only)
Kurt Straif (Section Head)
Jiri Zavadil (November meeting only)

Additional Contributors

The following people are co-authors of chapters but were not present at the Workshop.

Mélissa Billard
R. Samuel McLaughlin Centre for Population Health Risk Assessment
University of Ottawa
Ottawa, Ontario, Canada
Department of Epidemiology
Queens University
Kingston, Ontario, Canada

Nicholas Birkett
R. Samuel McLaughlin Centre for Population Health Risk Assessment
School of Epidemiology, Public Health and Preventive Medicine
University of Ottawa
Ottawa, Ontario, Canada

Brian Collins
Risk Sciences International
Ottawa, Ontario, Canada

Kate Z. Guyton
National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Washington, DC, USA

Julian Little
School of Epidemiology, Public Health and Preventive Medicine
University of Ottawa
Ottawa, Ontario, Canada

M. Matilde Marques
Department of Chemical and Biological Engineering
Graduate Technical Institute
Technical University of Lisbon
Lisbon, Portugal

Brittany Milton
Risk Sciences International
Ottawa, Ontario, Canada

Jan M. Zielinski (deceased)
R. Samuel McLaughlin Centre for Population Health Risk Assessment
School of Epidemiology, Public Health and Preventive Medicine
University of Ottawa
Healthy Environments and Consumer Safety Branch, Health Canada
Ottawa, Ontario, Canada

Administrative Assistance

Sandrine Egraz
Elisabeth Elbers
Brigitte Kajo
Helene Lorenzen-Augros
Annick Leroux
Karine Racinoux
Dorothy Russell

Production Team

Karen Müller
English Editor

Sylvia Lesage
Publishing Assistant

Elisabeth Elbers
Publications Technician

Fiona Gould
Publications Technician

Solène Quennehen
Publications Technician

Acknowledgements

The two-part IARC Workshop on Tumour Site Concordance and Mechanisms of Carcinogenesis (held in April and November 2012) was supported by grants from the European Commission (grant number VS/2012/0117) and from the United States National Institute of Environmental Health Sciences (NIEHS).

Introduction

Vincent J. Cogliano

The *IARC Monographs*

The *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, published by the International Agency for Research on Cancer (IARC) of the World Health Organization, are a series of scientific reviews that identify the causes of cancer in humans. Since its inception in the early 1970s, the *IARC Monographs Programme* has evaluated more than 1000 chemical, physical, and biological agents and classified almost 500 of these as *carcinogenic*, *probably carcinogenic*, or *possibly carcinogenic* to humans.

Agents are identified as subjects for *IARC Monographs* evaluations based on evidence of human exposure and some evidence or suspicion of carcinogenicity. Agents may be re-evaluated when substantial new information becomes available. Periodically, IARC convenes Advisory Groups of experts from national and international health agencies and from research institutions to recommend agents for evaluation or re-evaluation. Otherwise, agents may be reviewed in response to an urgent public health need.

International, interdisciplinary Working Groups of expert scientists develop each volume of the *IARC Monographs*. IARC selects participants in the Working Groups based on their knowledge and experience, and absence of conflicting interests. Working Group members generally have published research on the carcinogenicity of the agents under review. IARC also gives consideration to demographic diversity among Working Group members and to a fair balance of scientific findings and views. The *IARC Monographs* are a worldwide endeavour that, since 1971, has involved more than 1200 scientists from more than 50 countries.

For each agent, the Working Group writes a critical review of the pertinent studies of cancer in exposed humans, cancer after administration of the agent to experimental animals, and representative mechanistic and other relevant data, as well as general information on the agent and human exposure to it. The Working Group meets at IARC, in Lyon, France, for eight days to discuss the critical reviews and to develop consensus evaluations that classify each agent into one of the following categories:

- *carcinogenic to humans* (Group 1);
- *probably carcinogenic to humans* (Group 2A);
- *possibly carcinogenic to humans* (Group 2B);
- *not classifiable as to its carcinogenicity to humans* (Group 3);
- *probably not carcinogenic to humans* (Group 4).

Each volume of the *IARC Monographs* opens with the Preamble, which describes the objective and scope of the *IARC Monographs Programme*, the scientific principles and procedures used in developing a *Monograph*, the types of evidence considered, and the scientific criteria that guide the evaluations (IARC, 2006).

Volume 100: a review of human carcinogens

For Volume 100 of the *IARC Monographs*, a review was undertaken of relevant information on all the agents classified in Group 1 (*carcinogenic to humans*). There was value in such a comprehensive review, because about half of the agents classified in Group 1 had last been evaluated more than 20 years earlier. For advice on the development of Volume 100, IARC convened an Advisory Group (IARC, 2007) chaired by Dr Lorenzo Tomatis, who had founded the *IARC Monographs Programme* and later become the second IARC Director.

Volume 100 follows the practice within the *IARC Monographs Programme* of occasionally updating the evidence for a large number of agents from earlier Volumes. Supplement 1 updated the available data for 54 agents from Volumes 1–20 for which studies of cancer in humans were available, Supplement 4 updated the information for 155 agents from Volumes 1–29, and Supplement 7 reviewed 189 agents from Volumes 1–42 (IARC, 1979, 1982, 1987b). Supplement 7 was preceded by Supplement 6, which updated and summarized the findings from tests for genetic and related effects of the same agents (IARC, 1987a). More recently, Volume 71 updated the evidence for 121 agents, most of them classified in Groups 2A and 2B, by using a mini-*Monograph* format to present the findings for most of these agents (IARC, 1999).

Group 1 agents are diverse and include chemicals and chemical mixtures; occupations; metals, dusts, and fibres; ionizing and non-ionizing radiation; viruses and other biological agents; personal habits; and pharmaceuticals. The precise number of agents classified in Group 1 cannot be given, because generic categories such as "nickel compounds" and "human papillomaviruses" include multiple agents that were evaluated together.

IARC explored ways to strengthen the scientific outcome of Volume 100 (IARC, 2012a, b, c, d, e, f). For several prominent human carcinogens, of which asbestos and benzene are examples, active scientific debate was focused on the implications of mechanistic studies that had not been conceived when these agents had last been reviewed, more than 20 years earlier. For other agents, including alcoholic beverages and vinyl chloride, there were questions about whether additional cancer sites in humans had been established by more recent research. There were also cross-cutting questions about the relevance to humans of certain cancer sites or mechanistic pathways in animals. It was recognized that there would be scientific value in a systematic identification of the cancer sites observed in humans and those observed in experimental animals, and in a compilation of mechanistic events for agents known to cause cancer in humans. The outcome of Volume 100 would thus encompass a bridge from the past focus on cancer studies in humans and experimental animals to a future that promises increasing availability of mechanistic data. Therefore, IARC initially planned the project in two phases: (i) a review of human carcinogens that would accrue information on cancer sites and mechanistic events, followed by (ii) supplementary analyses of tumour site concordance between humans and experimental animals, and of mechanistic events deemed relevant to the carcinogenicity of these agents. The reviews and analyses were discussed during a two-part Workshop on Tumour Site Concordance and Mechanisms of Carcinogenesis, which was convened by IARC on 16–18 April 2012 and 28–30 November 2012 in Lyon. This Scientific Publication is the report of that Workshop.

Scientific Publication on tumour site concordance and mechanisms of carcinogenesis

This Scientific Publication analyses the information on cancer sites and mechanistic events that was documented for the more than 100 agents classified by IARC in Group 1 (*carcinogenic to humans*). The corresponding *Monographs* are organized by agent, with a separate *Monograph* for each agent or group of closely related agents. The chapters of this Scientific Publication are organized in other ways, in order to develop insights about larger groupings of agents, cancer sites, and mechanistic events.

Two data sets bring together information across all Group 1 agents evaluated up to and including Volume 109. The first data set contains the information about cancer sites in humans and in experimental animals (see Annex 1, by Grosse et al.). It is organized to facilitate the investigation of tumour site concordance across species (see the concordance analysis in Chapter 21,

by Krewski et al.). It has long been recognized that concordance between human and animal tumour sites is not evident for carcinogens of all types considered as a single category. Concordance can be analysed for a grouping of agents (e.g. aromatic amines) or for a cancer site (e.g. haematological cancers). Such analyses could explore the predictive value for human cancer of tumours in experimental animals, based on the information collected to date. These types of analyses may also identify human cancers for which there currently are no good animal models; for these cancers, it might be advantageous to focus on understanding mechanistic pathways to design new experimental models that could identify agents linked to these cancers.

The second data set contains the information about established and likely mechanistic events (see Al-Zoughool et al., 2019 and Birkett et al., 2019). It is organized to facilitate the investigation of patterns across mechanistic events and agents (see the mechanistic analysis in Chapter 22, by Krewski et al.). Data can be aggregated for groupings of agents that involve common mechanistic events or common cancer sites. Such analyses could identify biomarkers that could be incorporated into future epidemiological studies. They also could identify populations and developmental stages that may be especially susceptible to the occurrence of certain mechanistic events. Ultimately, they could lead to the confident identification of human carcinogens based on mechanistic information in the absence of adequate cancer studies in humans or experimental animals.

To accommodate the different degrees of precision with which cancer sites have been identified (e.g. "liver cancer" for one agent and "hepatocellular carcinoma" for another), the database uses designations that are more general in nature ("liver cancer" in this example). Likewise, a detailed list of 24 mechanistic events that was initially proposed was subsequently condensed to a set of 10 "key characteristics" (see below). This level of aggregation is necessary to avoid fragmenting the data into large numbers of categories with few data points; this makes it possible to conduct analyses across reasonable numbers of agents. Further research will change our understanding of mechanistic events and will establish additional associations of agents with mechanistic events in the future.

Five additional human carcinogens, identified after the completion of Volume 100, are included in the data set: (i) diesel engine exhaust (reviewed in Volume 105; IARC, 2013), (ii) trichloroethylene (evaluated in Volume 106; IARC, 2014), (iii) polychlorinated biphenyls (PCBs) and dioxin-like PCBs (reviewed in Volume 107; IARC, 2016b), and (iv) outdoor air pollution and (v) particulate matter in outdoor air pollution (both evaluated in Volume 109; IARC, 2016a).

The two data sets are linked. Concordance in the first data set may find support from the mechanistic information in the second. Lack of concordance in the first data set may or may not be explained by the mechanistic information in the second. Accordingly, the analyses in this Scientific Publication do not stop at a superficial analysis of concordance or discordance. They seek to determine whether there is *coherence*, which can be understood as concordance confirmed or discordance explained.

The chapters in this Scientific Publication address what we have learned about some major mechanisms for agents known to cause cancer in humans. The Consensus Statement was unanimously endorsed by the Workshop participants. The chapters in Part 1 discuss various groupings of carcinogenic agents, such as electrophilic agents, metals, constituents of tobacco smoke, and human tumour viruses. These chapters illustrate the types of analysis that can be undertaken for groups of carcinogenic agents, including those that act at a common site or through a common mechanism.

Chapter 10 (by Smith) discusses our observation that all human carcinogens evaluated in Volume 100 (and subsequent *Monographs* as mentioned above) display one or more of what are called key characteristics of carcinogens: is electrophilic or can be metabolically activated to electrophiles; is genotoxic; alters DNA repair or causes genomic instability; induces epigenetic alterations; induces oxidative stress; induces chronic inflammation; is immunosuppressive; modulates receptor-mediated effects; causes immortalization; and/or alters cell proliferation, cell death, or nutrient supply. Chapter 11 (by Stewart) places the key characteristics in the context of other viewpoints, such as the hallmarks of carcinogenesis. Subsequent chapters in Part 2 discuss the role of several of the key characteristics individually, followed by chapters that discuss susceptibility.

The analyses of concordance and mechanisms are presented in the chapters in Part 3. To facilitate similar analyses by cancer researchers worldwide, Annex 1 (by Grosse et al.), Al-Zoughool et al. (2019), and Birkett et al. (2019) provide a description of the databases of concordance and mechanisms that were developed from the information compiled for Volume 100 and for several subsequent Volumes.

We regard this Scientific Publication as the beginning, not the end of the lessons to be learned from the information in Volume 100 of the *IARC Monographs*. We encourage all scientists to continue to analyse these data and to develop further insights into the causes of cancer in humans.

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IARC (2013). Diesel and gasoline engine exhausts and some nitroarenes. IARC Monogr Eval Carcinog Risks Hum. 105:1–704. PMID:26442290. Available from: http://publications.iarc.fr/129.

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Consensus statement

This statement was unanimously endorsed by the participants in the Workshop on Tumour Site Concordance and Mechanisms of Carcinogenesis, which was convened by IARC on 16–18 April and 28–30 November 2012 in Lyon.

Introduction

The *IARC Monographs Programme* is an international consensus approach to the identification of chemicals and other agents that may present carcinogenic hazards to humans. The *Monographs* assess the strength of the published scientific evidence for such identifications, which are based primarily on epidemiological studies of cancer in humans and bioassays for carcinogenicity in laboratory animals. Information that may be relevant to the mechanisms by which the putative carcinogen acts is also considered in making an overall evaluation of the strength of the total evidence for carcinogenicity to humans.

The use of mechanistic data to identify human carcinogens is accelerating. Initially, the *IARC Monographs* required *sufficient evidence* in humans to classify an agent as *carcinogenic to humans* (Group 1). Scientific understanding of the mechanisms of carcinogenesis, accompanied by the development of assays for studying mechanistic events, has led to new ways of identifying human carcinogens. Some examples are the following agents that were classified as *carcinogenic to humans*: ethylene oxide (in 1994), based on strong evidence of genotoxicity and limited epidemiological evidence in exposed humans; 2,3,7,8-tetrachlorodibenzo-*para*-dioxin (in 1997), based on strong evidence of binding to the aryl hydrocarbon receptor and subsequent events; neutron radiation (in 2000), based on the underlying radiation physics; benzidine-based dyes (in 2010), because these substances are metabolized to a carcinogen in humans; and several compounds for which single-agent exposure does not exist because they are components of (complex) mixtures, for example tobacco-specific nitrosamines (in 2007), benzo[*a*]pyrene (in 2010), aristolochic acid (in 2012), and etoposide (in 2012). Mechanistic evidence was also important in classifying the carcinogenicity of several other agents between 2004 and 2010, and in revising the classification of carcinogenicity for several additional agents in Volume 100.

For Volume 100 of the *IARC Monographs*, a review was undertaken during 2008–2009 of relevant information on all the agents classified in Group 1 (*carcinogenic to humans*) in Volumes 1–99. There was value in a comprehensive review, because about half of the agents classified in Group 1 had last been reviewed more than 20 years earlier. Volume 100 was organized in six parts, each prepared by a separate Working Group, covering: pharmaceuticals (Volume 100A); biological agents (Volume 100B); arsenic, metals, fibres, and dusts (Volume 100C); radiation (Volume 100D); personal habits and indoor combustions (Volume 100E); and chemical agents and related occupations (Volume

100F). Volume 100: *A Review of Human Carcinogens* was published in 2012 as a six-book set.

IARC explored ways to strengthen the scientific value of Volume 100, and embarked on a two-phase project: (i) a review of the Group 1 human carcinogens with respect to cancer sites and mechanistic events, followed by (ii) supplementary analyses of tumour site concordance between humans and experimental animals, and of mechanistic events deemed relevant to the carcinogenicity of these agents. Accordingly, this Scientific Publication on Tumour Site Concordance and Mechanisms of Carcinogenesis was proposed.

To prepare for the supplementary analyses in this Scientific Publication, IARC had asked the six Working Groups for Volume 100 to collect additional information, not routinely developed before, on (i) cancer sites in humans for which there was *sufficient evidence* or *limited evidence* in epidemiological studies, (ii) cancer sites for which there was *sufficient evidence* in experimental animals, and (iii) established and likely mechanisms involved in the cancers observed in humans or experimental animals.

To further develop this Scientific Publication, the *IARC Monographs Programme* convened a group of international scientific experts in a two-part Workshop on Tumour Site Concordance and Mechanisms of Carcinogenesis, held in Lyon in April and November 2012. The Workshop participants used the lists of mechanistic events to develop a set of key characteristics to define the mechanistic profile of the agents classified in Group 1.

The main points of consensus, the conclusions, and the recommendations of the Workshop participants are described below.

Tumour site concordance

1. The results developed in Volume 100 of the *IARC Monographs* confirm that the induction of cancer in experimental animals is relevant to the identification of a carcinogenic hazard to humans: all human carcinogens identified to date that have been adequately tested in animals have also been shown to cause cancer in animals.
2. For many human carcinogens, there is tumour site concordance between humans and experimental animals; for many others, there is not. At present, the state of the science does not support tumour site concordance as a general principle. For example, there are four agents for which there is *sufficient evidence* for breast cancer in humans and seven agents for which there is *sufficient evidence* for breast cancer in experimental animals, but only one of these agents causes breast cancer in both humans and animals.
3. The analyses presented in this Scientific Publication are expected to underestimate concordance. One reason is the limited power and other limitations of many observational epidemiological studies that include populations and cancer sites that have not been adequately investigated. Another reason is that – for the purpose of this concordance analysis – an agent was considered to cause cancer at a site in animals only if positive results were replicated at the same specific site in another animal experiment (while recognizing the limitations of a single positive result in a cancer bioassay); however, metabolic or mechanistic considerations might explain tumour induction at different sites in separate animal models.
4. Descriptive statistics of tumour sites identified to date may not be representative of future evaluations or of the incompletely characterized "universe of human carcinogens". The carcinogens evaluated in Volume 100 include several classes of agents that have been relatively straightforward to investigate; some examples are: alkylating agents that were used in early cancer chemotherapy; viral agents that infect hundreds of millions of people; ionizing radiation, which affects multiple anatomical sites; widespread exposures, such as to tobacco smoke and its related agents and alcoholic beverages; and chemical agents with long histories of occupational exposure at high levels. Agents evaluated in the future may have more subtle effects and different characteristics. Evidence from sources other than human epidemiology will need to be relied upon to identify carcinogenic hazards to humans.
5. Past evaluations have noted cancer in experimental animals at approximately 40 tumour sites in 15 organ and tissue systems. Use of standard terminology for these sites can facilitate the development of databases and their analysis and linkage to other sources of information. The Workshop participants recommend that future *IARC Monographs* Working Groups consider the anatomically based taxonomy of tumour sites

that appears in this Scientific Publication in the analysis of concordance between sites where tumours arise in animals and in humans.

6. The Workshop participants also recommend that in future *IARC Monographs*, the Evaluation section for evidence of carcinogenicity in experimental animals be expanded to include additional information for agents evaluated as exhibiting *sufficient evidence*. For such agents, an additional sentence after the relevant evaluation should refer to the recognized site or sites of tumorigenesis, by using the specification system described in the chapter on concordance analysis (Chapter 21, by Krewski et al.).

Mechanisms involved in human carcinogenesis

7. With increasing scientific understanding and availability of information on mechanisms of carcinogenesis, it is expected that the *IARC Monographs* will make even greater use of mechanistic data in identifying human carcinogens.
8. Until now, there has been no generally accepted method for organizing mechanistic data pertinent to the identification of carcinogenic hazards to humans. The key characteristics presented here offer a promising foundation for the structured evaluation of mechanistic information, and this should increase the utility of mechanistic evidence in future identifications of carcinogenic hazards and the transparency of systematic reviews of such evidence. The Workshop participants recommend that the *IARC Monographs Programme* use the key characteristics in its evaluations of carcinogenicity.
9. It is notable that in vivo or in vitro mechanistic data are often available in humans. In most cases, when animal data are available for a key characteristic, human data for that characteristic are generally available, too. This supports the notion that carcinogens show their characteristics across species.
10. There should be no expectation that all, or even most, key characteristics operate for any human carcinogen. No key characteristic is necessary for carcinogenesis, and negative results for one or more key characteristics are not an argument against the potential carcinogenicity of an agent. Observation of one or more key characteristics in exposed humans can increase the biological plausibility of less-than-*sufficient evidence* in humans. Observation of one or more key characteristics in experimental animals can increase confidence in the human relevance of less-than-*sufficient evidence* in experimental animals. In interpreting the biological relevance of information pertaining to the key characteristics, it is important to consider aspects of metabolism and kinetics in extrapolating between in vitro and in vivo systems.
11. A human carcinogen may display multiple key characteristics that may interact with each other. The past practice of according greatest concern to those agents demonstrated to be genotoxic, relative to agents whose carcinogenicity appeared to be mediated by one or more other key characteristics, appears to be overly simplistic.
12. The objective of the *IARC Monographs Programme* is to identify carcinogenic hazards, not to exhaustively list all mechanistic events and pathways that might contribute to carcinogenesis. Future coverage of mechanistic data should increase as the retrieval of such data becomes more systematic and the key characteristics are used as a framework for organization and analysis of mechanistic data.
13. Descriptive statistics of mechanisms identified to date may not be representative of future evaluations. Although genotoxicity is the key characteristic most exhibited by the human carcinogens identified to date, this may reflect the relatively greater attention paid in the past to the investigation of genotoxic agents. Future evaluations of carcinogenic agents may involve a larger set of mechanistic events and pathways that are not yet well developed or understood. Accordingly, future shifts in the distribution of the key characteristics are to be expected. This does not detract from the value of using these characteristics now in evaluations of carcinogenic hazards.

Abbreviations

ADH	alcohol dehydrogenase
ADME	absorption, distribution, metabolism, and elimination
AFB_1	aflatoxin B_1
AhR	aryl hydrocarbon receptor
AIDS	acquired immune deficiency syndrome
ALDH2	aldehyde dehydrogenase 2
ALL	acute lymphoblastic leukaemia
AML	acute myeloid leukaemia
AP-1	activator protein 1
AR	androgen receptor
ARNT	aryl hydrocarbon receptor nuclear translocator
ATLL	adult T-cell leukaemia/lymphoma
ATP	adenosine triphosphate
B[*a*]P	benzo[*a*]pyrene
BCME	bis(chloromethyl)ether
BL	Burkitt lymphoma
BrdU	5-bromo-2′-deoxyuridine
bw	body weight
CA	chromosomal aberrations
CCL2	chemokine (C-C motif) ligand 2
CIN	cervical intraepithelial neoplasia
CLL	chronic lymphocytic leukaemia
CML	chronic myeloid leukaemia
CMME	chloromethyl methyl ether
COSMIC	Catalogue of Somatic Mutations in Cancer
COX-2	cyclooxygenase 2
CT	computed tomography

CYP450	cytochrome P450
DCVC	1,2-dichlorovinyl-cysteine
DCVG	(1,2-dichlorovinyl)glutathione
DDT	dichlorodiphenyltrichloroethane
DES	diethylstilbestrol
DMA(V)	dimethylarsinic acid
DMBA	7,12-dimethylbenz[*a*]anthracene
DOHaD	developmental origins of health and disease
DSBs	double-strand breaks
eBL	endemic Burkitt lymphoma
EBNA	EBV nuclear antigen 1
EBV	Epstein–Barr virus
EGF	epidermal growth factor
EH	epoxide hydrolase
ENCODE	Encyclopedia of DNA Elements
ENU	*N*-nitrosoethylurea
EPA	United States Environmental Protection Agency
ER	estrogen receptor
ERK	extracellular signal-regulated kinase
ERα	estrogen receptor alpha
F-344	Fischer 344
FANC/BRCA	Fanconi anaemia complementation groups/breast cancer A
FISH	fluorescence in situ hybridization
GLP	good laboratory practice
GSH	glutathione
GST	glutathione *S*-transferase
GSTM1	glutathione-*S*-transferase M1
GTP	guanosine-5′-triphosphate
GWAS	genome-wide association study
H_2O_2	hydrogen peroxide
Hb	haemoglobin
HBsAg	hepatitis B surface antigen
HBV	hepatitis B virus
HBx	HBV X protein
HCC	hepatocellular carcinoma
HCV	hepatitis C virus
HER	human epidermal growth factor receptor
HIF-1α	hypoxia-inducible transcription factor 1 alpha
HIV	human immunodeficiency virus
HIV-1	human immunodeficiency virus type 1
HMGB1	high-mobility group box 1 protein
HNSCC	head and neck squamous cell carcinoma
HPB	4-hydroxy-1-(3-pyridyl)-1-butanone
HPV	human papillomavirus
Hsp90	heat shock protein 90
HSV-1	herpes simplex virus type 1
HTLV-1	human T-cell lymphotropic virus type 1
IARC	International Agency for Research on Cancer
ICD	International Classification of Diseases
IGF-1	insulin-like growth factor 1

IgM	immunoglobulin M
IL-1α	interleukin 1 alpha
InChI	International Chemical Identifier
IRIS	Integrated Risk Information System
IUPAC	International Union of Pure and Applied Chemistry
JNK	c-Jun N-terminal kinase
K14	keratin 14
KEGG	Kyoto Encyclopedia of Genes and Genomes
KSHV	Kaposi sarcoma-associated herpesvirus
LANA	latency-associated nuclear antigen
LET	linear energy transfer
LMP	latent membrane protein
lncRNA	long non-coding RNA
MALT	mucosa-associated lymphoid tissue
MAPK	mitogen-activated protein kinase
methyl-CCNU	1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea
MHV-68	Murid herpesvirus 68
miRNA	microRNA
MMA	monomethylarsenic
MMA(III)	monomethylarsinous acid
MMPs	matrix metalloproteinases
MN	micronuclei
MNNG	*N*-methyl-*N*′-nitro-*N*-nitrosoguanidine
MOCA	4,4′-methylenebis(2-chloroaniline)
NAB	*N*′-nitrosoanabasine
NAD+	oxidized nicotinamide adenine dinucleotide
NADH	reduced nicotinamide adenine dinucleotide
NADPH	nicotinamide adenine dinucleotide phosphate
NAT	*N*′-nitrosoanatabine
NDEA	*N*-nitrosodiethylamine
NDMA	*N*-nitrosodimethylamine
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
NHL	non-Hodgkin lymphoma
NK	natural killer
NNAL	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol
NNK	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone
NNN	*N*′-nitrosonornicotine
·NO	nitric oxide
NPV	negative predictive value
NQO1	NAD(P)H:quinone oxidoreductase 1
Nrf_2	nuclear factor erythroid 2-related factor 2
$O_2^{\cdot-}$	superoxide
OAT	organic anion transporter
·OH	hydroxyl radical
PAHs	polycyclic aromatic hydrocarbons
PCB 77	3,3′,4,4′-tetrachlorobiphenyl
PCB 118	2,3′,4,4′,5-pentachlorobiphenyl
PCB 126	3,3′,4,4′,5-pentachlorobiphenyl
PCB 128	2,2′,3,3′,4,4′-hexachlorobiphenyl
PCB 153	2,2′,4,4′,5,5′-hexachlorobiphenyl

PCBs	polychlorinated biphenyls
PCNA	proliferating cell nuclear antigen
PeCDF	2,3,4,7,8-pentachlorodibenzofuran
PGHS-2	prostaglandin-endoperoxide synthase 2
PGRMC1	progesterone receptor membrane component 1
PI3K	phosphoinositide 3-kinase
PPAR	peroxisome proliferator-activated receptor
PPV	positive predictive value
PR	progesterone receptor
RET/PTC	rearranged during transfection/papillary thyroid carcinoma
SCC	squamous cell carcinoma
SCE	sister chromatid exchange
SCID	severe combined immunodeficiency
SIV	simian immunodeficiency virus
SREBP1c	sterol regulatory element-binding protein 1c
STAT3	signal transducer and activator of transcription 3
SULT1B1	sulfotransferase family cytosolic 1B member 1
SV40	simian virus 40
TCDD	2,3,7,8-tetrachlorodibenzo-*para*-dioxin
TCE	trichloroethylene
TCF8	transcription factor 8
TD_{50}	dose rate (in mg/kg body weight/day) that is estimated to reduce the proportion of tumour-free animals by 50%
TGF-β	transforming growth factor beta
TNF-α	tumour necrosis factor alpha
ToxCast	Toxicity Forecaster
TPA	12-*O*-tetradecanoylphorbol-13-acetate
UDP	uridine-5′-diphosphate
UV	ultraviolet
vFLIP	viral Fas-associated death domain-like IL-1-converting enzyme inhibitory protein
vGPCR	viral G protein-coupled receptor
WHO	World Health Organization

PART 1.

CONCORDANCE BETWEEN CANCER IN HUMANS AND IN EXPERIMENTAL ANIMALS

CHAPTER 1.

Electrophilic agents

James A. Bond and Ronald L. Melnick

Introduction

In this chapter, electrophilic agents include direct-acting electrophilic chemicals and chemicals that are metabolized to reactive electrophiles. All of the chemicals discussed here are IARC Group 1 agents and as such can be characterized as *carcinogenic to humans*. Relevant carcinogens discussed in this chapter do not include pharmaceutical drugs classified in Group 1, which otherwise typically include alkylating cytotoxic agents.

Tumour sites identified in previous IARC evaluations of the carcinogenicity of several non-pharmaceutical organic compounds in humans and laboratory animals are shown in Table 1.1. For each of these agents, there was *sufficient evidence* of carcinogenicity from studies in rats and/or mice and, except for ethylene oxide, *sufficient evidence* of carcinogenicity from studies of exposed humans. For ethylene oxide, there was *limited evidence* of carcinogenicity in humans, but the classification of this chemical was raised to *carcinogenic to humans* (Group 1) based on strong mechanistic evidence of mutagenicity and clastogenicity, including the induction of sister chromatid exchange (SCE), chromosomal aberrations (CA), and micronuclei (MN) in workers exposed to ethylene oxide.

Among this group of chemicals, there is remarkable concordance in tumour sites with *sufficient evidence* or *limited evidence* of carcinogenicity in humans and *sufficient evidence* of carcinogenicity in rats and/or mice, for example for the liver (aflatoxins, trichloroethylene [TCE], and vinyl chloride), the lung (sulfur mustard), the lymphohaematopoietic system (benzene, 1,3-butadiene, and ethylene oxide), nasal tissue (formaldehyde), and the kidney (TCE). For bis(chloromethyl)ether (BCME), the lung and the nasal cavity were identified as target organs in humans and rats, respectively. In addition, angiosarcomas of the liver, which are rare tumours, were identified in humans, rats, and mice exposed to vinyl chloride.

In several instances, tumour sites identified in animals were not detected in epidemiological studies of exposed workers. These apparent discrepancies may be due to differences in susceptibility between humans and certain animal models, differences in exposure conditions between studies in animals and in humans, or limitations in

Table 1.1. Tumour sites in humans, rats, and mice for electrophilic agents

Agent	Humans		Rats	Mice
	Sufficient evidence	Limited evidence	Sufficient evidence	Sufficient evidence
Aflatoxins	Liver: hepatocellular carcinoma		Liver: hepatocellular carcinoma	
Benzene	Acute myeloid leukaemia	Acute lymphoblastic leukaemia Chronic lymphocytic leukaemia Multiple myeloma Non-Hodgkin lymphoma	Oral cavity: carcinoma Forestomach: squamous cell carcinoma Skin: squamous cell carcinoma Zymbal gland: carcinoma	Lymphoid tissue: lymphoma Haematopoietic tissue: granulocytic leukaemia Mammary gland: adenocarcinoma Lung: bronchiolo-alveolar carcinoma Zymbal gland: carcinoma Preputial gland: squamous cell carcinoma
Bis(chloromethyl)ether (BCME)	Lung		Nasal cavity: olfactory neuroblastoma	Soft tissue: sarcoma Skin: fibrosarcoma
1,3-Butadiene	Lymphohaematopoietic			Lymphoid tissue: lymphoma Soft tissue: haemangiosarcoma Liver: hepatocellular carcinoma Mammary gland: adenocarcinoma Lung: bronchiolo-alveolar carcinoma Forestomach: squamous cell carcinoma Harderian gland: carcinoma Preputial gland: squamous cell carcinoma
Ethylene oxide		Lymphohaematopoietic (non-Hodgkin lymphoma, multiple myeloma, chronic lymphocytic leukaemia) Breast	Brain: glioma Lymphoid tissue: lymphoma Peritoneum: mesothelioma	Lung
Formaldehyde	Nasopharynx Leukaemia	Paranasal sinuses	Nasal cavity: squamous cell carcinoma	
Sulfur mustard	Lung	Larynx		Lung
Trichloroethylene (TCE)	Kidney	Non-Hodgkin lymphoma Liver	Kidney	Liver Lung
Vinyl chloride	Liver: angiosarcoma Liver: hepatocellular carcinoma		Liver and extrahepatic: angiosarcoma Liver: hepatocellular carcinoma Mammary gland: adenocarcinoma Zymbal gland: carcinoma	Liver and extrahepatic: angiosarcoma Lung: bronchiolo-alveolar carcinoma Mammary gland: adenocarcinoma

epidemiological studies that reduce their power to detect excess cancer risk at particular sites. For example, the finding that mammary gland tumours were induced in female mice exposed to benzene, 1,3-butadiene, or vinyl chloride, whereas breast cancer risk was not elevated in exposed workers may be due to the fact that women were not included in many occupational cohort or case–control studies of these agents, sometimes because there were very few or no women in relevant workforces, or because they were exposed to much lower concentrations. In contrast, for hospital-based sterilization workers exposed to ethylene oxide, among whom there is a high proportion of women, increases in breast cancer incidence were observed. Exposure to ethylene oxide increased the incidence of mammary gland tumours in female mice but not in male mice. Thus, limitations in human cancer databases and sex-specific tumours in animal studies need to be considered in evaluations of site concordance for tumour induction between humans and animals.

The carcinogenicity of organic compounds and their organ specificity in animal models and in humans are influenced by numerous factors. This chapter focuses on factors that affect tissue dosimetry, factors that contribute to the multistep carcinogenic process for each agent, and factors that might account for interspecies and inter-individual differences in susceptibility.

Chemical disposition

The role of metabolism in the formation of the putative carcinogenic intermediates of the non-pharmaceutical organic compounds that are *carcinogenic to humans* is summarized in Table 1.2. Common among these 10 agents is the electrophilic nature of the parent chemical or a metabolite thereof. All of these agents either exist as direct-acting electrophilic species or can be metabolized to reactive electrophilic species. It is generally accepted that the ability of these electrophiles, whether alkylating agents, epoxides, or quinones, to react with nucleophiles, such as DNA, is key to the carcinogenicity of this group of agents (Table 1.1 and Table 1.2).

Because of the diversity in the biochemical and physical properties of organic electrophilic compounds, it is useful to first examine, in a general way, factors that are important for their absorption, distribution, metabolism, and elimination. These factors include, but are not limited to, (i) physiological, (ii) chemical, and (iii) metabolic determinants. Examples of physiological factors are alveolar ventilation rate, cardiac output, blood flow to organs, organ volumes, and body mass and composition (e.g. percentage body fat). Examples of chemical factors include the stability and reactivity of the parent compound and its metabolites, partition coefficients that control the distribution of organic compounds between blood and air or blood and tissues, rates of uptake from the gastrointestinal tract, and absorption through the dermis. For some organic chemicals, metabolic determinants such as the cellular capacity for metabolism and the affinity of metabolic enzymes for the compound are critical, both in activating the parent compound to the putative carcinogenic electrophilic metabolite and in transforming the parent compound or the electrophilic intermediate into a metabolite that can be readily eliminated. Molecular interactions of organic compounds, or their metabolites, with DNA and other critical macromolecules or receptors are also important, because these interactions can initiate the carcinogenic process or affect its progression. Determinants for metabolism or molecular interactions are dependent on the exposure history (including the intensity, duration, frequency, and route or routes of exposure, and the life stage of the exposed individual) and may differ according to the specific genotype of the individual (i.e. genetic polymorphisms in metabolic enzymes).

Absorption

Organic compounds can be absorbed after inhalation, dermal exposure, or ingestion. The sites and extent of absorption depend on the physicochemical characteristics of the compound. Highly reactive organic chemicals typically interact with tissues at the portal of entry, and toxicity and carcinogenicity are often most evident at these sites. Formaldehyde, for example, is a water-soluble, reactive volatile organic compound. After inhalation, formaldehyde interacts with the highly aqueous nasal mucosa, the first respiratory tract tissue that is encountered upon inspiration. In rats chronically exposed to formaldehyde vapours, the anterior portion of the nasal cavity was the site of tumour induction.

Sulfur mustard (also known as mustard gas) is another example of a reactive volatile organic compound that can be absorbed after inhalation or through dermal exposure. It is a lipophilic substance that easily penetrates into the skin and mucosal surfaces (Drasch et al., 1987; Somani and Babu, 1989), resulting in a high

Table 1.2. Mechanisms for formation of electrophilic species

Agent	Mechanism	Electrophilic species
Bis(chloromethyl)ether (BCME)	Direct-acting	Chloroalkyl ether
Chloromethyl methyl ether (CMME)	Direct-acting	Chloroalkyl ether
Ethylene oxide	Direct-acting	Epoxide
Formaldehyde	Direct-acting	Aldehyde
Sulfur mustard	Direct-acting	Sulfonium ion
Aflatoxins	Metabolic activation	Epoxide
Benzene	Metabolic activation	Quinone, epoxide, aldehyde
1,3-Butadiene	Metabolic activation	Epoxides
Trichloroethylene (TCE)	Metabolic activation	Epoxide, thioketene
Vinyl chloride	Metabolic activation	Epoxide

degree of bioavailability. Sulfur mustard is a bifunctional alkylating agent (IARC, 1987). Like for formaldehyde, there is evidence that DNA alkylation by sulfur mustard leads to formation of cross-links, inhibition of DNA synthesis and repair, and induction of point mutations and chromosome-type and chromatid-type aberrations (ATSDR, 2003). Some of these changes are observed in nasal tissue, consistent with the nose being a target organ for sulfur mustard.

BCME and chloromethyl methyl ether (CMME) are highly reactive organic compounds that belong to the group of chloroalkyl ethers, which are flammable, volatile, colourless liquids with highly irritating odours. In water and aqueous biological fluids, these substances are rapidly hydrolysed to form hydrochloric acid, methanol, and formaldehyde (Nichols and Merritt, 1973; National Toxicology Program, 2004). The carcinogenic effects of BCME are restricted to the epithelial tissue where exposure occurs, namely the lung for humans and respiratory tract tissues for laboratory animals exposed to BCME by inhalation. This portal-of-entry effect is consistent with the short half-life of BCME in aqueous media (ATSDR, 1989). BCME is among the most potent animal and human carcinogens known. The fact that BCME and CMME are powerful alkylating agents provides moderate to strong evidence that they operate by a genotoxic mechanism. This mechanism is likely to be similar to that of other strong alkylating agents, involving modification of DNA and resultant mutations. BCME can also be absorbed through the skin, and studies in which mice were exposed to BCME by dermal application demonstrated that this agent is a potent complete skin carcinogen, producing both papillomas and squamous cell carcinomas (Van Duuren et al., 1969).

Ethylene oxide is a water-soluble volatile organic compound that is relatively stable in aqueous solutions at neutral pH (half-life, approximately 72 hours). Because it is completely miscible with water, inhaled ethylene oxide will dissolve in the aqueous lining of the respiratory tract and diffuse into the blood. Thus, uptake of this organic compound will be substantial throughout the respiratory tract.

Volatile organic compounds that are not reactive or water-soluble are generally absorbed into the blood, primarily in the pulmonary region of the respiratory tract because of the large blood–tissue interface of the alveoli in that region. The blood–air partition coefficient is a key factor in determining the maximum levels of the organic compound that can be attained in the blood at any given concentration of the compound in air. For example, the blood–air partition coefficients for the carcinogens vinyl chloride, 1,3-butadiene, benzene, and TCE are 1.2, 1.5, 7.8, and 10 respectively. At equivalent air concentrations, higher levels of benzene and TCE will be present in the blood at steady state, compared with vinyl chloride and 1,3-butadiene.

The organic compounds discussed here can also be absorbed after oral exposure. Ingestion and subsequent oral absorption represent the greatest potential route of exposure to non-volatile organic compounds. Organic chemicals may enter the body via food or in liquids. Aflatoxin is a compound for which ingestion is considered the most important route of exposure. For example, human uptake of aflatoxins in quantities of nanograms to micrograms per day occurs mainly through consumption of contaminated maize and peanuts, which are dietary staples in some tropical countries. Uptake of aflatoxin M_1 in quantities

of nanograms per day occurs mainly via consumption of aflatoxin-contaminated milk, including breast milk (IARC, 2002). Once absorbed from the gastrointestinal tract, aflatoxins are transported via the hepatic portal blood to the liver, where they are metabolized. As discussed below, metabolism is key to understanding the carcinogenicity of aflatoxins.

Metabolic activation or detoxification and elimination

Metabolism plays a key role in both the activation and the detoxification of many organic compounds. The first step, generally called phase I metabolism, is oxidation to a metabolic intermediate. This intermediate becomes the substrate for the second step, phase II, in which it is enzymatically hydrolysed or conjugated with one of a variety of biological substrates, such as sulfate, glucuronic acid, or glutathione (GSH). Phase II reactions increase the water solubility of the chemical, which facilitates its excretion in urine, or increase the molecular weight, so that the agent is more readily eliminated in bile. Phase I metabolism of organic compounds can also result in formation of reactive intermediates that can spontaneously interact with critical macromolecules. For many organic chemicals, this is the first key step in the carcinogenic process.

Cytochrome P450 (CYP450) is the collective name of the family of enzymes responsible for the initial phase I metabolism of many organic compounds. Metabolic activation by various CYP450 isozymes is a key first step in the carcinogenic process for aflatoxin, benzene, 1,3-butadiene, TCE, and vinyl chloride. For example, in the mechanism of carcinogenicity of aflatoxins, the key steps involve: metabolism to a reactive *exo*-epoxide; binding of the *exo*-epoxide to DNA, resulting in the formation of DNA adducts; and miscoding during DNA replication, which leads to development of mutations, with eventual progression to tumours. Aflatoxin B_1, the most common and potent of the aflatoxins, is metabolized predominantly in the liver. In humans, CYP1A2, CYP2B6, CYP3A4, CYP3A5, and CYP3A7, as well as the phase II enzyme glutathione-*S*-transferase M1 (GSTM1; see below) are hepatic enzymes that mediate aflatoxin metabolism. In humans, the relative contribution of these enzymes in vivo depends not only on their affinity but also on their expression level in the liver, where CYP3A4 is predominant, mediating the formation of the aflatoxin B_1 *exo*-epoxide and aflatoxin Q_1. In humans, as in other species, the DNA binding and carcinogenicity of aflatoxin B_1 result from its conversion to the *exo*-8,9-epoxide by CYP3A4 (Essigmann et al., 1982). This epoxide is highly reactive and is the main mediator of cellular injury (McLean and Dutton, 1995). In contrast, CYP1A2 can generate some *exo*-epoxide but also a high proportion of *endo*-epoxide and aflatoxin M_1. Aflatoxins M_1 and Q_1, produced from aflatoxin B_1 by CYP1A2 and CYP3A4, respectively, are present in the urine of individuals exposed to aflatoxins (Ross et al., 1992; Qian et al., 1994).

Benzene is also a substrate for CYP450 enzymes. In common with other compounds discussed in this section, benzene must be metabolized to become carcinogenic (Ross, 2000; Snyder, 2004). The initial metabolic step involves CYP450-dependent oxidation to benzene oxide, which exists in equilibrium with its tautomer oxepin. It has been reported that benzene is most likely to be metabolized via two CYP450 enzymes, rather than just one, and that the putative, high-affinity enzyme is active primarily at benzene concentrations below 1 ppm (Rappaport et al., 2009). Whereas CYP2E1 is the primary enzyme responsible for mammalian metabolism of benzene at higher levels of exposure (Valentine et al., 1996; Nedelcheva et al., 1999), CYP2F1 and CYP2A13 have been proposed as enzymes that metabolize benzene at environmental levels of exposure, i.e. below 1 ppm (Powley and Carlson, 2000; Sheets et al., 2004; Rappaport et al., 2009).

A large proportion of benzene oxide spontaneously rearranges to phenol, which is either eliminated via phase II conjugation or further metabolized to hydroquinone and 1,4-benzoquinone. The remaining benzene oxide is either hydrolysed to produce benzene-1,2-dihydrodiol (catechol), which is further oxidized to 1,2-benzoquinone, or conjugated with GSH to produce *S*-phenylmercapturic acid. Metabolism of the oxepin tautomer of benzene oxide is thought to open the aromatic ring; this yields the reactive muconaldehydes and muconic acid. Benzene oxide, the benzoquinones, the muconaldehydes, and the benzene dihydrodiol epoxides (formed from CYP450-mediated oxidation of benzene dihydrodiol) are electrophiles that react readily with peptides, proteins, and DNA (Bechtold et al., 1992; McDonald et al., 1993; Bodell et al., 1996; Gaskell et al., 2005; Henderson et al., 2005; Waidyanatha and Rappaport, 2005), thereby interfering with cellular function (Smith, 1996). The role of these different metabolites in the carcinogenicity of benzene remains unclear, but formation of benzoquinone from hydroquinone via myeloperoxidase in the bone marrow has been

suggested to be a key step (Smith, 1996). Indeed, there is considerable evidence for an important role of the metabolic pathway that leads to formation of benzoquinone, because the benzoquinone-detoxifying enzyme NAD(P)H:quinone oxidoreductase 1 (NQO1) protects mice against benzene-induced myelodysplasia (Long et al., 2002; Iskander and Jaiswal, 2005) and protects humans against the haematotoxicity of benzene (Rothman et al., 1997). However, this does not rule out adverse effects from other metabolites.

Similarly to the metabolism of benzene, the first step in 1,3-butadiene metabolism involves CYP450-mediated oxidation to epoxybutene (Himmelstein et al., 1997). At low concentrations of 1,3-butadiene, metabolism via the CYP2E1 isozyme predominates (IARC, 1999, 2008). Epoxybutene may be metabolized by conjugation with GSH mediated by glutathione *S*-transferase (GST) or by hydrolysis catalysed by epoxide hydrolase (EH) (Csanády et al., 1992; Himmelstein et al., 1997). It may also be oxidized to multiple diastereomers of diepoxybutane (Seaton et al., 1995; Krause and Elfarra, 1997), and dihydroxybutene formed by hydrolysis of epoxybutene may be oxidized to epoxybutanediol. Diepoxybutane and epoxybutanediol are also detoxified by GST or EH (Boogaard et al., 1996). Partial hydrolysis of diepoxybutane also produces epoxybutanediol. Bone marrow myeloperoxidase can also epoxidize 1,3-butadiene. Each of the epoxide intermediates may contribute to the mutagenicity and carcinogenicity of 1,3-butadiene. The formation of epoxybutanediol or diepoxybutane requires a second oxidation of butenediol or epoxybutene, respectively. At increasing exposure concentrations of 1,3-butadiene, competition between 1,3-butadiene and butenediol or epoxybutene for CYP450-mediated metabolism may limit the extent to which the second oxidation reaction may occur.

Vinyl chloride is another volatile organic compound that is primarily and rapidly metabolized in the liver (Reynolds et al., 1975; Ivanetich et al., 1977; Barbin and Bartsch, 1989; Lilly et al., 1998; Bolt, 2005) by CYP2E1 (WHO, 1999). The primary metabolites of vinyl chloride are the highly reactive chloroethylene oxide, which is formed in a dose-dependent process and has a half-life of 1.6 minutes in aqueous solution at neutral pH (Barbin et al., 1975; Dogliotti, 2006), and its rearrangement product chloroacetaldehyde (Bonse et al., 1975). Both can bind to proteins, RNA, and DNA and form etheno adducts in RNA and DNA. Chloroethylene oxide is more reactive with nucleotides than is chloroacetaldehyde (Guengerich and Watanabe, 1979).

Conjugation of chloroethylene oxide and chloroacetaldehyde with GSH eventually leads to the major urinary metabolites *N*-acetyl-*S*-(2-hydroxyethyl)cysteine and thiodiglycolic acid (Plugge and Safe, 1977). Chloroethylene oxide can also be detoxified to glycolaldehyde by microsomal EH, and chloroacetaldehyde can be converted to chloroacetic acid by aldehyde dehydrogenase 2 (ALDH2) (Guengerich and Watanabe, 1979; ATSDR, 2006; IARC, 2008).

Two metabolic pathways of TCE have been characterized in both humans and laboratory animals: oxidation and GSH conjugation (IARC, 2014). The major pathway is CYP450-mediated oxidation, resulting in the formation of a variety of short- and long-lived metabolites. Subsequent processing of oxidative metabolites involves reduction by alcohol dehydrogenase (ADH) and ALDH, and glucuronidation. The initial step in TCE oxidation in both humans and laboratory animals is catalysed by one of several CYP450 enzymes and results in the formation of an enzyme-bound intermediate (an oxygenated TCE-CYP450 transition state, TCE-O-CYP), which is chemically unstable. This intermediate can be converted to (i) the electrophile TCE epoxide, (ii) *N*-hydroxy-acetyl-aminoethanol, or (iii) chloral, which is in equilibrium with chloral hydrate. The majority of the conversion is directed towards chloral/chloral hydrate. TCE epoxide spontaneously generates dichloroacetyl chloride, another chemically unstable and reactive species, or oxalic acid, which is readily excreted in urine. Dichloroacetyl chloride undergoes spontaneous dechlorination to produce dichloroacetate. Chloral/chloral hydrate undergoes either reduction by ALDH or CYP450 to generate trichloroethanol, or oxidation by ALDH to form trichloroacetate. Trichloroacetate is typically the major urinary metabolite of TCE that is recovered, although trichloroethanol is also an important metabolite in urine. Trichloroethanol can be oxidized by CYP450 enzymes to yield trichloroacetate, or can undergo glucuronidation by uridine 5′-diphosphate (UDP)-glucuronosyltransferases to produce trichloroethanol glucuronide. In all species investigated, trichloroacetate and trichloroethanol/trichloroethanol glucuronide are formed in much larger quantities than other oxidative metabolites. There are quantitative differences in the extent of oxidative TCE metabolism among

species at higher exposures, but at lower exposures oxidation is limited by the hepatic blood flow.

Conjugation with GSH is another important metabolic pathway for TCE, resulting in the formation of short-lived and reactive metabolites. The initial conjugation reaction occurs primarily in the liver to form (1,2-dichlorovinyl)glutathione (DCVG). Subsequent processing of DCVG occurs primarily in the kidney, which is a tumour target site in rats and humans. In the kidney, DCVG can be hydrolysed by γ-glutamyltransferase and cysteinylglycine dipeptidase to 1,2-dichlorovinyl-cysteine (DCVC), which may be *N*-acetylated to form a mercapturate or converted by β-lyase to generate a reactive thiolate that rearranges to form either chlorothioketene or chlorothioacetyl chloride (Dekant et al., 1988; Völkel and Dekant, 1998). Chlorothioketene and chlorothioacetyl chloride are highly reactive and chemically unstable, and are thought to be the molecular forms responsible for adduct formation with nucleic acids in the kidney (Müller et al., 1998a, b). For both humans and rodents, the information on urinary excretion of stable end-products is much more extensive for the oxidative pathway than for the GSH conjugation pathway. However, this is not an accurate indication of the overall flux through each pathway, because it does not account for the formation of reactive and chemically unstable metabolites via the GSH conjugation pathway.

As noted above, CYP450 enzymes are not the only enzymes involved in the metabolism of organic compounds. Formaldehyde, an important intermediate in one-carbon metabolism, is a substrate for several enzymes, including cytosolic ADH, mitochondrial ALDH, cytosolic GSH-dependent formaldehyde dehydrogenase, and catalase, as well as CYP2E1. One-carbon metabolism not mediated by CYP450 is central to many biological processes. In aqueous solution, formaldehyde is rapidly converted to its dihydroxy form, methanediol ($CH_2(OH)_2$, also known as formaldehyde hydrate or methylene glycol), which is in dynamic equilibrium with formaldehyde.

Direct-acting compounds

Some organic compounds discussed here are sufficiently reactive that they do not require metabolic activation as the first step in the carcinogenic process. Formaldehyde reacts readily and reversibly with amino groups to form Schiff bases, and with sulfhydryl groups resulting in the formation of *S*-hydroxymethylglutathione, which is oxidized by ADH3 to *S*-formylglutathione. *S*-formylglutathione is further metabolized by *S*-formylglutathione hydrolase to generate formate and GSH. Formate can also be formed non-enzymatically (Hedberg et al., 2002). Because formaldehyde reacts non-enzymatically with critical macromolecules (DNA and others), many of these enzymatic processes can be viewed as detoxification steps, especially those that lead to incorporation of the compound into the one-carbon pool.

Ethylene oxide is another direct-acting alkylating agent that reacts with nucleophiles without the need for metabolic transformation. The direct reaction of ethylene oxide with DNA is thought to initiate the cascade of genetic and related events that lead to cancer (Swenberg et al., 1990). The pathways of ethylene oxide metabolism can thus be considered detoxification pathways that increase the elimination of the parent chemical. Ethylene oxide is converted (i) by enzymatic and non-enzymatic hydrolysis to ethylene glycol, which is partly excreted as such and partly metabolized further via glycolaldehyde, glycolic acid, and glyoxylic acid to oxalic acid, formic acid, and carbon dioxide; and (ii) by conjugation with GSH.

Sulfur mustard, BCME, and CMME can also react spontaneously with biological molecules without the need for metabolic activation. For example, the reactivity of sulfur mustard with a wide variety of cellular macromolecules is well documented (IARC, 1975, 1987; ATSDR, 2003). The presence of two chlorine atoms makes it a strong bifunctional alkylating agent with a high chemical reactivity (Dacre and Goldman, 1996). The chlorine atom is typically released under formation of a carbonium ion, which then undergoes intramolecular cyclization to create a highly reactive compound. Formation of the carbonium ion is facilitated in aqueous solutions (Somani and Babu, 1989); this explains the sensitivity of mucosal tissues, such as the eye, to its effect (Solberg et al., 1997). Elevated concentrations of thiodiglycol, the major hydrolysis product of mustard gas, were detected in human urine after exposure to mustard gas vapour and aerosol (Jakubowski et al., 2000). BCME and CMME are rapidly hydrolysed in water and in aqueous biological fluids to form hydrochloric acid, methanol, and formaldehyde (Nichols and Merritt, 1973; National Toxicology Program, 2004).

Molecular interactions (DNA adducts, genetic alterations, etc.)

A common feature of the above-mentioned agents is that they either are direct-acting electrophiles or are

metabolized to electrophiles. The carcinogenicity of these chemicals is considered to be initiated by reaction of the electrophile with nucleophilic sites in DNA, leading to the induction of mutations, DNA strand breaks, and/or CA. However, additional processes may also be involved, for example free-radical-mediated oxidative stress, inhibition of DNA repair, inhibition of topoisomerase II, and immunosuppression. In addition to time-dependent variation in tissue concentrations of DNA-reactive metabolites of the chemicals described above, the likelihood that these compounds or their metabolites will bind to DNA and induce site-specific genetic alterations that lead to tumour development is a function of the physicochemical properties of the reactive agent (e.g. binding affinity for DNA or protein), several cellular features (including tissue concentrations of alternative binding biomolecules such as GSH, rates of cell division and cell death, and the activities and effectiveness of repair enzymes for DNA adducts), other physiological characteristics (e.g. age, sex, health status, and immunocompetence), and lifestyle factors (e.g. other exposures). Thus, multiple factors and mechanistic processes affect the tissue and species specificity for tumour development associated with exposures to each of the carcinogenic chemicals discussed in this chapter.

Table 1.3 presents 10 key characteristics of carcinogens (see Chapter 10, by Smith) that have been identified in in vivo and/or in vitro studies on the electrophilic agents reviewed in this chapter. What is most evident from Table 1.3 is that all these compounds produce DNA adducts in humans and animals, and cause mutations and cytogenetic alterations. Entries with weak evidence may reflect the availability of few or no published studies for certain characteristics of particular agents in animal or human tissues, rather than negative responses (Table 1.3).

BCME and CMME

The chloroalkyl ethers BCME and CMME are often referred to as powerful alkylating agents. However, because these compounds are short-lived in aqueous solution and undergo rapid hydrolysis, genotoxicity studies of BCME and CMME are sparse and have produced mixed results (IARC, 1987). Both compounds were mutagenic in bacteria (Mukai and Hawryluk, 1973; Anderson and Styles, 1978) and caused an increase in the frequency of CA in peripheral lymphocytes of exposed workers (Srám et al., 1983). BCME binds to guanine and adenine residues of calf thymus DNA in vitro (Goldschmidt et al., 1975). Both compounds induced unscheduled DNA synthesis in cultured human cells (Agrelo and Severn, 1981; Perocco et al., 1983) and cell transformation in Syrian hamster embryo cells (Casto, 1983) and cultured human fibroblasts (Kurian et al., 1990). The carcinogenicity of BCME is widely thought to involve mutagenesis resulting from alkylation of DNA bases (Bernucci et al., 1997). BCME and CMME may act synergistically with formaldehyde, one of their hydrolysis products. The likelihood of BCME–DNA adducts leading to mutations depends on the cellular content and activity of DNA repair enzymes such as methylguanine methyltransferase, and enzymes involved in mismatch repair and excision repair (Bernucci et al., 1997).

Sulfur mustard

The elimination of a chloride ion from sulfur mustard creates a highly reactive cyclic sulfonium ion that can alkylate cellular macromolecules including DNA, RNA, and proteins. Because of the presence of two chlorine atoms, sulfur mustard can act as a bifunctional alkylating agent, producing DNA interstrand or intrastrand cross-links, for example by binding to guanines on opposite strands or to neighbouring guanines on the same strand (Roberts et al., 1971; Walker, 1971; Shahin et al., 2001; Saladi et al., 2006). Such cross-links can inhibit DNA synthesis and cell division. Sulfur mustard-specific 2-hydroxyethylthioethyl–DNA adducts have been detected in in vitro systems and in multiple tissues of exposed animals (Somani and Babu, 1989; Fidder et al., 1994; van der Schans et al., 1994; Niu et al., 1996). Similar to the binding pattern for other alkylating agents, sulfur mustard-derived DNA adducts have been identified at N7 of guanine, N3 of adenine, and O6 of guanine (Fidder et al., 1994). O^6-alkylguanine DNA alkyltransferase is ineffective in repairing O^6-ethylthioethylguanine adducts (Ludlum et al., 1986). Thus, sulfur mustard can inhibit cell division by cross-linking of DNA strands and can produce mutations by inducing errors in DNA replication or repair.

Sulfur mustard induced mutations and CA in exposed animals and in a variety of in vitro systems (IARC, 1987). Further, *TP53* mutations (predominantly G → A transitions) were detected in DNA extracted from lung tumours of individuals exposed to sulfur mustard (Hosseini-khalili

Table 1.3. Levels of evidence[a] in humans and animals of key characteristics of carcinogens

Characteristic	Aflatoxins		Benzene		1,3-Butadiene		Ethylene oxide	
	Humans	Animals	Humans	Animals	Humans	Animals	Humans	Animals
Is electrophilic or can be metabolically activated to electrophiles	2	2	2	2	2	2	2	2
Is genotoxic	2	2	2	2	2	2	2	2
Alters DNA repair or causes genomic instability	1	1	1	1				
Induces epigenetic alterations	0	0	1	0	0	0	1	0
Induces oxidative stress	0	0	1	1	0	0	0	0
Induces chronic inflammation			0	0				
Is immunosuppressive	0	0	2	2	0	0	0	0
Modulates receptor-mediated effects			1	1				
Causes immortalization	0	0	0	0	0	0	0	0
Alters cell proliferation, cell death, or nutrient supply	0	1	1	1	1	0	0	0

Characteristic	Formaldehyde		Sulfur mustard		Trichloroethylene		Vinyl chloride	
	Humans	Animals	Humans	Animals	Humans	Animals	Humans	Animals
Is electrophilic or can be metabolically activated to electrophiles	2	2	2	2	2	2	2	2
Is genotoxic	2	2	2	2	2	2	2	2
Alters DNA repair or causes genomic instability	2	1	1	1	0	0		
Induces epigenetic alterations	1	1	0	0	0	1	0	0
Induces oxidative stress	0	0	1	1	0	0	0	0
Induces chronic inflammation					0	0		
Is immunosuppressive	0	0	0	0	1	1	0	0
Modulates receptor-mediated effects					0	1		
Causes immortalization	0	0	0	0	0	0	0	0
Alters cell proliferation, cell death, or nutrient supply	2	2	1	1	0	1	1	1

[a] 2 = strong evidence, 1 = moderate evidence, 0 = weak evidence.

et al., 2009). The base excision repair and nucleotide excision repair pathways were activated in human lymphoblastoid cell lines exposed to the sulfur mustard analogue 2-chloroethyl-ethylsulphide (Jowsey et al., 2009).

Ethylene oxide

Ethylene oxide is a direct-acting alkylating agent that reacts with nucleophiles, resulting in the formation of a variety of adducts in DNA, RNA, and protein. Numerous studies have demonstrated that ethylene oxide induces gene mutations and chromosomal changes in in vitro systems and in prokaryotic and eukaryotic organisms. 2-Hydroxyethyl–DNA adducts formed upon exposure to ethylene oxide have been observed in vivo at N7 of guanine, N3 of adenine, and O6 of guanine (Walker et al., 1992) and in vitro at N1 and N6 of adenine and at the N3 position of cytosine, uracil, and thymine (Tates et al., 1999). Genotoxicity resulting from ethylene oxide-induced DNA adducts may involve mispairing of altered bases, formation of abasic sites upon depurination of the adducts at N7 of guanine followed by insertion of a different base during DNA synthesis, or DNA strand breaks and subsequent chromosome breakage (Tates et al., 1999; Houle et al., 2006). In mice, ethylene oxide induced large deletion mutations, base-pair substitutions, and frameshift mutations (Walker and Skopek, 1993; Walker et al., 1997a, b). In tumours obtained from mice exposed to ethylene oxide, increases in K-*Ras* mutations with frequent G → T transversions at codon 12 and G → C transversions at codon 13 were reported (Houle et al., 2006; Hong et al., 2007). Evidence was also provided for the involvement of mutations in *p53*.

The carcinogenicity of ethylene oxide is thought to be due to the induction of gene mutations and/or chromosomal changes resulting from the formation of ethylene oxide-derived DNA adducts. Although evidence for the carcinogenicity of ethylene oxide was *sufficient* in experimental animals and *limited* in humans, the observed increases in the frequencies of CA, SCE, and MN in lymphocytes of exposed workers served as the basis for raising the classification of this alkylating agent to *carcinogenic to humans* (Group 1) (IARC, 1994, 2008).

Formaldehyde

Formaldehyde can react directly with cellular macromolecules including proteins and nucleic acids. The formaldehyde-specific DNA adduct N^6-hydroxymethyl-deoxyadenosine has been identified in lymphocytes of smokers (Wang et al., 2009). The genotoxicity of formaldehyde is well established: it induces mutations (point mutations, deletions, and insertions), CA, SCE, MN, DNA strand breaks, and DNA–protein cross-links in several in vitro and in vivo systems, including CA, SCE, and MN in nasal mucosal cells and/or lymphocytes of exposed humans (IARC, 2006). In animals exposed to formaldehyde, genotoxic effects were more consistently found in nasal tissues than in blood lymphocytes. In addition, formaldehyde produces irritation of the nose and pharynx in humans and laboratory animals. Genotoxicity and increased cell proliferation appear to be the major determinants of the nasal carcinogenicity of formaldehyde in humans and laboratory animals.

A mechanism for formaldehyde-induced myeloid leukaemogenesis might involve pancytopenia caused by genotoxicity leading to damage of primitive progenitor cells in the bone marrow; mutation of myeloid progenitor cells by formaldehyde and subsequent growth of a mutant phenotype may then lead to myeloid leukaemia. Evidence of a mild pancytopenic effect of formaldehyde or changes in ratios of lymphocyte subsets has been reported in exposed workers (Kuo et al., 1997; Ye et al., 2005; Tang et al., 2009; Zhang et al., 2010). In addition, colony formation by cultured progenitor cells that give rise to myeloid cells is inhibited by low concentrations of formaldehyde (Zhang et al., 2010). The observation of increased monosomy (loss) of chromosome 7 and trisomy (gain) of chromosome 8 in cultured myeloid progenitor cells obtained from the blood of workers exposed to formaldehyde may be relevant to the potential involvement of formaldehyde in leukaemogenesis, because these types of cytogenetic changes are frequently seen in myeloid leukaemia and myelodysplastic syndromes (Zhang et al., 2010).

1,3-Butadiene

1,3-Butadiene can be metabolized to three different DNA-reactive epoxide intermediates, which are direct-acting mutagens (IARC, 2008). The major DNA adducts formed from these epoxide intermediates in rats and mice exposed to 1,3-butadiene are at the N7 position of guanine. These N7-guanine adducts can undergo spontaneous or glycosylase-mediated depurination, which leaves an apurinic site in the DNA. Epoxide

metabolites of 1,3-butadiene can also react at sites involved in base pairing and form adducts at the N3 position of cytosine, at N1 and N6 of adenine, and at N1 and N2 of guanine (Selzer and Elfarra, 1996a, b, 1997; Zhao et al., 1998; Zhang and Elfarra, 2004). An increase in the number of N1-trihydroxybutyladenine adducts was detected in lymphocytes of workers exposed to 1,3-butadiene (Zhao et al., 2000). Alkylation of N1-adenine by epoxybutene followed by hydrolytic deamination forms the highly mutagenic deoxyinosine (Rodriguez et al., 2001), which codes for incorporation of cytosine during DNA replication, leading to the generation of A → G mutations. Diepoxybutane is a bifunctional alkylating agent that can form monoadducts in DNA similar to those formed by epoxybutanediol, or DNA–DNA cross-links by binding at the N7 position of guanine of one DNA strand and at another site elsewhere in the DNA, such as the N7 of another guanine or the N1 of an adenine (Goggin et al., 2009). Depurination of these interstrand or intrastrand lesions can induce point mutations and large deletions. However, if diepoxybutane alkylates an adenine at N6 in DNA, an exocyclic adenine adduct is formed preferentially to DNA–DNA cross-linked products (Antsypovich et al., 2007).

1,3-Butadiene is genotoxic at multiple tissue sites in mice and rats, and its epoxide metabolites are mutagenic in a variety of in vitro systems. Deletion mutations and base substitution mutations induced by these alkylating agents are consistent with their DNA adduct profiles and include G → A transition mutations, G → C transversions, A → T transversions, and A → G transitions (Lee et al., 2002). Other genotoxic effects of 1,3-butadiene and its metabolites are induction of CA, SCE, and MN.

Genetic alterations in 1,3-butadiene-induced tumours in mice are of the same type as those frequently involved in the development of a variety of human cancers. The K-*Ras*, H-*Ras*, *p53*, *p16*/*p15*, and *β-catenin* mutations detected in tumours from exposed mice are probably the result of the DNA reactivity and the genotoxic effects of 1,3-butadiene-derived epoxides. Other DNA-alkylating metabolites of 1,3-butadiene (hydroxymethylvinylketone and crotonaldehyde) may also contribute to the mutagenicity and carcinogenicity of this compound. A consistent pattern of K-*Ras* mutations (G → C transversions at codon 13) was observed at multiple organ sites of 1,3-butadiene-induced cancers (Hong et al., 2000; Sills et al., 2001; Ton et al., 2007). Alterations in the *p53* gene in brain tumours in mice were mostly G → A transition mutations (Kim et al., 2005) that probably arose from miscoding at apurinic sites resulting from depurination of N7-guanine adducts. Inactivation of the tumour suppressor genes *p16* and *p15* may also be important in the development of 1,3-butadiene-induced lymphomas (Zhuang et al., 2000). Mammary gland adenocarcinomas induced by 1,3-butadiene in mice frequently had mutations in the *p53*, H-*Ras*, and *β-catenin* genes (Zhuang et al., 2002). Overall, these observations point to a genotoxic mechanism underlying the development of 1,3-butadiene-induced cancers.

Vinyl chloride

The carcinogenicity of vinyl chloride is probably caused by its highly reactive metabolite chloroethylene oxide and/or by the rearrangement product chloroacetaldehyde (Bonse et al., 1975). Both intermediates can bind to proteins, RNA, and DNA (Guengerich and Watanabe, 1979). Vinyl chloride is mutagenic in bacteria and mammalian cells. It is also clastogenic in vivo and in vitro, causing increases in the frequencies of CA, SCE, and MN (IARC, 2008). The major DNA adduct formed from chloroethylene oxide is at the N7 position of guanine. In addition, etheno DNA adducts (1,N^6-ethenoadenine, 3,N^4-ethenocytosine, N^2,3-ethenoguanine, and 1,N^2-ethenoguanine) have been identified after in vitro incubations with chloroethylene oxide, and levels of these adducts are increased in multiple organs of rats exposed to vinyl chloride by inhalation (Ciroussel et al., 1990; Guengerich, 1992; Swenberg et al., 2000). The etheno adducts, which may be involved in base-pair substitutions, are much more persistent than the N7-guanine adduct (Fedtke et al., 1990) and have demonstrated miscoding potential in vitro and in vivo, causing A → G transitions, A → T transversions, C → A transversions, C → T transitions, and G → A transitions (Singer et al., 1987; Cheng et al., 1991; Mroczkowska and Kuśmierek, 1991; Singer et al., 1991; Basu et al., 1993). The same types of mutation have been observed in the *TP53* and *RAS* genes in vinyl chloride-induced tumours. *TP53* mutations associated with exposure to vinyl chloride (frequently A → T transversions) were found in angiosarcomas in both humans and rats, and mutations in K-*RAS* were also associated with vinyl chloride-induced angiosarcomas in humans (IARC, 2008). Polymorphisms in *XRCC1*, a gene that encodes an enzyme that repairs etheno DNA adducts, may account

for inter-individual differences among exposed workers in susceptibility to genetic damage induced by vinyl chloride (Li et al., 2003).

Aflatoxins

Aflatoxin B_1 is genotoxic in prokaryotic and eukaryotic systems in vitro, including cultured human cells, and in vivo in humans and in a variety of animal species. Its metabolism to a reactive *exo*-8,9-epoxide results in DNA binding and formation of DNA adducts that lead to gene mutations, CA, SCE, MN, and mitotic recombination in a variety of in vivo and in vitro systems (IARC, 2002). Adduct formation in DNA at the N7 position of guanine represents more than 98% of the total adducts formed by the *exo*-8,9-epoxide (Guengerich et al., 1998). Depurination of this guanine adduct creates an apurinic site. Alternatively, the N7-guanine adduct may convert to the more stable ring-opened aflatoxin B_1–formamidopyrimidine adduct (Groopman et al., 1981). Differences in the mutational specificity of an apurinic site-containing genome (derived from depurination of aflatoxin B_1–N7-guanine) compared with that of a genome with the aflatoxin B_1–N7-guanine adduct itself, where mutations also occurred at the base 5′-adjacent to the site of the adducted guanine, suggest that intercalation of the aflatoxin moiety on the 5′ side of the modified guanine perturbs both the modified and the complementary DNA strands, causing interference with 5′ base pairing (Gopalakrishnan et al., 1990; Bailey et al., 1996). Thus, mutations resulting from aflatoxin B_1–N7-guanine adducts may not be due only to depurination.

A specific AGG → AGT transversion mutation at codon 249 of the *TP53* tumour suppressor gene in human hepatocellular carcinomas is associated with exposure to aflatoxin B_1 (Gomaa et al., 2008). G → T transversion mutations are predominant in cell culture systems and animal models and are consistent with the formation of the major aflatoxin B_1-derived N7-guanine adduct. This is because adenine is most commonly inserted opposite the apurinic site. However, other types of mutation have also been observed with aflatoxin B_1, including G → C transversions and G → A transitions in DNA repair-deficient xeroderma pigmentosum cells (Levy et al., 1992); this suggests that DNA repair deficiency may influence the frequency and distribution of mutations within a gene. Aflatoxin B_1 may contribute to genomic instability in hepatocellular carcinomas (Kaplanski et al., 1997) by inducing mitotic recombination and loss of heterozygosity. The concurrent presence of hepatitis B virus, which causes chronic active hepatitis and cirrhosis, increases the incidence of hepatocellular carcinomas caused by aflatoxins in humans (IARC, 2002).

Trichloroethylene

Data from human studies suggest that exposure to TCE increases the frequency of CA in peripheral lymphocytes (Tabrez and Ahmad, 2009) and leads to mutations in the von Hippel–Lindau tumour suppressor gene *VHL* in renal cell carcinoma (Brüning et al., 1997; Brauch et al., 1999), but these findings have been reported in only a limited number of studies. TCE exposure induced MN both in vitro (Wang et al., 2001; Robbiano et al., 2004; Hu et al., 2008) and in vivo (Hrelia et al., 1994; Kligerman et al., 1994; Robbiano et al., 2004). Although TCE itself appears to be incapable of inducing gene mutations, it has shown potential to affect DNA and chromosomal structure. The formation of DNA adducts (Mazzullo et al., 1992; Cai and Guengerich, 2001) and the mutagenicity of TCE in vitro are dependent on the presence of metabolic activation systems (IARC, 2014). There is strong evidence that the GSH-conjugated metabolites of TCE, particularly DCVC, are genotoxic, and some of the oxidative metabolites (TCE epoxide, dichloroacetate, and chloral/chloral hydrate) may also be genotoxic. Thus, biotransformation of TCE can produce genotoxic metabolites, particularly in the kidney, where in situ metabolism occurs (IARC, 2014).

Both genotoxic and non-genotoxic mechanisms may contribute to the carcinogenicity and toxicity of TCE at other sites, including the liver, the lung, and the haematopoietic system. In addition to genotoxicity, epigenetic alterations, oxidative stress, cytotoxicity, and altered rates of cell division or apoptosis may be involved in tumour induction in the liver or lung. The immunotoxicity of TCE may be involved in the development of haematopoietic cancers. However, the data are inadequate for reliable conclusions to be drawn about causal relationships between non-genotoxic mechanisms and TCE-induced tumours in humans or laboratory animals (IARC, 2014). From toxicity and carcinogenicity studies in humans and laboratory animals, there is strong evidence for the kidney as a target tissue for TCE-induced tumour formation. The database supporting the non-genotoxic mechanism of kidney carcinogenesis is *moderate*. However, the *strong evidence* of genotoxicity of DCVC, the kidney metabolite of TCE, supports the overall conclusion that the evidence for

a genotoxic mechanism of kidney carcinogenesis is *strong*. The evidence for the liver as a target tissue for TCE, from cancer assays and toxicity findings in laboratory animals, is *strong*. The evidence for non-genotoxic and/or genotoxic mechanisms of liver carcinogenesis is *moderate*. The available data suggest multiple non-genotoxic mechanisms and the potential for genotoxic mechanisms from the TCE metabolites dichloroacetate and chloral hydrate.

Benzene

Benzene induced CA, SCE, and MN in bone marrow cells of exposed mice, CA in bone marrow cells of exposed rats, and CA and mutations in human cells in vitro. CA in human peripheral lymphocytes have long been associated with occupational exposure to benzene (Forni, 1979; IARC, 1982; Eastmond, 1993; Zhang et al., 2002; Holecková et al., 2004). As noted above, metabolism of benzene produces several electrophilic agents (benzene oxide, in equilibrium with its tautomer oxepin, muconaldehyde, benzoquinone, and benzene dihydrodiol epoxide) that can react with DNA or proteins. DNA binding and adduct formation may not be the major steps in the development of benzene-induced leukaemias (Whysner et al., 2004). Although the mechanisms of benzene-induced carcinogenesis and the potential relative roles of each of these metabolites are not fully known, there is strong support for the involvement of clastogenic and aneugenic effects, such as formation of CA, MN, and DNA strand breaks.

Exposure to benzene has been associated with chromosomal changes that are commonly observed in acute myeloid leukaemia, including those comprising loss of various parts of the long arm of chromosome 5 or 7, or complete loss of these chromosomes, gain of the entire chromosome 8, and an increased frequency of translocations between chromosomes 8 and 21 in peripheral lymphocytes of exposed workers (Smith et al., 1998; Zhang et al., 1999, 2002). Benzene and its quinone metabolites are inhibitors of topoisomerase II, leading to increased frequencies of DNA cleavage complexes and DNA double-strand breaks; this effect can result in the formation of chromosome translocations and inversions (Hutt and Kalf, 1996; Lindsey et al., 2004, 2005; Deweese and Osheroff, 2009). Other potential pathways involved in benzene-induced acute myeloid leukaemia include mutagenesis (possibly through generation of reactive oxygen species), epigenetic changes due to altered methylation status, decreased immunosurveillance (Cho, 2008; Li et al., 2009), haematotoxicity and alterations in stem cell pool size (Rothman et al., 1997), and inhibition of gap-junction intercellular communication (Rivedal and Witz, 2005). Thus, multiple mechanisms are likely to be involved in benzene-induced leukaemogenesis. Benzene produces multiple cytogenetic abnormalities in human lymphocytes (Tough and Brown, 1965; Picciano, 1979; Smith and Zhang, 1998; Zhang et al., 2002) and induces specific chromosomal changes associated with non-Hodgkin lymphoma in human lymphocytes (Zhang et al., 2007). Induction of DNA double-strand breaks and chromosomal rearrangements in lymphoid cells in combination with immunosuppression by benzene might be the cause of lymphoma.

The carcinogenicity of the group of electrophilic chemicals discussed above is likely to be due to interaction between the parent electrophile or one or more electrophilic metabolites and nucleophilic DNA, leading to point mutations and induction of CA. These effects have been observed in humans, in animals, and in in vitro systems. In addition, production of reactive oxygen species, inhibition of DNA synthesis or repair, and cytotoxicity/cell proliferation could complement DNA modification to enhance DNA damage. Tumour outcome can result from certain DNA adducts leading to mutations and dysregulation initially described with reference to proto-oncogenes and tumour suppressor genes. For benzene, chromosomal translocations, in combination with haematotoxicity or immunosuppression, are associated with increased risk of haematopoietic cancer in humans. The extent to which other processes (inflammation, oxidative stress, immunosuppression, epigenetic alterations, and immortalization) might contribute to the carcinogenicity of this class of chemicals in general is limited by the availability of few or no published studies that address these effects.

Polymorphisms and susceptibility

Susceptibility to the carcinogenic effects of organic compounds may derive from acquired characteristics, such as altered expression of certain enzymes, or from genetic factors, such as enzyme polymorphisms. Polymorphisms of enzymes involved in the metabolism of organic compounds are likely to be responsible for individual differences in activation and detoxification reactions that control tissue levels of electrophilic intermediates. The enzymes that catalyse epoxide formation and elimination are polymorphic in human

populations, and some isozymes may be induced by a variety of environmental and pharmaceutical agents. For example, factors that explain differences in the response to aflatoxin between human individuals and between animal species and strains include the proportion of aflatoxin metabolized to the *exo*-8,9-epoxide (mainly by CYP450 enzymes) relative to other, much less toxic metabolites, and the prevalence of pathways that lead to the formation of non-toxic conjugates with reduced mutagenicity and cytotoxicity (Guengerich et al., 1998).

Similarly, the expression of enzymes involved in aflatoxin metabolism can be modulated with chemopreventive agents, resulting in inhibition of DNA adduct formation and hepatocarcinogenesis, as has been demonstrated in rats. Oltipraz is a chemopreventive agent that increases GSH conjugation and inhibits the activity of some CYP450 enzymes (e.g. CYP1A2). Results from clinical trials with oltipraz in China are consistent with experimental data in showing that after dietary exposure to aflatoxins, modulation of the metabolism of aflatoxins with oltipraz can lead to reduced levels of DNA adducts (IARC, 2002; Kensler et al., 2005).

Increased susceptibility to the toxic effects of benzene has been linked to genetic polymorphisms that increase the rate of metabolism of benzene to active intermediates or decrease the rate of detoxification of these active intermediates (Rothman et al., 1997; Xu et al., 1998; Kim et al., 2004).

Enzyme polymorphisms also affect the metabolism of 1,3-butadiene. Genetic polymorphisms in GST and microsomal EH affect the in vitro mutagenicity of 1,3-butadiene-derived epoxides or the in vivo mutagenicity of 1,3-butadiene in occupationally exposed workers (Wiencke et al., 1995; Abdel-Rahman et al., 2003). The extent to which these enzyme polymorphisms influence the carcinogenicity of 1,3-butadiene is not known. The genotoxic effects of 1,3-butadiene can be modulated by alterations in key determinants of its metabolism; this suggests that markers of individual susceptibility can be identified. For example, mice that lack a functional microsomal EH (*mEH*) gene were more susceptible than wild-type mice to the mutagenic effects of 1,3-butadiene or diepoxybutane (Wickliffe et al., 2003). EH activity varies considerably among humans. 1,3-Butadiene-exposed workers with the genotype for low-activity EH were reported to be more susceptible to 1,3-butadiene-induced genotoxicity (assessed by *HPRT* mutant frequency in lymphocytes) than individuals with the more common *EH* genotype (Abdel-Rahman et al., 2001, 2003). No significant effects were observed for induction of *HPRT* mutations or SCE in individuals with *GSTM1* or *GSTT1* polymorphisms (Abdel-Rahman et al., 2001). MN frequencies were higher among 1,3-butadiene-exposed workers in China with polymorphisms in *GSTM1* and/or *GSTT1* compared with workers with the wild-type genes (Cheng et al., 2013). These differences in response are consistent with the known important roles of EH and GST in the detoxification of 1,3-butadiene epoxides in tissues in which these intermediates are produced.

Ethylene oxide is a substrate of the GST isozyme T1 (Hayes et al., 2005). This detoxifying enzyme is polymorphic, and a relatively large proportion of the population (about 20% of Caucasians and almost 50% of Asians) has a homozygous deletion (*GSTT1*-null genotype) (Bolt and Thier, 2006). As expected, these individuals show a significantly elevated level of hydroxyethyl valine in their haemoglobin, due to the presence of endogenous ethylene oxide (Thier et al., 2001). Nevertheless, the influence of this genetic trait on the formation of this type of adduct as a result of exposure to exogenous ethylene oxide in the workplace is less clear.

In the cytoplasm of erythrocytes obtained from 36 individuals, ethylene oxide was eliminated 3–6 times as fast in samples from so-called conjugators (defined by a standardized conjugation reaction of methyl bromide and GSH; 75% of the population) as in samples from individuals who lack this GST-specific activity (the remaining 25%). In whole-blood samples incubated with ethylene oxide, an increase in the frequency of SCE was observed in lymphocytes from the non-conjugators but not in lymphocytes from the conjugators (Hallier et al., 1993).

The carcinogenicity and toxicity of TCE, particularly in the liver and kidney, are associated with its metabolism. There are inter-individual differences, both in humans and in rodents, in the formation of TCE metabolites that are thought to be responsible for the toxic and carcinogenic effects of TCE in the kidney and liver. The susceptibility to adverse health effects of TCE may be influenced by genetics, sex, life stage, and other conditions that influence the extent and nature of the metabolism of this chemical. Polymorphisms in metabolism genes in both oxidative (e.g. *CYP2E1*, *ADH*, and *ALDH*) and GSH conjugation (e.g. GSTs) pathways have been studied

in connection with susceptibility to TCE toxicity and carcinogenicity. Polymorphisms in genes for organic anion transporters (OAT1 and OAT3) in the kidney may also influence the rates of uptake and the extent of cellular accumulation of DCVG or DCVC. With respect to life-stage susceptibility, data are available to support the influence of differences in exposure (e.g. transplacental transfer or exposure through breast milk in early life stages) or life stage-specific differences in toxicokinetics. Lifestyle factors (e.g. consumption of alcoholic beverages) may also affect TCE metabolism, and nutrition or obesity may affect internal concentrations of TCE and its metabolites.

Genes involved in DNA repair act to maintain the integrity of the genome by removing lesions (i.e. adducts) that – if left unrepaired – could result in mutations or chromosomal damage. Individuals with defects in genes that encode DNA repair enzymes are at elevated risk for certain cancers (Poulsen et al., 1993). Heterozygous carriers may also have increased susceptibility, because of suboptimal levels of repair.

The reactive aflatoxin B_1 metabolite *exo*-8,9-epoxide induced a higher mutation frequency in a shuttle vector plasmid transfected into DNA repair-deficient xeroderma pigmentosum cells (human fibroblasts) compared with repair-proficient cells; the location of mutations was affected by repair proficiency (Levy et al., 1992).

Polymorphisms in genes involved in repair of DNA double-strand breaks (*WRN* [Werner syndrome], *TP53*, *BLM* [Bloom syndrome], *RAD51*, and *BRCA1*) can modify susceptibility to benzene-induced haematotoxicity in exposed workers (Shen et al., 2006; Lan et al., 2009; Ren et al., 2009).

Mice deficient in nucleotide excision repair were more susceptible than wild-type mice to the mutagenic effects of 1,3-butadiene or its reactive metabolites epoxybutene and diepoxybutane (Wickliffe et al., 2007).

Chicken cells deficient in the Fanconi anaemia complementation groups/breast cancer A (FANC/BRCA) pathway are hypersensitive (with reduced survival) to formaldehyde at levels measured in human plasma (Ridpath et al., 2007). This observation is consistent with an essential role for this pathway in the repair of DNA–protein cross-links caused by formaldehyde, and suggests that patients with Fanconi anaemia (a genetic disorder that is characterized by progressive pancytopenia) may have increased susceptibility to leukaemia from formaldehyde.

A common polymorphism in the DNA repair gene *XRCC1* is a biomarker of susceptibility to *TP53*-induced mutations in workers exposed to vinyl chloride (Li et al., 2003). In workers exposed to 1,3-butadiene, MN frequencies were higher in peripheral lymphocytes of individuals with polymorphisms in *XRCC1* compared with individuals carrying the wild-type repair gene (Wang et al., 2010).

In summary, genetic polymorphisms and variability in expression of enzymes due to induction or inhibition of constitutive enzyme levels can have considerable impact on the carcinogenic process. Determining the existence and functional role of genetic polymorphisms in cancer etiology is an active area of research in molecular epidemiology.

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PART 1.

CONCORDANCE BETWEEN CANCER IN HUMANS AND IN EXPERIMENTAL ANIMALS

CHAPTER 2.

Aromatic amines and aristolochic acids

Frederick A. Beland and M. Matilde Marques

Carcinogenicity in humans

Exposure to 4-aminobiphenyl, *o*-toluidine, 2-naphthylamine, and benzidine (Fig. 2.1) has been consistently associated with the induction of cancer of the urinary bladder in humans. This association is based upon occupational exposures, primarily of workers in the rubber and dye industries (IARC, 2010, 2012a). Similarly, occupational exposure to 4,4′-methylenebis(2-chloroaniline) (MOCA; Fig. 2.1), a curing agent for polyurethane pre-polymers, causes cancer of the bladder in humans, although the epidemiological data are not as strong as those for the other agents (IARC, 2010, 2012a). Certain azo dyes that are used in commercial products, for example, Direct Black 38, Direct Blue 6, and Direct Brown 95 (Fig. 2.2), are known to undergo azo reduction in vivo to yield the carcinogen benzidine. The overall evaluation for these dyes was raised to Group 1 based on this mechanistic information, although at present the corresponding epidemiological data are considered to provide *inadequate evidence* for the carcinogenicity of these dyes in humans (IARC, 2010, 2012a).

Cigarette smoke contains 4-aminobiphenyl, *o*-toluidine, and 2-naphthylamine, and tobacco smoking causes cancer of the bladder in humans (IARC, 1986, 2004, 2010, 2012a, b). The contribution of 4-aminobiphenyl, *o*-toluidine, and 2-naphthylamine to the induction of smoking-related cancer of the bladder is confounded by the presence of numerous other carcinogens, including carcinogenic aromatic amines, in tobacco smoke. Cigarette smoking also causes other cancers (e.g. cancer of the lung, oral cavity, and pancreas, and possibly breast cancer), but at present it is unclear whether these cancers can be attributed to 4-aminobiphenyl, *o*-toluidine, 2-naphthylamine, or other aromatic amines. Hair dyes are an additional source of exposure to 4-aminobiphenyl and *o*-toluidine (IARC, 2010, 2012a; Lizier and Boldrin Zanoni, 2012).

Exposure to phenacetin (Fig. 2.1), through its use as an analgesic, causes cancer of the kidney and ureter in humans (IARC, 2012c). Chlornaphazine (Fig. 2.1), a chemotherapeutic agent that has been used for the treatment of Hodgkin lymphoma and for the control of polycythaemia vera, causes cancer of the bladder in humans, presumably due to metabolism to 2-naphthylamine (IARC, 2012c). An additional source of human exposure to *o*-toluidine is from the anaesthetic prilocaine (Fig. 2.1) (IARC, 2010, 2012a).

Exposure to herbal remedies prepared from plant species of the genus *Aristolochia* has been causally

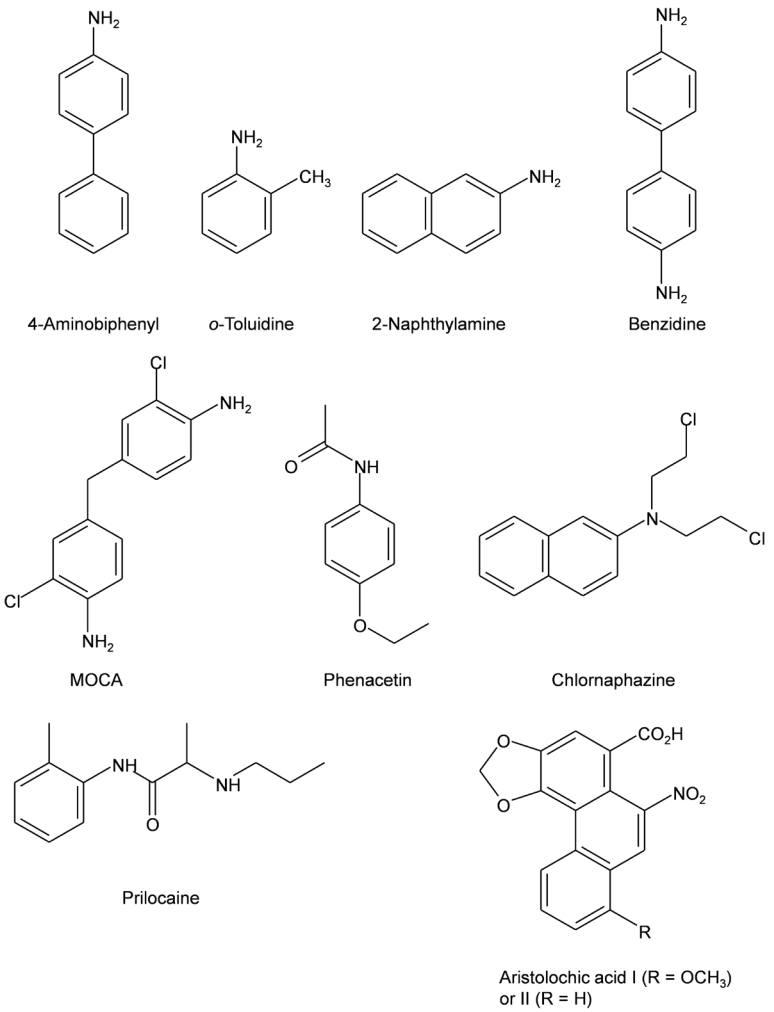
associated with the induction of urothelial cancer in humans (IARC, 2002, 2012c). The induction of urothelial tumours has been attributed to the presence of aristolochic acid I and aristolochic acid II (Fig. 2.1).

Metabolism and DNA adduct formation

4-Aminobiphenyl, *o*-toluidine, 2-naphthylamine, and MOCA are metabolized to electrophilic *N*-hydroxyarylamines by hepatic cytochrome P450 enzymes (IARC, 2010, 2012a, c). The *N*-hydroxyarylamines undergo acid-catalysed reactions with DNA to form a variety of DNA adducts. With the exception of MOCA, C8-substituted deoxyguanosine adducts are typically the major products, along with smaller amounts of N^2-substituted deoxyguanosine and N^6-substituted deoxyadenosine adducts (Fig. 2.3); with MOCA, only C8-substituted deoxyadenosine adducts have been detected (IARC, 2010, 2012a). These DNA adducts can also be formed from reactive esters of *N*-hydroxyarylamines (e.g. *N*-sulfoxyarylamines and *N*-acetoxyarylamines). Benzidine, which has two amino groups, also forms a C8-substituted deoxyguanosine adduct via a pathway involving an initial *N*-acetylation followed by *N*-hydroxylation of the remaining amino function (Fig. 2.3).

The carcinogenic activity of aromatic amines in the bladder in humans has been attributed to an initial *N*-hydroxylation, catalysed by hepatic cytochrome P450 enzymes, followed by transport of the *N*-hydroxyarylamines to the bladder as either aglycones or *N*-glucuronide conjugates (Bois et al., 1995). In the bladder lumen, the *N*-hydroxyarylamine *N*-glucuronides can undergo acid-catalysed hydrolysis to release the *N*-hydroxyarylamines, which can enter the bladder epithelium and react with DNA either directly or after esterification. DNA adducts derived from 4-aminobiphenyl, *o*-toluidine, benzidine, and MOCA have been detected in bladder tissue or exfoliated bladder cells from exposed individuals (IARC, 2010, 2012a; Böhm et al., 2011; Lee et al., 2014). With the exception of MOCA, which forms only C8-substituted deoxyadenosine adducts, the major – if not the only – adduct detected in each instance was a C8-substituted deoxyguanosine adduct. The importance of urinary acidity for the hydrolysis of the *N*-hydroxyarylamine *N*-glucuronides and perhaps for the reaction of the *N*-hydroxyarylamines with urothelial DNA has been demonstrated by the positive correlation between urinary acidity and the levels of benzidine DNA adducts in exfoliated bladder cells from exposed workers (Rothman et al., 1997). Additional support for this mechanism comes from the observation of a positive

Fig. 2.1. Structures of IARC Group 1 aromatic amines, drugs that are metabolized to Group 1 aromatic amines, and aristolochic acids. MOCA, 4,4′-methylenebis(2-chloroaniline).

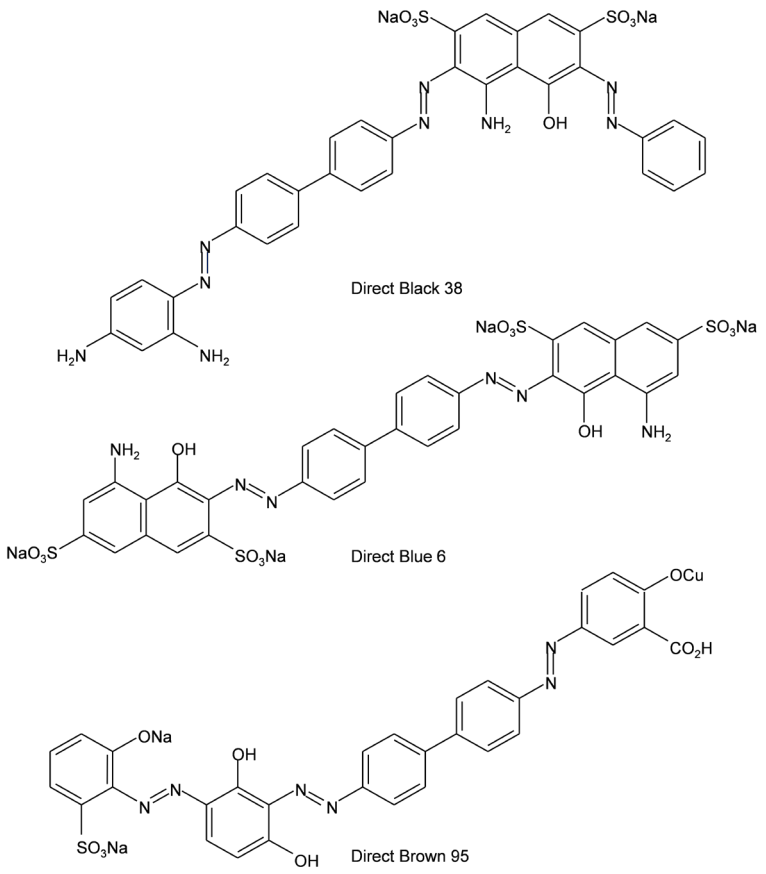


correlation between urinary acidity and the incidence of bladder cancer in smokers (Alguacil et al., 2011).

The major metabolic activation pathway for aristolochic acid I and aristolochic acid II involves nitro reduction, followed by cyclization to give *N*-hydroxyaristolactams, which – in contrast to other *N*-hydroxyarylamides – do not appear to require additional activation to react with DNA (Stiborová et al., 2011, 2013). Nonetheless, *N*-hydroxyaristolactams have been shown to serve as substrates for human sulfotransferases, particularly sulfotransferase family cytosolic 1B member 1 (SULT1B1), forming highly reactive *N*-sulfoxy derivatives (Sidorenko et al., 2014). The major adducts resulting from the *N*-hydroxyaristolactams are N^2-substituted deoxyguanosines and N^6-substituted deoxyadenosines (Fig. 2.4) (IARC, 2002, 2012c).

DNA adducts derived from aristolochic acids have been detected in renal tissue from patients who had been exposed to aristolochic acid-containing herbal products and from individuals who had consumed wheat grains contaminated with *Aristolochia* (IARC, 2002, 2012c; Chen et al., 2012; Jelaković et al., 2012; Schmeiser et al., 2012, 2014; Yun et al., 2013, 2014). Typically, the major lesion detected is an N^6-deoxyadenosine adduct derived from aristolochic acid I, accompanied by smaller amounts of a similar adduct derived from aristolochic acid II and an N^2-deoxyguanosine adduct derived from aristolochic acid I.

Fig. 2.2. Structures of benzidine-derived azo dyes.



Alterations in the *TP53* tumour suppressor gene in humans

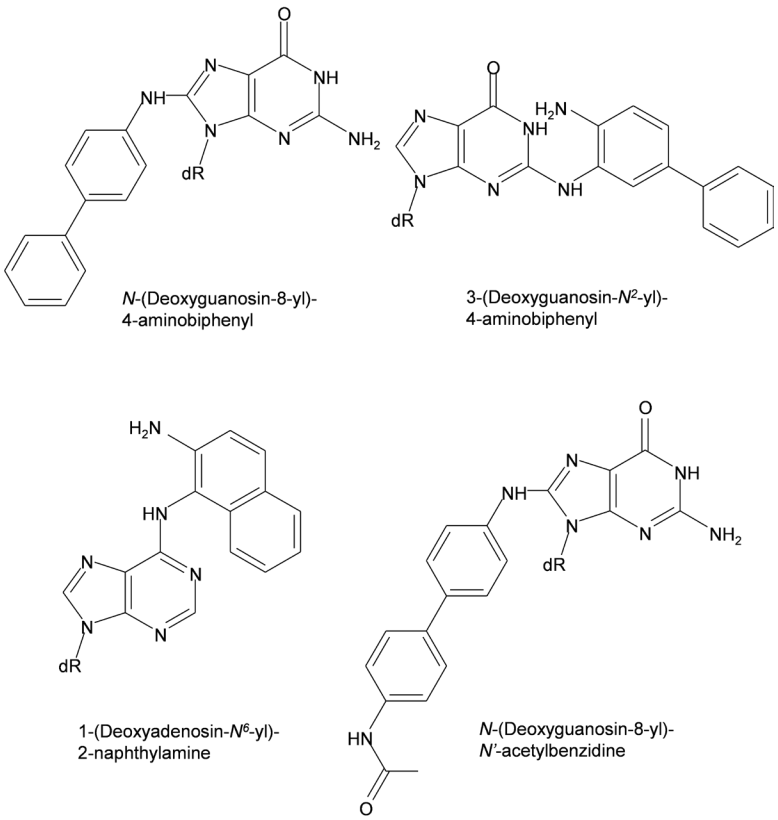
Mutations in the *TP53* tumour suppressor gene have been found in approximately 50% of all bladder cancers in humans (Petitjean et al., 2007), with G:C base substitution mutations occurring to a greater extent than A:T base substitution mutations.

TP53 gene mutations have been detected in bladder cancer patients exposed occupationally to 4-aminobiphenyl, 2-naphthylamine, and/or benzidine (Sørlie et al., 1998). The mutations occurred exclusively in higher-grade tumours (grades 2 or 3, i.e. moderately or poorly differentiated) and only at G:C base pairs.

Mutant p53 protein has also been detected in workers exposed occupationally to benzidine (Xiang et al., 2007). The occurrence and the amount of mutant protein were positively correlated with the level of benzidine exposure and the extent of neoplastic changes in exfoliated urothelial cells.

Urothelial tumours arising from exposure to aristolochic acids have been consistently shown to carry mutations in the *TP53* tumour suppressor gene, of which the most common mutation is an A → T transversion

Fig. 2.3. Structures of representative DNA adducts obtained from Group 1 aromatic amines. dR, deoxyribose.



mutation (reviewed in IARC, 2002, 2012c; Hollstein et al., 2013; see also Schmeiser et al., 2012; Chen et al., 2013; Hoang et al., 2013; Poon et al., 2013; Aydin et al., 2014). These mutations have been demonstrated in tumour tissue from patients who had consumed herbal preparations containing aristolochic acids and in urothelial cancer tissues of patients from regions with a high incidence of endemic (Balkan) nephropathy due to consumption of grains contaminated with *Aristolochia*. The presence of A → T transversion mutations is consistent with the observation that the major lesion detected in patients is an N^6-deoxyadenosine adduct derived from aristolochic acid I.

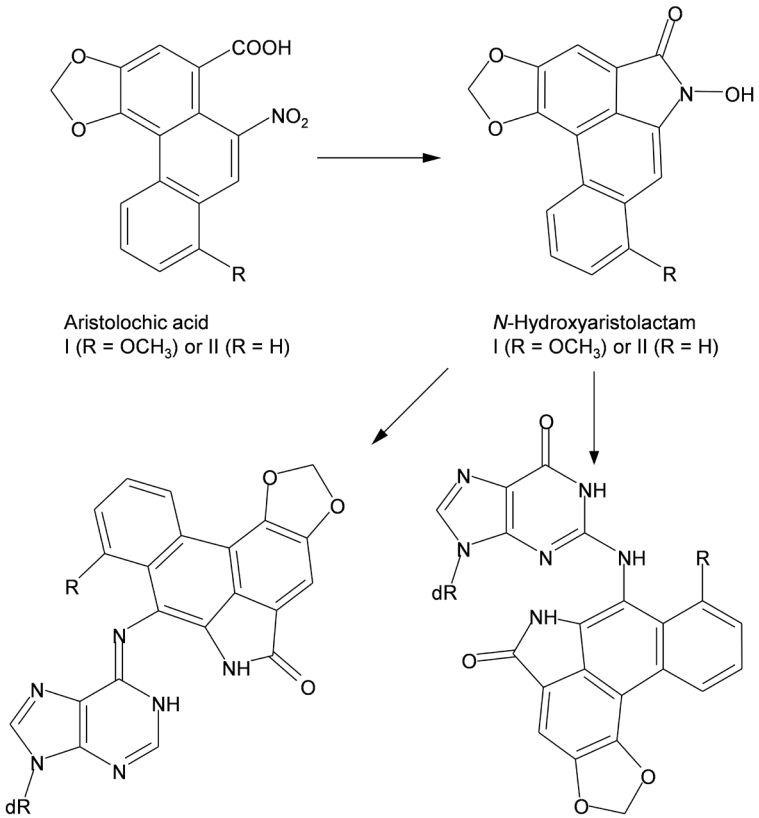
Tumour sites and the mechanism of tumour induction in experimental animals

4-Aminobiphenyl, 2-naphthylamine, and MOCA are bladder carcinogens in dogs (IARC, 2010, 2012a). Bladder tumours also occur in mice treated with 4-aminobiphenyl. As with humans, the induction of bladder tumours in dogs is thought to result from hepatic *N*-hydroxylation, transport of the *N*-hydroxyarylamines to the bladder as either aglycones or *N*-hydroxyarylamine *N*-glucuronides, and subsequent hydrolysis of the *N*-hydroxyarylamine *N*-glucuronides in the bladder lumen to release the *N*-hydroxyarylamines. Support for this mechanism comes from the observation that the DNA lesions detected in the bladders of dogs treated with 4-aminobiphenyl or MOCA appear to be C8-substituted deoxyguanosine and deoxyadenosine adducts that are identical to the DNA adducts detected in bladder tissues or exfoliated bladder cells from humans exposed to these carcinogens (IARC, 2010, 2012a). 2-Naphthylamine DNA adducts detected in the bladders of dogs exposed to 2-naphthylamine are entirely consistent with a mechanism involving the formation of *N*-hydroxy-2-naphthylamine (IARC, 2010).

In contrast to what is observed in humans, benzidine in not a bladder carcinogen in dogs. This lack of carcinogenicity has been attributed to the inability of dogs to *N*-acetylate aromatic amines (IARC, 2010). With most aromatic amines, *N*-acetylation is considered to be a detoxification event; however, with benzidine, *N*-acetylation appears to be required to give *N*-acetylbenzidine, which undergoes a subsequent *N*-hydroxylation of the second amino function. This metabolic pathway occurs in humans but not in dogs.

4-Aminobiphenyl, *o*-toluidine, 2-naphthylamine, benzidine, benzidine-based dyes, and MOCA induce hepatocellular tumours in mice (IARC, 2010, 2012a). DNA adducts derived from 4-aminobiphenyl and benzidine have been examined in liver tissue from exposed mice, and the major DNA lesions detected in each instance were C8-substituted deoxyguanosine adducts, consistent with formation of an *N*-hydroxyarylamine intermediate (IARC, 2010). In rats, DNA adducts derived from benzidine-based dyes and MOCA have been examined in the liver, which is also a target tissue for these

Fig. 2.4. Structures of DNA adducts derived from aristolochic acids through *N*-hydroxyaristolactam intermediates. dR, deoxyribose.



carcinogens, and again the major DNA adducts detected in each instance were consistent with formation of an *N*-hydroxyarylamine intermediate (IARC, 2010).

In mice, there appears to be a balance between hepatic *N*-acetylation, which is considered to be a detoxification step, and hepatic *N*-hydroxylation, which is considered to be an activation step. Should *N*-hydroxylation occur, the *N*-hydroxyarylamines can be further activated by hepatic *O*-acetylation to yield *O*-acetoxyarylamines, which can give rise to the DNA adducts detected in liver tissue (IARC, 2010). C8-substituted deoxyguanosine adducts have also been detected in the bladder DNA of mice treated with 4-aminobiphenyl (Poirier et al., 1995). These adducts presumably arise from hepatic *N*-hydroxylation and possibly *O*-acetylation of *N*-hydroxy-4-aminobiphenyl in the bladder epithelium.

The carcinogenicity of aristolochic acids has been assessed in rats and to a lesser extent in mice and rabbits, primarily by oral dosing (IARC, 2002, 2012c). Aristolochic acid I and mixtures of aristolochic acids I and II consistently induce tumours of the forestomach in rats. Tumours of the kidney have been reported to occur sporadically. Mice treated with mixtures of aristolochic acids I and II develop tumours of the forestomach, kidney, and lung. In rabbits, mixtures of aristolochic acids I and II administered intraperitoneally are associated with tumours of the kidney, ureter, and peritoneal cavity.

DNA adducts derived from aristolochic acid I and aristolochic acid II have been detected in target tissues in mice (forestomach, kidney, and lung), rats (forestomach and kidney), and rabbits (kidney) (IARC, 2002, 2012c; Debelle et al., 2003; Gillerot et al., 2003; Dong et al., 2006; Mei et al., 2006; Shibutani et al., 2007; Chan et al., 2008; Rosenquist et al., 2010; Shibutani et al., 2010; Baudoux et al., 2012; McDaniel et al., 2012; Wang et al., 2012a; Yun et al., 2013, 2014). Typically, three DNA adducts are detected: an N^6-deoxyadenosine adduct derived from aristolochic acid I, an N^6-deoxyadenosine adduct derived from aristolochic acid II, and an N^2-deoxyguanosine adduct derived from aristolochic acid I.

Oncogene alterations in experimental animals

Transversion mutations at codon 61 of the H-*Ras* oncogene (CAA → AAA) have been observed in the livers of mice treated with 4-aminobiphenyl (IARC, 2010, 2012a). G → T transversion mutations in the *cII* transgene have been detected in the livers and bladders of transgenic mice treated with 4-aminobiphenyl (Wang et al., 2012b; Yoon et al., 2012). The occurrence of these mutations at G:C base pairs is consistent with the observation that the major DNA adduct detected in target tissues

after exposure to 4-aminobiphenyl is a C8-substituted deoxyguanosine adduct.

Transversion mutations at codon 61 of the H-*Ras* oncogene (CAA → CTA) have been detected in tumours from rats and mice fed mixtures of aristolochic acids I and II and/or aristolochic acid I (IARC, 2002, 2012c; Wang et al., 2011, 2012a). A → T transversion mutations have also been detected in the *cII* transgene of rats and the *cII* and *lacZ* transgenes of mice treated with mixtures of aristolochic acids I and II, and in the *gpt* transgene of mice treated with aristolochic acid I or aristolochic acid II (IARC, 2012c; McDaniel et al., 2012; Xing et al., 2012). The occurrence of these mutations at A:T base pairs is consistent with the observation that the major DNA lesions detected in target tissues after exposure to aristolochic acids are N^6-substituted deoxyadenosine adducts.

Summary

In humans, exposure to aromatic amines and aristolochic acids that are IARC Group 1 carcinogens has been associated with induction of tumours of the urinary tract. With aromatic amines, the primary tumour site is the bladder; with aristolochic acids, the primary site for tumour formation is the kidney. Experimental animals treated with aromatic amines or aristolochic acids develop tumours of the urinary tract; tumours also arise in other tissues, primarily the liver.

Aromatic amines and aristolochic acids that are IARC Group 1 carcinogens are metabolized by amine oxidation (in the case of aromatic amines) or nitro reduction (in the case of aristolochic acids) to *N*-hydroxyarylamine metabolites in both humans and experimental animals. These *N*-hydroxyarylamine intermediates can react directly with DNA or be further activated by *O*-esterification to give rise to DNA adducts, predominantly at deoxyguanosine (primarily with aromatic amines) and deoxyadenosine (primarily with aristolochic acids), in tumour target tissues of humans and experimental animals.

Mutations of the *TP53* tumour suppressor gene consistent with the major DNA adducts derived from aromatic amines and aristolochic acids have been detected in tumours from exposed humans. Similarly, mutations of the H-*Ras* oncogene consistent with the major DNA adducts derived from aromatic amines and aristolochic acids have been found in target tissues of experimental animals.

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PART 1.

CONCORDANCE BETWEEN CANCER IN HUMANS AND IN EXPERIMENTAL ANIMALS

CHAPTER 3.

Arsenic and metals

Michael P. Waalkes

Arsenic and arsenic compounds together with various metals, specifically including beryllium and beryllium compounds, cadmium and cadmium compounds, chromium(VI) compounds, and nickel and nickel compounds, were re-evaluated in *IARC Monographs* Volume 100C (IARC, 2012) as *carcinogenic to humans* (Group 1). The most recent earlier evaluations appeared in Volume 84 (IARC, 2004) for arsenic, Volume 58 (IARC, 1993) for beryllium and cadmium, and Volume 49 (IARC, 1990) for chromium and nickel.

Arsenic and arsenic compounds

Carcinogenicity

Early on, exposure to inorganic arsenic via drinking-water or oral use of arsenic-based drugs was considered carcinogenic to the skin in humans, and exposure to inorganic arsenic via inhalation in occupational settings was evaluated as carcinogenic to the lung in humans (IARC, 1973). Arsenic was most recently assessed as a contaminant of drinking-water (IARC, 2004). The most prevalent source of human exposure to arsenic is now drinking-water, where it is found primarily as the inorganic forms of arsenite and arsenate. Inorganic arsenic can be metabolized by most mammals to form trivalent and pentavalent methylated metabolites through specific methyltransferases, with *S*-adenosylmethionine as the methyl donor (IARC, 2004, 2012). Questions remain about the relative contribution of the inorganic and the methylated arsenic species to the overall carcinogenic potential of exposure to arsenic. Arsenic and arsenic compounds in drinking-water are human carcinogens; there is *sufficient evidence* in humans for cancer of the lung, urinary bladder, and skin, as well as *limited evidence* for cancer of the kidney, liver, and prostate (IARC, 2004, 2012).

In rodents, transplacental arsenic exposure via maternal consumption of sodium arsenite in drinking-water during gestation induced bronchiolo-alveolar carcinoma in the adult offspring in C3H/HeNCr mice (two studies) and CD1 mice (IARC, 2012). In addition, in a study of CD1 mice with "whole-life" exposure to multiple levels of sodium arsenite in drinking-water from 2 weeks before breeding (male and female mice), during gestation and lactation (female mice), and after weaning until age 2 years (offspring, both sexes), bronchiolo-alveolar carcinoma occurred in a dose-related fashion in both male and female offspring (Tokar et al., 2011). In CD1 mice, in utero exposure via maternal consumption of sodium arsenite in drinking-water during gestation with or without subsequent dimethylarsinic acid (DMA(V)) in drinking-water of the offspring throughout adulthood induced bronchiolo-alveolar carcinoma in animals that received prenatal arsenite alone, DMA(V) alone, or the combination of prenatal arsenite and DMA(V) (Tokar et al., 2012a). In CD1 mice, in utero exposure to arsenic via maternal consumption of monomethylarsinous acid (MMA(III)) in drinking-water produced bronchiolo-alveolar

carcinoma in male offspring as adults, but not in a dose-related fashion (Tokar et al., 2012b). Multiple intratracheal instillations of inorganic arsenic produced lung tumours in hamsters (three studies) (IARC, 2012). In adult strain A/J mice, oral sodium arsenate increased the size and multiplicity of lung tumours (male mice), and oral DMA(V) increased the incidence and multiplicity of lung tumours (IARC, 2012). Oral exposure to DMA(V) increased the incidence of lung tumours in *Ogg*−/− mice, which cannot repair certain types of oxidative DNA damage, but not in *Ogg*+/+ mice (IARC, 2012).

Multiple studies in humans have found oral arsenic exposure to be carcinogenic to the urinary bladder, typically producing transitional cell carcinoma (IARC, 2004, 2012). Most oral exposure in humans would involve inorganic arsenic as the primary form. Multiple studies in rodents show that chronic oral exposure to DMA(V) causes transitional cell carcinoma of the urinary bladder in adult rats, but not in mice (IARC, 2012). DMA(V) exposure can be from the drinking-water or the feed. Exposure to inorganic arsenic has not been shown to be carcinogenic to the urinary bladder in rodents; the reasons for this are not clear.

Gallium arsenide is considered *carcinogenic to humans*, based largely on one robust study in rodents together with ancillary evidence (IARC, 2006). Chronic inhalation of gallium arsenide induced lung bronchiolo-alveolar adenoma or carcinoma in a dose-related fashion in female F344 rats, but not in male rats or male or female B6C3F1 mice (IARC, 2006, 2012). Male rats exposed to gallium arsenide did show dose-related increases in the number of pre-neoplastic lesions (atypical hyperplasia) of the lung epithelium. The role of the separate moieties of the inhaled compound (i.e. gallium and arsenic) in the carcinogenic response could not be defined by this one study in rodents, and it was concluded that either moiety alone or some combination of both could be active (IARC, 2006).

There is *limited evidence* that the liver is a target site for the carcinogenic effects of arsenic and arsenic compounds in humans (IARC, 2012). In rodents, multiple studies showed that in utero exposure to arsenic via maternal consumption of sodium arsenite in drinking-water during gestation induced hepatocellular carcinoma in the adult offspring of C3H/HeNCr mice (two studies) and CD1 mice (IARC, 2012). In addition, in a study of CD1 mice with "whole-life" exposure to multiple levels of sodium arsenite in drinking-water (see above), hepatocellular carcinoma occurred in a dose-related fashion in both male and female offspring (Tokar et al., 2011). In CD1 mice, in utero exposure via maternal consumption of sodium arsenite in drinking-water during gestation with or without subsequent DMA(V) in the drinking-water of the offspring throughout adulthood induced hepatocellular carcinoma in animals that received prenatal arsenite alone or the combination of prenatal arsenite and DMA(V). The combined treatment produced hepatocellular carcinoma at a significantly higher rate than prenatal arsenite alone or DMA(V) alone (Tokar et al., 2012a). In CD1 mice, in utero exposure to arsenic via maternal consumption of MMA(III) in drinking-water produced hepatocellular carcinoma in male offspring as adults (Tokar et al., 2012b).

In multiple studies in rodents, inorganic arsenic or DMA(V) given in drinking-water or by the transplacental route had initiating, promoting, or co-carcinogenic activity in the skin, kidney, and urinary bladder with other, non-arsenic-based compounds (IARC, 2004, 2012). Multiple studies in humans have found oral arsenic exposure to be carcinogenic to the skin and urinary bladder, and there is *limited evidence* that the kidney is a target site in humans (IARC, 2004, 2012). It is difficult to assess the relevance to humans of rodent studies that use multiple agents, one of which is an arsenic compound of concern.

Overall, the target sites for which there is *sufficient evidence* in humans for the carcinogenicity of arsenic and arsenic compounds include the urinary bladder and the lung, and there are multiple concordant rodent studies for these two sites (IARC, 2012). The skin is also a target site in humans for inorganic arsenic and arsenic compounds, but in rodents there is *insufficient evidence* that inorganic arsenic or arsenic compounds acting alone can cause cancer of the skin (IARC, 2012). There is *limited evidence* that the liver is a target site for the carcinogenic effects of arsenic and arsenic compounds in humans, and *sufficient evidence* that the liver is a target site in rodents (IARC, 2012). There is *limited evidence* that the kidney is a target site in humans (IARC, 2012), and one recent study in mice provided evidence that cancer of the kidney can be induced by a combination of inorganic arsenic (prenatal) and DMA(V) in adulthood (Tokar et al., 2012a). There is *limited evidence* that the prostate is a target site in humans, and there are no studies in rodents showing increased incidence of prostate cancer after

exposure to inorganic arsenic or arsenic compounds (IARC, 2012). Inorganic arsenic can cause lung cancer in humans after inhalation or ingestion, but there are no studies showing development of lung cancer in rodents after inhalation exposure (IARC, 1973, 2012). In fact, an adequate inhalation study in rodents with inorganic arsenic has never been performed, presumably because the agent had already been declared a human carcinogen and rodent research resources were directed elsewhere.

Mechanisms of carcinogenesis

Although a unifying mechanistic hypothesis for arsenic-induced carcinogenesis may seem reasonable, it is important to emphasize that given the multitude of toxic events at the subcellular level seen with inorganic arsenic and arsenic compounds (e.g. oxidative stress, altered DNA repair, altered DNA methylation, gene amplification, and altered growth factors), it is likely that multiple mechanisms are operative in arsenic-induced carcinogenesis (Kitchin and Conolly, 2010; Tokar et al., 2010; Hartwig, 2013). These multiple mechanisms are probably linked, at least in part, to the qualities of specific target tissue (e.g. high oxygen tension in the lung might favour oxidative stress, in contrast with the situation in the bladder) (Kitchin and Conolly, 2010). The target tissue-specific toxicokinetics of arsenic are likely to be key and may dictate that multiple toxic events combine into a target-specific carcinogenic mechanism (Kitchin and Conolly, 2010).

In humans, arsenic causes transitional cell carcinoma of the urinary bladder (IARC, 2012), which is the same tumour type induced in rats by chronic oral exposure to DMA(V) (IARC, 2012). Some researchers believe that the rat is a poor model for studying arsenic toxicology in humans, because the toxicokinetics of arsenic are dramatically different as a result of sequestration of arsenic in the blood of rats (Carter et al., 2003; Aposhian et al., 2004). However, for DMA(V) and cancer of the urinary bladder, there is clear site concordance between humans and rats. For bladder tumours induced by DMA(V) in the rat, the mechanism may involve sustained cytotoxicity, possibly from oxidative stress (Kitchin and Conolly, 2010), followed by cell proliferation and genomic instability. Specific methylated forms of arsenic may be involved in the sustained cytotoxicity (Cohen et al., 2007).

Inorganic arsenic and methylated arsenic metabolites generally show weak activity as mutagens. Low-dose exposure to inorganic arsenic can increase the number of mutations resulting from genomic instability, perhaps through production of reactive oxygen species, and cells that methylate inorganic arsenic show much more oxidative DNA damage than cells that poorly methylate the metalloid during in vitro malignant transformation (Kojima et al., 2009). MMA(III) may be one of the most deleterious arsenic methylation products, although the number of tumour end-point studies is very limited (only one study). The main cascade of mechanisms leading to carcinogenesis for inorganic arsenic and arsenic compounds after exposure to low concentrations could include the rapid induction of oxidative DNA damage and inhibition of DNA repair, followed by changes in DNA methylation patterns, aneuploidy, and gene amplification. Gene amplification, altered DNA methylation (epigenetic effects), or aneuploidy may cause alterations in gene expression that lead to genomic instability and cellular transformation. The metabolism of inorganic arsenic by methylation may contribute to its epigenetic effects, because the arsenic methylation pathway overlaps with DNA methylation by consumption of *S*-adenosylmethionine as the common methyl donor (Brocato and Costa, 2013). However, it is noteworthy that inorganic arsenic can cause malignant transformation in cells that do not methylate the metalloid, indicating that neither methylation nor a methylated metabolite are required for a cell to acquire a malignant phenotype after exposure to inorganic arsenic (Kojima et al., 2009). Thus, cell-specific, complex, multifaceted mechanisms are likely to be operative with arsenic (Kitchin and Conolly, 2010; Tokar et al., 2010).

Another important issue with mechanistic implications is the strong evidence that cancer can develop long after elevated arsenic exposure ends. For instance, a recent study on a human population in Chile measured rates of cancer of the bladder and lung in individuals who were highly exposed to inorganic arsenic in drinking-water during 1958–1970 but drank low-arsenic water thereafter. These subjects still showed very high risks of cancer even 40 years after the high exposures ended (Steinmaus et al., 2013). Similarly, the mouse transplacental model demonstrated that brief exposure to inorganic arsenic may result in tumours in adulthood (IARC, 2012). Given the time lag between arsenic exposure and development of cancer, the operative mechanisms would appear not to require concurrent high tissue levels of arsenic.

In discerning mechanisms of arsenic-induced carcinogenesis by use of in vitro model systems, the consideration that arsenic adversely alters many cellular physiological functions is critical; studies have frequently been carried out with levels of arsenic that would be highly unrealistic in vivo. Another common issue with in vitro arsenic studies is that many use short time frames, and very early responses to arsenic do not necessarily reflect in vivo exposures or take into account the adaptive capacities towards arsenic that are generally observed in vivo.

Beryllium and beryllium compounds

Carcinogenicity

There is *sufficient evidence* in humans that beryllium and beryllium compounds cause lung cancer (IARC, 2012). In rats, inhalation of beryllium metal, beryllium sulfate, or beryl ore dust produced bronchiolo-alveolar carcinoma, and intratracheal instillation of beryllium metal, beryllium hydroxide, or beryllium oxide produced bronchiolo-alveolar carcinoma.

Overall, for inhaled beryllium and beryllium compounds, the lung is the one identified cancer target site in humans, a response that has been repeatedly duplicated in rodent models (IARC, 2012).

Mechanisms of carcinogenesis

Multiple, related mechanisms are likely to be operative in beryllium-induced carcinogenesis (IARC, 2012). Although beryllium is inactive or weakly positive as a mutagen, chromosomal aberrations and aneuploidy can occur in vivo in rodents at non-toxic doses. Like many other inorganic carcinogens, beryllium produces oxidative stress, which can lead to damage in DNA or other key biomolecules and then produce gene activation and apoptosis. The cytotoxicity of beryllium in the lung may result in compensatory cell proliferation, along with chronic inflammation. The inflammatory processes induced by beryllium could contribute to the formation of reactive oxygen species, precipitate cell turnover, and activate or disrupt pulmonary cell signalling pathways. Beryllium can decrease DNA repair and recombination. The impairment of DNA repair by beryllium together with increased mitotic signalling may cooperate to induce error-prone cell proliferation (Beyersmann and Hartwig, 2008). The mechanisms of beryllium-induced carcinogenesis are probably complex, multifaceted, and interactive, as with other metals that are human carcinogens (Beyersmann and Hartwig, 2008; Kitchin and Conolly, 2010; Tokar et al., 2010; Brocato and Costa, 2013; Koedrith et al., 2013).

Cadmium and cadmium compounds

Carcinogenicity

There is *sufficient evidence* in humans that cadmium and cadmium compounds cause lung cancer, with positive associations between relevant exposure and cancer of the kidney and prostate (IARC, 2012). In rodents, there is *sufficient evidence* for the carcinogenicity of cadmium and cadmium compounds (IARC, 2012). In rats, inhalation of cadmium chloride, cadmium oxide, cadmium sulfide, or cadmium sulfate produced bronchiolo-alveolar carcinoma or squamous cell carcinoma of the lung, and intratracheal instillation of cadmium chloride and cadmium sulfide both induced primarily bronchiolo-alveolar carcinoma.

Multiple studies in rodents have established that various water-soluble and insoluble cadmium compounds can produce soft tissue sarcomas after repository injections (IARC, 1993, 2012). Studies that produced injection-site tumours have provided some evidence of the carcinogenic potential of cadmium, but there is no concordance with any specific target site in humans. Prostatic proliferative lesions can be produced in rats after oral administration or subcutaneous injection of cadmium chloride.

Overall, cadmium and cadmium compounds are *carcinogenic to humans* and target the lung after inhalation (IARC, 2012). Lung cancers have been repeatedly produced in rodents by either inhalation or intratracheal instillations of cadmium compounds. There is *limited evidence* in humans for prostate and renal carcinogenesis with cadmium or cadmium compounds. In rodents, prostatic proliferative lesions can be induced by cadmium, but there is no concordant evidence in rodents for the kidney.

Mechanisms of carcinogenesis

Cadmium-induced carcinogenesis may be attributable to various mechanisms (Beyersmann and Hartwig, 2008; Brocato and Costa, 2013; Hartwig, 2013; Koedrith et al., 2013). Direct interaction of cadmium with DNA appears to be limited, and cadmium is a weak mutagen. Cadmium can perturb DNA repair and affect tumour suppressor proteins, potentially causing genomic instability and chromosomal damage. Altered DNA methylation patterns

and disrupted signal transduction processes have been observed after exposure to cadmium; these factors could potentially contribute to aberrant cell growth, but their role in cadmium-induced carcinogenesis is unclear. Cadmium can induce reactive oxygen species, but this would be an indirect effect and its precise role in cadmium-induced carcinogenesis is not completely defined. Specific mechanisms of lung carcinogenesis after exposure to cadmium have not been elucidated fully. As with the other inorganic human carcinogens, the mechanisms of cadmium-induced carcinogenesis are probably multifaceted (Beyersmann and Hartwig, 2008; Brocato and Costa, 2013; Koedrith et al., 2013).

Chromium(VI) compounds

Carcinogenicity

There is *sufficient evidence* in humans for the carcinogenicity of chromium(VI) compounds, which cause cancer of the lung (IARC, 1990, 2012). Most of the epidemiological data come from occupational settings that would involve inhalation as the primary route of exposure. Also, positive associations have been observed between exposure to chromium(VI) compounds and cancer of the nose and nasal sinuses (IARC, 2012). In rodents, there is *sufficient evidence* for the carcinogenicity of chromium(VI) compounds (IARC, 1990, 2012). Calcium chromate induced lung tumours (adenomas) in mice, but only when data from male and female groups were combined (Nettesheim et al., 1971). The sex of a test animal is considered by IARC as a quantitative aspect of a tumour end-point study that potentially affects the outcome in terms of chemically induced tumours (IARC, 2012), and therefore the combination of data from male and female animals may be problematic. Male rats chronically exposed by inhalation to sodium dichromate developed lung tumours in one study, although the Working Group for that *Monograph* cautioned about small group sizes in that study (IARC, 1990). Nasal papilloma occurred after chronic inhalation of chromium trioxide in female mice in one study (IARC, 1990). Various compounds of chromium(VI), including calcium chromate, strontium chromate, and zinc chromate, produced local squamous cell carcinoma in rats when the test agent was first mixed with cholesterol and then used to fill or coat stainless steel wire baskets that were subsequently surgically implanted via tracheotomy into the bronchus (IARC, 1990). The coated basket acts as a point of release for the test agent. Implantation of a basket containing cholesterol alone caused notable squamous metaplasia (7% of controls) and bronchial inflammation (89% of controls), indicating that chronic, not agent-related, irritation is involved with this model even without a test agent. Although the tumours induced by this technique of exposure via implantation of a wire basket were histologically defined as bronchiolar squamous cell carcinoma, their relevance to human lung cancer induced by inhalation of chromium(VI), or in fact by any other agent, has not been rigorously validated. Repeated weekly intratracheal instillations of calcium chromate or sodium dichromate in male and female rats produced bronchiolo-alveolar carcinoma and squamous cell carcinoma in one study (Steinhoff et al., 1986). The authors noted that the chromium(VI)-induced tumours coexisted with extensive treatment-related scarring (confluent fibrosis) and chronic inflammatory changes indicative of high tissue burdens of the test agent and local toxicity.

Many studies have demonstrated carcinogenic activity for various chromium(VI) compounds in rodents, such as production of soft tissue sarcomas after repository injections (IARC, 1990, 2012). Studies that produced injection-site tumours have provided some evidence of the carcinogenic potential of chromium (VI), but there is no concordance with any specific target site in humans. Sodium dichromate dihydrate in drinking-water caused adenocarcinoma of the small intestine in mice and squamous cell carcinoma of the oral mucosa and tongue in rats (National Toxicology Program, 2008), but these tissues are not considered cancer target sites in humans. Given the response and the rarity of these tumours in rodents and the potential for oral exposure of humans to chromium(VI), these sites deserve additional focus in epidemiological studies.

The lung is a target site for the carcinogenic effects of chromium(VI) compounds in humans (IARC, 1990, 2012). From inhalation studies in rats and mice, there is *sufficient evidence* that chromium(VI) induces lung tumours, although all the available studies are considered to have some limitations (IARC, 1990). Lung tumours produced by surgically implanting stainless steel wire baskets containing chromium(VI) compounds in cholesterol into the bronchus of rats, like injection-site tumours, probably reflect carcinogenic activity, but the relevance of this exposure technique in modelling tumours produced in humans by inhalation of chromium(VI) compounds requires

further validation. Repeated intratracheal instillations of certain chromium(VI) compounds can produce malignant lung tumours (Steinhoff et al., 1986). There is *limited evidence* that the nose or the nasal sinuses are target sites for the carcinogenic effects of chromium(VI) in humans (IARC, 2012), and one study in mice showed nasal papillomas induced by inhalation of chromium(VI) (IARC, 2012).

Generally speaking, chromium(VI) compounds do show concordance between humans and rodents for the established target site in humans, the lung, on the basis of: (i) two positive inhalation studies in rodents, both with noted limitations (IARC, 1990); (ii) lung tumours induced in rodents by repeated intratracheal instillations; and (iii) activity in a rodent model with surgical implantation of wire baskets containing chromium(VI) compounds into the bronchus to produce lung tumours. However, additional state-of-the-art studies in experimental animals with inhaled chromium(VI) compounds appear to be needed. A high-quality, contemporary tumour end-point inhalation study in rodents would add greatly to the understanding of chromium(VI)-induced carcinogenesis and could be designed to significantly aid in elucidation of the mode of action of this compound (Proctor et al., 2014). Such a study is lacking, presumably because the agent had already been declared a human carcinogen and rodent research resources were directed elsewhere.

Mechanisms of carcinogenesis

In terms of general mechanisms, during in vitro conversion of chromium(VI) to chromium(III) by cellular reductants, various toxic intermediates, including radicals of chromium, oxygen, and sulfur, are likely to be generated, and they can react with key biomolecules relevant to carcinogenesis (IARC, 2012; Hartwig, 2013; Proctor et al., 2014). Some chromium(VI) reductants undergo Fenton-type reactions to produce hydroxyl radicals, which attack DNA. Chromium(VI) can stimulate formation of superoxide and nitric oxide in vitro. Chromium(VI) metabolites can be directly genotoxic, and the metal also causes inflammation and stimulates tumour growth pathways in cell culture systems. Aneuploidy has been observed after exposure to chromium(VI). Significant DNA methylation changes could be a contributing factor in chromium(VI)-induced carcinogenesis, particularly in the lung (Brocato and Costa, 2013). As with other inorganic human carcinogens, it is likely that multiple, probably interactive, mechanisms are operative in chromium(VI)-induced carcinogenesis, including DNA damage, oxidative stress, and aneuploidy, which lead to the acquisition of a malignant phenotype.

Nickel and nickel compounds

Carcinogenicity

There is *sufficient evidence* that mixtures of nickel compounds and metallic nickel cause cancers of the lung, nose and nasal cavity, and paranasal sinuses in humans (IARC, 2012). Epidemiological studies provided evidence for induction of lung cancer by specific nickel compounds, including water-soluble and insoluble substances. In rats, inhalation of nickel oxide, nickel subsulfide, or nickel carbonyl caused bronchiolo-alveolar carcinoma of the lung, whereas intratracheal instillation of nickel oxide, nickel subsulfide, or metallic nickel caused squamous cell carcinoma of the lung. In several well-performed experiments, the inhalation of various nickel compounds, both water-soluble and insoluble, including metallic nickel (in rats), nickel sulfate (in rats and mice), and nickel subsulfide (in mice) did not cause lung tumours. Oral exposure to nickel sulfate did not cause tumours in rats or mice.

Various water-soluble and insoluble nickel compounds and metallic nickel produced various types of sarcomas in rats, mice, or hamsters when administered by repository injections (subcutaneous, intramuscular, intraperitoneal, etc.) (IARC, 2012). Studies that produced injection-site tumours have provided some evidence of the carcinogenic potential of nickel and nickel compounds, but there is no concordance with any specific target site in humans.

There are multiple studies in rodents that recorded increased pheochromocytoma of the adrenal medulla after inhalation of nickel compounds, including metallic nickel and nickel subsulfide (IARC, 2012). These studies might imply the systemic bioavailability of inhaled nickel compounds, although there are no concordant data concerning the adrenal gland as a target of nickel-induced carcinogenesis in humans.

Overall, for nickel and nickel compounds, target site concordance exists between the human lung and the rodent lung for various nickel compounds and metallic nickel. There are no data in rodents on cancers of the nose, nasal cavity, and paranasal sinuses that would be concordant with data in humans.

Mechanisms of carcinogenesis

The nickel ion Ni(II) is considered to be the ultimate carcinogenic species in nickel-induced carcinogenesis (Beyersmann and Hartwig, 2008; IARC, 2012). Water-soluble and poorly water-soluble nickel compounds both enter the cell and reach the nucleus, although the soluble compounds do this by ion channels and transporters, whereas the poorly soluble compounds are taken up by phagocytosis. After phagocytosis, particulate nickel compounds gradually release nickel ions, making them available for interaction with key biomolecules. An increased level of nickel in the nucleus is evident after exposure to water-soluble or insoluble nickel compounds. Nickel compounds are weakly mutagenic in mammalian cells but induce DNA damage, chromosomal aberrations, and micronuclei in vitro and in vivo. Both water-soluble and insoluble nickel compounds induce malignant cell transformation in vitro. However, delayed mutagenicity and chromosomal instability have been observed long after treatment of cells with nickel. Nickel compounds induce epigenetic changes, including alteration in DNA methylation patterns and modification of histones (Brocato and Costa, 2013). An inflammatory component is also thought to contribute to nickel-induced carcinogenesis. Direct effects of nickel on DNA are probably limited, but oxidative stress and oxidative DNA damage have been found after nickel exposure. As with the other inorganic human carcinogens, it is likely that multiple, potentially interdependent, complex mechanisms are operative in nickel-induced carcinogenesis.

Comparative mechanisms of the inorganic human carcinogens

Among arsenic compounds and metals that are human carcinogens, only chromium(VI) seems to have the ability to interact with DNA directly, at least when it undergoes intracellular reduction, and to act as a direct genotoxin. Chromium(VI) does show human-to-rodent target site concordance with respect to the lung, although up-to-date inhalation studies in rodents would greatly aid in defining the mechanisms of chromium(VI)-induced carcinogenesis (see above; Proctor et al., 2014). With the inorganic human carcinogens other than chromium(VI), direct induction of DNA damage is not a key mechanism. All the metals that are human carcinogens seem to be able to cause oxidative stress (Beyersmann and Hartwig, 2008; Hartwig, 2013), mostly by indirect means. This may contribute to their carcinogenic potential because the resulting oxidative species could attack DNA. Arsenic, cadmium, chromium, and nickel can have epigenetic effects on DNA that alter critical gene expression and promote the acquisition of a malignant phenotype (Brocato and Costa, 2013).

With respect to carcinogenesis induced by inorganic chemicals, it is generally accepted that the ionic species is the most active species for the metals that are human carcinogens (i.e. beryllium, cadmium, chromium(VI), and nickel) (IARC, 2012). However, this scenario is not likely to be entirely true for the metalloid arsenic and its compounds. Of all the inorganic human carcinogens, inorganic arsenic alone can undergo conjugative biotransformation within the host. Methylation of inorganic arsenic generally produces monomethylarsenic (MMA) forms and then dimethylarsenic forms, but this process is not complete in most people (Melak et al., 2014). Incomplete methylation can result in the formation of very toxic, monomethylated arsenic metabolites, like MMA(III). Recent data in humans indicate that an increased MMA level, as a percentage of total urinary arsenic, strongly correlates with cancer of the lung and bladder in a population in northern Chile exposed to environmental inorganic arsenic (Melak et al., 2014). This indicates that arsenic metabolites generated by incomplete methylation are associated with increased carcinogenic risk after exposure to inorganic arsenic.

Stimulation of inflammation is also common among the group of inorganic human carcinogens. The role of inflammation in carcinogenesis may be secondary, through provision of oxidants or radical species produced by oxidation.

Definitive mechanisms have not been established for any inorganic human carcinogen. These agents are best considered to be multifaceted, interrelated, and complex carcinogens. For the inorganic carcinogens with multiple target sites, there is a strong possibility that the mechanism is target site-specific.

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PART 1.

CONCORDANCE BETWEEN CANCER IN HUMANS AND IN EXPERIMENTAL ANIMALS

CHAPTER 4.

Smokeless tobacco and its constituents

Stephen S. Hecht

Introduction

Smokeless tobacco is defined as follows in Volume 89 of the *IARC Monographs* (IARC, 2007): "Smokeless tobacco is used without burning the product, and can be used orally or nasally. Oral smokeless tobacco products are placed in the mouth, against the cheek or behind the lip and sucked (dipped) or chewed. Tobacco pastes or powders are used in a similar manner and applied to the gums or teeth. Fine tobacco mixtures are usually inhaled and absorbed in the nasal passages."

This chapter considers carcinogenicity studies, data on constituents, and mechanistic investigations on smokeless tobacco, to evaluate overall coherence between observations in humans and in experimental animals. Because of the differences between human use of smokeless tobacco and the exposure conditions in studies in experimental animals, the term "coherence", which means *logical consistency*, is more appropriate here than the term "concordance", which connotes a one-to-one agreement, with no conflicting data.

Coherence: carcinogenicity of smokeless tobacco in humans versus experimental animals

Evaluations of smokeless tobacco use by the *IARC Monographs* concluded that this practice is *carcinogenic to humans* (Group 1), causing cancers of the oral cavity, oesophagus, and pancreas (IARC, 1985, 2007, 2012). A meta-analysis of epidemiological data also concluded that use of smokeless tobacco significantly increased the risk of these cancers (Boffetta et al., 2008). A recent population-based case–control study, which was carried out in New England, USA, and was not included in the above-mentioned evaluations, demonstrated a statistically significant association between ever use of smokeless tobacco and the risk of head and neck squamous cell carcinoma (including cancers of the oral cavity, larynx, and pharynx) (Zhou et al., 2013). This section considers coherence between these conclusions and studies of the carcinogenicity of smokeless tobacco in experimental animals.

The use of smokeless tobacco by humans is a voluntary practice engaged in by hundreds of millions of people worldwide. There are great variations in use of smokeless tobacco: in Sweden, fine-cut tobacco, called *snus*, is placed between the upper lip and teeth; in North America, fine-cut tobacco, frequently in teabag-like sachets, is placed between the cheek and gums; and in South-East Asia and other parts of the world, there are vast arrays of different practices (IARC, 2007). Processed and fermented tobacco of varying types and blends are the common ingredients in all of these practices. Nicotine, perhaps along with other tobacco alkaloids and constituents, is the addictive substance

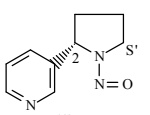
that drives the continuing use of these products (DHHS, 1988; Stolerman and Jarvis, 1995; Benowitz, 1999; IARC, 2007).

With reference to the use of smokeless tobacco by humans, it has not yet been possible to develop an experimental model in which laboratory animals *voluntarily and habitually* consume these products the way they are used by humans. Various approaches have been explored, including addition of tobacco to the diet, oral treatment of animals with tobacco extracts, exposure of animals to powdered tobacco by inhalation, placement of tobacco in the cheek pouch of hamsters, and surgical modification of the oral cavity. However, none of these methods faithfully replicate the human habit, and they have not always produced statistically significant results in carcinogenicity studies. The most consistent findings in animal carcinogenicity studies of smokeless tobacco have been reported in a model in which an artificial lip canal is created by surgery on rats. Several studies of this type produced tumours of the oral cavity, including squamous cell carcinomas, and their incidence was significantly increased compared with controls in some experiments (IARC, 2007). Also, in one study, insertion of snuff into the cheek pouch of hamsters infected with herpes simplex virus type 1 (HSV-1) or type 2 (HSV-2) significantly increased the incidence of squamous cell carcinoma compared with that in animals infected with HSV-1 or HSV-2 and not administered snuff (Park et al., 1986). This observation has not been replicated, and its relevance to human use of smokeless tobacco is unclear. Overall, there is some coherence between studies in experimental animals on the carcinogenicity of smokeless tobacco and cancer of the oral cavity in humans as induced by use of smokeless tobacco; the conclusion of the *IARC Monographs* that there is *sufficient evidence* in experimental animals for the carcinogenicity of smokeless tobacco followed from this evidence (IARC, 2012). However, the results are somewhat inconsistent and are limited by the requirement of surgery and other unnatural approaches in an attempt to replicate in laboratory animals the voluntary use of smokeless tobacco by humans.

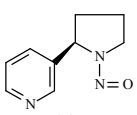
Coherence: carcinogenicity of smokeless tobacco in humans versus carcinogenicity of smokeless tobacco constituents in experimental animals

There is remarkable coherence between the carcinogenic activity in rats of tobacco-specific nitrosamines, which are constituents of smokeless tobacco, and observations in humans who use smokeless tobacco. Tobacco-specific nitrosamines – *N*′-nitrosonornicotine (NNN), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), *N*′-nitrosoanabasine (NAB), and *N*′-nitrosoanatabine (NAT) – are the most prevalent strong carcinogens in smokeless tobacco, generally found in the range of 1–10 µg per gram of product (IARC, 2007). No smokeless tobacco product analysed for these compounds has ever been reported to be free of them. Nitrosamine carcinogenesis was discovered by the pioneering work of Magee and Barnes on dimethylnitrosamine (Magee and Barnes, 1956). Subsequently, multiple studies by numerous investigators demonstrated that more than 200 nitrosamines are carcinogenic in laboratory animals, frequently inducing tumours in an organ-specific and systemic fashion, and in many cases after treatment of animals with very low doses (Preussmann and Stewart, 1984; Gray et al., 1991; Peto et al., 1991; Lijinsky, 1992). More than 30 different animal species develop cancer after treatment with various nitrosamines (Bogovski and Bogovski, 1981). Nitrosamines are genotoxic carcinogens that absolutely require metabolism to exert their carcinogenic effects (Hecht, 1998b). NNN and NNK, the most carcinogenic of the tobacco-specific nitrosamines, are typical members of the nitrosamine class of carcinogens (Hecht, 1998a). Amounts of NNN and NNK in many different types of smokeless tobacco products have been summarized based on the results of thousands of analyses; levels of NNN generally exceed those of NNK (IARC, 2007).

Multiple carcinogenicity studies of NNN have been reported (Hecht, 1998a). A recent investigation explored the carcinogenicity in rats of (*S*)-NNN, the enantiomer of NNN that is most prevalent in tobacco products, comprising 57–67%



(*S*)-NNN



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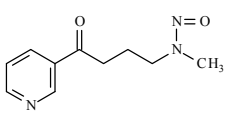

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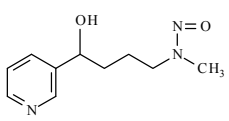

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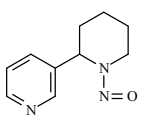

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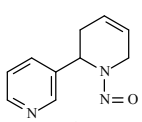

NAT

of total NNN in smokeless tobacco and cigarette tobacco (Balbo et al., 2013; Stepanov et al., 2013). (*S*)-NNN was administered in the drinking-water (15 ppm) to a group of 24 male Fischer 344 (F-344) rats. Two other groups of rats were given either (*R*)-NNN (15 ppm) or racemic NNN (30 ppm). The rats in the groups treated with (*S*)-NNN or racemic NNN began losing weight after 1 year of treatment and had died or were humanely killed by 17 months. All rats treated with (*S*)-NNN had tumours of the oral cavity. A total of 91 such tumours were observed in 20 rats that were necropsied, including tumours of the tongue, larynx, pharynx, oral mucosa, and soft palate. Some of the oral cavity tumours were large. The rats treated with (*S*)-NNN also had 122 oesophageal tumours. In contrast, (*R*)-NNN was only weakly tumorigenic. A highly significant carcinogenic response similar to that resulting from exposure to (*S*)-NNN was also observed in the rats treated with racemic NNN. The induction of tumours of the oral mucosa, tongue, larynx, and pharynx as well as oesophageal tumours in all rats treated with (*S*)-NNN or racemic NNN is remarkably consistent with the epidemiological studies of smokeless tobacco use summarized above. Although this was the first study to investigate the carcinogenicity of (*S*)-NNN, previous studies of racemic NNN administered in the drinking-water to rats uniformly produced high yields of oesophageal tumours, and oral cavity tumours were occasionally observed (Hecht, 1998a; IARC, 2007). The doses of NNN given in the earlier studies probably either were too low to observe a high incidence of oral cavity tumours in addition to oesophageal tumours, or were so high that they caused death from oesophageal tumours before oral cavity tumours could be observed.

Based on consumption of half a tin (17 g) per day of a popular smokeless tobacco product (Hecht et al., 2008a) containing about 3 µg per gram of NNN (Hecht et al., 2011) and an extraction efficiency of 60% (Hecht et al., 2008b), human exposure would be about 34 µg per day of NNN, or 20 µg per day of (*S*)-NNN; in 30 years of use, this would amount to about 220 mg (3 mg/kg body weight) of (*S*)-NNN. This compares to a dose of 150 mg (375 mg/kg body weight) of (*S*)-NNN in the drinking-water study described above. It is unclear whether a body weight correction is relevant, considering that smokeless tobacco is concentrated in the oral cavity and is frequently held at one site.

Whereas administration of NNN in the drinking-water to F-344 rats produces tumours of the oral cavity and the oesophagus, subcutaneous injection of NNN causes mainly tumours of the nasal mucosa, with malignant tumours arising predominantly in the olfactory epithelium (Hecht, 1998a). Treatment of mink with NNN by subcutaneous injection also produced malignant nasal tumours (Koppang et al., 1992; Koppang et al., 1997; IARC, 2007).

Carcinogenicity studies of NNN with Syrian golden hamsters have involved subcutaneous injection of NNN or swabbing of the cheek pouch. Tumours of the trachea and nasal cavity were observed upon subcutaneous injection; the cheek pouch was generally unresponsive (Hecht, 1998a). Treatment of various strains of mice with NNN by oral or intraperitoneal administration has resulted mainly in pulmonary adenomas (Hecht, 1998a). Thus, studies with Syrian golden hamsters and mice are generally less coherent with the epidemiology of smokeless tobacco use than are the studies in rats (IARC, 2007).

Swabbing the oral cavity and lips of rats with a mixture of NNN and NNK for 131 weeks produced 9 oral cavity tumours in 8 of 30 rats, which was statistically significant, but the result was not nearly as strong as that noted earlier, in part because the dose of racemic NNN in the swabbing study was about 40% of that described for the drinking-water study mentioned above (Hecht et al., 1986). NNK by itself did not induce oral cavity tumours when swabbed in the oral cavity of rats or hamsters (Hecht, 1998a). An interesting and unexplored observation in the swabbing study was that an extract of fine-cut moist snuff of the type used orally inhibited the oral cavity carcinogenicity of NNN and NNK.

Although the carcinogenicity studies of NNN administered orally to rats are in many respects remarkably consistent with the results of epidemiological studies of cancers of the oral cavity and the oesophagus in humans, they did not produce any pancreatic tumours. In another example of coherence, NNK and its metabolite 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) both produced significantly increased incidences of exocrine pancreatic tumours when administered in the drinking-water to male F-344 rats at doses of 1 ppm (NNK) or 5 ppm (NNAL) (Rivenson et al., 1988). It should be noted, however, that the lung was clearly the main target organ for NNK and NNAL in these studies – with significant increases in lung cancer for both agents ($P < 0.01$) – whereas the results of epidemiological studies on

smokeless tobacco use and lung cancer are, in aggregate, inconclusive at present (Boffetta et al., 2008).

Coherence: mechanistic studies of carcinogenicity of smokeless tobacco

Mechanistic studies can help bridge the gap between epidemiological investigations and experimental studies in laboratory animals. With respect to the role of tobacco-specific nitrosamines in carcinogenesis induced by smokeless tobacco products, as indicated by the animal studies described above, the first mechanistic question that arises concerns uptake of constituents. Many studies have demonstrated the presence of tobacco-specific nitrosamines in the saliva of smokeless tobacco users (IARC, 2007). In one study that quantified levels of NNK in a product before and after use, it was determined that approximately 59% of the NNK in a popular brand of smokeless tobacco was extracted during use (Hecht et al., 2008b). A second study of this type reported removal of 30% of the NNK and 23% of the NNN from an oral *snus* product during use (Caraway and Chen, 2013).

Analysis of the urine of smokeless tobacco users further demonstrates the uptake and metabolism of tobacco-specific nitrosamines. NNN, NNAL, NAT, and NAB as well as their glucuronides have all been detected in the urine of smokeless tobacco users at levels similar to or greater than those found in the urine of most smokers (Stepanov and Hecht, 2005; Hecht et al., 2007). It has been estimated that NNAL plus its glucuronides comprise 14–17% of the NNK dose in people who use a popular smokeless tobacco product, and that their uptake of NNK is about 6 µg per day (Hecht et al., 2008b). Furthermore, the level of NNAL plus its glucuronides in the urine of smokeless tobacco users is higher than that in controls and is also significantly correlated with years of use (Hecht et al., 2007).

Nitrosamines require metabolism to exert their carcinogenic effects, and the tobacco-specific nitrosamines NNN and NNK are no exception (Preussmann and Stewart, 1984; Hecht, 1998a). Many studies have conclusively demonstrated that α-hydroxylation of these compounds catalysed by cytochrome P450 enzymes leads to the formation of reactive metabolites and DNA adducts, and that these DNA adducts are crucial in the carcinogenic process. These studies have been reviewed in detail (Hecht, 1998a, 2008; IARC, 2007). As an example of the importance of DNA adducts in carcinogenesis by NNN, it is worth noting that the formation of NNN–DNA adducts in the oesophagus, oral cavity, and liver of rats treated chronically with 10 ppm of (*S*)-NNN or (*R*)-NNN in drinking-water correctly predicted cancer induction in the oral cavity and oesophagus of rats upon treatment with these enantiomers as described above (Lao et al., 2007; Zhang et al., 2009a). Thus, there is great coherence between mechanisms of NNN metabolism and DNA binding in rats and the corresponding carcinogenicity data. Less is known about mechanisms of pancreatic carcinogenesis by NNK, but DNA adducts of NNK and its metabolite NNAL have been characterized in the pancreas in rats (Zhang et al., 2009b).

In tandem with the formation of DNA adducts by NNN and NNK in experimental animals, the formation of haemoglobin (Hb) adducts occurs, because intermediates that react with DNA also react with Hb. These Hb adducts, when treated with base, release 4-hydroxy-1-(3-pyridyl)-1-butanone (HPB) and have therefore been termed HPB-releasing Hb adducts. Their formation and persistence in rats treated chronically with either NNK or NNN has been well documented (Hecht, 1998a; IARC, 2007).

Detection of NNN–DNA and NNK–DNA adducts as well as HPB-releasing Hb adducts would be anticipated in smokeless tobacco users, but there are no published studies on NNN–DNA and NNK–DNA adducts in this group. However, several studies have reported the presence of HPB-releasing Hb adducts in humans, with the highest levels consistently seen in smokeless tobacco users (Hecht, 1998a; IARC, 2007). These studies provide evidence that NNN and NNK are metabolically activated to form HPB-releasing Hb adducts in smokeless tobacco users, although it is possible that there could be other sources of these adducts as well. Collectively, these studies demonstrate coherence between mechanisms of NNN and NNK metabolic activation in rats and in smokeless tobacco users. Thus, there is coherence in the carcinogenicity data and in the mechanistic data available for these specific compounds and the observed cancer-causing effects of smokeless tobacco in humans.

There are still some noteworthy gaps that prevent the development of a completely coherent picture of NNN metabolism in laboratory animals and humans. Multiple studies, including some of those described above, indicate that in F-344 rats, 2′-hydroxylation of NNN is important in the formation of DNA adducts and in the expression of carcinogenicity

by NNN (Hecht, 1998a; IARC, 2007). It is not known which cytochrome P450 enzyme is responsible for NNN 2′-hydroxylation in the oral cavity and oesophagus in rats, or in humans. Two human cytochrome P450 enzymes that catalyse (*S*)-NNN metabolism by 5′-hydroxylation – cytochrome P450 2A6 and 2A13 enzymes – do not catalyse the 2′-hydroxylation (Wong et al., 2005). This raises some questions about the enzymology of (*S*)-NNN metabolic activation in humans. Further, in studies of NNN metabolism in patas monkeys, the major pathway appears to be 5′-hydroxylation (Upadhyaya et al., 2002). More research is needed to determine whether these observations reflect a lack of coherence between rats and humans or simply a lack of relevant data.

As noted above, the formation of DNA adducts is critical in the carcinogenic process induced by the agents discussed here. In contrast to the plethora of information available on DNA adduct formation in laboratory animals by smokeless tobacco constituents – most commonly NNN and NNK – there is a paucity of studies on DNA adduct formation by smokeless tobacco itself, both in laboratory animals and in humans (IARC, 2007). The few studies that have been reported either used non-specific techniques or did not find consistent effects of smokeless tobacco on DNA adduct formation. Similarly, there is at present no convincing published evidence that use of smokeless tobacco produces DNA adducts in the oral cavity, oesophagus, or pancreas in humans. This represents a significant gap in a mechanistically coherent pathway to cancer upon smokeless tobacco use as observed in epidemiological studies.

Nevertheless, many studies in human users of smokeless tobacco – but fewer in laboratory animals – demonstrate genetic effects that are consistent with the consequences of DNA adduct formation. Higher frequencies of micronuclei in buccal cells of smokeless tobacco users have been reported in multiple studies (Proia et al., 2006). Mutations in important growth control genes, such as *TP53* and *RAS*, from oral cavity tumours of smokeless tobacco users have also been observed frequently and are likely to be the result of DNA damage (IARC, 2007).

Conclusions

There is considerable coherence between established target tissues for the carcinogenicity of smokeless tobacco in humans – the oral cavity, oesophagus, and pancreas – and target tissues in rats treated orally with NNN or NNK, which are constituents of all smokeless tobacco products and are present in commonly used products at concentrations higher than those of other strong carcinogens. There is also coherence between the mechanisms by which NNN and NNK induce cancer in rats, via DNA adducts and their consequent effects, and observations in humans. There is less coherence between carcinogenicity and mechanistic aspects of smokeless tobacco exposure per se in laboratory animals and humans, in part because of operational difficulties in carrying out carcinogenicity studies, and perhaps because the right questions have not been addressed with respect to mechanisms.

Acknowledgement

This research on tobacco-specific nitrosamines is supported by grant CA-81301 from the United States National Cancer Institute.

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PART 1.

CONCORDANCE BETWEEN CANCER IN HUMANS AND IN EXPERIMENTAL ANIMALS

CHAPTER 5.

Tobacco smoke and its constituents

Stephen S. Hecht and David M. DeMarini

This chapter addresses the concordance in studies of carcinogenicity and mechanisms between experimental animals and humans for tobacco smoke and its constituents. Volume 100E of the *IARC Monographs* updated the literature on tobacco smoke and the evaluations of its carcinogenicity (IARC, 2012b). It concluded that there was *sufficient evidence* that tobacco smoking causes multiple types of cancer in humans, including (to varying extents) cancers of the lung, oral cavity, pharynx, oesophagus, stomach, colorectum, liver, pancreas, nasal cavity and paranasal sinuses, larynx, uterine cervix, ovary (mucinous), urinary bladder, kidney, ureter, and bone marrow (myeloid leukaemia).

Coherence: carcinogenicity of tobacco smoke in humans versus experimental animals

Volume 100E and previous *IARC Monographs* and certain reviews on tobacco smoke have summarized the literature on animal studies of the carcinogenicity of tobacco smoke (IARC, 1986, 2004, 2012b; Hecht, 2005). These studies demonstrated that cigarette smoke can induce tumours of the lung and nasal cavity in mice and rats and tumours of the larynx in hamsters. Some recent studies not included in the evaluations in Volume 100E have consistently established the carcinogenicity of cigarette smoke to the lung in the A/J mouse, where it produces adenoma and adenocarcinoma, as well as causing emphysema (Stinn et al., 2010, 2013). The A/J mouse, which is highly susceptible to lung tumour development, appears to present a relatively reproducible system for the induction of lung tumours, both benign and malignant, by cigarette smoke.

Although these studies have established animal models for the study of tobacco smoke carcinogenesis and, in aggregate, support the epidemiological findings that smoking is a cause of cancers of the lung and larynx in humans, specific problems associated with animal studies of tobacco smoke exposure have been recognized. Many of these issues result from the fact that most laboratory animals are obligate nose breathers and, thus, do not inhale tobacco smoke *voluntarily and habitually* in the same way in which humans smoke tobacco products (Wynder and Hoffman, 1967). Constant whole-body exposure of rodents to cigarette smoke, often at relatively high concentrations, produces avoidance reactions, stress, weight loss, and other indicators of toxicity that are widely different from

the human responses to voluntary inhalation driven by the desire for recurring small doses of nicotine.

There are some mechanistic differences as well. For example, mutations in the *KRAS* gene are frequently observed in lung adenocarcinoma in humans, as are K-*ras* mutations in lung adenocarcinoma in mice; however, the mutation frequency is not increased and the mutation spectrum is not altered in mice exposed to cigarette smoke (Hutt et al., 2005; DHHS, 2010; Stinn et al., 2013). Taken together, there is only moderate concordance between the carcinogenic and mechanistic effects of tobacco smoke evident in laboratory animals and epidemiological observations in humans (Witschi, 2007).

Concordance: carcinogenicity of tobacco smoke in humans versus carcinogenicity of tobacco smoke constituents in experimental animals

There is considerable concordance between the known carcinogenic properties of many tobacco smoke constituents and the multiple target tissues of tobacco smoke as demonstrated in epidemiological studies (IARC, 2012b).

It should be noted that carcinogenicity assays of pure compounds generally do not suffer from the above-mentioned operational difficulties with respect to tobacco smoke (Witschi, 2007).

With respect to lung cancer, multiple polycyclic aromatic hydrocarbons (PAHs) and the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) are found in the smoke of all cigarettes tested to date (IARC, 2004). There is abundant evidence attesting to the ability of PAHs to induce lung cancer in laboratory animals (IARC, 2010). Similarly, NNK is a powerful lung carcinogen, inducing adenocarcinoma of the lung in rats, mice, and hamsters independent of the route of administration and frequently at very low doses (Hecht, 1998). Thus, PAHs and NNK are widely considered to be causes of lung cancer in smokers. PAHs – and their diol epoxide metabolites in particular – produce mutations in *TP53* and *KRAS* that are similar to those observed in lung tumours from smokers (DHHS, 2010). In recent nested case–control studies within prospective cohorts, biomarkers of PAH and NNK exposure were associated with risk of lung cancer, after correction for duration and intensity of smoking (Hecht et al., 2013).

1,3-Butadiene is another compound likely to be involved in the etiology of lung cancer in smokers. It is found in relatively high concentrations in tobacco smoke and is a powerful lung carcinogen in mice, but not in rats (IARC, 2008).

Other tobacco smoke compounds with the lung as a target tissue/organ in some animal studies include isoprene, ethylene oxide, ethyl carbamate, benzene, and various metals (Hecht, 2011).

The oral cavity, pharynx, and oesophagus of rats are established target tissues of the tobacco-specific nitrosamine *N*′-nitrosonornicotine (NNN), and in particular its (*S*) enantiomer (Hecht, 1998; Balbo et al., 2013). NNN is found in the smoke of all tobacco products (IARC, 2007). *N*-nitrosodiethylamine is another tobacco smoke constituent that induces oesophageal tumours in rats, although its levels in smoke are considerably lower than those of NNN. One nested case–control study found a strong relationship between levels of NNN and its glucuronides in urine, collected years before diagnosis, and oesophageal cancer, but not lung cancer, in smokers, after correction for duration and intensity of smoking (Yuan et al., 2011). This indicates considerable concordance between target tissues of NNN and NNK in rats and observations of cancer incidence in smokers (Stepanov et al., 2014).

Carcinogenicity studies in laboratory animals and studies in humans exposed occupationally have established aromatic amines such as 4-aminobiphenyl and 2-naphthylamine as human bladder carcinogens (IARC, 1987, 2012a). These and other aromatic amines are components of mainstream cigarette smoke (Xie et al., 2013). There is also considerable mechanistic evidence from studies of haemoglobin adducts consistent with the proposal that 4-aminobiphenyl is responsible for bladder cancer in smokers (Castelao et al., 2001; IARC, 2012a).

Benzene is a leukaemogen in humans, and it occurs in considerable quantities in cigarette smoke (IARC, 1987, 2012a). The uptake of benzene by smokers has been demonstrated conclusively by biomarker studies (Hecht et al., 2010). Thus, it is likely that benzene is responsible for leukaemia in smokers, although it does not cause leukaemia in rodents (IARC, 2012a).

Multiple tobacco smoke carcinogens have produced tumours of the upper respiratory tract. Tumours of the larynx, nose, and trachea as well as of the pharynx and oesophagus were induced in inhalation studies with benzo[*a*]pyrene, an archetypal PAH, in hamsters (IARC, 2010). Tumours of the nose have also been observed in rats treated with tobacco-specific nitrosamines (Hecht, 1998; Balbo et al., 2013). Inhalation

studies with formaldehyde and acetaldehyde produced nasal tumours (IARC, 1985, 2006, 2012a).

Tobacco smoke contains compounds that are carcinogenic to the colorectum in rats, most notably certain heterocyclic aromatic amines (IARC, 2004, 2012b; Hecht, 2012). With respect to induction of liver cancer by tobacco smoke, there is coherence with furan and *N*-nitrosodimethylamine, which are liver carcinogens in rats (Peto et al., 1991; NTP, 1993), whereas NNK and its major metabolite 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) induce pancreatic cancer in rats (Hecht, 1998).

Collectively, there is considerable concordance between established sites of tobacco smoke carcinogenicity in humans and target tissues in experimental animals of individual carcinogens present in tobacco smoke.

Concordance: overall mechanism of cancer induction in humans versus laboratory studies

Fig. 5.1 presents a widely accepted mechanistic framework describing the events that occur in smokers and lead to the eventual development of lung cancer (IARC, 2004; DHHS, 2010, 2014). This scheme is for lung cancer because it is for this disease that the most data are available.

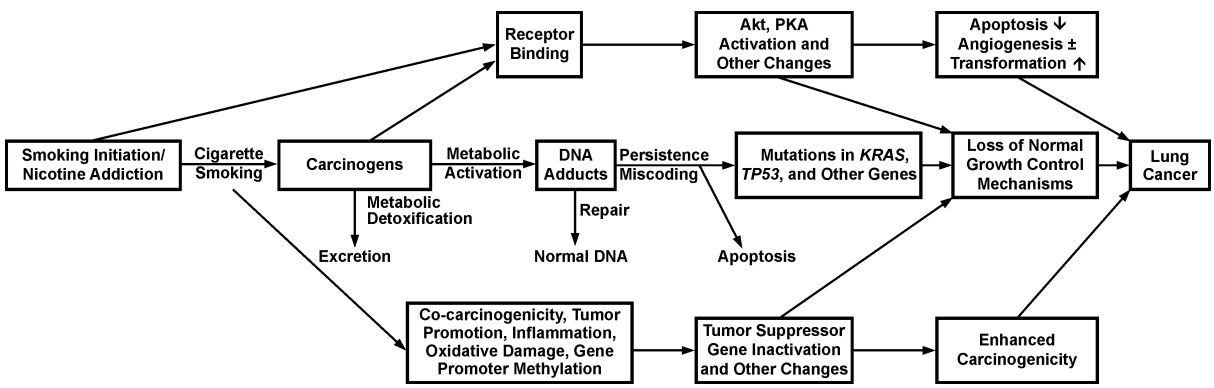
The central pathway of Fig. 5.1 in particular shows great coherence with established genotoxic mechanisms by which many carcinogens, including most of the more than 70 established carcinogens in cigarette smoke, drive the process of cancer induction. Thus, exhaustive mechanistic studies carried out both in vitro and in laboratory animals since the middle of the 20th century and continuing today provide solid evidence that most carcinogens, either directly or after metabolism catalysed by multiple cytochrome P450 enzymes, react with nucleophilic sites in DNA to form covalent binding products called DNA adducts.

There are cellular repair systems that have evolved to repair these DNA adducts and restore the normal structure of DNA. These repair systems are crucial because certain rare DNA repair-deficiency syndromes, such as xeroderma pigmentosum, lead to a high susceptibility to cancer development. Thus, DNA adducts, if left unrepaired, can persist and cause DNA replication errors that may lead to mutations. If these mutations occur in important regions of critical growth control genes, such as the oncogene *KRAS* or the tumour suppressor gene *TP53*, cellular growth processes become severely dysregulated, resulting in uncontrolled cell proliferation and cancer.

There are convincing data from studies of smokers and lung cancer patients that illustrate coherence of these observations in humans with the results observed in the plethora of mechanistic studies noted above. Carcinogen uptake by smokers has been unequivocally demonstrated by biomarker studies that compare levels of carcinogens and their metabolites in the urine of smokers and non-smokers (Hecht et al., 2010). These studies leave no doubt that exposure to multiple carcinogens, including tobacco-specific nitrosamines, PAHs, aromatic amines, and various volatile compounds including benzene and 1,3-butadiene, is significantly higher for smokers than for non-smokers.

These and related studies also show that virtually all of these carcinogens are metabolized by cytochrome P450 enzymes, resulting

Fig. 5.1. Mechanistic framework describing events involved in lung carcinogenesis in smokers. Adapted with permission from DHHS (2010).



in the formation of highly reactive metabolites that react with DNA to produce adducts. The induction of the cytochrome P450 1A1 enzyme in the lungs of smokers via activation of the aryl hydrocarbon receptor, resulting in the conversion of benzo[*a*]pyrene and related compounds to their DNA-reactive forms, is a frequently observed and consistent finding in the literature on the effects of cigarette smoking (DHHS, 2010).

Many studies have demonstrated the presence of multiple DNA adducts in the lungs of smokers, generally at higher levels than those in non-smokers. Although there is still room for further elaboration of the specific DNA adducts involved in this process, there can be little doubt about the higher levels of DNA damage in the lung tissue of smokers compared with non-smokers (IARC, 2004; Phillips and Venitt, 2012).

Consistent with these data are the common findings of mutagenicity in urine of smokers and sister chromatid exchange in peripheral lymphocytes of smokers (IARC, 2004).

Multiple recent studies with currently available DNA sequencing methods have demonstrated that DNA adducts in the lungs of smokers result in mutations.

Greenman et al. (2007) studied mutations in the coding exons of multiple protein kinase genes in lung cancer and other cancers. Lung cancers were among those with the most somatic mutations (4.21 per megabase); the authors attributed this to frequent exposure to exogenous mutagens (Greenman et al., 2007).

In another study, 188 primary lung adenocarcinomas were sequenced. Analysis of 247 megabases of tumour DNA sequence identified 1013 non-synonymous somatic mutations in 163 of the 188 tumours, including 915 point mutations, 12 dinucleotide mutations, 29 insertions, and 57 deletions. The analysis identified 26 genes mutated at significantly elevated frequencies, including *TP53*, *KRAS*, *CDKN2A*, and *STK11*, consistent with other studies and with the known involvement of these genes in growth control. Mutations were found most frequently in *TP53* and *KRAS* (Ding et al., 2008).

Another study examined a small cell lung cancer cell line. More than 22 000 somatic substitutions were identified, among which were 134 in coding exons. G → T transversions were the most common (34%), followed by G → A transitions (21%) and A → G transitions (19%), similar to earlier data in many studies (Pleasance et al., 2010).

Another investigation focused on a non-small cell lung cancer from a patient aged 51 years who had smoked 25 cigarettes per day for 15 years before excision of the tumour, which was histologically characterized as an adenocarcinoma. Single nucleotide variants were common, mostly at GC base pairs, frequently G → T transversions. Approximately 17.7 mutations per megabase were observed, for a total of more than 50 000 single nucleotide variants. At least eight genes in the *EGFR-RAS-RAF-MEK-ERK* pathway were either mutated or amplified (Lee et al., 2010).

These results are fully consistent with those reported earlier (DHHS, 2010) and with data in the Catalogue of Somatic Mutations in Cancer (COSMIC) database (http://www.sanger.ac.uk/genetics/CGP/cosmic/) and the IARC TP53 database (http://www-p53.iarc.fr/), which store and display somatic mutations in *TP53* and *KRAS* as well as other genes important in cancer. Overall, these results are coherent with the induction of multiple mutations in critical growth control genes by metabolically activated carcinogens in cigarette smoke, although it should be recognized that other processes downstream of carcinogen exposure probably also contribute to the mutation load.

In aggregate, these studies present a coherent mechanism based on multiple studies, including chemical analyses, measurements of mutation induction, and tests in laboratory animals as well as biochemical and molecular biological evaluations of human tissues, blood, and urine. The data are consistent and convincing with respect to the central track of Fig. 5.1.

It is clear that other processes are involved. Certain compounds in tobacco smoke, or their metabolites, may interact directly with cellular receptors. This can lead to activation of protein kinases, growth receptors, and other molecules that can contribute to carcinogenesis (Chen et al., 2011). It is well established that tobacco smoke contains inflammatory substances, resulting in enhanced pneumocyte proliferation, activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), and tumour promotion (Takahashi et al., 2010). There are also co-carcinogens that undoubtedly contribute to the overall mechanism of tobacco smoke carcinogenesis. Furthermore, cigarette smoke induces oxidative damage, altered gene promoter methylation, dysregulation of gene expression by microRNAs, and chronic cell injury and cytotoxicity with regenerative proliferation as an amplifying factor, all of which can

contribute to the overall carcinogenic effect (IARC, 2012b; Milara and Cortijo, 2012; Momi et al., 2014).

In summary, cigarette smoking represents a potent combination of biological effects associated with carcinogenesis, coherent with landmark publications dating back more than 60 years (Hecht and Szabo, 2014).

Disclaimer

We thank J.A. Ross and C.E. Wood for their helpful comments on this manuscript. This article was reviewed by the National Health and Environmental Effects Research Laboratory, United States Environmental Protection Agency and approved for publication. Approval does not signify that the contents reflect the views of the agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

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PART 1.

CONCORDANCE BETWEEN CANCER IN HUMANS AND IN EXPERIMENTAL ANIMALS

CHAPTER 6.

Anticancer agents: qualitative and quantitative aspects

Kari Hemminki and Harri Vainio

Introduction

Typically, regulation of human exposure to carcinogenic compounds is based mainly on qualitative considerations for compounds that cause cancer in experimental animals. This approach is based on the old paradigm of using animal models to understand human physiology and pathology; in the regulatory setting, no alternatives to this paradigm have been available specifically in relation to cancer (Maronpot et al., 2004).

Quantitative aspects of carcinogenesis, including estimates of carcinogenic potency in animals and in humans, would have both regulatory and scientific implications. For animals, a systematic assessment of potency based on bioassay data has been generated from values of TD_{50}, i.e. the chronic dose rate (in mg/kg body weight [bw]/day) that is estimated to reduce the proportion of tumour-free animals by 50% (Gold et al., 2005). For humans, exposures to ionizing radiation, occupational carcinogens, and tobacco smoke have been the primary sources of quantitative data on cancer risks, including considerations of dose, duration of exposure, and latency period (i.e. time from exposure to occurrence of cancer) (Breslow and Day, 1987; Moolgavkar et al., 1999; Brenner et al., 2003; Pierce and Vaeth, 2003; Preston et al., 2003, 2004; IARC, 2004). However, because of the complexities of these various human exposures, comparisons of potency assessments with data from animals have been hampered.

An additional source of quantitative data on cancer risks is the group of patients who are survivors of first primary cancers after treatment with anticancer therapy and who are monitored for treatment-related risks of second primary cancers (Travis, 2006; Travis et al., 2006). Active research on second cancers has been carried out since the 1980s, but because of the increasing numbers of patients enrolled and the extended periods of follow-up, the more recent studies provide the most comprehensive evidence on the magnitude of the effects

Table 6.1. Tumour sites and histological types of cancer induced in humans and in rodents after exposure to anticancer agents

Agent	Tumour sites and histological types		
	Humans	**Rats**	**Mice**
Cyclophosphamide	AML Bladder cancer	Lymphoma Leukaemia Mammary adenoma Transitional cell carcinoma of the bladder Neurogenic sarcoma	Lymphoma Acute lymphocytic leukaemia Mammary carcinoma Lung cancer Liver cancer
Chlorambucil	AML	Lymphoma Myeloid leukaemia Mammary carcinoma	Lymphoma Myeloid leukaemia
Melphalan	AML	Retroperitoneal sarcoma	Lymphoma
Thiotepa	Leukaemia	Lymphocytic leukaemia Uterine sarcoma Squamous cell cancers of the skin and the ear canal	Lymphoma Lymphocytic leukaemia

AML, acute myeloid leukaemia.
Source: Compiled from IARC (2012).

(Hijiya et al., 2007; Hodgson et al., 2007; Maule et al., 2007; Hemminki et al., 2008; Swerdlow et al., 2011). Although these studies present valuable data on many exposure-related aspects, relevant treatments are seldom based on single agents or single modalities, and individual carcinogens can rarely be singled out. Nevertheless, striking new data from these studies show increased risks of almost all site-specific cancers that emerge during the follow-up period. Such data challenge the "canonical" site-specificity of carcinogenesis. In their review of human carcinogens, Cogliano et al. indicate that no agents classified as *carcinogenic to humans* (Group 1) are identified as causing prostate cancer (Cogliano et al., 2011). However, some evidence is available. The risk of prostate cancer is significantly increased in survivors of non-Hodgkin lymphoma after chemotherapy, for those diagnosed at age 40–49 years (Hemminki et al., 2008). Anticancer agents are also used in some other cases, such as for certain autoimmune diseases, but even if a single anticancer agent is given, other medication and the inherent cancer risk of some autoimmune conditions may limit the applicability of the results.

In this chapter, data on anticancer agents from Volume 100A of the *IARC Monographs* are used to make qualitative comparisons between cancers induced in humans and in experimental animals, with reference to the possible underlying mechanisms. Furthermore, quantitative comparisons of carcinogens with respect to potency in humans and in experimental animals are discussed. This review is limited to anticancer agents for which the evidence of carcinogenicity was considered to be *sufficient* both in humans and in experimental animals: cyclophosphamide, chlorambucil, melphalan, and thiotepa (IARC, 2012). Of the anticancer agents included in Volume 100A, the current selection does not include busulfan, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea (methyl-CCNU), treosulfan, and some mixtures of anticancer agents for which evidence is *sufficient* in humans but evidence is *limited* or lacking in animals.

Therapeutic applications and trends in use

The four anticancer agents discussed here were first used in clinical practice in the 1960s, but since then the clinical indications have been narrowed and their therapeutic use has declined, with the possible exception of cyclophosphamide.

Cyclophosphamide may be used alone for the treatment of several types of cancer, but most often it is administered in combination with other drugs. Diseases for which cyclophosphamide is the recognized treatment include breast cancer,

Table 6.2. Values of TD_{50} (the chronic dose rate estimated to reduce the proportion of tumour-free animals by 50%) for anticancer agents in rodents

Agent	TD_{50} (mg/kg bw/day)			
	All tumours combined		Haematopoietic malignancies	
	Rats	Mice	Rats	Mice
Cyclophosphamide	2.2[a]	2.8	3.4[b]	7.9
Chlorambucil	0.7	0.1	1.6	0.6
Melphalan	0.6	0.1	0.9	0.5
Thiotepa	0.04	0.07	0.2	0.2

bw, body weight.
[a] Bladder tumours in rats: TD_{50} = 21 mg/kg bw/day (Gold et al., 1987).
[b] From Gold et al. (1987).
Source: Adapted with permission from Kaldor et al. (1988), copyright 1988, with permission from Elsevier.

lymphoma, leukaemia, sarcoma, and ovarian cancer. Cyclophosphamide is also used for treatment of diseases other than cancer, such as nephrotic syndrome and many autoimmune diseases, including Wegener granulomatosis, rheumatoid arthritis, lupus erythematosus, mycosis fungoides, and several forms of vasculitis.

The current clinical use for chlorambucil mainly involves treatment of chronic lymphocytic leukaemia. Chlorambucil may also be used for treatment of non-Hodgkin lymphoma, Waldenström macroglobulinaemia, polycythaemia vera, trophoblastic neoplasms, and ovarian cancer. Chlorambucil has also been applied as an immunosuppressive drug for various autoimmune and inflammatory conditions.

The use of melphalan has declined for treatment of most cancers, but since about 2000 it has been given in high doses to patients with myeloma in combination with autologous stem cell transplantation.

Thiotepa has previously been used in the palliation of a wide variety of neoplastic diseases. It may still be prescribed in intravesical chemotherapy for bladder cancer.

Tumour sites

Tumour sites and histological types of cancer induced in humans and in rodents by the four anticancer drugs are listed in Table 6.1. In humans, cyclophosphamide causes acute myeloid leukaemia and bladder cancer of undefined histology. Lymphoma, leukaemia, and mammary carcinoma have been detected in rats and mice after administration of cyclophosphamide. In rats, transitional cell carcinoma of the bladder and neurogenic sarcoma have been reported. In mice, cancers of the lung and liver have been detected. Chlorambucil causes acute myeloid leukaemia in humans. Lymphoma and leukaemia have been detected in rats and mice, as well as mammary carcinoma in rats. Melphalan causes acute myeloid leukaemia in humans, retroperitoneal sarcoma in rats, and lymphoma in mice. Thiotepa causes leukaemia in humans and lymphocytic leukaemia in rats and mice. It has been reported to induce uterine sarcoma and squamous cell cancers of the skin and the ear canal in rats and lymphoma in mice.

Carcinogenic potency

Gold et al. have systematically analysed the carcinogenic potency of compounds tested in animal experiments in the context of the United States National Toxicology Program (Gold et al., 1987, 2005). These data were used by Kaldor et al. to quantify the carcinogenic potency of anticancer agents (Kaldor et al., 1988). With respect to potency, it should be noted that low daily doses producing cancer (i.e. low TD_{50} values) indicate high carcinogenic potency.

Data on rats and mice from Kaldor et al. (1988) are collected in Table 6.2. For ease of analysis, results for male and female rodents were averaged. When information was lacking in the paper by Kaldor et al., data were taken from other sources, as indicated. The TD_{50} values for all tumours combined are lower than those for haematopoietic malignancies. According to these data, thiotepa is the most potent carcinogen, with TD_{50} values of 0.04 mg/kg bw/day in rats and 0.07 mg/kg bw/day in mice for all tumours combined. Chlorambucil and melphalan

Table 6.3. Estimated carcinogenic potency (10-year cumulative incidence [%] divided by total dose in grams) in humans of anticancer agents

Agent	Leukaemia			Bladder cancer
	From Kaldor et al. (1988)	Calculated[a]		
		Low dose	High dose	
Cyclophosphamide	0.28	–	0.04	0.02[b], 0.1[c]
Chlorambucil	4.2, 1.8	16.5	1.4	–
Melphalan	18.7, 3.3	14.1	11.5	–
Thiotepa	–	55.3	3.2	–

[a] Calculated from Kaldor et al. (1990).
[b] Calculated from Kaldor et al. (1995).
[c] Calculated from Travis et al. (1995).

are equally potent as carcinogens, whereas cyclophosphamide is weaker by approximately an order of magnitude. After treatment of rats with cyclophosphamide, bladder cancer was detected with a TD_{50} of 21 mg/kg bw/day, i.e. an order of magnitude lower than the value for all tumours combined (Gold et al., 1987).

The measure of potency used by Kaldor et al. was the 10-year cumulative incidence of leukaemia (a percentage) divided by the total administered dose in grams; thus, a large number indicates high potency (Kaldor et al., 1988). These data are shown in Table 6.3. Information was lacking for thiotepa, but of the remaining compounds melphalan was the most potent, with values of 18.7 and 3.3 from two separate studies. Chlorambucil showed an intermediate potency, which was an order of magnitude higher than that of cyclophosphamide.

More recent data were added to Table 6.3 from studies in which the anticancer agent was the principal drug used and no radiotherapy was applied. Kaldor et al. published a multinational study of secondary leukaemias in women after treatment for primary ovarian cancer (Kaldor et al., 1990). The potency according to Kaldor et al. was calculated from the cumulative baseline incidence of leukaemia in Sweden of 0.2 per 10 years, multiplied by the relative risk given in the relevant paper; the product was then divided by the median doses cited for the low dose and the high dose (Kaldor et al., 1988). The low and high doses differed widely, and the calculated potency values were clearly higher for the low dose than for the high dose. Thiotepa and melphalan were the most potent drugs, followed by chlorambucil and the much weaker cyclophosphamide. The potency of cyclophosphamide to induce bladder cancer was also calculated, according to the data from two studies; in the first study, bladder cancer was diagnosed in women after treatment for ovarian cancer (Kaldor et al., 1995), and in the second study, bladder cancer was diagnosed in survivors of non-Hodgkin lymphoma (Travis et al., 1995). The use of a cumulative baseline incidence of bladder cancer in women of 0.4 per 10 years for the data of the first study and a sex-adjusted incidence of 0.9 per 10 years for the second study resulted in potency values of 0.02 and 0.1, respectively (Table 6.3). Thus, the potency of cyclophosphamide determined in relation to bladder cancer was lower than its potency in the haematopoietic system. A similar outcome was evident in the rodent studies (Table 6.2).

Mechanisms of action

Cyclophosphamide is activated through a cytochrome P450-mediated reaction to yield phosphoramide mustard and acrolein, both of which can bind to DNA. Phosphoramide mustard undergoes rapid dephosphoramidation, which in neutral in vitro conditions proceeds with a half-life of 8 minutes, resulting in the formation of nornitrogen mustard (Hemminki, 1985). Because most of the metabolic activation of cyclophosphamide takes place in the liver, it seems likely that a considerable proportion of DNA binding in peripheral tissues is in fact mediated by nornitrogen mustard (Hemminki, 1985). As summarized in Volume 100A of the *IARC Monographs*, cyclophosphamide has several endpoints indicative of genotoxic effects

in humans, including DNA damage as measured by the comet assay, mutations at the *HPRT* locus, and sister chromatid exchange. Historically, cyclophosphamide has been included in several genetic structure–activity studies (Vogel et al., 1996, 1998).

Chlorambucil and melphalan are direct-acting derivatives of nitrogen mustard, and thiotepa is a direct-acting trifunctional derivative of aziridine. These compounds bind to DNA and give a positive response in a wide spectrum of assays for genomic injury, including tests for cytogenetic damage, specifically as indicated by chromosomal aberrations and sister chromatid exchange in patients. These drugs have also been included in several genetic structure–activity studies (Vogel et al., 1996, 1998).

Conclusions

For the anticancer drugs cyclophosphamide, chlorambucil, melphalan, and thiotepa, the data summarized in this chapter show that the target sites for which there is *sufficient evidence* of carcinogenicity are generally similar in rodents and humans, particularly for bladder cancer induced by cyclophosphamide.

Anticancer agents allow unique comparisons of carcinogenic potency among species, because the doses administered to humans and animals are known. Cancer treatment has become increasingly multimodal and involves the use of multiple drugs; this makes it difficult to single out individual agents. Also, survival rates have risen and the probability of detecting second tumours has increased. It is unclear why there is not more research activity to follow up other patient groups who receive anticancer agents, such as patients with autoimmune diseases. The current potency data for four anticancer drugs suggest that the TD_{50} values for rats and mice are reasonably homogeneous and consistent. As a carcinogen, cyclophosphamide was the least potent and thiotepa was the most potent agent in any of the rodent models analysed. In humans, cyclophosphamide was the least potent and thiotepa and melphalan were the most potent compounds to induce secondary cancers.

Acknowledgements

This research was supported by the Deutsche Krebshilfe, the Swedish Council for Working Life and Social Research, and the EU FP7 grant number 260715.

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PART 1.

CONCORDANCE BETWEEN CANCER IN HUMANS AND IN EXPERIMENTAL ANIMALS

CHAPTER 7.

Polycyclic aromatic hydrocarbons and associated occupational exposures

Charles William Jameson

Properties of PAHs

Most polycyclic aromatic hydrocarbons (PAHs) with potential biological activity have been determined to have a molecular structure that ranges in size from two to six fused aromatic rings (IARC, 2010). The physicochemical properties of these PAHs that are critical to their biological activity vary greatly, because their molecular weights cover a vast range.

Aqueous solubility of PAHs decreases approximately logarithmically with increasing molecular mass (Johnsen et al., 2005). Two-ring PAHs, and, to a lesser extent, three-ring PAHs, dissolve in water; this makes them more readily available for biological uptake and degradation (Mackay and Callcott, 1998; Choi et al., 2010). Furthermore, two- to four-ring PAHs volatilize sufficiently to appear in the atmosphere predominantly in gaseous form, although the physical state of four-ring PAHs can depend on temperature (Atkinson and Arey, 1994; Srogi, 2007).

In contrast, PAHs with five or more rings have low solubility in water and low volatility. They therefore occur predominantly in solid form, bound to particulates in polluted air, soil, or sediment (Choi et al., 2010). In the solid state, these compounds are less accessible for biological uptake or degradation, which means that their persistence in the environment is increased (Johnsen et al., 2005; Haritash and Kaushik, 2009).

The properties that influence the biological activity of PAHs include their vapour pressure, their adsorption on surfaces of solid carrier particles, their absorption into liquid carriers, their lipid–water partition coefficient in tissues, and their limits of solubility in the lipid and aqueous phases of tissues. These properties are linked with the metabolic activation of PAHs, as well as their deposition and disposition.

PAHs share a similar mechanism of carcinogenic action in both humans and experimental animals.

This includes metabolic conversion to oxides and dihydrodiols, which in turn are oxidized to diol epoxides. These oxides and diol epoxides are the ultimate DNA-reactive metabolites of PAHs. The oxides form stable DNA adducts, and the diol epoxides form stable adducts but also unstable adducts (so-called depurinating adducts) with DNA through formation of electrophilic carbonium ions.

Occupational exposure to PAHs

Occupational exposure to PAHs occurs predominantly through inhalation and dermal contact. Industrial processes that involve the pyrolysis or combustion of coal and the production and use of coal-derived products, including coal tar and coal tar-derived products, are major sources of occupational exposure to PAHs. Workers at coal-tar production plants, coking plants, bitumen production plants, coal-gasification sites, smokehouses, aluminium production plants, coal-tarring facilities, and municipal waste incinerators are exposed to PAHs. Exposure may also result from inhalation of engine exhaust and from use of products that contain PAHs in a variety of other industries, such as mining, oil refining, metalworking, chemical production, transportation, and the electrical industry (Vanrooij et al., 1992).

Studies in Germany measured concentrations of PAHs in the breathing zone of chimney sweeps during "black work"; the PAHs in the air samples varied depending on the type of fuel burned (oil, oil/solid, or solid) (Knecht et al., 1989). Concentrations of PAHs in coal-tar products may range from less than 1% to 70% or more (ATSDR, 2002). Occupational exposure can lead to PAH body burdens among exposed workers that are considerably higher than those in the general population.

There is growing awareness that uptake of PAHs through the skin is substantial (Jongeneelen, 2001). Dermal uptake has been shown to contribute to the internal exposure of workers to PAHs; a study in the creosote industry found that the total internal dose of PAHs did not necessarily correlate with levels of inhalation exposure alone, and that dermal exposure contributed significantly (Vanrooij et al., 1992).

Classification of PAHs

The *IARC Monographs Programme* has reviewed experimental data for 60 individual PAHs (IARC, 2010). Of these 60 PAHs, one, benzo[*a*]pyrene, is classified as *carcinogenic to humans* (Group 1). Other PAHs reviewed by IARC include cyclopenta[*cd*]pyrene, dibenz[*a,h*]anthracene, and dibenzo[*a,l*]pyrene, which are classified as *probably carcinogenic to humans* (Group 2A), and benz[*j*]aceanthrylene, benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, benzo[*k*]fluoranthene, benzo[*c*]phenanthrene, chrysene, dibenzo[*a,h*]pyrene, dibenzo[*a,i*]pyrene, indeno[1,2,3-*cd*]pyrene, and 5-methylchrysene, which are classified as *possibly carcinogenic to humans* (Group 2B). It should be noted that in the evaluations of benz[*j*]aceanthrylene, benzo[*c*]phenanthrene, benzo[*a*]pyrene, cyclopenta[*cd*]pyrene, dibenzo[*a,h*]anthracene, and dibenzo[*a,l*]pyrene, the mechanistic data available for these compounds were critical for determining the overall evaluation for each one (IARC, 2010).

The remaining 45 PAHs reviewed by IARC were acenaphthene, acepyrene (3,4-dihydrocyclopenta[*cd*]pyrene), anthanthrene, anthracene, 11*H*-benz[*bc*]aceanthrylene, benz[*l*]aceanthrylene, benzo[*b*]chrysene, benzo[*g*]chrysene, benzo[*a*]fluoranthene, benzo[*ghi*]fluoranthene, benzo[*a*]fluorene, benzo[*b*]fluorene, benzo[*c*]fluorene, benzo[*ghi*]perylene, benzo[*e*]pyrene, coronene, 4*H*-cyclopenta[*def*]chrysene, 5,6-cyclopenteno-1,2-benzanthracene, dibenz[*a,c*]anthracene, dibenz[*a,j*]anthracene, dibenzo[*a,e*]fluoranthene, 13*H*-dibenzo[*a,g*]fluorene, dibenzo[*h,rst*]pentaphene, dibenzo[*a,e*]pyrene, dibenzo[*e,l*]pyrene, 1,2-dihydroaceanthrylene, 1,4-dimethylphenanthrene, fluoranthene, fluorene, 1-methylchrysene, 2-methylchrysene, 3-methylchrysene, 4-methylchrysene, 6-methylchrysene, 2-methylfluoranthene, 3-methylfluoranthene, 1-methylphenanthrene, naphtho[1,2-*b*]fluoranthene, naphtho[2,1-*a*]fluoranthene, naphtho[2,3-*e*]pyrene, perylene, phenanthrene, picene, pyrene, and triphenylene. These compounds were determined to be *not classifiable as to their carcinogenicity to humans* (Group 3), because of limited or inadequate experimental evidence (IARC, 2010).

As noted above, benzo[*a*]pyrene is the only PAH classified by IARC in Group 1. A review of the data available for this PAH indicates that the complete sequence of steps in the metabolic activation pathway of benzo[*a*]pyrene to mutagenic and carcinogenic diol epoxides has been demonstrated in humans, in human tissues, and in experimental animals. After exposure, humans

Table 7.1. Group 1 agents associated with dermal exposures to polycyclic aromatic hydrocarbons (PAHs) that cause squamous cell carcinoma of the skin in humans and in rodents

Agent	Target organ		
	Humans	**Mice**	**Rats**
Benzo[*a*]pyrene	No data	Skin	Skin
Chimney sweep soots	Skin, including scrotum	Skin	No data
Coal tar	Skin, including scrotum	Skin	No data
Coal-tar pitch	Skin, including scrotum	Skin	No data
Mineral oils, untreated and mildly treated	Skin, including scrotum	Skin	No data
Shale oils	Skin, including scrotum	Skin	No data

metabolically activate benzo[*a*]pyrene to benzo[*a*]pyrene diol epoxides that form DNA adducts. The *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide–deoxyguanosine adduct has been measured in populations (e.g. coke-oven workers and chimney sweeps) exposed to PAH mixtures that contain benzo[*a*]pyrene. The reactive *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide induces mutations in rodent and human cells. Mutations (G → T transversions) in the K-*ras* proto-oncogene in lung tumours from mice treated with benzo[*a*]pyrene are associated with *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide–deoxyguanosine adducts. Similar mutations in the *KRAS* proto-oncogene and mutations in the tumour suppressor gene *TP53* were found in lung tumours from non-smokers exposed to PAH-rich products of coal combustion that are known to contain benzo[*a*]pyrene (as well as many other PAHs). In an in vitro study, the codons in *TP53* that are most frequently mutated in lung cancer in humans were shown to be targets for DNA adduct formation and mutation induced by benzo[*a*]pyrene. The strong and extensive experimental evidence for the carcinogenicity of benzo[*a*]pyrene in many animal species, supported by the consistent and coherent mechanistic evidence from studies in exposed humans and in experimentally exposed animals, and from in vitro studies in human and animal tissues and cells, provides biological plausibility to support the overall classification of benzo[*a*]pyrene as *carcinogenic to humans* (Group 1) (IARC, 2010, 2012).

Studies of cancer in humans

There are no epidemiological studies on human exposure to individual PAHs, because these chemicals never occur in isolation in the environment but are present as components of complex chemical mixtures. PAHs are very widespread environmental contaminants, because they are formed during incomplete combustion of materials such as coal, oil, gas, wood, or waste, or during pyrolysis of other organic materials, such as tobacco. Data on the carcinogenicity of PAHs to humans are available primarily from studies in occupational settings where workers are exposed to mixtures containing PAHs. It is difficult to ascertain the carcinogenicity of the component PAHs in these mixtures, because of potential chemical interactions and the presence of other carcinogenic substances.

Certain occupations associated with high exposure to PAHs have been classified by IARC as *carcinogenic to humans* (Group 1); these include coal gasification, coke production, coal-tar distillation, chimney sweeping, paving and roofing with coal-tar pitch, and work involving mineral oils, shale-oil production, and aluminium production. In most cases the classification is based on epidemiological studies of increased cancer incidence without reference to supporting evidence from bioassays in experimental animals. The roles of individual PAHs in the genesis of cancer observed in these occupations could not be defined (IARC, 2010).

Tumour site concordance

There are six IARC Group 1 agents that cause non-melanoma tumours of the skin (Rice, 2005). Five of these are related to occupations where PAH exposures are high and are believed to be the causative agents (Table 7.1). There is a precise correlation between carcinogenicity to human skin and carcinogenicity to mouse skin for these five

Table 7.2. Group 1 carcinogens associated with inhalation exposures to polycyclic aromatic hydrocarbons (PAHs) that cause lung cancer in humans and in rodents

Agent	Target organ	Route/target organ	
	Humans	**Mice**	**Rats**
Benzo[*a*]pyrene	No data	Intraperitoneal injection of and oral exposure to soot extracts/ lung	Intratracheal and intrapulmonary instillation of soot extracts/lung
Chimney sweep soots	Lung	No data	Intratracheal instillation of soot extracts/ lung
Coal-tar vapours from coke ovens	Lung	Inhalation/lung	Inhalation/lung
Soots and vapours from aluminium production	Lung, bladder	No data	No data

PAH-associated exposures when the complex mixtures isolated from the occupational environment are applied topically.

In 1775, Pott made the pioneering observation that cancer of the scrotum in chimney sweeps was an occupational disease resulting from direct contact with soot (Pott, 1775). All five established PAH-based chemical carcinogens for human skin to which exposures occur by direct dermal contact are complex mixtures: coal tar, coal-tar pitch, untreated and mildly treated mineral oils, shale oils, and soots. Because these mixtures contain PAHs, all have a genotoxic component to their mode of action in rodents. Most of the individual PAHs classified by IARC as either *probably carcinogenic to humans* (Group 2A) or *possibly carcinogenic to humans* (Group 2B) (listed above) are genotoxic and have been shown to cause skin cancer and/or be initiators of skin cancer in rodents (IARC, 1983, 2010).

Soots and vapours from coke production, aluminium production, and related industries also cause lung cancer in humans, but only extracts of soot and vapours from coke production have been tested in rodents by an appropriate route (Table 7.2). Both mice and rats developed lung tumours after inhalation of coal-tar vapours from coke ovens. Soot extracts caused lung tumours in rats after intratracheal instillation. There appears to be a good correlation between lung cancer in humans and in rodents for these two mixtures when studied by an appropriate route in mice and rats. All these complex mixtures have genotoxic activity, which is recognized to underlie their carcinogenic activity in the lung. In summary, many of the individual PAHs in these complex mixtures that have been classified by IARC as either *probably carcinogenic* or *possibly carcinogenic* to humans are also genotoxic and have been shown to cause lung tumours in rodents when administered by an appropriate route (IARC, 2010).

The various tissue compartments of the respiratory tract are metabolically active towards exogenous chemicals in both humans and experimental animals and are clearly capable of transforming many metabolism-dependent chemicals, including carcinogenic PAHs, to their chemically reactive metabolites (Rice, 2005). In the lung, metabolically active cell types include pulmonary macrophages as well as epithelial cells.

Benzo[*a*]pyrene is the only PAH that has been classified by IARC as *carcinogenic to humans* (Group 1). As indicated above, the basis for this classification is the extensive knowledge of the mechanism of carcinogenic action of benzo[*a*]pyrene in humans and experimental animals. None of the many remaining PAHs shown to be carcinogens in animals have been classified as an IARC Group 1 carcinogen, most likely because much less mechanistic information is available for these agents than for benzo[*a*]pyrene. These other PAHs are classified as either *probably carcinogenic to humans* (Group 2A) or *possibly carcinogenic to humans* (Group 2B). Most marked human occupational exposure to these compounds involves complex mixtures that contain more than one of these PAHs and that often contain other, non-PAH carcinogens. Therefore, the carcinogenic activity of these mixtures cannot confidently be ascribed to any one of their individual components.

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PART 1.

CONCORDANCE BETWEEN CANCER IN HUMANS AND IN EXPERIMENTAL ANIMALS

CHAPTER 8.

Benzene and haematological cancers

Bernard D. Goldstein and Martyn T. Smith

Introduction

Haematological cancers present an example of seeming discordance between epidemiological data and animal data, which is apparently resolved by mechanistic information. Known causes of haematological cancers in humans are ionizing radiation, chemotherapeutic agents, infectious agents such as human immunodeficiency virus (HIV), and occupational exposures to chemical agents such as formaldehyde and benzene. These agents are recognized as causes of acute myeloid leukaemia (AML), and all have been implicated in the causation of other forms of haematological cancers.

This chapter focuses on the issue of whether benzene can be considered to cause lymphoproliferative disorders in humans, as distinct from AML. Whereas epidemiological evidence establishes that benzene is a cause of human AML (IARC, 1987), long-term studies in experimental animals exposed to benzene generally have not indicated an increased risk of AML. In contrast, increased incidence of lymphoma has been readily evident in such studies, but the corresponding epidemiological evidence can be debated. However, recent mechanistic data, as well as information that has led to a reclassification of haematological neoplasms, are consistent with benzene being recognized as a cause of human lymphoproliferative disorders (Smith et al., 2007, Goldstein, 2010; Smith, 2010). Recent epidemiological evidence also strongly supports this notion (Bassig et al., 2015; Linet et al., 2015).

Studies in experimental animals

In 1974, IARC concluded that studies in experimental animals did not show evidence of carcinogenesis from exposure to benzene (IARC, 1974). Subsequently, results from two long-term studies of experimental animals exposed to benzene that did report cancer were published by a research group at New York University (NYU) in the USA (Snyder et al., 1980, cited in IARC, 1982), who exposed C57BL/6J and AKR/J mice to benzene by inhalation, and by Maltoni et al. (1989) in Bologna, Italy, who

exposed Sprague-Dawley rats by gavage. Both studies reported an increased incidence of lymphoma as well as a variety of other tumours. There are now at least seven studies, including long-term animal bioassays by the United States National Toxicology Program and by others, showing an increased incidence of lymphomas of various types (Huff et al., 1989; Farris et al., 1993; Huff, 2007; National Toxicology Program, 2007; Kawasaki et al., 2009).

In contrast, evidence that AML results from exposure to benzene has been difficult to obtain in experimental animals, and has generally been lacking in the same studies that readily showed an increase in the incidence of lymphatic cancers. In other NYU studies, 3 of 40 CD1 mice were found to have myeloproliferative disorders, and 1 of 40 Sprague-Dawley rats was found to have AML; the results are notable only because these disorders are rare in both species (Goldstein et al., 1982). The NYU group also noted an 8-fold increase in the number of early precursor cells in the bone marrow of CD1 mice exposed to benzene (Snyder et al., 1981). An increase in the number of haematopoietic progenitor cells was also noted by Cronkite et al. (1989), who also observed AML in CBA/Ca mice exposed to benzene. More recent inhalation studies by Kawasaki et al. (2009) found an increase in the incidence of AML in leukaemia-prone C3H/He mice exposed to benzene, particularly if they were *Trp53*-deficient. Thymic lymphomas and non-Hodgkin lymphoma (NHL) were also noted.

Epidemiological studies

The causal relationship between exposure to benzene and AML was accepted by the medical community long before cohort-based epidemiological studies provided unequivocal evidence. Despite several studies evaluating AML in shoe workers in Italy (Vigliani and Saita, 1964) and leather workers in Turkey (Aksoy, 1989), the relationship between benzene and AML required a conventional cohort study in a well-defined worker population (Infante et al., 1977) before causality was fully accepted.

Numerous epidemiological studies evaluating the relationship between exposure to benzene and different forms of lymphoma have yielded inconsistent results. Some studies suggest a relationship (e.g. Arnetz et al., 1991; Hayes et al., 1997; Fabbro-Peray et al., 2001; Kristensen et al., 2008; Wong et al., 2010), whereas others do not (e.g. Raabe et al., 1998; Divine et al., 1999; Bloemen et al., 2004).

Recent meta-analyses suggest an association of exposure to benzene with NHL, multiple myeloma, chronic lymphocytic leukaemia (CLL), and acute lymphoblastic leukaemia (ALL) (Infante, 2006; Smith et al., 2007; Steinmaus et al., 2008; Vlaanderen et al., 2011). However, at the Working Group meeting for Volume 100F of the *IARC Monographs*, the epidemiological evidence relating exposure to benzene to NHL or multiple myeloma was not deemed to establish causality. As discussed below, recent changes in diagnostic criteria complicate epidemiological evaluation of lymphoproliferative disorders, as does the recognition that the criteria will continue to evolve. Recently published epidemiological studies also conclude that exposure to benzene is associated with NHL and other lymphoproliferative disorders (Bassig et al., 2015; Linet et al., 2015).

Changing diagnostic criteria for haematological disorders

The classification of haematological disorders continues to evolve, including relatively recent modifications that have major implications for studying and understanding causality. These reclassifications are largely based on advances in understanding the pathological and molecular basis for haematological diseases, and on the development of assays that permit differentiation among the various haematological cell types. Myeloid leukaemias are no longer divided simply into AML and chronic myeloid leukaemia (CML). The French–American–British classification has eight distinct subtypes of AML (Bennett et al., 1989). The more recent World Health Organization (WHO) classification makes greater use of cytogenetic findings to subclassify AML (Swerdlow et al., 2008). Myelodysplasia has also been subdivided into various types, enabling recognition of the different pathways and rates of transformation to AML (Bennett et al., 1989; Swerdlow et al., 2008).

Similarly, the newer approaches to classifying NHL have divided this entity into more than 40 subtypes, while at the same time moving previously separate diseases into this general classification. NHL now includes CLL, ALL, and multiple myeloma, as well as the multitude of lymphocytic disorders that were formerly included within the NHL diagnostic rubric (Swerdlow et al., 2008). It should be noted that both CLL and ALL are no longer standalone diagnoses (Swerdlow et al.,

2008). This is not surprising, because clinicians have long known that there was little distinction in clinical course, prognosis, and response to chemotherapy between CLL and lymphomas with similar mature lymphocytes involving other organs but not blood, or between ALL and more aggressive lymphomas with similar primitive lymphocytes involving other organs but not blood. However, the ability to use molecular markers to build on this clinical insight permits reclassification.

Evidence for an overlap between myeloid and lymphoid cancers in humans

There are two lines of evidence that myeloid and lymphoid haematological cancers are closely related: (i) evidence that has led to the general acceptance that there is a common haematological stem cell, and (ii) clinical evidence based on the use of newer biological markers that show the overlap between the two types, which were previously considered to be separate. The clinical evidence includes recognition of the following: perhaps 10% of new cases of acute leukaemia have both lymphoblastic and myeloid characteristics; leukaemia that occurs in individuals with Down syndrome can be lymphoid or myeloid, as can leukaemia resulting from chemotherapy for cancer; and blast transformation of CML can be lymphoid rather than the more usual myeloid (Gassmann et al., 1997; Calabretta and Perrotti, 2004; Lee et al., 2009; Xavier et al., 2009).

Mechanism of benzene-induced haematotoxicity

Benzene, which has been a known cause of human bone marrow toxicity since the 19th century, produces its effects through metabolism to one or more haematotoxic metabolites. Like other well-established causes of AML, including ionizing radiation and chemotherapeutic agents, benzene has the ability to cause aplastic anaemia at high doses and a pancytopenic effect at lower doses, indicating toxicity to the pluripotent haematopoietic stem cell population responsible for the production of red blood cells, white blood cells, and platelets.

Lymphocytes are affected by benzene, as is evident from a decreased lymphocyte count in exposed workers (Goldstein, 1988; Rothman et al., 1996), and from the severe loss of function of lymphatic organs in experimental animals as a result of longer-term high-level exposure. Particularly pertinent to the mechanistic considerations with respect to whether benzene is a cause of lymphatic tumours is the finding in many studies of genotoxic effects in circulating lymphocytes of exposed humans or experimental animals (Vigliani and Forni, 1969; Forni, 1979; Zhang et al., 1996, 2002, 2005, 2011; Navasumrit et al., 2005). These effects are seen in bone marrow precursor cells and in circulating lymphocytes and include overt chromosomal abnormalities, translocations, deletions, and aneuploidy of several different chromosomes (Zhang et al., 2007, 2011, 2012). The evidence is consistent with exposure to benzene potentially affecting multiple sites within the genetic material of pluripotent stem cells.

Conclusions

Both IARC and the United States National Toxicology Program have in recent years changed their approach to classification of carcinogens to give additional emphasis to the role of mechanistic understanding. IARC now allows a chemical to be classified as *carcinogenic to humans* (Group 1) "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" (IARC, 2006).

In the case of benzene and lymphoproliferative cancers, there is clearly *sufficient evidence* in experimental animals and strong evidence in humans of a relevant mechanism of carcinogenicity. This evidence includes the recognition that the stem cell known to be at risk for benzene-induced AML is also responsible for lymphoproliferation, the well-known vulnerability of human lymphocytes to benzene toxicity, and the demonstration of increased chromosomal abnormalities in the circulating lymphocytes of workers exposed to benzene. It also includes the promiscuous DNA damage caused by benzene metabolites and the observation of aberrations in multiple chromosomes among workers who have been heavily exposed to benzene or who have haematological cancers attributable to benzene. These mechanistic findings bridging lymphoproliferative and myeloproliferative cancers appear to explain the seeming lack of congruence between epidemiological data and animal data for myeloid and lymphoid cancers observed with benzene, and with other agents.

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PART 1.

CONCORDANCE BETWEEN CANCER IN HUMANS AND IN EXPERIMENTAL ANIMALS

CHAPTER 9.

Human tumour viruses

Paul F. Lambert and Lawrence Banks

Among the biological agents reviewed in Volume 100B of the *IARC Monographs* (IARC, 2012) are several oncogenic viruses that are strictly species-specific, causing cancer in humans only. For this reason, the question about tumour site concordance between humans and experimental animals is not easy to answer for these agents, because cancer bioassays in animals are often lacking, and hence a proper comparison between data in humans and in experimental animals is not obvious. In this chapter, some aspects of this issue are discussed.

The use of animals as surrogate hosts for the study of human tumour viruses is often problematic, because species specificity limits the feasibility of this approach for most of these viruses. One exception is human T-cell lymphotropic virus type 1 (HTLV-1): in addition to its ability to infect humans, this virus can infect several other species – including rabbits, rats, and monkeys – and it does induce adult T-cell leukaemia/lymphoma (ATLL), albeit in monkeys only.

For other human tumour viruses, the use of humanized severe combined immunodeficiency (SCID) mice, in which the human target cell for the virus is placed in a murine host, can provide a platform for in vivo infection. However, except for Epstein–Barr virus (EBV), which causes lymphoproliferative diseases in New World monkeys and in humanized SCID mice, the use of surrogate hosts has not proven very useful for defining tumour site concordance between humans and experimental animals.

Animal models for human tumour viruses that make use of animal viruses are scarce. In fact, although many animal viruses that infect non-human primate species are related to the human tumour viruses, the incidence of cancer is low in these species (as it is in humans), which renders cancer studies costly and difficult. Moreover, animal models for tumour viruses in non-primate species often do not accurately reflect the mechanism of

the disease caused by the cognate human tumour virus. For instance, woodchuck hepatitis virus induces hepatocellular carcinoma (HCC) that is histopathologically very similar to that caused by hepatitis B virus (HBV) in humans, but it does so through a different mechanism.

Transgenic mouse models provide a powerful tool in mechanistic studies on the role of individual viral genes in cancer. Indeed, for several of the human tumour viruses described in Volume 100B of the *IARC Monographs*, transgenic mouse studies provide important mechanistic evidence. However, such transgenic models are inadequate for understanding the cancer etiology in the context of natural viral infection.

For several of the human tumour viruses classified by IARC in Group 1 (*carcinogenic to humans*), a number of studies with surrogate hosts, with cognate animal viruses, and with transgenic mouse models are reviewed below.

Epstein–Barr virus (EBV)

Human peripheral blood leukocytes injected into SCID mice increase in number and survive for at least 6 months. These mice secrete human immunoglobulin and show a specific human antibody response after immunization. The major cell populations present in peripheral blood leukocytes are found in the lymphoid tissue and blood of the SCID mice recipients, although relative proportions may differ. Mice injected with peripheral blood leukocytes from EBV-seropositive donors often develop EBV-positive B-cell lymphomas (Mosier et al., 1988).

In a later study, a highly immunodeficient mouse strain (NOG) was injected with haematopoietic stem cells from human cord blood. These mice are able to reconstitute most major components of the human haematolymphoid system including T cells, B cells, natural killer cells, macrophages, and dendritic cells, and this humanized mouse model can simulate key aspects of EBV infection. Inoculation with a high dose of EBV caused a B-cell lymphoproliferative disorder in these mice, with histopathological findings and latent EBV gene expression similar to those in immunocompromised patients. Inoculation with a low dose of EBV resulted in apparently asymptomatic persistent infection. The number of activated CD8-positive T cells increased considerably in the peripheral blood of the infected mice, and various assays demonstrated an EBV-specific T-cell response. In addition, immunoglobulin M (IgM) antibodies specific to the EBV-encoded protein BFRF3 were detected in serum from infected animals (Yajima et al., 2008).

Inoculation of cotton-top tamarins – a type of New World monkey – with EBV induced multiple EBV genome-positive malignant large-cell lymphomas that closely resembled the EBV genome-positive B-cell lymphomas observed in human allograft recipients (Young et al., 1989a). The tumours in tamarins expressed EBV nuclear antigen 1 (EBNA1) and EBNA2, EBNA leader protein, and the latent membrane protein (LMP). The expression of EBNA2 and LMP in these lymphomas in tamarins strengthens their resemblance to lymphomas observed in human transplant recipients, because these tumours in humans are also EBNA2-positive and LMP-positive (Young et al., 1989b). Both proteins are important effector molecules of EBV-induced B-cell transformation in vitro, and their expression in these lymphomas in monkeys and humans provides strong support for a direct oncogenic role of EBV in vivo.

LMP1 of EBV can transform rodent fibroblasts and is expressed in most of the human cancers associated with EBV infection. Three strains of *LMP1* transgenic mice were established that express LMP1 under the control of the immunoglobulin heavy chain promoter/enhancer. Mice of all three strains developed lymphoma, the incidence of which increased considerably with age: after 18 months, 42% of the transgenic mice had developed lymphoma. LMP1 was strongly expressed in the lymphoma tissues but was hardly expressed in normal lymphoid tissues. These results show that *LMP1*, without the expression of other EBV genes, is oncogenic in vivo, and indicate that the LMP1 protein is a major contributing factor to the development of EBV-associated lymphomas (Kulwichit et al., 1998).

The role of LMP1 was also studied in the epidermis of *LMP1* transgenic mice. Epidermal cells that carried the transgene showed a 2–3-fold increase in the mitotic index and an increased expression of proliferative cytokeratin markers. This is direct evidence that LMP1 induces proliferation in otherwise normal epithelial cells in vivo. When treated topically with 7,12-dimethylbenz[*a*]anthracene, *LMP1* transgenic mice developed small papillomas more rapidly and in larger numbers than did non-transgenic controls. Furthermore, LMP1 could replace 12-*O*-tetradecanoylphorbol-13-acetate in promoting tumour formation, but it inhibited expansion and did not stimulate progression of the papillomas to carcinomas, or to more malignant spindle cell carcinomas.

These data show that early in the carcinogenic process, LMP1 acts as a tumour promoter after chemical initiation, but it may also block expansion or progression of benign lesions (Curran et al., 2001).

A further study on *LMP1* transgenic mice showed that they have a higher incidence of lymphoma and that the progression to lymphoma correlates with higher expression levels of LMP1, compared with non-transgenic controls. Although LMP1 is expressed in all B lymphocytes of the transgenic mice, lymphoma develops in a specific subset, the B-1a lymphocytes, which is a population predisposed to clonal expansion with age. The malignant lymphocytes show constitutively active Stat3 signalling, have decreased levels of p27, and display activated Akt and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathways, properties that are associated with promoting growth and survival of B lymphocytes. The transgenic lymphomas mirror multiple aspects of EBV-induced tumours; this suggests that these properties of LMP1 are major factors in cancer development (Shair et al., 2007).

Transcripts of *LMP2A* can be expressed in resting virus-carrying B lymphocytes in healthy individuals – the reservoir of persistently latent EBV. In *LMP2A* transgenic mice, a block in surface immunoglobulin rearrangement results in the generation of B-cell receptor-negative cells, which normally would undergo apoptosis. *LMP2A* transgenic B cells develop and survive without a B-cell receptor. These data indicate that LMP2A imparts developmental and survival signals to B cells in vivo (Merchant et al., 2000). Furthermore, when co-expressed with human MYC, LMP2A accelerates the development of B-cell lymphomas in a transgenic mouse model (Bieging et al., 2009; Bultema et al., 2009). A more recent study has also shown that LMP1 and LMP2 can cooperate in the induction of epithelial squamous cell carcinomas (SCCs) when co-expressed under the control of a keratin 14 (K14) promoter in transgenic mouse models (Shair et al., 2012).

Establishment of long-term latent infections with EBV was possible in a humanized mouse model that was challenged either with wild-type EBV or with a replication-defective virus. B-cell lymphomas developed in both cases, but at a higher frequency after infection with the wild-type virus, indicating a potential role for lytic virus infection in the development of malignancy (Ma et al., 2011).

For EBV, the overall concordance between the animal models and humans with respect to the types of tumour and the identity and function of the major oncogenes and oncogene products is high.

Hepatitis B virus (HBV)

Interactive viral–chemical hepatocarcinogenesis was studied in woodchucks (*Marmota monax*) inoculated as newborns with woodchuck hepatitis virus, which is closely related to the human HBV. Starting at age 12 months, the woodchucks were fed a diet containing aflatoxin B_1 (AFB_1) (at a high dose for 4 months and then at a lower dose for lifetime). Carriers of woodchuck hepatitis virus with or without treatment with the carcinogen AFB_1 developed a high incidence of pre-neoplastic hepatic foci, hepatocellular adenomas, and HCCs, but AFB_1 treatment resulted in a much earlier appearance of hepatic neoplasms and a higher incidence of HCCs. No hepatocellular adenomas or HCCs were seen in non-infected woodchucks that received AFB_1 (but pre-neoplastic hepatic foci were seen), and no pre-neoplastic or neoplastic lesions were found in untreated controls. These results provide strong evidence of a synergistic hepatocarcinogenic effect of viral infection and dietary AFB_1 intake (Bannasch et al., 1995).

Transgenic mice that contain the *BglIIA* fragment of HBV under the transcriptional control of the mouse albumin promoter overexpress the HBV large envelope polypeptide and accumulate toxic quantities of hepatitis B surface antigen (HBsAg) in their hepatocytes. As a result, the mice develop severe, prolonged hepatocellular injury associated with an inflammatory response, followed by chronic hepatocellular regeneration, transcriptional deregulation, dysplasia, aneuploidy, hepatocellular adenoma, and eventually HCC. The incidence of HCC depends on the frequency, severity, and age of onset of the liver cell injury, which itself depends on the intrahepatic concentration of HBsAg and is influenced by genetic background and sex. Thus, the excessive expression of a single structural viral gene is sufficient to cause malignant transformation in this model. Similar events may be responsible for the development of HCC in humans after HBV infection, irrespective of the mechanism or mechanisms involved in the initial induction of liver cell injury (Chisari et al., 1989).

Transgenic mice overexpressing the HBV large envelope polypeptide suffer from hepatic injury as a result of accumulation of HBsAg (see the previous study). When treated with the hepatocarcinogens AFB_1

or diethylnitrosamine, these mice showed more rapid and more extensive formation of nodules, proliferation of oval cells, and development of adenomas and primary HCCs than did non-exposed transgenic mice. This suggests that the chronic liver damage and repair caused by overexpression of the HBV large envelope polypeptide act synergistically with chemical hepatocarcinogens to produce liver neoplasia (Sell et al., 1991).

To explain the synergistic hepatocarcinogenic effect of viral infection and dietary AFB_1 intake, it was suggested that infection with HBV and associated liver injury might alter the expression of carcinogen-metabolizing enzymes. This hypothesis was tested in the HBV transgenic mouse model described in the previous study. In these mice, the expression levels of the cytochrome P450 isozymes Cyp2a5 and Cyp3a – both involved in AFB_1 metabolism – were examined. Increases in the expression of and alterations in the distribution of Cyp2a5 were age-dependent in these mice and were associated with the extent of liver injury. Cyp3a expression was also increased, but this was less clearly related to age. These data show that expression of specific cytochrome P450 isozymes is altered in association with overexpression of the HBV large envelope polypeptide and the ensuing liver injury in this mouse model. This may have general relevance to human HCC, the etiology of which is associated with a diverse range of liver-damaging agents (Kirby et al., 1994).

The HBV X protein (HBx) is highly multifunctional. In transgenic mouse models, the expression of HBx promotes HCC (Yu et al., 1999). More recent studies have also shown cooperation between HBx and K-*ras* mutation in the development of HCC in transgenic mice (e.g. Ye et al., 2014). A truncated form of HBx that is commonly found in human HCCs also exhibits tumour-forming activity in association with exposure to diethylnitrosamine in transgenic mouse models of HCC (Quetier et al., 2015).

For HBV, the overall concordance between the animal models and humans with respect to the types of tumour is high.

Hepatitis C virus (HCV)

Role of the HCV core protein in HCV-induced steatosis

Several histological features in the liver are characteristic of chronic hepatitis C: bile duct damage, lymphoid follicles, and steatosis (fatty change). It has been suggested that the core protein of HCV functions as a transcriptional regulator that induces phenotypic changes in hepatocytes. Two independent strains of transgenic mice carrying the HCV core gene developed progressive hepatic steatosis, confirming that the core protein plays a direct role in the development of steatosis, which characterizes hepatitis C. These mice express the core protein in the liver at concentrations similar to those in the liver of patients with chronic hepatitis C. This transgenic mouse system may be a good animal model for the study of pathogenesis in human HCV infection (Moriya et al., 1997).

In a further study by the same group, several parameters of oxidative stress and redox homeostasis were measured in these transgenic mice. At age 3–12 months, the mice showed similar concentrations of phosphatidylcholine hydroperoxides and phosphatidylethanolamine in liver tissue homogenates as the non-transgenic controls. In contrast, the level of phosphatidylcholine hydroperoxides was increased nearly 2-fold in transgenic mice after age 16 months. In addition, catalase activity was increased and the concentrations of total and reduced glutathione were decreased. These mice show steatosis without inflammation early in life, and finally develop HCC from age 16 months. The HCV core protein thus alters the oxidant–antioxidant status in the liver in the absence of inflammation and may thereby contribute to or facilitate the development of HCC after HCV infection (Moriya et al., 2001).

Transgenic mice carrying the complete HCV polyprotein

In a study to determine whether expression of HCV proteins alters hepatic morphology or function in the absence of inflammation, transgenic C57BL/6 mice carrying the complete viral polyprotein (*FL-N* transgene) or viral structural proteins (*S-N* transgene) were compared with non-transgenic littermates for altered liver morphology and function. No inflammation was seen in the livers of transgenic mice, but mice expressing either transgene developed age-related hepatic steatosis. The numbers of apoptotic or proliferating hepatocytes were not increased significantly. Hepatocellular adenoma or HCC developed in older male mice expressing the *FL-N* or the *S-N* transgene, but the incidence was increased significantly only in *FL-N* transgenic mice. Neither of these tumours was observed in age-matched non-transgenic mice. Expression of viral proteins gave rise to common pathological features of hepatitis C in the absence of a specific antiviral immune response, which suggests a

metabolic or genetic host susceptibility for HCV-associated HCC (Lerat et al., 2002).

In a subsequent study by the same group, the mechanisms underlying HCV-induced defects in lipid metabolism were studied in transgenic mice that expressed the full viral protein repertoire at levels corresponding to those seen in natural human HCV infection. Expression of the full-length HCV open reading frame was associated with hepatocellular steatosis, impaired triglyceride secretion, and nuclear translocation of sterol regulatory element-binding protein 1c (SREBP1c), followed by increased transcription of lipogenic enzymes. Stress markers in the endoplasmic reticulum were expressed at similar levels in HCV transgenic mice and in non-transgenic controls. Transgenic mice expressing the full-length HCV polyprotein have reduced plasma triglyceride concentrations and develop hepatocellular steatosis in the same way as do patients with HCV infection (Lerat et al., 2009).

Effect of peroxisome proliferator-activated receptors

Peroxisome proliferator-activated receptor alpha (PPARα) is a central regulator of triglyceride homeostasis and a mediator of hepatocarcinogenesis in rodents. In a study to determine the role of PPARα in HCV core protein-induced disease, double transgenic mice were generated that carried *Pparα* (homozygous, heterozygous, and null) and the HCV core protein gene (*HCVcp*) as transgenes. Severe steatosis was observed only in *Pparα*$^{+/+}$*:HCVcp* mice, as a result of higher fatty acid uptake and decreased mitochondrial β-oxidation due to breakdown of mitochondrial outer membranes. HCC developed in about 35% of *Pparα*$^{+/+}$*:HCVcp* mice by age 24 months, but no tumours were seen in the other genotypes. These phenomena were closely associated with sustained PPARα activation: in *Pparα*$^{+/-}$*:HCVcp* mice, PPARα activation and the related changes did not occur despite the presence of a functional *Pparα* allele. Persistent activation of PPARα is essential for the pathogenesis of hepatic steatosis and HCC induced by HCV infection (Tanaka et al., 2008).

Conclusions

In the absence of animal models that develop HCC in the context of an HCV infection, various groups have reported the use of transgenic mouse models. Studies with mice expressing HCV replicons, polyproteins, or single HCV proteins as transgenes, alone or in combination, under the control of liver-specific promoters have been described by several groups (Dorner et al., 2011, 2013). There is good concordance between the outcomes observed in these mice and those in humans with HCV infection; however, questions remain about to what extent the results obtained with these experimental approaches reflect the pathological consequences of human HCV infection that contribute to HCC.

It remains a matter of considerable debate whether HCV causes HCC through a direct mechanism in which virally encoded genes contribute to carcinogenesis, or through an indirect mechanism, where the injury to the liver caused by HCV infection and host immune responses to that infection, such as inflammation, contribute to the onset of cancer. This issue may be resolved with the development of more physiological models that permit chronic and productive HCV replication. A recently developed model holds promise in this regard. Genetically engineered mice expressing two host restriction factors – human CD81 and occludin – can be infected with HCV, and in these mice sustained viraemia and infectivity can be observed for more than 12 months after infection, with the expected fibrotic and cirrhotic progression in the liver (Chen et al., 2014).

Kaposi sarcoma-associated herpesvirus (KSHV)

Species specificity of KSHV

After injection, KSHV can infect non-human primates (Renne et al., 2004), NOD/SCID mice (Parsons et al., 2006), and humanized SCID mice (Dittmer et al., 1999; Foreman et al., 2001; Wu et al., 2006; Wang et al., 2014). These infections do not result in the formation of tumours, but they confirm the viral tropism (with KSHV targeting B cells and endothelial cells) and drug susceptibility (to ganciclovir) in vivo.

In one report of KSHV infection in marmosets (*Callithrix jacchus*), viral replication was apparent in peripheral blood mononuclear cells, and a Kaposi sarcoma-like lesion developed on one of the animals (Chang et al., 2009). Viruses that are homologous to KSHV exist in the bank vole (*Myodes glareolus*), as Murid herpesvirus 68 (MHV-68), and in virtually all non-human primates (Fleckenstein and Ensser, 2007). The infection of macaques (*Macaca mulatta*) with rhesus rhadinovirus in the context of simian immunodeficiency virus (SIV) induces B-cell lymphoma and endothelial cell

hyperplasia (Mansfield et al., 1999; Wong et al., 1999). Several tumour graft models of Kaposi sarcoma and primary effusion lymphoma have been established (Boshoff et al., 1998; Staudt et al., 2004; Wu et al., 2005; An et al., 2006; Mutlu et al., 2007; Sin et al., 2007).

In humans, KSHV is associated with Kaposi sarcoma and primary effusion lymphoma. The transforming capacity of several individual KSHV proteins with respect to these two tumour types has been studied in experimental systems, particularly in transgenic mice.

Studies in transgenic mice

Transgenic mice in which individual KSHV proteins are expressed are often used to replicate aspects of the pathogenesis of KSHV. However, single transgenic models have some limitations. Whereas lymphoproliferative lesions and lymphomas in mice are easily classified on the basis of histology and marker gene expression, this is more difficult for endothelial cell tumours, which are often referred to as Kaposi sarcoma-like lesions but can easily be mistaken for fibrosarcomas.

LANA

The KSHV latency-associated nuclear antigen (LANA) is consistently expressed in all KSHV-associated tumour cells and was shown to bind the tumour suppressor proteins p53 and pRb. The contribution of this antigen to lymphomagenesis in vivo was investigated in transgenic mice that expressed LANA under the control of its own B-cell-specific promoter. All of the mice developed splenic follicular hyperplasia due to an expansion of IgM-positive, IgD-positive B cells, and 11% developed different types of lymphoma, among which were plasmacytoma, follicular B-cell lymphoma, small lymphocytic lymphoma, and composite lymphoma. These results imply that LANA can activate B cells and trigger the first step towards lymphomagenesis (Fakhari et al., 2006). The authors of the study speculated that in asymptomatic human carriers, infected B cells remain organ-resident and sustain latent persistence of KSHV; this would be consistent with the low frequency of KSHV genome-positive cells in peripheral blood lymphocytes of LANA-seropositive individuals (Whitby et al., 1995).

vFLIP

The KSHV-encoded viral Fas-associated death domain-like IL-1-converting enzyme inhibitory protein (vFLIP) is expressed in latently infected cells and plays an important role in survival and proliferation of primary effusion lymphoma cells. One function ascribed to this protein is activation of NF-κB. Transgenic mice expressing vFLIP display constitutive activation of NF-κB pathways, show an enhanced response to mitogenic stimuli, and have an increased incidence of lymphoma. These results demonstrate that the KSHV-encoded vFLIP is an oncoprotein that could contribute to the development of lymphoproliferative disorders via constitutive NF-κB activation (Chugh et al., 2005).

K1 protein

The K1 protein of KSHV is a transmembrane signalling protein. In transgenic mice that express the KSHV *K1* gene under the control of the simian virus 40 (SV40) promoter, tumours were observed that showed features of spindle cell (sarcomatoid) cancers and malignant plasmablastic lymphoma. The enhanced NF-κB activity in non-malignant lymphocytes of *K1* transgenic mice and the persistence of this activity in the lymphoma tumours that these mice develop suggest that the KSHV *K1* transgenic mouse may be a model of premalignancy (Prakash et al., 2002).

K cyclin

KSHV encodes a cyclin D homologue, K cyclin, which is thought to promote viral oncogenesis. In cultured cells, expression of K cyclin not only triggers cell-cycle progression but also engages the p53 tumour suppressor pathway, which probably restricts the oncogenic potential of K cyclin (Verschuren et al., 2002). The tumorigenic properties of K cyclin were assessed in transgenic mice in which expression of K cyclin was targeted to B and T lymphocytes via the Eμ promoter/enhancer. About 17% of K cyclin transgenic mice had developed lymphoma by age 9 months, and all lymphomas had lost p53 activity. The critical role of p53 in suppressing K cyclin-induced lymphomagenesis was confirmed by the greatly accelerated onset of B and T lymphomagenesis in all K cyclin/$p53^{-/-}$ mice compared with K cyclin/$p53^{+/-}$ and K cyclin/$p53^{+/+}$ mice, but suppression of apoptosis does not appear to be the underlying mechanism, given the very high numbers of apoptotic cells observed in all Eμ-K cyclin/$p53^{-/-}$ thymic lymphomas (Verschuren et al., 2004).

vGPCR

In transgenic mice, the expression of viral G protein-coupled receptor (vGPCR) by cells of endothelial origin triggers the development of an angioproliferative disease that resembles Kaposi sarcoma. It includes expression of angiogenic factors such

as placental growth factor, platelet-derived growth factor B, and inducible nitric oxide synthase by the vGPCR-expressing cells. Finally, continued vGPCR expression is essential for progression of the Kaposi sarcoma-like phenotype, and downregulation of vGPCR expression results in reduced expression of angiogenic factors and regression of the lesions. These findings implicate vGPCR as a key element in the pathogenesis of Kaposi sarcoma (Jensen et al., 2005).

Further support for a role of vGPCR in tumorigenesis has come from studies with MHV-68. In mice, this virus can replicate transiently before entering a latent state, but no lymphoproliferative disorders arise in immunocompetent animals. However, when a recombinant MHV-68 in which the vGPCR is replaced with the KSHV-derived vGPCR was used to infect mice, angiogenic lesions formed that had features characteristic of those seen in human Kaposi sarcoma lesions. The difference in activity between the wild-type MHV-68 and the recombinant MHV-68 was linked to differences in activation: the KSHV-derived vGPCR was constitutively active, whereas the murine vGPCR in MHV-68 was not (Zhang et al., 2015). This study provides compelling evidence for a direct role of vGPCR in the development of Kaposi sarcoma in a non-transgenic animal model.

For KSHV, the overall concordance between the animal models and humans with respect to the types of tumour and the identity and function of the major oncogenes and oncogene products is high.

Human papillomaviruses

The cell-transforming capacity of human papillomavirus (HPV)-encoded proteins has been demonstrated in various cell lines. In particular, HPV type 16 (HPV16) had transforming potential in established rodent cells (Yasumoto et al., 1986), with the principal activity residing in the E7 oncoprotein (Vousden et al., 1988). The E5 and E6 proteins of both HPV16 and HPV18 also showed transforming potential in such assays (Pim et al., 1992). The E6 protein can inactivate p53 (Scheffner et al., 1990), and as a result, E6 can abrogate cell-cycle arrest induced by a variety of DNA-damaging agents, such as actinomycin D. The normal response to DNA damage, i.e. inhibition of DNA synthesis and increase in p53 protein levels, did not occur after treatment with actinomycin D of keratinocytes that had been immortalized with HPV16 E6/E7 (Kessis et al., 1993).

Transgenic models for HPV-associated cancers

The first germline transgenic mouse model for HPV-associated cervical cancer was developed with a K14-HPV16 construct that contained the early genes of the virus under the control of the K14 transcriptional promoter, which directs the expression of these genes to the stratified epithelium of the oral cavity and the lower female reproductive tract. These mice did not develop cervical cancers spontaneously, but treatment with estrogen, sufficient to induce continuous estrus, led to a highly penetrant cervical cancer phenotype in the context of a progressive disease much like that seen in women, given that it was preceded by the onset of cervical intraepithelial neoplasia (CIN) of grades 1–3. As in women, the cancers preferentially arose in the transformation zone (Arbeit et al., 1994, 1996). All *K14-E7* transgenic mice treated with estrogen for 6 months developed high-grade dysplasia and/or cervical cancer, but *K14-E6* mice only developed cervical cancer after treatment with estrogen for 9 months (Riley et al., 2003; Shai et al., 2007, 2008).

An additional activity of HPV16 E7 that contributes to its oncogenicity is the ability to inactivate p21 (Shin et al., 2009). In transgenic mice carrying a tetracycline-regulated HPV16 *E7* transgene, the continued expression of E7 was found to be critical for the maintenance not only of cervical cancer but also of the dysplastic neoplasia that is recognized as its precursor lesion (Jabbar et al., 2009). This dependence on continued expression of E7 was observed even in the context of constitutive expression of HPV16 E6 (Jabbar et al., 2012).

Like E7, the E6 protein of HPV appears to contribute to cervical carcinogenesis through multiple activities. This protein is known to degrade p53 and other cellular targets through its interaction with the ubiquitin ligase E6AP. This enzyme was found to be critical for E6-mediated oncogenesis in the cervix (Shai et al., 2010). E6 is known to bind to several cellular proteins that contain PDZ domains (common structural regions of 80–90 amino acids in proteins involved in signalling). Transgenic mice expressing a mutant form of HPV16 E6 that is unable to bind to PDZ-domain proteins (Nguyen et al., 2003) had a reduced susceptibility to cervical cancer compared with mice expressing the wild-type E6 protein (Shai et al., 2007). It remains unclear which of the interactions with PDZ-domain proteins contribute to E6-mediated carcinogenesis in vivo, and how E7 is involved in this process (Simonson et al., 2005; Shai et al., 2007).

The same high-risk HPV types associated with cervical cancer are also linked to the development of anal cancer. In the same K14-HPV16 transgenic mouse models, the expression of the *E6/E7* transgenes gave rise to anal cancers in mice treated with 7,12-dimethylbenz[*a*] anthracene (Stelzer et al., 2010). As with the cervical lesions, the E7 oncoprotein also plays a dominant role in this case (Thomas et al., 2011).

Role of estrogen in HPV-mediated cervical carcinogenesis

Estrogen is an important cofactor in the development of cervical cancers in HPV transgenic mouse models (Arbeit et al., 1996). Tumours arising in HPV16 transgenic mice – carrying *E7* or *E6/E7* as transgenes – treated with estrogen for 9 months were much larger than those observed after 6 months of treatment. When these mice were treated with estrogen for 6 months and then kept without treatment for 3 months, they had significantly fewer, smaller, and less aggressive tumours at 9 months than those seen in mice treated for the full 9 months; thus, tumour regression was seen in the 3-month period after treatment. Estrogen therefore plays a critical role not only in the genesis of cervical cancer but also in its persistence and continued development (Brake and Lambert, 2005).

In a later study, estrogen receptor alpha (ERα) was found to be necessary for development of cervical carcinogenesis in *K14-E7* transgenic mice: exogenous estrogen failed to promote either dysplasia or cervical cancer in *K14-E7/ERα*$^{-/-}$ mice (Chung et al., 2008). Interestingly, expression of ERα in the cervical stroma was required for cervical carcinogenesis in HPV transgenic mice (Chung et al., 2013); evidence for a role of stromal ERα has also been obtained in the context of human cervical cancer (den Boon et al., 2015). ERα antagonists were effective in eliminating cervical cancer and the precancerous lesions in this animal model (Chung and Lambert, 2009). The cervical cancers in these mouse models are strictly associated with atypical squamous metaplasia, which is believed to be the precursor of cervical cancer in women.

High-risk HPV types and cancers of the head and neck

The same high-risk HPV types that are etiologically associated with anogenital cancers, particularly HPV16, are also associated with a subset of human head and neck squamous cell carcinomas (HNSCCs), most notably of the oropharynx (e.g. tonsils), the base of the tongue, and the upper oesophagus. The role of the HPV16 proteins E6 and E7 in HNSCC has been evaluated in HPV transgenic mice that express the two oncogenes *E6* and *E7* in the relevant tissues. These mice do not spontaneously develop HNSCC, but when treated with the synthetic carcinogen 4-nitroquinoline-*N*-oxide, they become more susceptible to head and neck cancers (Strati et al., 2006). The progressive disease observed in the mice treated with 4-nitroquinoline-*N*-oxide was similar to that seen in humans, and the cancers that occurred were primarily high-grade HNSCC, as observed in HPV-positive HNSCCs in humans. As with cervical cancer, *E7* proved to be the more potent oncogene (Jabbar et al., 2010), and the inactivation of pRb could not fully account for the role of the E7 protein (Strati and Lambert, 2007), whereas inactivation of both pRb and p107 could fully recapitulate the oncogenicity of E7 in HNSCC (Shin et al., 2012).

Carcinogenic potential of epidermodysplasia verruciformis-associated beta HPV types in the skin

HPV types of the genus beta, specifically HPV8 and HPV38, are associated with a rare familial benign disease termed epidermodysplasia verruciformis. Patients with this disease are at an increased risk of SCCs of the skin at sun-exposed areas. K14-HPV8 transgenic mice expressing the early genes of HPV8 in the epidermis were susceptible to spontaneous development of both benign and malignant skin cancers (Schaper et al., 2005). Unlike what is seen for the mucosal HPV types, the viral E2 protein seems to play a major role in these HPV8-induced cancers, because expression of E2 alone also results in the development of skin cancer, a process that is accelerated after irradiation with ultraviolet (UV) light (Pfefferle et al., 2008).

In the case of HPV38, *K10-E6/E7* transgenic mice were highly susceptible to multistage skin carcinogenesis, specifically when treated with UVB radiation or chemical carcinogens (Dong et al., 2005). The synergy between cutaneous HPV types and UV radiation in the development of SCCs of the skin has also been studied in transgenic SKH-hr1 hairless mice expressing in their epidermis the *E6* and *E7* genes of HPV20, which is commonly associated with SCC observed in renal transplant recipients, or of HPV27, which is only associated with benign papillomas. Upon UV irradiation, both HPV20 *E6/E7* and HPV27 *E6/E7* transgenic mice were more

susceptible to tumours compared with non-transgenic mice, and the HPV20 *E6/E7* transgenic mice had an increased incidence of malignant tumours. Alterations in the expression of both p53 and p63 were noted in the transgenic mice exposed to UV radiation (Michel et al., 2006).

For HPV, the overall concordance between the animal models and humans with respect to the types of tumour caused by mucosal HPV types and the identity and function of the major oncogenes and oncogene products is high. There are important context-dependent differences in the function of the E6 oncoprotein, depending on the anatomical site.

Human immunodeficiency virus type 1 (HIV-1)

Infectious agents can act as indirect carcinogens by causing immunosuppression. This has been shown for infection with HIV-1, which strongly increases the incidence of several human cancers. Strikingly, the majority of cancers associated with HIV-1 have another known infectious etiology, and HIV-1 infection increases their incidence considerably. Among these cancers, those associated with the herpesviruses KSHV and EBV are most strongly enhanced by immunosuppression. The same cancers are also enhanced by iatrogenic immunosuppression, as shown by their increased incidence in transplant recipients, which lends additional support to the notion that HIV-1 acts as a carcinogen mainly through this indirect effect. The most common cancers in individuals with HIV-1 infection are Kaposi sarcoma (caused by KSHV), lymphomas (many of which are EBV-positive), and cervical and anogenital carcinomas associated with HPV infection.

Because HIV-1 is species-specific, like the oncogenic herpesviruses EBV and KSHV, there are no ideal animal models for HIV-1-associated cancers. In contrast to what is observed with infectious agents that are directly oncogenic, the HIV-1 genome is not present in cancer cells. Therefore, any interaction between virus and host is indirect. Although none of the HIV-1-encoded proteins has been unequivocally shown to be directly oncogenic, some are associated with immunodeficiency, thereby indirectly promoting cancer development. In addition, there is evidence that some of the HIV-1-encoded proteins may promote cancer by other indirect mechanisms that are not dependent on immunodeficiency. There are also reports of transgenic mouse models containing HIV proviral transgenes. In some cases these animals develop lymphoproliferative disorders, including B-cell lymphomas, which show characteristics similar to those of B-cell lymphomas arising in patients with HIV infection (Curreli et al., 2013). Virus-encoded proteins including Nef, gp120, p17, and Tat have all been implicated in promoting B-cell hyperproliferation, although the strongest association in animals comes from work on the viral Tat protein.

Tat protein

The multifunctional Tat protein is the only HIV-1 protein for which there is experimental evidence of a potential role in Kaposi sarcoma. Tat is an important regulator of viral transcription; it recruits cellular transcription factors to the HIV-1 promoter, strongly stimulates HIV-1 DNA transcription, and interacts with protein–kinase complexes (Cdk9/cyclin T1, Cdk2/cyclin E), protein phosphatases, and multiple other cellular proteins (Gatignol, 2007). Tat also affects the course of HIV-1-associated disease indirectly, because it is secreted by infected cells and can enter non-infected cells (Gupta and Mitra, 2007).

Evidence that Tat is involved in oncogenesis includes its ability to induce apoptosis in neighbouring non-infected cells when secreted from infected cells, thereby increasing the susceptibility of bystander CD4-positive T cells to death induced by cross-linking (Alimonti et al., 2003). This may contribute to the massive depletion of CD4-positive T cells by apoptosis, leading to the severe immunodeficiency seen in the acquired immune deficiency syndrome (AIDS). Tat has also been shown to stimulate the growth of Kaposi sarcoma cells (Aoki and Tosato, 2007). However, when cells are removed from Kaposi sarcoma lesions and expanded in vitro, they lose the KSHV genome, and the question remains whether the “Kaposi sarcoma” cells lacking KSHV used in most of these studies represent a valid model for Kaposi sarcoma.

To investigate the role of Tat in carcinogenesis, several studies have been carried out in vivo, in transgenic mice. Transgenic mice carrying a recombinant DNA sequence containing the early region of the BK virus and the HIV-1 *Tat* gene developed skin leiomyosarcomas, squamous cell papillomas and carcinomas, adenocarcinomas of skin adnexal glands, and B-cell lymphomas. Although the incidence of HCC was low, most animals showed liver cell dysplasia of variable degree. These mice were also affected by skin lesions resembling the early stages of Kaposi sarcoma (see also Vogel et al., 1988). The *Tat* transgene was

detected intact in all the organs of the transgenic mice, and the Tat protein was expressed in essentially all tissues and organs of these animals. These BK virus/*Tat* transgenic mice may be useful in studies of the role of Tat in AIDS-associated malignancies and of the pathogenesis of Kaposi sarcoma (Corallini et al., 1993).

Because the Tat protein stimulates cell proliferation, inhibits apoptosis, displays angiogenic functions, and may be involved in the pathogenesis of Kaposi sarcoma and other tumours arising in patients with AIDS, the BK/*Tat* transgenic mice (see the previous study) may be predisposed to tumour formation. When *Tat* transgenic mice were treated with urethane, the incidence of lung tumours and lymphomas was not different between the transgenic mice and the controls, whereas the incidence of pre-neoplastic lesions and tumours in the liver was significantly higher in *Tat* transgenic mice than in control mice. This remarkable effect of urethane observed in the liver may be due to a Tat-induced predisposition, manifested as a liver cell dysplasia, spontaneously affecting most of the *Tat* transgenic mice. Liver cell dysplasia may exert a promoting effect by stimulating proliferation of cell clones initiated by the mutagenic effect of urethane. In addition, liver cell dysplasia may enhance the progression to malignancy of the pre-neoplastic lesions induced by urethane. This study suggests a role of Tat in the promotion and progression of carcinogen-initiated tumours in patients with HIV-1 infection (Altavilla et al., 2004).

For HIV-1, the overall concordance between the animal models and humans with respect to the types of tumour is low.

Human T-cell lymphotropic virus type 1 (HTLV-1)

HTLV-1 naturally infects humans, but the virus can be inoculated into different animals, including rabbits, rats, mice, and New World monkeys, with various effects (Lairmore et al., 2005). In rabbits and rats, HTLV-1 infection is persistent but does not lead to definite diseases.

Different monkey species are naturally infected with STLV-1, the simian analogue of HTLV-1, and several cases of adult T-cell leukaemia/lymphoma (ATLL) have been described in African green monkeys (Tsujimoto et al., 1987; Akari et al., 1998). Experimental infection with HTLV-1 of squirrel monkeys (*Saimiri sciureus*) caused a strong reduction in the proliferation rate of the CD4-positive T-cell population in those infected animals that were affected by a pathology similar to ATLL in humans (Debacq et al., 2005). Co-infection of rhesus macaques (*Macaca mulatta*) with HTLV-1 and SIV type 1 (SIV-1) increased the number of multilobulated lymphocytes ("flower" cells) in the circulation; this cell type is also seen in patients with ATLL. SIV-1 may have the potential to upregulate HTLV-1 and enhance expression of disease (Traina-Dorge et al., 2007). So far, non-human primates represent the only suitable animal model to study human ATLL.

The pX region of HTLV-1 encodes the regulatory genes *Tax* and *Rex*, and several accessory genes. Tax, a 40-kD phosphoprotein, is found mainly in the nucleus but also in the cytoplasm (Meertens et al., 2004). Interaction of Tax with several host factors results in transactivation of some genes, transrepression of others, modulation of the cell cycle, and dysregulation of apoptosis (Matsuoka and Jeang, 2007). The transforming ability of Tax was demonstrated in the Rat-1 fibroblast cell line in vitro in a soft agar assay, and in vivo in nude mice (Tanaka et al., 1990). These findings clearly show that Tax is oncogenic.

Several studies with animals transgenic for Tax clearly demonstrated that Tax expression leads to the induction of tumours, confirming that Tax is oncogenic in vivo. In *Tax* transgenic animals, the Tax protein was shown to be oncogenic, with the tumour type depending on the promoter used in each study. Mice that expressed Tax under the control of the granzyme B promoter developed tumours of natural killer cells (Grossman et al., 1995), and mice that expressed Tax via the lck promoter developed a disease that resembles ATLL (Hasegawa et al., 2006). The major difference between most of these animal tumours and ATLL in humans is the fact that a subset of human ATLL cells do not express Tax.

More recently, different models of humanized mice have been used to assess the effects of infection with HTLV-1 (Villaudy et al., 2011; Tezuka et al., 2014). In these cases, the mice developed ATLL-like leukaemic symptoms, including splenomegaly and lymphoma.

Cytogenetic analysis of ATLL cells has shown a common breakpoint cluster region in chromosome 10p11.2. Further analyses have shown that the transcription factor 8 (TCF8) is frequently disrupted by several mechanisms, including epigenetic silencing. Suppressed expression of TCF8 is associated with resistance to transforming growth factor beta (TGF-β). Mice carrying a mutation in TCF8 frequently developed thymic T-cell lymphoma, indicating that *TCF8* is

a tumour suppressor gene (Hidaka et al., 2008). There have been only few reports of cellular oncogenes in ATLL cells. When complementary DNA expression libraries derived from leukaemic cells of patients with ATLL were screened for the potential to transform NIH 3T3 mouse fibroblasts, a novel transforming gene, *Tgat*, was identified. Expression of *Tgat* in NIH 3T3 cells resulted in cellular transformation, indicated by anchorage-independent growth in semi-solid medium, and tumour formation in nude mice (Yoshizuka et al., 2004).

For HTLV-1, the overall concordance between the animal models and humans with respect to the types of tumour and the identity and function of the major oncogenes and oncogene products is high.

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CHAPTER 10.

Key characteristics of carcinogens

Martyn T. Smith

Introduction

The agents documented and listed as *carcinogenic to humans* (Group 1) in Volume 100 of the *IARC Monographs* show several key characteristics that distinguish them as carcinogenic agents. Many appear to act via multiple mechanisms, causing various biological changes in the multistage process of carcinogenesis. Others appear to act by a single predominant mechanism.

The participants in the IARC Workshop on Tumour Site Concordance and Mechanisms of Carcinogenesis, after considering previously published options for classification of carcinogens and related matters (see Chapter 11, by Stewart, and Chapter 12, by DeMarini), extensively debated the mechanisms by which carcinogens produce cancer. The Workshop participants concluded that carcinogens commonly show one or more of 10 key characteristics (Table 10.1).

To achieve wide dissemination and assessment, these key characteristics have been described in an open access journal publication (Smith et al., 2016) that also delineates their application, and hence complements material presented in this chapter and, to a broader extent, this Scientific Publication. Here, these key characteristics are defined, and reference is made to subsequent chapters where these particular characteristic properties are extensively discussed.

The Workshop participants also discussed several modulating factors that, along with mechanistic differences, may explain the lack of concordance or coherence between tumour sites in humans and experimental animals. Neither the list given in Table 10.1 nor the set of modulating factors mentioned by the Workshop participants is meant to be exhaustive, but they were agreed upon as being established characteristics or modulating factors. It is hoped that they will assist future *IARC Monographs* Working Groups in evaluating additional potential human carcinogens.

Characteristic 1: Is electrophilic or can be metabolically activated to electrophiles

Electrophiles are electron-seeking molecules that commonly form addition products, generally referred

Table 10.1. Key characteristics of carcinogens

1. Is electrophilic or can be metabolically activated to electrophiles
2. Is genotoxic
3. Alters DNA repair or causes genomic instability
4. Induces epigenetic alterations
5. Induces oxidative stress
6. Induces chronic inflammation
7. Is immunosuppressive
8. Modulates receptor-mediated effects
9. Causes immortalization
10. Alters cell proliferation, cell death, or nutrient supply

to as adducts, with cellular macromolecules including DNA, RNA, lipids, and proteins. Some chemical carcinogens are direct-acting electrophiles, whereas others require biotransformation by enzymes in a process termed metabolic activation (Miller, 1970).

Examples of direct-acting electrophilic carcinogens are formaldehyde, sulfur mustard, and ethylene oxide (see Chapter 1, by Bond and Melnick). The classic examples of chemical agents that require metabolic activation to become carcinogenic are polycyclic aromatic hydrocarbons and benzene, which by themselves are relatively inert chemically. This lack of reactivity puzzled chemists working on experimental carcinogenesis for many years until the Millers discovered metabolic activation by the mixed-function oxidase system (Conney et al., 1957). It is now known that several human enzymes can biotransform relatively inert chemical compounds to potent toxic and carcinogenic metabolites or reactive intermediates. These enzymes include cytochrome P450 isozymes, flavin mono-oxygenase, prostaglandin synthase, and various peroxidases (O'Brien, 2000; Hecht, 2012). The ability to form adducts with DNA and protein is a common property of these electrophilic and metabolically activated human carcinogens.

Characteristic 2: Is genotoxic

The term "genotoxic" refers to an agent that induces DNA damage, but this damage may or may not necessarily be processed by the cell into a mutation (see Chapter 12, by DeMarini). Thus, if an agent is found to induce DNA damage, it can be called a genotoxicant or a genotoxin, and if it is shown that the agent also induces mutations in a mutagenicity assay, it can be classified as a mutagen. Most of the IARC Group 1 carcinogens are considered to be genotoxic, and many are mutagenic (Waters et al., 2010), although this may not be their primary mechanism of carcinogenesis.

DNA damage from genotoxicity may be in the form of DNA adducts or single- or double-strand breaks. Other types of DNA damage are oxidized or fragmented bases or the intercalation of a molecule between two bases. The DNA damage is by itself not a mutation and generally does not alter the linear sequence of nucleotides (or bases) in the DNA, whereas a mutation is defined as a change in the DNA sequence, which usually arises as the cell attempts to repair the DNA damage.

Characteristic 3: Alters DNA repair or causes genomic instability

Normal cells try to avoid deleterious mutations by replicating their genomes with high accuracy. However, the fidelity of DNA replication can vary widely depending on the DNA polymerase involved, and this introduces the possibility of error. Indeed, most spontaneous mutations are caused by polymerase error (Preston et al., 2010). The nature of the mistake, the flanking sequence, the presence of DNA damage, and the ability to correct errors all have an impact on the outcome of this process (Arana and Kunkel, 2010). As a consequence, defects in processes that determine DNA replication fidelity can confer strong mutator phenotypes that result in genomic instability. Thus, carcinogens may act not only by producing DNA damage directly but also by altering the processes that control normal DNA replication.

Similarly, the major DNA repair pathways – such as base excision repair, nucleotide excision repair, and double-strand break repair – involved in the removal of DNA adducts and other lesions may also be altered by environmental exposures. Furthermore, whereas especially excision repair pathways are predominantly error-free and thus protective, double-strand break repair is largely error-prone and may contribute to genomic instability.

Genomic instability is a well-recognized feature of many cancers (Bielas et al., 2006). Studies of cultured cells exposed to ionizing radiation have shown that instability is a relatively late-occurring event that appears several cell generations after irradiation and results in a reduced ability to replicate the genotype faithfully (see Chapter 18, by Hill and Ullrich). The events that indicate genomic instability include chromosomal aberrations, gene mutations, microsatellite instability, and apoptosis. The instability phenotype may play a major role in radiation-induced cancers and other forms of cancer by providing the cell and its progeny with a constantly elevated rate of any of the various genetic and epigenetic changes that may occur in multistage carcinogenesis (Aypar et al., 2011).

Characteristic 4: Induces epigenetic alterations

The term "epigenetic" refers to all stable changes in gene expression and chromatin organization that are independent of the DNA sequence itself and that can be mitotically inherited over cell divisions. Epigenetic phenomena, including genomic imprinting, X-chromosome inactivation, global reconfiguration of the DNA methylome, and changes in chromatin compaction states and histone modification patterns, occur during development and contribute to the lineage-specific epigenome that is maintained over the lifetime of an organism. Many of these same phenomena are altered during carcinogenesis (see Chapter 20, by Rice and Herceg).

A wide range of known and suspected carcinogens (including chemical, physical, and biological agents) have been shown to deregulate the epigenome, and it has been suggested that their mode of action may involve disruption of epigenetic mechanisms. Because the evidence for a truly causal role of epigenetic changes in cancer produced by Group 1 agents was deemed to be limited in Volume 100, for many agents their impact on the epigenome was not considered to be a secondary mechanism of carcinogenesis. However, it should be noted that most carcinogens (even those considered for Volume 100 in 2008 and 2009) were evaluated by IARC Working Groups before new data on their epigenetic effects became available. Many recent studies have demonstrated the impact of several Group 1 agents included in Volume 100 on epigenetic mechanisms (Koturbash et al., 2011; Ravegnini et al., 2015; Chappell et al., 2016). This rapidly evolving area will generate new mechanistic data on carcinogens in the next few years.

Characteristic 5: Induces oxidative stress

Many human and animal carcinogens are capable of influencing redox processes and redox balance within target cells (see Chapter 15, by Bucher). An imbalance between formation of reactive oxygen and/or nitrogen species and their detoxification is commonly referred to as oxidative stress. Reactive oxygen species, which can arise from inflammation, may contribute to genomic instability and – along with other free radical species – play key roles in many of the processes identified as being necessary for the conversion of normal cells to cancer cells. Oxidative damage is considered a major factor in the generation of mutations in DNA, and more than 100 different oxidative DNA adducts have been identified (Klaunig et al., 2011). Reactive oxygen species produce at least 24 base modifications, as well as DNA–protein cross-links and other lesions (Berquist and Wilson, 2012), all potentially leading to genomic instability. Oxidative damage to DNA can lead to point mutations, deletions, insertions, or chromosomal translocations, which may cause activation of oncogenes and inactivation of tumour suppressor genes, potentially leading to initiation of carcinogenesis (Klaunig et al., 2011; Berquist and Wilson, 2012). Thus, agents that generate and promote oxygen radical-induced cellular injury may be carcinogenic.

Characteristic 6: Induces chronic inflammation

Chronic inflammation from persistent infections, such as that caused by *Helicobacter pylori* as well as that produced by silica or asbestos fibres, has been associated with several forms of cancer (see Chapter 17, by Kane). Indeed, inflammation is an "enabling characteristic" of cancer (Hanahan and Weinberg, 2011), and it has been hypothesized to contribute to cancer initiation, promotion, and progression (Trinchieri, 2012).

Inflammation acts by both intrinsic and extrinsic pathways. Persistent infection and chronic inflammation disrupt local tissue homeostasis and alter cell signalling, leading to the recruitment and activation of inflammatory cells. These constitute extrinsic pathways linking inflammation to cancer (Multhoff and Radons, 2012). In contrast, intrinsic pathways driven by activation of proto-oncogenes in pre-neoplastic and neoplastic cells recruit host-derived inflammatory cells that accelerate tumour promotion and progression (Grivennikov et al., 2010). Strong links exist between inflammation and

the induction of oxidative stress and genomic instability; this makes it difficult to separate out the importance of each of these mechanisms.

Characteristic 7: Is immunosuppressive

Immunosuppression is a reduction in the capacity of the immune system to respond effectively to foreign antigens, including antigens on tumour cells. Persistent immunosuppression presents a risk of cancer, especially excess risk of lymphoma when it is accompanied by continuing exposure to foreign antigens such as after organ transplantation, or when it occurs in individuals who are latently infected with an oncogenic virus (Gutierrez-Dalmau and Campistol, 2007; Münz and Moormann, 2008; Shelton et al., 2016).

Immunosuppression differs from other mechanisms of carcinogenesis in that agents that cause immunosuppression may not directly transform normal cells into potential tumour cells. Potentially neoplastic cells that arise naturally, or that have been transformed by other carcinogens acting by a mechanism such as genotoxicity or by the various mechanisms of action associated with oncogenic viruses, escape immune surveillance in immunosuppressed individuals. As a result, survival of these cells and their replication to form tumours is greatly facilitated by immunosuppression.

Several Group 1 agents included in Volume 100 act entirely or largely by immunosuppression, often in concert with other Group 1 agents, especially oncogenic infectious agents. The Group 1 agents that act by immunosuppression include human immunodeficiency virus type 1 (HIV-1) and the immunosuppressive drugs ciclosporin and azathioprine (IARC, 2012a, c).

Characteristic 8: Modulates receptor-mediated effects

Hormonally active agents that are associated with carcinogenic effects typically act as ligands via nuclear receptors, and in some cases via receptors located on the cell surface. There are many other agents that may be carcinogenic by acting on receptor proteins, even though some of these also have genotoxic effects, for example polycyclic aromatic hydrocarbons such as benzo[*a*]pyrene. Receptor activation falls into two broad categories: (i) activation of intracellular receptors that translocate into the nucleus and act on DNA as transcription factors, and (ii) activation of cell surface receptors and some intracellular receptors that activate signal transduction pathways, resulting in biological responses (see Chapter 14, by Bosland).

The predominant effect of receptor activation is on gene transcription. Although some exogenous ligands act as agonists by competing for binding with an endogenous ligand, others may bind but lack intrinsic activating activity for the receptor they bind to and have an antagonistic effect. There are also receptors for which no endogenous ligand has been identified, such as the aryl hydrocarbon receptor. One other important type of potential effect of exogenous agents on receptor-mediated mechanisms involves modulation of the amount of endogenous ligand available for binding and activating its receptor, by affecting biosynthesis, bioavailability, bioactivation, and/or degradation of the ligand (Rushmore and Kong, 2002).

Characteristic 9: Causes immortalization

Volume 100 of the *IARC Monographs* identifies several human DNA and RNA viruses that are *carcinogenic to humans* (Group 1); these include various types of human papillomavirus (HPV), Epstein–Barr virus (EBV), Kaposi sarcoma-associated herpesvirus (KSHV), hepatitis B virus (HBV), hepatitis C virus (HCV), and HIV-1. These viruses have evolved multiple molecular mechanisms to disrupt specific cellular pathways to facilitate aberrant replication.

Although oncogenic viruses belong to different families, their strategies in human cancer development show many similarities and involve viral-encoded oncoproteins targeting the key cellular proteins that regulate cell growth. Recent studies have shown that virus and host interactions also occur at the epigenetic level (Allday, 2013). The result of these viral effects is to immortalize the cells of the target tissue such that they are not subject to the Hayflick limit, the point at which cells can no longer divide due to DNA damage or shortened telomeres.

For example, the HPV type 16 (HPV16) *E6* and *E7* oncogenes are selectively retained and expressed in cervical carcinomas, and expression of *E6* and *E7* is sufficient to immortalize human cervical epithelial cells (Yugawa and Kiyono, 2009). E6 and E7 proteins do not possess intrinsic enzymatic activities but instead function through several direct and indirect interactions with cellular proteins, some of which are well-known cellular tumour suppressors, including p53 and Rb.

Characteristic 10: Alters cell proliferation, cell death, or nutrient supply

There are at least three scenarios related to cancer and cancer mechanisms in which alterations in cellular replication and/or cell-cycle control have been described. The first

invokes the predisposition for unrepaired DNA damage to lead to cancer-initiating mutations in replicating cells. The second has attempted to identify sustained replication as a key mechanistic event, and the third describes the ability of a transformed cell to escape normal growth control and to continue replication. A component common to all three scenarios is the evasion of apoptosis or other terminal programming, including autophagy, in at least a proportion of the cell population (Ryter et al., 2014).

Sustained cellular proliferation has been argued to be a factor in increased cancer susceptibility. As summarized in the United States Environmental Protection Agency guidance assessing risk of cancer from early-life exposures (EPA, 2005), more frequent cell division during development can result in enhanced fixation of mutations because of the reduced time available for repair of DNA lesions, while clonal expansion of a mutated cell produces a larger population of mutant cells. For mature organisms, sustained proliferation has also been postulated to increase risk of cancer, based on the same rationale.

The mechanism by which necrosis may enable cancer induction is also part of the description of the hallmarks of cancer. In contrast to apoptosis and autophagy, necrotic cell death releases pro-inflammatory signals into the surrounding tissue microenvironment, resulting in recruitment of inflammatory cells of the immune system that can participate in tumour promotion through their influence on cancer cell proliferation and invasiveness.

In addition to cell death caused by direct toxicity of an agent, cells within a tumour may die as a result of impaired nutrient supply. The exponentially increasing number of neoplastic cells may quickly outstrip the supply capabilities of the existing tissue vasculature. Neo-angiogenesis, in which new blood vessels grow into a tumour, is key to providing this supply of nutrients. Thus, agents that promote or inhibit angiogenesis, such as arsenic, will promote or delay tumour growth (Yang et al., 2014).

Multiple mechanisms of action of human carcinogens

The number of mechanisms by which chemicals are known to contribute to carcinogenesis can be extensive if one includes all biochemical or molecular end-points, but the mechanisms can be grouped into a limited number of categories (genotoxicity, immunosuppression, etc.). Guyton et al. described 15 types of key events associated with carcinogenesis, which collectively represent the majority of known carcinogenic modes of action (Guyton et al., 2009).

The IARC Workshop participants initially identified 24 mechanistic end-points, with several subcategories in each. This was considered too many categories, and it was determined that several of them could be merged. The Workshop participants then concluded that carcinogens commonly show one or more of the 10 key characteristics described above (see Table 10.1). These represent the majority of known carcinogenic mechanisms of action.

It is increasingly evident that multiple biological alterations or sets of different perturbations are necessary to convert a normal cell into a transformed cell, and ultimately a tumour (Hanahan and Weinberg, 2011). Carcinogens appear to have an impact on this complex process in multiple ways and can act through multiple mechanisms of action to induce cancer and other adverse health outcomes. As an illustration of this point, the evidence has been evaluated for which key characteristics contribute to the carcinogenicity of benzene, an IARC Group 1 carcinogen, in humans and in experimental animals. The results are shown in Table 10.2, where the level of evidence for a particular characteristic is classified on a scale with 2 = strong evidence, 1 = moderate evidence, and 0 = weak evidence. For benzene, there is strong evidence in my view that metabolic activation, genotoxicity, and immunosuppression are established mechanisms of carcinogenicity in both animals and humans (McHale et al., 2012). There is weak or no evidence that inflammation and immortalization play a role in the carcinogenicity of benzene. However, moderate evidence exists for the other five key characteristics or mechanisms in humans. This suggests that there is strong or moderate evidence that eight of the key characteristics of carcinogens contribute to the carcinogenicity of benzene and that they are consistently observed, for the most part, both in humans and in experimental animals (Table 10.2).

Factors modulating human carcinogenesis

Lack of concordance or coherence between tumour sites in humans and experimental animals may be explained by several modulating factors that, along with mechanistic effects, cause discordance between the findings. For example, physiological differences exist between animals and humans, including the fact that rodents are nose-only breathers,

Table 10.2. Key characteristics of the carcinogen benzene[a]

Characteristic	Level of evidence[b]		
	IARC (2012b)	Humans	Animals
1. Is electrophilic or can be metabolically activated to electrophiles	2	2	2
2. Is genotoxic	2	2	2
3. Alters DNA repair or causes genomic instability	1	1	1
4. Induces epigenetic alterations	1	1	0
5. Induces oxidative stress	1	1	1
6. Induces chronic inflammation	0	0	0
7. Is immunosuppressive	2	2	2
8. Modulates receptor-mediated effects	1	1	1
9. Causes immortalization	0	0	0
10. Alters cell proliferation, cell death, or nutrient supply	1	1	1

[a] This table shows the overall weight of evidence as stated in Volume 100F of the *IARC Monographs* (IARC, 2012b), and the levels of evidence from studies in humans and animals, respectively.
[b] 2 = strong evidence, 1 = moderate evidence, 0 = weak evidence.

whereas humans breathe through both the nose and mouth. Rodents do not retain their urine as humans and dogs do, perhaps explaining the lack of carcinogenicity of aromatic amines to rodents (see Chapter 2, by Beland and Marques).

Experimental animals may also exhibit differences in the pharmacokinetics or toxicokinetics of a chemical: they may absorb, distribute, metabolize, and excrete a compound in ways that are different to those seen in humans. There are many examples of this kind. For instance, mice hydrolyse 6-propylthiopurine to mercaptopurine, which has a potent carcinogenic effect, whereas humans oxidize the drug at two positions in the molecule without hydrolysis, and the end products are not carcinogenic. With regard to infectious agents, it is clear that a human infectious agent, for example a human tumour virus that is not infectious to other animal species, will not produce carcinogenic effects in these species (see Chapter 9, by Lambert and Banks).

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CHAPTER 11.

Mechanisms of carcinogenesis: from initiation and promotion to the hallmarks

Bernard W. Stewart

Introduction

For many decades, a corollary to the contemporary understanding of the nature of cancer and of carcinogenesis has been the recognition of causative agents. Since the 1950s, many agents that contribute to the development of cancer have been categorized as initiators or promoters, on the basis of studies of chemical carcinogenesis in mouse skin (Berenblum and Shubik, 1947).

Cancer was described with reference to causative agents. Thus, a 1970s pathology text (Cappell and Anderson, 1974) introduced malignancy by describing a tumour as "an abnormal mass of tissue, the growth of which exceeds and is uncoordinated with that of the surrounding tissue, and that continues to grow in the same excessive manner after cessation of the stimulus that caused it". According to the same textbook, development of tumours of the skin, the alimentary canal, or the respiratory tract was to be expected among individuals exposed "to various noxious agents in the environment".

Causation of cancer in humans or animals by certain chemicals, radiation, and biological agents was recognized by early in the 20th century. The types of biological agents and of radiation now recognized by IARC as *carcinogenic to humans* (Group 1) are few compared with the number of chemicals in this category (IARC 2012a, b, c, d, e, f); there is a much larger number of chemicals for which at least some evidence of carcinogenicity is available (see Volumes 1–105 of the *IARC Monographs*, available from http://publications.iarc.fr).

Research has established how many carcinogenic chemicals cause, or are likely to cause, malignant transformation, but the biological processes involved are diverse, and

Table 11.1. A selection of proposals for the categorization of chemical carcinogens[a]

Mode of action	Exposure context	Chemistry	Human relevance of bioassay data	Agent type
Genotoxic	**Tobacco smoke**	**PAHs**	**DNA binding**	**Atmospheric pollutants**
Direct-acting	**Alcoholic beverages**	***N*-nitroso compounds**	PPARα activation	**Pesticides**
Pro-carcinogen	**Occupation**	**Aromatic amines**	α_{2u}-Globulin nephropathy	Organic solvents
Inorganic carcinogen	**Pollution**	**Halogenated organic compounds**	Urinary tract calculi	Endocrine disruptors
Non-genotoxic	**Diet**	**Naturally occurring compounds**		Disinfection by-products
Solid-state carcinogen	**Pharmaceutical drugs**	**Inorganic compounds**		**Pharmacological steroids**
Hormone	**Exogenous hormones**			
Immunosuppressant				
Promoter				

PAHs, polycyclic aromatic hydrocarbons; PPAR, peroxisome proliferator-activated receptor.
[a] Knowledge about chemical carcinogens is presented from a variety of perspectives apart from that of mechanism of action. The listings indicate those used in particular publications (e.g. Searle, 1984; Tomatis et al., 1990; Vainio et al., 1992; Vainio and Hietanen, 2003; Hsu and Stedeford, 2010) as ways of ordering data, as indicated by chapter headings in many cases, and are not necessarily comprehensive. Categories shown in **bold** involve or include at least one Volume 100 (Group 1) agent.

there is no generally accepted mechanistic basis for classifying chemical carcinogens (Loeb and Harris, 2008), beyond categorization according to genotoxicity (Weisburger and Williams, 1981). There is no single comprehensive basis for categorization; chemical carcinogens are sometimes ordered according to the context in which information is presented, with genotoxicity ordered according to mutational signatures, or agents categorized in relation to differing classes of receptors. There have been many proposals for the categorization of chemical carcinogens according to various criteria. A selection of these is shown in Table 11.1; others include the categorization of chemical carcinogens on the basis of the organ affected (Warshawsky and Landolph, 2006).

Currently, the most widely recognized description of the nature of cancer is that presented by Hanahan and Weinberg in two reviews – published more than a decade apart – that identify the "hallmarks" of cancer (Hanahan and Weinberg, 2000, 2011). These papers have been so influential that others refer to "the hallmarks" without further qualification, for example in the title of a recent perspective on tumour metabolism (Cantor and Sabatini, 2012).

Since 2000, about 200 cancer research papers with a title including "hallmark" or "hallmarks" have been published. These papers typically describe signal transduction pathways and their therapeutic implications. Although the characterization by Hanahan and Weinberg (2011) of the hallmarks of cancer did not refer to chemical carcinogens or causative agents in general, recently the hallmarks have been used to characterize chemical carcinogens (Kleinstreuer et al., 2013).

These considerations give rise to two questions: (i) whether previously used mechanism-based descriptions of chemical carcinogens may be recast in relation to the hallmarks; and (ii) whether, and to what extent, the hallmarks provide opportunities

to characterize agents apart from currently known carcinogens as contributing to the development of cancer. Both of these matters are addressed in this chapter.

Multistage carcinogenesis

Exogenous agents

The widely accepted paradigm of carcinogenesis as involving a multistage process is generally recognized to have been developed from the two-stage model of carcinogenesis in mouse skin (Berenblum and Shubik, 1947), which typically involves a polycyclic aromatic hydrocarbon (PAH) and a phorbol ester (identified as the active agent in the irritant croton oil). Because tumorigenesis in animals is amenable to histological examination at all stages, morphological criteria can be used to characterize the process. With the production of malignant tumours as the end-point, two-stage or multistage carcinogenesis was readily described in various organ sites in animals, including the liver and the bladder (Slaga et al., 1978).

Thus, in relation to hepatocarcinogenesis, agents such as phenobarbital, dichlorodiphenyltrichloroethane, polychlorinated biphenyls, butylated hydroxytoluene, and estradiol benzoate were identified as promoters (Dohi et al., 1996). The relevant experimental observations, in addition to indicating the possible risk to humans presented by the relevant chemicals, also led to the contemporary understanding of the nature of malignancy itself. That understanding was based on the identification of particular abnormal cell populations, specifically including chemically induced hyperplastic nodules in rat liver (Farber, 1973).

Morphological and genetic changes

Within 20 years of the publications cited above, the identification of multistage carcinogenesis with particular carcinogens or other exogenous agents had become irrelevant to an understanding of cancer development. Over the same decades, the context in which carcinogenesis was best understood changed from rodents to humans. Critical to this transition was the identification of multistage carcinogenesis with alterations in gene structure or expression rather than with the impact of exogenous agents.

A key development was the correlation by Vogelstein et al. (1988) of morphological change during the development of colon cancer in humans with particular genetic change. The concept was applicable to all tumour types. Thus, in a diagram illustrating multistage carcinogenesis with respect to human lung cancer, Harris (1992) made no reference to any particular exogenous agents as mediating specific stages in tumorigenesis, and showed the transitions between stages as being mediated by alterations in the structure or expression of oncogenes and tumour suppressor genes.

Oncogenes and tumour suppressor genes mediate altered proliferative activity in a positive and negative sense, respectively. Classically, increased proliferative activity due to oncogene expression accounted for the transformation of NIH 3T3 cells by DNA isolated from tumours and not by DNA from normal tissue (Shih et al., 1981). Oncogene activation (e.g. mutation of *Ras*) has shown that although binding of many chemical carcinogens to diverse biological macromolecules had been variously demonstrated over decades, carcinogen adducts in DNA were crucial.

Alkylation of DNA by *N*-nitroso compounds was shown by Magee and Farber (1962), with tumorigenesis attributable to the pro-mutagenic O^6-methylguanine product, which mispairs with thymine. In rats, activation of H-*Ras* in mammary gland tumours induced by *N*-methyl-*N*′-nitrosourea was correlated with H-*Ras* mutation at codons 12, 13, and 61 (Sukumar et al., 1983). However, although this insight had been gained, it was clear that the etiology of some types of cancer, such as breast cancer in humans, did not primarily involve alkylating agents. Thus, in human cancer *RAS* activation is a relevant genetic change in tumour tissue, without reference to exogenous agents (Bos et al., 1987).

Although the concept of multistage carcinogenesis was established through the use of exogenous agents that target particular organ sites in animals, by 1990 multistage carcinogenesis was primarily identified with altered structure or expression of genes associated with cell proliferation, specifically as described in human tumours. However, the focus of that research has not involved the specification of genetic change over time in a manner that might account for the emergence of a metastatic cell population from within normal tissue. Rather, the relevant research has involved the identification of disordered signal transduction pathways, with a view to developing targeted therapies. The archetype of such research is that establishing the transforming role of the tyrosine kinase BCR-ABL in chronic myeloid leukaemia, and its inhibition

– to the great benefit of patients – by the low-molecular-weight inhibitor STI-571 (imatinib) (Bilanges and Stokoe, 2007; Rosa et al., 2008).

Molecular changes

Among a series of reviews marking the publication of the 100th volume of the journal *Cell*, Hanahan and Weinberg (2000) delineated the very wide (even then) spectrum of studies addressing the genetics of cancer by reference to phenotype. Six characteristics of how cancer cells behave could be identified in relation to particular genes or classes of genes. The phenotypic characteristics were: uncontrolled proliferative activity (Hall, 1984), tumour growth attributable to familial risk (Hussain and Harris, 1998), survival of cancer cells (Vaux et al., 1988), immortalization of cancer cells (Sedivy, 1998), growth of blood vessels in tumours (angiogenesis) (Cavallaro and Christofori, 2000), and metastatic growth (Webb and Vande Woude, 2000). Accordingly, the hallmarks of cancer were initially identified as follows:

- self-sufficiency in growth signals;
- insensitivity to anti-growth signals;
- evasion of apoptosis;
- sustained angiogenesis;
- limitless replicative potential; and
- tissue invasion and metastasis.

The 2000 "hallmarks" review was concerned primarily with the characterization of the genes and associated signal transduction pathways that mediate these respective activities in malignant cells and tumours. In that paper, hypothetical patterns of multistage carcinogenesis were illustrated by a linear arrangement of the pictograms for the hallmarks, without reference to any morphological criteria. From that diagram, it can be inferred that some hallmarks – such as self-sufficiency in growth signals – emerge early, whereas others – sustained angiogenesis, and tissue invasion and metastasis – are seen later.

Although hallmarks such as sustained angiogenesis and metastasis involve morphological change, all of the hallmarks were identified with reference to changes in gene expression and not by reference to, or necessarily in correlation with, a change in morphology. Diversity between tumour types and within a given tumour type was noted, and no reference was made to any particular type of neoplasm for illustrative purposes.

In such a description of the manifestation of essential alterations that collectively characterize malignant growth, there is no requirement to identify exogenous agents as acting on normal or premalignant cells to cause the change. The focus is on the nature of tumours and how they may be distinguished from relevant normal tissue. Finally, Hanahan and Weinberg (2000) identified an enabling characteristic: genomic instability, which is equated with increased mutability evident during the process of tumour progression (Loeb, 1994).

A decade on: "the next generation"

In 2011, Hanahan and Weinberg provided a new assessment of the hallmarks (Hanahan and Weinberg, 2011). They commented, "The past decade has witnessed remarkable progress towards understanding the mechanistic underpinnings of each hallmark." One indication of progress is that the original hallmarks were rebadged as follows:

- sustaining proliferative signalling;
- evading growth suppressors;
- resisting cell death;
- inducing angiogenesis;
- enabling replicative immortality; and
- activating invasion and metastasis.

It is notable that, in almost every instance, the hallmark is not the name of a phenotype but refers to a dynamic process. Consistent with this perception, the authors wrote, "The hallmarks of cancer comprise six biological capabilities acquired during the multistep development of human tumours. The hallmarks constitute an organizing principle for rationalizing the complexities of neoplastic disease."

In addition, a decade of progress had enabled the specification of two "emerging hallmarks":

- deregulating cellular energetics; and
- evading immune destruction.

The enabling characteristic identified in 2000 as "genomic instability" was described in 2011 as "genomic instability and mutation", and a second enabling characteristic was identified as "tumour-promoting inflammation". Superficially, such reference to mutation and to promotion might be seen as implying, if not specifying, the roles that DNA-damaging and proliferation-inducing agents have in carcinogenesis. However, this is not the case.

In this context, "mutation" refers to an acceleration of the accumulation of mutations, due to, among other things, defects in the DNA maintenance machinery (Kinzler and Vogelstein, 1997). As a result, mutation occurs more readily, irrespective of whether it is mediated by exogenous or endogenous agents. Accordingly, DNA adducts, strand breakage, and related phenomena are not to be identified with this enabling characteristic and do not account for, or are not properly identified with, a particular hallmark. Mutation, in the context of

carcinogenesis, identifies a mechanism whereby a chemical carcinogen may cause the emergence of any of the hallmarks, and almost certainly of several of them, or perhaps of all of them. The enabling characteristic "genomic instability and mutation" renders such outcomes more likely (Wang et al., 2012), rather than referring to the mechanism through which the change occurs.

The identification of "tumour-promoting inflammation" as the second enabling characteristic recognizes that inflammation causes the emergence of several of the hallmarks, including sustaining proliferative signalling and inducing angiogenesis. In their discussion of this enabling characteristic, Hanahan and Weinberg (2011) were concerned primarily with cellular infiltration by cells of both the innate and the adaptive arms of the immune response. They made scant, if any, reference to exogenous agents provoking an inflammatory response.

From a broad perspective, reference to the multistep development of human tumours provides a way to consider the particular impact of carcinogens and other exogenous agents that may contribute to cancer development. However, in identifying the hallmarks, Hanahan and Weinberg did not pursue this matter.

Identifying mechanisms of carcinogenesis

As mentioned above, chemical carcinogens have been categorized primarily with reference to whether they exhibit genotoxicity. This mechanistic distinction began with many then-known carcinogens being identified as mutagens in vitro by use of particular bacterial strains and after metabolic activation (the Ames test) (McCann et al., 1975). The term "genotoxic" indicated, among other things, that the covalent binding of a carcinogen adduct to DNA, when evident, might account for carcinogenesis. Thus, Weisburger and Williams (1981) categorized carcinogens primarily on the basis of genotoxicity. Research over the subsequent 30 years did not alter that approach (Hsu and Stedeford, 2010).

The multiplicity of agents and the relatively limited understanding of their respective mechanisms of action have precluded the adoption of a scheme for categorizing carcinogens beyond the consideration of genotoxicity. Arguably, until the present IARC Scientific Publication, the most authoritative assessment on how carcinogens act was the 35-page consensus report in the publication *Mechanisms of Carcinogenesis in Risk Identification* (Vainio et al., 1992); this was the agreed position of a Working Group of more than 40 scientists in 1991. The consensus report did not centre on a scheme for classifying carcinogens according to their mechanism of action.

Across decades, commentaries on chemical carcinogens (Van Duuren, 1980; Pitot, 1990; Xue and Warshawsky, 2006; Cohen and Arnold, 2011) have not been based on any generally agreed categorization according to mechanism of action. Rather, the common theme has been the enumeration of biological parameters that may determine whether tumours develop in response to carcinogens in general.

Genotoxicity: progress and problems

Multiple indicators of genotoxicity have been recognized and categorized as involving data generated either in vitro or in vivo (Montesano et al., 1976). In vitro test systems include bacterial, mammalian, and other cells, with weight being given to the extent to which the test system has been "validated", as summarized by sensitivity and specificity in relation to known carcinogens and non-carcinogens. In vivo indicators of genotoxicity include, among others, (i) metabolism of a chemical to produce reactive, typically electrophilic, intermediates, which are the source of adducts bound to DNA and other macromolecules, and (ii) evidence of subsequent DNA repair and/or mutation.

This description of indicators of genotoxicity also summarizes the relevant mechanism of chemical carcinogenesis as currently understood (Cohen and Arnold, 2011). Thus, carcinogen metabolism and DNA repair processes have been used to identify candidate genes for lung cancer susceptibility studies (Yokota et al., 2010). Compared with the relatively modest number of genes that account for the absorption, metabolism, and elimination of a carcinogen, together with the repair of corresponding DNA adducts, the hallmarks (Hanahan and Weinberg, 2011) enable the specification of tens – if not hundreds – of genes whose expression contributes to the malignant phenotype.

At the single-gene level, mutation of *TP53*, specified with reference to particular transitions and transversions, is attributable to miscoding, which in turn is a consequence of DNA adduct formation from relevant carcinogens, including those in tobacco smoke (Soussi, 2011). The data provide evidence of particular exposures, but it remains unclear how tumorigenesis is enhanced by such mutation, beyond the consideration that a functional p53

Table 11.2. Chemicals cited by Ashby (1992) and Eastmond (2012) as examples of compounds with equivocal genotoxicity

Chemicals identified by Ashby (1992)	Chemicals identified by Eastmond (2012)
3-Amino-4-ethoxyacetanilide	Bromate
3-Amino-9-ethylcarbazole.HCl	Captan
Chlorinated paraffins	Carbon tetrachloride
CI Acid Orange 3	Chloroprene
CI Basic Red 9.HCl	Chromium(III)
Cinnamyl anthranilate	Chromium(VI)
1,2-Dibromo-3-chloropropane	1,3-Dichloro-2-propanol
di-Menthol	1,4-Dioxane
Methyldopa sesquihydrate	Ethylene glycol monobutyl ether
5-Nitroacenaphthene	Hydroquinone
4-Nitro-*o*-phenylenediamine	2-Nitrotoluene
Piperonyl butoxide	Trichloroacetic acid
Piperonyl sulfoxide	1,2,3-Trichloropropane
1,2-Propylene	
Sulfallate	

protein induces apoptosis, cell-cycle arrest, and senescence, and that these processes are compromised after *TP53* mutation (Bieging and Attardi, 2012). The hallmarks offer a broadened perspective as to signalling pathways that may be affected by mutation of *TP53* or any tumour suppressor gene.

In the first such determination made, genotoxic injury by tobacco smoke in one individual case of lung cancer accounted for 22 910 somatic base substitutions, of which 134 were in coding sequences (Pleasance et al., 2010). The role of tobacco smoke as a determinant of the genomic landscape of lung cancer has been confirmed, with an average mutation frequency in lung tumours from smokers of more than 10 times that in lung tumours from never-smokers (Govindan et al., 2012).

However, analysis of lung cancer genomics does not require immediate reference to smokers and never-smokers to present relevant data (Liu et al., 2012; Peifer et al., 2012). Moreover, the recognition of tobacco-induced genomic injury does not necessarily extend to other sites; for example, on the basis of individual genomic analysis, it is not possible to differentiate between cases of pancreatic cancer in smokers and in never-smokers (Wei et al., 2012).

More generally, although mutation of *TP53* is highly relevant to colorectal cancer, the impact of exogenous influences or causal factors on the development of this tumour type is not evident from genomic analysis (Muzny et al., 2012). In short, the role of mutation as contributing to cancer development may be elucidated without reference to any genotoxic agent, even when the role of such an agent has been otherwise established.

Distinguishing genotoxic from non-genotoxic carcinogens

Even though molecular processes associated with genotoxicity are being defined in steadily greater detail, it is not always possible to immediately discriminate between individual chemicals on the basis of whether particular substances should be categorized as genotoxic. Difficulties are evident when relevant chemicals are considered on a case-by-case basis. More than

Table 11.3. Examples of categories of non-genotoxic carcinogens as variously proposed over more than three decades[a]

Weisburger and Williams (1981)	Weisburger (1989)	Marquardt (1999)	Hernández et al. (2009)	Benigni et al. (2013)
Solid-state carcinogens	Halogenated compounds	Cytotoxic carcinogens	Endocrine modifiers	Peroxisome proliferators
Hormones	Immunosuppressants	Tumour promoters	Receptor-mediated	Gap-junction inhibitors
Immunosuppressants	Hormones	Hormones	Non-receptor-mediated	DNA-methylating agents
Co-carcinogens	Solid-state materials	Immunosuppressants	Promoters	Agonists/antagonists of the aryl hydrocarbon receptor
Promoters	Certain hypolipidaemic carcinogens	Peroxisome proliferators	Tissue-specific toxicity and inflammation inducers	Oxidative stress inducers
	Phthalate ester plasticizers	Solid bodies or particles	Cytotoxic agents and immunosuppressants	Hormonal imbalance inducers
			Gap-junction inhibitors	

[a] Typically, the listings have been provided by the respective authors for illustrative purposes, without necessarily specifying an intent to be comprehensive.

20 years ago, Ashby (1992) reported on "practical examples of instances in which the term genotoxic is both needed and capable of having different meanings". Two decades later, Eastmond (2012) provided insight by summarizing data for another set of chemicals, different from those discussed by Ashby (Table 11.2).

Hence, there are some chemicals that are not readily categorized in relation to genotoxicity because, for example, they produce positive results when assessed by use of in vitro genotoxicity tests but after their administration to intact animals, they do not cause structural DNA damage or other manifestations of genotoxicity. As described by Eastmond (2012), apparently contradictory findings can be reconciled when, for different individual chemicals, account is taken of:

- the chemical properties of the agent, its metabolites, and/or its degradation products;
- the agent's metabolism and toxicokinetics;
- structural similarities to recognized mutagenic carcinogens;
- the origin of or mechanisms underlying the observed effects; and
- in vivo data, particularly in the target organ.

Eastmond (2012) illustrated each of these points with two or more examples.

Specifying genotoxicity is complex, as becomes evident when all available mechanistic data are identified, as occurs, for example, in *IARC Monographs* evaluations. In some instances, the totality of available mechanistic data may indicate that the categorization of a carcinogen as genotoxic is equivocal. There does not appear to be a context in which awareness of the hallmarks would provide an improved basis for identifying genotoxic carcinogens specifically.

Non-genotoxicity: multiple mechanisms and pathways

Regardless of any difficulty with particular agents as discussed in the previous section, the conceptual basis of genotoxicity is unequivocally focused on a particular pathway to malignant transformation. No such single focus is available for non-genotoxic carcinogens, as illustrated by the designation "epigenetic", which, although previously applied to these agents (Weisburger and Williams, 1981; Benigni et al., 2013), can no longer be unequivocally used in this context.

Epigenetic processes are relevant to both genotoxic and non-genotoxic agents (Pogribny et al., 2008), and epigenetic change may be determined by mutation (You and Jones, 2012). From a different perspective, when discussing non-genotoxic carcinogens, Meza et al. (2010) identified tobacco smoke and radon in

this context. Despite such ambiguity, 45 non-genotoxic carcinogens were recognized in 2009 among 371 agents classified by IARC in Group 1, Group 2A (*probably carcinogenic to humans*), and Group 2B (*possibly carcinogenic to humans*) (Hernández et al., 2009).

Grouping agents on the basis of a default criterion – i.e. that the agent is not genotoxic – implies uncertainty. The scope of uncertainty can be seen from differences between reports indicating categories of agents that are reasonably considered to be non-genotoxic carcinogens; Table 11.3 shows selected examples from 1981 to 2013.

Parameters used to identify non-genotoxic carcinogens include either the nature of the agent or some indicator of a putative mechanism of action. The terminology is far from definitive. Thus, while the term "promoter" may be used to identify a non-carcinogen that contributes to tumour development, tumour promotion may be identified with the action of many non-genotoxic carcinogens (Schulte-Hermann et al., 1999).

The role of receptors has long been recognized as key to the carcinogenicity of many non-genotoxic agents (Lucier, 1992) and underpins current commentaries (Klaunig, 2010). Relevant receptors include the aryl hydrocarbon receptor (AhR), the peroxisome proliferator-activated receptor (PPAR), and various hormone receptors.

Arguably, AhR is recognized mainly as mediating the carcinogenicity of 2,3,7,8-tetrachlorodibenzo-*para*-dioxin (TCDD). However, as specified by Matsumura et al. (2009), apart from mediating toxic effects of some pollutants, AhR is involved in development, regulation of cell differentiation and cycling, hormonal and nutritional homeostasis, coordination of cellular stress responses (including inflammation and apoptosis), immune responses, and ageing. Therefore, it is difficult to identify AhR-mediated processes with a specific hallmark.

The adoption of a mechanistic approach to categorize non-genotoxic carcinogens leads to incongruities if definitive and exclusive specifications are sought. Thus, TCDD may be readily identified as a promoter (Ray and Swanson, 2009) while also being recognized as a complete carcinogen on the basis of bioassay and epidemiological data (Baan et al., 2009). Similarly, although PAHs can be identified with the genotoxicity of, for example, tobacco smoke, Puga et al. (2009) noted that exposure to toxic PAHs raises several toxic and carcinogenic responses in experimental animals and humans, mediated for the most part by AhR. Such apparent paradoxes indicate that although mechanistic categorization of many genotoxic carcinogens is definitive and exclusive, the same process applied to non-genotoxic agents may lead to outcomes determined by context. The relevant agents cannot be identified with a single path to malignancy.

The role of cell proliferation in relation to non-genotoxic agents also depends on the context (Preston-Martin et al., 1990; Marquardt, 1999). With respect to chemicals, the original focus was on mitogens, including peroxisome-proliferating carcinogens (Butterworth et al., 1992). This approach now identifies inflammation as contributing to cancer development, and auto-inflammatory disease and the impact of various cancer-causing infectious agents are equally recognized (Schetter et al., 2010). Cell proliferation in this context does not pertain to proliferation after toxic injury by genotoxic agents. Proliferative activity induced by genomic injury may be considered in relation to the pluripotent stem cells (Cohen and Arnold, 2011), further indicating how a characteristic – such as the hallmark "sustaining proliferative signalling" – cannot readily be assigned or restricted to a particular category of carcinogens.

Public health decision-making: the definitive consideration

This IARC Scientific Publication is based on evaluations made in Volume 100 of the *IARC Monographs*. Two broad issues are addressed: (i) the extent to which the occurrence and anatomical site of agent-attributable cancer in humans may be correlated with the occurrence and, where relevant, organ site of tumours in animals treated with the same agent; and (ii) whether known mechanisms of action of the carcinogenic agents in question, considered together with current knowledge of cancer etiology, reveal options for categorizing carcinogens, so as to better indicate the risk posed to humans by exposure.

These two considerations are intimately related. Thus, the occurrence or absence of tumours in rodents treated with particular agents may be wholly dependent on biological mechanisms operating, or not operating, in particular species. Until now, mechanistic assessment of carcinogens has not established a comprehensive basis for determining whether particular agents are capable of causing cancer in humans. This situation confirms that evaluations of the

IARC Monographs are appropriate for hazard identification, as distinct from any simple categorization of relevant agents. The fact that agents may be classified into Groups does not alter the need to make evaluations on a case-by-case basis.

The determination of whether a chemical induces cancer through a genotoxic mechanism frequently plays an important role in evaluating the risks associated with low exposures (Eastmond, 2012). For low levels of exposure to non-genotoxic carcinogens, there is expected to be a dose–response threshold for the carcinogenic effects; this does not apply to genotoxic carcinogens (Klaunig, 2010). Low-dose models of liver cancer induction in fish by genotoxic carcinogens indicate further levels of complexity (Williams, 2012), and ongoing controversy about non-monotonic responses means that such issues remain pertinent (Fagin, 2012). Mechanisms that underpin, for example, dose–response curves may become amenable to genomic and related analyses.

Systematic appraisal of mechanisms of carcinogenesis

Information about mechanisms of carcinogenesis for the Group 1 agents in the *IARC Monographs* is summarized in this Scientific Publication with initial reference to 24 mechanistic end-points, which were then merged into 10 key characteristics (see Chapter 10, by Smith). These end-points – which include DNA damage, changes in gene expression, receptor-mediated effects, and inhibition of gap junctional intercellular communication – have been adopted on the basis of their wide use to investigate mechanisms of carcinogenesis. Once the available data are ordered according to these end-points, it is evident that for many agents, simple categorization according to a single mechanism is not possible or appropriate.

An important consideration is the discrepancy between the extents to which end-points have been assessed. DNA damage and gene mutations have been studied most extensively, and agents for which there is unequivocal evidence of genotoxicity across in vitro and in vivo systems have rarely been studied in relation to, for example, epigenetic alterations. Epigenetic alterations have been described for estrogenic hormones (Imamura, 2011), arsenic (Jensen et al., 2008), and nickel (Costa et al., 2005), although each of these agents had also been characterized as causing DNA damage. Evidence of immunosuppression may have been considered as a singular mechanism of carcinogenesis, but while azathioprine can be characterized as immunosuppressive, this agent also causes DNA damage.

Having been adopted as described, the 10 key characteristics warrant review with reference to the hallmarks as cataloguing a broad biological basis for malignancy (Hanahan and Weinberg, 2011). One hallmark, "activating invasion and metastasis", is not recognized as a mechanistic end-point because few, if any, agents are identified primarily with metastatic growth, given that no such hazard needs to be established over and above carcinogenicity. Some hallmarks are singularly identified as mechanistic end-points or enabling characteristics, i.e. those corresponding to chronic inflammation, immune effects, cell death, and angiogenic effects. Arguably, the end-point "DNA repair alteration" correlates with the enabling characteristic "genomic instability and mutation". The end-points "alterations in telomere length" and "immortalization" address the hallmark "enabling replicative immortality".

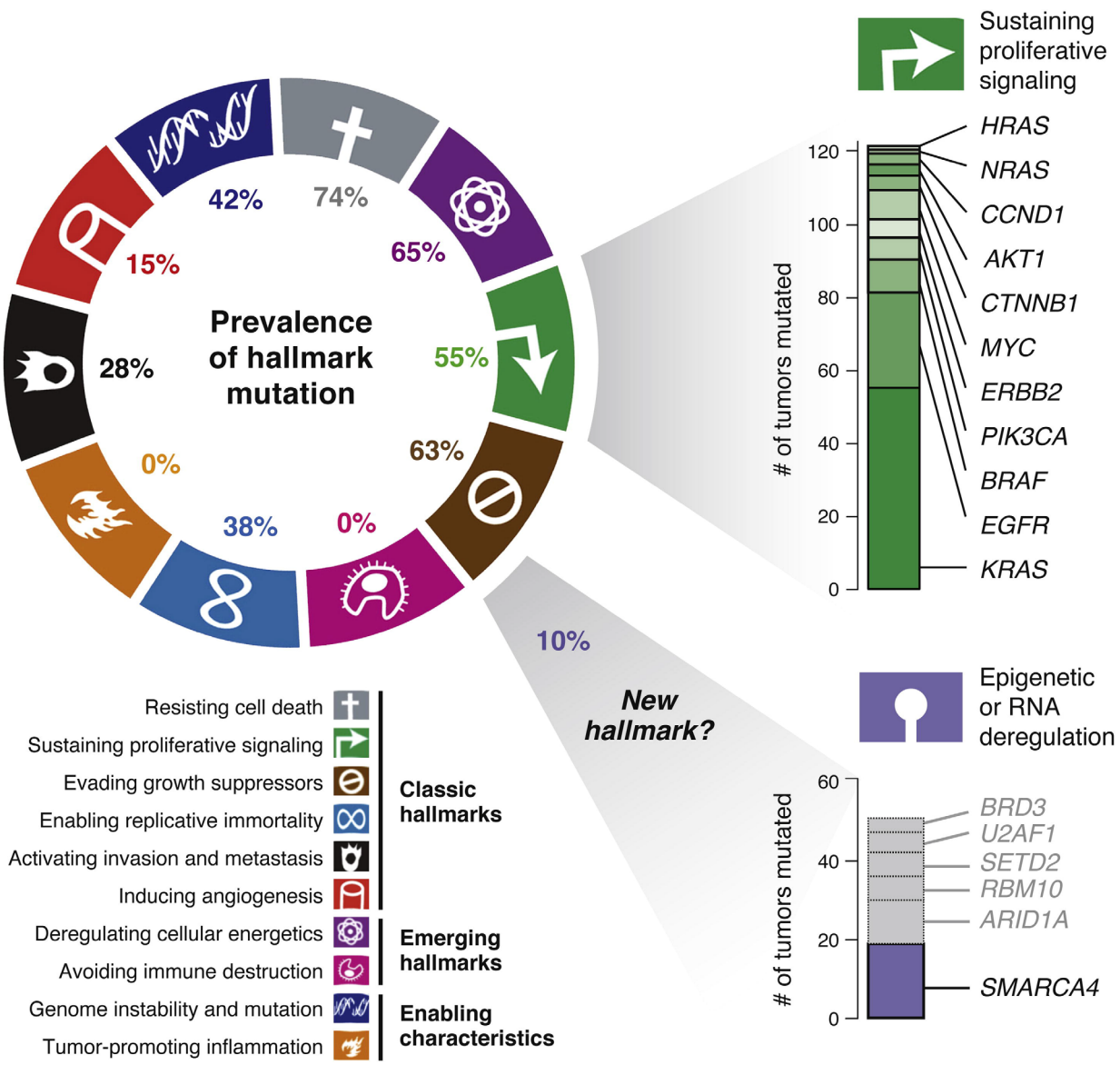
It would appear that the hallmark "evading growth suppressors" corresponds to end-points identified by cell-cycle effects taken together with a subset within the end-point "gene mutations": the subset of mutation of tumour suppressor genes as distinguished from mutation of oncogenes or other genes. The default position would then be to identify "sustaining proliferative signalling" – arguably the premier hallmark – with the remaining end-points. However, reference to those end-points leads to the recognition that end-points such as "epigenetic alterations" are the means through which many, if not all, of the hallmarks may emerge.

Finally, "deregulating cellular energetics" remains as the hallmark not addressed through the characteristics identified, because this parameter has not been recognized in systematic efforts to characterize mechanisms of carcinogenesis. Overall, no particular insight appears to be gained by attempting to relate the 10 key characteristics with specific hallmarks.

Tobacco smoke, cancer of the lung, and the hallmarks

Generalizing across tumour types, genomic and comparable analyses are concerned little, if at all, with exogenous agents that mediate malignant transformation. Paradoxically, the first tumour genome documented was described with a total focus on mutations attributable to tobacco smoke (Pleasance et al., 2010). Although genomic analysis revealed

Fig. 11.1. Hallmarks of lung adenocarcinoma. Left: The prevalence of mutation or somatic copy number alterations of genes mapping to cancer hallmarks defined by Hanahan and Weinberg (2011) based on tumour specimens from a cohort of 183 patients of whom more than 85% had a history of smoking. Top right: Genes comprising the mutated genes in the hallmark "sustaining proliferative signalling" are shown. Bottom right: A proposed new hallmark of "epigenetic or RNA deregulation" is shown, depicted as above. Genes shown in grey are candidate lung adenocarcinoma genes identified in the study of Imielinski et al. (2012) that may additionally contribute to the hallmark. Reprinted from Imielinski et al. (2012), copyright 2012, with permission from Elsevier.



an average mutation frequency in lung tumours from smokers of more than 10 times that in lung tumours from never-smokers (Govindan et al., 2012), the genomic pattern of squamous cell lung cancer, established from 178 patients of whom 96% had a history of smoking, was presented with no overt reference to tobacco use (Hammerman et al., 2012).

The genomic profile of lung adenocarcinoma, involving a cohort of patients of whom more than 85% had a history of smoking, was presented with reference to the hallmarks, documenting the prevalence of the enabling characteristic "genomic instability and mutation" in 25 adenoma genes adopted as indicators (Imielinski et al., 2012). The findings were not presented with reference to smoking status but indicated markedly different fractions of mutation (Fig. 11.1), including 42% with respect to "genomic instability and mutation". This result indicates the requirement to distinguish between gene mutation being relevant to etiology, whether or not it is caused by an exogenous agent, and frequency of mutation being an indicator of genomic instability and thus a characteristic of malignancy. Also of note, only 6% of tumours had alterations assigned to all six original hallmarks.

Mutation of genes that mediate particular hallmarks and are attributable to, among other agents, *N*-nitroso derivatives of nicotine and related compounds, and PAHs, is to be expected. However, beyond lung cancer, there are only few references to genomic analyses that enable individual tumours attributable to smoking to be distinguished from others. Thus, genomic analysis did not reveal likely tobacco causation for particular pancreatic cancers (Biankin et al., 2012).

Possible inferences from hallmark-based studies

Any malignancy is expected to exhibit the hallmarks, whether it arises spontaneously or upon exposure to a carcinogen. Insight into mechanisms of carcinogenesis is gained by the demonstration of biological change, which may be aligned with a hallmark (He et al., 2014). The public health implications of such a discovery may apply to agents not recognized as carcinogenic but shown to be promoters and/or inducers of inflammation or angiogenesis. Nicotine is an example of such an agent (Cardinale et al., 2012; Schaal and Chellappan, 2014). In addition to its contribution to a better understanding of tobacco smoke carcinogenesis, this information about the properties of nicotine is relevant to appropriate regulation of electronic cigarettes (also known as electronic nicotine delivery systems) (Dutra and Glantz, 2014). Nicotine may contribute to cancer development, for example by stimulating angiogenesis, in a manner not likely to result in the compound being designated a carcinogen.

During the past 50 years, the understanding and use of the term "carcinogenesis" has changed from that involving a necessary reference to one or more exogenous carcinogens to that involving intracellular processes leading to malignant transformation, with no necessary or implied reference to exogenous agents. This understanding has recently included the description of random mutations arising from DNA replication in normal non-cancerous stem cells as accounting for sporadic disease (Tomasetti and Vogelstein, 2015). However, another recent development is the identification of different mutational landscapes between classes of K-*ras*-driven tumours, depending on whether oncogene activation was achieved by genetic manipulation or after exposure to an alkylating *N*-nitroso compound (Westcott et al., 2015). Hence, genomic analysis may reveal distinct patterns of tumour-associated changes that are dependent on etiology and relevant to the full scope of tumour-associated signal transduction as identified by the hallmarks.

Apart from any mechanistic categorization of carcinogens in relation to particular hallmarks, the hallmarks do provide a basis for innovation. Genes identified from the perspective of each hallmark provide a basis on which to analyse both known carcinogens and agents of unknown status in that regard. An indication of agents worthy of attention may well be achieved by adding hallmark-related targets in the context of high-throughput screening assays, as described by Kavlock and colleagues (Kleinstreuer et al., 2013). The outcome may be the recognition of new classes of toxins that contribute to increased risk of cancer.

Summary

Cancer was once described with reference to causative agents, and multistage development of tumours was characterized through the impact of particular chemicals. Subsequently, multistage development of cancer was identified with morphological change being correlated with altered genetic makeup. The more recent description of eight hallmarks of malignancy is based not on morphology or on the impact of carcinogens but on changes in gene expression, sometimes mediated by mutation, and on selection for growth.

In parallel to this evolution of our understanding of cancer, no generally recognized mechanism-based scheme for classifying carcinogens

has evolved beyond categorization of chemical carcinogens according to genotoxicity. When appropriately studied, both genotoxic and non-genotoxic agents may mediate genetic and epigenetic change, variously resulting in emergence of the hallmarks, with the relevant processes being facilitated by genomic instability and inflammation. Enhancing –omics-based screening procedures to specifically include signal transduction pathways associated with particular hallmarks may provide new understanding of agent-related carcinogenesis.

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CHAPTER 12.

The role of genotoxicity in carcinogenesis

David M. DeMarini

The process of mutagenesis

The process of agent-induced mutagenesis consists of three parts: the induction of DNA damage, the sensing of the DNA damage by the cell (the DNA damage response), and the processing of the DNA damage by the cell, which may or may not result in a mutation. A key underlying concept is that mutagenesis is a cellular process, frequently involving DNA replication. Another key concept is that there is a distinct difference between DNA damage and mutation. Thus, mutagens, despite what their name suggests, generally do not produce mutations; instead, mutagens produce DNA damage, and they might more appropriately be called DNA-damaging agents. Instead, it is the cell that produces the mutation – either through faulty DNA repair of the mutagen-induced or spontaneous DNA damage, or by replicating past the unrepaired DNA damage, thereby introducing a replication error (Shaughnessy and DeMarini, 2009).

A description of the process of mutagenesis begins with the induction of DNA damage by an endogenous or exogenous event. Examples of DNA damage are DNA adducts (i.e. a molecule bound covalently to DNA) and single- or double-strand breaks (i.e. breakage of the phosphodiester backbone). Other types of DNA damage are oxidized or fragmented bases and the intercalation of a molecule between a pair of bases. Again, DNA damage is itself not a mutation and generally does not alter the linear sequence of nucleotides. A mutation is defined as a change in the sequence or number of nucleotides in the DNA.

When DNA damage occurs, the cell detects it by means of the DNA damage response system and determines how it will be processed; the DNA damage response includes DNA repair and apoptosis pathways, which are described in detail by Ciccia and Elledge (2010). The DNA damage response can mediate the repair of the damage, attempt to repair the damage but instead process it into a mutation, or direct the cell to undergo apoptosis. Another

possibility is that the damage is not repaired at all, and when the cell replicates, the DNA polymerase correctly bypasses the damage, resulting in a normal DNA sequence.

The cell can process DNA damage into three general classes or types of mutation: gene mutation (mutations that occur within a gene), chromosomal mutation (mutations involving more than one gene, typically called chromosomal aberrations), and genomic mutation (mutations involving the whole genome – generally aneuploidy, which is the gain or loss of a whole chromosome). The standard definition of a gene refers to a segment of DNA that codes for an mRNA that codes for a protein. The recent Encyclopedia of DNA Elements (ENCODE) project indicates that at least 80% of the human genome is transcriptionally active, but only a small proportion of the expressed regions code for protein (Maurano et al., 2012).

In the context of the process of mutagenesis described above, the term "mutagen" refers to an agent that can induce DNA damage that the cell processes into a mutation. The more general term "genotoxin" refers to an agent that induces DNA damage that may or may not be processed by the cell into a mutation. Some assays for genotoxicity, for example, measure only DNA damage, such as ^{32}P-postlabelling and the comet assay, whereas other assays measure mutation, such as the *Salmonella typhimurium* reverse mutation test, the *Hprt* gene mutation assay in Chinese hamster ovary cells, and transgenic mouse mutation assays. Thus, finding that an agent induces DNA damage would permit it to be called genotoxic, and showing that the agent also induces mutations in a mutation assay permits it to be classified as mutagenic.

A brief history of the nexus between mutagens and carcinogens

As reviewed by Claxton et al. (2010), there was little direct evidence for the role of mutagenesis in carcinogenesis until the early 1970s, and before that time only a few carcinogens had been shown to be mutagens (Burdette, 1955). Indeed, it is surprising to recall that at the time it was somewhat bold to propose that there was any direct connection between the two processes (Miller and Miller, 1971; Knudson, 1973). Many studies in the 1950s and 1960s showed binding of carcinogens to nucleic acids (Wiest and Heidelberger, 1953; Brookes and Lawley, 1964). However, before 1972 there was no direct proof that the electrophilicity of some chemical carcinogens had a necessary role in the potential mutagenic activity of such compounds, or even that DNA, as opposed to protein, was the ultimate target of carcinogens (Miller, 1970).

Although sound theoretical reasons had been proposed to support the notion that carcinogens might act through a mutagenic mechanism, a clear demonstration of this connection did not yet exist (Miller and Miller, 1971). Thus, binding to DNA of metabolites of carcinogens had been identified, but there were no data to show that these DNA adducts were processed into mutations or that mutations themselves played a role in carcinogenesis. Consequently, mutagenesis was viewed at that time as an equally plausible mechanism for carcinogenesis, along with epigenetic changes (Miller, 1970; Miller and Miller, 1971), altered expression of an integrated viral genome (Todaro and Huebner, 1972), and alteration of immunological factors by carcinogens, permitting the formation and growth of tumours (Baldwin, 1973). As time has shown, all of the above-mentioned mechanisms can play a role in the carcinogenic process, especially in the light of the accumulating evidence for the important role of epigenetic mechanisms (Baylin and Jones, 2011).

How did the paradigm shift occur that showed a connection between mutagenesis and carcinogenesis? The first screening studies to test the hypothesis that some carcinogens might also be mutagens were performed by Demerec et al. (1951) in *Escherichia coli* and then by Szybalski (1958), who tested more than 400 compounds in *E. coli*. Although additional testing proceeded throughout the 1960s in a variety of systems in bacteria, fungi, and mammalian cells, few carcinogens other than the direct-acting alkylating agents were found to be mutagens, leading to the conclusion that carcinogens were generally not mutagenic.

However, this view began to shift when Malling (1966) combined a chemical hydroxylating mixture with the carcinogens diethylnitrosamine and dimethylnitrosamine, which were not mutagenic in vitro, and showed that the resulting metabolites were mutagenic in the fungus *Neurospora crassa*. Malling (1971) then prepared an enzymatic activation system composed of the supernatant from mouse liver homogenate centrifuged at 30 000*g* (microsomes) plus cofactors, and showed that dimethylnitrosamine was mutagenic in *S. typhimurium* in a liquid suspension assay in the presence of this activation mixture. Additional evidence that carcinogens could be mutagens

after mammalian metabolism was provided by Legator and Malling (1971) with the host-mediated assay.

Ames et al. (1972) introduced the use of the plate incorporation assay in *Salmonella* and demonstrated that DNA-reactive metabolites of known carcinogens were direct-acting mutagens. The connection between mutagenesis and carcinogenesis was extended when Ames et al. (1973) combined a rat liver homogenate centrifuged at 9000*g* (S9 fraction) plus cofactors prepared as described by Garner et al. (1972) with *Salmonella* and a variety of rodent carcinogens then considered to be non-mutagenic in the plate incorporation assay and showed that these carcinogens were, in fact, mutagenic. Additional refinements of the *Salmonella* tester strains and the conduct of multiple testing studies, involving not only *Salmonella* but also other test systems (Tennant et al., 1987), resulted in the current recognition that many carcinogens, by themselves or after metabolic activation, are mutagens, and that mutagenesis is a critical feature of carcinogenesis.

Despite the recognized importance of mutagenicity as a part of cancer induction and progression, by the 1990s it appeared that many rodent and human carcinogens were, in fact, not clearly mutagenic or genotoxic. Some operate through receptor binding, which can result in an alteration in gene expression, often leading to increased cell replication. However, an analysis of a set of so-called non-genotoxic carcinogens found that most of them were, in fact, genotoxic (inducing DNA damage and/or mutation) when tested adequately for both gene, chromosomal, or genomic (aneuploidy) damage and mutation (Jackson et al., 1993). Indeed, a comprehensive analysis showed that more than 90% of the IARC Group 1 chemical carcinogens are genotoxic (Waters et al., 1999).

The current genetic toxicity test battery is based on this relationship between mutagenesis and carcinogenesis. Consequently, mutagenicity assays continue to be used as a potential screen for carcinogens, and the results are used for regulatory purposes throughout the world (Eastmond et al., 2009). For example, a positive result in the *Salmonella* mutagenicity assay indicates a 70% probability that the test chemical is a rodent carcinogen (Zeiger, 1998). When a randomly selected set of 100 organic compounds was tested in the *Salmonella* mutagenicity assay, about 20% of them were positive (Zeiger and Margolin, 2000). Thus, out of an estimated 80 000 such compounds in commercial use, 16 000 (20%) may be positive for mutagenicity in the *Salmonella* mutagenicity assay, and 11 200 (70%) of those may be potential rodent carcinogens.

Mutations in tumours

Soon after the discovery of the correct number of human chromosomes (46) by Tjio and Levan in 1956 (Gartler, 2006; Harper, 2006), cytogenetic studies began to show that tumours (specifically leukaemic cells) had higher frequencies of chromosomal aberrations than did normal cells (Nowell and Hungerford, 1960). A decade later, the development of quinacrine fluorescence and Giemsa staining enabled the first discovery that a specific chromosomal aberration was associated with a specific type of leukaemia (Rowley, 1973). As evidence accumulated that chromosomal aberrations were present at high frequencies in tumours, DNA sequencing methods were introduced in 1977 (Pettersson et al., 2009), which provided the technical means to directly determine the presence and types of mutations in any gene or chromosome.

DNA sequencing of mutations induced in selected genes by a limited number of mutagenic carcinogens in microbes in the 1980s and in mammalian cells and tumours in the 1990s began to show that any particular mutagen produced an array of mutations and that these varied among the genes and cells examined. A variety of mutagens produce similar mutation spectra, and the predominant base substitution that an agent induces in one system is generally the same one that the agent produces predominantly in all other systems across the phylogenetic scale, from bacteria to humans (DeMarini, 1998, 2000). Thus, in terms of the predominant base substitution produced by agents, there is concordance across species in that the DNA damage induced by a particular agent is processed similarly by a wide range of species.

With regard to mutations in tumours, generally elucidated without reference to any exogenous causative agent, the technology in use from the 1980s until the early 2000s permitted the determination of mutations in only a few cancer-related genes, such as *TP53* and *KRAS*. The first gene mutation in a human tumour was determined in 1982 (Reddy et al., 1982), and by the end of the 20th century, there was clear evidence that some tumours had mutations in certain oncogenes and tumour suppressor genes that could be associated with the types of mutations produced by the carcinogen associated with the induction of the

tumour – both in rodents and in humans (Dogliotti et al., 1998; Hainaut and Wiman, 2009). Examples include CC → TT mutations in the *TP53* gene in skin tumours associated with exposure to sunlight, G → T mutations in codon 259 of the *TP53* gene in liver tumours associated with exposure to aflatoxin B_1 (Ceccaroli et al., 2015), A → T mutations in the *TP53* gene in tumours of the upper urinary tract associated with exposure to aristolochic acid (Grollman, 2013), and the different locations of G → T mutations in the *TP53* and *KRAS* genes in lung tumours associated with exposure to cigarette smoke (IARC, 2012) or to emissions from smoky coal (DeMarini et al., 2001). There are about 20 carcinogens that are known to produce either unique or distinctive mutation spectra in tumours linked epidemiologically to specific exposures in humans (Ceccaroli et al., 2015).

With the advent of next-generation DNA sequencing, exome and genome sequencing of tumours was first reported by Wood et al. (2007) and Parsons et al. (2008). These initial studies revealed that tumours had large numbers of mutated genes; however, it appeared that only a few (six to eight) genes were involved directly in the carcinogenic process. As few as three driver gene mutations are required for the development of lung cancer or colorectal cancer (Tomasetti et al., 2015). Large-scale sequence analysis of the genomes of thousands of human tumours has identified new genes that are important for cancer and new mutational signatures that are specific to particular tumour types and subtypes (Hoang et al., 2013; Alexandrov and Stratton, 2014).

As discussed elsewhere in this Scientific Publication (see Chapter 11, by Stewart, and Chapter 19, by Caldwell et al.), cancer is a genetic disease that proceeds by a type of Darwinian evolution (Hanahan and Weinberg, 2011). In this way, changes in gene function (by mutation) and in gene expression (by epigenetic mechanisms) that result in a cell having a specific growth advantage may be selected for in certain tissue microenvironments (Hanahan and Weinberg, 2011; Solomon et al., 2011; Whitfield and Soucek, 2012).

Stratton (2011) estimated that most human tumours contain 1000 to 10 000 base substitution mutations; tissues exposed more directly to the environment, such as the lung and the skin, have tumours with more than 100 000 mutations. However, only about 400 genes (~2% of the coding genome) appeared to be involved directly in tumorigenesis; the rest were likely to be passenger mutations, i.e. mutations not related to the carcinogenic process and possibly resulting from the genomic instability of the tumour (Bozic et al., 2010).

Genome or exome sequencing has identified 20 distinct mutational signatures among human tumours and confirmed that tumours of the most "protected" organs (e.g. the brain) have only a few mutations, whereas those in organs exposed more directly to the environment (e.g. the lung and the skin) have thousands of mutations (Alexandrov et al., 2013). The vast majority of mutations in tumours are base substitutions (Vogelstein et al., 2013). Only one or two mutated genes appear to be required for some haematopoietic tumours, whereas at least five or six are required for solid tumours (Stratton, 2011). In addition, all tumours have many genes with altered gene expression (Baylin and Jones, 2011).

A recent discovery is "shattered" chromosomes in tumours, a phenomenon termed chromothripsis, which results in massive chromosomal rearrangements in 1–3% of human tumours (Stephens et al., 2011). Studies indicate that these shattered, highly rearranged chromosomes may appear exclusively in micronuclei (Crasta et al., 2012; Maher and Wilson, 2012), providing a new insight into the potential role of micronuclei in tumours (Hatch and Hetzer, 2015).

As evidence had accumulated that mutation and mutagenesis are essential features of carcinogenesis, the notion that tumours might be monoclonal became popular, because of the monoclonality of haematopoietic malignancies and because this notion appeared to support the prevalent initiation–promotion model of carcinogenesis. However, as molecular analyses of tumours became more sophisticated, it soon became clear that tumours are not monoclonal and that they are, in fact, highly heterogeneous (Parsons, 2008).

The most exquisite evidence for this has been provided by Gerlinger et al. (2012), who showed that approximately two thirds of all somatic mutations were not present in all regions of a set of kidney tumours analysed by a combination of exon sequencing, chromosomal aberration analysis, and mRNA expression analysis. Recently, Martincorena et al. (2015) demonstrated that this heterogeneity is established early on, by showing that physiologically

normal human skin contains a patchwork of thousands of evolving clones, with more than one quarter of such cells having cancer-causing mutations.

Although there is now also overwhelming evidence for the essential role of epigenetic changes in the carcinogenic process (Grønbaek et al., 2007; Baylin and Jones, 2011) and for the fact that many carcinogens can induce such changes (Ceccaroli et al., 2015; Nicolaidou and Koufaris, 2015), as discussed below there is emerging information that mutation itself might underlie some, if not most, of these epigenetic changes.

There are three primary epigenetic mechanisms by which cells regulate gene expression: methylation of DNA (Hsiao et al., 2009), modifications of histones (Ellis et al., 2009), and binding of microRNAs and other non-coding RNAs to the genome or to other RNAs (Garzon et al., 2009). However, studies have shown that mutations in genes involved in these three processes may be the basis for many of the epigenetic events mediated by these mechanisms (You and Jones, 2012). For example, mutations in specific chromatin-modifying genes appear to occur in specific cancers, such as in *JARID1C* in renal cancer, in *SMARCA4/BRG1* in lung cancer, and in *ARID1A* in ovarian cancer (Jones et al., 2010). Also, mutations in the DNA methyltransferase genes *DNMT1* and *DNMT3A* are found in colorectal cancer or acute myeloid leukaemia, the histone lysine methyltransferases or demethylases *HK4*, *H3K9*, and *H3IK27* are mutated in kidney cancer and colon cancer, and the histone acetyltransferases *H3K18* and *H3K27* are mutated in acute lymphoblastic leukaemia (Peltomäki, 2012; Ryan and Bernstein, 2012). Although epigenetic changes per se are not mutations because the sequence of nucleotides has not been changed, as evidenced above, mutation may be the basis for some epigenetic events.

Models of agent-induced carcinogenesis

Data generated in recent years have led to a reconsideration of the dichotomy between so-called non-genotoxic versus genotoxic carcinogens (Waters et al., 1999) and indicate that some epigenetic events may have a mutational basis (You and Jones, 2012). In addition, chronic inflammation, which is associated with increased cancer risk (Colotta et al., 2009), causes DNA damage (etheno-base lesions and other exocyclic DNA adducts) that appears to be the basis for the increased risk, as demonstrated by the fact that repair of the damage by base excision repair enzymes (alkyl glycosylases) reduces the risk of cancer (Calvo et al., 2012). Indeed, an analysis of a dozen human studies found strongly increased risks of cancer among individuals with high levels of DNA adducts relative to those with low levels, and the cancer risks were even higher for the group with high adduct levels when other risk factors, such as infection and inflammation, were taken into account (Poirier, 2012). As noted in Chapter 19, by Caldwell et al., host susceptibility factors modulate all of these events and are a critical element in the overall cancer risk.

Within the context of both the initiation–promotion model of carcinogenesis and the "hallmarks" of cancer (Hanahan and Weinberg, 2011), these data have led to the view that (i) cancer is essentially a genetic disease and (ii) an agent that causes cancer induces alterations in gene function (by mutation) and/or gene expression (by epigenetic changes), either by direct interaction with DNA or chromatin or by indirect mechanisms, such as through generation of reactive oxygen species, inflammation, and/or receptor-mediated interactions. These considerations suggest that carcinogens must be genotoxic in the broadest sense of the term, i.e. they damage DNA or alter its expression either directly or indirectly, leading to a change in function or expression of genes. Such changes in the appropriate genes with promotion through cell replication and selective pressure can then lead to a tumour. For colorectal tumours this concept has been characterized as the "Big Bang" model for tumour growth, in which tumours start early on producing mixed subclones that are not subject to stringent selection, thus explaining the heterogeneity of tumours (Sottoriva et al., 2015).

This greater appreciation for how chemical, physical, and biological agents may induce cancer leads to a model for agent-induced carcinogenesis that integrates portions of the classic initiation–promotion model with elements of the hallmarks of cancer. Such a model would envision a carcinogenic agent establishing the process by either genetic or epigenetic mechanisms that cause changes in gene function and expression, resulting in the plethora of characteristics of cancer cells, i.e. the hallmarks of cancer: mutations in key oncogenes, altered gene expression, changes in cell signalling, altered cell growth, evasion of apoptosis, sustained angiogenesis, increased genomic instability, and eventual metastasis. Much of this can be modulated by various sus-

ceptibility factors, including genetic or epigenetic factors, as well as by a large number of environmental and lifestyle factors (see Chapter 19, by Caldwell et al.). However, a genotoxic carcinogen may not necessarily cause cancer via a genotoxicity mechanism alone or predominantly, and further mechanistic studies are needed to delineate the carcinogenic mechanisms of any particular agent.

This generalized model no longer makes a distinction between initiation and promotion, which was an operational model derived largely from mouse skin-painting studies. Similarly, it does not divide carcinogens into genotoxic and non-genotoxic categories. Instead, an integrated model of agent-induced carcinogenesis as described above emphasizes the ability of the carcinogen (chemical, physical, or biological) to alter gene structure (by mutation) and/or gene expression (by genetic or epigenetic changes), leading to functional changes in the genome that manifest themselves through changes in cell signalling, altered cell growth, and genomic instability, resulting in the hallmarks of cancer, with susceptibility factors modifying various aspects of these processes and outcomes. The importance of epigenetic changes, cell signalling, and tissue–cell interactions have suggested alternative models to the somatic mutation theory of cancer, such as tissue organization field theory (Baker, 2015) and tissue programming theory (Burgio and Migliore, 2015).

Summary

Work since the 1970s demonstrated that many carcinogens are either directly or indirectly genotoxic or mutagenic and/or alter gene expression. Analyses of tumours, first by cytogenetic methods in the 1970s, then by single-gene analysis in the 1990s, and most recently by exome or whole-genome sequencing, have demonstrated clearly that mutagenesis is a central feature of carcinogenesis. Thus, it is not surprising that more than 90% of the known human chemical carcinogens (IARC Group 1) are positive in conventional short-term tests for genotoxicity (Waters et al., 1999).

Cancer is now recognized as an essentially genetic disease, with carcinogens causing genetic damage and/or changes in gene expression either directly or indirectly. This recognition should prompt a reconsideration of the distinction between genotoxic and non-genotoxic carcinogens. A generalized model of agent-induced carcinogenesis would no longer make a distinction between initiation and promotion but would instead emphasize the initial effects of the agent that then lead to a series of changes in cell signalling that result in the hallmarks of cancer, with the entire process being modified by a variety of susceptibility factors.

The ability of carcinogenic agents to induce mutation and/or alter gene expression, with either ability being sufficient to initiate the process of tumour formation (Grønbaek et al., 2007; Halazonetis et al., 2008), is now an established feature of agent-induced carcinogenesis. This deeper understanding of the relationship between genotoxicity and carcinogenicity is the culmination of research that provided the first evidence for such a relationship only about 40 years ago.

Acknowledgements

The author thanks J.A. Ross and C.E. Wood for their helpful comments on this manuscript.

Disclaimer

This article was reviewed by the National Health and Environmental Effects Research Laboratory, United States Environmental Protection Agency and approved for publication. Approval does not signify that the contents reflect the views of the agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

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CHAPTER 13.

Alterations in cell proliferation, cell death, or nutrient supply

Jane C. Caldwell

Introduction

Mechanistic data have been included in Volume 100 of the *IARC Monographs*, and they vary with the agent studied. These data are especially dependent on the type of study and the contemporary understanding of the state of the science at the time of publication of the study.

As an outcome of the two-part IARC Workshop on Tumour Site Concordance and Mechanisms of Carcinogenesis, a mechanistic database was assembled for the IARC Group 1 carcinogens (see Chapter 22, by Krewski et al.). These agents were examined with regard to 10 key characteristics – one or more of which are commonly exhibited by these agents – that can be used to identify and organize mechanistic information related to cancer induction (see Chapter 10, by Smith; see also Smith et al., 2016). One of these 10 mechanistic categories of data is a composite that includes information on the ability of a carcinogen to alter cell proliferation, cell death, or nutrient supply.

Alteration of cell proliferation is identified through assays that detect replicative DNA synthesis, 5-bromo-2′-deoxyuridine (BrdU) labelling, proliferating cell nuclear antigen (PCNA) labelling, and hyperplasia or the occurrence of multinucleated cells by light microscopy, and through analysis of some of these end-points by use of flow cytometry (Gray et al., 1986; Jayat and Ratinaud, 1993; Stacey and Hitomi, 2008; Irish and Doxie, 2014). Although for many of the human carcinogens Volume 100 of the *IARC Monographs* contains more descriptive data under this category, primarily as changes in cell proliferation, these changes are inherently related to alterations in cell signalling and/or cell-cycle control.

Many challenges are associated with the use of data described for alterations in cell proliferation, cell death, or nutrient supply to examine mechanistic and tumour site concordance between humans and experimental animals. Several key mechanistic characteristics can result in or arise from changes in cell signalling

(e.g. inflammation, genotoxicity, and epigenetic alterations) and can have both genetic and epigenetic origins.

There are other levels of interdependence between the key mechanistic characteristics. Inflammation, excessive oxidative stress, and genomic instability are related (see Chapter 17, by Kane). Mutagenesis may also underlie some epigenetic events that change cell signalling. For example, mutations in genes involved in the methylation of DNA, modification of histones, and binding of microRNAs to the genome or to other RNAs may initiate epigenetic changes (see Chapter 12, by DeMarini, and Chapter 20, by Rice and Herceg).

Cell signalling pathways that regulate cell proliferation are not independent of those associated with other key mechanistic characteristics of IARC Group 1 carcinogens. Many of the genes associated with cell proliferation are also linked with apoptosis, inflammation, and several pleiotropic responses. Dysregulation in the mitogen-activated protein kinase (MAPK) pathway affects most, if not all, processes involved in cancer (Dhillon et al., 2007). The extracellular signal-regulated kinase (ERK) pathway of the MAPK family is most commonly associated with regulation of cell proliferation (Reuter et al., 2010).

The ability to use this characteristic to evaluate both mechanistic and tumour site concordance is influenced by recent developments in cancer research, by the overarching issue of how carcinogens may express certain characteristics, and by the question as to the biological basis of the differences between species, strains, target organs, and target cells in cell signalling and cell-cycle control.

This chapter focuses on issues associated with the understanding and interpretation of available data for this key mechanistic characteristic.

Genetic drivers of cell proliferation and apoptosis: complex relationships and pleiotropic roles of cell signalling molecules

Cell signalling is a process whereby proteins or other chemical messengers activate receptors at the cell surface and then transmit signals inside the cell via membrane-to-nucleus pathways. In healthy adults, cell proliferation, cell differentiation, and cell death determine the size of the proliferating cell population in soft tissues, for example surface epithelia, mucosal lining cells of excretory ducts, columnar epithelia lining the gastrointestinal tract and uterus, transitional epithelium of the urinary tract, and bone marrow and haematopoietic cells. These proliferating cells replace dead cells throughout life. Pathological effects (e.g. injury resulting from hepatocellular necrosis or partial hepatectomy) and physiological conditions (e.g. estrogen-induced effects on the endometrium during the menstrual cycle) can involve stimulation of cell proliferation (Engström et al., 2015).

The relationships between cell signalling molecules and pathways that control cell proliferation and programmed cell death (apoptosis) are complex. Numerous enzymes and cell signalling pathways are modulated during apoptosis, and dysfunction of cell death pathways is associated with initiation and progression of tumorigenesis; the products of proto-oncogenes (genes that encode proteins that stimulate cell proliferation, inhibit apoptosis, or do both) include transcription factors, chromatin remodellers, growth factors, growth factor receptors, signal transducers, and apoptosis regulators (Narayanan et al., 2015). In response to mitogens, cell proliferation is triggered by increased translocation into the nucleus of ERK 1 and 2 (ERK1/2), the last proteins in the MAPK/ERK cascade. Activating mutations upstream or within the ERK1/2 cascade are present in several human cancers, but ERK1/2 activation also occurs in cancers without mutation of components of the cascade (Plotnikov et al., 2011).

Ras, a small upstream guanosine-5′-triphosphate (GTP)-binding protein in several signalling pathways, has two isoforms, H-Ras and K-Ras, with different potencies to activate the MAPK/ERK pathway; the *KRAS* gene is more frequently mutated in human cancer, which can result in constitutive activation (McCubrey et al., 2007). H-Ras has been implicated as contributing to the cancer phenotype, through evasion of anti-growth signalling, angiogenesis, genetic instability, tissue invasion and metastasis, tumour-promoting inflammation, and changes in the tumour microenvironment (Engström et al., 2015). K-Ras promotes metabolic reprogramming, activation of proliferative signalling pathways, glycolysis, reduction of oxidative metabolism in the tricarboxylic acid cycle, and channelling of glucose intermediates into anabolic pathways, such as the hexosamine biosynthesis pathway.

The tumour suppressor protein p63, which is activated by DNA damage, cellular stress, and oncogenic signal transduction, has pleiotropic anti-proliferative and metabolic effects that include metabolic cell-cycle arrest (Robey et al., 2015). Numerous pathways have been

identified to be involved in disruption of resistance to cell death, and p53 has been described as implicated in cell-cycle arrest, apoptosis, regulation of metabolism, DNA repair, and every pathway linked to these processes (Narayanan et al., 2015). Disruption of the MAPK cascades, which are central signalling pathways that regulate a wide variety of cellular processes, is associated with induction and progression of various diseases, including not only cancer but also diabetes, autoimmune disease, and developmental abnormalities (Plotnikov et al., 2011).

The role of gene activation in carcinogenesis is also complex and evolving. Activation of protein kinase C, which acts as a catalyst for several cellular functions that are related to cancer (e.g. cell survival, proliferation, apoptosis, and migration), has been thought to enable tumour development. However, protein kinase C isozymes have recently been reported to be suppressed in human cancers, possibly through loss of function that suppresses other oncogenic signals (Antal et al., 2015). The gene for an RNA-binding protein that is highly active in blood cancers (i.e. RNA-binding protein Musashi homolog 2) is not directly mutated in tumours, but its activation affects the ability of RNA to be translated into proteins (Wang et al., 2015), and consequently its role in cancer has not been identified through mutation or gene expression patterns.

Genetic variability in cell signalling between species, strains, and target organs

Genetic variability between species has been described in terms of their genomic content and the regulation and expression of their genes. Both the genetic code, which specifies trinucleotides that identify amino acids, and the regulatory code that determines how DNA sequences direct gene expression are highly conserved between species. However, species differ in the composition and the length of the DNA sequences that use this language in the regulatory regions of their genes (Nitta et al., 2015).

The Mouse ENCODE (Encyclopedia of DNA Elements) Consortium reported that the degree of conservation is high: the mouse genome is similar to the human genome in size, structure, and sequence composition, and more than 80% of mouse genes have human orthologues. The chromatin landscape in a cell lineage is relatively stable in both humans and mice, transcription factor networks are substantially more conserved, and both the human and mouse genomes are pervasively transcribed (Vierstra et al., 2014; Yue et al., 2014). The pattern of chromatin states (defined by histone modifications) and the large-scale chromatin domains are highly similar between mice and humans, but there is a divergence in the regulatory landscape that confers plasticity both between cell types and between species (Yue et al., 2014).

Organ-specific genes are more highly expressed than housekeeping genes (i.e. those present in all tissues), and the highest organ-specific gene expression is observed in the testes, brain, liver, muscle (cardiac and/or skeletal), and kidney (Lin et al., 2014). Comparisons of gene expression between human and murine tissues showed similarities in gene expression profiles at the tissue and organ level. However, there were greater similarities within each species for non-coding and conserved protein-coding genes, which are likely to mediate species differences (Lin et al., 2014).

Human-to-mouse transgenic experiments demonstrate recapitulation of human gene regulation in mice, even in the case of human genes that lack murine orthologues. However, for distinct biological pathways, the expression profiles of many mouse genes diverged from those of human orthologues (Yue et al., 2014). A core set of candidate regulatory sequences were conserved and display similar activity profiles in humans and mice: expression patterns for genes that encode proteins in the nuclear and intracellular organelle compartments, and genes involved in RNA processing, nucleic acid metabolism, chromatin organization, and other intracellular processes. However, less interspecies concordance was observed for genes involved with the extracellular matrix, cellular adhesion, signalling receptors, and immune responses (Yue et al., 2014).

Within orthologous mouse and human cell types, there is conservation across species of the global fraction of regulatory DNA sequences that encode recognition sites for each transcription factor (Vierstra et al., 2014). However, between humans and mice there is variation in regulatory regions that govern individual gene systems and the occupancy pattern of transcription factors, with extensive *cis*-regulatory "rewiring", mediated by elements that recognize transcription factors. Although they have a common language in regulation, active elements in one species may be reassigned to a different tissue in another species (Vierstra et al., 2014). Thus, differences in the regulation of gene expression and

cell signalling between species and tissues may affect mechanistic and tumour site concordance.

Variability in mutation targets and cell signalling across tissues and in tumours

Effects from activation of the MAPK/ERK pathway, such as cell growth, prevention of apoptosis, and cell-cycle arrest, depend on the cell lineage; for example, activation of this pathway is associated with proliferation and drug resistance in haematopoietic cells, but the activation is suppressed in some prostate cancer cell lines (McCubrey et al., 2007).

The difficulty in predicting cell-specific effects on cell signalling is also illustrated by differences in certain biological responses between histological subtypes of lung cancer, i.e. adenocarcinoma versus squamous cell carcinoma, in human patients and in chemically induced lung cancer in mouse models. In A/J mice treated with urethane to induce adenocarcinoma, or with *N*-nitrosotris-(2-chloroethyl)urea to induce squamous cell carcinoma, inhibition of vascular endothelial growth factor has the opposite effect in these two tumour subtypes (increased apoptosis vs increased proliferation) (Larrayoz et al., 2014).

In another example, gene expression profiling was poor at distinguishing histological subtype and cell type of origin for human breast cancer, but a mouse model could demonstrate the correct genetic lesion and cell type to model human disease, by confirming that the origin of *BRCA1* mutation-associated breast cancer is a luminal estrogen receptor (ER)-negative mammary epithelial progenitor (Molyneux and Smalley, 2011). However, different mouse models with the same K-*ras* mutations, one chemically induced and the other genetically engineered transgenic, produced tumours with different gene expression patterns (Westcott et al., 2015) and showed differences not only in tumour susceptibility but also in model-dependent signalling pathways. (See Chapter 19, by Caldwell et al., for a discussion of host susceptibility factors that influence tumour site concordance.)

As noted above, human tumours carry mutations in genes that encode components of cell signalling pathways associated with cell proliferation and cell death. High mutation frequencies have been associated with tumours induced by particular carcinogens or mixtures of carcinogens, for example melanoma induced by exposure to ultraviolet light and lung cancer induced by exposure to tobacco smoke (Lawrence et al., 2013).

However, cancer is not a disease of uniform origin, progression, or cell biology. Different types of cancer show variation in overall mutation rate, predominant mutation type, and distribution of mutations along the genome. Epigenetic patterns of chromatin accessibility, histone modification, gene expression, and DNA replication timing are also cell lineage-specific (Polak et al., 2015). A study of 173 cancer genomes from eight different types of cancer, representing a wide range of tissues of origin, carcinogenic mechanisms, and mutational signatures, showed that chromatin features of the cell type of origin, and not of matched cancer cell lines, were the best predictors of local frequency of somatic mutations. Mutation density was associated with epigenomic features, i.e. it was lower in areas of active chromatin and transcription (Polak et al., 2015).

To create a comprehensive catalogue of genes responsible for the initiation and progression of cancer, 27 types of cancer were studied through sequencing of matched tumour and normal tissue samples as part of the Cancer Genome Atlas and the International Cancer Genome Consortium (Lawrence et al., 2013). However, as sample sizes increase, the number of putative significant genes also increases and the risk of false positives resulting from tissue heterogeneity between cancer types in mutation type, distribution, and frequency is highly variable. Across the 27 types of cancer, the median frequency of non-synonymous mutations varied over more than three orders of magnitude; half of the variation in mutation frequency was associated with tissue type of origin. Within cancer types, patient-specific mutation frequencies also spanned three orders of magnitude. This mutational heterogeneity was strongly correlated with DNA replication timing and with transcriptional activity, i.e. it was higher in late-replicating DNA regions and lower in highly expressed genes. Higher mutation frequencies occurring in late-replicating genes may be responsible for potentially false-positive putative cancer genes, such as the olfactory receptor genes and some genes cited in association with lung cancer (Lawrence et al., 2013). Thus, the tissue of origin greatly affects mutation patterns and is linked to DNA replication timing and tissue-specific transcriptional activity.

Genomic sequencing of established tumours to study their causes has its limitations, because such an approach is unable to study the

evolution of the clones, the accumulation of mutations in normal somatic cells, the variability among individuals in driver mutation profiles, and the variability among cancer genes in clonal dynamics. In a study of 74 cancer genes in sun-exposed skin, i.e. a polyclonal quilt of mutations in key cancer genes consistent with damage from ultraviolet light, multiple cancer genes were found to be under strong positive selection even in physiologically normal skin (Martincorena et al., 2015). Positively selected mutations were found in 18–32% of normal skin cells. The size of clonal expansions varied across genes, and gene size was not necessarily correlated with the potential of the somatic mutation to induce malignant transformation. Consistent with findings in tumours, the mutation rate also varied along the genome, with higher rates found in less frequently expressed genes and in repressed chromatin. These findings were inconsistent with the assumption that driver mutations occur infrequently in long-lived cell lineages and that those arising in cancer are too small to be detected clinically (Martincorena et al., 2015).

If a gene signature – a group of genes with a characteristic combined expression pattern – is associated with a prognosis, it is assumed to be likely to encode a biological signature driving carcinogenesis. However, this assumption has been questioned in view of findings that random changes in gene sets are associated with prognosis and that prognostic signatures in ER-negative breast cancer – associated with hypoxia and angiogenesis – are more similar to those in ovarian cancer than to those in ER-positive breast cancer, which are driven by proliferation pathways (Beck et al., 2013). Two distinct expression arrays of breast cancer cells, with almost no genes – and thus no protein changes – in common, can be equally useful for predicting clinical behaviour, and analyses with gene expression arrays may not provide a true understanding of cancer biology (Weinberg, 2014).

Sequencing of entire tumour genomes has yet to demonstrate definitively the number of somatic mutations required to create a human tumour. A few conceptual insights into cell and tissue behaviour have resulted from elaborate maps of interacting signalling components and computer models of signalling (Weinberg, 2010). The paradigm of somatic evolution and multistep tumorigenesis does not provide a logical reason why oncogenesis recapitulates ontogenesis (Huang et al., 2009).

Alterations in nutrient supply

Although dysregulated metabolism is one of the most common and recognizable features of cancer and is associated with other phenotypic indicators, the results of a recent literature review attempting to link cancer development and dysregulated metabolism suggested that there are major gaps in the understanding of exposure-related carcinogenesis and metabolic reprogramming, for example with respect to the specific causal and temporal relationships between exposures, dysregulated metabolism, and the development of cancer and the associated phenotypic hallmarks of cancer (Robey et al., 2015). This review did not consider lifestyle-related exposures and IARC Group 1 carcinogens.

It is difficult to identify associations that directly support a primary metabolic link between environmental exposures and cancer, for several reasons: metabolic control does not occur in a single step in a metabolic pathway; controlling factors differ between intact cells and in vitro cell-free systems; observed changes in individual pathway elements do not always lead to changes in metabolic flux; and cancer cell phenotypes are neither fixed nor cancer-specific. The review also noted the functional interdependence of dysregulated metabolism and other hallmarks of cancer, considering that, for example, proliferating cancer cells have shared regulatory factors associated with the fundamental anabolic and catabolic demands of the hallmark "sustaining proliferative signalling" (Robey et al., 2015).

Increased body mass index has been associated with increased risk of cancer: obesogens – chemicals that disrupt normal development and the balance of lipid metabolism – are able to cause permanent changes in metabolism that may render the subject more susceptible to cancer later in life (see Chapter 19, by Caldwell et al.). Inflammation is associated with metabolic changes and has been linked with several chronic diseases, including cancer. Extracellular pro-inflammatory metabolic signals are adenine nucleotides, succinate, oxidized nicotinamide adenine dinucleotide (NAD^+), and urate (McGettrick and O'Neill, 2013; Tannahill et al., 2013). The gut microbiome has an important role in carbohydrate absorption and metabolism in humans and plays a significant part in inflammatory responses as well (see Chapter 19, by Caldwell et al.).

The transgenerational character of metabolic disturbances and effects on cell signalling is demonstrated by studies of multigenerational undernutrition in rats (i.e. for 50 generations). The undernourished rats were predisposed to insulin resistance, had altered levels of several metabolic regulators (e.g. circulating insulin, homocysteine, endotoxin, leptin, adiponectin, vitamin B_{12}, and folate), and had a higher susceptibility to streptozotocin-induced diabetes compared with properly fed control rats. These changes were not reversed by feeding rats a normal diet in the two subsequent generations (Hardikar et al., 2015).

Studies on altered cell signalling have traditionally been performed on putative target cells of cancer, but the contribution of the gut microbiome (i.e. the microbiota living on and in humans) has only recently been investigated as a factor in cancer susceptibility (see Chapter 19, by Caldwell et al.). The microbiome plays a role in the control of nutrient supply (e.g. the gut microbiome is highly enriched in carbohydrate metabolism genes, compared with the human genome overall; Bultman, 2014), in metabolic pathways, and in host susceptibility to metabolic disease (Suez et al., 2014).

Cell proliferation as a component or cause of cancer

There are at least three scenarios related to cancer and mechanisms of cancer induction in which alterations in cellular replication and/or cell-cycle control have been described. The first invokes the predisposition of replicating cells with unrepaired DNA damage to develop into cancer cells. The second identifies sustained replication as a key event in various modes of action, and the third describes the ability of a transformed cell to escape normal cell-cycle control and to continue replication. The interpretation of mechanistic data for cell proliferation and cell death is dependent on the development of appropriate animal models (see Chapter 19, by Caldwell et al.), and although cell proliferation has been used in descriptions of hypothesized modes of action (Wood et al., 2015), it should be viewed in the context of the newer understandings of cancer mechanisms.

For DNA damage to lead to a mutation, DNA replication and cell division are typically required (see Chapter 12, by DeMarini). As noted in the United States Environmental Protection Agency guidance assessing the risk of cancer from early-life exposures (EPA, 2005), more frequent cell division during development can result in enhanced fixation of mutations because of the reduced time available for repair of DNA lesions, and clonal expansion of a mutated cell produces a larger population of mutant cells. For adult organisms, sustained cell proliferation has also been postulated to increase risk of cancer, based on the same rationale, and it has been proposed as a factor in increased cancer susceptibility. Sustained cell proliferation is a feature of several hypothesized modes of action for cancer development, for example the induction of kidney cancer via $alpha_{2u}$-globulin accumulation (EPA, 1991).

Although alterations in cellular replication or cell-cycle control are important features of carcinogenesis, cell proliferation in and of itself is not able to induce cancer. Several carcinogenic substances can cause cancer in humans after perinatal or prenatal exposure without the need for either continued exposure or sustained proliferation during exposure (see Chapter 19, by Caldwell et al.). It has been noted for some time that enhanced cell division does not always predict carcinogenesis (Melnick et al., 1993). After exposure to a carcinogen, the development of cancer in experimental animal models is influenced by the circumstances under which exposure occurs (e.g. sustained vs transient) and by the presence or absence of inflammatory mediators or DNA damage.

Liver cancer

The complex interactions between proliferation, mutation, and inflammatory cell signalling have been studied extensively for liver cancer. In humans, hepatocellular carcinoma (HCC) is markedly heterogeneous, both histomorphologically (Yeh et al., 2007) and genetically, with a wide diversity in gene expression patterns (Chen et al., 2002). Histopathological variability is also associated with geographical region: slow-growing, differentiated HCC nodules surrounded by a fibrous capsule are common in this type of cancer in Japanese patients, whereas a febrile form of HCC, characterized by leukocytosis, fever, and necrosis within a poorly differentiated tumour, is common in this cancer type among black people in South Africa (Feitelson et al., 2002).

HCC signature genes vary considerably and depend on etiological and accompanying pathological conditions, such as viral infection, cirrhosis, inflammation, and fibrosis. The study of tumour formation in the liver is also affected by continuous changes in the transcriptome that accompany hepatectomy and age (Colak et al., 2010). A comparison of

conserved genes between rats and humans (human orthologues) with respect to early expression profiles of HCC signature genes showed some conservation between species for components of the MAPK/ERK, phosphoinositide 3-kinase (P13K)/Akt, and transforming growth factor beta (TGF-β) pathways (Colak et al., 2010).

Development of liver cancer after exposure to carcinogens is more common in rodents, especially in the mouse, than in humans. There are obvious differences between rodents, non-human primates, and humans in background susceptibility to hepatocarcinogenesis and, as noted above, in the regulation of gene expression and cell signalling. With respect to the ability to respond to a mitogenic stimulus such as partial hepatectomy, the liver responds differently and much more slowly in non-human primates and in humans compared with rodents (Gaglio et al., 2002).

Global gene expression patterns in HCCs from seven different mouse models were compared with those in human HCCs from groups with poorer survival and better survival. Expression patterns in HCCs from *Myc-Tgfa* transgenic mice and in diethylnitrosamine-induced HCCs in mice were most similar to those in human HCCs from the group with poorer survival, whereas the patterns in HCCs from *Myc*, *E2f1*, and *Myc-E2f1* transgenic mice were most similar to those in human HCCs from the group with better survival (Lee et al., 2004).

Many factors, such as diet, hormones, oncogene activation, methylation, imprinting, and cell proliferation or apoptosis, are modulators of spontaneous and induced murine hepatocarcinogenesis. There is no simple paradigm to explain the differences in strain sensitivity, for example between C3H/HeJ and C57BL/6J mice, which show a difference of up to 40-fold in multiplicity of liver tumours (Hanigan et al., 1988; Maronpot, 2009).

The activation of oncogenic pathways appears to be more heterogeneous in human HCC than in other types of cancer (El-Serag and Rudolph, 2007). The high degree of heterogeneity in the ways in which cell signalling is disturbed before hepatocellular neoplasia may make induction of liver cancer a useful marker for changes that can lead to cancer elsewhere, depending on cellular context and target (Vogelstein and Kinzler, 2004).

Thus, pathway concordance between species may not always result in site concordance for expression of cancer. The analysis of liver tumour site concordance is complicated by the heterogeneity of disease in humans, as well as by rodent susceptibility.

For induction of cancer in the liver in rodents, the nature of cell proliferation also determines the risk of cancer (Caldwell et al., 2008). When both necrosis and inflammation are present, the resultant hepatocellular proliferation is fundamentally different from the transient proliferation caused by peroxisome proliferators or other primary mitogens. After treatment with a mutagenic agent, transient proliferation induced by primary mitogens has not been shown to lead to cancer induction, whereas partial hepatectomy or necrogenic treatments with carbon tetrachloride have (Ledda-Columbano et al., 1993; Gelderblom et al., 2001).

The mechanism by which necrosis may enable cancer development involves concurrent inflammatory cell signalling and is consistent with inflammation contributing to cancer development. After exposure of rodents to trichloroethylene, hepatocyte proliferation is confined to only a small population of cells without regenerative hyperplasia, sustained hepatocellular proliferation, and hepatocellular necrosis. Any transient DNA synthesis, peroxisome proliferation, or cytotoxicity is not correlated with trichloroethylene-induced liver carcinogenicity (EPA, 2011). Thus, induction of liver cancer by trichloroethylene is not a result of sustained cell proliferation.

Exposure to one of the most studied carcinogens, 2,3,7,8-tetrachlorodibenzo-*para*-dioxin (TCDD), induces liver cancer in rodents, but short-term effects do not include induction of hepatocellular proliferation. Rather than simply inducing cell proliferation, TCDD is thought to cause cancer by altering the cellular ability to proliferate, migrate, undergo apoptosis, senesce, and terminally differentiate in a multistep process focused on the accumulation of mutations and heritable epigenetic changes (Safe, 2001; Marlowe and Puga, 2005; Ray and Swanson, 2009). In addition, the upregulation of drug-metabolizing enzymes by TCDD may enhance the formation of highly reactive intermediates during metabolic activation and/or transformation of several key hormones (e.g. enzyme induction as a source of reactive oxygen species formation, which is linked to decoupling of the cytochrome P450 catalytic cycle) and result in DNA damage and mutations (IARC, 2012c).

Although liver data provide an example of the role of inflammatory signals under some circumstances, inflammation in itself may not induce cancer without other concurrent

cofactors. For many of the agents discussed in Volume 100C of the *IARC Monographs* (IARC, 2012a), inflammation is a key characteristic of their effects (see Chapter 17, by Kane). One of the most recognized examples of how inflammation contributes to neoplastic development is the induction of mucosa-associated lymphoid tissue (MALT) lymphoma and gastric adenocarcinoma associated with exposure to *Helicobacter pylori.* The MALT proliferations of B-cell lymphoid follicles are the precursor of a low-grade lymphoma of B cells. A large proportion (98%) of patients with gastric MALT lymphoma are also infected with *H. pylori*; however, only a small percentage of *H. pylori*-positive individuals develop MALT lymphoma (Bassig et al., 2012). Little is known about the possible role of environmental cofactors in the predisposition to *H. pylori*-induced gastric lymphomagenesis. Other factors are certainly involved, including susceptibility (IARC, 2012b).

Inflammation

Certain types of inflammatory processes in skin, and possibly in other tissues, may serve a tumour suppressor function. Some clinical conditions show that inflammation is a critical component of tumour progression, for example reflux esophagitis before oesophageal cancer, or inflammatory bowel disease that precedes colorectal cancer. However, a condition such as psoriasis is known as a chronic cutaneous inflammatory disease that is seldom, if ever, accompanied by cancer. Similarly, despite extensive inflammation, activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), and abundant proliferation of bile ducts in portal spaces, *Mdr2* knockout mice rarely develop tumours of the bile duct (Nickoloff et al., 2005).

The relationship between chronic inflammation and cancer is complex: inflammation may have roles in initial genetic mutations or epigenetic changes that not only drive cell transformation but also provide a microenvironment that enables progression and metastasis and prevents immune responses against the tumour. Chronic inflammation favours accumulation of DNA damage and chromosomal damage (see Chapter 12, by DeMarini, and Chapter 17, by Kane).

The hallmarks of cancer

In their updated paper, Hanahan and Weinberg (2011) noted that the most fundamental trait of cancer cells involves their ability to sustain chronic proliferation. As part of the "hallmarks" of cancer, alterations in cellular replication and/or cell-cycle control figure prominently in the discussions of cell proliferation, inflammation, and changes in cell signalling that are part of cancer cell physiology. The authors noted that the precise identities and sources of the proliferative signals in general remain poorly understood, but that mitogenic signalling in cancer cells is characterized and known in somewhat more detail. The mechanism by which necrosis enables cancer induction was also described in terms of the release of pro-inflammatory signals by necrotic cells and the influence of cytokines on proliferation and invasiveness of cancer cells. Thus, tumour-promoting inflammation was considered by Hanahan and Weinberg (2011) to be an enabling characteristic for acquisition of core hallmark capabilities.

Several key mechanistic characteristics of IARC Group 1 carcinogens induce traits of cancer cells described as the hallmarks of cancer, including effects on cell proliferation, cell death, and nutritional status (see Chapter 11, by Stewart). The description of the hallmarks attempts to bring together a fundamental understanding of how cancer cells manifest a distinct phenotype. More recently, a series of reviews in *Carcinogenesis* reported the findings from an international team of cancer biologists and toxicologists who participated in the Halifax Project (Harris, 2015). They reviewed the literature on each of the hallmarks of cancer to examine the carcinogenic potential of exposure to low doses and mixtures of chemicals. Relevant reviews for alterations in cell proliferation, cell death, or nutrient supply included the potential of chemical mixtures to enable sustained proliferative signalling (Engström et al., 2015), to confer resistance to cell death (Narayanan et al., 2015), and to induce metabolic reprogramming and dysregulated metabolism (Robey et al., 2015). A related review that encompasses many aspects of cell signalling reported on environmental immune disruptors, inflammation, and risk of cancer (Thompson et al., 2015). The overlap in the descriptions of pathway disruption and functions of cell signalling molecules between these papers is striking and is consistent with the discussion presented above with regard to the complex relationship and pleiotropic roles of cell signalling molecules involved in cell proliferation and apoptosis.

The paradigms of cytotoxicity, cell proliferation, and initiation–promotion as mechanisms of carcinogenesis have been superseded by a more nuanced understanding of the

process of carcinogenesis (Hanahan and Weinberg, 2011). As stated above, cell proliferation, inflammation, or cytotoxicity alone do not lead to cancer, and they are interrelated through changes in cell signalling. The hallmarks of cancer describe well the characteristics that are manifested after the development of cancer. However, by the time of diagnosis, tumour cells already carry large numbers of mutations and are also very heterogeneous in gene product profiles; the multiple cell divisions and the consequent damage processing obscure the initial lesion, rendering it difficult, if not impossible, to make a distinction between causal and consequential events in carcinogenesis.

Conclusions

Among the 10 mechanistic characteristics more commonly observed for IARC Group 1 carcinogens is a composite that includes information on alteration of cell proliferation, cell death, and nutrient supply. This chapter examines many of the challenges associated with the use of this type of information to determine mechanistic and tumour site concordance between humans and experimental animals, and discusses how this mechanistic characteristic shows interdependence with others, such as genotoxicity and inflammation. Many of the indicators of changes in cell proliferation or cell death are non-specific for the induction of cancer, and although they result primarily from effects on the MAPK/ERK cell signalling pathway, they are influenced by a large array of cell signalling molecules with pleiotropic effects.

The biological basis for how cell signalling and cell-cycle control differ between species, organs, and tumour cells, as well as the variability in mutation targets, are also discussed here. Determining the causes of cancer through examination of gene expression profiles in tumours is difficult, especially in terms of increased and sustained cell proliferation, which is a characteristic of cancer itself. Alteration in nutrient supply is a common and recognizable feature of cancer, and is also not independent of activities associated with increased cell proliferation or the hallmarks of cancer.

Some of the information collected in Volume 100 of the *IARC Monographs* was presented in the context of the older hypotheses for mechanisms of cancer induction. The understanding of cancer mechanisms and the descriptive data associated with them continues to evolve (see Chapter 11, by Stewart). The discussion of mechanistic data for ionizing radiation in Volume 100D of the *IARC Monographs* (IARC, 2012d) and Chapter 12, by DeMarini, provide more current discussions about the understanding of cancer and the key mechanistic characteristics of IARC Group 1 carcinogens. With the present state of knowledge, carcinogenesis cannot be confidently attributed to an underlying purely genetic or purely epigenetic process. Mechanistically, it is probably a mixture of the two.

Epigenetic alterations may precede DNA sequence mutations, with subsequent mutations occurring not in a random fashion but in response to specific types of epigenetic changes induced by the environment (Karpinets and Foy, 2005). This selection for enhanced growth has been suggested to explain both the delayed cancer induction after exposure to toxicants and the bystander effect of radiation on tumour development. With regard to cell signalling, spontaneous or environmentally induced epigenetic alterations are increasingly recognized as early molecular events in cancer formation, and these alterations may potentially be more adverse than nucleotide mutations, because their effects on regional chromatin structure can spread out, thereby affecting multiple genetic loci (Weidman et al., 2007).

The key characteristics of the IARC Group 1 carcinogens have some overlap with the hallmarks of cancer and perhaps can provide insight into the "environment" that creates the neoplastic cell. The elucidation and understanding of susceptibility factors may help determine what parts of that environment are already present in individuals, species, or target tissues where cancer develops as a result of exposure to environmental carcinogenic agents. This may also help in evaluating mechanistic and tumour site concordance between species. However, the mechanistic data provided on agents identified as *carcinogenic to humans* need to be examined in the context of more recent information on carcinogenesis. Cancer is a heterogeneous disease, even within the same target site. Because, among other considerations, some epigenetic events may have a mutational basis, the dichotomy once identified between genotoxic and non-genotoxic carcinogens should be reconsidered.

Enhanced cell proliferation and reduced cell death are key mechanistic characteristics of IARC Group 1 carcinogens, are a hallmark of cancer, and are necessary for DNA damage to be processed into a mutation.

However, such changes alone are not sufficient to induce cancer. A key mechanistic question emerges as to what events or cellular environment may precede or cause such changes and may stimulate the formation and selection of DNA sequence mutations and epigenetic changes that induce a cell and its descendants to acquire the hallmarks of cancer, including increased cell proliferation and evasion of apoptosis.

Acknowledgements

The author thanks D.M. DeMarini, M.V. Evans, and S. Vulimiri for their helpful comments on this manuscript.

Disclaimer

This article was reviewed and approved for publication by the National Center for Environmental Assessment, United States Environmental Protection Agency. Approval does not signify that the contents reflect the views of the agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

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CHAPTER 14.

Receptor-mediated mechanisms

Maarten C. Bosland

Introduction

Carcinogenic agents can cause cancer by inducing DNA damage through various genotoxic mechanisms that result in mutations. Many of the agents classified as *carcinogenic to humans* (Group 1) by the *IARC Monographs* are not directly or indirectly genotoxic but cause cancer via mechanisms that, by themselves, do not generate or involve DNA damage. One non-genotoxic mechanism involves receptor mediation (Pitot, 1995).

Hormonally active agents that are associated with carcinogenic effects typically act as ligands for nuclear receptors and, in some cases, for receptors located at the cell surface. There are also agents that are considered to be carcinogenic through receptor mediation but in addition have genotoxic effects; polycyclic aromatic hydrocarbons (PAHs), such as benzo[*a*]pyrene, are examples of this group. Yet other agents have cancer-enhancing or tumour-promoting effects through pathways involving receptors but also cause formation of reactive oxygen species or enhance the bioactivation of pro-carcinogens to ultimate carcinogens, both of which can potentially damage DNA and result in mutations; 2,3,7,8-tetrachlorodibenzo-*para*-dioxin (TCDD) is an example of this group.

Thus, the distinction between genotoxic carcinogens and carcinogens that act through receptor-mediated mechanisms is often not black and white. Insight continues to evolve about the mechanisms by which some carcinogens act through receptors. For example, there is now evidence that certain polychlorinated biphenyls (PCBs) can be metabolized to reactive, DNA-damaging intermediates that may contribute to their receptor-mediated carcinogenicity (Ludewig and Robertson, 2012; Lauby-Secretan et al., 2013; IARC, 2016).

The purpose of this chapter is to provide an overview of receptor-mediated mechanisms thought to be involved in carcinogenesis, followed by a discussion reflecting on the complexity of these mechanisms and how such mechanistic information can be used for rational hazard identification of carcinogenic agents. Receptor-mediated carcinogenesis was often discussed in the 1990s as a major mechanism of cancer induction by non-genotoxic carcinogens (Pitot, 1995). However, carcinogenesis research has since moved beyond these receptors to the

downstream mechanisms involved in their action or has often focused on just one agent's effects. Indeed, a recent PubMed search for articles with "receptor-mediated carcinogenesis" in the title yielded only eight hits, four of which are reviews, all published between 1992 and 1999, and one journal issue devoted to this topic (volume 333 of *Mutation Research*, in 1995). Despite a very large number of relevant primary publications, there have been no major general reviews on this subject since the mid-1990s. Other reviews identified by the keywords "receptor-mediated" and "carcinogenesis" or "toxicity" are devoted to specific carcinogens, tumour types, or target tissues, such as the liver (Andersen et al., 2014). Yet, several of the IARC Group 1 carcinogens act entirely or in part via receptor-mediated mechanisms, including benzo[*a*]pyrene, TCDD, 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126), 2,3,4,7,8-pentachlorodibenzofuran (PeCDF), and the hormonal agents that are used in combined oral contraceptives and in treatment for menopausal symptoms (IARC, 2012a, b).

General comments

Biological mechanisms involving receptor activation fall into two broad categories: (i) those that involve intracellular receptors that translocate into the nucleus and act as transcription factors regulating gene expression (genomic action), and (ii) those that involve cell surface receptors and some intracellular receptors that activate signal transduction pathways, resulting in biological responses, including gene transcription (non-genomic action). The effects on gene transcription of the first group of receptors typically have a slow onset and a long duration, whereas the effects of the second group of receptors are typically rapid and transient. Both classes of receptors can be involved in mechanisms of carcinogenesis.

Although some exogenous ligands act as agonists by competing for binding with an endogenous ligand, others may bind but lack the intrinsic activity to activate the receptor they bind to and thus have an antagonist effect. There are also receptors for which no endogenous ligand has been identified with certainty, such as the aryl hydrocarbon receptor (AhR); these receptors are conceivably activated only by exogenous agents, because they are often involved in detoxification processes that have evolved as protective mechanisms. Exogenous agents may also indirectly affect receptor-mediated mechanisms by modulating the amount of endogenous ligand available for receptor binding and activation, through effects on the biosynthesis, bioavailability, bioactivation, and degradation of the bioactive ligand. In addition, exogenous agents may influence the abundance of receptors by modifying receptor biosynthesis and degradation. Finally, exogenous agents may indirectly affect receptor-mediated mechanisms by having effects on or acting as co-activators and co-repressors of nuclear receptors.

The effects of agents that act via receptor mediation depend in many cases on the dose, duration and route of exposure, and timing of exposure during the life of an organism. For example, the effects in animals of exposure to diethylstilbestrol in utero or neonatally are known to differ from those after exposure in adult life (IARC, 2012b) (see also Chapter 20, by Rice and Herceg). The duration of internal exposure depends in part on the half-life of the agent in vivo and on its bioaccumulation. Notorious in this regard are compounds such as TCDD and PCBs, which are stored in fat tissue and have a long half-life (6–10 years); consequently, even a short-term exposure can result in a sustained internal dose (IARC, 1997).

Importantly, many agents that act by receptor mediation display non-linear dose–response relationships for end-points such as cell proliferation, similar to the dose–response of steroid hormones (Reddel and Sutherland, 1987; Pitot, 1995; Sewall and Lucier, 1995; Gaido et al., 1997; Toyoshiba et al., 2004; Walker et al., 2005); androgenic and estrogenic steroid hormones typically stimulate in vitro proliferation of receptor-positive cells at low, physiological concentrations but are inhibitory at supraphysiological and pharmacological concentrations (Lee et al., 1995; Almstrup et al., 2002; Simons, 2008). This phenomenon has important implications for quantitative risk assessment. However, this is outside the scope of this chapter, which, like the *IARC Monographs*, focuses on hazard identification.

Oral exposure can result in a first-pass effect if elimination of the agent by the liver is efficient, thereby reducing the dose to organs beyond the liver. When hepatic elimination is slow, the systemic dose of a carcinogen may be high and may lead to cancer induction. This was demonstrated in mouse strains that differed in inducibility of CYP1A1/2 enzymes – members of the cytochrome P450 (CYP450) family of enzymes – by the AhR mechanism when treated with benzo[*a*]pyrene (Nebert et al., 1979).

The downstream biological effect of receptor binding is really what determines the potential for

carcinogenicity of an agent that acts via a direct receptor-mediated mechanism. Effects that are generally considered to be associated with carcinogenic activity of such agents include, but are not limited to, the following: enhancement of carcinogen bioactivation; reduction of DNA repair; induction of oxidative stress; stimulation of cell proliferation, angiogenesis, and invasive or migratory cell properties (including epithelial–mesenchymal transition); inhibition of cellular apoptosis, senescence, and/or differentiation; evasion of immune surveillance; and epigenetic effects (Hanahan and Weinberg, 2011) (see Chapter 10, by Smith).

Finding any one such effect, or even a combination of several of these effects, as a result of exposure to an agent that binds to cellular receptors is not by itself sufficient evidence that the agent is a carcinogen, but such findings do generate suspicion that the agent might cause cancer. If such receptor binding is associated with the mechanism by which a recognized human carcinogen operates, the evidence for carcinogenicity in humans may be sufficient. An example of this is the recent upgrading by IARC of PCB 126 and PeCDF to Group 1 carcinogens, based on the similarity of their binding to AhR (IARC, 2012a, 2016).

Proof that a receptor-mediated mechanism is involved in carcinogenesis in mammals would require evidence that blocking the activation of a specific receptor, or genetic removal of the receptor or reduction of its expression in mouse models, interferes with tumour induction by the carcinogen in question. The latter approach was used to demonstrate that several of the toxic effects of TCDD are indeed mediated by AhR in vivo (Poland and Glover, 1980; Birnbaum et al., 1990; Fernandez-Salguero et al., 1995). Although some of these studies evaluated the involvement of AhR in effects of TCDD that are mechanistically associated with carcinogenesis, this approach has not been applied to determine whether AhR is necessary for TCDD-induced tumour formation. Studies with mice that overexpress or lack estrogen receptor alpha (ER-α) have demonstrated that this receptor is involved in many of the developmental and carcinogenic effects of neonatal treatment with diethylstilbestrol in female animals (see below) (Couse et al., 1997, 2001; Couse and Korach, 2004).

Non-genomic receptor-mediated mechanisms and carcinogenesis

Non-genomic regulation of gene expression and cellular function involves signal transduction pathways that are activated by a wide range of receptor molecules located at the plasma membrane. These receptor modules are activated by an equally large number of ligands, ranging from locally produced autocrine and paracrine growth factors to circulating cytokines and hormones. The major groups of these receptors include tyrosine kinase receptors such as the epidermal growth factor (EGF) receptor, serine/threonine kinase receptors such as the protein kinase C receptor, G protein-coupled receptors, and receptors for cytokines, chemokines, and other ligands. They all catalyse phosphorylation of downstream signalling molecules upon ligand binding, some via activation of G protein signalling, ultimately resulting in regulation of expression of specific genes or sets of genes that control cellular function and behaviour and potentially influence carcinogenic processes (Pitot, 1995; Shawver et al., 1995).

12-*O*-tetradecanoylphorbol-13-acetate (TPA) may act as a tumour promoter through activation of membrane-located protein kinase C receptors, activating downstream signalling pathways that contribute to the enhancing effects of TPA on formation of skin tumours (Niedel et al., 1983; Nishizuka, 1984). However, conclusive evidence for this mechanism is still lacking (Gschwendt et al., 1995; Marks and Gschwendt, 1995). Moreover, TPA is a complete carcinogen for the skin in mice when given at sufficiently high doses with a sufficiently long period of observation (Iversen and Iversen, 1979). The involvement of protein kinase C in TPA-induced tumour promotion is highly complex and is only partially understood (Griner and Kazanietz, 2007), and more recent research is focused on downstream signalling effects (Hsu et al., 2000; Lu et al., 2007). TPA has also been shown to act through other receptor mechanisms (Marks and Gschwendt, 1995) and, when applied to the skin in mice, to induce inflammation, which in itself can stimulate cell proliferation and generate reactive oxygen species (Aldaz et al., 1985; Wei and Frenkel, 1993).

Receptors for several steroid hormones are not only intracellular nuclear receptors (see below) but may also be present at the plasma membrane, where their activation by agonists causes rapid non-genomic signal transduction-mediated effects. These non-classical receptor-mediated effects have been shown to occur for the androgen receptor (AR) (Bennett et al., 2010; Lang et al., 2013), the glucocorticoid receptor (Matthews et al., 2008; Samarasinghe et al., 2012), the progesterone receptor (PR)

(Wheeler et al., 2000; Peluso, 2011), and the estrogen receptor (ER) (Acconcia and Kumar, 2006; Song and Santen, 2006).

Activation of rapid membrane-initiated estrogen signalling by estrogenic agonists causes non-genomic signal transduction-mediated effects (Acconcia and Kumar, 2006; Song and Santen, 2006). The biological significance and contribution of these effects to carcinogenesis are uncertain at present. One of the two rapidly acting non-genomic membrane-located forms of PR, PR membrane component 1 (PGRMC1), is expressed in human ovarian cells and tumours (Wendler et al., 2012). PGRMC1 expression is associated with cell proliferation in ovarian cancers and may have anti-apoptotic effects (Wheeler et al., 2000; Peluso et al., 2008). PGRMC1 also interacts with and changes the function of some CYP450 enzymes and may affect carcinogen metabolism (Rohe et al., 2009; Szczesna-Skorupa and Kemper, 2011). There are no published studies to date indicating a contribution of these non-genomic ER- and PR-mediated effects to cancers associated with exposure to estrogens and progestins.

None of the IARC Group 1 carcinogens are known to be carcinogenic by acting via non-genomic receptor mechanisms, but this cannot be ruled out, for several reasons. Both estrogens and progestins can act via transmembrane receptors, and TCDD can affect signalling pathways via mechanisms that do not involve AhR (Tijet et al., 2006; Biswas et al., 2008; Boutros et al., 2009; Kim et al., 2009; Denison et al., 2011). Thus, it is conceivable that carcinogenicity associated with exposure to estrogens, progestins, or dioxins may involve mediation by non-genomic action of these agents. In addition, indirect non-genomic activity of carcinogenic agents could conceivably affect receptor abundance or stimulate signal transduction pathways that lead to pro-carcinogenic effects.

Nuclear receptor-mediated mechanisms and carcinogenesis

Nuclear receptor-mediated mechanisms involve intracellular receptors, most of which belong to the so-called nuclear receptor superfamily (Evans, 1988; Mangelsdorf et al., 1995). There are a large number and a wide variety of nuclear receptors that act via genomic mechanisms involving direct binding to specific DNA sequences (response elements) or indirect binding to AP1 or Sp1 sites in the promoter regions of the specific genes they regulate (Kushner et al., 2000; Aranda and Pascual, 2001; Safe and Kim, 2008). Once the nuclear receptor is bound to a response element, various co-regulator molecules are recruited that are critical for regulation of the initiation of gene transcription in a cell type-specific manner (Edwards, 2000; Lonard and O'Malley, 2012). Many members of the nuclear receptor superfamily are involved in physiological functions that mediate the effects of steroid hormones and other endogenous ligands (Evans, 1988; Tsai and O'Malley, 1994; Whitfield et al., 1999; Jacobsen and Horwitz, 2012).

ER, PR, and AR are examples of receptors that bind to and are activated by steroid hormones, whereas the retinoic acid receptors and the retinoid X receptors bind to vitamin A metabolites (Rochette-Egly and Germain, 2009; Duong and Rochette-Egly, 2011; Dawson and Xia, 2012). There are also nuclear receptors for which no endogenous ligands have been identified, but they do bind to exogenous ligands. These receptors are thought to mediate protection against harmful xenobiotics, predominantly by inducing expression of specific drug-metabolizing CYP450 enzymes (Boutros et al., 2009). Examples of the latter category are the pregnane X receptor and the constitutive androstane receptor (Nebert and Dalton, 2006; Tompkins and Wallace, 2007; Elcombe et al., 2014), both of which frequently engage in cross-talk with various other nuclear receptors and transcription factors (Lim and Huang, 2008).

Several other nuclear receptors with endogenous ligands are involved in physiological functions, but they are also activated by xenobiotics. An example of this category is the group of peroxisome proliferator-activated receptors (PPARs), which are involved in lipid metabolism but are also activated by xenobiotics with peroxisome proliferating activity; the PPARα subtype has been implicated in the hepatocarcinogenicity in rats of some of these xenobiotics (Green, 1995; Cattley, 2004; Corton et al., 2014), which are probably not human hepatocarcinogens (IARC, 1994, 1996). Many nuclear receptors need to homodimerize before they can bind to response elements, and several others heterodimerize with the retinoid X receptor before binding (Dawson and Xia, 2012).

AhR, which binds to TCDD and ligands that are structurally similar to TCDD, does not belong to the nuclear receptor superfamily and is in a class by itself. There are no established physiological endogenous ligands for AhR, even though this receptor has physiological functions, because mice that lack AhR expression show developmental abnormalities (Gonzalez and

Fernandez-Salguero, 1998; Carlson and Perdew, 2002). However, some endogenous compounds that bind to and activate AhR have been identified, such as certain tryptophan derivatives, but their physiological role has not yet been firmly established (Denison and Nagy, 2003; Henry et al., 2006). The tryptophan metabolite kynurenine has been identified as an endogenous ligand of human AhR. After receptor binding, downstream effects are the suppression of anticancer immune mechanisms and the promotion of cancer cell survival and motility in human brain tumours (Opitz et al., 2011), but the role of kynurenine in carcinogenesis has not been established. To exert its effects, AhR heterodimerizes with a unique molecule, the AhR nuclear translocator (ARNT).

AhR, ER, and PR mediate effects of several agents that are classified as IARC Group 1 human carcinogens (IARC, 2012a, b) and are discussed in more detail below. Although AR has been implicated in the causation of prostate cancer in humans, this has not been firmly established, and there is only *limited evidence* of the carcinogenicity of androgenic steroids in humans; they are classified by IARC as *probably carcinogenic to humans* (Group 2A), because there is *sufficient evidence* of their carcinogenicity in experimental animals (IARC, 1979, 1987). In rats, testosterone is a weak complete carcinogen and a strong tumour promoter in the prostate (Bosland, 2014).

Estrogen receptor (ER) and progesterone receptor (PR)

ER was discovered by Jensen (Jensen et al., 1968; Jensen and DeSombre, 1973), and its gene was cloned in 1986 (Greene et al., 1986). A second form of ER was discovered in 1996 and was named ER-β to distinguish it from the receptor previously cloned, ER-α (Kuiper et al., 1996).

Genomic activity of ER, also termed nuclear-initiated steroid signalling, is achieved through two main mechanisms. The first involves the direct binding of ER to its target gene. Estrogen binding to its receptor in the cytosol triggers a series of events, starting with loss of chaperone molecules such as heat shock protein 90 (Hsp90), and followed by migration of the receptor from the cytosol into the nucleus. Dimerization of the receptor then induces a conformational change that allows subsequent binding of the receptor dimer to specific sequences of DNA known as estrogen response elements. The DNA–receptor complex recruits other proteins such as co-activators, which are responsible for the initiation of transcription of downstream DNA into mRNA, resulting in translation to proteins to produce changes in cellular function (Dickson and Stancel, 2000; Aranda and Pascual, 2001). The second mechanism of transcriptional regulation does not involve estrogen response elements but is based on interaction of ER with the transcription factors Fos and Jun to bind to AP1 sites, or with Sp1 to bind to Sp1 sites (Nilsson et al., 2001).

The two basic forms of ER, ER-α and ER-β, show some sequence homology and are widely distributed in tissues of the body, including target tissues of estrogen carcinogenicity, but they differ greatly in cell type-specific abundance. In addition, although their basic molecular mechanism of action is similar, they differ in specificity and binding affinity for ligands (Matthews and Gustafsson, 2003; Thomas and Gustafsson, 2011) and often have opposite activity in breast, prostate, and colon cancer cells (Bardin et al., 2004; Roger et al., 2008; Chen and Iverson, 2012; Nelson et al., 2014).

There are several isoforms of ER-α and ER-β that result from alternative mRNA splicing (Moore et al., 1998; Flouriot et al., 2000; Wang et al., 2005). Of these variants, ER-α36 and ER-α46 are predominantly localized to the plasma membrane and cytoplasm, mediating membrane-initiated rapid non-genomic signalling (Flouriot et al., 2000; Wang et al., 2005). Of note, the so-called estrogen-related receptors show a high degree of sequence homology with the ERs, but they are orphan receptors for which no ligand has been identified (Deblois and Giguère, 2011, 2013). Although activation of the α-isoform of estrogen-related receptors resulted in suppression of growth of xenografted human breast cancer cells in nude mice (Chisamore et al., 2009), there is no evidence that these nuclear receptors are involved in estrogen-induced carcinogenesis.

Two isoforms of PR have been identified, PR-A and PR-B, which are encoded by the same gene but controlled by different estrogen-regulated promoter regions and different translation initiation events (Conneely et al., 1989; Kastner et al., 1990; Kraus et al., 1993). These two receptor forms play different roles in various tissues and cell types and may have opposite molecular effects (Jacobsen and Horwitz, 2012). Although PRs are in many respects similar to ERs in the way they initiate transcription, dimerization may not always be necessary, and PR monomers can bind to progesterone response element half-sites (Jacobsen et al.,

2009; Jacobsen and Horwitz, 2012). The gene expression and other biological responses mediated by receptor isoforms are often progestin- and PR subtype-specific (Graham and Clarke, 2002; Jacobsen and Horwitz, 2012; Moore et al., 2012). The expression of both isoforms is induced by estrogens, but progestins downregulate PRs (Jacobsen and Horwitz, 2012).

Breast cancer induced by hormonal therapies

Combined estrogen–progestin treatments, as therapy for menopausal symptoms or as oral contraceptives, are carcinogenic to the female breast, but only the menopausal therapy is carcinogenic to the endometrium, whereas only the contraceptive treatment is carcinogenic to the uterine cervix and liver (IARC, 2012b). The mechanisms by which these hormonal regimens cause these cancers in women are not clear.

The outcome of the estrogen-alone arm of the Women's Health Initiative randomized clinical trial was remarkable in that breast cancer incidence was reduced (Anderson et al., 2012), whereas breast cancer incidence was increased in women treated with combined estrogen–progestin (Chlebowski et al., 2010). These findings clearly indicate that the addition of progestins (in the form of continuous medroxyprogesterone) to treatment with estrogens (conjugated equine estrogens) is a determining factor in breast carcinogenesis induced by these hormones.

In the prospective Million Women Study, estrogen-only treatment increased risk of breast cancer, and combined estrogen–progestin treatment was associated with a greater increase in breast cancer risk than that observed with estrogen alone (Beral, 2003). The stimulating effect of adding progestin treatment to estrogen exposure in the induction of breast cancer has also been demonstrated in a rat model (Blank et al., 2008). Furthermore, the pure anti-estrogen ICI 182780 inhibited growth stimulation of transplanted ER-positive mouse mammary tumours by medroxyprogesterone in intact mice (Giulianelli et al., 2012).

However, precisely how these two hormones and their respective receptors interact in increasing breast cancer risk is far from understood. Both ER-α and ER-β can mediate the growth stimulatory and, at higher doses, inhibitory effects of estrogen, and the ratio of abundance of the two ER isoforms and the cellular context in which they operate appear to be critical determinants of the eventual effect (Sotoca et al., 2008; Soldati et al., 2010). In addition, these ERs may cross-talk with signalling pathways and interact with PR (Giulianelli et al., 2012; Cotrim et al., 2013).

Initially, ER- and PR-mediated induction of cell proliferation had been postulated to be responsible for the carcinogenic effects of estrogens and progestins (Anderson et al., 1989; King, 1991; Yager et al., 1991; Pike et al., 1993; Feigelson and Henderson, 1996). However, this may be too simple a proposition, because these hormonal factors also have a wide range of other biological effects. Although cell proliferation is no doubt a necessary component of the carcinogenic process, is it probably not sufficient, and additional factors, some of which may also be receptor-mediated, are likely to be required to induce malignant cell transformation (Dickson and Stancel, 2000) (see also below). Enhanced cell proliferation has been found in some substructures of the breast of women treated with estrogen–progestin menopausal therapy (Hofseth et al., 1999), and similar effects have been observed in mice (Raafat et al., 2001; Haslam et al., 2002). However, there are partially conflicting data in humans (Harvey et al., 2008), and progesterone has been found to inhibit estrogen-stimulated proliferation of breast cancer cells in vitro, depending on dose and timing (Groshong et al., 1997; Lippert et al., 2000).

There are convincing data indicating that the hormonal changes during the normal menstrual cycle are associated with cyclic changes in the rate of proliferation of epithelial cells in the breast, which reaches a maximum during the luteal phase, when circulating levels of both estradiol and progesterone are high (Anderson et al., 1989; Pike et al., 1993). The cell proliferation rate of the breast epithelium is increased during both the follicular phase and the late luteal phase (Anderson et al., 1989). These findings suggest that cyclicity in circulating hormone levels may be a driving force in stimulating cell proliferation in the normal breast, resulting in a higher risk of breast cancer with a higher number of years during which a woman menstruates (Henderson et al., 1982). If this notion were correct, one would expect that continuous exposure to estrogen plus progestin would not increase breast cancer risk, but the Women's Health Initiative randomized clinical trial with continuous exposure to both agents demonstrated that this is clearly not the case (Chlebowski et al., 2010). Furthermore, similar effects in increasing breast cancer risk were found for both sequential and continuous combined estrogen–progestin treatment in the prospective Million Women Study (Beral, 2003).

The breast cells that proliferate are not the cells that strongly express ER or PR, suggesting that paracrine mechanisms, possibly involving breast stromal cells, play a major role. Breast density (i.e. the relative proportion of the stromal component of the breast) has been found to be associated with the rate of cell proliferation (Clarke et al., 1997; Russo et al., 1999; Harvey et al., 2008). Thus, in vitro studies with benign or malignant breast epithelial cells exposed to the sex hormones contained in estrogen–progestin treatments have limited significance, because they do not involve a stromal component, although once they are malignantly transformed, breast cancer cells may become independent of paracrine mediation and regulate their growth by autocrine mechanisms (Obr and Edwards, 2012).

Also, cell culture-based studies have yielded a somewhat confusing overall picture of how estrogen and progestin treatments interact in affecting cell proliferation. Progestins can inhibit estrogen-stimulated proliferation of breast cells (Seeger et al., 2003a, b), but they also increase the ratio of apoptosis to proliferation (Krämer et al., 2005; Seeger et al., 2005). Dose and type of progestin appear to be critical in determining the eventual effect (Seeger et al., 2003b, 2005). Despite their limitations, these in vitro studies have shown that ER- and PR-mediated apoptosis must be considered as an important effect of combined estrogen–progestin treatment, and they have provided evidence that growth factors can be determinants of these effects (Krämer et al., 2006).

More recent observations in normal human breast tissue have provided evidence that ER-α is predominantly expressed in luminal cells, whereas PR expression is enriched in the basal cell compartment, which may contain progenitors of luminal cells and possibly cancer progenitor cells (Hilton et al., 2012). This observation may have a bearing on the notion that there are four basic breast cancer types (and possibly subtypes of these) (Sorlie et al., 2001; Guiu et al., 2012; Elsawaf et al., 2013). Although it is currently not known which of these four breast cancer types are associated with estrogen–progestin treatment, breast cancer risk associated with hormonal menopausal therapy varied among different histological types of invasive and in situ breast cancer in the Million Women Study (Reeves et al., 2006). Of interest in this respect is the observation that there may be cross-talk between PR subtypes and human epidermal growth factor receptor (HER)/ErbB receptors in HER-positive breast cancers (Lindet et al., 2012).

Genotoxicity of estrogen metabolites potentially plays a role in the carcinogenicity of estrogen and estrogen–progestin treatments (Yager and Liehr, 1996; Cavalieri et al., 2000; Yager and Davidson, 2006). In addition, some estrogen metabolites (the 4- and 16α-hydroxylated but not the 2-hydroxylated metabolites) can also have cell proliferative effects through poorly defined mechanisms that may involve ER mediation (Seeger et al., 2006).

Overall, there is little doubt that ER- and PR-mediated mechanisms, including stimulation of breast cell proliferation, are involved in the carcinogenicity of estrogen–progestin treatments (Sutherland et al., 1998; Anderson and Clarke, 2004). However, other mechanisms are clearly also operational and are probably critically important as well; the interplay of these molecularly diverse mechanisms is likely to be highly complex and is still poorly understood (Yager and Davidson, 2006). In addition, the understanding of how these receptors function is still evolving in many ways. For example, it has become apparent that microRNAs regulate ER and PR expression and in turn are regulated by these receptors as well (Adams et al., 2007; Maillot et al., 2009; Pandey and Picard, 2010; Cochrane et al., 2012), but it is not known whether or how these mechanisms are involved in breast carcinogenesis.

Cancer of the endometrium and ovary

The reduction of risk of cancer of the endometrium and ovary by use of combined oral contraceptives is well established, whereas menopausal estrogen therapy unopposed by progestins causes cancer of the endometrium (IARC, 2012b). In the normal premenopausal endometrium, cell proliferation rates are high during the follicular phase, when estradiol levels peak, but they decline as progesterone levels rise in the luteal phase (Whitehead et al., 1981; Key and Pike, 1988).

It is well established that the longer a woman menstruates, the higher the risk of cancer of the endometrium (Key and Pike, 1988; Karageorgi et al., 2010). This relationship is similar for cancers that differ in the degree of genomic instability (Amankwah et al., 2013) and for both the common endometrioid form of endometrial cancer (type I) and the less common type II endometrial cancers (including serous, clear cell, and mixed Müllerian tumours) (Setiawan et al., 2013).

Estrogen therapy during menopause also increases endometrial cell proliferation, and this effect

is counteracted by progesterone (Whitehead et al., 1981), whereas progestin-only contraceptives suppress cell proliferation in the endometrium (Landgren et al., 1990; Moyer and Felix, 1998). It is not clear whether changes in cell proliferation are the only explanation for the induction of endometrial cancer by estrogens and for the preventive effects of combined oral contraceptives on endometrial and ovarian cancer and the preventive effects of progestins on endometrial cancer induced by estrogens. Even less is known about hormonal effects on the ovary that may be involved in ovarian carcinogenesis.

Diethylstilbestrol

The human transplacental carcinogen diethylstilbestrol is not only genotoxic but is also probably the strongest known estrogen. Its genotoxic effects in human and rodent tissues and cells are unlikely to be receptor-mediated. Non-genotoxic effects of diethylstilbestrol mediated by ERs are hormonal in nature and involve estrogenic stimulation of cell proliferation, which has a biphasic dose–response relationship in breast cancer cells in vitro (Reddel and Sutherland, 1987).

Diethylstilbestrol induces epigenetic changes in DNA methylation in target tissues in rodents (Li et al., 2003; Newbold et al., 2007; Tang et al., 2008). These changes may be heritable (Anway and Skinner, 2006) and conceivably underlie the mechanism responsible for the developmental and carcinogenic effects observed in the second, and possibly the third, generation of rodents after in utero exposure to diethylstilbestrol (Newbold et al., 1998, 2000); these effects have also been reported in female and male offspring of women exposed to this synthetic estrogen during pregnancy (Titus-Ernstoff et al., 2008; Kalfa et al., 2011). Whether these epigenetic effects are ER-mediated is unknown (Newbold et al., 2006). It is also unclear whether the immunosuppressive effects of diethylstilbestrol are ER-mediated (Brown et al., 2006). Overall, the contribution of ER-mediated mechanisms in the carcinogenic effects of in utero exposure to diethylstilbestrol is not clear. It seems likely that any ER-mediated effect of prenatal exposure to diethylstilbestrol facilitates its genotoxic effects by stimulating cell proliferation in target cells, or otherwise by increasing the induction of the DNA alterations that underlie the carcinogenicity of diethylstilbestrol (IARC, 2012b).

Studies with mice that overexpress or lack ER-α suggest that endogenous estrogen in the adult animal acts as a tumour promoter via ER mediation, after induction of permanent genotoxic and epigenetic effects by exposure to diethylstilbestrol early in life (Couse et al., 1997, 2001; Couse and Korach, 2004). This notion would be consistent with the observation that malignancies induced by diethylstilbestrol in women exposed in utero do not appear until after menarche (Couse and Korach, 2004). The role of ER in the increased risk of breast cancer observed in women exposed to diethylstilbestrol during pregnancy is not clear, but a combination of genotoxic effects and stimulation of cell proliferation is likely (IARC, 2012b).

In conclusion, cell proliferation appears to be involved in how hormones used in menopausal therapy cause cancer of the breast and endometrium and possibly cancer of the ovary, and how combined oral contraceptives cause malignancies of the liver, uterine cervix, and breast, but the precise mechanisms by which these tumours are caused and how exactly these hormonal treatments are involved mechanistically is far from clear. Most recent research has focused on molecular alterations in cancers at these sites and the mechanisms by which they can develop, but how hormonal factors intersect with these processes remains unknown (Merritt and Cramer, 2010).

Even more nebulous is how combined oral contraceptives provide lasting protection against cancer of the endometrium and ovary, and how precisely progestins protect the endometrium against estrogens used in menopausal therapy. Downregulation of PR may in part explain these protective effects, because it may result in reduction of cell proliferation, but other mechanisms are also likely to be involved. Similarly, just evoking stimulation of cell proliferation as the mechanism by which unopposed estrogen causes endometrial cancer is probably an oversimplification as well. Involvement of ER in the mechanism by which diethylstilbestrol causes cancer in women after prenatal exposure may be limited to the effects of endogenous estrogens acting as tumour promoters.

Aryl hydrocarbon receptor (AhR)

AhR is a member of the PER-ARNT-SIM family of basic helix–loop–helix transcription factors (Burbach et al., 1992). This receptor is induced by and binds to a very large number of exogenous ligands, including PAHs such as benzo[*a*]pyrene, planar PCBs, polychlorinated dibenzofurans, and its most potent

ligand, TCDD (Denison et al., 2002; Tsuchiya et al., 2003; Flaveny et al., 2009).

Endogenous ligands for AhR have not been identified with certainty, but it is likely that these exist and that AhR has a physiological role (Gonzalez and Fernandez-Salguero, 1998; Carlson and Perdew, 2002; Bock and Köhle, 2009). Certain endogenously formed tryptophan derivatives that bind to and activate AhR have been identified, but their physiological role has not yet been firmly established (Denison and Nagy, 2003; Henry et al., 2006; Perdew et al., 2015). Factors that can activate AhR in cultured liver cells have been found in the serum of knockout mice that lack AhR (McMillan and Bradfield, 2007), but whether these are the elusive endogenous ligands is uncertain.

This cytosolic receptor, once bound to a ligand, translocates to the nucleus, loses various chaperone molecules, such as Hsp90, and heterodimerizes with ARNT. The AhR–ARNT complex then binds to xenobiotic response elements, also termed dioxin response elements, in the promoter regions of the genes it regulates, including those encoding CYP1A1, CYP1A2, CYP1B1, glutathione-*S*-transferase M, and nicotinamide adenine dinucleotide phosphate NAD(P)H:quinone oxidoreductase, all of which are involved in metabolism of xenobiotics (Beischlag et al., 2008; Denison et al., 2011). Involvement of AhR in immune regulation, the cell cycle, the function of mucosal barriers, and development has recently been identified (Hubbard et al., 2015).

In addition, AhR can interact with molecules other than ARNT and can engage extensively in cross-talk with various signalling pathways. It can interact directly with phosphorylated retinoblastoma protein, leading to cell-cycle arrest, and with ER-α and ER-β in various ways, resulting in repression of ER-mediated signalling and anti-estrogenic effects (Safe et al., 1998; Safe and Wormke, 2003; Dietrich and Kaina, 2010; Denison et al., 2011). Interestingly, ARNT by itself can activate ER-α, and particularly ER-β (Rüegg et al., 2008). AhR may activate cyclin A via JunD and ATF2, thereby inhibiting contact inhibition in vitro, which would be a pro-carcinogenic effect (Weiss et al., 2008; Dietrich and Kaina, 2010).

The great diversity of genes regulated by AhR and the many mechanisms involved are remarkable and illustrate the considerable complexity in how AhR functions (Denison et al., 2011). This complexity is exacerbated because (i) ligand-dependent differences have been observed in co-activator recruitment and in binding specificity of AhR towards sequence variants in xenobiotic response elements, (ii) cell type-specific co-activators and co-repressors can modulate DNA binding specificity of the AhR–ARNT complex, (iii) AhR and the AhR–ARNT complex may themselves act as co-activators for other transcription factors, and (iv) extensive cross-talk exists with important signalling pathways (Beischlag et al., 2008; Denison et al., 2011).

It is not surprising that activation of AhR results in distinct species- and tissue-specific changes in gene expression profiles and that these may be subject to temporal changes and can be dependent on, as well as independent of, TCDD and dioxin-like compounds such as PCBs and dibenzofurans (Vezina et al., 2004; Tijet et al., 2006; Kopec et al., 2008, 2013; N'Jai et al., 2008; Dere et al., 2011a, b). In this regard, it is relevant that there are interspecies and rat and mouse strain-specific differences in AhR binding affinity for TCDD, as well as in functional AhR polymorphisms, AhR abundance, and cofactors required for AhR activity, which may all be critical to the carcinogenic process (Barrett, 1995; De Souza et al., 2009; Flaveny et al., 2009).

The binding affinity of human AhR for dioxin is much weaker than that found for AhR in some rodent species (Ema et al., 1994; Ramadoss and Perdew, 2004). Rodents have a high degree of AhR sequence homology (Korkalainen et al., 2001), but there is less homology with human AhR (Ema et al., 1994; Flaveny et al., 2008). There are also differences between humans and rodents in the response of hepatic gene expression to TCDD, but little difference in inducibility of CYP450 enzymes (Mandal, 2005).

Mice that carry AhR variants with different dioxin-binding affinities vary in their response towards TCDD toxicity and PAH-induced carcinogenesis (Garte and Sogawa, 1999; De Souza et al., 2009; Flaveny et al., 2010). Thus, there are differences between humans and rodents in AhR binding affinity that may account for interspecies variation in AhR-mediated carcinogenicity. Toxicogenomic comparisons of human, rat, and mouse cells or tissues exposed to TCDD indicate considerable interspecies differences in the response to dioxin (Boverhof et al., 2006; Black et al., 2012). Of note, there are AhR polymorphisms in humans, some of which could conceivably be associated with differences in toxic effects and cancer risk in response to exposure to TCDD (Garte and Sogawa, 1999; Hung et al., 2013; Luo et al., 2013).

AhR-mediated carcinogenesis

TCDD and dioxin-like PCBs are considered to be AhR-mediated carcinogens. PCBs as a group have recently been designated by IARC as Group 1 carcinogens (Lauby-Secretan et al., 2013; IARC, 2016). TCDD is a multisite human carcinogen (Warner et al., 2011; IARC, 2012a, 2016). There is *sufficient evidence* from epidemiological studies that exposure to PCBs causes melanoma (Loomis et al., 1997; Gallagher et al., 2011; IARC, 2012a, 2016). For non-Hodgkin lymphoma, there are mixed data, suggesting that risk may be elevated in men but not in women (Bertrand et al., 2010; Laden et al., 2010; Maifredi et al., 2011; Bräuner et al., 2012; Kramer et al., 2012; IARC, 2016). The most recent update of the follow-up study on people affected by the poisoning incident in 1968 in Japan, where rice oil was contaminated with PCBs and polychlorinated dibenzofurans, indicated small but statistically significant associations with risk of all cancers and risk of cancer of the liver and lung (Onozuka et al., 2009). The epidemiology for PeCDF is very sparse (IARC, 2012a).

TCDD has an extremely wide range of biological and toxic effects, many, but not all, of which are mediated by AhR (Poland and Glover, 1980; Birnbaum et al., 1990; Fernandez-Salguero et al., 1995). AhR is critical to the carcinogenicity of TCDD and at least some dioxin-like compounds, such as certain PCBs and polychlorinated dibenzofurans, as summarized in Volume 69 (IARC, 1997), Volume 100F (IARC, 2012a), and Volume 107 (IARC, 2016) of the *IARC Monographs*. In Volume 69, it was stated that "even though Ah receptor activation is likely to be required for the carcinogenicity of 2,3,7,8-TCDD, its precise role in this process remains unclear." Ten years later, Walker (2007) concluded that "despite … decades of research, we still do not have a clear mechanism of action leading from ligand binding of TCDD or a related ligand to the AhR and the ultimate development of toxicities [including carcinogenesis]". Research from the past several years does not change this conclusion (Budinsky et al., 2014).

TCDD, PCB 126, and PeCDF are complete carcinogens as well as tumour promoters in rodents, and some other PCBs and polychlorinated dibenzofurans have tumour-promoting activity as well (Hébert et al., 1990; Anderson et al., 1991; Waern et al., 1991; Hemming et al., 1995; IARC, 1997). Induction of xenobiotic-metabolizing CYP450 enzymes has been recognized as one of the major effects of activation of AhR relevant to its contribution to the carcinogenic effects of planar PAHs, TCDD, and dioxin-like compounds (IARC, 1997, 2012a). However, many other major effects of AhR activation can conceivably also contribute to the carcinogenicity of these AhR ligands, including induction of cell proliferation, oxidative stress, cross-talk with signalling pathways involved in carcinogenic mechanisms, induction of cell proliferation, and alteration of cell–cell interactions (Schwarz and Appel, 2005; Dere et al., 2006; Dietrich and Kaina, 2010). Sustained activation of AhR has been postulated to be necessary for its carcinogenic activity in the liver in rodents (Budinsky et al., 2014). Carcinogenicity of PCBs is likely to involve not only AhR activation but also other such mechanisms (IARC, 2016).

Many of these effects and mechanisms may be ligand-specific, as indicated by comparative toxicogenomic studies of the liver of mice in vivo and of primary rat hepatocytes in vitro after short-term exposure to dioxin-like compounds (Kopec et al., 2008, 2010; N'Jai et al., 2008; Rowlands et al., 2011). In toxicogenomic studies, longer-term in vivo exposure (13 or 52 weeks) of rats to the AhR ligands TCDD, PeCDF, and PCB 126, as well as the non-AhR ligand 2,2′,4,4′,5,5′-hexachlorobiphenyl (PCB 153), revealed considerable ligand specificity (Vezina et al., 2004; Ovando et al., 2010).

Nevertheless, these gene expression profiling studies did show partial overlap of genes regulated by TCDD, PCB 126, and PeCDF (Vezina et al., 2004; Kopec et al., 2008; Ovando et al., 2010). PeCDF and PCB 126 appeared to share more gene responses than either compound shared with TCDD (Vezina et al., 2004). These studies used equipotent doses on the basis of so-called toxic equivalency factors, and showed AhR-mediated gene expression responses that were shared among TCDD, PCB 126, and PeCDF or 2,3,7,8-tetrachlorodibenzofuran. However, the non-coplanar PCB 153, which does not activate AhR but may antagonize it (Suh et al., 2003), also shared some gene responses with TCDD and PCB 126 (Kopec et al., 2010; Ovando et al., 2010).

Exposure to TCDD, and possibly to 2,2′,3,3′,4,4′-hexachlorobiphenyl (PCB 128) and PeCDF, affects various metabolic genes and cell regulatory pathways known to be involved in cancer, such as genes of the G protein-coupled receptor protein signalling pathway and genes of the polyamine and glutathione pathways (Jennen et al., 2011). Exposures to

combinations of PCBs have not been studied but would be of interest, given that, for example, PCB 153 significantly increased the carcinogenic potency of PCB 126 (NTP, 2006c). Overall, the mechanism of action of TCDD and dioxin-like compounds appears to be highly complex and to be dependent on ligand, dose, duration of exposure, and sex, and it is only partly understood (Silkworth et al., 2008; Budinsky et al., 2014).

Oxidative stress and oxidative DNA damage have long been recognized as important factors in carcinogenesis (Klaunig et al., 2011) (see Chapter 15, by Bucher). Oxidative stress has been demonstrated to occur after in vivo and in vitro exposure to AhR ligands including TCDD, PCB 126, and PeCDF (Yoshida and Ogawa, 2000; Hennig et al., 2002). Upregulation of CYP1A1 and CYP1B1 by activated AhR increases the chance of production of reactive oxygen species upon uncoupling of the catalytic cycle of these enzymes, as has been shown for exposure to dioxin-like coplanar PCBs (Schlezinger et al., 2006; Green et al., 2008). In addition, in experiments with mice, AhR activation has been associated with mitochondrial production of reactive oxygen species in a process that does not appear to involve CYP450 enzymes (Senft et al., 2002).

Many signalling pathways are mechanistically involved in carcinogenesis, including those involved in regulation of cell proliferation, apoptosis, senescence, and angiogenesis (Hanahan and Weinberg, 2011) (see Chapter 10, by Smith). There is considerable evidence of extensive cross-talk of AhR with several of these and other pathways, including various nuclear transcription factors (Haarmann-Stemmann et al., 2009; Puga et al., 2009) and the transforming growth factor beta (TGF-β) (Gomez-Duran et al., 2009) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathways (Tian et al., 1999; Vogel and Matsumura, 2009). There is also evidence for cross-talk with the constitutive androstane receptor and one of its target genes in mice, *Cyp2b10* (Patel et al., 2007), and with cyclin G2 (Ahmed et al., 2012).

The extensive cross-talk with ER signalling mentioned above explains the anti-estrogenic properties of TCDD (Safe and Wormke, 2003; Denison et al., 2011). There is evidence that this AhR–ER cross-talk can occur at different levels of AhR involvement in the regulation or dysregulation of gene expression, including non-classical modes with AhR–ARNT or just AhR acting as co-repressor (Ohtake et al., 2003; Labrecque et al., 2012).

Mediated by AhR, TCDD downregulates the EGF receptor and inhibits binding of EGF to its receptor in several tissue types (Madhukar et al., 1984; Kitamura et al., 2006; Haarmann-Stemmann et al., 2009). This reduces EGF signalling, which may be an anti-carcinogenic rather than a pro-carcinogenic effect. Conversely, EGF receptor signalling appears to inhibit AhR action at the DNA level (Sutter et al., 2009), indicating significant cross-talk with EGF pathways.

Induction of cell proliferation has long been considered to be a causal factor in carcinogenesis in humans and experimental animals (Preston-Martin et al., 1990; Schwarz et al., 1995), with receptor mediation and an imbalance between proliferation and apoptosis in favour of cell growth as major mechanisms (Roberts et al., 1997; Oliver and Roberts, 2002). TCDD has been found to induce hepatocellular replicative DNA synthesis in vivo in some studies (Lucier et al., 1991) but not in others (Fox et al., 1993; Bauman et al., 1995), and it inhibits growth of primary hepatocytes in vitro (Hushka and Greenlee, 1995). Lack of AhR in knockout mice resulted in increased hepatocellular apoptosis (Zaher et al., 1998), but TCDD treatment of rats that express AhR also resulted in increased apoptosis in the liver during hepatocarcinogenesis (Stinchcombe et al., 1995). In human skin cells in vitro, TCDD caused inhibition of cellular senescence, presumably via AhR, which may explain in part the tumour-promoting activity of TCDD in initiated skin in mice (Ray and Swanson, 2009). However, whether this mechanism occurs in vivo and in the liver is not known. PCBs can also stimulate hepatic cell proliferation, but this is not consistently found and may or may not be AhR-dependent.

The coplanar PCB 126 and the non-coplanar PCB 153 both induced proliferation, but only after a tumour-initiating dose of diethylnitrosamine, and both had liver tumour-promoting activity (Rignall et al., 2013). However, in other studies, PCB 153 stimulated hepatocellular proliferation in rats but not when preceded by treatment with diethylnitrosamine, whereas the coplanar 3,3′,4,4′-tetrachlorobiphenyl (PCB 77) inhibited or stimulated hepatocellular proliferation (Tharappel et al., 2002; Lu et al., 2003, 2004). PCB 126, but not PCB 153, stimulated proliferation of cultured liver cells (Vondrácek et al., 2005). In studies with exposures of up to 2 years, PCB 153 did not stimulate hepatic cell proliferation and

had equivocal carcinogenic activity, whereas PCB 126 did stimulate proliferation after exposure for 1 year and was carcinogenic (NTP, 2006a, b). In similar studies, PeCDF and the coplanar 2,3′,4,4′,5-pentachlorobiphenyl (PCB 118) also stimulated hepatic cell proliferation and had carcinogenic activity (NTP, 2006c, 2010). Overall, the involvement of AhR-mediated changes in cell proliferation and apoptosis in TCDD carcinogenesis is not clear and may depend on context (in vivo or in vitro), tumour induction protocol (initiation–promotion or continuous treatment), tissue, dose and duration of TCDD treatment, and species (Budinsky et al., 2014). This may also be the case for dioxin-like PCBs and dibenzofurans.

AhR appears also to be critical to the carcinogenicity of PAHs that are preferentially metabolized by CYP1A1 and CYP1A2, such as benzo[*a*]pyrene and dibenzo[*a,l*]pyrene (Shimizu et al., 2000; Nakatsuru et al., 2004), but not 7,12-dimethylbenz[*a*]anthracene (DMBA) (Ide et al., 2004). This is presumably because DMBA is metabolically activated by CYP1B1, which does not need induction by AhR but is constitutively expressed in rodent target tissues, and is required for the carcinogenicity of DMBA (Buters et al., 1999; Ide et al., 2004). Diethylnitrosamine, which is not an AhR ligand and is metabolically activated not by CYP1A1 but by CYP2E1, induced liver tumours in mice, and this effect was markedly enhanced in mice that lack AhR (Fan et al., 2010).

TRAMP (transgenic adenocarcinoma mouse prostate) mice, which have disrupted retinoblastoma and p53 function in the prostate, resulting in tumour development in this organ, displayed increased tumour formation when crossed with AhR-null mice (Fritz et al., 2007). These findings suggest that AhR can function as a tumour suppressor, which is supported by the observation of increased cell proliferation and reduced apoptosis in liver tumours induced by diethylnitrosamine in AhR-null mice (Fan et al., 2010).

There is some evidence from studies with human tumour cells in culture to support the idea that AhR has tumour-suppressive potential (Zudaire et al., 2008). This notion may appear to be in conflict with the reduced or absent tumour development in mice that lack AhR in response to treatment with benzo[*a*]pyrene and dibenzo[*a,l*]pyrene (Shimizu et al., 2000; Nakatsuru et al., 2004). However, mice that lack the *Arnt* gene specifically in the skin did not develop skin cancer when treated with benzo[*a*]pyrene plus TPA, whereas they did so when treated with a carcinogen that does not require AhR mediation, such as *N*-methyl-*N*-nitrosourea, followed by TPA (Shi et al., 2009). This finding strongly suggests that CYP1A1 induction by the AhR–ARNT complex in response to benzo[*a*]pyrene treatment is required for benzo[*a*]pyrene to be carcinogenic.

In conclusion, AhR is activated by many chemical agents; some of these are carcinogens, and others are not. Most or all carcinogenic compounds that activate AhR are likely to also act via other mechanisms to cause cancer. Thus, AhR activation is probably best considered as an important mechanistic effect of carcinogens rather than as the sole or predominant carcinogenic mechanism of such compounds.

In view of the complexity of AhR-mediated mechanistic events, it is likely that there are many ways in which this important receptor can be involved in the carcinogenic process. Nonetheless, it is clear that AhR is critical to the carcinogenicity of TCDD and some or many dioxin-like compounds as well as some important PAHs, and it is evident that this receptor can be involved in cancer-initiating as well as tumour-promoting mechanisms.

Summary

How do we evaluate – on the basis of mechanistic data – the carcinogenicity to humans of chemicals that have not been tested in chronic animal bioassays or adequate epidemiological studies? Are all chemicals that bind to and activate AhR, or all agents that activate both ER and PR, human carcinogens?

A case in point is the designation of PCB 126 and PeCDF as *carcinogenic to humans* (Group 1) in Volume 100F of the *IARC Monographs* (IARC, 2012a). This classification was based on the ability of these chemicals to activate AhR, induce CYP1A1, CYP1A2, and other drug-metabolizing enzymes, stimulate cell proliferation, induce oxidative stress, and have carcinogenic and tumour-promoting activities in ways similar to those observed with TCDD. A critical point was the ability of these agents to produce a spectrum of neoplasms that is similar to the tumours found after treatment with TCDD in the same rodent species. This designation would probably not have been justified in the absence of such animal bioassay data.

There may be compounds that activate AhR and induce CYP1A1 and CYP1A2 but are not carcinogenic. β-Naphthoflavone is an example of such a compound that activates AhR and induces CYP1A1 but is not a carcinogen (Denison et al.,

2011), even though it is a liver tumour promoter after treatment with diethylnitrosamine (Hayashi et al., 2012). Omeprazole also activates AhR and induces CYP1A1 but is not carcinogenic (Kleeberg et al., 1999). However, many other agents that activate AhR and induce CYP1A1 and CYP1A2 are carcinogenic. Furthermore, induction of oxidative stress by TCDD and dioxin-like compounds is non-specific and is – like various other pleiotropic effects of these agents – perhaps not required for AhR-mediated carcinogenesis. Such effects can only be considered additional evidence suggesting that a compound that activates AhR and induces CYP1A1 and CYP1A2 is carcinogenic to humans or experimental animals.

Stimulation of cell proliferation is often invoked as an effect that indicates carcinogenic potential of chemicals and as a cause of human cancer (Preston-Martin et al., 1990; Oliver and Roberts, 2002). But one can ask whether receptor-mediated effects resulting in enhancement of cell proliferation and/or reduction of apoptosis are sufficient for carcinogenicity, or whether more is needed.

Induction of cell proliferation by estrogens and progestins or by TCDD and dioxin-like compounds is not a simple effect but is highly complex and dependent on a wide variety of factors, as was pointed out above. Only in certain circumstances will these agents stimulate cell proliferation in target tissues, whereas in other situations they may inhibit proliferation (Hushka and Greenlee, 1995). Furthermore, there is only scant direct evidence that increased cell proliferation by itself causes tumours or malignant transformation. Most likely, genetic damage is required in combination with cell proliferation or reduction of apoptosis; in that concept, receptor-mediated carcinogens are tumour promoters, co-carcinogens, enhancers of susceptibility, or indirect inducers of genotoxicity, for example via induction of oxidative stress. For example, increased cell proliferation has been shown to neoplastically transform rat hepatocytes only after treatment with a genotoxic agent (Lee et al., 1989). NIH 3T3 cells will undergo complete neoplastic transformation under favourable conditions after sustained proliferation, but these cells are already immortalized (Yao et al., 1990; Chow and Rubin, 2000).

Thus, it appears that stimulation of cell proliferation by itself is not sufficient to induce cancer, but that it can be a powerful enhancer of carcinogenesis by facilitating initiating events, for example through fixation of pro-mutagenic DNA lesions, affecting DNA repair, inducing error-prone DNA synthesis (Mimura and Fujii-Kuriyama, 2003), and/or by acting as a driving force during tumour promotion and progression. These notions obviously have implications for the use of cell proliferation as a criterion in hazard identification of suspected carcinogens and in carcinogen risk assessment (Melnick et al., 1996).

Receptor-mediated events are typically dose-dependent, and dose–response relationships between ligands and receptor-mediated effects are often nonlinear. This has led to the concept of a dose threshold for receptor-mediated carcinogens that is used in carcinogen risk assessment. However, non-threshold dose–responses also occur, and there are many factors that can affect the shape of dose–responses of non-genotoxic or receptor-mediated carcinogens (Melnick et al., 1996). Moreover, for steroid hormone-like compounds, such as those used in combined oral contraceptives and hormonal menopausal therapy, the dose–response is typically biphasic (inverted U-shaped), and the effect threshold is observed at very low doses (Reddel and Sutherland, 1987; Groshong et al., 1997).

A further complicating issue is the evidence for a wide range of modifying factors that can affect the receptor-mediated action of suspected carcinogenic agents; not only is the dose critical, but species, sex, age, tissue or cell context, duration and timing of exposure, and route of exposure are all crucially important modifying factors, as pointed out above. Often neglected are co-exposures that may be important in determining the ultimate effect; for example, exposure to cigarette smoke and its constituents may influence the outcome of exposure to TCDD or other dioxin-like compounds (Kitamura and Kasai, 2007).

The diversity of ER-, PR-, and AhR-mediated effects observed across species, sexes, tissues, and cell types and across exposure conditions (dose, duration, timing, and route of exposure) poses considerable obstacles to rational testing for carcinogenic and mechanistic effects that predict human carcinogenicity of agents that act via these receptors. Whole-animal models are essential to sort out these effects, and animal bioassays that are designed to detect relevant carcinogenic effects remain indispensable. However, no good animal models exist for exposures to some of the important receptor-mediated carcinogens. It is not clear how to best test for effects of agents that are used or intended as oral contraceptives, and there are no truly relevant animal models reflecting conditions found

during and after human menopause. Mice or rats ovariectomized at age 1–1.5 years may be useful as a model for menopause, but this approach has not been used much. More research will be needed to develop and validate approaches with appropriate animal models for these important exposure conditions in humans.

As indicated above, there are also no validated models to evaluate the protective effects of combined oral contraceptives and hormonal agents used in menopausal therapy. Although standard animal models for cancer of the colon and breast could be used, there is a paucity of models for cancer of the endometrium, ovary, and cervix. To investigate effects of environmental exposures to receptor-mediated dioxin-like compounds, the Harlan Sprague-Dawley rat has proven to be suitable and sensitive in a large number of studies with these agents. Mice that lack or overexpress AhR have been useful to examine mechanisms underlying the effects of TCDD, but differences related to genetic background and strain hamper the generalization of the results of such research. Studies with diethylstilbestrol have demonstrated that for exposure to hormonal agents in early life, particularly in utero, neonatal treatment of CD-1 mice and Sprague-Dawley rats is informative and relevant for effects observed in humans. However, in vitro models may not be very relevant for studying the effects of most carcinogens that act via receptor mediation, because of the likely importance of paracrine mechanisms involving stromal cells, as shown for ER- and PR-mediated events. However, as a screening tool, approaches based on the use of cell cultures will remain very important for initial investigations and studies that are not feasible in whole animals.

Perhaps the most difficult aspect of evaluating the carcinogenic potential of agents that act via receptors, particularly AhR, ER, and PR, is the sheer complexity of how these receptors are involved in mediating the effects of chemical agents (Pandey and Picard, 2010; Denison et al., 2011; Jennen et al., 2011; Ruiz-Aracama et al., 2011; Thomas and Gustafsson, 2011; Cochrane et al., 2012; Jacobsen and Horwitz, 2012). Signature changes in gene expression induced in target cells or tissues by suspected carcinogens would be a most useful tool for carcinogen identification if they would suggest or predict the carcinogenicity of the agents. However, no consensus alterations in gene expression profiles have yet been identified for groups of agents that are thought to act via similar mechanisms, such as dioxin-like compounds or estrogenic substances. In fact, as pointed out above, even within the group of dioxin-like compounds there is considerable variation in the gene expression changes they induce (Vezina et al., 2004; Kopec et al., 2008, 2010; Ovando et al., 2010; Jennen et al., 2011). In addition, knowledge about the mechanisms by which these chemicals act continues to evolve in major ways. For example, recent data indicate that some PCBs can be genotoxic because they are metabolized to DNA-damaging reactive intermediates, overturning the notion that PCBs are purely receptor-mediated agents (Ludewig and Robertson, 2012; IARC, 2016).

In conclusion, receptor-mediated mechanisms are central to the carcinogenicity of important groups of human carcinogens, but they are often incompletely or poorly understood. Research during the past two decades has revealed a high degree of complexity as to how such carcinogenic agents mechanistically act via receptors, and insight into these mechanisms continues to evolve. Receptor binding and activation by chemical agents or the induction of cell proliferation or oxidative stress are by themselves insufficient as evidence for carcinogenicity in animals or humans.

To make progress in the identification of receptor-mediated carcinogens, it will be important (i) to identify signature changes in gene expression in relevant tissues and cells in validated animal models that are predictive of carcinogenic activity of groups of structurally and functionally related chemical agents, and (ii) to further develop and validate such in vivo models. It also remains critical to continue generating data that allow evaluation of coherence in mechanisms and concordance in exposure-associated tumour types and target sites between animal models and humans.

An improved understanding is still needed of the relationships between carcinogenic activity and timing, dose, and duration of exposure to receptor-mediated carcinogens, and of the cancer-inhibitory activity of exposure to some of these agents for some tissue sites, such as that demonstrated for the reduction of risk of cancer of the endometrium, ovary, and colorectum by combined oral contraceptives and of risk of cancer of the colon by estrogen-only menopausal therapy (IARC, 2012b).

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CHAPTER 15.

Oxidative stress and radical-induced signalling

John R. Bucher

Throughout evolution, aerobic organisms have developed multiple defence systems to protect themselves against oxygen radicals (Benzie, 2000). One-, two-, and three-electron reductions of molecular oxygen give rise to, respectively, superoxide ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2, a radical precursor), and the highly reactive hydroxyl radical ($^{\cdot}OH$) or equivalent transition metal–oxygen complexes (Miller et al., 1990). Reactions of oxygen radicals with cellular components can deplete antioxidants, can cause direct oxidative damage to lipids, proteins, RNA, and DNA, and can result in the formation of a variety of other reactants with varying oxidative potentials, including carbon- or nitrogen-centred radicals (West and Marnett, 2006). A growing body of literature presents radicals as mediators of various cell signalling processes (Ma, 2010). An imbalance between the normal production of oxygen radicals and their capture and disposal by protective enzyme systems and antioxidants results in oxidative stress, and this condition has been proposed to be the basis of many deleterious chronic health conditions and diseases, including cancer.

Sources of oxygen radicals

Mitochondrial oxidative phosphorylation is a major source of oxygen radicals of endogenous origin. Mitochondrial complex I (reduced nicotinamide adenine dinucleotide [NADH]:ubiquinone oxidoreductase) and complex III (ubiquinol:cytochrome c oxidoreductase) are sites of superoxide production, with as much as 1–2% of the electron flux shunted through one-electron reduction of molecular oxygen (St-Pierre et al., 2002). Peroxisomes are a source of H_2O_2, through reactions involving acyl-CoA oxidase (which is involved in oxidation of long-chain fatty acids), D-amino acid oxidase, and other oxidases (Schrader and Fahimi, 2006).

When stimulated, inflammatory cells such as neutrophils, eosinophils, and macrophages produce oxygen radicals during the associated respiratory burst (the rapid release of reactive oxygen species from cells) that involves nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (Babior, 1999). This reaction produces superoxide, which is converted by superoxide dismutase to the more readily diffusible oxidant H_2O_2 and is involved in cell killing functions. Inflammatory cells such as macrophages are also capable of producing nitric oxide ($^{\cdot}NO$), through an inducible form of nitric

oxide synthase (Hibbs et al., 1988). ·NO is also involved in cell killing but can also react with superoxide at diffusion-limited rates to form peroxynitrite, a potent oxidant with a longer half-life and diffusion distance than the hydroxyl radical (Beckman, 1996).

Exogenous agents are also implicated in the generation of reactive oxygen. Metals such as cadmium and arsenic can participate in reactions that generate oxygen radicals (Liu et al., 2008; Kojima et al., 2009). Miller et al. (1990) presented a list of endogenous and exogenous agents that are capable of reducing oxygen to superoxide or that "autoxidize", probably through reactions catalysed by transition metals. Metabolism of many exogenous agents through cytochrome P450-mediated reactions can also result in the release of oxygen radicals (Hrycay and Bandiera, 2015), as can exposure to ionizing radiation. In addition, several lifestyle factors, such as obesity, tobacco smoking, and alcohol consumption, as well as chronic inflammatory conditions and viral infections are thought to involve radical-induced injury (Mena et al., 2009).

Oxidative damage

The hydroxyl radical or equivalent transition metal–oxygen complexes (Bucher et al., 1983) are highly reactive entities, capable of abstracting electrons from lipids, proteins, or DNA (Miller et al., 1990), and the resulting target molecule radical can then combine with molecular oxygen to participate in subsequent radical reactions, such as propagation of lipid peroxidation. Radical reactions with DNA result in single- and double-strand breaks (Toyokuni and Sagripanti, 1996), cross-links, and modified bases. The oxidation product 8-oxo-2′-deoxyguanosine is often used as a marker of oxidative DNA damage, although other bases are also susceptible to oxidation. DNA bases can be modified by lipid peroxidation reaction products (*trans*-4-hydroxy-2-nonenal, 4-hydroperoxy-2-nonenal, and malondialdehyde) to form various pro-mutagenic exocyclic adducts (Bartsch and Nair, 2006).

Defence mechanisms

Cytosolic and mitochondrial forms of superoxide dismutase catalyse the reduction of superoxide to H_2O_2, and when coupled with catalase within peroxisomes or with cytosolic glutathione peroxidase, can further convert these reactive species to water (Benzie, 2000). Sequestration of transition metals, principally iron and copper, in their oxidized forms through deposition in transport or storage proteins, or as chelates that do not support redox reactions, also limits radical reactions (Hatcher et al., 2009).

Dietary and endogenously produced antioxidants also contribute in the defence against radical damage by serving as radical scavengers. Theoretically any oxidizable substrate can act as a radical scavenger; ascorbic acid, tocopherols, uric acid, and sulfhydryl-containing amino acids provide considerable scavenging capacity (Benzie, 2000).

Interestingly, high concentrations of antioxidants in the presence of transition metals can actually drive formation of oxygen radicals (Tien et al., 1982). The importance of a balance between pro- and antioxidant capacities is also emphasized by an emerging understanding of the role of radical species in cellular signal transduction.

Oxygen radicals in cancer

Hanahan and Weinberg (2011), in their landmark review "Hallmarks of cancer: the next generation", identified sustaining proliferative signalling, reprogramming of energy metabolism, evading growth suppressors and immune destruction, resisting cell death, enabling replicative immortality, inducing angiogenesis, and activating invasion and metastasis as signal transduction pathways key to unravelling the cancer phenotype. They also described how genomic instability and tumour-promoting inflammation are principal drivers of these events. Oxygen radicals clearly contribute to genomic instability, are produced by inflammation, and – along with other radical species – play key roles in many of the processes identified above as necessary for conversion of normal cells into cancer cells.

Oxidative damage is considered to be a major factor in the generation of mutations, which are estimated to occur at a frequency of 10 000 per cell per day in humans (Lu et al., 2001). More than 100 different oxidative DNA lesions (Klaunig et al., 2011) and at least 24 base modifications (Wilson et al., 2003) have been identified, along with DNA–protein cross-links (Cadet et al., 1997), all of which potentially lead to genomic instability. RNA has also been shown to be susceptible to radical attack (Li et al., 2006) but may be less chemically susceptible than DNA (Thorp, 2000). Oxidative damage to DNA can lead to point mutations, deletions, insertions, or chromosomal translocations, which may cause activation of oncogenes and inactivation of tumour suppressor genes and may lead to initiation of carcinogenesis (reviewed by Bartsch and Nair, 2006; Klaunig et al., 2011).

Clearly, high levels of oxygen radicals can be fatal to the cell through overt necrosis or the induction of apoptosis, but lower levels may also contribute to the process of carcinogenesis through stimulation of cellular proliferation and alterations in other cellular functions. There appear to be a myriad of potential mechanisms for these effects, involving induction of transcription factors for numerous signalling pathways, particularly nuclear factor erythroid 2-related factor 2 (Nrf_2), mitogen-activated protein kinase (MAPK)/AP1, nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), and hypoxia-inducible transcription factor 1 alpha (HIF-1α) (reviewed by Klaunig et al., 2011). Protein kinase C, which is also susceptible to activation by cellular oxidants, is a family of serine/threonine kinases that is central to the regulation of many cellular functions, including proliferation, cell-cycle control, differentiation, cytoskeletal organization, cell migration, and apoptosis (Wu, 2006). There is also evidence that the activated oncogene v-Ha-*Ras* may act as a sustained proliferative stimulus in transformed fibroblasts through superoxide-mediated signalling pathways (Irani et al., 1997).

Vulnerable tissue sites and selected carcinogenic exposures

The synthesis of thyroid hormones requires iodination of thyroglobulin in a peroxidase-catalysed reaction that is dependent on H_2O_2. Krohn et al. (2007) reviewed evidence suggesting that the thyroid is particularly sensitive to formation of malignant nodules induced by oxidative stress. During active hormone synthesis, H_2O_2 levels are held in check with increased concentrations of glutathione peroxidases along with thyroid peroxidase, and high levels of glutathione peroxidases can interfere with the synthesis of thyroid hormones. Immunostaining for 8-oxo-2′-deoxyguanosine shows greater intensity in thyroid follicular cells near the lumen where H_2O_2 is generated than in the spleen, liver, or lung, suggesting a high level of oxidative DNA damage in the thyroid (Maier et al., 2006).

Environmental insults may augment oxidative DNA damage in the thyroid. Thyroid uptake of iodine-131 released during the accident with the Chernobyl Nuclear Power Plant in Ukraine is thought to be responsible for the high rate of papillary carcinoma observed in exposed children (Bennett et al., 2006). Rats and mice exposed to iodine-131 develop follicular cell tumours (IARC, 2012b). Thiocyanate from cigarette smoke may, by inhibiting uptake of iodine, cause oxidative damage through iodine deficiency. Production of thyroid-stimulating hormone is increased during periods of iodine deficiency, and this hormone stimulates H_2O_2 production in the thyroid. Levels of antioxidant enzymes have been shown to be elevated by iodine deficiency (Krohn et al., 2007).

The lung is also a vulnerable target for oxidative damage, by virtue of its exposure to air, which contains 21% oxygen, as opposed to the much lower oxygen concentrations in systemic tissues (Carreau et al., 2011). A key role of oxygen radicals in the pulmonary toxicity resulting from prolonged exposure to hyperoxia is demonstrated by the dramatic difference in the sensitivity of adult animals of various species, which succumb to oxygen toxicity after less than a week of exposure to 100% oxygen, compared with the ability of neonates of certain of these species to survive such exposures with little evidence of injury. The neonates of species resistant to pulmonary injury are capable of increasing their levels of antioxidant enzymes in response to hyperoxia, in contrast to the adults, which are incapable of mounting a similar response (Frank et al., 1978).

Several metals, metalloids, and fibres that contain metals or are frequently contaminated with metals have been demonstrated to cause cancer of the lung in humans and experimental animals (IARC, 2012a). These include certain forms of arsenic, asbestos, beryllium, cadmium, chromium, and nickel. Although carcinogenesis induced by metals appears to involve many mechanisms common to the process for other carcinogens, oxidative stress has been implicated as an important contributing factor for several metals (reviewed by Beyersmann and Hartwig, 2008).

Certain metals may undergo direct redox cycling, as demonstrated by the participation of nickel(II) in a Fenton-like reaction with H_2O_2, or in the metabolism of trivalent to pentavalent arsenic. Other metals, such as cadmium, may inhibit antioxidant enzymes or deplete antioxidants (cadmium and arsenic bind with sulfhydryl groups in glutathione) or potentially delocalize iron or copper from protected storage sites (e.g. trivalent arsenic can release iron from ferritin), making them available for participation in oxygen radical reactions. Still other metals, such as arsenic (reviewed by Shi et al., 2004), may activate signalling pathways through increased production of oxygen radicals, and potentially promote radical reactions through a variety of mechanisms. Finally, many studies have shown that asbestos or

asbestos-like materials (respirable elongated mineral particles or fibres) are capable of generating oxygen radicals, primarily through reactions catalysed by iron that is present in coordination bonding within the mineral structure, is associated with the surface, or is chelated and released from the fibre by various intracellular organic acids, such as citrate (Aust et al., 2011).

Summary

Many substances recognized as carcinogens in both humans and experimental animals are capable of influencing redox processes and redox balance within target cells. Oxygen radicals are capable of interacting with and influencing many cellular processes believed to be involved in the dysregulation of normal cellular physiology, thus sending the cells down the pathway to cancer. Oxygen radical reactions are intimately involved in many well-recognized mechanisms of carcinogenesis, such as inflammation, genomic instability, and cell proliferation. Because of the fundamental involvement of the oxygen radical in many of these processes, substances that have been shown to promote cellular injury induced by oxygen radicals should be considered as putative human carcinogens until it has been adequately demonstrated otherwise (Bucher and Portier, 2004).

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CHAPTER 16.

Immunosuppression

Jerry M. Rice

Introduction

Immunosuppression is a reduction in the capacity of the immune system to respond effectively to foreign antigens, including surface antigens on tumour cells. Immunosuppression can result from killing of immune effector cells or from blockage of intracellular pathways essential for antigen recognition or of other elements of the immune response.

Persistent immunosuppression presents a risk of cancer. Individuals who are latently infected with an oncogenic virus are at greatly increased risk for developing virus-related cancers when they become immunosuppressed (Grulich et al., 2007; Schulz, 2009; Wieland et al., 2014), and there is excess risk of B-cell non-Hodgkin lymphoma (NHL) when immunosuppression is accompanied by continuing immune stimulation from exposure to non-viral antigens, such as after organ transplantation (Ponce et al., 2014).

Potentially neoplastic cells that arise naturally, or that have been transformed by carcinogens acting by a mechanism such as genotoxicity or by the various mechanisms of action associated with oncogenic viruses, may escape immune surveillance in immunosuppressed individuals. As a result, survival of these cells and their replication to form tumours is greatly facilitated.

Certain pharmaceutical drugs, ionizing and ultraviolet radiation, or infection with certain viruses and parasites can cause immunosuppression. After exposure to X-rays or other types of ionizing radiation, immunosuppression is most pronounced if the entire body, rather than a limited area, is irradiated. Immunosuppression by pharmaceutical drugs or by ionizing or ultraviolet radiation is dose-dependent – the intensity and duration of the effect increases with increasing dose or continuing exposure – and is usually transient: immune function generally recovers after cessation of exposure. In contrast, infection with certain pathogens, such as human immunodeficiency virus type 1 (HIV-1) or malaria parasites, is persistent, and the immune deficiency that results is progressive unless the infection is effectively treated.

Immunosuppression as a medical therapy is used to treat autoimmune diseases such as lupus erythematosus or rheumatoid arthritis. Immunosuppressive drugs, usually in much higher dosage, are used to maintain the functional and anatomical integrity of foreign tissues grafted onto another individual, such as a kidney or heart transplant. A graft

from any individual except oneself or an identical twin will provoke an immune reaction against the grafted tissues, the intensity of which varies with the degree of antigenic difference between graft and host. In the absence of adequate immunosuppression, the host will destroy the graft. Whole organs (e.g. kidney, heart, liver, or lung) can be transplanted with maintenance of function that may continue for a normal lifetime when appropriate levels of immunosuppression are maintained. However, the risk of primary cancer in the transplant recipient increases with increasing intensity and duration of immunosuppression (Kinlen, 1996; Yu et al., 2014).

An uncommon but potentially dangerous side effect of immunosuppression to support organ transplants is that suppression of the immune response can allow occult tumours or metastatic tumour cells within the transplanted tissues or organs to survive, grow, and metastasize in the transplant recipient. Occult metastatic melanoma in the donated organ is especially dangerous for the transplant recipient (Penn, 1996; Loren et al., 2003). Such transplanted cancers regress when immunosuppressive therapy is withdrawn (Wilson et al., 1968; Loren et al., 2003).

Immunosuppression and genotoxicity

The fact that a carcinogen has immunosuppressive properties does not necessarily mean that this is the mechanism by which it causes human cancer. DNA-damaging agents are generally also immunosuppressants, especially at high levels of exposure; these include external ionizing radiation (X-rays and γ-rays), ultraviolet and solar radiation, and most of the chemical alkylating agents used in anticancer chemotherapy. Radiation and chemical alkylating agents are considered to cause cancer primarily by inducing DNA damage, rather than by immunosuppression.

Cyclophosphamide is an antineoplastic drug and is classified as *carcinogenic to humans* (Group 1). This drug has very marked immunosuppressive properties. In addition to its application in anticancer chemotherapy, cyclophosphamide is used clinically as an immunosuppressant to treat certain autoimmune diseases, such as severe systemic lupus erythematosus (Valeri et al., 1994). The drug, which must be metabolized to act as an alkylating agent, causes acute myeloid leukaemia and carcinoma of the urinary bladder in patients in whom it has been used as an antineoplastic agent (IARC, 2012b). All available evidence, including the organ sites of tumour development and the specific kinds of neoplasms induced, indicates that cyclophosphamide exerts its carcinogenic activity via a genotoxic mechanism (McCarroll et al., 2008), rather than via immunosuppression.

Chlorambucil, like cyclophosphamide, is a bifunctional alkylating agent that also is an antineoplastic drug and is classified as *carcinogenic to humans* (Group 1). It is used clinically as an immunosuppressant to treat childhood nephrotic syndrome (Neuhaus et al., 1994), rheumatoid arthritis, and other autoimmune diseases. It has been used to treat polycythaemia vera (a malignancy) and is used, often alone, as initial therapy for chronic lymphocytic leukaemia and in combination with other drugs to treat other cancers. Chlorambucil, like other antineoplastic alkylating agents, can cause acute myeloid leukaemia by a genotoxic mechanism after its use in anticancer chemotherapy (IARC, 2012b).

Immunosuppressive carcinogens

Several Group 1 agents reviewed in Volume 100 of the *IARC Monographs* act entirely or largely by immunosuppression, often in concert with other Group 1 agents, especially oncogenic infectious agents. The Group 1 agents that act by immunosuppression are HIV-1 and the pharmaceutical drugs ciclosporin and azathioprine.

HIV-1 infection

Infection with HIV-1 is the cause of the acquired immune deficiency syndrome (AIDS). The severe immune deficiency that is characteristic of AIDS results from a deficiency in CD4-positive T lymphocytes and a severe loss of memory B cells (IARC, 2012a). In addition to severe infections, several cancers occur at high frequency in patients with AIDS. NHL, especially primary brain NHL, as well as Kaposi sarcoma and cervical carcinoma are AIDS-defining conditions in severely immunosuppressed patients.

There is no evidence that HIV-1 causes NHL or other cancers through a direct effect. Unlike what is known about other cancer-associated viruses, there is no evidence that HIV-1 infection by itself leads to cell transformation or immortalization. The HIV-1 genome is not present in cancer cells, in contrast to what is observed with infectious agents that are directly oncogenic (IARC, 2012a).

Kaposi sarcoma, which is caused by Kaposi sarcoma herpesvirus (KSHV), is the most common cancer

in patients with HIV-1 infection. Its occurrence is highly correlated with the severity of suppression of CD4-positive T lymphocytes. The standardized incidence ratio for Kaposi sarcoma in a Swiss cohort was more than 500 in patients with a CD4-positive lymphocyte count of less than 100 cells/mm^3 but approximately 76 in patients with a CD4-positive lymphocyte count of greater than 500 cells/mm^3 (Clifford et al., 2005; IARC, 2012a).

NHL, chiefly of the B-cell type, is the second most common malignancy in patients with AIDS. In a meta-analysis of six studies, NHL had a standardized incidence ratio of 77 in patients with HIV-1 infection relative to the general population (Grulich et al., 2007), and NHL is frequently associated with Epstein–Barr virus (EBV) co-infection. The severe depletion of CD4-positive T lymphocytes induced by HIV-1 leads to dysregulated control of B lymphocytes and to the expression of co-infecting lymphotropic viruses (Engels, 2007).

The third most common malignancy in HIV-1-positive individuals, and also an AIDS-defining condition, is cervical carcinoma associated with human papillomavirus (HPV) infection. Anogenital intraepithelial neoplasms and carcinomas are also increased in frequency, and so are skin cancers associated with HPV infection (IARC, 2012a). In addition to NHL and Kaposi sarcoma, infection with HIV-1 causes cancer of the cervix, anus, and conjunctiva, as well as of the vulva, vagina, and penis (IARC, 2012a). The primary cause of these squamous epithelial neoplasms is co-infection with HPV. Finally, individuals with HIV-1 infection have a greatly increased incidence of infection with hepatitis B virus and hepatitis C virus, and are therefore at elevated risk for hepatocellular carcinoma (Grulich et al., 2007).

Therapeutic immunosuppression

Therapeutic immunosuppression, generally by various combinations of drugs such as ciclosporin and azathioprine, is administered to organ transplant recipients to maintain their transplanted organ or organs. Recipients are at high risk for some of the same cancers that occur in patients with AIDS. A comparison of AIDS-related and transplantation-associated tumours, from which this text is excerpted, is presented in IARC (2012a).

Although individuals with AIDS and those with iatrogenic immunosuppression after organ transplantation have immunodeficiency in common, the immunological abnormalities appear to differ considerably between these two conditions. However, the spectra of neoplasms that occur in patients with AIDS and in organ transplant recipients largely overlap. An obvious similarity between organ transplant recipients and patients with AIDS is the increased incidence of B-cell NHL associated with EBV infection. Specific differences include more frequent high-grade lymphomas in patients with AIDS and a more frequent EBV association and polymorphic lesions in organ transplant recipients.

The second important malignancy that is greatly increased in incidence in both individuals with HIV-1 infection and transplant recipients is Kaposi sarcoma (Zattra et al., 2014). A study of renal transplant recipients reported a more than 20-fold increase in the incidence of Kaposi sarcoma compared with the general population (Kasiske et al., 2004). Non-melanoma skin cancers other than Kaposi sarcoma also occur at high frequency in organ transplant recipients (Forchetti et al., 2014). There is a 65-fold increase in the incidence of squamous cell carcinoma and a 10-fold increase in the incidence of basal cell carcinoma in organ transplant recipients relative to the general population (Yu et al., 2014).

Ciclosporin

Ciclosporin, a cyclic lipophilic undecapeptide, is a calcineurin inhibitor and a potent immunosuppressant that is virtually non-myelotoxic but is markedly nephrotoxic. It is used in organ and tissue transplantation to prevent graft rejection after bone marrow, kidney, liver, pancreas, heart, lung, and heart–lung transplantation, and for prophylaxis and treatment of graft-versus-host disease (IARC, 2012b).

The immunosuppressive activity of ciclosporin is consistent with an increased risk of cancer as a result of impaired immune surveillance, particularly for virus-related cancers such as EBV-related NHL and HPV-related cervical cancer (IARC, 2012b). Patients who receive ciclosporin also are at increased risk for squamous cell tumours of the skin, which may be due in part to effects of the drug other than immunosuppression. Ciclosporin has the ability to generate reactive oxygen species, and this is probably relevant to its carcinogenicity (IARC, 2012b).

Azathioprine

Azathioprine, a substituted 6-mercaptopurine, is used in immunosuppressive treatments to prevent rejection of kidney allografts. The drug is usually used in conjunction with

other immunosuppressive therapy, including local radiation therapy and treatment with corticosteroids and other cytotoxic agents.

One large prospective cohort study (Kinlen et al., 1979) on renal transplant recipients who received azathioprine examined the incidence of and mortality from different types of cancer compared with the numbers expected on the basis of the incidence and mortality rates for the relevant country (Australia, New Zealand, and the United Kingdom). An almost 60-fold increase in the risk of NHL was observed for all countries combined (34 observed, 0.58 expected), as well as a 30-fold increase in the risk of squamous cell skin cancer in patients from the United Kingdom (3 observed, 0.13 expected) (IARC, 2012b).

Azathioprine is used more often in individuals with autoimmune conditions than in transplant recipients. For example, azathioprine is given for management of the signs and symptoms of rheumatoid arthritis in adults (IARC, 2012b). Excesses in the risk of NHL (relative risk, 10.9) and of squamous cell skin cancer (relative risk, 5.0) were found in non-transplant patients receiving azathioprine, although these excesses are smaller than those in transplant recipients (Kinlen, 1985). Azathioprine is carcinogenic via two mechanisms: (i) as an immunosuppressant, it is associated with post-transplant lymphoproliferative disorders that generally have a viral etiology; and (ii) because it causes 6-thioguanine to accumulate in patients' DNA, it also contributes to cancer development by induction of DNA damage (IARC, 2012b).

Often, milder therapy and less potently immunosuppressive drugs (e.g. steroids such as prednisone) are used for autoimmune conditions than for maintenance of organ transplants. Prednisone and related immunosuppressive steroid drugs have not been shown to be carcinogenic.

Malaria, a probable human carcinogen

In addition to the IARC Group 1 agents that are carcinogenic largely or entirely by an immunosuppressive mechanism, infection with *Plasmodium falciparum* malaria in holoendemic areas is *probably carcinogenic to humans* (Group 2A), at least in part by immunosuppression (Bouvard et al., 2012; IARC, 2014). Infection with *P. falciparum* malaria has immunosuppressive effects, as reflected by impairment of macrophage function and antigen presentation (dendritic cell inhibition), reduction in specific T-cell response, induction of regulatory T cells, and high plasma levels of pro-inflammatory cytokines (interleukin 6 [IL-6] and tumour necrosis factor alpha [TNF-α]) and regulatory cytokines (IL-10 and tumour growth factor beta [TGF-β]) (reviewed by Cunnington and Riley, 2010). Impaired humoral immune protection associated with prenatal or chronic exposure to *P. falciparum* is common in children living in malaria-endemic regions (Chelimo et al., 2005; Scott et al., 2005).

Children in certain regions of Africa become infected with EBV early in life, and nearly all have seroconverted by age 3 years (Biggar et al., 1978). EBV is activated when the immune system is compromised (reviewed by Hopwood and Crawford, 2000). Endemic Burkitt lymphoma (eBL), the most common paediatric cancer in sub-Saharan Africa, is a high-grade B-cell lymphoma characterized by the consistent presence of EBV (Epstein et al., 1964, 1965; zur Hausen et al., 1970). eBL occurs only where malaria transmission intensity is high, for example in the so-called lymphoma belt of sub-Saharan Africa and in the high-transmission areas of Papua New Guinea. Furthermore, within areas and countries where eBL occurs, it arises only among those living in regions with the highest transmission intensity, the so-called holoendemic or hyperendemic areas. *P. falciparum* can disturb the immature immune system in young children by expanding the B-cell pool in which eBL arises, and can reactivate latent EBV. Infection with both EBV and *P. falciparum* is required for the development of eBL (Bouvard et al., 2012; IARC, 2012a).

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CHAPTER 17.

Inflammation

Agnes B. Kane

Introduction

In 1863, Virchow proposed that cancer originates at sites of inflammatory responses (Virchov 1863a, b; Balkwill and Mantovani, 2001). Based on extensive epidemiological studies in the 20th century (de Martel et al., 2012; Elinav et al., 2013; Okada, 2014), the association between persistent infections, inflammation, and the development of human cancers was conclusively established for several types of carcinomas and lymphomas (Table 17.1 and Table 17.2).

Worldwide, infections have been linked with 16.1% of human cancers, accounting for 22.9% of cancers in developing countries (de Martel et al., 2012). Sterile inflammation associated with inhalation of crystalline silica or asbestos fibres (Volume 100C of the *IARC Monographs*; IARC, 2012a) has also been associated with development of lung cancer and malignant mesothelioma (Table 17.1). Cancer-related inflammation has been defined as "the seventh hallmark of cancer" (Colotta et al., 2009), and Hanahan and Weinberg (2011) added "tumour-promoting inflammation" as an enabling characteristic of human tumours.

This chapter focuses on the contribution of inflammation to multiple steps during the evolution of cancer, including genetic and epigenetic alterations, disruption of tissue organization and homeostasis, and establishment of a local microenvironment that contributes to tumour growth, invasion, and metastasis (Fig. 17.1). The key mechanistic pathways and mediators involved in inflammation-associated carcinogenesis are summarized.

Intrinsic and extrinsic pathways linking inflammation and cancer

Acute inflammation is a beneficial host response against tissue injury and microbial invasion that usually resolves after killing of the invading organisms, followed by tissue regeneration or repair. Persistent infections or inadequate resolution of acute inflammatory responses perpetuate tissue injury and lead to prolonged chronic inflammation accompanied by fibrosis or scarring (Kundu and Surh, 2012).

Persistent infection and inflammation disrupt local tissue homeostasis and dysregulate cell signalling pathways, leading to recruitment and activation of inflammatory cells (Balkwill, 2012). Persistent inflammatory conditions triggered by infectious or environmental agents are defined

Table 17.1. Human carcinomas associated with infections and chronic or persistent inflammation

Agent	Cancer sites
Hepatitis B and C viruses	Liver
Human papillomaviruses	Cervix, oral cavity, larynx, vulva, penis, anus
Clonorchis sinensis	Bile duct
Helicobacter pylori	Stomach
Opisthorchis viverrini	Bile duct
Schistosoma haematobium	Bladder
Asbestos fibres	Lung, mesothelium, larynx, ovary
Crystalline silica	Lung
Erionite fibres	Mesothelium

Source: Compiled from de Martel et al. (2012), IARC (2012a, b), and Trinchieri (2012).

as extrinsic pathways leading to the development of cancer (Mantovani et al., 2010; Multhoff et al., 2011). Examples of human carcinomas and lymphomas associated with exogenous infections or environmental exposures are listed in Table 17.1 and Table 17.2.

Intrinsic pathways driven by activation of proto-oncogenes and signalling pathways in pre-neoplastic and neoplastic cells also recruit host-derived inflammatory cells that stimulate tumour growth and progression (Grivennikov et al., 2010), changes that may be evident in the absence of any obvious exogenous infectious exposure. For example, the *RAS* oncogene is frequently activated by point mutation in malignant epithelial cells, which leads to activation of intracellular signalling cascades and increased expression of the pro-inflammatory cytokines interleukin 1 alpha (IL-1α), IL-1β, and IL-6 (Trinchieri, 2012).

In papillary thyroid cancer, rearrangement of the RET/PTC (rearranged during transfection/papillary thyroid carcinoma) tyrosine kinase activates expression of the chemokines IL-8, chemokine (C-C motif) ligand 2 (CCL2), and CCL20 that attract inflammatory cells, and induces expression of the chemokine receptor CXCR4 by malignant thyroid epithelial cells (Bozec et al., 2010). In addition to CXCR4, which is overexpressed in many malignant cells as well as in cancer stem cells (Trautmann et al., 2014), multiple chemokine receptors are expressed in leukaemias and lymphomas as well as in cancers of the lung, ovary, stomach, bladder, and prostate, which may contribute to tumour invasion and metastasis (Balkwill, 2012).

The pathways that are activated by persistent or repeated episodes of acute inflammation or by chronic inflammation and that contribute to tumour growth, invasion, and metastasis are illustrated in Fig. 17.1. Continued recruitment of host inflammatory cells to the evolving tumour microenvironment in response to persistent infections, ongoing tissue injury, or upregulated production of cytokines and chemokines drives cancer development (Grivennikov et al., 2010; Chai et al., 2015).

Activated inflammatory cells, including neutrophils and macrophages, produce reactive oxygen species and reactive nitrogen species. These potent chemical mediators are important in killing invading pathogens; however, their prolonged release can cause local tissue injury, damage to proteins, lipids, and DNA (the DNA damage may be mutagenic if not repaired correctly), and upregulation of signalling pathways, especially of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), that amplify pro-inflammatory responses (Lu et al., 2006; DiDonato et al., 2012). Key pro-inflammatory mediators that initiate and amplify tumour-associated inflammation include prostaglandins, cytokines, chemokines, and heat shock proteins (Fig. 17.1).

Prostaglandins are synthesized from arachidonic acid and contribute to tumour cell proliferation, survival, angiogenesis, and invasion. The rate-limiting enzyme in prostaglandin synthesis is prostaglandin-endoperoxide synthase 2 (PGHS-2), also known as cyclooxygenase 2 (COX-2), induced by NF-κB (Chai et al., 2015). Key cytokines in tumour-associated inflammation are IL-1 and tumour necrosis factor alpha (TNF-α). IL-1 is produced by tumour, endothelial, and inflammatory cells and activates intracellular signalling pathways, including those of NF-κB, activator protein 1 (AP-1), and p38 mitogen-activated protein kinase (MAPK) and c-Jun N-terminal kinase (JNK), that stimulate production of mediators involved in inflammation, invasion, and angiogenesis (Multhoff et al., 2011). TNF-α is a pro-inflammatory mediator produced by activated macrophages and tumour cells, leading to activation of NF-κB and signal transducer and activator

Table 17.2. Human lymphomas associated with persistent infections and inflammation

Agent	Malignancy
Borrelia burgdorferi	Cutaneous lymphoma
Chlamydia psittaci	Ocular adnexal lymphoma
Helicobacter pylori	Gastric lymphoma
Epstein–Barr virus	Burkitt lymphoma Large B-cell lymphoma Hodgkin lymphoma
Human T-cell lymphotropic virus type 1	Adult T-cell lymphoma

Source: Compiled from de Martel et al. (2012) and IARC (2012b).

of transcription 3 (STAT3) (Lin and Karin, 2007). IL-6 also promotes tumour angiogenesis and invasion (Multhoff et al., 2011). Constitutive activation of NF-κB and STAT3 is found in several human tumours, and these factors act synergistically to sustain and enhance tumour-associated inflammation (Chai et al., 2015).

The ultimate impact of intratumoural inflammation on tumour growth is to stimulate proliferation and survival of tumour cells, resistance to apoptosis, evasion of host immune attack, angiogenesis, and invasion, which are all major hallmarks of cancer (Hanahan and Weinberg, 2011). These authors identified tumour-promoting inflammation as an enabling characteristic of cancer, in addition to genomic instability, which is discussed later in this chapter.

How do exogenous infectious agents and environmental exposures trigger tumour-associated inflammation?

Exogenous agents responsible for inflammation cause persistent tissue injury, aberrant tissue regeneration and healing, and a favourable environment for tumour growth (Trinchieri, 2012). Although apoptosis is usually not associated with an inflammatory response, extensive apoptosis and necrosis do trigger inflammation by releasing damage-associated molecular factors, including adenosine triphosphate (ATP), nucleic acids, heat shock proteins, S100 proteins, and the high-mobility group box 1 protein (HMGB1) (Pandey et al., 2015).

Pyroptotic cell death – which is similar to apoptosis but is dependent on a different set of initiator caspases – is associated with necrosis and depends on activation of the inflammasome, a multiprotein cytoplasmic complex assembled in response to generation of reactive oxygen species, potassium ion efflux, or permeabilization of lysosomes, resulting in cytoplasmic release of neutral proteases, such as cathepsin B (Kuraishy et al., 2011; Zitvogel et al., 2012).

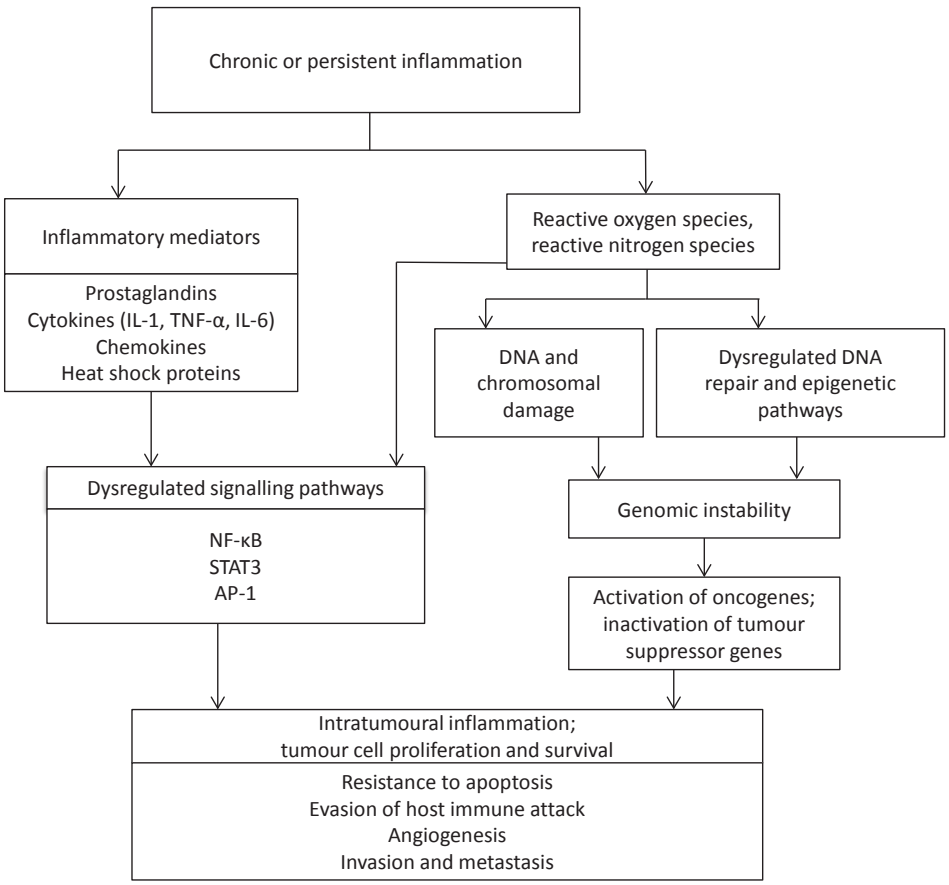
Environmental exposures to, for example, crystalline silica and asbestos fibres also trigger activation of the inflammasome, which results in lung epithelial injury, release of pro-inflammatory mediators, and lung fibrosis (Fig. 17.2; Dostert et al., 2008, 2013; Luna-Gomes et al., 2015). Crystalline silica and asbestos fibres are phagocytosed by macrophages, resulting in activation of the NLRP3 inflammasome (Biswas et al., 2011; Li et al., 2012).

Amphibole asbestos fibres, after inhalation into the lungs, translocate to the pleural cavity, where they may also activate the inflammasome in the mesothelial cell lining, leading to sustained inflammation that contributes to the development of diffuse malignant pleural mesothelioma (Broaddus et al., 2011; Mossman et al., 2013). Particles of carbon black have also been shown to induce activation of the inflammasome and pyroptosis of alveolar macrophages at high doses (Reisetter et al., 2011).

After assembly of the inflammasome or release of cathepsin B from lysosomes, pro-caspase-1 is activated by proteolytic cleavage to produce active caspase-1 that cleaves pro-IL-1β and pro-IL-18 to their active forms, which initiate and amplify inflammation after release in the local environment (Fig. 17.2). Active caspase-1 can also cleave pro-caspase-7, triggering cell death by pyroptosis (Zitvogel et al., 2012). Active IL-1β and IL-18 can suppress immune surveillance in addition to promoting growth of tumour stromal cells by paracrine signalling pathways (Fig. 17.2). Persistent inflammation and pyroptosis may enhance damage to epithelial barriers and thus contribute to gastric cancer associated with *Helicobacter pylori* infection (Grivennikov et al., 2010). Persistent infection with hepatitis B or C virus can also lead to chronic release of pro-inflammatory cytokines (Grivennikov et al., 2010; Read and Douglas, 2014).

Chronic exposure to irritants present in tobacco smoke, or to acetaldehyde generated by ethanol metabolism after consumption of alcoholic beverages, induces epithelial

Fig. 17.1. Pathways linking inflammation to cancer. AP-1, activator protein 1; IL-1, interleukin 1; IL-6, interleukin 6; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; STAT3, signal transducer and activator of transcription 3; TNF-α, tumour necrosis factor alpha. Source: Compiled from Colotta et al. (2009), Grivennikov et al. (2010), Multhoff et al. (2011), Kundu and Surh (2012), and Cooks et al. (2014).



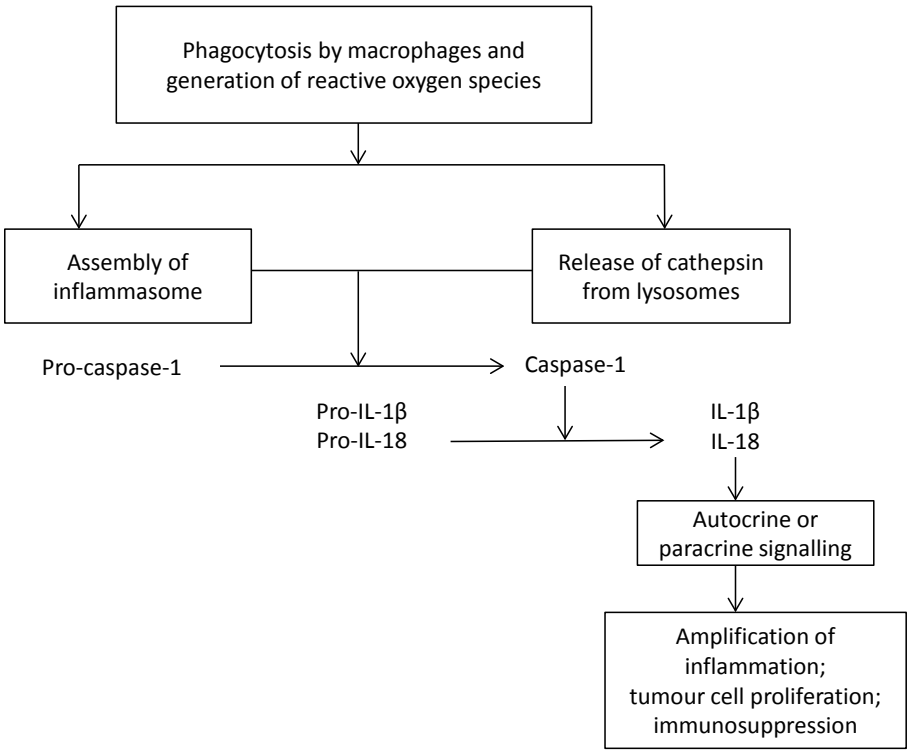
cell injury in the oral cavity and the upper respiratory tract. These lesions synergize with potent chemical carcinogens in tobacco smoke and smokeless tobacco, thereby increasing the risk of cancers of the oral cavity, larynx, and oesophagus (Smith et al., 2006). Persistent chronic inflammation associated with hepatitis B viral infection acts synergistically with aflatoxin, a genotoxic carcinogen, in the development of hepatocellular carcinoma (Kew, 2003; Cougot et al., 2005).

Inflammation and genomic instability

Activation of the NF-κB and STAT3 signalling pathways and activation of macrophages by TNF-α are the key contributors to sustained generation of reactive oxygen species and reactive nitrogen species in cancer-related inflammation (Colotta et al., 2009). Activated macrophages release large quantities of reactive oxygen species, and NF-κB upregulates nitric oxide synthase, which generates excess reactive nitrogen species (Laskin et al., 2011). Constitutive activation of STAT3 in cancers maintains NF-κB activation, creating a self-sustaining amplification loop (Chai et al., 2015).

NF-κB activity is prolonged in the presence of mutant p53 protein and upregulates activation-induced cytidine deaminase, an error-prone DNA repair enzyme (Cooks et al., 2014). Reactive oxygen species inactivate mismatch DNA repair enzymes by inducing oxidative damage; although

Fig. 17.2. Crystalline silica and asbestos fibres activate the NLRP3 inflammasome. IL-1β, interleukin 1 beta; IL-18, interleukin 18. Source: Compiled from Dostert et al. (2008), Biswas et al. (2011), Zitvogel et al. (2012), and Mossman et al. (2013).



reactive oxygen species induce the base excision repair pathway, overexpression of these repair enzymes enhances microsatellite instability (Colotta et al., 2009). Upregulation of NF-κB and sustained production of cytokines cooperate with mutated p53 protein to enhance genomic instability (Fig. 17.1; Grivennikov et al., 2010; Cooks et al., 2014).

Tumour-associated inflammation is also linked with epigenetic alterations that lead to silencing of key tumour suppressor genes, such as *INK4a* or *p16* in lung cancers and malignant mesothelioma (Blanco et al., 2007; Christensen et al., 2009, 2010; Nelson et al., 2012). Oxidative damage to cytosine and chlorination or bromination have been proposed to induce heritable epigenetic alterations due to cytosine methylation (Valinluck and Sowers, 2007). Chronic infection with *Helicobacter pylori* is also associated with hypermethylation and silencing of *p16* as well as *E-cadherin* (Kundu and Surh, 2012).

In summary, chronic or persistent inflammation favours accumulation of DNA lesions and chromosomal damage induced by persistent production of reactive oxygen species and reactive nitrogen species, impaired DNA repair pathways, and heritable epigenetic alterations, leading to activation of oncogenes and inactivation of tumour suppressor genes in developing tumours. Tumour-associated inflammation and genomic instability drive tumour growth and progression, which enable acquisition of the six core hallmarks of cancer (Hanahan and Weinberg, 2011).

Inflammation, fibrosis, and cancer

In 1986, Dvorak described cancers as “wounds that do not heal”, based on evidence of inflammatory cells infiltrating into tumours, accompanied by angiogenesis and fibrosis, similar to wound healing (Dvorak, 1986; see also Dvorak, 2015). Persistent infections accompanied by parenchymal cell injury and chronic inflammation, and fibrosis associated with

inhalation of crystalline silica or asbestos fibres promote tumorigenesis (Kuraishy et al., 2011). For example, persistent infection with hepatitis B or C virus can produce fibrotic scarring or cirrhosis in the liver, accompanied by nodules of regenerating hepatocytes, leading to development of hepatocellular carcinoma (El-Serag and Rudolph, 2007). Nodular fibrotic scarring of the lungs is characteristic of silicosis (Leung et al., 2012), and inhalation of asbestos fibres can cause diffuse fibrosis or asbestosis (Mossman et al., 2011). Pulmonary fibrosis has been associated with development of lung cancer (IARC, 2002; Laskin et al., 2011). Several mechanistic links between fibrosis and tumour development and progression have been proposed (Hanahan and Coussens, 2012).

Tissue fibrosis changes the normal architecture and compliance of the extracellular matrix, resulting in dense cross-linked connective tissue and increased stiffness (Liu et al., 2010). Excess extracellular matrix components, including heparan sulfate proteoglycans that bind to the CD44 receptor, are produced during cancer development and contribute to enhanced growth factor signalling and cell proliferation (Nasser, 2008). Disrupted assembly and disorganization of the extracellular matrix can alter polarity and differentiation of pre-neoplastic epithelial cells, facilitating their proliferation, migration, and invasion through the basement membrane (Lu et al., 2012).

Resident fibroblasts in connective tissues and mesenchymal stem cells, in local stem cell niches or recruited from the bone marrow, secrete epithelial and fibroblast growth factors as well as pro-inflammatory cytokines and chemokines that contribute to the tumour microenvironment (Hanahan and Coussens, 2012). They are also a source of paracrine growth factors, including insulin-like growth factor 1 (IGF-1) and IGF-2, that promote survival of cancer cells, as well as a source of angiogenic factors that promote tumour angiogenesis (Lu et al., 2012).

Matrix metalloproteinases (MMPs), which accelerate the degradation or remodelling of the tumour stroma and the release of immobilized growth factors and cytokines, are frequently overexpressed in tumours. The gelatinases MMP-2 and MMP-9 are overexpressed by malignant tumour cells or stromal cells in a wide range of carcinomas, as well as by leukaemias and lymphomas. Activation of MMPs in the tumour microenvironment promotes tumour cell migration and invasion, release of sequestered growth factors, and activation of latent forms of cytokines including IL-1β (Bauvois, 2012).

Tumour-associated macrophages and lymphocytes are major sources of cytokines in the tumour environment (Laskin et al., 2011; Balkwill, 2012). The phenotype of tumour-associated macrophages is shifted to a pro-fibrotic, pro-angiogenic phenotype, M2, characterized by production of arginase, IL-10, and TGF-β (Sica and Mantovani, 2012) as well as IL-23 (Grivennikov et al., 2010). The T-lymphocyte subset TH17 produces IL-17, which upregulates IL-23 expression in the tumour microenvironment. IL-23 is a key cytokine in tumour growth and invasion; it causes upregulation of MMP-9 expression and increases angiogenesis and fibrosis (Langowski et al., 2006). IL-17 also promotes liver fibrosis and activates hepatic stellate cells to produce collagen in murine models of toxic liver cell injury (Meng et al., 2012).

Resident tissue stem cells in adults occupy a specialized niche to maintain their polarity, self-renewal, and differentiation by anchoring to receptors in the basement membrane or in the extracellular matrix. Altered extracellular matrix organization and stiffness may disrupt this contact, allowing local expansion of the stem cell pool (Lu et al., 2012). These locally proliferating stem cells may give rise to cancer stem cells or tumour-initiating cells. Cancer stem cells express cell surface markers, including CD24, CD44, and CD133, which are thought to enhance growth and invasion as well as resistance to apoptosis (Keysar and Jimeno, 2010).

Summary and conclusions

Inflammation has been hypothesized to contribute to multiple stages in cancer development (Trinchieri, 2012). Sustained or persistent inflammation releases mediators that can damage DNA and hamper DNA repair, leading to cell transformation; it establishes a local microenvironment that allows the tumour to grow and metastasize, and to avoid immune destruction, thus preventing an effective immune response against the tumour (Mantovani et al., 2010).

Persistent or chronic inflammation, frequently in association with oxidative stress, is considered to be an established or likely mechanistic event contributing to human cancers associated with exposure to crystalline silica and asbestos fibres (IARC 2002; Shukla et al., 2003; Valavanidis et al., 2013) as well as diesel engine emissions and indoor coal combustion (Sood, 2012; Carlsten and Georas, 2014; Vermeulen et al., 2014). These inhalation exposures

are associated with lung cancer in humans and experimental animals, and asbestos fibres may also induce cancer at distant sites, including the mesothelial lining and the ovary (IARC, 2012a).

Persistent bacterial, viral, and parasitic infections (IARC, 2012b) are also associated with a variety of human carcinomas as well as leukaemias and lymphomas (Table 17.1 and Table 17.2). Persistent or repeated episodes of tissue injury and inflammation may also synergize with viral oncoproteins and the fungal contaminant aflatoxin in the development of hepatocellular carcinoma (Kew, 2003; IARC, 2012b; Simet et al., 2012). In combination with potent carcinogens in tobacco smoke and smokeless tobacco (e.g. arylamines, polycyclic aromatic hydrocarbons, and nitrosamines), ethanol consumption may increase epithelial permeability and injury, which together with oxidative stress enhance the development of cancers of the oral cavity, larynx, and oesophagus (Bor and Capanoglu, 2009; IARC, 2012c). Exogenous environmental, occupational, personal, and infectious exposures resulting in persistent or chronic inflammation are preventable, and modifying these circumstances of exposure would diminish the worldwide burden of cancer.

Acknowledgement

The author's research is supported by a National Institute of Environmental Health Sciences (NIEHS) Superfund Research Program grant, P42 ES013660.

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CHAPTER 18.

Ionizing radiation

Mark A. Hill and Robert L. Ullrich

Introduction

The carcinogenic risk associated with exposure to ionizing radiation has been evaluated previously in the *IARC Monographs*: radon in Volume 43 (IARC, 1988), X-rays, γ-rays, and neutrons in Volume 75 (IARC, 2000), and some internally deposited radionuclides in Volume 78 (IARC, 2001). An updated review on all carcinogenic types of radiation, also including solar and ultraviolet radiation, was published as Volume 100D (IARC, 2012).

For certain types of ionizing radiation, the evidence of carcinogenicity in humans is clear, but in other cases the data are few or non-existent. However, the overall conclusion reached in Volume 100D of the *IARC Monographs* was that all types of ionizing radiation should be considered as *carcinogenic to humans* (Group 1). The rationale for this was that all types of ionizing radiation transfer their energy to biological material in clusters of ionization and excitation events, primarily through a mechanism mediated by free electrons. In addition, DNA damage is a common biological outcome of exposure to all ionizing radiation; energy deposition results in a wide variety of molecular damage, such as base damage and single- and double-strand breaks, some of which may be clustered to form complex lesions. Subsequent processing of these lesions may lead to chromosomal aberrations and mutations. The generality of induction of and response to radiation damage is discussed for all types of ionizing radiation in greater depth later in this chapter.

In addition to the above-mentioned reviews in the *IARC Monographs*, there have been many major national and international reviews of the literature on radiation, as well as radiation risk estimates. These include the publications of the United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR, 2000, 2008, 2010) and the reports from the United States National Research Council (NRC, 1999, 2006), the United States National Council on Radiation Protection and Measurements (NCRP, 1993, 1999, 2001, 2005), and the International Commission on Radiological Protection (ICRP, 2003, 2007; Valentin, 2005).

Two major issues faced when studying radiation carcinogenesis is that radiation-induced cancers are indistinguishable from those that occur naturally, and that risk estimates rely on epidemiological data for which statistical significance is reached only at high doses. The existing data

are not powerful enough to enable comment on the shape of the dose–response curve and the associated risks at doses associated with typical human exposures. Many of the in vitro and in vivo studies investigating the mechanisms underlying cancer risk from exposure to ionizing radiation have concentrated on low-dose exposures, typically of 0.1 Gy (= 0.1 J/kg) and below.

The nature of ionizing radiation

Ionizing radiation is a term used for any radiation that is capable of ionizing (i.e. removing electrons from) atoms or molecules of the medium being traversed. Ionizing radiations are usually classified as either electromagnetic or particulate.

X-rays and γ-rays are both electromagnetic radiations. They do not differ in nature, but their designation reflects their origin; X-rays are produced by extranuclear processes and γ-rays by intranuclear processes. These types of radiation are often classified as indirectly ionizing, because the chemical and biological damage is dominated by the charged particles (mainly electrons) produced as a result of interactions within the medium. Neutrons are also classified as indirectly ionizing. They deposit energy and cause damage through recoil protons, α-particles, and nuclear fragments that result from neutron interactions.

Particulate radiations include electrons, positrons, protons, neutrons, α-particles, and other ions. With the exception of neutrons, all of these particles are charged and are classified as directly ionizing (if they have sufficient energy) because they directly ionize the medium they are traversing, producing chemical and biological damage.

The human body can be irradiated either from external sources or through internal exposure as a result of ingestion, inhalation, dermal absorption, or injection of radionuclides. The effects of radiation are directly related to the dose received by individual cells or organs, and by the radiation quality. Therefore, these effects can vary significantly, depending on the resulting dose distribution or distribution of radionuclides throughout the body. The dose distribution may vary from being essentially uniform after whole-body exposure to being highly heterogeneous in the case of non-uniform distribution of internal radionuclides that emit short-range α-particles or β-particles. Medium- to high-energy X-rays, γ-rays, and neutrons are typically highly penetrating and will traverse the body, whereas α-particles and β-particles typically have a short range (for α-particles, less than 100 μm, and for β-particles, from less than 1 μm to several millimetres). In general, the penetration range of charged particles can vary significantly depending on their energy and the type of particle.

Genotoxicity and the importance of radiation track structure

Ionizing radiation interacts within cells and tissues by depositing energy in highly structured tracks of ionization and excitation events that are stochastic in nature. On average, these events are relatively sparsely distributed for high-energy X-rays and γ-rays, which deposit energy via electrons with relatively low linear energy transfer (LET), where LET corresponds to the energy loss per unit track length. For example, cobalt-60 γ-rays have an LET of about 0.25 keV/μm (where 1 eV = 1.602×10^{-19} J). The ionization and excitation events are much closer together for low-energy charged particles, which are considered to be high-LET radiation. For example, an α-particle with an energy of 2 MeV has an LET of about 180 keV/μm.

All types of ionizing radiation induce a wide range of damage and effects, including DNA damage, chromosomal aberrations, mutations, cell transformation, and cell killing (NRC, 1999, 2006; UNSCEAR, 2000; ICRP, 2003, 2007). The efficiency in causing damage and subsequent biological effects is related not only to the amount of energy transferred per unit mass (the absorbed dose, expressed in units of gray, where 1 Gy = 1 J/kg) and the rate of energy transfer (the dose rate) but also to the microdistribution of energy, which is determined by the type of radiation and the associated track structure.

The relative biological effectiveness is defined as the inverse ratio of the dose required to produce a given biological effect to the dose required by a reference radiation to produce the same effect. The relative biological effectiveness typically increases with the LET value of the radiation, and it reaches a peak at about 100–200 keV/μm for a range of biological end-points. Whereas the absorbed dose unadjusted for attenuation by the body is expressed in units of gray (Gy), the weighted organ dose (the equivalent or effective dose) is expressed in sieverts (Sv) or millisieverts (mSv), which are also the units in which radiation exposure limits are given.

For many biological effects, nuclear DNA is a critical target of ionizing radiation (UNSCEAR, 1993).

Ionizing radiation can cause DNA damage either by direct ionization of the constituent atoms in the DNA or indirectly by reactions with free radicals produced by interactions with water molecules (most notably the hydroxyl radical, which can induce DNA strand breakage or base damage), or by a combination of direct and indirect effects. In the cell, hydroxyl radicals will typically only diffuse a few nanometres (< 6 nm), thus preserving the spatial structure of the radiation tracks.

Ionizing radiation can thus induce a range of different types of molecular damage in DNA, such as base damage (including apurinic/apyrimidinic sites), strand breaks, DNA–protein cross-links, and combinations of these within a few base pairs of each other. Examples are double-strand breaks (DSBs) and non-DSB clusters (two or more base damages and/or strand breaks within about 10 base pairs, but not resulting in a DSB). The pattern and frequency of these lesions are determined by the clustering of ionization and excitation events on the nanometre scale, which ultimately produces clustering of damage over the dimensions of the DNA helix and larger.

Theoretical analyses show that clustered DNA damage that is more complex than a single-strand break can occur at biologically relevant frequencies with all types of ionizing radiation (Goodhead 1987, 1994; Brenner and Ward, 1992). Such clustered damage in DNA is produced mainly within a single track, with a probability that increases with increasing ionization density (LET). Calculations show that a dose of greater than 10 000 Gy is required for a second track to have a reasonable chance of contributing to the local complexity of DNA damage (Nikjoo and Goodhead, 1991). These more complex forms of damage are essentially unique to ionizing radiation and are not seen spontaneously or with other DNA-damaging agents.

The number of DSBs induced in DNA is approximately 20–40 per cell per gray for low-LET X-rays and γ-rays, and a similar number is observed for α-particles in standard assays. However, the percentage of complex DSBs (with extra strand breaks and/or associated base damage within 10 base pairs) is about 30–50% for electrons (similar to the percentage produced by X-rays and γ-rays) based on Monte Carlo calculations, and this percentage increases with increasing ionization density (LET) of the radiation, to about 90% for 0.3 MeV protons and about 96% for high-LET 2 MeV α-particles (Nikjoo et al., 1991; Goodhead, 2006). In addition to this increase in the frequency of complex DSBs with increasing LET, there is also an increase in the overall complexity of the damage spectrum produced. Clustering of damage is not confined to DNA but can occur in all biomolecules.

Complex non-DSB damage has been shown to be a significant component of the lesions induced by radiation, occurring 4–8 times as frequently as direct DSB formation. Whereas isolated lesions (e.g. base damage or single-strand breaks) are repaired quickly and generally with high fidelity, for non-DSB clusters the rate of repair is typically impaired by the presence of additional lesions within the cluster. The delay and the ultimate consequence depend on the types of lesion and their relative positions. The longer lifetime of these clusters also results in an increased probability that the damage will be present during DNA replication, which ultimately leads to stalled replication forks that may give rise to DSBs or mutations. Therefore, non-DSB clusters are potentially highly mutagenic and are likely to play a more important role at low doses of low-LET radiation; because non-DSB damage is produced at a higher frequency than DSBs at these lower doses, more cells will contain non-DSB clustered damage compared with DSBs (reviewed by Eccles et al., 2011).

DNA is wrapped around histone proteins to form nucleosomes, which are organized into 30 nm chromatin fibres that are typically arranged in loops. As a result of the sequence of ionization events along individual radiation tracks, especially in the case of densely ionizing high-LET particles such as α-particles, these tracks can lead to multiple correlated DSBs over short sections of DNA arranged in these structures. Conventional DSB assays (e.g. pulsed-field gel electrophoresis and γH2AX assays) are not able to resolve these additional DSBs and therefore typically underestimate the absolute yields (Friedland et al., 2008). However, experimental and theoretical data have demonstrated the existence of these short fragments for these particles, showing a significant deviation from a random distribution (Rydberg et al., 1998; Friedland et al., 2008). Whereas viable radiation-induced mutations are rarely associated with visible chromosomal exchanges observed by use of fluorescence in situ hybridization (FISH), molecular analysis of these sites shows that high-LET particles can induce gene mutations of greater complexity than simple deletions or point mutations,

consistent with the correlation of damage along the radiation track (Singleton et al., 2002).

The pattern of energy deposition is also important on the cellular or nuclear scale (over distances in the micrometre range). When an α-particle traverses a cell, the dose distribution of the energy deposited is highly heterogeneous across the cell, with a greater probability of correlated damage and DSBs within a single chromosome or adjacent chromosomes. Studies with multiplex FISH (mFISH) have shown that commonly four and up to a maximum of eight different chromosomes may be involved in rearrangements after the nuclear traversal of a human peripheral blood lymphocyte by an α-particle (Anderson et al., 2002, 2006); a similar response was seen in human CD34-positive haematopoietic stem cells (Anderson et al., 2007). This is in contrast to the production of mainly simple rearrangements between two chromosomes observed for low doses of low-LET X-rays. Complex rearrangements have been observed in radiation workers with a large body burden of α-particle-emitting plutonium (Anderson et al., 2005). Stable intrachromosomal rearrangements were also found in lymphocytes of former nuclear weapons workers who were exposed to plutonium (Hande et al., 2003), although not consistently for all cases of in vivo high-LET exposures (reviewed by Hada et al., 2011).

Other potential mechanisms for modifying cancer risk from radiation exposure

Ionizing radiation also produces a whole range of effects with potential implications for carcinogenesis (UNSCEAR, 2012). For example, the patterns of gene and protein expression are critical in determining cellular function and response. Ionizing radiation has been shown to modulate protein phosphorylation (Yang et al., 2006) and gene expression in a dose- and dose rate-dependent manner (Ding et al., 2005; Fachin et al., 2009). Epigenetic changes can also result in modifications in gene expression, and ionizing radiation produces DNA methylation (Kovalchuk et al., 2004), histone methylation (Pogribny et al., 2005), and chromatin modification (Kim et al., 2009; Luijsterburg et al., 2009; Nagarajan et al., 2009; Pandita and Richardson, 2009), along with modulation of microRNA expression (Templin et al., 2011).

Intercellular communication and the bystander effect

Within tissues of multicellular organisms, cells do not act in isolation; intercellular signalling is vital for maintaining the multicellular organization of the tissue and for normal functioning of the constituent cells (Park et al., 2003). These cellular interactions and the microenvironment are also important in influencing the growth and development of cancer cells.

Radiation can initiate stress-inducible signals, which can perturb this signalling and affect not only irradiated cells but also non-irradiated cells. Many studies have shown a wide range of responses in non-irradiated “bystander” cells, including induction of DNA damage, chromosomal aberrations, delayed genomic instability, mutations, oncogenic transformation, and cell killing (Morgan, 2003a, b).

Signalling has been demonstrated to occur via intercellular gap junctions and media-borne factors. Several signalling pathways have been implicated, and these typically result in the modulation of reactive oxygen species and reactive nitrogen species as a result of signalling through molecules such as nitric oxide, peroxidase, and the cytokine transforming growth factor beta (TGF-β) and other inflammatory markers (Burdak-Rothkamm et al., 2007; Han et al., 2007; Portess et al., 2007; Coates et al., 2008). Radiation is capable of perturbing intercellular signalling down to very low doses (on the order of 2 mGy for γ-rays and 0.3 mGy for α-particles), which are directly relevant to typical human exposures (Portess et al., 2007).

Reactive oxygen species are expected to be important in initiating and maintaining the inflammatory process (Barcellos-Hoff et al., 2005; Mantovani et al., 2008). In addition, radiation can lead to a modification in the immune response; at high whole-body doses, this results in immunosuppression, whereas at low doses and dose rates, this can lead to either suppression or stimulation of the immune response (UNSCEAR, 2008).

There is increasing evidence to suggest that radiation-induced perturbation of intercellular signalling and of the microenvironment may play a role in modulating cancer risk. However, the relative importance of these effects to cancer induction after human exposure is unclear, and it is not generally known whether the dominant consequences of these effects are beneficial or detrimental.

Radiation-induced genomic instability

In addition to being capable of producing mutations directly in the irradiated cell, ionizing radiation can also lead to genomic instability, resulting in the cell and its progeny having a

reduced ability to replicate the genotype faithfully and therefore showing a permanently increased rate of acquisition of alterations in the genome (Kadhim et al., 1992, 1994; Little, 2000; Morgan, 2003a, b; Barcellos-Hoff et al., 2005). This may lead to an increased probability that the cell and its progeny will undergo the various genetic and epigenetic changes necessary in multistage carcinogenesis. It is thus possible that the instability phenotype plays a major role in radiation-induced cancer, especially because genomic instability is a well-recognized feature in many tumours (Bielas et al., 2006).

Radiation-induced genomic instability typically becomes manifest several cell generations after irradiation and can be detected via a range of end-points, including chromosomal and chromatid aberrations, micronuclei, changes in ploidy, gene mutations and amplifications, and mini- and microsatellite instabilities. The frequency of genomic instability was observed to be too high to be explained by the induction of a mutator genotype. Several mechanisms have been proposed, including dysfunctional telomeres (Goytisolo et al., 2000; McIlrath et al., 2001; Williams et al., 2009) and inflammatory (free radical) responses (Barcellos-Hoff et al., 2005; Natarajan et al., 2007; Coates et al., 2008; Lorimore et al., 2008), along with DNA damage and response, for example long-term response to directly induced DNA damage and reduced ability to handle subsequent damage or cell division (Snyder and Morgan, 2005; Maxwell et al., 2008; Toyokuni et al., 2009).

Epigenetic modification has been implicated as playing an important role in the promotion and maintenance of transmissible instability (Kadhim et al., 2004; Barber et al., 2009; Filkowski et al., 2010; Rugo et al., 2011). Genomic instability has also been observed in non-irradiated cells that were in the neighbourhood of irradiated cells, demonstrating the importance of intercellular signalling in initiating this instability response (Lorimore et al., 1998). Although genomic instability is a plausible mechanism for cancer induction, its precise role, if any, remains to be proven.

The importance of dose distribution with respect to tumour sites

The passage of ionizing radiation through the body results in the deposition of energy within the irradiated tissue volume. External irradiation with photons is typically highly penetrating and will often result in all cells and tissues in the radiation field being irradiated. In contrast, emission from internalized radionuclides typically occurs from specified locations occupied by the emitting nuclide source. This will often lead to a non-uniform dose distribution in the body, especially if the emitted radiation has only a short range (e.g. for α-particles and β-particles).

The biological effects of deposited radionuclides in the body depend on the amount and activity of the radionuclide deposited, the type of radiation emitted, the physical half-life of the isotope, the mode of entry, the organs and tissues in which the radionuclide is retained, the duration of retention, and the rate of excretion from the body. The chemical characteristics of the radionuclide (or the compound in which it is incorporated) along with its physical properties (such as size and shape) determine its behaviour, including absorption and transport within the body, elimination route and rate, and uptake and retention in organs. In some cases, for example for radioactive heavy metals, the health effects and carcinogenic potential may also be related to, and potentially dominated by, the chemical properties rather than the radiation emitted.

In some cases, a radionuclide may spread throughout the whole body; in other cases, it will concentrate in specific organs or locations within the body. If the emitted radiation has a short range (e.g. for α-particles and β-particles), this can lead to significant heterogeneity in the resulting dose distribution, with certain organs receiving a significant dose while for others the radiation dose is minimal. Biokinetic models (ICRP, 1989, 1993, 1994, 1995a, b, c, 2001) are used to estimate the spatial and temporal uptake of radionuclides as well as their subsequent distribution and ultimate excretion. Dosimetry models (Eckerman, 1994) are then used to calculate the resulting dose distribution over the body and organs, based on the physical characteristics of the radionuclides.

The ability of internal radionuclides to produce a biological response and ultimately cancer in various organs is related to the biodistribution of these emitters within the body (which will depend on the chemical and physical properties of the particles and the route of entry). Examples are iodine-131, which concentrates largely in the thyroid, and strontium-90 and plutonium-239, which are deposited mainly in the bone. The same radionuclide may result in a different range of tumours if it is delivered in such a way as to produce a different biodistribution pattern. In addition, there may be

confounding factors, such as chemical toxicity, that may contribute to or even dominate the cancer response.

Human exposures to ionizing radiation typically occur at low dose and low dose rate

The effects of radiation are most notable at the high doses (above a few gray) that are usually associated with significant radiation accidents and radiotherapy treatments, and that are observed in atomic bomb survivors. These effects include erythema, oedema, ulceration, necrosis, fibrosis, telangiectasia, inflammation, immunosuppression (through bone marrow depletion), and pneumonitis (HPA, 2007; Stewart et al., 2012). Although there is clear evidence from epidemiological data for significant cancer risks associated with high-dose exposures, the existing data for the low-dose range are limited, such that below approximately 0.1 Gy – doses associated with typical human exposures – the data are not powerful enough to enable comment on the shape of the dose–response curve and the associated risks.

For an average annual environmental background exposure of approximately 0.001 Gy for low-LET radiation, individual cells may receive no track at all or only single tracks, well isolated in time. The nucleus of each cell in a tissue will experience on average one electron track per year from background radiation, assuming a spherical nucleus of 8 μm diameter. Exposure from diagnostic procedures can vary from 0.005 Gy for dental exposures to approximately 0.01 Gy for typical exposures from computed tomography (CT), or occasionally up to 0.1 Gy for some procedures over a short period (Brenner and Hall, 2007, 2012). Individuals are also exposed to high-LET α-particles as a result of naturally occurring radon gas. With typical residential levels of radon gas, the cell nuclei in the bronchial epithelium of the inhabitants are estimated to receive on average between 0.15 and 0.6 α-particle traversals per year (NRC, 1999). However, for those cell nuclei that are occasionally traversed, the dose to the traversed nucleus is significant (on the order of 0.1–0.5 Gy).

For the high doses associated with radiotherapy or significant accidental exposures, it is expected that classical direct effects of radiation are likely to dominate the response, as a result of radiation-induced DNA damage. However, at the very low doses associated with typical human exposures, where only a small fraction of cells have a DNA DSB, it is possible that other mechanisms for cancer induction or modulation of cancer incidence (such as radiation-induced genomic instability or effects associated with perturbation of intercellular signalling) may play a more important role.

Generality of response after exposure to different types of ionizing radiation

All types of ionizing radiation ultimately lead to clusters of ionization and excitation events, along with the production of electrons, through which energy is deposited. Interaction of X-rays and γ-rays with tissues generates fast electrons that interact with atoms or nuclei, producing additional electrons as they slow down and deposit energy. Charged particles such as α-particles and protons also interact with tissue, producing primary ionization and excitation events, and also a trail of secondary electrons along the path of the primary particle. Uncharged neutrons also interact with tissue and deposit energy via lower-energy charged particles such as protons, deuterons, α-particles, and heavy-ion recoils, ultimately leading to energy deposition via secondary electrons.

Therefore, energy deposition by way of electrons is common to all ionizing radiation. Indeed, isolated track ends of low-energy electrons (produced by all ionizing radiations) have been shown not only to be capable of affecting a wide range of genotoxic end-points but to do so with a high efficiency per unit dose (Goodhead and Nikjoo, 1990; Hill et al., 2001; Hill, 2004; HPA, 2007). Because of their increased local ionization density, these track ends of low-energy (0.1–5.0 keV) electrons have been proposed as the biologically critical component of low-LET radiation, rather than the isolated ionization and excitation events along the path of fast electrons (Goodhead and Nikjoo, 1990; Botchway et al., 1997).

In addition, α-particles emitted by radionuclides, irrespective of their source, produce the same pattern of secondary ionizations and the same pattern of localized damage to biological molecules, including DNA, and ultimately the same biological effects. Therefore, due to the communality in their interactions within the body and in the biological responses induced, all types of ionizing radiation have been classified by IARC as *carcinogenic to humans* (Group 1), even though in some cases direct evidence is weak or non-existent, with the risk of cancer depending on dose and radiation quality. Although internal radionuclides can vary significantly in the range of cancers and cancer sites observed, the cancer response is ultimately dominated by the biodistribution of these emitters within the body.

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CHAPTER 19.

Host susceptibility

Jane C. Caldwell, Ronald L. Melnick, and Lauren Zeise

Introduction

For several tumour sites (i.e. lung, lymphoid tissue, and digestive tract), concordance has often been observed among different species after exposure to a given IARC Group 1 human carcinogen (see Chapter 21, by Krewski et al.). When reported in epidemiological studies, these tumour sites are also noted in some or all of the animal species tested experimentally. There are several other tumour sites with fewer, or less common, tumour site concordances among the species studied.

Evaluation of concordance of cancer development in specific target organs between and within species is dependent on several factors. There are various limitations in epidemiological studies (e.g. statistical power, exposure assessment, follow-up, and misclassification), in experimental design of animal bioassays, in methods used to identify concordance, and in the degree to which the animal model captures the range of potential human response to the particular agent tested. An integral consideration for the development and use of these models is host susceptibility – the intrinsic and extrinsic factors that have an impact on variable response to carcinogens: genetic variation, health status, life stage, lifestyle, sex, and the impact of co-exposures. The microbiome can also play a critical part in host susceptibility.

This chapter focuses on examples in the *IARC Monographs* and in recent literature on how well animal models reflect the range of human susceptibility, how host susceptibility factors may modulate the impact of mechanistic events leading to tumour development between species and within species, and how host susceptibility factors may affect evaluations of tumour site concordance.

It can be difficult to parse out reasons for lack of tumour site concordance (i.e. lack of response or common responses between species). The factors alluded to above that are not strongly associated with host susceptibility include the following. Competing causes of mortality may prevent the development of late-developing tumours, or studies may lack statistical power to detect an increase in tumour incidence at sites with high background rates. Limitations in how the database on tumour site concordance was constructed may affect the types of responses observed (e.g. some studies may focus only on specific tumour outcomes or may not be designed to detect some types of tumours). Also, when the concordance database

was constructed, the identification of a site in animals required a significant response in multiple species or in both sexes even though one sex may be more susceptible at a particular site (i.e. the mammary gland in females). In addition, there may be mechanistic concordance between species in how an agent elicits effects (e.g. is able to induce genotoxicity or affect similar pathways), but host susceptibility factors may result in different site-specific neoplastic responses. Thus, host susceptibility may determine how and whether specific individual sites or target organ systems are influenced by mechanistic events associated with cancer induction.

Although host susceptibility factors have a modulating role in carcinogenesis and can affect underlying mechanistic events, they should not be confused with concepts such as modes of action and adverse outcome pathways. A mode of action is a well-defined and biologically plausible series of key events leading to an adverse effect (EPA, 2005a); an adverse outcome pathway is a construct that attempts to link an initiating event with an adverse outcome at a biological level of organization relevant to risk assessment (Ankley et al., 2010). Both the mode of action and the adverse outcome pathway concepts can have limitations for the determination of mechanistic concordance between species. For example, leukaemia induced by exposure to benzene appears to result from multiple mechanistic events, some of which are not well characterized and are difficult to quantitate or quantify, and they do not occur in an ordered sequence; these features limit the applicability of an approach based on mode of action to assess risk of leukaemia (McHale et al., 2012).

The mechanistic database assembled for IARC Group 1 carcinogens contains information on mechanistic characteristics, one or more of which are commonly exhibited, that can be used to identify and organize mechanistic information related to cancer induction. The database is organized in terms of whether an agent displays these key characteristics of carcinogens: (1) is electrophilic or can be metabolically activated to electrophiles, (2) is genotoxic, (3) alters DNA repair or causes genomic instability, (4) induces epigenetic alterations, (5) induces oxidative stress, (6) induces chronic inflammation, (7) is immunosuppressive, (8) modulates receptor-mediated effects, (9) causes immortalization, and/or (10) alters cell proliferation, cell death, or nutrient supply (see Chapter 10, by Smith; see also Smith et al., 2016).

These characteristics are not in themselves sufficient to explain all aspects of carcinogenesis (see Chapter 13, by Caldwell) but are indicative of multiple mechanisms and associated biological changes observed after exposure to carcinogenic agents. Similar to the limitations mentioned above for the database on tumour site concordance, the mechanistic database may present what was studied and reported, and reflect the depth to which an agent was treated in each review in the *IARC Monographs*. Identification and categorization of mechanistic data by use of these key characteristics cannot always predict tumour site concordance, because the information is at best collected in a way that provides only partial evidence on differences in host susceptibility.

Advances in the understanding of host susceptibility in tandem with the evolution of the knowledge on the mechanisms of carcinogenesis allow for greater understanding of both. The mechanistic data for all types of ionizing radiation (IARC, 2012f) are particularly informative with regard to mechanisms of genetic damage (mutation and epigenetic changes, and bystander effects; see Chapter 18, by Hill and Ullrich), as well as other host susceptibility factors from this large and rich database. The classic mutation theory of cancer no longer fully encompasses the mechanistic data for several carcinogens (e.g. benzene) that induce not only mutations but also a variety of epigenetic changes. With the present state of knowledge, the carcinogenic process cannot be confidently attributed to either a purely genetic or epigenetic process but probably involves both (see Chapter 12, by DeMarini).

Differences and similarities in apparent tumour types and targets between rodents and humans can result from a variety of factors that affect absorption, distribution, metabolism, and elimination (ADME) of the agent, as well as the wide range of inherent susceptibility elements associated with toxicodynamic factors. Differences in the expression of genes coding for enzymes that regulate these processes can contribute to differences in cancer susceptibility and tumour targets within a species, between species, and at various life stages when exposure occurs. In some instances, the apparent discordance between cancer outcomes in different species may be explained when these genetic factors are identified, when relevant animal models

are tested, and when more susceptible human populations are studied for carcinogenic effects.

Analogous transgenic, strain-specific, or species-specific animal models

Human and animal studies on cancer may be more effective in discerning tumour responses when those most at risk are studied, such as a susceptible subgroup within a human cohort and susceptible animal species and strains in a bioassay. Concordance of response between species may increase for a particular agent when mechanisms of carcinogenesis and subpopulations at risk are identified and more analogous transgenic or strain-specific animal models are examined. However, these are generally not known before an animal study is conducted. There may be gaps in the understanding of the impact of inherent variability on tumorigenesis, in the identification of susceptible human populations, and in the development of adequate animal models to detect a carcinogenic risk from an agent or exposure condition.

Inherent variability

Most animal studies used to identify a potential carcinogenic risk in humans are conducted in rodents under standard conditions (e.g. 2-year cancer bioassays) with one particular agent. However, humans are exposed throughout their lifetime to a mixture of agents (see below), and inherent biological variability among individuals is due to epigenetic and genetic variance (Zeise et al., 2013). The contribution of the inherited predisposition to diseases, such as cancer, has been an active area of research and has an impact on susceptibility analyses. Two proposed hypotheses for the inherited basis of complex genetic traits are that they result from "common disease–common variant" (i.e. many common alleles of small effect) or "common disease–multiple rare variants" (i.e. few rare alleles of large effect). Although genome-wide association study (GWAS) approaches have been based on the "common disease–common variant" hypothesis, they have not been successful in explaining genetic predisposition to disease (Zhang et al., 2011).

The understanding of the scope of human genetic variability now shows that although there is a great deal of similarity in the DNA sequences between individuals, rare gene mutations are abundant, are geographically localized across the world, are difficult to catalogue, and are possibly a consequence of the rapid spread and growth of the human population and weak purifying selection (Nelson et al., 2012). The breadth and scope of rare mutations have also been illustrated through studies of asthma that have attempted to discover the role of rare mutations in the "missing heritability" of the genetic contributions to disease. Although common variants at many loci have been associated with asthma, they do not account for overall genetic risk. A study of rare and low-frequency variants has reported ethnic specificity but was unable to account for the missing heritability of the disease (Igartua et al., 2015), which is relevant to that associated with cancer risk.

Whole-genome (i.e. exome) sequencing of common (i.e. minor allele frequency > 5%) and rare (i.e. minor allele frequency < 1%) alleles across 12 cytochrome P450 genes has identified many polymorphisms with pharmacogenetic effects, as well as 730 novel non-synonymous alleles with uncommon deleterious variations that, although individually rare, were present in 7.6–11.7% of the population studied (Gordon et al., 2014). Genetic variability in cell signalling and gene expression may be the result of the variants in regulatory regions of the genes, rather than being a consequence of variants in the genetic code that instructs how to build proteins, or in the regulatory code itself (see Chapter 13, by Caldwell).

Just as there is genetic variability in the human population that has an impact on host susceptibility to cancer, there is also variability within rodent strains and species. Genetic heterogeneity resulting from crossing different mouse strains has long been recognized as an issue of concern in the development of experimental mouse models, and has been used as an argument to create genetically inbred strains. Transgenic mice carrying exogenous DNA and gene-targeted knockout mice have both been used as models for studies on cancer and for identifying carcinogenic properties of chemicals (Lunardi et al., 2014).

Two examples illustrate the complexity and usefulness of studying cancer susceptibility with such models. Although they carried exactly the same mutation in K-*Ras*, mouse lung tumours that resulted from carcinogen-induced versus genetically engineered models appeared to develop along different mechanistic pathways (Westcott et al., 2015). Exposure of two strains of mice of different susceptibility (i.e. A/J and BALB/cBy) to the same treatment (3-methylcholanthrene, a polycyclic aromatic hydrocarbon found in tobacco smoke, and butylated hydroxytoluene) produced lung tumours

with different K-*Ras* mutations in codon 12, one resembling human tumours from smokers and the other resembling human tumours from non-smokers (Fritz et al., 2010). Thus, the same carcinogenic treatment given to different strains of mice produced tumours at the same site but with different K-*Ras* mutations. These examples highlight the utility of using different animal models to understand the mechanistic basis for tumour induction in diverse human populations, but they also demonstrate that studies in multiple animal models are needed.

Concerns have been raised about the sensitivity and design of accelerated cancer bioassays that use genetically modified mice. Design features (e.g. sample size, study duration, reproducibility, and genetic stability of the animals), pathway dependency of effects, and potentially different carcinogenic mechanisms render their utility for predicting human health risks uncertain, especially in terms of dose–response (Eastmond et al., 2013).

Although the use of isogenic mice to detect carcinogenicity of an agent should reduce the within-group variance and the number of animals required to detect a response, such mice fail to model the influence of genetic diversity. The genetically diverse inbred Collaborative Cross mouse strains and the heterogeneous Diversity Outbred mice derived from the same eight founder strains as the Collaborative Cross were developed to more accurately capture the impact of human variability on tumour responses (Threadgill et al., 2011; Churchill et al., 2012; French et al., 2015). Because they more accurately reflect human susceptibility, an order of magnitude greater sensitivity to chromosomal damage induced by benzene was observed in Diversity Outbred mice compared with the inbred B6C3F_1 mice (French et al., 2015). Other groups have used genetically diverse panels of inbred mice to better predict liver toxicity (Bradford et al., 2011) and kidney toxicity (Harrill et al., 2012).

Genetic polymorphisms

There are several examples of organic compounds where polymorphisms in metabolizing genes in the human population may cause an increased risk of cancer within certain subpopulations exposed to such agents (see Chapter 1, by Bond and Melnick). Inherited mutations in cancer-related genes (e.g. *TP53*, *BRCA1*, *APC*, and mismatch repair enzymes) have a low frequency in the population but can confer a high individual risk of cancer (Melnick, 2001). In such cases there may be more concordance of response between species when analogous transgenic or strain-specific animal models are also tested with that carcinogenic agent.

Transgenic mice that lack a functional epoxide hydrolase gene are more susceptible to the mutagenicity of butadiene, as are workers with low-activity epoxide hydrolase polymorphisms (see Chapter 1, by Bond and Melnick). Thus, a transgenic mouse model with a reduced ability to eliminate a mutagenic metabolite more closely simulates a susceptible human subpopulation. Polymorphisms in genes that encode enzymes involved in metabolism of aromatic amines (*N*-acetyltransferases: NAT1 and NAT2) have also been noted to have an impact on inter- and intraspecies differences in risk for cancer of the bladder; conflicting findings between studies are potentially a consequence of the interdependence of pathways to activate the parent compound and detoxify reactive metabolites at different rates in different tissues (IARC, 2012c).

Polymorphism in the human aldehyde dehydrogenase enzyme is related to risk of cancer from alcohol consumption in a complex way. Individuals who do not express the enzyme at all may have lower risk of cancer, because the acute effects they experience from alcohol intake (e.g. facial flushing and physical discomfort) cause them to abstain from alcohol consumption, whereas individuals with reduced expression of the enzyme would be able to drink alcohol and consequently would have a higher blood concentration of acetaldehyde (IARC, 2012d). The development of animal models to reflect this response is dependent on recognition of the role of these metabolic polymorphisms in forming or eliminating cancer-causing intermediates. Cancers related to alcohol consumption were first detected without consideration of enzyme polymorphisms. Later on, differential risks were associated with specific polymorphisms.

The main focus in pharmacogenetics has been on polymorphisms of genes encoding drug-metabolizing enzymes, based on the supposition that inter-individual differences in response were determined by such genetic differences and that the main genomic hazard was mutagenesis or physical damage to DNA (Szyf, 2007). However, human variability and susceptibility are influenced not only by genetic polymorphisms but also by differences in the epigenome and its regulatory features.

Time-dependent changes in global DNA methylation have been demonstrated in the same individuals in separate populations in widely

separated geographic locations, with familial clustering for both increased and decreased methylation (Bjornsson et al., 2008). The same study also showed considerable inter-individual variation with age, with differences in DNA methylation accruing over time among individuals who would be missed by studies that apply group averaging. Thus, a focus only on genetic polymorphisms does not consider the fact that epigenetic programming plays an equally important part in generating inter-individual differences in phenotype (Szyf, 2007), and that it should be taken into account in the analysis of such phenotypic diversity. Such inter-individual differences would also not be readily observed with conventional rodent models, for several reasons (see below).

Strain- and species-specific differences in ADME and susceptibility to biological agents

Most cancer bioassays have been conducted in rodents (see Chapter 21, by Krewski et al.). The genetic code has been described as conserved between humans and mice in terms of genome size, structure, and sequence composition, and although candidate regulatory sequences have been conserved and the chromatin landscape in cell lineages is relatively stable, there are interspecies differences in gene expression and regulation (see Chapter 13, by Caldwell), which may account for some apparent differences in susceptibility or specific tumour site concordance after exposure to a carcinogenic agent or condition. Other factors described for some agents in Volume 100 of the *IARC Monographs* focus primarily on ADME considerations and species-specific vulnerabilities to biological agents.

For example, with regard to induction of cancer of the bladder by aromatic amines, increased risks are consistently found in humans and in dogs exposed to, for example, 4-aminobiphenyl, benzidine, 4,4′-methylenebis(2-chloroaniline), and 2-naphthylamine. Several aromatic amines (e.g. *o*-toluidine and 2-naphthylamine) induce bladder tumours in rats (IARC, 2012c; see also Chapter 2, by Beland and Marques).

Multiple organ site carcinogenicity of aromatic amines in experimental animals is associated with metabolic activation of these agents to DNA-reactive intermediates via multiple pathways in target organs. For dogs, lack of *N*-acetylation of aromatic amines reduces elimination of the parent compound via a detoxification pathway (IARC, 2010), and their ability to store urine – as humans do – increases exposure to urinary metabolites that are hydrolysed in the bladder lumen epithelium to reactive electrophilic metabolites (IARC, 2012c). Indeed, infrequent voiding has been associated with increased DNA adduct formation in the bladder in dogs (Kadlubar et al., 1991). Thus, similarities between the metabolism of aromatic amines in dogs and metabolic polymorphisms in susceptible humans, and physiological similarities (i.e. the ability to store urine) between dogs and humans contribute to a stronger correspondence with respect to the target organ.

The mechanism of tumour induction by aromatic amines is similar between humans and rodents, but the target organ is not always the same; in rodents, there are multi-organ targets for exposure to these agents through similar effects on DNA from electrophilic metabolites. Cancer of the bladder is associated with exposure to 2-naphthylamine in humans, rats, dogs, hamsters, and monkeys, as well as with exposure to *o*-toluidine in humans and rats. In mice, however, tumours are seen in other tissues but not in the bladder. Exposure to benzidine is associated with cancer of the bladder in humans, but in rodents liver tumours, not bladder tumours, are observed. Conflicting findings between studies are potentially a consequence of the interdependence of pathways to activate the parent compound and detoxify reactive metabolites at different rates in different tissues (IARC, 2012c).

Human exposure to asbestos has resulted in lung cancer, pleural and peritoneal mesothelioma, and cancer of the larynx and ovary (IARC, 2012a). The targets of this carcinogen are associated with its distribution. After inhalation, fibres may penetrate into the interstitium and translocate to the pleura or peritoneum or more distant sites. Asbestos has been shown to accumulate in the ovary in women (IARC, 2012a). Bronchial carcinomas and pleural mesotheliomas have been observed in rats after exposure to asbestos fibres, with no consistent increases reported for tumours at other sites. However, the Working Group for Volume 100C of the *IARC Monographs* (IARC, 2012a) noted that in many studies complete histopathology was not done, so it was not possible to observe a similar tumour pattern associated with carcinogen distribution.

The complexity of developing an appropriate animal model that takes into account similar distribution factors is further illustrated by the example of cancer induced by asbestos.

After inhalation of asbestos or synthetic fibres, Syrian golden hamsters are more susceptible than rats to induction of malignant pleural mesothelioma. More rapid translocation of synthetic vitreous fibres to the pleural space of hamsters compared with that in rats has been proposed as the reason for interspecies differences in susceptibility (Gelzleichter et al., 1999). Rats and hamsters are equally susceptible after direct intrapleural or intraperitoneal injection of the fibres, which circumvents differences in distribution.

Because rodents do not smoke, it is difficult to develop rodent models that mimic human smoking patterns and exposure to mainstream tobacco (see Chapter 5, by Hecht and DeMarini). However, rodents and other species have been used to study some of the carcinogenic components in cigarette smoke (e.g. polycyclic aromatic hydrocarbons, nitrosamines, aromatic amines, benzene, and butadiene). Although studies of the individual components of cigarette smoke have demonstrated genotoxicity, the development of murine models that reflect the induction of analogous forms of human lung cancer from smoking involves not only ADME considerations but also strain susceptibility (see above).

Standard animal cancer bioassays (i.e. 2-year testing in rats and mice) are not used to study biological agents that are specific to humans. Biological agents have evolved to preferentially target specific host species, specific organs or cell types within those species, and cell types with a specific differentiation status. There are data on the development and use of transgenic models to study biological agents with critical mechanistic evidence (see Chapter 9, by Lambert and Banks). With the exception of lymphoproliferative disease associated with Epstein–Barr virus (EBV), the use of surrogate hosts has not proven useful for assessing the carcinogenicity of human tumour viruses, and for several of them (e.g. EBV, Kaposi sarcoma-associated herpesvirus, and human papillomavirus), there is no understanding of cancer etiology in the context of natural viral infection (IARC, 2012b). Thus, determinations of interspecies concordance are hampered by the species specificity of most human tumour viruses.

In addition, human susceptibility and the identification of tumour targets of virally induced cancers involve many factors. The type of tumour induced is not only associated with the age of the subject but also related to stages of latency of the viral agent and the presence of susceptibility cofactors (e.g. variants or subtypes of the virus, gene polymorphisms and the immune status of the host, and environmental co-exposures that may lead to viral reactivation) (IARC, 2012b).

For EBV, specific latency transcription programmes that arise at specific stages in the viral life-cycle have been associated with specific tumours, i.e. latency I with EBV-related Burkitt lymphoma (BL), latency II with Hodgkin lymphoma and T-cell non-Hodgkin lymphoma, and latency III in immunocompromised individuals with lymphoproliferative disorders. In addition, three subtypes of BL are associated with EBV (endemic, sporadic, and immunodeficiency-associated), two of which primarily involve children (i.e. endemic and sporadic BL) (IARC, 2012b). Thus, the complexity of identifying target sites and susceptibility factors for these agents in humans also renders the analysis of tumour site and mechanistic concordance problematic.

Life stage

The timing of exposure to an agent during one's lifetime can affect the specific type of tumour that may arise, as well as the degree of cancer risk from such an exposure. Life stage as a susceptibility factor has been recognized and included in guidelines used by regulatory agencies in assessing cancer hazards and risks (EPA, 2005a, b). Although puberty and its associated biological changes could lead to changes in cancer susceptibility, exposures during that critical period and in that age group are seldom the subject of epidemiological studies; historically, the focus on cancer has been as a disease associated with ageing after extended exposure duration, with prolonged latency periods before the cancers appear (EPA, 2005b).

Cancer studies in rodents are generally designed to last somewhat less than a lifetime (2 years), beginning in early adulthood, and to mimic mostly occupational exposure circumstances (Melnick et al., 2008). With the exception of biological agents, radiation, or household exposures (IARC, 2012b, d, f), many data in cancer epidemiology come from exposures that occur in the workplace or upon the use of certain pharmaceuticals. Thus, these studies may not reveal the potential of exposures during the sensitive early-life period to induce childhood tumours, nor do they detect tumours with long latency periods.

Although similarities between childhood and adult cancers have been noted, childhood cancers generally are embryonic cell tumours (i.e. leukaemias, tumours of the brain

and the central nervous system, lymphomas, bone cancers, soft-tissue sarcomas, kidney cancers, eye cancers, and adrenal gland cancers), whereas adults generally develop more carcinomas (i.e. cancers of the skin, prostate, breast, lung, and colorectum). In addition, some tumours appear to be unique to the young, for example tumours of the kidney (Wilms tumour) or eye (retinoblastoma) (EPA, 2005b). Thus, another aspect of tumour site concordance between species is the difference in tumour types that may be observed in children versus adults.

A full assessment of cancer risks from childhood exposure to chemicals in the environment has been impeded by the relative rarity of childhood cancers, the lack of studies of the late effects of childhood exposure with sufficiently long follow-up, and the lack of relevant animal testing guidelines and assays focused on early-life or perinatal exposures (EPA, 2005b). However, some human carcinogens listed in Volume 100 of the *IARC Monographs* have been specifically identified as associated with increased risk of childhood cancer (i.e. radiation and certain pharmaceutical agents used in chemotherapy), as well as cancers occurring later in life after exposure during childhood.

In animals, several agents induce a higher incidence of tumours occurring later in life after perinatal exposure, for example diethylnitrosamine, benzidine, polybrominated biphenyls, and dichlorodiphenyltrichloroethane (DDT). For vinyl chloride, there appears to be greater susceptibility of weanling animals to the formation of DNA adducts (EPA, 2005b).

Along with the potential for more tumour types occurring after early-life exposure, the strength of the response (i.e. potency) may also be increased. There are examples of IARC Group 1 carcinogens for which potency is greatly increased in the young. For example, vinyl chloride is an agent for which young rodents are more susceptible for the target site and cell types (i.e. rare liver angiosarcomas and more common hepatocellular carcinomas) that are also observed in humans (see Chapter 1, by Bond and Melnick). The literature on cancer induced by exposure of animals to vinyl chloride is extensive and includes transplacental and perinatal exposures (IARC, 2012c). Barton et al. (2005) estimated the increase in potency of vinyl chloride for liver angiosarcomas to be 30-fold and for hepatomas to be about 50-fold in female rats after early-life exposure compared with exposure as adults.

As noted above, exposure to benzidine is associated with bladder cancer in humans and liver tumours in mice (IARC, 2012c). The ratio of potency after early-life versus adult exposure in studies with repeat exposures of juvenile and adult animals to benzidine is about 100 for liver cancer induction in male mice (Barton et al., 2005). This example illustrates an increased susceptibility in the young but an apparent lack of site concordance between humans and mice.

For most of the IARC Group 1 human carcinogens, there are data indicating genotoxicity as defined by the toxicological end-point of DNA damage (see Chapter 12, by DeMarini, and Chapter 22, by Krewski et al.). DNA damage has been noted to potentially exhibit a greater effect after early-life versus later-life exposure; this increased susceptibility has been attributed to more frequent cell divisions during development, which may enhance fixation of mutations, and the absence of key DNA repair enzymes in some embryonic cells, such as brain cells. In addition, increased risk may result from lack of fully functional components of the immune system during development, different functional operation of hormonal systems during different life stages, and induction of developmental abnormalities that can result in a predisposition to carcinogenic effects later in life (e.g. diethylstilbestrol) (EPA, 2005b). However, several other factors may also increase susceptibility in the young. The developmental origins of health and disease (DOHaD) hypothesis posits that environmental exposures during development increase susceptibility to cancer in adulthood through epigenomic reprogramming (Walker and Ho, 2012).

In some cases, the newborn or young rodent may be a better model to assess human cancer risk for either children or adults. Components of diesel exhaust, an IARC Group 1 carcinogen (Benbrahim-Tallaa et al., 2012; IARC, 2013), appear to be metabolized in a similar fashion in rodents and humans at different stages of development. Nitroarenes (and, by extension, diesel exhaust) are activated to mutagens in humans and young rodents. Concordance of lung cancer risk is observed between young rodents and humans (see Chapter 5, by Hecht and DeMarini). Specifically, 1-nitropyrene (a component of diesel exhaust) is a compound that lacks evidence of carcinogenicity when exposure occurs in adult rodents, but it is carcinogenic in young adult or newborn rodents because of its more extensive metabolism to mutagens. Metabolism of 1-nitropyrene by adult humans resembles that of newborn

rodents. Accordingly, examination of bioassay data for exposures of adult rodents only would miss any similarity of cancer response between the two species.

In addition to the difficulty of developing adult rodent models that mimic human adult smoking patterns, the use of rodent models exposed in adulthood may not reflect susceptibility. Lung tumours can be induced in Swiss mice if exposure to mainstream cigarette smoke begins within 12 hours after birth, but not if exposures are delayed (Balansky et al., 2007; IARC, 2012d).

Diethylstilbestrol is an important example of a transplacental carcinogen where in utero exposure causes vaginal and uterine cancer in daughters but not in exposed mothers (IARC, 2012e; see Chapter 20, by Rice and Herceg). The effects of diethylstilbestrol on the developing reproductive tract of rodents are species- and strain-specific; neonatal exposure to diethylstilbestrol results in uterine adenocarcinomas in CD1 mice but not in C57BL/6 mice. Increased incidence of uterine tumours is seen in Eker rats (i.e. a strain that is tumour-prone because of a germline defect in the *Tsc2* tumour suppressor gene) but not in wild-type rats. CD1 mice exposed to diethylstilbestrol also exhibit permanent estrogen imprinting, morphological changes in the reproductive tract, and persistent expression of the *Ltf* (lactoferrin) and *c-Fos* genes (Cook et al., 2005).

In humans, exposure to inorganic arsenic compounds causes cancer of the lung, bladder, and skin, with limited evidence for cancer of the kidney, liver, and prostate (IARC, 2012a). Transplacental exposure to arsenic from oral intake by pregnant female mice induces lung bronchiolo-alveolar carcinomas and liver hepatocellular carcinomas in the offspring when they become adults; continuous exposure during adulthood was not required, and exposure only in adulthood did not induce these tumours (see Chapter 3, by Waalkes; IARC, 2004, 2012a). However, a recent study in male mice reported that low doses of arsenic in drinking-water given according to a scheme that more closely resembles human exposure (i.e. to parents before conception and throughout pregnancy and lactation, and to offspring after weaning and throughout adulthood) caused lung cancer, a response that has also been reported for in utero or early-life exposures in humans (Waalkes et al., 2014).

Bladder cancer has been induced in adult rats after chronic exposure to arsenic. The skin, kidney, and bladder have been reported as cancer targets in multiple rodent studies of inorganic arsenic upon co-exposure with other carcinogens, via drinking-water or transplacentally (IARC, 2004, 2012a). There is no identified rodent model for arsenic-induced cancer of the skin or lung after exposure by inhalation. Thus, arsenic is an example where carcinogenicity and tumour site concordance are dependent on experimental design, and especially on the impact of early-life exposures.

For some carcinogens, there may not be an appropriate animal model for human cancer risk from later-life exposures. The timing of exposure determines tumour patterns and is critical for tumour concordance relationships for estrogens. The Working Group for Volume 100A of the *IARC Monographs* (IARC, 2012e) cautioned that "estrogen products given with or without a progestogen have markedly different carcinogenic or anti-carcinogenic effects, and the same regimens may have markedly different effects in different organs and at different stages of women's lives."

To date, there are no mouse models for ovarian cancer that reflect the genetics and histology of human serous ovarian cancer, which is most often diagnosed in postmenopausal women (Smith et al., 2014). Tamoxifen has been given to these women to treat metastatic breast cancer, or to women who are at high risk of developing the disease. There is a concordant decrease in risk of breast cancer in such women and in female rodents treated chronically as adults. However, tamoxifen treatment also causes an increase in risk of endometrial cancer in postmenopausal women. In female mice and rats, perinatal exposure to tamoxifen is required to produce tumours of the reproductive tract (IARC, 2012e).

In children, several types of ionizing radiation show life stage-related differences in susceptibility that affect target sites and cancer risk later in life (see Chapter 18, by Hill and Ullrich; IARC, 2012f). Low-dose radiation at background levels has recently been reported to contribute to the risk of leukaemia and tumours of the central nervous system in children (Spycher et al., 2015). Children exposed to ionizing radiation from the atomic bombs in Japan and from the accident with the Chernobyl Nuclear Power Plant in Ukraine had an increased risk of thyroid cancer attributable to iodine-131 and its accumulation in the thyroid.

Ultraviolet radiation from tanning beds increases the risk of skin cancer, especially when exposure occurs at a younger age, i.e. an increased risk for malignant melanoma when first exposure occurs before

age 30 years, and for squamous cell carcinoma when first exposure occurs before age 20 years (IARC, 2012f). In mice exposed to this type of radiation, squamous cell carcinoma is regularly observed, but no malignant melanoma has been reported. However, transgenic mice that spontaneously develop malignant melanomas or that have melanocyte hyperplasia can develop early-onset malignant melanoma if exposure to ultraviolet radiation occurs neonatally, but not after the age of 6 weeks (IARC, 2012f). This example illustrates the complexity of developing an animal model that mimics human susceptibility. Target site susceptibility as well as age at which exposure occurs must be taken into account when evaluating tumour site concordance between species.

Influence of study design on determination of site concordance

Host susceptibility as well as the type of information collected in either human or animal studies influence the degree of tumour site concordance that can be identified and evaluated. Epidemiological research is often done in men, especially for occupational exposures. This can limit or preclude the detection of female-specific cancers in humans and thus site concordance between species.

The Working Group for Volume 100F of the *IARC Monographs* (IARC, 2012c) specifically noted that many plausible tumour sites identified in rodents have not been reported in humans, and gave the example of rats treated with aromatic amines that developed tumours in the mammary gland, an organ that has not been studied adequately as a potential target site in humans for cancer induced by aromatic amines. Epidemiological studies of aromatic amines have not considered breast cancer, because industrial cohorts were generally small and the relevant workforce did not include women. The lack of studies involving female subjects not only affects species concordance but also influences the weight of evidence of an effect when data on both sexes are required to identify a target site in experimental animals. For example, in the construction of the animal database to assess tumour site concordance (see Annex 1, by Grosse et al.), the same neoplastic effect is required in two animal species or in both sexes of one species. Breast cancer is rare in male rats as well as in men. Thus, limitations in epidemiological studies and sex differences in cancer response can also account for lack of tumour site concordance between humans and experimental animals.

Inaccurate diagnoses of disease or incorrect entries on death certificates can affect concordance determinations, especially for myeloproliferative and lymphoproliferative disorders, which can be described as extranodal or predominantly nodal, precursor or mature neoplasms, and which may have multiple cellular phenotypes. Changing codes in the International Classification of Diseases (ICD) can make it difficult to develop a conclusion from human studies. However, a multipotent haematopoietic stem cell is the precursor of myeloid or lymphoid progenitors that further give rise to several cell types (Greaves, 2004). Although the disease induced by an agent may be considered a "lymphoma or leukaemia", the common progenitors overlap in haematopoietic cancers and complicate determinations of "target organ or target cell".

For studies in humans, changes in classification schemes for haematopoietic cancers can present difficulties in target organ identification from different studies. Modern classifications of leukaemia and other lymphatic and haematopoietic malignancies are based on cytogenetic and molecular principles (Swerdlow et al., 2008) that do not always coincide with those of the ICD (IARC, 2012f). Although there may be concordance of haematopoietic cancers between or within species, the manifestation of disease may differ. Thus, the determination of tumour site concordance can be dependent on the definition and level of specificity of the target.

The highest likelihood of identifying a human cancer risk may come from the study of sensitive subgroups with increased susceptibility to an agent or groups of agents. If multiple disease categories are lumped together and sensitive subpopulations are not distinguished, it may be difficult to detect a subtle but real cancer response in epidemiological studies. For example, taking into account the influence of genetic polymorphisms or the heterogeneity of tumour phenotypes will improve the ability to determine the risk to specific subpopulations for colon cancer after excessive alcohol consumption (Schernhammer et al., 2010).

Similarly, designing animal studies in such a way that rare tumours can be detected may increase the ability to determine a response and establish site concordance or mechanistic concordance between species. In epidemiology, rare tumours are considered a special type of finding, and they constitute a data set

that is different from the tumours that occur more commonly. For example, asbestos-induced mesothelioma is a rare tumour associated with exposure to a specific agent. In addition to the role of organ distribution of asbestos fibres in the determination of tumour site concordance, asbestos carcinogenicity also provides an example of the importance of tumour rarity for the determination of a response after exposure, either in humans or in animal bioassays: untreated controls from lifetime studies of asbestos exposure in five strains of rats and Syrian hamsters showed zero incidence of mesothelioma in 1175 rats and 253 hamsters (IARC, 2012a).

Many reports of animal bioassay data only highlight statistical significance to identify a positive tumour finding. Because of the relatively small number of animals involved in rodent bioassays, these studies may lack statistical power to identify rare tumours induced by a specific agent. As noted above, the use of a genetically heterogeneous strain of mice increased the ability to determine a genotoxic response to benzene (French et al., 2015).

Use of multiple strains of rats and mice in chronic studies of trichloroethylene enhanced the likelihood of observing increases in the incidence of rare kidney tumours and improved the probability of showing concordance with the finding of increased risk of kidney cancer through epidemiology. The epidemiological database for the current Scientific Publication also includes a study showing lower risk of kidney cancer among individuals with genetic polymorphisms that reduced their ability to produce mutagenic metabolites from trichloroethylene. Thus, tumour site concordance was more easily observed when rare tumours were detected in multiple strains of rats and mice, and when genetic polymorphisms were taken into account in human studies (Guha et al., 2012; Chiu et al., 2013; IARC, 2014).

In some cases, tumour site concordance between humans and experimental animals may be more evident when studies use rodent strains in which there is a lower background rate of more common tumours as well (e.g. the use of mice with lower body weight and decreased background tumour rates; see the discussion below), but with enough sensitivity to detect a response.

As illustrated by the example of exposure to ultraviolet radiation, a specific cell type (i.e. the melanocyte) at the origin of skin cancer in humans may not lead to skin cancer in wild-type mice (IARC, 2012f). Different cell types within a target organ may have different mechanisms of tumour development, susceptibilities, and cancer phenotypes that depend on the life stage at which exposure occurs. The determination of tumour site concordance between species can depend on the degree of specificity of the target description (i.e. cellular vs organ) in addition to cancer phenotype.

The risk of liver cancer from cigarette smoking illustrates how timing of exposure and interspecies differences in susceptibility are related to specific phenotypes of hepatocellular tumours. There is an increased risk of hepatocellular carcinoma in adult humans who smoke cigarettes. However, the strongest risk of hepatoblastoma (an embryonal hepatocellular tumour) is associated with paternal smoking before conception and a median age at diagnosis of 12 months; the timing of exposure for the cancer response is consistent with the identification of cigarette smoking as a germ cell mutagen in humans (IARC, 2012d).

Like in humans, hepatocellular adenomas and carcinomas occur in aged rodents, and background occurrence rates depend on species, strain, and sex. However, hepatoblastoma is extremely rare in rodents and, unlike in humans, this tumour usually occurs in aged rather than in young animals (Turusov et al., 2002). Therefore, at the organ level the liver is a similar target for cancer induction from exposure to cigarette smoke for adults and children. However, interspecies tumour site concordance is more difficult to demonstrate if cellular phenotype, life stage susceptibility, and age and timing of exposure are not taken into account.

Lifestyle, disease status, and co-exposures

Cancer susceptibility involves not only genetic predisposition but also the myriad of exposures experienced over a lifetime, at home and at work, and the various other microenvironments in which voluntary and involuntary choices affect cancer risk. Genetic and environmental interactions involving complex pathways, multiple genes, and multiple exposures have been suggested to provide an explanation for the inability of GWAS approaches to account for the missing heritability of most complex diseases, and for the failure of analyses of rare mutations to account for asthma (Schadt and Björkegren, 2012).

The “exposome” concept encompasses the totality of exposures from conception onwards, complementing the genome, instead of focusing on single exposure–health effect relationships (Vrijheid et al.,

2014). The exposome includes three broad domains of non-genetic exposures: the internal environment (e.g. endogenous hormones, the gut microflora, and ageing), specific external exposures (e.g. chemical contaminants, lifestyle factors such as tobacco use, and occupation), and the general external domain (which includes influences such as stress, the urban–rural environment, and climate) (Wild, 2012).

Tumour site concordance can be affected by the inherent nature of the conditions under which each species is studied to assess cancer risk. Human study subjects have a wide and varied range of co-exposures, whereas studies in experimental animals involve relatively uniform exposures in highly controlled environments. As noted above, changes in expression levels of metabolizing enzymes through genetic polymorphisms have been a focus of research, but metabolism is also affected by environmental co-exposures, which are less well studied. Also, many solvents have similar exposure targets, and in humans these exposures often occur together with co-exposures that have the potential to increase the effects of solvents (Caldwell et al., 2008). However, studies of solvents in general may mask effects of specific agents, for example trichloroethylene (Vermeulen et al., 2012). Lifestyle and co-exposures (e.g. obesity, alcoholism, nutritional status, a compromised immune system, and viral infections) can affect environmental cancer risk, but they are often not considered in animal models of carcinogenicity, nor are they typically addressed in human studies.

In humans, lifestyle choices and previous exposure during development that may change set points in genetic control and cell signalling can affect cancer susceptibility. In addition, exposures to preceding generations have been identified as affecting susceptibility to cancer. In experimental studies, transgenerational endocrine effects have been identified in the third generation of mice after the exposure to diethylstilbestrol (Ziv-Gal et al., 2015).

Obesogens have not been evaluated for carcinogenicity by the *IARC Monographs*. However, prenatal exposure to obesogens that activate the constitutive androstane receptor in neonates may affect susceptibility by causing permanent changes in enzyme expression and metabolism of environmental agents encountered as adults (Caldwell, 2012). Consequently, obesity associated with prenatal environmental exposures may render the subject more susceptible to cancer later in life. Increased background levels of all cancers have been observed in conjunction with increased body weight and obesity in rodent bioassays (Rao et al., 1987; Leakey et al., 2003).

Such changes in background tumour incidence and altered susceptibility will affect the detection of site concordance, especially across data sets that span many decades of research. Site concordance may be more difficult to detect, because exposure-induced and background tumours are harder to distinguish in small groups of animals. Site concordance may be detected more frequently between animal models and humans when obesity status is taken into account. Such is the case with liver cancer (Caldwell, 2012).

The proportion of the population that is overweight or obese has increased substantially over the past few decades. Type 2 diabetes, non-alcoholic fatty liver disease, cardiovascular disease, and increased body mass index are risk factors for liver cancer, and diabetes induces synergistic actions with other variables, such as viral hepatitis and alcohol consumption (Fan et al., 2009).

Immune system status can affect human responses to carcinogen exposures, and thus influence the ability to determine site concordance between species (e.g. lack of concordance in responses because animal models are used that do not also take immunosuppression into account). With the increasing survival of patients with the acquired immune deficiency syndrome (AIDS), associated cancers in West Africa have been reported to be Kaposi sarcoma, non-Hodgkin lymphoma, cervical cancer, anogenital cancer, and liver cancer (Tanon et al., 2012). Infection with human immunodeficiency virus (HIV) or immunosuppression causes a higher risk for lymphomas, i.e. a 400-fold increase in risk of non-Hodgkin lymphoma in the presence of HIV infection (Bassig et al., 2012). However, the increases in the incidence of non-Hodgkin lymphoma can only be partially explained by the HIV epidemic (Bassig et al., 2012).

An example of a common co-exposure that affects cancer risk is that of aflatoxin B_1 contamination of food supplies, which tends to be highest in areas with high prevalence rates of infection with hepatitis B and C viruses. While aflatoxin B_1 and particularly its epoxide metabolite are potent mutagens by themselves, infection with hepatitis B virus greatly amplifies the risk of liver cancer from aflatoxin exposure (IARC, 2012b), i.e. from 4-fold with aflatoxin alone to 60-fold in the

presence of infection with hepatitis B virus (Wu-Williams et al., 1992; Yu and Yuan, 2004). As discussed previously, aflatoxin metabolism and the attendant risk are also affected by polymorphisms of detoxification or activation pathways (IARC, 2012c).

Aflatoxin is one of several agents for which carcinogenicity in experimental animals was established or highly suspected before epidemiological studies confirmed its carcinogenicity in humans (IARC, 2012d). Aflatoxin B_1 is a liver carcinogen in humans, rats, tree shrews, trout, and several types of transgenic mice, but not in wild-type mice (IARC, 2012c). The resistance of adult mice to aflatoxin carcinogenesis has been suggested to result from constitutive hepatic expression of an α-class glutathione-*S*-transferase, mGSTA3-3, a detoxifying enzyme with a high affinity for aflatoxin B_1 8,9-epoxide (IARC, 2012c). However, aflatoxin is a liver carcinogen in newborn mice (Vesselinovitch et al., 1972). Therefore, risk of aflatoxin-induced liver cancer serves as an example not only of the effects of co-exposure but also of the influence of genetic polymorphism and age as susceptibility factors.

Finally, using the framework of the hallmarks of cancer (Hanahan and Weinberg, 2011), a task force of 174 scientists from 28 countries who participated in the Halifax Project ("Getting to know cancer") published a series of reviews that evaluated exposures to mixtures in the environment that may have the potential to contribute to cancer risk (Harris, 2015). Cumulative effects of individual chemicals that had not been identified as carcinogens were reviewed for actions on key pathways and mechanisms related to carcinogenesis and were reported to plausibly produce carcinogenic synergies (Goodson et al., 2015). The modification of human responses to carcinogens from co-exposures would not be reflected in current animal cancer bioassays of individual agents, and would thus affect the demonstration of tumour site concordance.

Microbiome effects

Included in the exposome concept is a more recently described component of gene–environment interactions that influence cancer susceptibility in humans and experimental animals: the contribution to cancer risk of the microbiota living on and in humans. These microbiota include 100 trillion (10^{14}) microbial cells, outnumbering human somatic and germ cells combined by 10-fold (Bultman, 2014), and a quadrillion (10^{15}) viruses that interact with one another and with the host immune system in ways that influence disease outcome. As humans age and develop, so do their microbiota. These microbiota and the genes they encode are collectively known as the microbiome (Clemente et al., 2012). The microbiome differs across species and individuals, and its effects on tumour site concordance have yet to be determined. However, its potential effect on human susceptibility to many chronic diseases, as well as cancer, is an emerging subject of research.

The composition of the microbiome varies across anatomical sites; the gut microbiome is highly enriched in genes involved in carbohydrate metabolism, in contrast to the relatively few genes in the human genome that encode carbohydrate-metabolizing enzymes (Bultman, 2014). The microbiome not only alters metabolic pathways in the human gut but is also linked to host susceptibility to metabolic diseases (Suez et al., 2014) and other multifactorial diseases. Microbial imbalance (dysbiosis) usually involves shifts in the relative abundance of commensal microbes. Inter-individual differences in arsenic-induced disease are associated with differences in arsenic metabolism; disturbances of the gut microbiome phenotype have also been reported to affect the biotransformation of arsenic (Lu et al., 2014).

Shifts in the microbiome have also been associated with several types of cancer, and two dominant phyla normally associated with healthy individuals (i.e. the gram-negative Bacteroidetes and the gram-positive Firmicutes) were underrepresented in colorectal tumour tissue compared with adjacent normal colonic tissue from the same individuals (Bultman, 2014).

Modulation of microbiota in mouse models of cancer has demonstrated that cancer susceptibility and progression are affected by concurrent changes in inflammation, the genomic stability of the host cell, and the production of metabolites that function as histone deacetylase inhibitors to epigenetically regulate host gene expression. Specific diets associated with changes in cancer susceptibility (e.g. increased consumption of red meat and higher intake of dietary fibres) have also been associated with corresponding changes in the microbiome (Bultman, 2014).

Altering the composition of the microbiota in transgenic mice prone to colorectal cancer led to a lower cancer incidence as a result of reduced provision of carbohydrate-derived metabolites that fuel hyperproliferation of colon epithelial cells, without changes in inflammation or DNA damage induction (Belcheva et al., 2014). Microbiota have also been implicated in the activation of the innate

immune response against tumours (Iida et al., 2013; Viaud et al., 2013). The interplay between human carcinogenic pathogens and the microbiome as well as the linkage between dysbiosis and carcinogenesis have recently been reviewed (Dzutsev et al., 2015).

Conclusions

This chapter discusses the importance of considering host susceptibility factors and their modulation of tumour response in interpreting findings of tumour site concordance between species, or lack thereof. Examples are given of how discordance can result from lack of studies covering sensitive sexes, subgroups, or life stages. Examples are also provided in which polymorphisms in metabolizing genes were associated with sensitive subpopulations, and where experimentally sensitive rodent strains were studied that also had sensitivity because of similar capacity for increased activation or reduced detoxification (e.g. in the case of butadiene, aromatic amines, and alcohol consumption). For aromatic amines, anatomical and physiological similarity (infrequent voiding of the bladder) between humans and dogs increases DNA adduct formation and ultimately tumour development at the same site, i.e. the bladder. More challenging in study design is to account for lifestyle, with its attendant co-exposures to exogenous chemicals and its influences on the microbiome.

The analyses of tumour site concordance are dependent on the types of information and databases available at present. Such analyses are limited by the underlying available studies, which may not provide adequate coverage of host susceptibilities. Animal models that cannot reflect the intrinsic and extrinsic factors that have an impact on biological variability in humans may not have adequate sensitivity to detect all targets of carcinogenicity occurring in humans. Similarly, limitations in epidemiological studies affect their ability to detect many tumour responses observed in animals.

Transgenic animal models as well as highly diverse outbred mouse strains and panels of diverse inbred strains have been developed as an approach to model the genetically highly diverse human species. Diversity Outbred mouse models may be used for future bioassays to obtain a better direct estimate of genetic contribution to variance, and these assays may detect potential human tumour sites missed by studies in genetically homogeneous strains. These models may also provide a platform to study other susceptibility factors, such as co-exposures or obesity. However, the use of such mouse models will involve greater expense; heritability estimates suggest that sample sizes should be increased by a factor of 3 to obtain the same precision as with isogenic mice (French et al., 2015). Other issues to be considered for Diversity Outbred models would be the percentage survival, the tumour rates in the controls, and the limited historical database that is used in the interpretation of data from current animal models. When these models are applied to address these issues, they may prove to be an invaluable resource for determining the impact of host susceptibility and of the intrinsic and extrinsic factors on variable responses to carcinogens.

Acknowledgements

The authors wish to thank D.M. DeMarini, M. Marty, S. Vulimiri, V.J. Cogliano, and N. Keshava for their helpful comments on this manuscript.

Disclaimer

This article was reviewed and approved for publication by the National Center for Environmental Assessment, United States Environmental Protection Agency. Approval does not signify that the contents reflect the views of the agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use. The views expressed are those of the authors and do not necessarily reflect those of the California Office of Environmental Health Hazard Assessment.

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CHAPTER 20

Age and susceptibility

Jerry M. Rice and Zdenko Herceg

Introduction

There is abundant experimental evidence from studies in animals, especially rats and mice, that susceptibility to certain chemical carcinogens is higher, and sometimes much higher, during prenatal and early postnatal life than in adulthood. There is also an extensive epidemiological literature on the differential effects of a wide variety of carcinogens in humans at different stages of life, including various forms of radiation, carcinogenic infectious agents, and chemicals and chemical mixtures. This chapter summarizes the literature that documents this high susceptibility of the fetus, infant, and child to many potentially carcinogenic exposures.

Studies in experimental animals

Most experimental studies of carcinogenesis during prenatal life and infancy have been conducted with chemical agents. Experimental evidence for susceptibility in utero and during infancy to chemical carcinogens and, to a lesser extent, to various sources of ionizing radiation has been summarized in reviews and symposium proceedings (Tomatis and Mohr, 1973; Rice, 1979; Napalkov et al., 1989; Rice, 2004).

At least in experimental animals, greater susceptibility to chemical carcinogens in utero and during early postnatal life is usually manifested as a higher incidence of the same kinds of tumours that occur in exposed adults, with a shorter latency period from the time of exposure to the carcinogen until the appearance of the tumour. In bioassays for carcinogenicity in adult rodents, the incidence and multiplicity of tumours increase and the latency period decreases with increasing dose. Thus, the predominant results of early-life exposure are what would be expected from a higher effective dose to the fetus or infant than that experienced by the mother.

However, in some cases the tumours that result from prenatal or perinatal exposures are different from those that occur in exposed adults. Tumours induced prenatally become manifest only during adult life in rats and mice, except in certain genetically modified strains, because in these species the interval between birth and sexual maturity is only a few weeks. Therefore, the types of tumours that occur during childhood in humans, including various embryonal solid tumours, are observed as tumours of adult life in conventional rodents. An example is the development of nephroblastomas – embryonal kidney tumours that correspond to Wilms tumour in humans – in the adult rat after perinatal exposure to a chemical carcinogen. Such tumours do not develop in rats exposed to the same carcinogen during adult life (Diwan and Rice, 1995).

Chemical carcinogens that reach the fetus via the maternal circulation must have crossed the placenta, and consequently are generally referred to as transplacental carcinogens. All but a few known transplacental carcinogens are organic compounds that act principally or entirely by a genotoxic mode of action. Factors that contribute to fetal susceptibility to these agents include maternal, placental, and fetal metabolism, the immature state of fetal DNA repair capability, the high rate of cell division during prenatal development, and the rapidly changing patterns of gene expression in fetal target tissues, which may render the genetic material of fetal cells highly accessible to carcinogens.

Different organs and tissues are not equally susceptible to transplacental carcinogens: in experimental animals, some fetal organs, notably the developing nervous system, are exceptionally susceptible to a wide range of agents. Although differences between mother and fetus in absorption and distribution of a carcinogen may well exist and may contribute to greater apparent effects of an administered dose in the offspring, these differences are likely to be less important than certain other physiological differences between mother and fetus, including differences in metabolic competence and in DNA repair capacity.

Most chemicals that have a genotoxic mode of action must be biotransformed to chemically reactive metabolites to initiate carcinogenicity. The requisite enzymes are often expressed in fetal tissues only late in gestation, and then at low levels of activity; thus, only small amounts of reactive metabolites are generated in fetal tissues. Metabolites of maternal or placental origin may contribute to carcinogenic effects in utero, but when the reactive metabolites formed in maternal tissues are too unstable to circulate in the maternal bloodstream, cross the placenta, and reach the fetus, a carcinogenic chemical may have no transplacental carcinogenic activity or may only cause a low incidence of tumours near the end of gestation, in offspring that were exposed transplacentally. This pattern can be seen in the transplacental carcinogenicity of single doses of *N*-nitrosodimethylamine (NDMA) in rats (Alexandrov, 1968). In both the fetus and the pregnant female rat, the target organ for single doses of NDMA is the kidney, but a much lower incidence of tumours is observed in the offspring. For many other compounds whose reactive metabolites are longer-lived in vivo, the maternal contribution of reactive metabolites to fetal tissue burden may be substantial, and the resulting susceptibility of the fetus may be greater than that of the mother.

Short-chain alkylnitrosourea compounds are chemically highly reactive and are extremely potent, direct-acting transplacental carcinogens. In rats, a single exposure to one of these short-lived agents can cause a high incidence and multiplicity of tumours of the nervous system in the offspring of females treated during the second half of gestation (Ivankovic and Druckrey, 1968). These substances are direct-acting, and the simplest members of this chemical class, especially *N*-ethyl-*N*-nitrosourea (ENU), have been used to probe changing susceptibility to carcinogenesis during prenatal life due to factors other than carcinogen metabolism. Tumours are induced in offspring exposed once transplacentally to ENU, beginning at approximately 12 days of gestation in the rat, when organogenesis starts. Tumour multiplicity rises to a maximum in offspring exposed at approximately 21 days of gestation, a few days before birth. The susceptibility of the fetus relative to that of adult rats is measured as the incidence and multiplicity of tumours that develop in offspring after birth, compared with the incidence and multiplicity of the same types of tumours in their directly exposed mothers. By that measure, the susceptibility of the rat fetus to induction of brain tumours by ENU during the final week of gestation is approximately 50 times that of the mother.

Transplacental carcinogenesis studies with ENU in non-human primates, although far less extensive than studies in rats, also indicate that the susceptibility of the fetus is greater than that of the mother. Tumours have been induced in the offspring of rhesus and patas monkeys exposed to ENU during the first trimester of pregnancy (Rice et al., 1989).

Ionizing radiation, both from external sources and from internalized radionuclides, is capable of producing mutations, mainly by large-scale gene deletions, as well as gross chromosomal damage, and thus is similar in its effects to direct-acting genotoxic chemical carcinogens, because there is no metabolic biotransformation of the agent (IARC, 2012e). There are many examples of carcinogenesis by different forms of ionizing radiation, in experimental animals and in humans and at all ages, from prenatal life to adulthood. Solar and ultraviolet radiation also are carcinogens that directly damage DNA, but they are less penetrating than the more highly energetic forms of ionizing radiation (e.g. X-rays), and thus exert their carcinogenic effects primarily on the skin,

causing distinctive mutations in DNA (Agar et al., 2004; IARC, 2012e).

Results from experiments with genetically engineered transgenic and knockout mice, especially double knockouts, provide information about the significance of individual genes and gene combinations in susceptibility to and pathogenesis of specific tumours, including embryonal neurogenic tumours of childhood such as medulloblastoma (reviewed in Rice, 2004), and offer some insight into why embryonal tumours appear relatively later in life in mice than in humans.

For example, the gene *PTCH1*, the human homologue of the *Drosophila* segment polarity gene *patched*, is a tumour suppressor gene associated with nevoid basal cell carcinoma syndrome. Patients with this syndrome are predisposed to develop primitive neuroectodermal tumours of the central nervous system, including medulloblastomas, and mutations in *PTCH1* have been identified in a subset of sporadic primitive neuroectodermal tumours. Genetically engineered knockout mice with only a single normal allele of *Ptc1*, the mouse homologue of *PTCH1*, develop medulloblastoma-like cerebellar tumours (7–14% incidence). Neonatal exposure of these *Ptc1*$^{+/-}$ mice to 3 Gy X-radiation increased this incidence to 50%, but irradiation in adulthood had no effect on medulloblastoma incidence (Pazzaglia et al., 2002).

Dramatically, 95% of *Ptc1*$^{+/-}$ mice that had also been genetically engineered to remove both alleles of the tumour suppressor gene *p53* developed medulloblastomas, and did so very early in life, at younger than 12 weeks (Wetmore et al., 2001). This combination of inactivating gene mutations is not seen in conventional mice exposed to transplacental carcinogens, presumably because the probability of such a combination of events without concomitant lethal genetic damage is immeasurably low.

Although the importance of specific genetic events, including mutations and chromosomal alterations, in the genesis of cancers is clear, evidence is accumulating that many carcinogens also cause intracellular changes that may contribute to the carcinogenic process but do not involve carcinogen-induced alterations in genetic sequences. These changes, which may occur several cell generations after exposure to the carcinogen, are termed epigenetic and can be caused by ionizing radiation, chemicals, and ultraviolet light. They include genomic instability, a reduced ability to replicate the genotype faithfully (Barcellos-Hoff, 2005), and various other effects (IARC, 2012e). It is not yet clear how epigenetic events in carcinogenesis may vary with age at time of exposure to the carcinogen.

Epidemiological findings in humans

The consequences of environmental exposures to chemicals and radiation during childhood for the risk of cancer later in life have been reviewed (Carpenter and Bushkin-Bedient, 2013). In patients who receive anticancer therapies, the exposures are much more intense, and consequently the risk of cancer is higher.

Anticancer therapy

Non-surgical therapy for cancer in childhood and adolescence – by ionizing radiation, combination chemotherapy, or both – has become increasingly effective and in many cases is curative, but it imposes a long-term risk of second cancers in survivors. The most intense exposure of children to ionizing radiation and to genotoxic chemicals most commonly occurs in the context of anticancer therapy. Carcinogenic effects resulting from early-life exposures are most clearly seen among the long-term survivors of childhood cancers who were successfully treated with high doses of radiation and/or chemotherapy. The examples given here and in the next section are representative rather than comprehensive.

The risk of acute myeloid leukaemia, non-Hodgkin lymphoma, and solid cancers of the breast, thyroid, bone, central nervous system, colorectum, and stomach increased significantly in survivors of Hodgkin lymphoma diagnosed before age 16 years and successfully treated with radiation, chemotherapy with alkylating agents, or both. Breast cancer occurred only in women who had received X-radiation alone or chemotherapy and X-radiation combined to treat Hodgkin lymphoma. Breast cancers developed usually within the radiation field, and the risk of breast cancer was 75 times as great as that in the general population. Second cancers occurred at increased rates in patients originally treated with chemotherapy alone, X-radiation alone, or chemotherapy and X-radiation combined, but at different sites; breast cancers occurred only in patients who had received X-radiation with or without chemotherapy, and leukaemia was observed only in patients who had received chemotherapy (Bhatia et al., 1996). In survivors of childhood cancers overall, the risk of gastrointestinal second cancers increased

significantly with abdominal radiation and after high-dose chemotherapy with procarbazine and platinum drugs (Henderson et al., 2012a).

Radiation from nuclear weapons and nuclear reactor accidents

There is a statistically significant excess risk of solid cancers in people who were exposed to ionizing radiation from the atomic bombs in Japan either in utero or during early childhood (age < 6 years). Cancers developed in both children and adults (age 12–55 years at the time of diagnosis) and included leukaemia and a variety of solid tumours (Preston et al., 2008; IARC, 2012e). Cancers of the thyroid are notable in this cohort in the context of an exceptional susceptibility to develop cancer during early life, because they occurred almost exclusively in survivors who were younger than 14 years at the time of the bombings.

Short-lived radionuclides of iodine, especially iodine-131, were released into the atmosphere in enormous quantities during the accident with the Chernobyl Nuclear Power Plant in Ukraine, in 1986. Children in Ukraine and in neighbouring countries who were exposed to this radiation at an early age developed papillary adenocarcinoma of the thyroid later in childhood, beginning only a few years after the event (Bennett et al., 2006; IARC, 2012e). Thyroid cancer has also been observed in children from highly contaminated areas who were in utero at the time of the accident (Hatch et al., 2009). Children exposed to radioisotopes of iodine from the Chernobyl accident were at much higher risk of thyroid cancer than adults who were similarly exposed.

Medical radiation

People who were exposed to diagnostic X-rays in utero and in childhood during the 1950s are at increased risk of cancer, as documented in the Oxford Survey of Childhood Cancers (Wakeford and Little, 2003). However, a more recent meta-analysis (Schulze-Rath et al., 2008) of studies published after 1990 did not find any association between in utero exposure to medical radiation and the risk of childhood cancer, probably because in utero diagnostic doses for single-film X-rays are now substantially lower than those that were used previously and that formed the database for earlier reports of increased cancer risk.

Computed tomography (CT) diagnostic scans, for which much higher doses of radiation are used than for single-film X-rays, have come into common use for diagnostic procedures in both adults and children. CT scans have recently been reported to increase the risk of leukaemia and brain tumours in a dose-dependent fashion in patients who received their first scan when younger than 22 years (Pearce et al., 2012).

Therapeutic X-radiation of the head and neck during childhood for non-neoplastic conditions, most commonly to treat fungal infections of the scalp, caused a statistically significant increase in the incidence of intracranial meningiomas and nerve sheath tumours and a smaller increase in the incidence of brain tumours (Ron et al., 1988; Sadetzki et al., 2005). Thyroid carcinoma also occurred in irradiated children, who were much more sensitive to X-ray-induced thyroid cancer than were adults (Ron et al., 1995; IARC, 2012e). The risk of thyroid cancer in survivors of various childhood cancers who had received radiotherapy for their first malignancy increased linearly with radiation dose to the thyroid up to 20 Gy; the relative risk peaked at 14.6-fold (Bhatti et al., 2010).

Therapeutic anti-tumour X-radia tion to the chest during childhood or adolescence for Hodgkin lymphoma, and to a lesser extent for non-Hodgkin lymphoma, Wilms tumour, leukaemia, bone cancer, neuroblastoma, and soft tissue sarcoma, greatly increased the risk of breast cancer in female survivors, who tended to develop the second malignancy at a comparatively early age, during young adulthood (Henderson et al., 2010). Secondary sarcomas are associated in a dose-dependent fashion with radiation therapy for childhood tumours; radiation exposure was the most important factor for development of secondary sarcomas in survivors of childhood cancer (Henderson et al., 2012b).

Solar radiation

Solar radiation and sunburn during childhood are significant risk factors for malignant melanoma of the skin. Duration of residence in Australia – and the associated exposure to intense solar radiation – is associated with the risk of developing malignant melanoma, and childhood is an especially vulnerable life stage (Holman and Armstrong, 1984). A history of sunburn, especially during childhood, is also correlated with the risk of cutaneous melanoma. A study in England concluded that the strongest association with elevated melanoma risk was for sunburn that occurred in children aged 8–12 years (Elwood et al., 1990).

Chemicals and chemical mixtures other than cytotoxic anti-tumour agents

Diethylstilbestrol (DES) is a synthetic non-steroidal estrogen that was administered to pregnant women during the 1950s and 1960s in an effort to maintain high-risk pregnancies. Although DES is rarely used now, it has been estimated that 5–10 million women in the USA were treated with DES during pregnancy or were exposed to the drug in utero (Giusti et al., 1995).

Female offspring of women treated with DES developed an unusual cancer of the vagina and cervix, clear cell adenocarcinoma, which became clinically evident during adolescence and early adulthood (Herbst et al., 1971). DES caused breast cancer and is positively associated with the risk of endometrial cancer in women who were exposed while pregnant. In addition, a positive association has been observed between prenatal exposure to DES and squamous cell carcinoma of the cervix in female offspring and cancer of the testis in male offspring (IARC, 2012d). DES is the only chemical carcinogen known to have caused cancer in humans by transplacental exposure.

The mechanism of action of DES as a carcinogen is complex. DES is a potent estrogen, and some of its effects are mediated, at least in large part, by estrogen receptor alpha. DES can also undergo oxidative metabolism. In fetal mouse tissues, it causes aneuploidy, chromosomal breaks, and other chromosomal aberrations; it binds covalently to DNA and thus probably acts in part through a DNA-reactive genotoxic mechanism. In mice, neonatal exposure to DES also causes persistent changes in gene expression in target tissues (Newbold et al., 2006). These changes were found in specific genes (*Fos* and *Ltf* [lactoferrin]) and persisted even after cessation of treatment. Interestingly, changes in gene expression were associated with epigenetic alterations: specifically, the genes that were differentially expressed in animals treated with DES also exhibited abnormal DNA methylation (Newbold et al., 2000, 2006). These findings, although limited in genome coverage, strongly suggest that exposure to DES may have a significant and long-term effect on gene expression through epigenetic mechanisms. More recent studies that used microarray-based transcriptome analysis in both rats and mice identified DES-induced changes in expression of a wide range of genes (Hsu et al., 2009; Warita et al., 2010; Lee et al., 2011). Whether these changes are caused by epigenetic deregulation has not been tested.

Another interesting feature of exposure to DES is its potential impact on cancer incidence in subsequent generations. In addition to an increased cancer susceptibility associated with epigenetic changes in parents treated with DES, an epigenetic mechanism may operate in subsequent generations of mice (the second generation) (Newbold et al., 2006). These findings further support the notion that DES-induced carcinogenesis may operate in part through an epigenetic mechanism, although studies extending to the third generation are needed to establish a true transgenerational epigenetic inheritance.

Parental cigarette smoking causes hepatoblastoma, an embryonal tumour of the liver, in children. The effects of prenatal and postnatal exposures to parental cigarette smoke cannot be evaluated separately, although the weight of evidence favours the greater importance of prenatal exposures. Also, a positive association has been observed between parental smoking and risk of childhood leukaemia (particularly acute lymphoblastic leukaemia) (IARC, 2012c).

A plethora of experimental studies indicate that chemical components in tobacco smoke induce a wide range of genetic changes (Hainaut and Pfeifer, 2001; Pfeifer et al., 2002; Wistuba et al., 2002; Lea et al., 2007). More recent studies also implicate epigenetic events in human cancer associated with tobacco exposure (Herceg, 2007; Lin et al., 2010; Huang et al., 2011).

In a study of the methylome in cord blood of newborns in connection with maternal smoking during pregnancy, differential DNA methylation changes in a specific set of genes were associated with tobacco exposure (Joubert et al., 2012). A genome-wide methylomics approach and measurement of cotinine (a validated and objective biomarker of smoking) were used to identify methylation alterations in newborn cord blood samples from a mother–child cohort in relation to maternal smoking. Maternal smoking during pregnancy influenced methylation changes in specific genes. *CYP1A1* and *AHRR*, which encode proteins involved in the detoxification of chemicals in tobacco smoke, were among the differentially methylated genes (Joubert et al., 2012), suggesting a potential epigenetic mechanism involved in adverse effects associated with in utero exposure to tobacco smoke.

Various forms of inorganic arsenic have been collectively classified as *carcinogenic to humans* (Group 1). These compounds cause cancer of

the skin, bladder, and lung, and possibly of the liver and kidney, in exposed humans. Arsenic compounds have been notoriously difficult to evaluate in conventional animal bioassays for carcinogenicity. However, during the past decade sodium arsenite has been shown in several studies to be a transplacental carcinogen for the lung, liver, ovary, and adrenal cortex in mice (Waalkes et al., 2007; IARC, 2012a; see also Chapter 3, by Waalkes). Sodium arsenite is unique in this respect among inorganic carcinogens.

Also, recent epidemiological studies indicate that early-life exposure of humans to inorganic arsenic, most commonly in drinking-water but also in contaminated food products (Yorifuji et al., 2011), can lead to liver cancer during childhood (Liaw et al., 2008), to lung cancer in young adulthood (Smith et al., 2006), and to kidney cancer decades later (Yuan et al., 2010).

The possible transplacental effects of other inorganic Group 1 agents, such as nickel, cadmium, and chromium(VI), in animals or humans have not been well established.

Infectious agents

The factors that underlie the high susceptibility to oncogenic infectious agents during early life are different from those that govern susceptibility to chemical carcinogens and radiation. Lack of immunity to the agents in infants and immature immune responses to infection in infancy and during childhood are major contributors to susceptibility to these agents early in life, in common with the well-known susceptibility of children to other, non-oncogenic infections.

Several oncogenic infectious agents readily establish persistent infections in young children, setting in motion pathogenic processes that may lead to overt cancer development during childhood. Examples are Epstein–Barr virus (EBV) and hepatitis B virus (HBV). Other oncogenic pathogens, including Kaposi sarcoma-associated herpesvirus (KSHV), the bacterium *Helicobacter pylori*, and the bladder fluke *Schistosoma haematobium*, establish primary infection during childhood, but the resulting cancers appear after the paediatric age (> 18 years) and may require cofactors, especially immunosuppression. Suppression of the immune response may result either from co-infection with a second agent, generally the malarial parasite *Plasmodium falciparum* or human immunodeficiency virus type 1 (HIV-1), or by iatrogenic immunosuppression before and after organ or tissue transplantation.

EBV is a ubiquitous oncogenic gamma herpesvirus that infects and persists for life in more than 90% of the adult population worldwide. Children in certain regions of Africa become infected with EBV early in life, and nearly all have seroconverted by age 3 years, whereas in affluent countries primary infection is often delayed until adolescence (Biggar et al., 1978a, b). Primary EBV infection in early childhood, unlike that in adolescence, is usually asymptomatic (Chan et al., 2001).

EBV coexists for a lifetime in most human hosts without causing overt disease, but viral replication can be reactivated in several ways, including malaria infection, specifically *P. falciparum* malaria. Children living in areas endemic for malaria, notably in tropical regions of sub-Saharan Africa, have an elevated EBV viral load and a diminished EBV-specific immunosurveillance at ages 5–9 years. As a result of this combined infection, they are at high risk of developing endemic Burkitt lymphoma (eBL) during that period (IARC, 2012b). eBL is a high-grade B-cell lymphoma characterized by the consistent presence of EBV (zur Hausen et al., 1970) and is the most common paediatric cancer in sub-Saharan Africa (Greenwood et al., 1970). The determining factors that bring about eBL are, as far as is now known, the malaria parasite *P. falciparum* and EBV.

HBV readily infects young children by percutaneous and permucosal exposure to infected blood and other body fluids. The infection causes chronic active hepatitis that leads to a high incidence of hepatocellular carcinoma (HCC) in young children in Asian and African countries where the prevalence of HBV infection is high. Perinatal transmission from HBV surface antigen-positive mothers to their newborn babies, or transmission from one child to another, is a major source of HBV infection in many areas of the world (WHO, 2001). In utero transmission is relatively rare. Most (80–90%) of infected infants and 30–50% of children infected at ages 1–4 years develop a chronic infection, and about 25% of those who become chronically infected during childhood develop either cirrhosis or HCC. HCC can become clinically evident in chronically HBV-infected children during – or even before – adolescence (IARC, 2012b).

KSHV is transmitted primarily by saliva; in geographical areas where the virus is highly prevalent, infection occurs during childhood, and the peak age of acquisition is generally 6–10 years (Whitby et al., 2000; Dedicoat et al., 2004; Malope et al., 2007). KSHV infection is necessary

but not sufficient to cause Kaposi sarcoma or other cancers in the absence of severe immunosuppression, for example by co-infection with HIV-1 (IARC, 2012b).

The bacterium *H. pylori* typically establishes infection of the human stomach during childhood, and untreated infections may persist for life (Malaty and Graham, 1994; Goodman et al., 1996; Brown, 2000). The infection evolves to cause chronic atrophic gastritis, a pre-neoplastic condition that leads to development of gastric adenocarcinoma and gastric mucosa-associated lymphoid tissue lymphoma later in life (IARC, 2012b).

Infestation with the bladder fluke *S. haematobium* causes squamous cell carcinoma of the bladder as a result of chronic inflammation. The parasite has a complex life-cycle that includes an infective cercaria form present in freshwater bodies in sub-Saharan Africa, the Nile valley in Egypt and Sudan, and the Arabian Peninsula. Infections are percutaneous and result from direct contact with contaminated water. Maintenance of transmission of the infection depends on contamination of fresh water with excreta containing schistosome eggs, the presence of snails as intermediate hosts, and human contact with contaminated water (Jordan and Webbe, 1993). Children start to accumulate worms as soon as they are old enough to have contact with water, and they may be continuously reinfected and remain infected throughout their lives (IARC, 2012b). The incidence of schistosome-related bladder cancer in Africa peaks at ages 40–49 years, whereas infection with *S. haematobium* begins as early as age 6 months and usually peaks at ages 5–15 years (Mostafa et al., 1999).

Children whose mothers are infected with HIV-1 can be infected during gestation and at birth, and during infancy by nursing (IARC, 2012b). In the absence of any intervention, transmission of HIV-1 in utero and during birth is estimated to occur in approximately 25% of infants born to HIV-1-positive women (Connor et al., 1994). The risk of mother-to-child transmission increases steadily towards the late stages of pregnancy; almost 80% of new HIV-1 infections occur during the period from 36 weeks of pregnancy to delivery (Kourtis et al., 2006).

In summary, infants and children are exceptionally susceptible to many carcinogenic infectious agents. Some infections can result in the onset of malignancy within the first decade of life. In children, HBV infection causes HCC, and EBV accompanied by *P. falciparum* malaria infection results in eBL. Infections with KSHV, *H. pylori*, and *S. haematobium* typically occur within the first few years of life but result in development of cancer – Kaposi sarcoma, gastric adenocarcinoma and gastric mucosa-associated lymphoid tissue lymphoma, and bladder carcinoma, respectively – decades later. On a global scale, in terms of the numbers of children exposed and the numbers of cancer cases that result, oncogenic infectious agents pose the greatest cancer risks during childhood.

Summary

Treatment of childhood cancers with high doses of ionizing radiation and combinations of cytotoxic drugs, many of which are *carcinogenic to humans* (Group 1), has been very successful in recent years, but survivors are at high risk of second cancers, including acute myeloid leukaemia, non-Hodgkin lymphoma, and solid cancers of the breast, thyroid, bone, central nervous system, colorectum, and stomach. Certain tissues are extremely radiosensitive during childhood and adolescence, including the thyroid and the female breast. Cancers of these and other tissues occur at increased frequency not only among survivors of childhood cancer but also in individuals exposed as children and adolescents to diagnostic X-rays (including CT scans) and to ionizing radiation from nuclear weapons and nuclear reactor accidents.

Other high-dosage circumstances early in life that pose increased cancer risks include transplacental exposure to the non-steroidal estrogen DES, which causes distinctive carcinomas of the reproductive tract in female offspring of women treated with DES during pregnancy. Intense and repeated exposures to solar radiation during childhood, including sunburn, predispose to development of cutaneous malignant melanoma. Evidence is beginning to accumulate that exposure to inorganic arsenic in utero and during childhood can cause cancer of the liver during childhood and of the lung or kidney decades later.

The consequences of exposures to lower doses or concentrations of other carcinogens during prenatal and early postnatal life have been more difficult to establish (Carpenter and Bushkin-Bedient, 2013). Parental cigarette smoking can cause hepatoblastoma in children, an extreme case of the danger of second-hand tobacco smoke. Possible environmental causes of other embryonal tumours of childhood continue to be investigate.

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CHAPTER 21.

Analysis of tumour site concordance

Daniel Krewski, Jerry M. Rice, Michael Bird, Brittany Milton, Brian Collins, Pascale Lajoie, Mélissa Billard, Yann Grosse, Vincent J. Cogliano, Jane C. Caldwell, Ivan I. Rusyn, Christopher J. Portier, Ronald L. Melnick, Julian Little, and Jan M. Zielinski (deceased)
in collaboration with other participants (see the Contributors list) in the Workshop on Tumour Site Concordance and Mechanisms of Carcinogenesis, which was convened by IARC in April and November 2012 in Lyon

Introduction

Since its establishment in the early 1970s, the *IARC Monographs Programme* has evaluated more than 1000 agents with evidence of human exposure for which some suspicion exists of an increased cancer risk to humans. The *IARC Monographs Programme* has developed detailed criteria against which to evaluate the available scientific evidence on the carcinogenic potential of such agents. These criteria, which are described in the Preamble to the *IARC Monographs* (Cogliano et al., 2004; IARC, 2006), are used to evaluate and integrate the evidence provided by human epidemiological studies, animal cancer bioassays, and information on possible biological mechanisms of action, to classify agents into one of the following categories: *carcinogenic to humans* (Group 1), *probably carcinogenic to humans* (Group 2A), *possibly carcinogenic to humans* (Group 2B), *not classifiable as to its carcinogenicity to humans* (Group 3), and *probably not carcinogenic to humans* (Group 4). These evaluations involve classifying the data from both the human and the animal studies as providing *sufficient evidence of carcinogenicity*, *limited evidence of carcinogenicity*, *inadequate evidence of carcinogenicity*, or *evidence suggesting lack of carcinogenicity*. The information on biological mechanisms of action may be evaluated as *strong*, *moderate*, or *weak*, and is taken into consideration in the overall evaluation.

To date, IARC has developed 119 *Monographs* Volumes on more than 1000 agents for which there exists

some evidence of cancer risk to humans; of these, 120 agents met the criteria for Group 1. Volume 100 of the *IARC Monographs* provided a review and update of the 107 Group 1 agents identified as of 2009. Volume 100 is divided into six parts, focusing on pharmaceuticals (Volume 100A; IARC, 2012e); biological agents (Volume 100B; IARC, 2012b); arsenic, metals, fibres, and dusts (Volume 100C; IARC, 2012a); radiation (Volume 100D; IARC, 2012f); personal habits and indoor combustions (Volume 100E; IARC, 2012d); and chemical agents and related occupations (Volume 100F; IARC, 2012c). Since the publication of Volume 100, five additional agents had been added to Group 1 at the time the present analysis was undertaken: (i) diesel engine exhaust (reviewed in Volume 105; IARC, 2013), (ii) trichloroethylene (TCE) (evaluated in Volume 106; IARC, 2014), (iii) polychlorinated biphenyls (PCBs) and dioxin-like PCBs (reviewed in Volume 107; IARC, 2016b), and (iv) outdoor air pollution and (v) particulate matter in outdoor air pollution (both evaluated in Volume 109; IARC, 2016a). Had these five agents been evaluated within Volume 100, they would have been included in Volume 100F; for ease of reference, these agents are included in an expanded group of chemical agents and related occupations, denoted by Volume 100F*.

The 113 agents classified by IARC as known causes of cancer in humans up to and including Volume 109 of the *IARC Monographs* are listed in Table 21.1. Note that although 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126) was evaluated as a separate Group 1 agent in Volume 100F, it is included within the group of agents consisting of PCBs and dioxin-like PCBs, which were determined to be Group 1 agents in Volume 107. For the purposes of the present analysis, PCBs and dioxin-like PCBs were considered as a single group of PCBs, resulting in 113 – 2 = 111 distinct agents for analysis. Including the five Group 1 agents identified since Volume 100, there are 23, 11, 10, 18, 12, and 37 Group 1 agents in Volumes 100A to 100F*, respectively.

Because both animal and human data are considered in evaluating the weight of evidence for human carcinogenicity, the degree of concordance between species for tumour induction by carcinogenic agents is important. A high degree of site concordance between species supports the ability of studies in experimental animals to predict not only a potential cancer risk to humans but also the specific sites of cancer induction expected from human exposure to carcinogenic agents. In contrast, lack of concordance may indicate the need for further research to make sure that all cancer sites have been identified in sensitive human subpopulations or in appropriate experimental animal models, and to identify the underlying mechanisms that different species may or may not have in common.

This chapter uses the data set assembled by Grosse et al. (Annex 1) derived from the available information on the agents classified by IARC as *carcinogenic to humans* (Group 1) in Volume 100 to Volume 109, the last *Monograph* for which final data were available at the time this analysis was conducted. This database includes all tumour sites identified in the *IARC Monographs* for which agents presented *sufficient evidence* of carcinogenicity in humans and/or animals, and includes internationally peer-reviewed and published data from studies in humans and experimental animals to support analyses of tumour sites seen in humans and animals. Although the database also includes human tumour sites for which there is *limited evidence* of carcinogenicity of the agent, such sites were not systematically identified in the *IARC Monographs*. Likewise, animal tumour sites were generally not identified in the case of *limited evidence* of carcinogenicity in animals.

The next section describes how information was retrieved and assembled from the data set compiled by Grosse et al., as well as the approach used to evaluate tumour site concordance between animals and humans. A detailed description of the results of the analysis of these data is then presented both in the text of this chapter and in online supplemental material (see below). A discussion of the results of these analyses and the conclusions drawn from this work are presented in the last two sections of this chapter.

Methods

Tumour nomenclature in animals and humans

Although human tumours can be coded in a standardized manner by use of the *International Classification of Diseases* coding system (WHO, 1977, 2011), a comparable nomenclature system does not exist for animal tumours. To render the animal and human tumours identified in the *IARC Monographs* comparable, a taxonomy of tumour sites was constructed (Table 21.2). As detailed in Supplemental Material I (online only; available from: http://publications.iarc.fr/578), this

Table 21.1. Group 1 agents included in Volumes 100A–F, 105, 106, 107, and 109[a]

Volume	Type of agent	Number of agents	Agents
100A	Pharmaceuticals	23	Aristolochic acid; Aristolochic acid, plants containing; Azathioprine; Busulfan; Chlorambucil; Chlornaphazine; Ciclosporin; Cyclophosphamide; Diethylstilbestrol; Estrogen-only menopausal therapy; Estrogen–progestogen menopausal therapy (combined); Estrogen–progestogen oral contraceptives (combined); Etoposide; Etoposide in combination with cisplatin and bleomycin; Melphalan; Methoxsalen in combination with UVA; MOPP; Phenacetin; Phenacetin, analgesic mixtures containing; 1-(2-Chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea (Methyl-CCNU); Tamoxifen; Thiotepa; Treosulfan
100B	Biological agents	11	*Clonorchis sinensis* (infection with); Epstein–Barr virus; *Helicobacter pylori* (infection with); Hepatitis B virus; Hepatitis C virus; Human immunodeficiency virus type 1; Human papillomaviruses[b]; Human T-cell lymphotropic virus type 1; Kaposi sarcoma-associated herpesvirus; *Opisthorchis viverrini* (infection with); *Schistosoma haematobium* (infection with)
100C	Arsenic, metals, fibres, and dusts	10	Arsenic and inorganic arsenic compounds; Asbestos (all forms, including actinolite, amosite, anthophyllite, chrysotile, crocidolite, and tremolite); Beryllium and beryllium compounds; Cadmium and cadmium compounds; Chromium(VI) compounds; Erionite; Leather dust; Nickel compounds; Silica dust, crystalline, in the form of quartz or cristobalite; Wood dust
100D	Radiation	18	Fission products including strontium-90; Haematite mining with exposure to radon (underground); Ionizing radiation (all types); Neutron radiation; Phosphorus-32, as phosphate; Plutonium-239; Radioiodines, including iodine-131; Internalized radionuclides that emit α-particles; Internalized radionuclides that emit β-particles; Radium-224 and its decay products; Radium-226 and its decay products; Radium-228 and its decay products; Radon-222 and its decay products; Solar radiation; Thorium-232 (as Thorotrast); UV radiation (bandwidth 100–400 nm, encompassing UVC, UVB, and UVA); UV-emitting tanning devices; X- and γ-radiation
100E	Personal habits and indoor combustions	12	Acetaldehyde associated with consumption of alcoholic beverages; Alcoholic beverages; Areca nut; Betel quid with tobacco; Betel quid without tobacco; Coal, indoor emissions from household combustion of; Ethanol in alcoholic beverages; *N*′-Nitrosonornicotine (NNN) and 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone NNK); Salted fish, Chinese-style; Second-hand tobacco smoke; Tobacco smoking; Tobacco, smokeless
100F	Chemical agents and related occupations	32	Acid mists, strong inorganic; Aflatoxins; Aluminium production; 4-Aminobiphenyl; Auramine production; Benzene; Benzidine; Benzidine, dyes metabolized to; Benzo[*a*]pyrene; Bis(chloromethyl)ether; Chloromethyl methyl ether (technical grade); 1,3-Butadiene; Coal gasification; Coal-tar distillation; Coal-tar pitch; Coke production; Ethylene oxide; Formaldehyde; Iron and steel founding, occupational exposure during; Isopropyl alcohol manufacture using strong acids; Magenta production; 4,4′-Methylenebis(2-chloroaniline) (MOCA); Mineral oils, untreated or mildly treated; 2-Naphthylamine; *ortho*-Toluidine; Painter, occupational exposure as a; 3,3′,4,4′,5-Pentachlorobiphenyl (PCB 126)[a]; 2,3,4,7,8-Pentachlorodibenzofuran (PeCDF); Rubber manufacturing industry, occupational exposures in the; Shale oils; Soot (as found in occupational exposure of chimney sweeps); Sulfur mustard; 2,3,7,8-Tetrachlorodibenzo-*para*-dioxin; Vinyl chloride

Table 21.1. Group 1 agents included in Volumes 100A–F, 105, 106, 107, and 109[a] (continued)

Volume	Type of agent	Number of agents	Agents
105[c]	Diesel and gasoline engine exhausts and some nitroarenes	1	Engine exhaust, diesel
106[c]	Trichloroethylene and some chlorinated agents	1	Trichloroethylene
107[c]	Polychlorinated biphenyls and polybrominated biphenyls	1	Polychlorinated biphenyls (PCBs) and dioxin-like PCBs[a]
109[c]	Outdoor air pollution	2	Outdoor air pollution; Particulate matter in outdoor air pollution

UV, ultraviolet.

[a] Although 113 Group 1 agents have been identified up to and including *Monographs* Volume 109, the present analysis is based on 111 distinct agents remaining after considering PCBs and dioxin-like PCBs within the broader category of PCBs, and including PCB 126 within the broader category of PCBs.

[b] Human papillomavirus (HPV) types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59 were evaluated as *carcinogenic to humans*.

[c] During the concordance analyses, the Group 1 agents in these Volumes were included with “chemical agents and related occupations” in Volume 100F*.

Table 21.2. Anatomically based taxonomy of tumour sites/organ systems in animals and humans

Organ system	Sites coded from Volume 100 (A, B, C, D, E, and F*)[a]
Upper aerodigestive tract	Nasal cavity and paranasal sinuses Nasopharynx Oral cavity Pharynx Tongue Tonsil Salivary gland
Respiratory system	Larynx Lung Lower respiratory tract
Mesothelium	Mesothelium
Digestive tract	Oesophagus Stomach Intestine (including colon and rectum)
Digestive organs	Liver parenchyma and bile ducts Pancreas NOS Gallbladder
Nervous system and eye	Brain and spinal cord (CNS) Eye
Endocrine system	Thyroid, follicular epithelium Adrenal gland (medulla, cortex, NOS) Pituitary gland
Kidney	Kidney (renal cortex, renal medulla, kidney NOS)
Urothelium	Urothelium (renal pelvis, ureter, or bladder)
Lymphoid and haematopoietic tissues	Haematopoietic tissue Lymphoid tissue
Skin	Skin and adnexae Cutaneous melanocytes
Connective tissues	Soft connective tissue Blood vasculature (endothelium) Hard connective tissue (bone, cartilage)
Female breast, female reproductive organs, and female reproductive tract	Breast Ovary Uterine cervix Uterus Vulva/vagina
Other groupings	All cancers combined All solid cancers Exocrine glands NOS

CNS, central nervous system; NOS, not otherwise specified.
[a] These sites are derived from all site descriptors used in *IARC Monographs* to describe human and experimental animal cancer data (see Supplemental Table 1. Animal and human tumour sites for 111 Group 1 agents identified up to and including Volume 109 of the *IARC Monographs*).

taxonomy is anatomically based and includes 47 tumour sites grouped within 15 organ and tissue systems. There are 39 distinct animal and human tumour sites specified for Group 1 agents in Volume 100A–F*, and eight additional tumour sites were considered to be important, even though they did not appear in the tumour site concordance data set developed by Grosse et al. (Annex 1). The individual tumour sites seen in either animals or humans up to and including Volume 109 of the *IARC Monographs* are listed in Table 21.2. The category "other groupings" includes the three sites ("all cancers combined", "all solid cancers", and "exocrine glands not otherwise specified") that do not fit into any of the other 14 groupings of organ and tissue systems. All analyses reported in this chapter are based on the 39 individual tumour sites within the 14 organ and tissue systems listed in Table 21.2 (excluding tumours of the male reproductive tract, for which the data do not show *sufficient evidence* in both humans and animals).

Aggregation of tumour sites within an organ and tissue system was guided by several factors, including anatomical and functional relatedness. The specialized epithelia of the upper aerodigestive tract, respiratory system, digestive tract, and digestive organs are found for the most part in a single or a few anatomical sites, which are precisely captured by the available epidemiological and experimental data. In contrast, both the kidney and the urothelium are data-rich sites, and carcinogenic agents for either site display little or no overlap in target organ. Accordingly, the kidney and the urothelium were analysed separately rather than being aggregated as "urinary tract". Cancers of soft connective tissues, lymphoid and haematopoietic tissues, and bone and cartilage can arise wherever in the body their progenitor tissues occur, and are aggregated according to tissue of origin without regard to anatomical location. Likewise, skin cancers are aggregated irrespective of anatomical location, with the exception that malignant melanoma as it occurs in humans is unknown in rats or mice; cutaneous melanocytes are thus included separately in Table 21.2 as a human tumour site only for the sake of completeness. Estrogen-producing and estrogen-responsive tissues are aggregated in the organ system "female breast, female reproductive organs, and female reproductive tract". In contrast to the female reproductive system, no carcinogens are known with *sufficient evidence* for the male reproductive system in humans, despite the high prevalence in humans of prostate and testicular germ cell cancers.

Retrieval of data on tumour occurrence from the IARC Monographs

Grosse et al. (Annex 1) extracted data from Volumes 100, 105, 106, 107, and 109 on tumour sites reported in humans or animals for the 111 distinct Group 1 agents considered here. This information is illustrated in Table 21.3, with one compound from each of Volumes 100A–F, as well as diesel engine exhaust (Volume 105), TCE (Volume 106), PCBs (Volume 107), and particulate matter in outdoor air pollution (Volume 109). Table 21.3 gives the tumour sites for which the agents provide *sufficient evidence* of carcinogenicity in humans, as well as sites for which there is *limited evidence.* Tumour sites for which *sufficient evidence* of carcinogenicity exists in specific animal species are also noted. Information on the histology of animal lesions, when available, is also recorded in Table 21.3; however, because this information is not generally available in the *IARC Monographs* for human studies, it was not considered in the comparative analyses reported here.

Although tumour sites for which agents show *limited evidence* of carcinogenicity in humans are included in Table 21.3, this information is not considered in the present analysis. In fact, although the original intent was to consider tumour sites with *sufficient* or *limited evidence* in humans when evaluating concordance with animal tumour sites with *sufficient evidence*, there are only two Group 1 agents with *limited*, but not *sufficient*, evidence of carcinogenicity in humans.

Effects of sex, strain, and route of administration

The last column in Table 21.3 provides details on animal studies relevant to the evaluation of the agent of interest, including the sex and strain of the test animals and the route of administration of the test agent. Although this information has been recorded where available, it is difficult to examine concordance with respect to these important factors for a variety of reasons, as outlined below.

Because many epidemiological studies are based on predominantly male occupational cohorts, men tend to be over-represented in the human studies on Group 1 agents. Other agents, such as hormonal oral contraceptives, are evaluated only in women. Certain lesions, notably breast cancer and prostate cancer, are largely sex-specific. Also, some animal studies use only one sex, and others do not specify whether male

Table 21.3. Information on animal and human tumours and tumour sites for Group 1 agents in the *IARC Monographs* (adapted from Annex 1, by Grosse et al.)

Volume Agent number	Agent	Sites with *sufficient evidence* in humans	Site with *limited evidence* in humans	Agent tested in experimental animals	Species Site	Histology	Study/sex/strain/ exposure route	Comments
100A 3	Azathioprine	Non-Hodgkin lymphoma, skin (squamous cell carcinoma)		Azathioprine	Mouse Lymphoid tissue	Lymphoma	Mitrou et al. (1979a) (Volume 26), F, New Zealand Black and New Zealand White, s.c.; Mitrou et al. (1979b) (Volume 26), F, New Zealand Black and New Zealand White, s.c.; Ito et al. (1989), F, B6C3F1, p.o.; Brambilla et al. (1971), MF, Swiss, i.p.	
100B 25	Epstein–Barr virus	Burkitt lymphoma, immunosuppression-related non-Hodgkin lymphoma, extranodal NK/T-cell lymphoma (nasal type), Hodgkin lymphoma, nasopharyngeal carcinoma	Lymphoepithelioma-like carcinoma, gastric carcinoma					No data on animal studies listed; humans are the only natural hosts for Epstein–Barr virus
100C 35	Arsenic and inorganic arsenic compounds	Lung, bladder, skin	Kidney, liver, prostate	Dimethylarsinic acid [DMA(V)], Monomethylarsinous acid [MMA(III)], Sodium arsenite	Mouse Lung	Bronchiolo-alveolar carcinoma	DMA(V): Tokar et al. (2012a), M, CD1, d.w.; Sodium arsenite: Waalkes et al. (2003), F, C3H/HeNCr, in utero; Waalkes et al. (2006), M, CD1, in utero; Tokar et al. (2011), MF, CD1, in utero + p.o.; Tokar et al. (2012a), M, CD1, in utero; MMA(III): Tokar et al. (2012b), M, CD1, in utero	

Table 21.3. Information on animal and human tumours and tumour sites for Group 1 agents in the *IARC Monographs* (adapted from Annex 1, by Grosse et al.) (continued)

Volume Agent number	Agent	Sites with *sufficient evidence* in humans	Site with *limited evidence* in humans	Agent tested in experimental animals	Species Site	Histology	Study/sex/strain/ exposure route	Comments
100D 45	Fission products including strontium-90	Solid cancers, leukaemia		Strontium-90	Mouse Bone	Osteosarcoma	Nilsson (1970, 1971), M, CBA, i.p.; Nilsson et al. (1980), F, CBA, i.p.	
100E 68	Coal, indoor emissions from household combustion of	Lung		Coal smoke	Mouse Lung	Bronchiolo-alveolar carcinoma	Liang et al. (1988), MF, Kunming, inh.; Lin et al. (1995), MF, Kunming, inh.	
100F 80	Benzene	Acute myeloid leukaemia, acute non-lymphoblastic leukaemia	Acute lymphoblastic leukaemia, chronic lymphocytic leukaemia, multiple myeloma, non-Hodgkin lymphoma	Benzene	Mouse Thymus	Lymphoma	Snyder et al. (1980), M, C57Bl/6J, inh.; Cronkite et al. (1984), F, C57Bl/6 BNL, inh.	
105 107	Engine exhaust, diesel	Lung	Bladder	Whole diesel engine exhaust	Rat Lung	Bronchiolo-alveolar carcinoma	Ishinishi et al. (1986),MF, F344, inh.: Mauderly et al. (1986, 1987), MF F344, inh.: Iwai et al. (1986), F, F344, inh.: Heinrich et al. (1995), F, Wistar, inh.: Nikula et al.. (1995), F, F344, inh.: Iwai et al. (2000), F, F344, inh.	
106 108	Trichloroethylene	Kidney	Non-Hodgkin lymphoma, liver	Trichloroethylene	Rat Kidney	Renal cell carcinoma	National Toxicology Program (1990), M, F344/N, g.; National Toxicology Program (1988), M, Osborne-Mendel, g.; National Toxicology Program (1988), F, ACI, g.	

Table 21.3. Information on animal and human tumours and tumour sites for Group 1 agents in the *IARC Monographs* (adapted from Annex 1, by Grosse et al.) (continued)

Volume Agent number	Agent	Sites with *sufficient evidence* in humans	Site with *limited evidence* in humans	Agent tested in experimental animals	Species Site	Histology	Study/sex/strain/ exposure route	Comments
107 109	Polychlorinated biphenyls	Skin (melanoma)	Non-Hodgkin lymphoma, breast	Aroclor 1260	Rat Liver	Hepatocellular carcinoma	Mayes et al. (1998), F, Sprague-Dawley, p.o.; Norback and Weltman (1985), F, Sprague-Dawley, p.o.; Kimbrough et al. (1975), F, Sherman, p.o.	
109 111	Particulate matter in outdoor air pollution	Lung						*Sufficient evidence* in experimental animals, but no organ sites identified due to the absence of two (or more) studies of adequate design and quality pointing at the same organ site (with a similar histological origin) in the same species

F, female; d.w., drinking-water; g., gavage; inh., inhalation; i.p., intraperitoneally; M, male; MF, male and female; NK, natural killer; p.o., orally; s.c., subcutaneously.

or female animals – or both – were used. For these reasons, separate analyses of species concordance across the spectrum of Group 1 agents are difficult to conduct. Separate concordance analyses by strain are also difficult, because of the sparseness of studies on specific strains of experimental animals. Indeed, in many cases information on strain is unavailable, precluding the possibility of strain-specific analyses.

Human exposure to carcinogens can occur by oral ingestion, inhalation, or dermal absorption, as well as via other routes, such as injection of pharmaceutical agents for therapeutic purposes. Animal studies may involve other routes of exposure, such as intraperitoneal injection or intratracheal instillation. In many cases, the route of exposure used in animal studies may not correspond to the predominant route by which humans are exposed; in such cases, the dose of the reactive metabolite reaching critical target tissues may be quite different, depending on the route of administration. Differences in routes of exposure between animals and humans could thus contribute to lack of concordance between tumour sites observed in animals and humans. However, because data on cancer outcomes for a given route of exposure are not available across the entire set of Group 1 agents, a systematic evaluation of concordance for specific exposure routes is not possible.

Species-specific tumour site profiles

Before the concordance analyses were conducted, the organ distribution was examined of the tumours caused by the 111 distinct Group 1 carcinogens identified by IARC to date, both in humans and in animal species. These distributions are of value in demonstrating the spectrum of tumours caused by these agents in different species, including the identification of the most common tumours caused in humans. Human tumours caused by the human tumour viruses reported in Volume 100B were included in these distributions, so that these results reflect the tumours caused by all 111 distinct Group 1 carcinogens considered here.

Organization of concordance analyses

Analytical results are presented first for the 39 tumour sites and then for the 14 organ and tissue systems. Because the present database involves only a moderate number of agents with comparable data in animals and humans, results aggregated by organ and tissue system may be expected to be more stable.

Results

The concordance data set assembled by Grosse et al. (Annex 1) and summarized in Table 21.1 includes 111 distinct Group 1 agents identified in the *IARC Monographs* up to and including Volume 109. Nine of these 111 agents were placed in Group 1 in the absence of *sufficient evidence* of carcinogenicity in humans (Table 21.4). These determinations were made on the basis of mechanistic upgrades according to the evaluation criteria outlined in the Preamble to the *IARC Monographs* (IARC, 2006). For example, benzo[*a*]pyrene (B[*a*]P) was placed in Group 1 on the basis of epidemiological data on exposure to mixtures of polycyclic aromatic hydrocarbons (PAHs) containing B[*a*]P that provided *sufficient evidence* for cancer of the lung or skin in humans, coupled with extensive mechanistic data on B[*a*]P, suggesting that the mechanisms by which this agent causes tumours in animals would also be expected to operate in humans; no data in humans on B[*a*]P alone were available for evaluation (IARC, 2010). An important aspect of such mechanistic upgrades for purposes of the present analysis is the general lack of identification of a human tumour site.

Of the nine agents in Table 21.4 placed in Group 1 on the basis of mechanistic upgrades, all but one – etoposide – demonstrated *sufficient evidence* of carcinogenicity in animals. In the assignment of etoposide to Group 1 in the absence of *sufficient evidence* in animals, the *Monograph* noted the *limited evidence* of carcinogenicity in humans on the basis of the induction of acute myeloid leukaemias with distinctive chromosomal translocations by drugs, including etoposide, that target topoisomerase II (IARC, 2012e). Of the nine mechanistic upgrades, three showed *limited evidence* in humans, and six had *inadequate evidence* in humans or no epidemiological data were available, for example for B[*a*]P and 2,3,4,7,8-pentachlorodibenzofuran (PeCDF).

Apart from the nine Group 1 mechanistic upgrades for which no human tumour sites were identified, there are four other agents for which the same is true (Table 21.5): ionizing radiation (all types), internalized radionuclides that emit α-particles, internalized radionuclides that emit β-particles, and ultraviolet (UV) radiation (bandwidth 100–400 nm, encompassing UVC, UVB, and UVA). These were generic evaluations across a range of agents falling in these categories. In addition, no human tumour site was specified for the agents areca nut and ethanol in

alcoholic beverages, because no epidemiological data were available for areca nut alone or for ethanol in alcoholic beverages alone (see Annex 1, by Grosse et al.).

No animal tumour sites were identified for 38 of the 111 agents considered here (Table 21.6). These included 20 agents with *inadequate evidence* in animals: seven agents representing occupational exposures that would be difficult to replicate in the laboratory; two pharmaceutical agents used in combination for which no animal data were available on the mixture; seven biological agents (all viruses) for which the selection of an appropriate animal model was problematic; two agents, etoposide and wood dust, for which the available animal tests were considered inadequate; and two agents, treosulfan and leather dust, for which no animal data were available. Although the two agents that lack any animal test data – treosulfan and leather dust – clearly do not permit an evaluation of concordance between animals and humans, the two agents for which inadequate animal data were available – etoposide and wood dust – warrant some further discussion to distinguish between the case in which well-conducted animal studies have failed to demonstrate carcinogenicity and the case in which the animal data are largely uninformative because of inadequate testing: Volume 76 (IARC, 2000) and Volume 100A (IARC, 2012e) of the *IARC Monographs* noted that etoposide was tested in only one experiment with wild-type and heterozygous neurofibromatosis type 1 (*Nf1*) knockout mice that were treated by gastric intubation for 6 weeks with etoposide at 100 mg/kg body weight/week (Mahgoub et al., 1999). This single short-duration study was judged as providing *inadequate evidence* of carcinogenicity in animals. The available studies with wood dust originally considered in Volume 62 (IARC, 1995) did not show significant carcinogenic or co-carcinogenic potential of beech wood dust, but these studies were subject to several limitations as well as inadequacies in data reporting. Upon re-evaluation of wood dust in Volume 100C (IARC, 2012a), it was concluded that most of the studies conducted with wood dust (nearly all with beech wood dust) had small numbers of animals or were of short duration, thus providing *inadequate evidence* of carcinogenicity in animals. These considerations suggest that neither etoposide nor wood dust have been subject to adequate animal testing, therefore precluding a determination of their carcinogenic potential in animals.

Ten agents, including six pharmaceutical products (busulfan, chlornaphazine, cyclosporine; combined estrogen–progestogen menopausal therapy, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea [methyl-CCNU], and analgesic mixtures containing phenacetin), three biological agents (infections with *Clonorchis sinensis*, *Opisthorchis viverrini*, and *Schistosoma haematobium*), and one chemical agent (sulfur mustard), provided *limited*, but not *sufficient*, evidence of carcinogenicity in animals. As mentioned above, tumour sites are not specified in the *IARC Monographs* for agents that demonstrate only *limited evidence* in animals.

The reasons that these 10 agents were judged as providing only *limited evidence* of carcinogenicity in animals varied. For example, treatment with busulfan resulted in a significant increase in the incidence of thymic and ovarian tumours in BALB/c mice, which was found difficult to interpret, whereas in another study busulfan, when given to rats during gestation, affected the incidence of uterine adenocarcinomas in the offspring upon intrauterine treatment with *N*-ethyl-*N*′-nitro-*N*-nitrosoguanidine (IARC, 2012e). As a second example, sulfur mustard significantly increased the incidence of lung tumours (not otherwise specified) in mice after exposure by inhalation for 15 minutes, and of pulmonary tumours (not otherwise specified) after intravenous injection; a significant increase in the incidence of mammary tumours was seen after subcutaneous injection of sulfur mustard in rats, relative to an external control group, whereas forestomach tumours were numerically, but not significantly, elevated in rats treated by oral gavage (IARC, 2012c). The exposure by subcutaneous and intravascular injection was considered to be of limited relevance to the most common human routes of exposure. Although not meeting the stringent criterion for *sufficient evidence* of carcinogenicity in animals, the *limited evidence* provided by busulfan, as well as by the other six chemicals with only *limited evidence* of carcinogenicity in animals, does suggest that these agents have the potential to cause cancer in animals.

No tumour sites were specified for eight agents demonstrating *sufficient evidence* of carcinogenicity in animals, because reproducible results were unavailable in two or more studies of adequate design in the same species for any of these agents. Although melphalan showed evidence of a statistically significant increase in the incidence of tumours of the forestomach, skin, and lung in mice, as well as lymphosarcoma, these results were not replicated in a second, independent study (IARC,

Table 21.4. Agents placed in Group 1 on the basis of mechanistic upgrades[a]

Agent	Level of evidence in humans/ animals	Human tumour site	Basis for mechanistic upgrade
Aristolochic acid	*Limited*/ *Sufficient*	Not specified	Herbal remedies containing aristolochic acid provide *sufficient evidence* for upper urinary tract cancer in humans; genotoxic mechanistic data
Benzo[*a*]pyrene (B[*a*]P)	[No epidemiological data]/ *Sufficient*	Not specified	PAH mixtures containing B[*a*]P provide *sufficient evidence* for lung or skin cancer in humans; extensive mechanistic data on B[*a*]P linking animal and human biology
Dyes metabolized to benzidine	*Inadequate*/ *Sufficient*	Not specified	Benzidine provides *sufficient evidence* of being a human bladder carcinogen
Ethylene oxide	*Limited*/ *Sufficient*	Not specified	*Limited evidence* for non-Hodgkin lymphoma, breast cancer in humans; genotoxic mechanistic data
Etoposide	*Limited*/ *Inadequate*	Not specified	*Limited evidence* of acute myeloid leukaemia in humans, with distinctive chromosomal translocations
4,4′-Methylenebis(2-chloroaniline) (MOCA)	*Inadequate*/ *Sufficient*	Not specified	Bladder cancer expected in humans, based on mechanistic data and human case report
Neutron radiation	*Inadequate*/ *Sufficient*	Not specified	Biophysics of radiation damage induction similar across different types of radiation
N′-Nitrosonornicotine (NNN) and 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)	*Inadequate*/ *Sufficient*	Not specified	Target sites correspond to those of smokeless tobacco; mechanistic data on tobacco smoke
2,3,4,7,8-Pentachlorodibenzofuran (PeCDF)	[No epidemiological data]/ *Sufficient*	Not specified	*Sufficient evidence* in experimental animals combined with strong mechanistic support for receptor-mediated mechanism, with biological activity identical to that of 2,3,7,8-tetrachlorodibenzo-*para*-dioxin (TCDD) for every mechanistic step

PAH, polycyclic aromatic hydrocarbon.

[a] Although dioxin-like PCBs evaluated in Volume 107 were also upgraded to Group 1 on the basis of support for receptor-mediated mechanisms and analogies with TCDD (IARC, 2016b), dioxin-like PCBs have been subsumed within the broader category of PCBs for the purposes of the present analysis of 111 distinct Group 1 agents, and are therefore not included in this table.

Table 21.5. Group 1 agents with no human tumour sites specified (15 agents)

Nature of evidence in humans (number of agents)	Volume: Agent(s)
Mechanistic upgrades	
Mechanistic upgrade with no human tumour site specified (9 agents)	**Volume 100A:** Aristolochic acid; Etoposide. **Volume 100D:** Neutron radiation. **Volume 100E:** *N′*-Nitrosonornicotine (NNN) and 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). **Volume 100F:** Benzo[*a*]pyrene (B[*a*]P); Dyes metabolized to benzidine; Ethylene oxide; 4,4′-Methylenebis(2-chloroaniline) (MOCA); 2,3,4,7,8-Pentachlorodibenzofuran (PeCDF)
Generic evaluations	
Generic evaluation, of all types of ionizing radiation; internalized radionuclides that emit α-particles; internalized radionuclides that emit β-particles; and the UV region (100–400 nm) of the electromagnetic spectrum (4 agents)	**Volume 100D:** Ionizing radiation (all types); Internalized radionuclides that emit α-particles; Internalized radionuclides that emit β-particles; UV radiation (bandwidth 100–400 nm, encompassing UVC, UVB, and UVA)
Absence of epidemiological data on the agent alone	
No epidemiological data available for agent alone (2 agents)	**Volume 100E:** Areca nut; Ethanol in alcoholic beverages

2012c). In rats, melphalan also produced mammary gland tumours and peritoneal sarcoma, but these findings were again not replicated in independent studies. Phosphorous-32 caused leukaemia in mice and osteogenic sarcomas in rats in single studies. Similarly, acetaldehyde in drinking-water induced pancreatic adenomas, combined lymphomas and leukaemias, uterine and mammary gland adenocarcinomas, and head osteosarcomas in rats, but without replication. Betel quid with tobacco produced malignant forestomach and cheek pouch tumours in a single study in hamsters. *Sufficient evidence* of carcinogenicity in animals of aluminium refining was based on a single limited skin application study in mice with PAH-containing particulates from aluminium production plants, in conjunction with *sufficient evidence* of carcinogenicity in experimental animals for many of the PAHs detected in air samples from such plants, and previously evaluated in Volume 92 (IARC, 2010). Had the animal evidence for the agents mentioned above been eligible for inclusion in the tumour site concordance database, additional concordant results would have been noted, including concordance between lymphoid and haematopoietic tissues in mice and humans for both melphalan and phosphorous-32, and concordance between tumours of the upper aerodigestive tract in hamsters and humans for betel quid with tobacco.

Although PeCDF provided *sufficient evidence* of carcinogenicity in animals, no animal site was identified. PeCDF was tested by the United States National Toxicology Program in a 2-year animal bioassay (female rats only) with exposure by oral gavage (National Toxicology Program, 2006). There was some evidence of carcinogenic activity of PeCDF, based on increased incidences of hepatocellular adenoma and cholangiocarcinoma of the liver and gingival squamous cell carcinoma of the oral mucosa. The occurrence of cystic keratinizing epithelioma of the lung, neoplasms of the pancreatic acinus, and carcinoma of the uterus may have been related to administration of PeCDF. There were also three rat studies of PeCDF in combination with *N*-methyl-*N′*-nitro-*N*-nitrosoguanidine (MNNG) and *N*-nitrosodiethylamine (NDEA), where increased tumour multiplicity was observed in each case (IARC, 2012c). These observations led to the conclusion that there is *sufficient evidence* for the carcinogenicity of PeCDF in animals, although there is no specific organ site that can be designated as responsible for this *sufficient evidence*. Because of the absence of a specific tumour site in

Table 21.6. Group 1 agents with no animal tumour sites specified (38 agents)

Nature of evidence in animals (number of agents)	Volume: Agent(s)
Agents with inadequate evidence in animals	
Occupational exposures are complex and probably could not be reliably replicated in the laboratory (7 agents)	**Volume 100F:** Acid mists, strong inorganic; Auramine production; Iron and steel founding, occupational exposure during; Isopropyl alcohol manufacture using strong acids; Magenta production; Painter, occupational exposure as a; Rubber manufacturing industry, occupational exposures in the.
Used in combination; no animal data available on mixture (2 agents)	**Volume 100A:** Etoposide in combination with cisplatin and bleomycin; MOPP.
Use of animal models problematic because of species specificity and other limitations (7 agents)	**Volume 100B:** Infection with Epstein–Barr virus; Hepatitis B virus; Hepatitis C virus; Human immunodeficiency virus type 1; Human papillomaviruses; Human T-cell lymphotropic virus type 1; Kaposi sarcoma-associated herpesvirus.
Animal tests conducted but considered inadequate (2 agents)	**Volume 100A:** Etoposide. **Volume 100C:** Wood dust.
No animal data available (2 agents)	**Volume 100A:** Treosulfan. **Volume 100C:** Leather dust.
Agents with limited evidence in animals	
Evidence of carcinogenicity in animals judged as *limited* for various reasons (10 agents)	**Volume 100A:** Busulfan; Chlornaphazine; Ciclosporin; Estrogen–progestogen menopausal therapy (combined); 1-(2-Chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea (Methyl-CCNU); Phenacetin, analgesic mixtures containing. **Volume 100B:** *Clonorchis sinensis* (infection with); *Opisthorchis viverrini* (infection with); *Schistosoma haematobium* (infection with). **Volume 100F:** Sulfur mustard.
Agents with sufficient evidence in animals	
Sufficient evidence in animals, but no tumour sites specified[a] (8 agents)	**Volume 100A:** Melphalan. **Volume 100D:** Phosphorus-32, as phosphate. **Volume 100E:** Acetaldehyde associated with the consumption of alcoholic beverages; Betel quid with tobacco. **Volume 100F:** Aluminium production; 2,3,4,7,8-pentachlorodibenzofuran (PeCDF); **Volume 109:** Outdoor air pollution; Particulate matter in outdoor air pollution.

[a] *Sufficient evidence* in experimental animals, but no organ sites identified due to the absence of at least two studies of adequate design and quality showing tumours at the same organ site with a similar histological origin in the same species.

animals, PeCDF is not included in the concordance analyses.

A component of four Group 1 agents, but not the agents themselves, demonstrated *sufficient evidence* of carcinogenicity in animals. These are: fission products including strontium-90, where strontium-90 demonstrated *sufficient evidence* of carcinogenicity in animals (IARC, 2012f); haematite mining with exposure to radon (underground), where radon demonstrated *sufficient evidence* of carcinogenicity in animals (IARC, 2012f); acetaldehyde associated with consumption of alcoholic beverages, where acetaldehyde demonstrated *sufficient evidence* of carcinogenicity in animals (IARC, 2012d); and occupational exposures during aluminium production, where airborne particulate polynuclear organic matter from aluminium production plants demonstrated *sufficient evidence* of carcinogenicity in animals (IARC, 2012c). Although this animal evidence is consistent with the *sufficient evidence* for the carcinogenicity of these four agents in humans, the animal evidence represents only a component of these agents.

Excluding the 20 agents in Table 21.5 that lack appropriate animal data, i.e. seven occupational exposures not reproducible in the laboratory, two agents used in combination with no animal data available on the mixture, seven agents where the use of animal models is problematic because of species specificity or other limitations, and four agents for which animal tests were inadequate (two agents) or unavailable (two agents), all 91 distinct Group 1 agents identified by IARC up to and including Volume 109 of the *IARC Monographs* provided either *sufficient evidence* (82 agents) or *limited evidence* (nine agents) of carcinogenicity in animals. This observation provides support for the use of animal data in human cancer risk assessment.

To further explore the correspondence between sites where tumours are seen in animals and humans among the 111 distinct Group 1 agents considered here, descriptive statistics are presented on tumour site profiles by species, followed by an evaluation of concordance between tumour sites seen in animals and humans. Results are presented first for the 39 tumour sites included in the anatomically based tumour nomenclature system seen in either animals or humans, followed by the data for the 14 organ and tissue systems.

Tumour site profiles by species

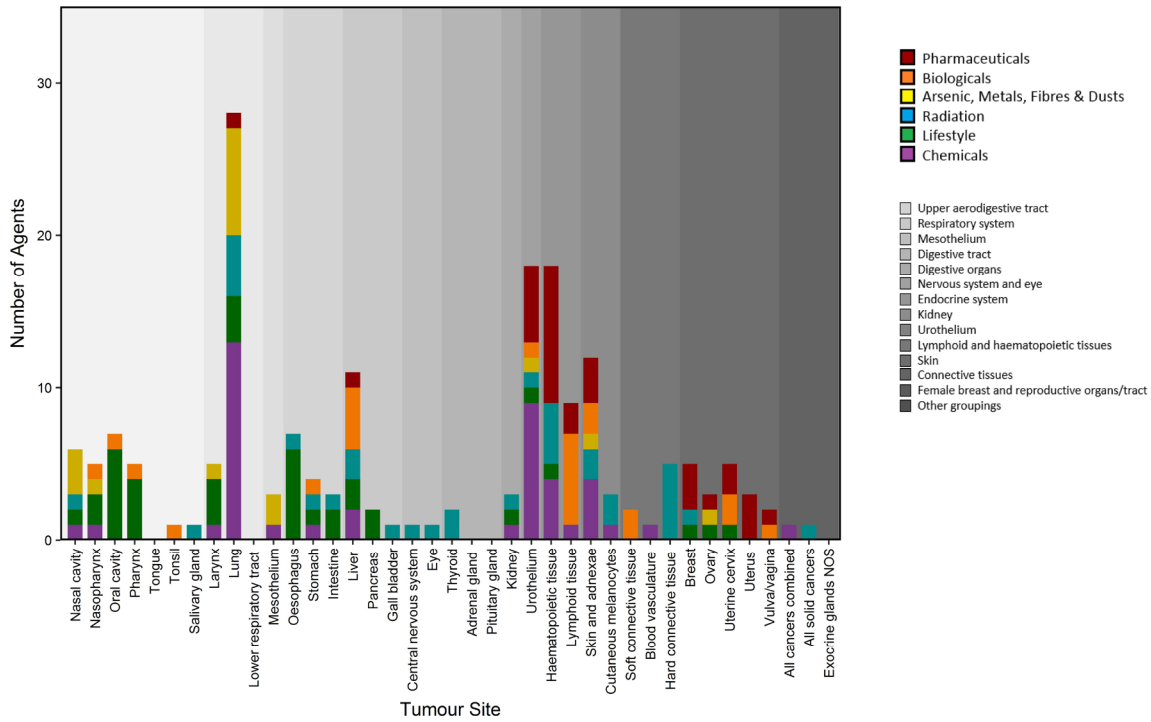
The number of agents that induce tumours in humans at each of the 39 tumour sites is shown in Fig. 21.1 by type of agent (pharmaceuticals; biological agents; arsenic, metals, fibres, and dusts; radiation; personal habits and indoor combustions; and chemical agents and related occupations). Lung tumours are the most common tumour seen in humans, with 28 of the 111 known human carcinogens inducing lesions at this site; of these, 13 are associated with exposure to chemical agents and related occupations and seven are in the category of arsenic, metals, fibres, and dusts. Tumours of the haematopoietic tissues are associated with exposure to 18 agents, urothelial tumours with 18 agents, skin tumours with 12 agents, and liver and bile duct tumours with 11 agents. The category chemical agents and related occupations accounts for half (9 of 18) of the agents that cause urothelial tumours, and pharmaceuticals account for half (9 of 18) of the agents that cause tumours in haematopoietic tissues.

The number of agents that induce tumours in one or more animal species at each of the 39 tumour sites is shown in Fig. 21.2 by type of agent. As in humans, lung tumours are the most common tumour in animals, with 29 of the 111 known human carcinogens inducing lesions at this site, mostly from the categories of chemical agents and related occupations (10 agents), arsenic, metals, fibres, and dusts (7 agents), and radiation (7 agents). After the lung, the animal sites associated with the largest number of carcinogenic agents are the liver parenchyma and bile ducts (19 agents), the skin and adnexae (18 agents), lymphoid tissue (14 agents), the breast (12 agents), and soft connective tissue (11 agents). Separate tumour profiles are shown for agents that cause tumours in mice (48 agents) and rats (49 agents) in Fig. 21.3 and Fig. 21.4, respectively. In rodents (mice and rats combined), the lung is the site associated with the largest number of carcinogens.

Organ and tissue system profiles by species

The number of agents that induce tumours in humans in each of the 14 aggregate organ and tissue systems is shown in Fig. 21.5 by type of agent. Tumours of the respiratory system are caused by 31 of the 111 human carcinogens, mostly from the categories of chemical agents and related occupations (14 agents), arsenic, metals, fibres, and dusts (7 agents), and personal habits and indoor combustions (5 agents). After the respiratory system, the organ and tissue systems associated with the largest number of agents are lymphoid and haematopoietic tissues (26 agents),

Fig. 21.1. Number of agents that induce tumours in humans in each of 39 tumour sites, by type of agent.



the urothelium (18 agents), and the upper aerodigestive tract (16 agents). Pharmaceuticals are the largest group of agents associated with tumours of the lymphoid and haematopoietic tissues (11 of 26 agents), and chemical agents and related occupations are most often associated with tumours of the urothelium (9 of 18 agents). Personal habits and indoor combustions are most commonly associated with tumours of the upper aerodigestive tract (7 of 16 agents).

The number of agents that induce tumours in one or more animal species at each of the 14 organ and tissue systems is given in Fig. 21.6 by type of agent. Tumours of the respiratory system are caused by 29 of the 111 agents, mostly from the categories of chemical agents and related occupations (10 agents), arsenic, metals, fibres, and dusts (7 agents), and radiation (7 agents). Tumours of the digestive organs are caused by 19 agents, mostly from the categories of chemical agents and related occupations (12 agents) and radiation (4 agents). Skin tumours are caused by 18 agents, mostly from the category of chemical agents and related occupations (12 agents). Connective tissue tumours are associated with 17 agents, mostly from the categories of radiation (8 agents) and chemical agents and related occupations (5 agents).

In mice (Fig. 21.7), tumours of the skin and connective tissues are caused by 29 agents, consisting mostly of tumours caused by chemical agents and related occupations (14) and radiation (10). In rats (Fig. 21.8), tumours of the respiratory system are caused by 19 agents, including those in the categories of arsenic, metals, fibres, and dusts (6 agents), radiation (6 agents), and chemical agents and related occupations (5 agents).

Qualitative assessment of concordance

Of the 111 distinct Group 1 agents identified up to and including Volume 109 (see Table 21.1), for 60 agents both a human tumour site and an animal tumour site have been identified, 15 agents had no human tumour site specified (Table 21.5), and 38 agents had no animal tumour site identified (Table 21.6). Because two agents – etoposide and PeCDF – have neither a human nor an animal tumour site specified, there are 111 − 15 − 38 + 2 = 60 agents with at least one tumour site identified in both humans and animals. These 60 agents have been used to evaluate concordance between tumour sites seen in animals and humans, because at least one tumour site has been identified in both.

Fig. 21.2. Number of agents that induce tumours in animals in each of 39 tumour sites, by type of agent.

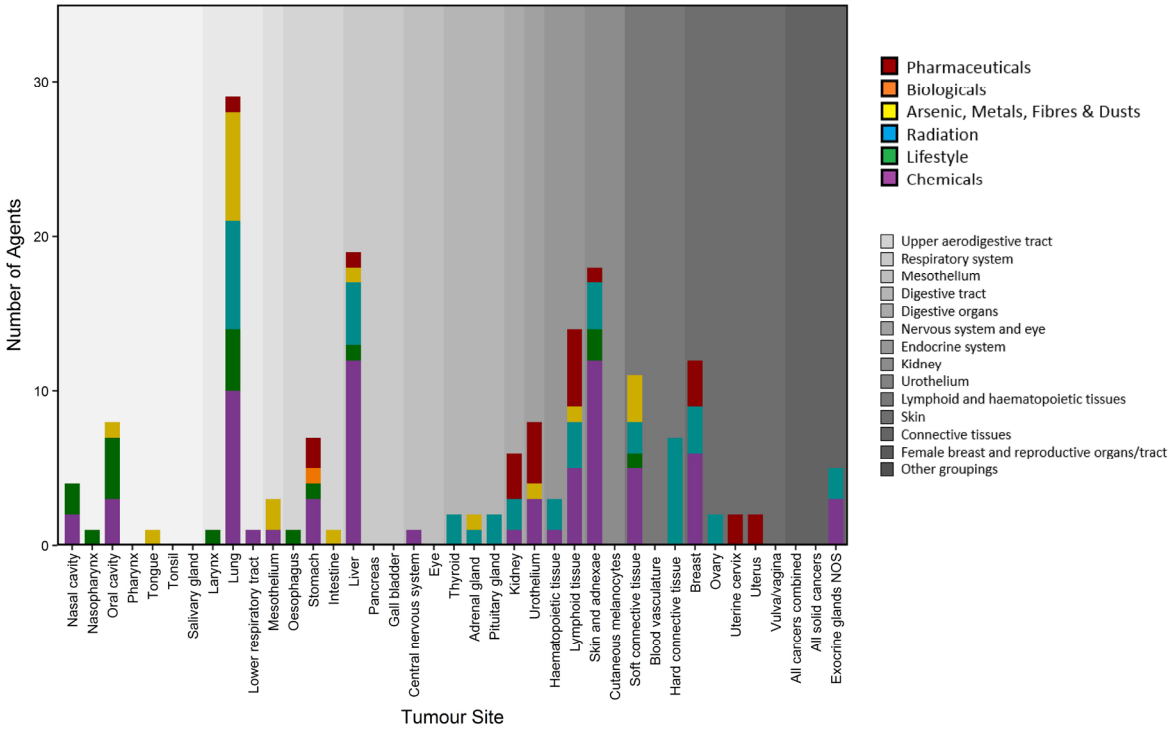


Fig. 21.3. Number of agents that induce tumours in mice in each of 39 tumour sites, by type of agent.

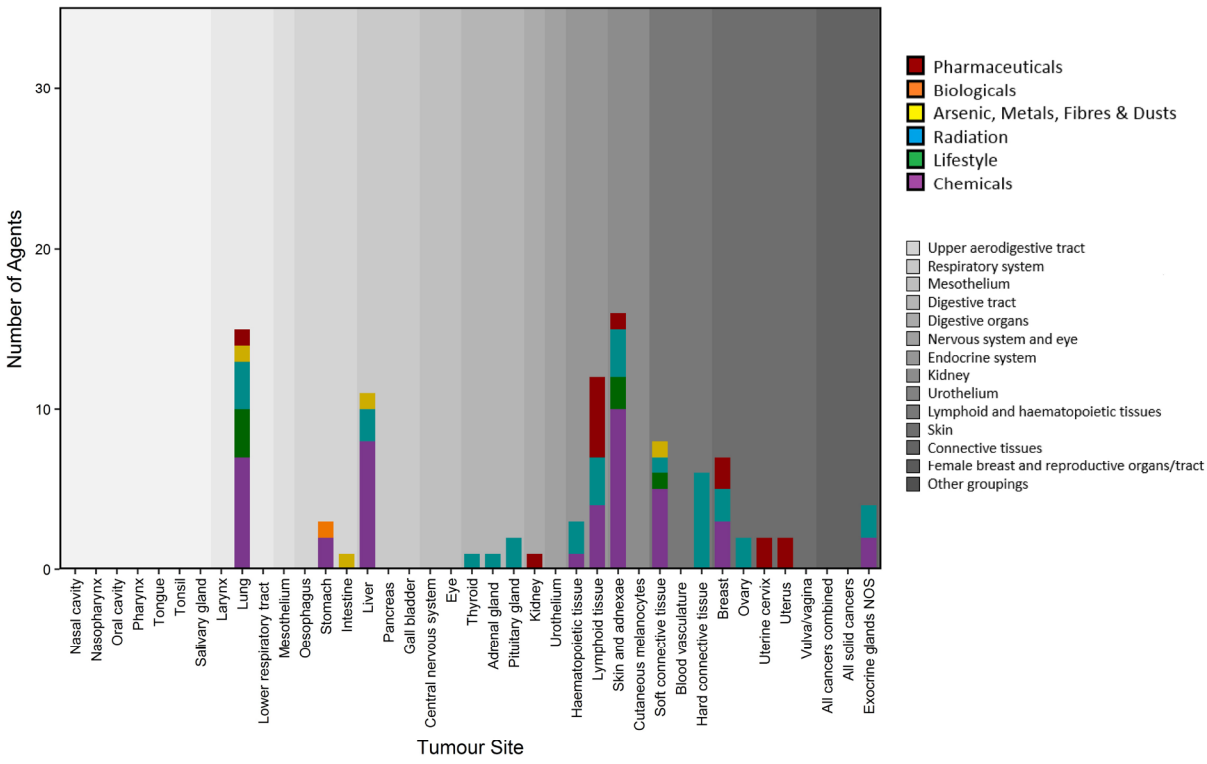
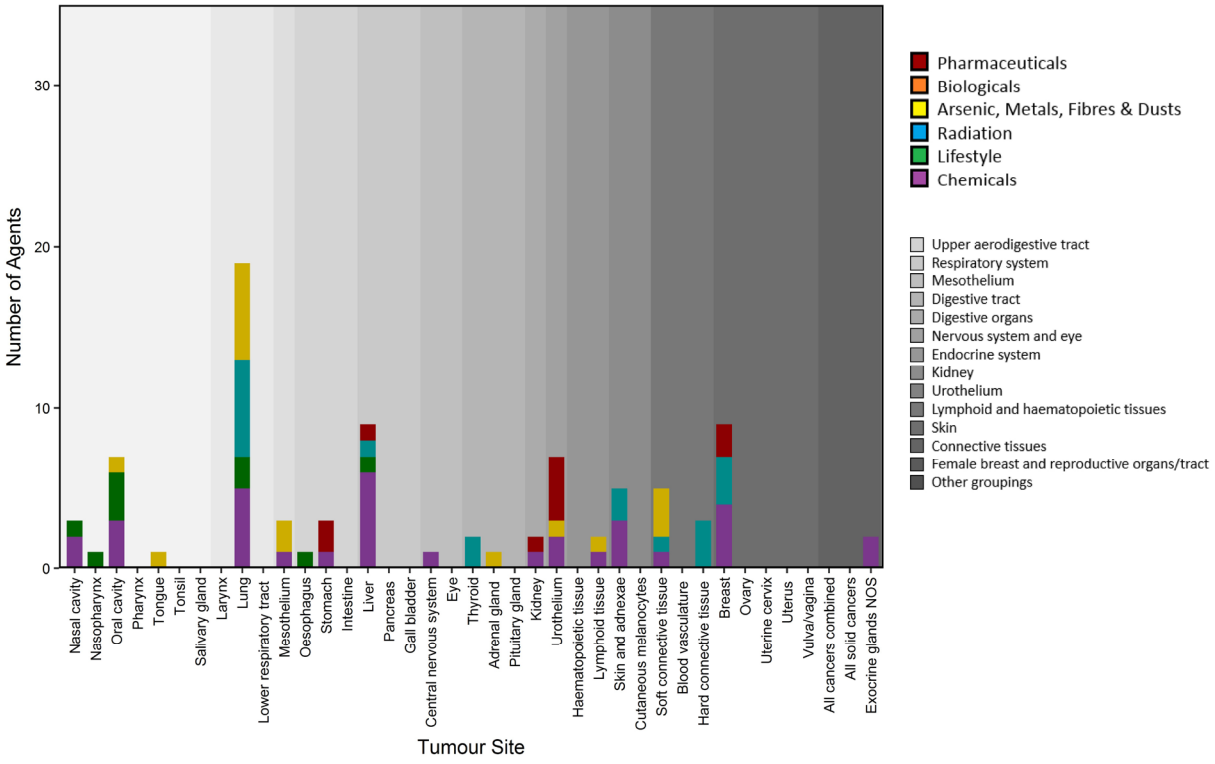


Fig. 21.4. Number of agents that induce tumours in rats in each of 39 tumour sites, by type of agent.



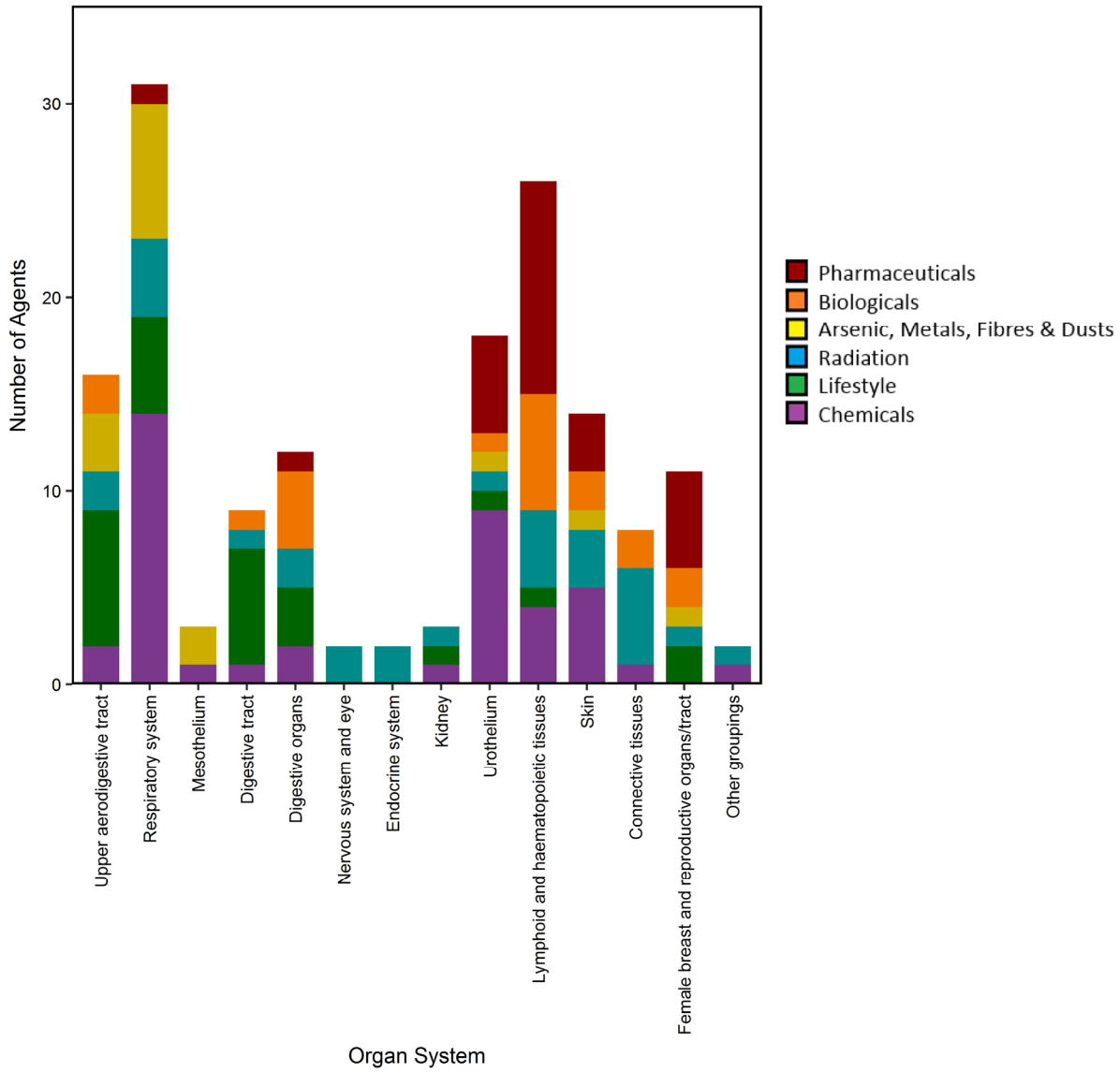
The overlap between human and animal tumour sites targeted by these 60 agents is summarized in Table 21.7 by organ and tissue system and tumour site. The category "other groupings" of tumours – which comprises "all cancers combined", "all solid cancers", and "exocrine glands not otherwise specified" – was created to accommodate tumour sites reported in the *IARC Monographs* that did not fall into any of the other categories in Table 21.2. The only human site identified for 2,3,7,8-tetrachlorodibenzo-*para*-dioxin (TCDD) is "all cancers combined"; fission products including strontium-90 are associated with "all solid cancers" in humans, but also with tumours in haematopoietic tissue. Because this category lacks biological cohesiveness, "other groupings" of tumours were not considered in the concordance analysis.

Nine agents cause tumours of the upper aerodigestive tract in humans, and nine agents cause tumours in this organ and tissue system in animals; four agents cause tumours in this system in both humans and animals. There are 9 + 9 − 4 = 14 distinct agents that cause tumours in this system in either humans or animals, for an overlap of 4 of 14, or 29%. Within the upper aerodigestive tract, there are three agents that cause tumours in the nasal cavity and paranasal sinuses in humans and three agents that cause tumours at this site in animals, with no overlap. Of the three agents that induce tumours in the nasopharynx, one agent causes tumours in both humans and animals, for an overlap of 33%. In the oral cavity, overlap is 25%. Overlap is not calculated when there are no agents that cause tumours in either humans or animals, as in the pharynx, tongue, and salivary gland.

The lung is the most common site at which tumours are observed, with 62% overlap among the 26 agents that cause lung tumours in humans or animals. Among the 10 agents that cause tumours in the urothelium (renal pelvis, ureter, or bladder), there is 70% overlap between agents that cause tumours in humans or animals.

Because results for individual tumour sites are often based on small numbers, emphasis is placed on interpretation of results at the organ and tissue system level, where the sample size is generally larger than for individual tumour sites within organ and tissue systems. Overlap varies among the organ and tissue systems, ranging from 20% (based on 10 agents) in the digestive tract

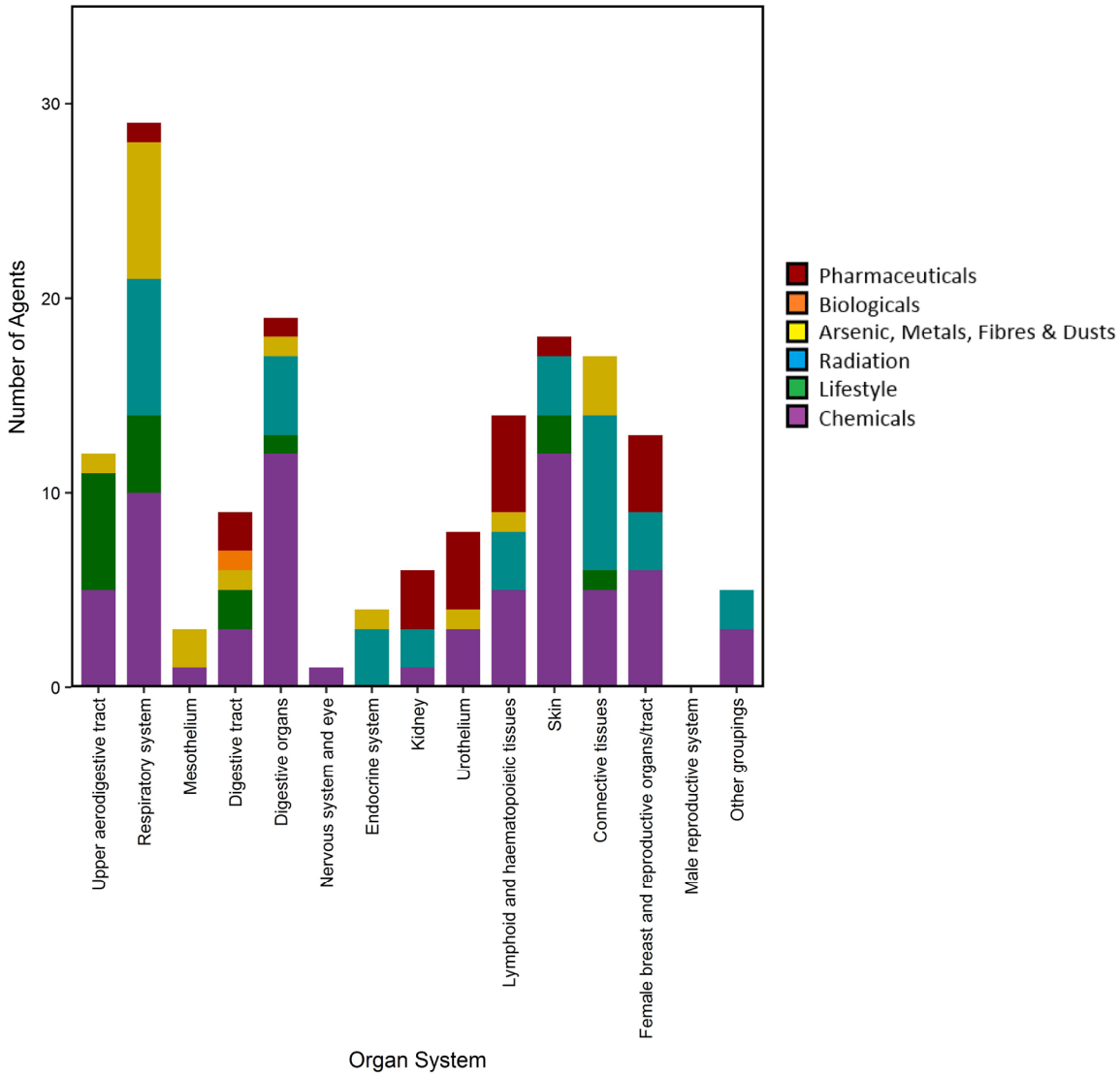
Fig. 21.5. Number of agents that induce tumours in humans in each of 14 organ and tissue systems, by type of agent.



to 100% in the mesothelium. Overall, high overlap is seen for some organ and tissue systems but not for others. Some caution is needed in interpreting concordance at sites where the sample size is particularly small: although 100% concordance was noted for agents that cause tumours of the mesothelium, only two Group 1 agents – asbestos and erionite – meeting the criteria for inclusion in the concordance analysis caused tumours at this site.

The results in Table 21.7 are depicted in graphical form in Fig. 21.9. As noted above, of the 14 Group 1 agents that cause tumours of the upper aerodigestive tract in either humans or animals, nine agents cause tumours in the upper aerodigestive tract in humans (and not in animals), nine agents cause tumours in this system in animals (and not in humans), and four agents cause tumours in this system in both humans and animals, for an overlap of 29%. Of the 27 agents that cause tumours of the respiratory system in either humans or animals, 21 agents cause respiratory tumours in humans, 22 agents cause respiratory tumours in animals, and 16 agents cause respiratory tumours in both humans

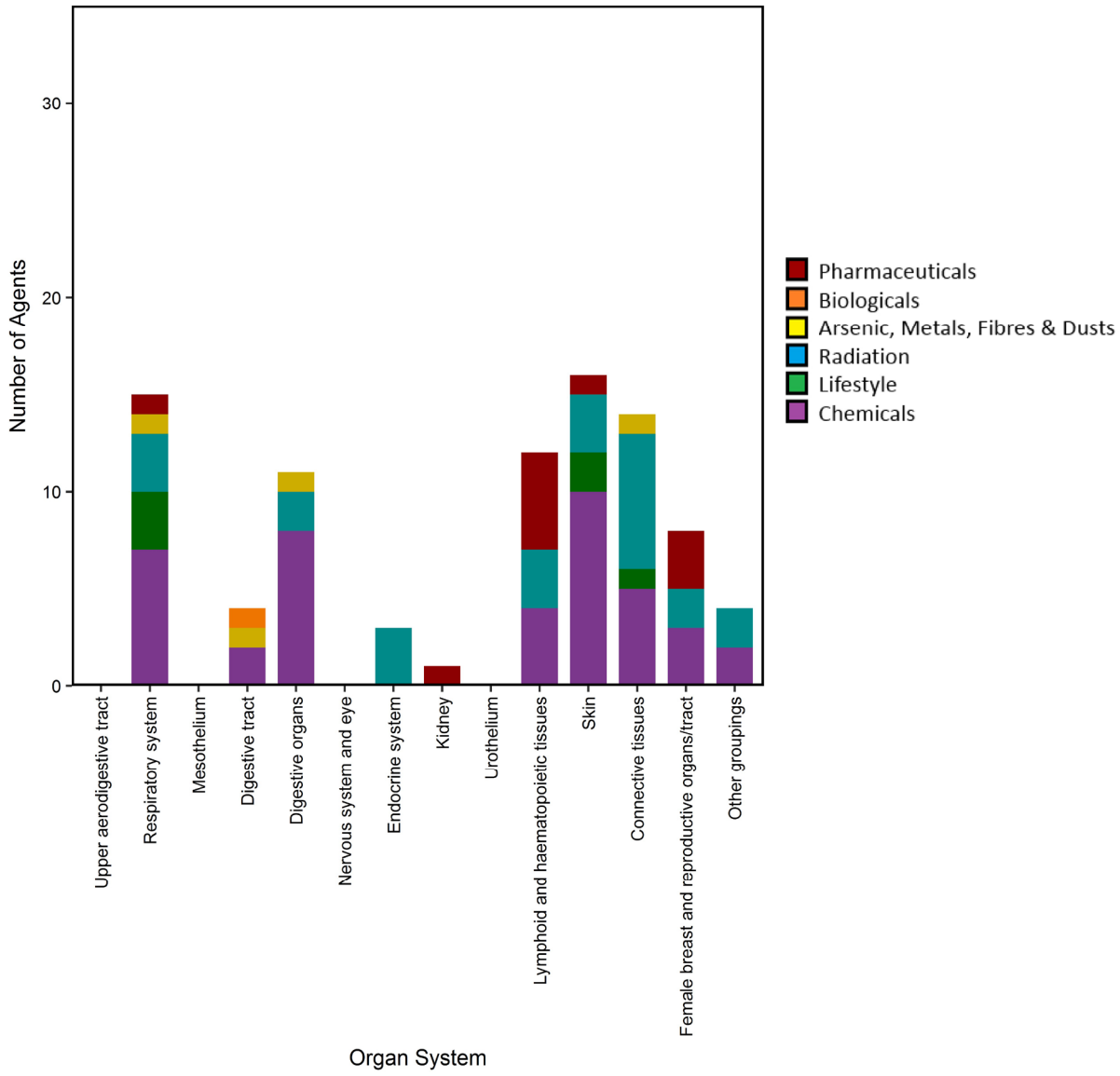
Fig. 21.6. Number of agents that induce tumours in animals in each of 14 organ and tissue systems, by type of agent.



and animals, for an overlap of 59%. Although they present the same data as shown in Table 21.7, the graphical representations of these results in Fig. 21.9 for all organ and tissue systems also illustrate the large variation in sample size among the organ and tissue systems; the area of the circles is proportional to sample size.

The results presented in Table 21.7 are based on concordance between tumour sites seen in humans and all animal species tested, reflecting the interest in evaluating the extent to which tumours caused by Group 1 agents occur in similar organ and tissue systems in humans and in animals. The animal data included in this analysis are dominated by results obtained in studies with rats and mice: of the 60 Group 1 agents included in the analysis, 40, 38, 8, 7, and 3 agents cause tumours in mice, rats, hamsters, dogs, and monkeys, respectively. Therefore, including only mice and rats in the analysis yielded results similar to those in Table 21.7 (see details in Supplemental Material II [online only; available from: http://publications.iarc.fr/578], where Supplemental Table 6 presents results for all animal species tested

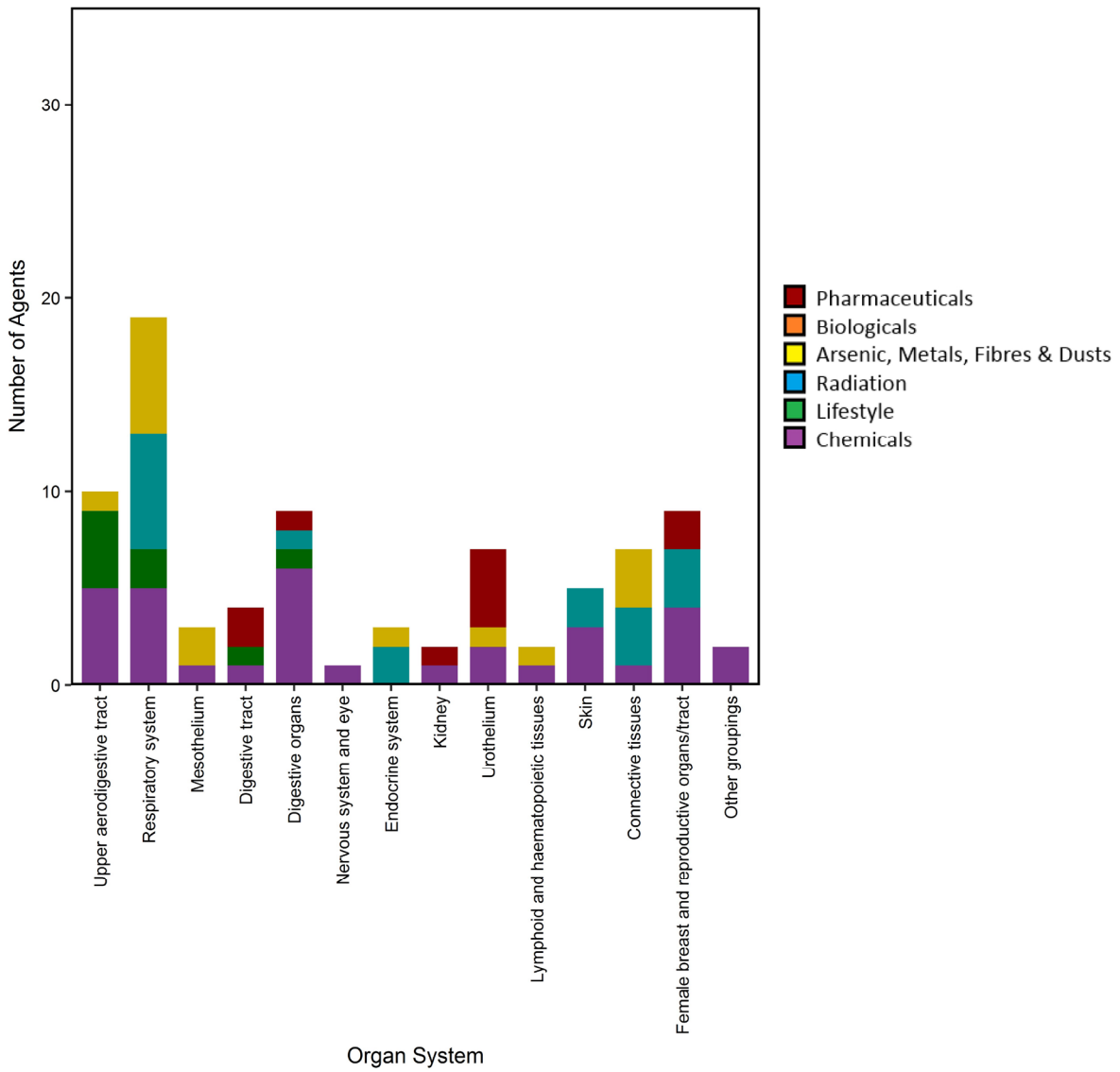
Fig. 21.7. Number of agents that induce tumours in mice in each of 14 organ and tissue systems, by type of agent.



and Supplemental Table 7 presents results for mice and rats only).

Fig. 21.10 shows the percentage of Group 1 agents that cause tumours in specific organ and tissue systems in humans that are also associated with tumours in animals (panel A), as well as the percentage of agents that cause tumours in specific organ and tissue systems in animals that are also associated with tumours in humans (panel B). As detailed in Supplemental Material II (online only; available from: http://publications.iarc.fr/578), it is important to note that the measures of concordance presented in Fig. 21.10 differ from those in Table 21.7. The percentage overlap in Table 21.7 (and Fig. 21.9) reflects the number of agents that cause tumours in a specific organ and tissue system in *both* humans *and* animals, relative to the number of agents that cause tumours in that system in *either* humans *or* animals, providing an overall measure of overlap between animal and human carcinogens in a specific organ and tissue system. The percentage overlap in panel A of Fig. 21.10 provides a measure of the overlap between agents that cause tumours in a specific organ and tissue system in animals with agents that cause tumours in that system in humans. Conversely, the percentage

Fig. 21.8. Number of agents that induce tumours in rats in each of 14 organ and tissue systems, by type of agent.



overlap in panel B of Fig. 21.10 provides a measure of the overlap between agents that cause tumours in a specific organ and tissue system in humans with agents that cause tumours in that system in animals. Note that unless the numbers of agents that cause tumours in humans and animals in a specific organ and tissue system are the same (as is the case for tumours of the upper aerodigestive tract), the results in panel A, where human carcinogens constitute the reference set against which animal carcinogens are compared, will differ from those in panel B, where animal carcinogens constitute the reference set for comparison with human carcinogens.

As indicated in panel A of Fig. 21.10, all agents (100%) that cause tumours of the mesothelium, endocrine system, and connective tissues in humans also cause tumours in those organ and tissue systems in animals. Overlap of at least 50% is observed for all other organ and tissue systems, with the exception of the upper aerodigestive tract (44%) and the digestive tract (33%). Conversely, there is less overlap between agents that cause tumours in specific organ and tissue systems in animals with results in humans (Fig. 21.10, panel B), possibly reflecting the larger number of studies

Table 21.7. Concordance between tumours seen in humans and animals for 60 Group 1 agents by organ and tissue system and tumour site

Organ and tissue system[a] *Tumour site*[a]	Number of agents			Overlap[b] (%)
	Humans	Animals	Both	
Upper aerodigestive tract	9	9	4	29
Nasal cavity and paranasal sinuses	*3*	*3*	*0*	*0*
Nasopharynx	*3*	*1*	*1*	*33*
Oral cavity	*4*	*6*	*2*	*25*
Pharynx	*2*	*0*	*0*	*N/A*
Tongue	*0*	*1*	*0*	*N/A*
Salivary gland	*1*	*0*	*0*	*N/A*
Respiratory system	21	22	16	59
Larynx	*3*	*1*	*1*	*33*
Lung	*20*	*22*	*16*	*62*
Mesothelium	2	2	2	100
Mesothelium	*2*	*2*	*2*	*100*
Digestive tract	6	6	2	20
Oesophagus	*5*	*0*	*0*	*N/A*
Stomach	*3*	*5*	*1*	*14*
Intestine (including colon and rectum)	*3*	*1*	*0*	*0*
Digestive organs	8	14	4	22
Liver parenchyma and bile ducts	*7*	*14*	*4*	*24*
Pancreas NOS	*2*	*0*	*0*	*N/A*
Gall bladder	*1*	*0*	*0*	*N/A*
Nervous system and eye	2	0	0	N/A
Brain and spinal cord (CNS)	*1*	*0*	*0*	*N/A*
Eye	*1*	*0*	*0*	*N/A*
Endocrine system	2	3	2	67
Thyroid, follicular epithelium	*2*	*2*	*2*	*100*
Adrenal gland (medulla, cortex, NOS)	*0*	*1*	*0*	*N/A*
Pituitary gland	*0*	*1*	*0*	*N/A*
Kidney	3	5	2	33
Kidney (renal cortex, renal medulla, kidney NOS)	*3*	*5*	*2*	*33*
Urothelium	10	7	7	70
Urothelium (renal pelvis, ureter, or bladder)	*10*	*7*	*7*	*70*

Table 21.7. Concordance between tumours seen in humans and animals for 60 Group 1 agents by organ and tissue system and tumour site (continued)

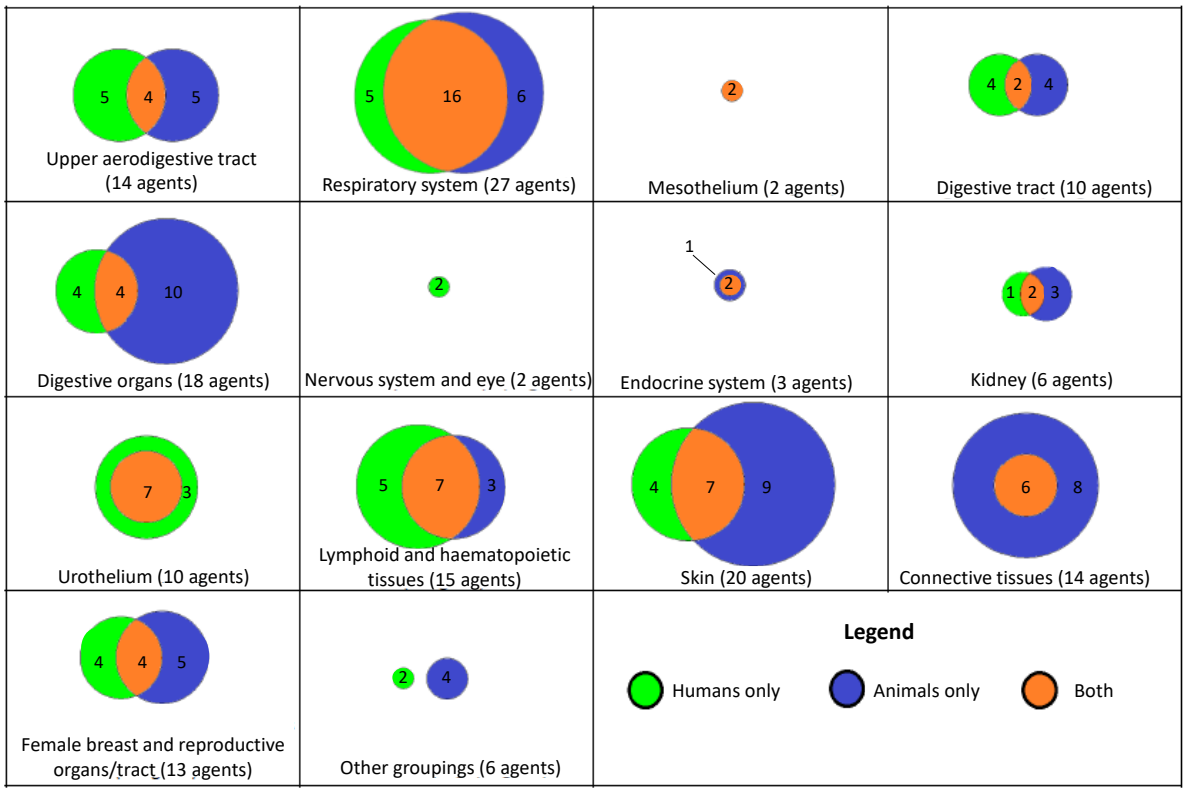
Organ and tissue system[a] *Tumour site*[a]	Number of agents			Overlap[b] (%)
	Humans	Animals	Both	
Lymphoid and haematopoietic tissues	12	10	7	47
Haematopoietic tissues	*10*	*2*	*2*	*20*
Lymphoid tissue	*2*	*10*	*1*	*9*
Skin	11	16	7	35
Skin and adnexae	*9*	*16*	*6*	*32*
Cutaneous melanocytes	*3*	*0*	*0*	*N/A*
Connective tissues	6	14	6	43
Soft connective tissue	*0*	*9*	*0*	*N/A*
Blood vasculature (endothelium)	*1*	*0*	*0*	*N/A*
Hard connective tissue (bone, cartilage)	*5*	*5*	*4*	*67*
Female breast, female reproductive organs, and female reproductive tract	8	9	4	31
Breast	*4*	*8*	*2*	*20*
Ovary	*3*	*1*	*0*	*0*
Uterine cervix	*3*	*2*	*1*	*25*
Uterus	*2*	*2*	*1*	*33*
Vulva/vagina	*1*	*0*	*0*	*N/A*
Other groupings	2	4	0	0
All cancers combined	*1*	*0*	*0*	*N/A*
All solid cancers	*1*	*0*	*0*	*N/A*
Exocrine glands NOS	*0*	*4*	*0*	*N/A*

CNS, central nervous system; N/A, not applicable: assigned to sites/systems when overlap is not possible (positive data are available in either humans or animals, but not in both); NOS, not otherwise specified.

[a] Systems/sites in the anatomically based tumour nomenclature system (see Table 21.2) that lack *sufficient evidence* in both humans and animals not shown. For example, there were insufficient data on tumours of the male reproductive tract in both humans and animals.

[b] Percentage overlap calculated as $[N_b/(N_h + N_a - N_b)] \times 100\%$, where N_h, N_a, and N_b denote the number of agents with *sufficient evidence* of carcinogenicity in humans, animals, or both humans and animals, respectively.

Fig. 21.9. Concordance between tumour sites seen in humans and animals for 60 Group 1 agents by organ and tissue system.



conducted in animals compared with humans, the broader spectrum of tissues (potential tumour sites) examined in animal studies than in human studies, or the limitations associated with the conduct of human studies at environmental exposure levels. As is the case with the concordance results focusing on overall overlap, as presented in Table 21.7, caution is needed in interpreting results where there are few agents for comparison in Fig. 21.10 (both panels A and B).

The 60 agents included in the present concordance analysis are listed in Table 21.8. This table presents the tumour site data for humans and animals at the organ and tissue system level only, because results for individual tumour sites are too sparse to support meaningful comparisons. The human data are presented in the column on the left, the animal data in the column on the right, and the overlap in the middle column. With this display, potential relationships among agents that cause tumours within the same organ and tissue system can be examined. Overlap between human and animal carcinogens acting within the same organ and tissue system can also be examined both for individual agents and for groups of agents. Of the 60 agents for which there is *sufficient evidence* of carcinogenicity in at least one tumour site in both humans and animals, 52 (87%) cause tumours within at least one of the same organ and tissue systems in Table 21.8.

To permit a more complete comparison between animal and human tumour sites, tumour sites with only *limited evidence* in humans are included in Table 21.8 (in *italics*). For agents such as diethylstilbestrol (a synthetic non-steroidal estrogen that was widely prescribed in the USA between the 1940s and the 1970s but is rarely used now), there is difficulty in generating newer data on human exposure. Because men exposed to diethylstilbestrol in utero

Fig. 21.10. Overlap between Group 1 agents with *sufficient evidence* of carcinogenicity in humans and animals that cause tumours in specific organ and tissue systems. (A) Overlap between animals and humans; the number of Group 1 agents that cause tumours in specific organ and tissue systems in humans is shown. (B) Overlap between humans and animals; the number of Group 1 agents that cause tumours in specific organ and tissue systems in animals is shown.

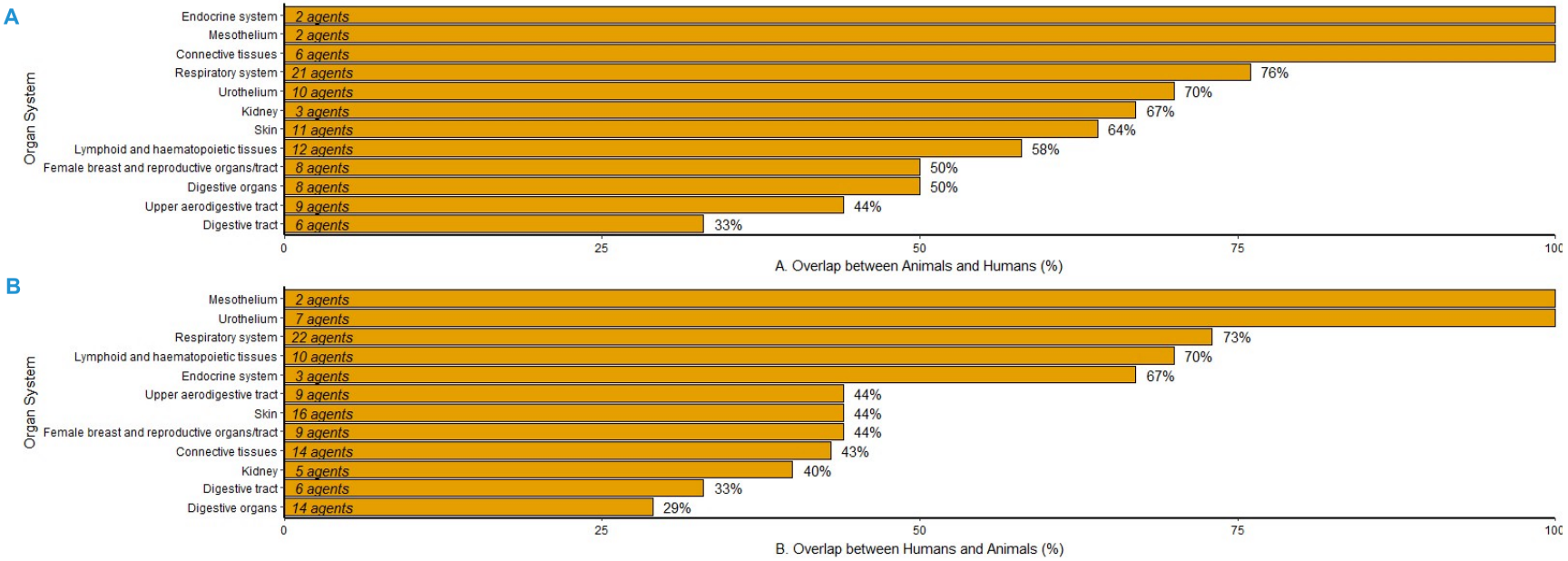


Table 21.8. Comparison of 60 Group 1 agents with *sufficient* or *limited evidence* of carcinogenicity in humans and *sufficient evidence* of carcinogenicity in animals in specific organ and tissue systems[a]

Humans[b] **Agent (*Monographs* Volume[c])**	**Humans and animals[b]** **Agent (*Monographs* Volume)**	**Animals[b]** **Agent (*Monographs* Volume)**
Upper aerodigestive tract (29% overlap[d])		
Chromium(VI) compounds (100C) Nickel compounds (100C) Radium-226 and decay products (100D) X- and γ-radiation (100D) *Radioiodines, including iodine-131(100D)* Betel quid without tobacco (100E) Alcoholic beverages (100E) Salted fish, Chinese-style (100E) *Second-hand tobacco smoke (100E)* Tobacco, smokeless (100E) Tobacco smoking (100E) Formaldehyde (100F)	Alcoholic beverages (100E) Salted fish, Chinese-style (100E) Tobacco, smokeless (100E) Formaldehyde (100F) *Chromium(VI) compounds (100C)*	Chromium(VI) (100C) Alcoholic beverages (100E) Salted fish, Chinese-style (100E) Tobacco, smokeless (100E) Formaldehyde (100F) Benzene (100F) 2,3,7,8-Tetrachlorodibenzo-*para*-dioxin (100F) Polychlorinated biphenyls (100F) Bis(chloromethyl)ether; Chloromethyl methyl ether (100F)

Table 21.8. Comparison of 60 Group 1 agents with *sufficient* or *limited evidence* of carcinogenicity in humans and *sufficient evidence* of carcinogenicity in animals in specific organ and tissue systems[a] (continued)

Humans[b] **Agent (*Monographs* Volume[c])**	**Humans and animals[b]** **Agent (*Monographs* Volume)**	**Animals[b]** **Agent (*Monographs* Volume)**
Respiratory system (59% overlap)		
Arsenic and inorganic arsenic compounds (100C) Asbestos (all forms, including actinolite, amosite, anthophyllite, chrysotile, crocidolite, and tremolite) (100C) Beryllium and beryllium compounds (100C) Cadmium and cadmium compounds (100C) Chromium(VI) compounds (100C) Nickel compounds (100C) Silica dust, crystalline, in the form of quartz or cristobalite (100C) Haematite mining with exposure to radon (underground) (100D) Plutonium-239 (100D) Radon-222 and its decay products (100D) X- and γ-radiation (100D) Alcoholic beverages (100E) Coal, indoor emissions from household combustion of (100E) Second-hand tobacco smoke (100E) Tobacco smoking (100E) Bis(chloromethyl)ether; Chloromethyl methyl ether (technical grade) (100F) Coal gasification (100F) Coal-tar pitch (100F) Coke production (100F) Soot (as found in occupational exposure of chimney sweeps) (100F) 2,3,7,8-Tetrachlorodibenzo-*para*-dioxin (100F) Engine exhaust, diesel (100F)	Arsenic and inorganic arsenic compounds (100C) Asbestos (all forms, including actinolite, amosite, anthophyllite, chrysotile, crocidolite, and tremolite) (100C) Beryllium and beryllium compounds (100C) Cadmium and cadmium compounds (100C) Chromium(VI) compounds (100C) Nickel compounds (100C) Silica dust, crystalline, in the form of quartz or cristobalite (100C) Haematite mining with exposure to radon (underground) (100D) Plutonium-239 (100D) Radon-222 and its decay products (100D) X- and γ-radiation (100D) Coal, indoor emissions from household combustion of (100E) Second-hand tobacco smoke (100E) Tobacco smoking (100E) Coke production (100F) Engine exhaust, diesel (100F) 2,3,7,8-Tetrachlorodibenzo-*para*-dioxin (100F)	Cyclophosphamide (100A) Arsenic and inorganic arsenic compounds (100C) Asbestos (all forms, including actinolite, amosite, anthophyllite, chrysotile, crocidolite, and tremolite) (100C) Beryllium and beryllium compounds (100C) Cadmium and cadmium compounds (100C) Chromium(VI) compounds (100C) Nickel compounds (100C) Silica dust, crystalline, in the form of quartz or cristobalite (100C) Haematite mining with exposure to radon (underground) (100D) Plutonium-239 (100D) Radon-222 and its decay products (100D) X- and γ-radiation (100D) Coal, indoor emissions from household combustion of (100E) Second-hand tobacco smoke (100E) Tobacco smoking (100E) Benzene (100F) 1,3-Butadiene (100F) Coke production (100F) Vinyl chloride (100F) Engine exhaust, diesel (100F*) 2,3,7,8-Tetrachlorodibenzo-*para*-dioxin (100F*) Trichloroethylene (100F*)

Table 21.8. Comparison of 60 Group 1 agents with *sufficient* or *limited evidence* of carcinogenicity in humans and *sufficient evidence* of carcinogenicity in animals in specific organ and tissue systems[a] (continued)

Humans[b] **Agent (*Monographs* Volume[c])**	**Humans and animals[b]** **Agent (*Monographs* Volume)**	**Animals[b]** **Agent (*Monographs* Volume)**
Mesothelium (100% overlap)		
Asbestos (all forms, including actinolite, amosite, anthophyllite, chrysotile, crocidolite, and tremolite) (100C) Erionite (100C)	Asbestos (all forms, including actinolite, amosite, anthophyllite, chrysotile, crocidolite, and tremolite) (100C) Erionite (100C)	Asbestos (all forms, including actinolite, amosite, anthophyllite, chrysotile, crocidolite, and tremolite) (100C) Erionite (100C)
Digestive tract (20% overlap)		
Helicobacter pylori (infection with) (100B) X- and γ-radiation (100D) *Radioiodines, including iodine-131 (100D)* Alcoholic beverages (100E) Betel quid without tobacco (100E) *Salted fish, Chinese-style (100E)* Tobacco smoking (100E) Tobacco, smokeless (100E)	*Helicobacter pylori* (infection with) (100B) Betel quid without tobacco (100E)	Aristolochic acid, plants containing (100A) *Helicobacter pylori* (infection with) (100B) Chromium(VI) compounds (100C) Betel quid without tobacco (100E) Benzene (100F) 1,3-Butadiene (100F)
Digestive organs (22% overlap)		
Estrogen–progestogen oral contraceptives (combined) (100A) *Arsenic and inorganic arsenic compounds (100C)* *Cadmium and cadmium compounds (100C)* Thorium-232 (as Thorotrast) (100D) Plutonium-239 (100D) *X- and γ-radiation (100D)* Alcoholic beverages (100E) *Betel quid without tobacco (100E)* Tobacco smoking (100E) Tobacco, smokeless (100E) Aflatoxins (100F) Vinyl chloride (100F) *Trichloroethylene (100F*)*	Arsenic and inorganic arsenic compounds (100C) Plutonium-239 (100D) Thorium-232 (as Thorotrast) (100D) X- and γ-radiation (100D) Aflatoxins (100F) Vinyl chloride (100F) Trichloroethylene (100F*)	Tamoxifen (100A) Arsenic and inorganic arsenic compounds (100C) Thorium-232 (as Thorotrast) (100D) Plutonium-239 (100D) X- and γ-radiation (100D) Aflatoxins (100F) 4-Aminobiphenyl (100F) Benzidine (100F) 1,3-Butadiene (100F) 2-Naphthylamine (100F) 2,3,7,8-Tetrachlorodibenzo-*para*-dioxin (100F) Vinyl chloride (100F) Trichloroethylene (100F*) Polychlorinated biphenyls (100F)

Table 21.8. Comparison of 60 Group 1 agents with *sufficient* or *limited evidence* of carcinogenicity in humans and *sufficient evidence* of carcinogenicity in animals in specific organ and tissue systems[a] (continued)

Humans[b] **Agent (*Monographs* Volume[c])**	**Humans and animals[b]** **Agent (*Monographs* Volume)**	**Animals[b]** **Agent (*Monographs* Volume)**
Nervous system and eye (N/A)		
UV-emitting tanning devices (100D) **X- and γ-radiation (100D)** *Solar radiation (100D)*		
Endocrine system (67% overlap)		
Radioiodines, including iodine-131 (100D) X- and γ-radiation (100D)	Radioiodines, including iodine-131 (100D) X- and γ-radiation (100D)	Nickel compounds (100C) Radioiodines, including iodine-131 (100D) X- and γ-radiation (100D)
Kidney (33% overlap)		
Arsenic and inorganic arsenic (100C) *Cadmium and cadmium compounds (100C)* X- and γ-radiation (100D) Tobacco smoking (100E) Trichloroethylene (100F*)	X- and γ-radiation (100D) Trichloroethylene (100F*)	Diethylstilbestrol (100A) Estrogen-only menopausal therapy (100A) Phenacetin (100A) X- and γ-radiation (100D) Trichloroethylene (100F*)
Urothelium (70% overlap)		
Aristolochic acid, plants containing (100A) Cyclophosphamide (100A) Phenacetin (100A) Arsenic and inorganic arsenic compounds (100C) X- and γ-radiation (100D) Tobacco smoking (100E) *Coal-tar pitch (100F)* *Soot (as found in occupational exposure of chimney sweeps) (100F)* 4-Aminobiphenyl (100F) Benzidine (100F) 2-Naphthylamine (100F) *ortho*-Toluidine (100F) *Engine exhaust, diesel (100F*)*	Aristolochic acid, plants containing (100A) Cyclophosphamide (100A) Phenacetin (100A) Arsenic and inorganic arsenic compounds (100C) 4-Aminobiphenyl (100F) 2-Naphthylamine (100F) *ortho*-Toluidine (100F)	Aristolochic acid, plants containing (100A) Cyclophosphamide (100A) Phenacetin (100A) Arsenic and inorganic arsenic compounds (100C) 2-Naphthylamine (100F) 4-Aminobiphenyl (100F) *ortho*-Toluidine (100F)

Table 21.8. Comparison of 60 Group 1 agents with *sufficient* or *limited evidence* of carcinogenicity in humans and *sufficient evidence* of carcinogenicity in animals in specific organ and tissue systems[a] (continued)

Humans[b] **Agent (*Monographs* Volume[c])**	**Humans and animals[b]** **Agent (*Monographs* Volume)**	**Animals[b]** **Agent (*Monographs* Volume)**
Lymphoid and haematopoietic tissues (47% overlap)		
Azathioprine (100A) Chlorambucil (100A) Cyclophosphamide (100A) Thiotepa (100A) *Helicobacter pylori* (infection with) (100B) Fission products including strontium-90 (100D) Thorium-232 (as Thorotrast) (100D) X- and γ-radiation (100D) *Radioiodines, including iodine-131(100D)* *Radon-222 and its decay products (100D)* Tobacco smoking (100E) *Ethylene oxide (100F)* Benzene (100F) 1,3-Butadiene (100F) Formaldehyde (100F) *Trichloroethylene (100F*)* *2,3,7,8-Tetrachlorodibenzo*-para-*dioxin (100F)* *Polychlorinated biphenyls (100F*)*	Azathioprine (100A) Chlorambucil (100A) Cyclophosphamide (100A) Thiotepa (100A) X- and γ-radiation (100D) Benzene (100F) 1,3-Butadiene (100F) 2,3,7,8-Tetrachlorodibenzo-*para*-dioxin (100F)	Azathioprine (100A) Chlorambucil (100A) Cyclophosphamide (100A) Estrogen-only menopausal therapy (100A) Thiotepa (100A) Silica dust, crystalline, in the form of quartz or cristobalite (100C) X- and γ-radiation (100D) Ethylene oxide (100F) Benzene (100F) 1,3-Butadiene (100F) 2,3,7,8-Tetrachlorodibenzo-*para*-dioxin (100F)

Table 21.8. Comparison of 60 Group 1 agents with *sufficient* or *limited evidence* of carcinogenicity in humans and *sufficient evidence* of carcinogenicity in animals in specific organ and tissue systems[a] (continued)

Humans[b] **Agent (*Monographs* Volume[c])**	**Humans and animals[b]** **Agent (*Monographs* Volume)**	**Animals[b]** **Agent (*Monographs* Volume)**
Skin (35% overlap)		
Azathioprine (100A) Methoxsalen in combination with UVA (100A) Arsenic and inorganic arsenic compounds (100C) Solar radiation (100D) UV-emitting tanning devices (100D) X- and γ-radiation (100D) Coal-tar distillation (100F) Mineral oils, untreated or mildly treated (100F) Shale oils (100F) Soot (as found in occupational exposure of chimney sweeps) (100F) Polychlorinated biphenyls (100F*)	Methoxsalen in combination with UVA (100A) Solar radiation (100D) UV-emitting tanning devices (100D) Coal-tar distillation (100F) Mineral oils, untreated or mildly treated (100F) Shale oils (100F) Soot (as found in occupational exposure of chimney sweeps) (100F)	Methoxsalen in combination with UVA (100A) Solar radiation (100D) UV-emitting tanning devices (100D) Coal, indoor emissions from household combustion of (100E) Tobacco smoking (100E) Benzene (100F) Bis(chloromethyl)ether; Chloromethyl methyl ether (technical grade) (100F) Coal gasification (100F) Coal-tar distillation (100F) Coal-tar pitch (100F) Coke production (100F) Mineral oils, untreated or mildly treated (100F) Shale oils (100F) Soot (as found in occupational exposure of chimney sweeps) (100F) 2,3,7,8-Tetrachlorodibenzo-*para*-dioxin (100F) *ortho*-Toluidine (100F)

Table 21.8. Comparison of 60 Group 1 agents with *sufficient* or *limited evidence* of carcinogenicity in humans and *sufficient evidence* of carcinogenicity in animals in specific organ and tissue systems[a] (continued)

Humans[b] **Agent (*Monographs* Volume[c])**	**Humans and animals[b]** **Agent (*Monographs* Volume)**	**Animals[b]** **Agent (*Monographs* Volume)**
Connective tissues (43% overlap)		
Plutonium-239 (100D) Radium-224 and its decay products (100D) Radium-226 and its decay products (100D) Radium-228 and its decay products (100D) X- and γ-radiation (100D) *Radioiodines, including iodine-131 (100D)* Vinyl chloride (100F) *2,3,7,8-Tetrachlorodibenzo*-para-*dioxin (100F)*	Plutonium-239 (100D) Radium-224 and its decay products (100D) Radium-226 and its decay products (100D) Radium-228 and its decay products (100D) X- and γ-radiation (100D) Vinyl chloride (100F)	Cadmium and cadmium compounds (100C) Chromium(VI) compounds (100C) Nickel compounds (100C) Fission products including strontium-90 (100D) Plutonium-239 (100D) Radium-224 and its decay products (100D) Radium-226 and its decay products (100D) Radium-228 and its decay products (100D) X- and γ-radiation (100D) 4-Aminobiphenyl (100F) Bis(chloromethyl)ether; Chloromethyl methyl ether (technical grade) (100F) 1,3-Butadiene (100F) *ortho*-Toluidine (100F) Vinyl chloride (100F)
Female breast, female reproductive organs, and female reproductive tract (31% overlap)		
Diethylstilbestrol (100A) Estrogen-only menopausal therapy (100A) Estrogen–progestogen oral contraceptives (combined) (100A) Tamoxifen (100A) Asbestos (all forms, including actinolite, amosite, anthophyllite, chrysotile, crocidolite, and tremolite) (100C) X- and γ-radiation (100D) Alcoholic beverages (100E) Tobacco smoking (100E) *Ethylene oxide (100F)* *Polychlorinated biphenyls (100F*)*	Diethylstilbestrol (100A) Estrogen-only menopausal therapy (100A) Estrogen–progestogen oral contraceptives (combined) (100A) X- and γ-radiation (100D)	Cyclophosphamide (100A) Diethylstilbestrol (100A) Estrogen-only menopausal therapy (100A) Estrogen–progestogen oral contraceptives (combined) (100A) X- and γ-radiation (100D) Benzene (100F) Benzidine (100F) 1,3-Butadiene (100F) Vinyl chloride (100F)

Table 21.8. Comparison of 60 Group 1 agents with *sufficient* or *limited evidence* of carcinogenicity in humans and *sufficient evidence* of carcinogenicity in animals in specific organ and tissue systems[a] (continued)

Humans[b] **Agent (*Monographs* Volume[c])**	**Humans and animals[b]** **Agent (*Monographs* Volume)**	**Animals[b]** **Agent (*Monographs* Volume)**
Male reproductive organs including prostate and testes (overlap N/A)		
Diethylstilbestrol (100A) *Arsenic and inorganic arsenic compounds (100C)* *Cadmium and cadmium compounds (100C)* *Thorium-232 (as Thorotrast) (100D)* *X- and γ-radiation (100D)*		
Other groupings (0%)		
2,3,7,8-Tetrachlorodibenzo-*para*-dioxin (100F) [all cancers combined] Fission products including strontium-90 (100D) [all solid cancers] *Plutonium-239 (100D)*		X- and γ-radiation (100D) [exocrine glands NOS] Benzene (100F) [exocrine glands NOS] 1,3-Butadiene (100F) [exocrine glands NOS] Vinyl chloride (100F) [exocrine glands NOS]

N/A, not applicable: denotes organ and tissue systems when overlap is not possible (positive data are available in either humans or animals, but not in both); UV, ultraviolet.

[a] Organ and tissue systems in the anatomically based tumour nomenclature system (see Supplemental Table 1. Animal and human tumour sites for 111 Group 1 agents identified up to and including Volume 109 of the *IARC Monographs*). Data inputs for human and animal data with *sufficient evidence* of carcinogenicity are from Supplemental Table 2. Database of animal and human tumour sites for 111 distinct Group 1 agents up to and including Volume 109 of the *IARC Monographs*. Agents that lack *sufficient evidence* in both humans and animals are not shown, with the exception of limited additional data inputs for *limited evidence* of human sites from Volumes 100A–F, Volume 107, and Volume 109 (in *italics*) and included data for ethylene oxide, estrogen–progestogen oral contraceptives, and diethylstilbestrol. Data for male reproductive organs are also included, although they are not part of the concordance analyses. 2,3,7,8-Tetrachlorodibenzo-*para*-dioxin is included, but its designation of "all cancers combined" for human data precludes specific site analyses between species.

[b] Agents with *sufficient evidence* in humans, animals, and both humans and animals.

[c] Part A, B, C, D, E, or F in Volume 100 of the *IARC Monographs* in which the agent is included. Volume 100F* denotes chemical agents and related occupations identified as Group 1 agents after the publication of Volume 100.

[d] Number of agents with *sufficient evidence* in both humans and animals, as a percentage of the total number of agents that cause tumours in either humans or animals (or both) in the specified organ and tissue system (see Table 21.7).

have passed the age of highest risk for testicular cancer, further study cannot clarify the association between this exposure and this type of cancer (IARC, 2012e). Human data for this agent will remain limited for this end-point, although supported by the induction of testicular tumours in rodents.

With ongoing studies, more evidence can be gathered that provides increasing certainty about potential cancer risks to humans. Although IARC had previously evaluated TCE in 1979, 1987, and 1995, this substance was not declared to be *carcinogenic to humans* – causing kidney cancer – until 2012, after the emergence of new data (IARC, 2014). Although it was noted that a positive association had been observed between liver cancer and exposure to TCE, the lack of data was cited as the rationale for its designation as demonstrating only *limited evidence* of carcinogenicity in humans in the previous evaluations. In 2013, an updated pooled analysis of three Nordic studies with 10–15 years of additional follow-up demonstrated that human exposure to TCE was associated with a possibly increased risk of liver cancer (Hansen et al., 2013). Inclusion of the limited data for TCE-induced liver cancer in humans allows for the observation of overlap between animals and humans for this end-point.

This example illustrates that the inclusion of agents with *limited evidence* of carcinogenicity in humans enhances the ability to identify concordant relationships. Comparison between Table 21.7, which mentions only sites with *sufficient evidence* in humans, and Table 21.8, which also lists sites with *limited evidence* in humans, illustrates increased coherence, when limited human data are considered, among agents that have similar chemical and mechanistic characteristics. For example, if the *limited evidence* of tumours of the upper aerodigestive tract for chromium(VI) compounds in humans noted in Table 21.8 were admitted as evidence of carcinogenicity in humans, concordance between animals and humans would be established within this organ and tissue system.

Concordance may also be increased if less stringent criteria are applied than are used by IARC for determining *sufficient evidence* of carcinogenicity in animals. In evaluating the available animal data on estrogen–progestogen oral contraceptives (IARC, 2012e), it was concluded that "the data evaluated showed a consistent carcinogenic effect of several estrogen–progestogen combinations across different animal models in several organs." Similarly, the synthesis statement in the evaluation of diethylstilbestrol (IARC, 2012e) notes: "The oral administration of diethylstilbestrol induced tumours of the ovary, endometrium, and cervix, and mammary adenocarcinomas in female mice. Osteosarcomas and Leydig cell tumours were induced in rasH2 [transgenic] and Xpa/p53 [knockout] male mice, respectively. Subcutaneous implantation of diethylstilbestrol induced mammary tumours in female Wistar rats. Perinatal exposure to diethylstilbestrol induces lymphoma, uterine sarcomas, adenocarcinomas, and pituitary, vaginal, and ovarian tumours in female mice. Uterine adenocarcinomas and mammary and vaginal tumours were also induced in female rats. In hamsters, diethylstilbestrol perinatal exposure induced kidney tumours."

Although agents affecting male reproductive organs are included in Table 21.8, they are not part of the concordance analyses in Table 21.7, because of a lack of *sufficient evidence* in either humans or animals. TCDD is included in Table 21.8, but its designation as an agent affecting "all cancers combined" in humans precludes site-specific tumour concordance analyses. Nevertheless, the *limited evidence* of carcinogenicity of TCDD in humans in the respiratory system and lymphoid and hematopoietic tissues is consistent with the *sufficient evidence* of carcinogenicity in animals in these two organ and tissue systems. These examples illustrate increased site concordance by applying less stringent criteria than those applied for the concordance analysis presented in Table 21.7.

Table 21.8 shows human data indicating biological plausibility for the upper aerodigestive tract and lung to be targets for agents for which the portal of entry is the lung (as with dusts, particles, and particles that serve as a vehicle for a mixture of other carcinogens, such as during tobacco smoking and coke production). Lymphohaematopoietic cancers are a consistent end-point for antineoplastic alkylating agents that induce these cancers after their use in chemotherapy to eradicate other neoplasms (IARC, 2012e), for radioactive materials (IARC, 2012f), and for several chemical agents and related compounds that are metabolized to or are in themselves agents that are reactive with DNA (IARC, 2012c).

Table 21.8 also illustrates some of the potential relationships between agents that may act in a similar fashion in humans. Tobacco smoke and its related agents (smokeless tobacco and second-hand tobacco smoke) affect several similar organ

and tissue systems. For radioactive materials, almost all organs and sites are affected by ionizing radiation; these agents affect multiple target tissues because they are able to reach the nucleus and cause a variety of DNA lesions and other effects reflected by the key characteristics of human carcinogens (see Chapter 10, by Smith, and Chapter 22, by Krewski et al.; see also Smith et al., 2016).

Radioactive materials also do not require metabolism in order to induce cancer. Several dyes are associated with urothelial cancer in humans and act through a similar mechanism (IARC, 2012c). Agents that disrupt the endocrine system and related organs (e.g. PCBs, diethylstilbestrol, estrogen-only menopausal therapy, combined estrogen–progestogen oral contraceptives, and tamoxifen) induce cancer at similar sites, including the female reproductive organs and the breast. Metals appear to have many target sites in common, including the upper aerodigestive tract, the respiratory system, the kidney, and the prostate.

As noted previously, the animal database is predominantly populated by results from studies in rodents. Respiratory tract tumours are induced in rodents by many of the same agents that cause such tumours in humans. For the mesothelium, where tumour formation in humans or animals is rare and is specifically induced by a small number of agents, there is good agreement between the human and animal databases. Many agents metabolized in the liver to reactive compounds induce liver cancer in animal models, with less apparent overlap with the human data (see digestive organs, Table 21.8). Susceptibility of the liver in rodents to cancer induction is species-, sex-, and strain-specific and varies widely. Nonetheless, all agents that induce liver cancer in rodents induce cancer at some other site in humans. In some instances the apparent lack of overlap between the animal and human databases can still reflect mechanistic concordance for similar agents. Dyes such as magenta, 4-aminobiphenyl, benzidine, and 2-naphthylamine all cause liver cancer in rodents and urothelial cancer in humans. TCDD and PCBs are both associated with liver cancer in rodents and tumours of the lymphoid and haematopoietic tissues in humans.

Human exposures to diethylstilbestrol, estrogen-only menopausal therapy, and combined estrogen–progestogen oral contraceptives are all associated with cancers of the female breast, female reproductive organs, and female reproductive tract. Kidney cancer is induced in male hamsters upon exposure to either diethylstilbestrol or estrogens used in menopausal therapy. Data from a control group that received only estrogen, presented in the *Monograph* on combined estrogen–progestogen oral contraceptives, indicate a similar result (IARC, 2012e). Although there appears to be concordance in rodents for the tumours induced by these agents, there does not appear to be overlap with humans: rodent kidney versus female breast and reproductive organs. However, there may be mechanistic concordance between these two end-points, because both diethylstilbestrol and estrogen may damage DNA through oxidative damage, formation of unstable adducts, and induction of apurinic sites. In male Syrian hamsters the major metabolites of diethylstilbestrol are catechols that easily oxidize to catechol *o*-quinones, which are DNA-reactive. Implantation of estrone or estradiol in castrated male hamsters results in the induction of renal carcinomas exclusively (Li et al., 1983). Metabolic activation of estrogens by cytochrome P450 may also be related to a mechanism similar to that for PAHs (Cavalieri and Rogan, 2014). Thus, diethylstilbestrol and estrogen may have mechanistic similarities that result in an apparent lack of organ and tissue system overlap, with the hamster kidney being indicative of human risk.

Discussion

Since the early 1970s, the *IARC Monographs Programme* has been evaluating potential cancer risks to humans (Saracci and Wild, 2015). Separate evaluations of the available animal and human evidence are made, and these are then combined to make an overall evaluation of the strength of evidence of carcinogenicity to humans. At the time of this analysis, 120 distinct agents have met the IARC criteria for determining causality and for designation of these agents as *carcinogenic to humans* (Group 1). Of these, 111 distinct Group 1 agents were included in the data set of tumours and tumour sites in animals and humans developed by Grosse et al. (Annex 1).

The well-established weight-of-evidence criteria for the evaluation of the available human, animal, mechanistic, and exposure data used by IARC are detailed in the Preamble to the *IARC Monographs* (IARC, 2006) and provide clear guidance to the Working Groups convened to review agents. If the criteria for *sufficient evidence* of carcinogenicity in both animals and humans are satisfied, then causality can be reasonably inferred, and this can be strengthened by mechanistic considerations.

However, an immediate challenge in making comparisons for tumour site concordance between species was how to compare tumours in animals and in humans. A detailed historical discussion of approaches to the coding of human tumours was provided by Muir and Percy (1991), considering the topographical, morphological, and histological characteristics of the lesion to be classified. In the absence of a common coding system for animal and human tumours, an anatomically based tumour taxonomy system was developed during the course of the work presented here.

Although this system worked well for the purposes of the present concordance analysis, there are some animal sites that do not have a human counterpart, including the Harderian gland and the Zymbal gland. Tumours at these unique sites occurred rarely and were included within the category of "other groupings" in the anatomically based tumour nomenclature system used here. Other sites that are unique to animals but are, however, closely related to a similar human site were aligned with the corresponding human tumour site; for example, the forestomach was considered as part of the stomach in the anatomically based taxonomy system.

This tool, developed for tumour comparisons across and within species, included 39 individual tumour sites for which agents showed *sufficient evidence* of carcinogenicity in humans and/or animals, which were further aggregated into 14 organ and tissue systems. This aggregation allows comparisons to be made at a higher level of organization, reflecting anatomical and physiological similarities among certain tumour sites; for example, the lung and lower respiratory tract are considered together as the respiratory system. Aggregation also allows more data to be considered for analysis, which increases the robustness of the ensuing conclusions. For the concordance analyses, data at both the individual tumour site level and the organ and tissue system level were examined.

Although the present analysis demonstrates generally good agreement between tumour sites in animals and in humans after exposure to Group 1 carcinogens, concordance was not demonstrated with every agent and tumour site. There are several factors and important limitations that may result in lack of tumour concordance based on these data. For many of the 111 agents, relevant and reliable data to support a complete analysis of concordance are unavailable for either animals or humans. For some agents, notably the human tumour viruses, relevant animal models are lacking, thereby precluding the possibility of obtaining results on concordance. There may also be little motivation for conducting animal tests for other agents, such as leather dust in occupational environments or acetaldehyde associated with consumption of alcoholic beverages. Mixtures such as those in combined estrogen–progestogen menopausal therapy may also not have been evaluated in animals, particularly if the components of the mixture had been previously evaluated separately. Relevant animal tests may still provide only *limited* or *inadequate* evidence of carcinogenicity through limitations in study design or conduct, or if the mechanism of action of the agent of interest was specific to humans and not easily replicated in an experimental animal model. Animal studies may also show tumours that are species- and/or sex-specific.

As part of the determination of weight of evidence, agents that induce tumours at multiple sites and across multiple species are considered to present a more robust cancer hazard to humans. However, the experimental animal database used for the analysis consists primarily of rodent data. It is notable that of the 111 Group 1 agents examined here, three agents caused tumours in humans and in four animal species (mice, rats, hamsters, and non-human primates): asbestos, which causes lung tumours in all five species; plutonium-239, which causes skin tumours in these species; and 2-naphthylamine, which causes urinary tract/uroendothelial tumours in these species. These agents are examples of carcinogens that cause the same type of tumour in multiple species, thereby demonstrating a high degree of interspecies tumour site concordance.

The present analyses exclude the human tumour viruses evaluated in Volume 100B, because, with the possible exception of human T-cell lymphotropic virus type 1 (HTLV-1), the use of animals to assess the potential cancer risks of human tumour viruses is problematic (IARC, 2012b). The best animal models to study human viruses are non-human primates, which are difficult to use experimentally both because of the time and expense involved in conducting studies with long-lived species and because the incidence of cancer is low in non-human primates. Although transgenic mouse models have been developed for evaluating human cancer viruses, such models are considered more informative for understanding cancer

mechanisms than for human cancer risk assessment (see Chapter 9, by Lambert and Banks).

The criteria for *sufficient evidence* of carcinogenicity in animals as outlined in the Preamble to the *IARC Monographs* (IARC, 2006) generally require independent replication in two different animal species, or particularly strong results in a single species. The *IARC Monographs* generally do not identify animal tumour sites for agents with only *limited evidence* of carcinogenicity in animals. The criteria developed by Grosse et al. (Annex 1) further restrict the use of tumour data for agents with *sufficient evidence* in experimental animals (e.g. tumour sites were not identified in the absence of two or more animal studies of adequate design and quality pointing at the same tumour site with a similar histological origin in the same species). Although melphalan produced tumours of the forestomach, skin, and lung as well as lymphosarcomas in mice and mammary gland tumours and peritoneal sarcomas in rats (IARC, 2012c), none of these tumour sites were replicated in a second animal species, and hence are not included in the data set of Grosse et al. (Annex 1).

Human evidence is also subject to limitations. As noted above, the opportunity may no longer be available to conduct further informative studies in humans of a substance like diethylstilbestrol. The absence of *sufficient evidence* in humans may be due to a lack of evidence in appropriate epidemiological or clinical studies, or to the inability of existing studies to detect an association between exposure to the agent of interest (including exposures early or later in life) and a tumour outcome. Study limitations may also include inadequate power as a result of small sample size. If human exposures to the agent of interest are extremely low, a particularly large, well-conducted study would be required to achieve reasonable sensitivity.

Failure of human studies to identify tumour sites can occur when these studies do not consider all possible sites. Most case–control studies focus on only one or a limited number of tumour sites. Human studies that fail to identify a relevant tumour site may have low sensitivity, possibly because they do not focus on the most appropriate study population. As noted above for TCE, evidence on specific tumour sites may not yet have accrued at the time of an evaluation. After the first evaluation of tobacco smoking in Volume 38 of the *IARC Monographs* (IARC, 1986), cigarette smoking was subsequently shown – in Volume 83 – to cause cancer at a much larger number of tumour sites, including cancers of the nasal cavities and nasal sinuses, oesophagus, stomach, liver, kidney, and uterine cervix, and myeloid leukaemia (IARC, 2004). Thus, the potential for underestimation of interspecies tumour site concordance may result from missing tumour sites for agents for which *sufficient evidence* of carcinogenicity in humans already exists.

How human study data are reported in the *Monographs* may also affect the ability to conduct analyses to establish tumour site concordance. A specific example of this constraint is ionizing radiation. No specific human tumour sites were identified for ionizing radiation (all types), internalized radionuclides that emit α-particles, internalized radionuclides that emit β-particles, and UV radiation (bandwidth 100–400 nm, encompassing UVC, UVB, and UVA). Although the skin was not explicitly mentioned as a human tumour site for UV radiation in Volume 100D, the skin is implicitly suggested as being a human tumour site for this agent. In the present analysis, the lack of explicit designation of the skin as a human tumour site for UV radiation precluded its use. A similar situation occurred for areca nut, for which the oral cavity might have been considered as a human tumour site, although this site was not explicitly designated in the *Monograph*.

An agent can be categorized by IARC as a Group 1 carcinogen in the absence of *sufficient evidence* for carcinogenicity in humans when it is clear that the mechanisms by which the agent causes cancer in animals also operate in humans. Such "mechanistic upgrades" have occurred with various levels of human evidence, including for aristolochic acid (*limited evidence* of carcinogenicity in humans; IARC, 2012e), B[*a*]P (*inadequate evidence* in humans; IARC, 2012c), ethylene oxide (*limited evidence* in humans; IARC, 2012c), 4,4′-methylenebis(2-chloroaniline) (MOCA) (*inadequate evidence* in humans; IARC, 2012c); and neutron radiation (*inadequate evidence* in humans; IARC, 2012f).

For further discussion of mechanistic upgrades and key characteristics of Group 1 agents developed for this analysis, see Chapter 10, by Smith, Chapter 22, by Krewski et al., Smith et al. (2016), and Birkett et al. (2019). Ten key characteristics of human carcinogens described by Smith et al. (2016) focus on whether the agent (1) is electrophilic or can be metabolically activated to electrophiles, (2) is genotoxic, (3) alters DNA repair or causes genomic instability, (4) induces epigenetic alterations, (5) induces oxidative

stress, (6) induces chronic inflammation, (7) is immunosuppressive, (8) modulates receptor-mediated effects, (9) causes immortalization, and/or (10) alters cell proliferation, cell death, or nutrient supply. These considerations will be relevant in planned future analyses of coherence between tumours in animals and humans, taking into account key characteristics of carcinogens. However, mechanistic upgrades limit the ability to identify tumour site concordance when human tumour sites are not identified.

Exposure assessment is one of the most difficult aspects of epidemiological investigations (Nieuwenhuijsen, 2003). In some cases, such as ecological studies that compare two population groups subject to notably different exposure circumstances, exposure may not be measured at all. In other cases, however, exposures may be very well determined, as with the use of personal dosimeters to measure exposures to agents such as ambient air pollution or ionizing radiation, or in the dose regimens of pharmaceutical drugs or medical radiation. In the future, enhanced exposure assessment methodologies may serve to strengthen the ability of epidemiological studies to identify Group 1 agents (Cohen-Hubal et al., 2010; National Research Council, 2012). Biomarkers of exposure are expected to play an important part in the future of exposure science (Gurusankar et al., 2017).

The data set assembled and evaluated by Grosse et al. (Annex 1) was retrieved from the *IARC Monographs*. Thus, these agents do not represent a "random sample" of all potential human carcinogens, and the data set is populated by the available animal and human evidence that was the focus of the *Monographs* from which they were drawn. The ability to determine concordance may change as additional Group 1 agents are identified, or as additional animal or human evidence on current Group 1 agents becomes available. New mechanistic data could affect IARC evaluations of agents currently classified in Group 2A (*probably carcinogenic to humans*) and Group 2B (*possibly carcinogenic to humans*), and hence affect the concordance estimates reported here. Birkett et al. (2019) noted that additional information on the 10 mechanistic key characteristics of human carcinogens described by Smith et al. (2016) is available in the general scientific literature, beyond what is summarized in the *IARC Monographs*.

In addition to the restrictions used by Grosse et al. (Annex 1) for inclusion of certain experimental animal data, other limitations of the database affect the ability to determine tumour site concordance, including incomplete information on tumour histology, limited information on the effects of sex, strain, and route of exposure, and limited information on dose-dependent effects. These and other limitations are discussed briefly below.

Incomplete information on tumour histology

Because of incomplete information on the histology of lesions in both animal and human studies, it was not possible to conduct concordance analyses for specific histological subtypes of cancers at a given site (such as adenocarcinoma or squamous cell carcinoma of the lung). The concordance analyses reported here are necessarily restricted to tumours occurring in a given organ or tissue (such as lung cancer) or in a more broadly defined organ and tissue system (such as the upper aerodigestive tract and the respiratory system). The concordance analyses reported here are based either on 39 tumour sites or on the broader classification of 14 organ and tissue systems.

Effects of sex, strain, and route of exposure

Risks of cancer can differ between male and female animals, among different strains of the same animal species, and by route of exposure. Because of incomplete information on these three factors in the database used in the present analysis, it was not possible to evaluate how concordance might vary by sex, strain, or exposure route.

Effects of dose

Because the primary objective of the *IARC Monographs Programme* is to identify agents with the potential to cause cancer in humans in qualitative terms, rather than to quantify the level of risk at a given dose, information on dose dependence in cancer risk is not systematically collected in the *Monographs*, although this is currently under review by IARC (IARC Advisory Group to Recommend on Quantitative Risk Characterization, 2013). Therefore, analyses of concordance considering dose–response relationships seen in animals and humans were not attempted at this time.

Multisite/multiorgan carcinogenicity

Several agents, notably radiation and tobacco smoke, induce malignant lesions at multiple sites or in multiple organ and tissue systems. Volume 100F (IARC, 2012c) summarizes the evidence that 1,3-butadiene induces haemangiosarcomas of the heart, malignant lymphomas, bronchiolo-alveolar neoplasms, and squamous cell neoplasms of

the forestomach in male and female B6C3F1 mice, and acinar cell carcinomas of the mammary gland, granulosa cell neoplasms of the ovary, and hepatocellular neoplasms in female mice. Assessing species concordance with multisite carcinogens is inherently more difficult than with carcinogens that affect a single organ or tissue. Understanding the mechanistic and other attributes of such multisite carcinogens will be useful in translating results in experimental animals to humans.

Measures of concordance

For simplicity of presentation, concordance was evaluated here in terms of the "overlap" between tumour sites seen in animals and humans. Although more formal statistical analyses of concordance as described in Supplemental Material II (online only; available from: http://publications.iarc.fr/578) were considered during the course of this work, the consensus of the Working Group was to represent concordance in terms of the simpler, more directly interpretable, indicators of "overlap" in Table 21.7 and Fig. 21.10.

Small sample size

After the 111 Group 1 agents tabulated by Grosse et al. (Annex 1) up to and including Volume 109 of the *IARC Monographs* were filtered to include only agents that provided *sufficient evidence* of carcinogenicity in at least one tumour site in humans and at least one tumour site in animals, 60 agents remained eligible for concordance analysis. Because the sample size for some tumour sites is small (only two agents – asbestos and erionite – caused tumours of the mesothelium), caution is needed in interpreting the concordance results presented in this chapter for these sites.

Predictive value of animal tests for carcinogenicity

Using a database comprising 150 agents tested for toxicity in animals and humans, Olson et al. (2000) estimated the positive predictive value (PPV) and the negative predictive value (NPV) for human toxicity (excluding cancer). In this context, the PPV is defined as the probability of observing human toxicity in clinical testing, given that toxicity has been observed in animal tests. The PPV for human toxicity was estimated to be 71% for rodent and non-rodent species combined, 63% for non-rodents alone, and 43% for rodents alone. Although a statement of the PPV and the NPV of animal cancer tests for human carcinogenicity may be desirable, this cannot be done on the basis of the IARC concordance database considered in this chapter. This is because both the PPV and the NPV depend on the prevalence of true positives in the database (Altman and Bland, 1994). Because the IARC concordance database comprises Group 1 agents that are known causes of cancer in humans, the PPV of animal cancer tests will artificially be calculated as 100%, whereas a lower PPV would be obtained with a more representative database that includes agents that do not cause cancer in humans. However, identifying agents that do not cause cancer in humans is not the focus of the *IARC Monographs Programme*; at present, only one agent – caprolactam – is classified as *probably not carcinogenic to humans* (Group 4).

In considering the relevance of animal data in the context of the *IARC Monographs*, it is important to keep in mind how animal data are used in the identification of Group 1 agents, according to the criteria outlined in the Preamble to the *IARC Monographs* (IARC, 2006). Most Group 1 agents are identified on the basis of *sufficient evidence* in humans, and for the purpose of the overall evaluation, there is no immediate recourse to animal data. Of the 111 Group 1 agents considered in this chapter, 102 demonstrated *sufficient evidence* of carcinogenicity in humans; the remaining nine agents were placed in Group 1 because the mechanisms by which tumours occurred in animals were considered to be directly relevant to humans, or on the basis of other relevant mechanistic considerations. For example, neutron radiation was placed in Group 1 despite *inadequate evidence* in humans, because the biophysics of radiation damage is similar for different types of ionizing radiation.

Bearing in mind the contribution of animal data to the identification of Group 1 agents in the *IARC Monographs*, it is possible with the present IARC concordance database to make a statement about the likelihood of positive results in animals among the Group 1 agents that have been shown to cause cancer in humans. Excluding mechanistic upgrades (nine agents) and Group 1 agents that lack appropriate animal data (20 agents), *all* Group 1 agents with *sufficient evidence* of carcinogenicity in humans have also provided *sufficient* or *limited evidence* of carcinogenicity in one or more animal species.

Conclusions

The *IARC Monographs Programme* is widely recognized as one of the most authoritative sources of information on the identification of agents that may be carcinogenic to humans. The *Monographs* are prepared with the involvement of

leading scientific experts worldwide, who apply the guidance provided in the Preamble to the *IARC Monographs* (IARC, 2006) to evaluate the weight of evidence that an agent may present a cancer risk to humans. Up to and including Volume 109, more than 2000 scientists have contributed to the development of the *IARC Monographs*; nearly 200 scientists were involved in Volume 100 alone. Since its beginning in 1971–1972 (Saracci and Wild, 2015), the *IARC Monographs Programme* has evaluated more than 1000 agents for their potential to cause cancer in humans, with 120 of these agents assigned to Group 1, indicating that the weight of evidence supports the conclusion that the agent is *carcinogenic to humans*.

A noteworthy aspect of the process used by IARC to identify the causes of cancer in humans is the reliance on leading experts in the Working Groups that conduct the evaluations documented in the *Monographs* to interpret the data according to the weight-of-evidence guidelines provided in the Preamble to the *IARC Monographs* (IARC, 2006). With the trend towards greater reliance on systematic review (National Research Council, 2014) and structured weight-of-evidence approaches to the evaluation of toxic substances (Rhomberg et al., 2013), the continued involvement of international experts in the *IARC Monographs* to interpret the often extensive human, animal, and mechanistic data is a major strength of the *IARC Monographs Programme*.

Collectively, the *IARC Monographs* provide a rich source of information on the causes of cancer in humans. In particular, Volume 100 presents a review and update of 107 Group 1 agents identified in the previous 99 Volumes of the *IARC Monographs*, providing a veritable "encyclopaedia of carcinogens". This information, supplemented with data on Group 1 agents identified in Volumes 101 to 109, formed the basis for the analyses included in this chapter. After both PCB 126 and dioxin-like PCBs were subsumed within the broader category of PCBs, 113 – 2 = 111 district Group 1 agents were included in the concordance analyses presented in this chapter. The importance of human data in the IARC carcinogen evaluation process is highlighted by the observation that 102 of the 111 distinct Group 1 agents identified at the time this analysis was done demonstrated *sufficient evidence* of carcinogenicity in humans.

Analysis of concordance between tumour sites in animals and humans was restricted to 60 Group 1 agents demonstrating *sufficient evidence* for at least one tumour site in animals and in humans. Substantial overlap between animal and human tumours was seen in some organ and tissue systems but not in others. This analysis focused on tumours seen in the 14 organ and tissue systems in the anatomically based tumour classification system rather than 39 individual tumour sites, because of the sparseness of data at the individual tumour site level.

The principle that agents that are carcinogenic in experimental animals should be regarded as presenting a carcinogenic risk to humans was further confirmed in the course of this investigation. Excluding agents for which animal data are lacking or otherwise uninformative, all agents that cause cancer in humans also cause cancer in one more animal species, a finding consistent with an earlier evaluation of results from the *IARC Monographs Programme* (Wilbourn et al., 1986) and commented upon by other authors (Tomatis et al., 1989; Huff, 1994; Maronpot et al., 2004). However, it is important to note that the present database cannot be used to estimate the predictive value of animal cancer tests for humans, because it comprised by design only Group 1 agents; the PPV and the NPV of the animal data for humans would be 100% and 0%, respectively (an artefact of a database that comprises human carcinogens only).

Despite the challenges in evaluating concordance between tumour sites in animals and humans, the IARC concordance database is a useful source of information for comparing animal and human data with respect to the tumours caused in different species by the 111 distinct Group 1 agents identified by IARC up to and including Volume 109 of the *IARC Monographs*. Future *Monographs* may benefit from a more systematic summary of the animal and human data on agents evaluated within the *IARC Monographs Programme*, including data on the types of tumours seen in animal and human studies, possibly using the anatomically based tumour nomenclature system introduced in this chapter to facilitate comparisons between animals and humans. Data on route of exposure, sex, and animal strain would also support comparisons of animal and human tumours at a finer level of biological resolution. Data on the exposure or dose levels at which tumours are seen in animals and humans would further support evaluation of the relative carcinogenic potency of agents evaluated in animals and humans. Information on tumour sites affected by agents evaluated within the *IARC Monographs Programme* should be

recorded in as much detail as possible to facilitate future evaluations of the concordance between tumours seen in animals and humans on a site-specific basis.

Summary

Since its inception in the early 1970s, the *IARC Monographs Programme* has developed 119 *Monographs* Volumes on more than 1000 agents for which there exists some evidence of cancer risk to humans; of these, 120 agents met the criteria for classification as *carcinogenic to humans* (Group 1). Volume 100 of the *IARC Monographs*, compiled in 2008–2009 and published in 2012, provided a review and update of the 107 Group 1 agents identified as of 2009. These agents were divided into six broad categories: pharmaceuticals; biological agents; arsenic, metals, fibres, and dusts; radiation; personal habits and indoor combustions; and chemical agents and related occupations. The data set developed by Grosse et al. (Annex 1) for human and animal tumours and tumour sites associated with exposure to these agents, as well as five additional Group 1 agents defined in subsequent Volumes of the *Monographs*, were used to analyse the degree of concordance between sites where tumours arise in humans and in experimental animals (mice, rats, hamsters, dogs, and non-human primates). An anatomically based tumour nomenclature system, representing 39 tumour sites and 14 organ and tissue systems for which agents presented *sufficient evidence* of carcinogenicity in humans and/or in experimental animals, was developed and used as the basis for interspecies comparison. The present analysis identified 91 Group 1 agents with *sufficient evidence* (82 agents) or *limited evidence* (9 agents) of carcinogenicity in animals. The most common tumours observed in both humans and animals were those of the respiratory system (including larynx, lung, and lower respiratory tract). In humans, such tumours were observed for 31 of the 111 distinct Group 1 carcinogens identified up to and including Volume 109 of the *IARC Monographs*, comprising mostly chemical agents and related occupations (14 agents), arsenic, metals, fibres, and dusts (7 agents), and personal habits and indoor combustions (5 agents). After tumours in the respiratory system, those in lymphoid and haematopoietic tissues (26 agents), the urothelium (18 agents), and the upper aerodigestive tract (16 agents) were most often seen in humans, and tumours in digestive organs (19 agents), the skin (18 agents), and connective tissues (17 agents) were most often seen in animals. Exposures to radiation (particularly X- and γ-radiation) and tobacco smoke were associated with tumours at multiple sites in humans. Although the *IARC Monographs* do not emphasize tumour site concordance between animals and humans, substantial concordance was observed for several organ and tissue systems, even under the stringent criteria for *sufficient evidence* of carcinogenicity used by IARC. Of the 60 agents for which at least one tumour site had been identified in both humans and animals, 52 (87%) cause tumours in at least one of the same organ and tissue systems in humans and animals. It should be noted that some caution is needed in interpreting concordance at sites where the sample size is particularly small: although perfect (100%) concordance was noted for agents that cause tumours of the mesothelium, only two Group 1 agents meeting the criteria for inclusion in the concordance analysis caused tumours at this site. Although the present analysis demonstrates good concordance between animals and humans for many, but not all, tumour sites, limitations of the available data may result in underestimation of concordance.

Acknowledgements

Pascal Lajoie assembled the tumour site concordance database analysed in this chapter while working as a Visiting Scientist under the direction of Yann Grosse at IARC during the summers of 2011 and 2012. Mélissa Billard also contributed to the development of the concordance database while working as a Visiting Scientist under the direction of Yann Grosse and Robert Baan at IARC during the summers of 2013 and 2014. Daniel Krewski is the Natural Sciences and Engineering Research Council of Canada Chair in Risk Science at the University of Ottawa. Julian Little is the Canada Research Chair in Human Genome Epidemiology at the University of Ottawa. The authors would like to thank Nawal Farhat and Mohamed Taher for their assistance in reviewing and checking the final draft of this chapter. This chapter is dedicated to the memory of Jan M. Zielinski, who succumbed to cancer in 2016; before becoming ill, Dr Zielinski led the team that conducted the analyses reported here.

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CHAPTER 22.

Analysis of key characteristics of human carcinogens

Daniel Krewski, Mustafa Al-Zoughool, Michael Bird, Nicholas Birkett, Mélissa Billard, Brittany Milton, Jerry M. Rice, Vincent J. Cogliano, Mark A. Hill, Julian Little, and Jan M. Zielinski (deceased) in collaboration with other participants (see the Contributors list) in the Workshop on Tumour Site Concordance and Mechanisms of Carcinogenesis, which was convened by IARC in April and November 2012 in Lyon

Introduction

Since its establishment in the early 1970s, the *IARC Monographs Programme* has evaluated more than 1000 agents with evidence of human exposure and for which some suspicion exists of an increased cancer risk to humans. The *IARC Monographs Programme* has developed detailed criteria against which to evaluate the available scientific evidence on the carcinogenic potential of such agents. These criteria, which are described in the Preamble to the *IARC Monographs* (Cogliano et al., 2004; IARC, 2006), are used to evaluate and integrate the evidence provided by human epidemiological studies, animal cancer bioassays, and information on possible biological mechanisms of action, to classify agents into one of the following categories: *carcinogenic to humans* (Group 1), *probably carcinogenic to humans* (Group 2A), *possibly carcinogenic to humans* (Group 2B), *not classifiable as to its carcinogenicity to humans* (Group 3), and *probably not carcinogenic to humans* (Group 4). These evaluations involve classifying the data from both the human and the animal studies as providing *sufficient evidence of carcinogenicity*, *limited evidence of carcinogenicity*, *inadequate evidence of carcinogenicity*, or *evidence suggesting lack of carcinogenicity*. The information on biological mechanisms of action may be evaluated as *strong*, *moderate*, or *weak*, and is taken into consideration in the overall evaluation.

The role of mechanistic information in evaluating carcinogenicity has increased substantially during the history of the *IARC Monographs Programme*. In 1991, IARC convened a Working Group on the Use of Data on Mechanisms of Carcinogenesis in Risk Identification, to explore how mechanistic data could be used to

identify agents with the potential to cause cancer in humans. The consensus report of the Working Group documented several mechanisms that were considered to be relevant to human carcinogenesis at that time, including genotoxicity, cell proliferation, receptor mechanisms in mitogenesis, alterations in DNA repair, intercellular communication, and immune defects and immunosuppression (Vainio et al., 1992). Toxicokinetic and other variables were also identified as factors affecting multistage carcinogenesis. Since 1991, IARC (2006) and other organizations – for example, the United States National Toxicology Program (National Toxicology Program, 2014) and the United States Environmental Protection Agency (EPA) (EPA, 2005) – have stressed the increasing importance of mechanistic information in cancer risk assessment. Related risk assessment practices concern mode of action (Meek et al., 2014) and pathways of toxicity (Krewski et al., 2014; Bourdon-Lacombe et al., 2015; Cote et al., 2016), as well as dosimetric considerations (Gurusankar et al., 2017).

This chapter examines the available data on mechanisms of action of the Group 1 agents identified up to and including Volume 106 of the *IARC Monographs* (Table 22.1). The present analysis is based on a review of human cancer mechanisms, conducted by the participants in the two-part Workshop on Tumour Site Concordance and Mechanisms of Carcinogenesis, which was convened by IARC in April and November 2012 in Lyon. This approach initially involved retrieval of information from the *IARC Monographs* on 24 toxicological end-points identified as likely indicators of biological processes at the cellular and molecular level and thought to be relevant to carcinogenesis. Information on these 24 end-points was derived from human in vivo, human in vitro, animal in vivo, and animal in vitro studies (see Al-Zoughool et al., 2019). During the November 2012 meeting, the Workshop participants identified 10 broader key characteristics of carcinogens (see Chapter 10, by Smith, and Smith et al., 2016). Information on these characteristics was extracted from the *IARC Monographs* and used to develop a database of key characteristics for Group 1 agents (see Al-Zoughool et al., 2019). This chapter focuses on the key characteristics of the Group 1 agents identified in the *IARC Monographs* up to and including Volume 106, and presents the results of an exploratory analysis of this database.

Methods

Key characteristics

As mentioned above, Chapter 10, by Smith, and Smith et al. (2016) describe 10 key characteristics of human carcinogens, as listed in Table 22.2. The toxicological end-points initially considered by the Workshop participants and used as indicators of these characteristics are also noted in Table 22.2. A brief summary of each of these characteristics and the associated toxicological end-points is provided below.

Characteristic 1: Is electrophilic or can be metabolically activated to electrophiles

The first characteristic refers to agents that act as electrophiles themselves or that can be metabolized to form electrophiles. An electrophile can react with cellular macromolecules such as DNA, RNA, and proteins to form adducts. Some chemical carcinogens are direct-acting electrophiles (e.g. formaldehyde; sulfur mustard, and ethylene oxide), whereas others require biotransformation by enzymes in a process termed metabolic activation (e.g. polycyclic aromatic hydrocarbons and benzene) (Miller, 1970).

Characteristic 2: Is genotoxic

Genotoxicity is the ability to induce DNA damage or other chromosomal alterations, as measured by three associated toxicological end-points: (i) DNA damage: an alteration in the chemical structure or integrity of DNA, including a break in a DNA strand, and/or chemical modifications such as covalent binding to the nucleotide bases (Hoeijmakers, 2009); (ii) gene mutations: changes in the normal nucleotide sequence of cellular DNA that may have a central role in human carcinogenesis (Ding et al., 2008); (iii) clastogenic effects reflect damage to chromosomes, including DNA breakage, or the rearrangement, gain, or loss of chromosome fragments (Snyder, 2010).

Characteristic 3: Alters DNA repair or causes genomic instability

Alterations in DNA repair result in defects in processes that monitor and correct DNA replication fidelity. Such defects can confer strong mutator phenotypes that result in genomic instability.

Characteristic 4: Induces epigenetic alterations

Induced epigenetic alterations are stable changes in gene expression and chromatin organization that are independent of mutation and that

Table 22.1. Number of Group 1 agents in Volumes 100–118 of the *IARC Monographs*, by type of agent[a]

Type of agent	Volume											Total
	100	105	106	107	109	110	111	113	114	117	118	
Pharmaceuticals	23	–	–	–	–	–	–	–	–	–	–	23
Biological agents	11	–	–	–	–	–	–	–	–	–	–	11
Arsenic, metals, fibres, and dusts	10	–	–	–	–	–	2[b]	–	–	–	–	12
Radiation	18	–	–	–	–	–	–	–	–	–	1[c]	19
Personal habits and indoor combustions	12	–	–	–	–	–	–	–	1[d]	–	–	13
Chemical agents and related occupations	33	1[e]	1[f]	2[g]	2[h]	1[i]	–	1[j]	–	1[k]	1[l]	43
Total	**107**	**1**	**1**	**2**	**2**	**1**	**2**	**1**	**1**	**1**	**2**	**121**

[a] At the time that the present analysis was conducted, mechanistic information was available only for the 109 Group 1 agents evaluated in the *IARC Monographs* up to and including Volume 106.
[b] Fluoro-edenite fibrous amphibole; occupational exposures associated with the Acheson process in the manufacture of silicon carbide fibres.
[c] Ultraviolet radiation from welding.
[d] Processed meat.
[e] Diesel engine exhaust.
[f] Trichloroethylene.
[g] Polychlorinated biphenyls (PCBs); dioxin-like PCBs.
[h] Outdoor air pollution; particulate matter in outdoor air pollution.
[i] 1,2-Dichloropropane.
[j] Lindane.
[k] Pentachlorophenol (PCP).
[l] Welding fumes.

can be inherited through cell division. Epigenetic phenomena include genomic imprinting, X-chromosome inactivation, global reconfiguration of the DNA methylome, changes in chromatin compaction states and histone modification patterns, and altered expression of microRNAs (miRNAs). These phenomena occur during organ development and contribute to the lineage-specific epigenome that is maintained over the lifetime of an organism.

Characteristic 5: Induces oxidative stress

Oxidative stress results from an imbalance between formation of reactive oxygen and detoxification of the radical species within cells and tissues. Reactive oxygen species induce a cascade of events that can include DNA mutation and oxidative DNA damage. Both are key events in carcinogenesis (Klaunig et al., 2011).

Characteristic 6: Induces chronic inflammation

Chronic inflammation can arise from persistent infection (e.g. with human papillomavirus or with *Helicobacter pylori*) as well as from exogenous irritants (e.g. silica or asbestos fibres). Persistent infection and chronic inflammation disrupt local tissue homeostasis and alter cell signalling, leading to the recruitment and activation of inflammatory cells. Strong links exist between inflammation and the induction of oxidative stress and genomic instability; this makes it difficult to separate out the relative importance of each of these mechanisms. These linkages might be the basis of the relationship between chronic inflammation and cancer (Multhoff and Radons, 2012).

Characteristic 7: Is immunosuppressive

Immunosuppression is an induced reduction in the capacity of the immune system to respond effectively to foreign antigens, including

Table 22.2. Key characteristics and toxicological end-points demonstrated by agents known to cause cancer in humans (adapted from Al-Zoughool et al., 2019)

Key characteristic	Corresponding toxicological end-points
Is electrophilic or can be metabolically activated to electrophiles	Metabolic activation Protein adducts ADME (differences in absorption, distribution, metabolism, and elimination)
Is genotoxic	DNA damage Cytogenetic/clastogenic effects Gene mutations
Alters DNA repair or causes genomic instability	DNA repair alteration, leading to genomic instability
Induces epigenetic alterations	Epigenetic alterations (DNA methylation, histone modification, and altered expression of microRNAs)
Induces oxidative stress	Oxidative stress
Induces chronic inflammation	Chronic inflammation Chronic irritation
Is immunosuppressive	Immune effects
Modulates receptor-mediated effects	Receptor-mediated effects Hormonal effects
Causes immortalization	Immortalization Alterations in telomere length
Alters cell proliferation, cell death, or nutrient supply	Cell-cycle effects Bystander effects Alterations in cell signalling pathways Angiogenic effects Cell death Inhibition of gap-junctional intercellular communication

antigens on tumour cells. In contrast to other key characteristics, immunosuppression does not play a direct part in transforming normal cells into tumour cells, but enables them to escape immune surveillance. Among other roles, the immune system also plays a major part in the inflammatory response to injury.

Characteristic 8: Modulates receptor-mediated effects

Modulation of receptor-mediated effects can occur when agents mimic the structure of endogenous ligands that bind to cells and activate cell surface receptors or intracellular receptors, thereby inducing or modifying a plethora of signal transduction pathways that, among other responses, stimulate cell proliferation. Receptor-mediated effects can induce hormonal effects whereby external agents can interfere with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body. These external factors can also demonstrate reactivity similar to endogenously produced hormones, which can lead to them mediating changes in homeostasis, reproduction, development, or behaviour.

Characteristic 9: Causes immortalization

Immortalization refers to a cell evading normal cellular senescence and proliferating indefinitely. In culture, normal cells have a fixed number of replication cycles before they enter cellular senescence and stop replicating. Evasion of senescence is frequently associated with activation of telomerase (Willeit et al., 2010) and plays a critical part in carcinogenesis (Reddel, 2000). Carcinogenesis may involve activation of a telomerase that prevents loss of telomere length, leading to immortalization of cells (Willeit et al., 2010).

Characteristic 10: Alters cell proliferation, cell death, or nutrient supply

Cell proliferation is affected by alterations in the rates of cell growth within a tissue. It may be a direct effect or a secondary regenerative effect after induction of cell death by cytotoxic agents. Two associated toxicological end-points are (i) cell-cycle effects, i.e. alterations in the functioning of the complex series of factors that control the cell cycle and cell division, which have been associated with carcinogenesis (Diaz-Moralli et al., 2013), and (ii) alterations in cell signalling pathways, which relate to the ability of the agent to interfere with cell signalling pathways, leading to expression of a carcinogenic trait or phenotype in the cell.

For cell death, necrosis triggers the invasion of cells such as macrophages into the affected area, and enhances the proliferation and spread of cancer cells. Defects in programmed cell death can cause cancer; evasion of apoptosis is a requirement for both neoplastic transformation and sustained growth of cancer cells.

Adequate cell nutrition is essential to proliferating cancer cells, and agents that promote or inhibit the growth of blood vessels (angiogenesis) will affect tumour growth.

Group 1 agents included in the analysis

Volume 100 of the *IARC Monographs* provided a review and update of the 107 Group 1 agents identified as of 2009. Since the publication of Volume 100, mechanistic information has become available on two additional Group 1 agents: diesel engine exhaust (reviewed in Volume 105; Benbrahim-Tallaa et al., 2012; IARC, 2013) and trichloroethylene (evaluated in Volume 106; Guha et al., 2012; IARC, 2014). Had these two agents been evaluated within Volume 100, they would have been included in Volume 100F; they have therefore been listed with other chemical agents and related occupations in Volume 100F*.

Although additional Group 1 agents have since been identified (Table 22.1), the present analysis is restricted to Group 1 agents identified in the *IARC Monographs* up to and including Volume 106, the most recent volume for which mechanistic information was available at the time of the present analysis. Group 1 agents not included in the present analysis are (i) polychlorinated biphenyls (PCBs) and dioxin-like PCBs (reviewed in Volume 107; Lauby-Secretan et al., 2013; IARC, 2016b), (ii) outdoor air pollution and (iii) particulate matter in outdoor air pollution (both evaluated in Volume 109; Loomis et al., 2013; IARC, 2016a), (iv) 1,2-dichloropropane (reviewed in Volume 110; Benbrahim-Tallaa et al., 2014; IARC, 2017a), (v) fluoro-edenite fibrous amphibole and (vi) occupational exposures associated with the Acheson process used in the manufacture of silicon carbide fibres (both evaluated in Volume 111; Grosse et al., 2014; IARC, 2017b); (vii) lindane (Volume 113; Loomis et al., 2015), and (viii) processed meat (Volume 114; Bouvard et al., 2015).

In some cases, the discussion of mechanisms of action in Section 4 ("Other relevant data") of the *IARC Monographs* is based on groups of agents that act via the same mechanism. For example, internalized radionuclides that emit α-particles are discussed in the *Monographs* as a group with the same mechanism of action. Birkett et al. (2019) reviewed the mechanistic information for 109 Group 1 agents identified in the *IARC Monographs* up to and including Volume 106. The 86 Group 1 agents for which separate mechanistic summaries are provided in the *IARC Monographs* up to and including Volume 106 are listed in Table 22.3, along with their relationship to the 111 distinct agents identified up to and including Volume 109 used by Krewski et al. (Chapter 21) in a parallel analysis of overlap between tumours and tumour sites in animals and humans.

Database of mechanistic characteristics

A database of toxicological end-points was assembled for the 86 Group 1 agents identified up to and including Volume 106 of the *IARC Monographs*. The database includes information from in vivo and in vitro studies in humans and animals. Information on the 24 toxicological end-points was retrieved from Section 4 ("Other relevant data") of the *IARC Monographs* (Al-Zoughool et al., 2019).

Recognizing that, among other limitations, new data may have become available since 2009, when the various parts of Volume 100 were compiled, PubMed searches were conducted to identify evidence on any of the 24 toxicological end-points linked to these agents that was not recorded in the *IARC Monographs* (Birkett et al., 2019). The mechanistic database distinguishes information derived from the *Monographs* from that found in the PubMed search, thereby permitting an assessment of the extent to which Section 4 ("Other relevant data") of the *IARC Monographs* captured all relevant information on these end-points. The analyses in the present chapter are restricted to information taken

Table 22.3. Relationship between 86 agents used in the analysis of key characteristics of human carcinogens and 111 agents used in the analysis of concordance between tumours and tumour sites in humans and animals

Volume[a]	Agent number	86 agents used in the analysis of key characteristics	111 agents used in the analysis of concordance between tumours and tumour sites in humans and animals
100A	1	Aristolochic acid	Aristolochic acid Aristolochic acid, plants containing
100A	2	Azathioprine	Azathioprine
100A	3	Busulfan	Busulfan
100A	4	Chlorambucil	Chlorambucil
100A	5	Chlornaphazine	Chlornaphazine
100A	6	Cyclophosphamide	Cyclophosphamide
100A	7	Ciclosporin	Ciclosporin
100A	8	Diethylstilbestrol	Diethylstilbestrol
100A	9	Estrogen-only menopausal therapy	Estrogen-only menopausal therapy
100A	10	Estrogen–progestogen menopausal therapy (combined)	Estrogen–progestogen menopausal therapy (combined)
100A	11	Estrogen–progestogen oral contraceptives (combined)	Estrogen–progestogen oral contraceptives (combined)
100A	12	Etoposide in combination with cisplatin (Group 2A) and bleomycin (Group 2B)	Etoposide Etoposide in combination with cisplatin and bleomycin
100A	13	Melphalan	Melphalan
100A	14	PUVA (psoralen–UVA photochemotherapy)	Methoxsalen in combination with UVA
100A	15	MOPP	MOPP
100A	16	Phenacetin	Phenacetin Phenacetin, analgesic mixtures containing
100A	17	1-(2-Chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea (Methyl-CCNU)	1-(2-Chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea (Methyl-CCNU)
100A	18	Tamoxifen	Tamoxifen
100A	19	Thiotepa	Thiotepa
100A	20	Treosulfan	Treosulfan
100B	21	*Opisthorchis viverrini* and *Clonorchis sinensis*	*Clonorchis sinensis* (infection with) *Opisthorchis viverrini* (infection with)
100B	22	Epstein–Barr virus	Epstein–Barr virus
100B	23	*Helicobacter pylori*	*Helicobacter pylori* (infection with)
100B	24	Hepatitis B virus	Hepatitis B virus
100B	25	Hepatitis C virus	Hepatitis C virus
100B	26	Human immunodeficiency virus type 1	Human immunodeficiency virus type 1
100B	27	Human papillomavirus	Human papillomavirus

Table 22.3. Relationship between 86 agents used in the analysis of key characteristics of human carcinogens and 111 agents used in the analysis of concordance between tumours and tumour sites in humans and animals (continued)

Volume[a]	Agent number	86 agents used in the analysis of key characteristics	111 agents used in the analysis of concordance between tumours and tumour sites in humans and animals
100B	28	Human T-cell lymphotropic virus type 1	Human T-cell lymphotropic virus type 1
100B	29	Kaposi sarcoma-associated herpesvirus	Kaposi sarcoma-associated herpesvirus
100B	30	*Schistosoma haematobium*	*Schistosoma haematobium* (infection with)
100C	31	Arsenic and inorganic arsenic compounds	Arsenic and inorganic arsenic compounds
100C	32	Asbestos (all forms, including actinolite, amosite, anthophyllite, chrysotile, crocidolite, and tremolite)	Asbestos (all forms, including actinolite, amosite, anthophyllite, chrysotile, crocidolite, and tremolite)
100C	33	Beryllium and beryllium compounds	Beryllium and beryllium compounds
100C	34	Cadmium and cadmium compounds	Cadmium and cadmium compounds
100C	35	Chromium(VI) compounds	Chromium(VI) compounds
100C	36	Erionite	Erionite
100C	37	Leather dust	Leather dust
100C	38	Nickel and nickel compounds	Nickel compounds
100C	39	Silica dust, crystalline, in the form of quartz or cristobalite	Silica dust, crystalline, in the form of quartz or cristobalite
100C	40	Wood dust	Wood dust
100D	41	Solar and UV radiation	UV radiation (bandwidth 100–400 nm, encompassing UVC, UVB, and UVA) UV-emitting tanning devices Solar radiation
100D	42	X- and γ-radiation	X- and γ-radiation Ionizing radiation (all types)
100D	43	Neutron radiation	Neutron radiation
100D	44	Internalized radionuclides that emit α-particles	Haematite mining with exposure to radon (underground) Plutonium-239 Internalized radionuclides that emit α-particles Thorium-232 (as Thorotrast) Radium-224 and its decay products Radium-226 and its decay products Radium-228 and its decay products Radon-222 and its decay products
100D	45	Internalized radionuclides that emit β-particles	Fission products including Sr-90 Radioiodines, including iodine-131 Phosphorus-32, as phosphate Internalized radionuclides that emit β-particles

Table 22.3. Relationship between 86 agents used in the analysis of key characteristics of human carcinogens and 111 agents used in the analysis of concordance between tumours and tumour sites in humans and animals (continued)

Volume[a]	Agent number	86 agents used in the analysis of key characteristics	111 agents used in the analysis of concordance between tumours and tumour sites in humans and animals
100E	46	Consumption of alcoholic beverages	Acetaldehyde associated with consumption of alcoholic beverages Alcoholic beverages Ethanol in alcoholic beverages
100E	47	Betel quid and areca nut	Areca nut Betel quid with tobacco Betel quid without tobacco
100E	48	Coal, indoor emissions from household combustion of	Coal, indoor emissions from household combustion of
100E	49	*N′*-Nitrosonornicotine (NNN) and 4-(Methyl-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK)	*N′*-Nitrosonornicotine (NNN) and 4-(Methyl-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK)
100E	50	Salted fish, Chinese-style	Salted fish, Chinese-style
100E	51	Second-hand tobacco smoke	Second-hand tobacco smoke
100E	52	Tobacco smoking	Tobacco smoking
100E	53	Tobacco, smokeless	Tobacco, smokeless
100F	54	Acid mists, strong inorganic	Acid mists, strong inorganic
100F	55	Aflatoxins	Aflatoxins
100F	56	Aluminium production	Aluminium production
100F	57	4-Aminobiphenyl	4-Aminobiphenyl
100F	58	Auramine production	Auramine production
100F	59	Benzene	Benzene
100F	60	Benzidine	Benzidine
100F	61	Benzidine, dyes metabolized to	Benzidine, dyes metabolized to
100F	62	Benzo[*a*]pyrene	Benzo[*a*]pyrene
100F	63	Bis(chloromethyl)ether; Chloromethyl methyl ether (technical grade)	Bis(chloromethyl)ether; Chloromethyl methyl ether (technical grade)
100F	64	1,3-Butadiene	1,3-Butadiene
100F	65	Coal gasification	Coal gasification
100F	66	Coal-tar distillation	Coal-tar distillation
100F	67	Coal-tar pitch	Coal-tar pitch
100F	68	Coke production	Coke production
100F	69	Ethylene oxide	Ethylene oxide
100F	70	Formaldehyde	Formaldehyde
100F	71	Iron and steel founding, occupational exposure during	Iron and steel founding, occupational exposure during

Table 22.3. Relationship between 86 agents used in the analysis of key characteristics of human carcinogens and 111 agents used in the analysis of concordance between tumours and tumour sites in humans and animals (continued)

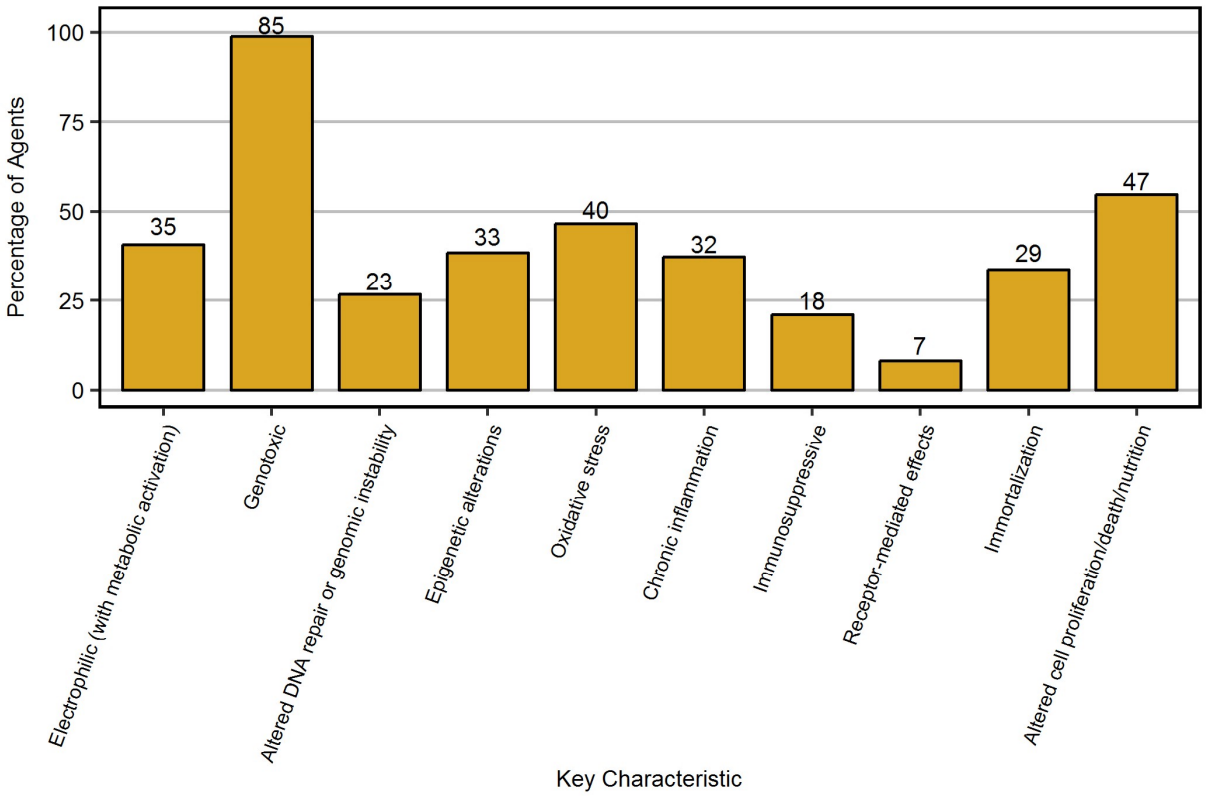
Volume[a]	Agent number	86 agents used in the analysis of key characteristics	111 agents used in the analysis of concordance between tumours and tumour sites in humans and animals
100F	72	Isopropyl alcohol manufacture using strong acids	Isopropyl alcohol manufacture using strong acids
100F	73	Magenta production	Magenta production
100F	74	4,4′-Methylenebis(2-chloroaniline) (MOCA)	4,4′-Methylenebis(2-chloroaniline) (MOCA)
100F	75	Mineral oils, untreated or mildly treated	Mineral oils, untreated or mildly treated
100F	76	2-Naphthylamine	2-Naphthylamine
100F	77	*ortho*-Toluidine	*ortho*-Toluidine
100F	78	Painter, occupational exposure as a	Painter, occupational exposure as a
100F	79	2,3,7,8-Tetrachlorodibenzo-*para*-dioxin, 2,3,4,7,8-Pentachlorodibenzofuran, 3,3′,4,4′,5-Pentachlorobiphenyl	2,3,4,7,8-Pentachlorodibenzofuran 2,3,7,8-Tetrachlorodibenzo-*para*-dioxin 3,3′,4,4′,5-Pentachlorobiphenyl
100F	80	Rubber manufacturing industry, occupational exposures in the	Rubber manufacturing industry, occupational exposures in the
100F	81	Shale oils	Shale oils
100F	82	Soot (as found in occupational exposure of chimney sweeps)	Soot (as found in occupational exposure of chimney sweeps)
100F	83	Sulfur mustard	Sulfur mustard
100F	84	Vinyl chloride	Vinyl chloride
105	85	Diesel and gasoline engine exhausts	Engine exhaust, diesel
106	86	Trichloroethylene	Trichloroethylene
107			Polychlorinated biphenyls[b]
109			Outdoor air pollution[b]
109			Particulate matter in outdoor air pollution[b]

UV, ultraviolet.

[a] *IARC Monographs* Volumes 100A (IARC, 2012e), 100B (IARC, 2012b), 100C (IARC, 2012a), 100D (IARC, 2012f), 100E (IARC, 2012d), 100F (IARC, 2012c), 105 (IARC, 2013), 106 (IARC, 2014), 107 (IARC, 2016b), and 109 (IARC, 2016a).

[b] Because the mechanistic sections for *Monographs* Volumes 107–109 were not available for review at the time that the present analysis was conducted, Group 1 agents in these volumes were not included in the present analysis.

Fig. 22.1. Key characteristics of 86 Group 1 agents. The number of agents is shown above each characteristic.



directly from the *IARC Monographs*: Birkett et al. (2019) present the results of a sensitivity analysis incorporating the additional information obtained through the PubMed search.

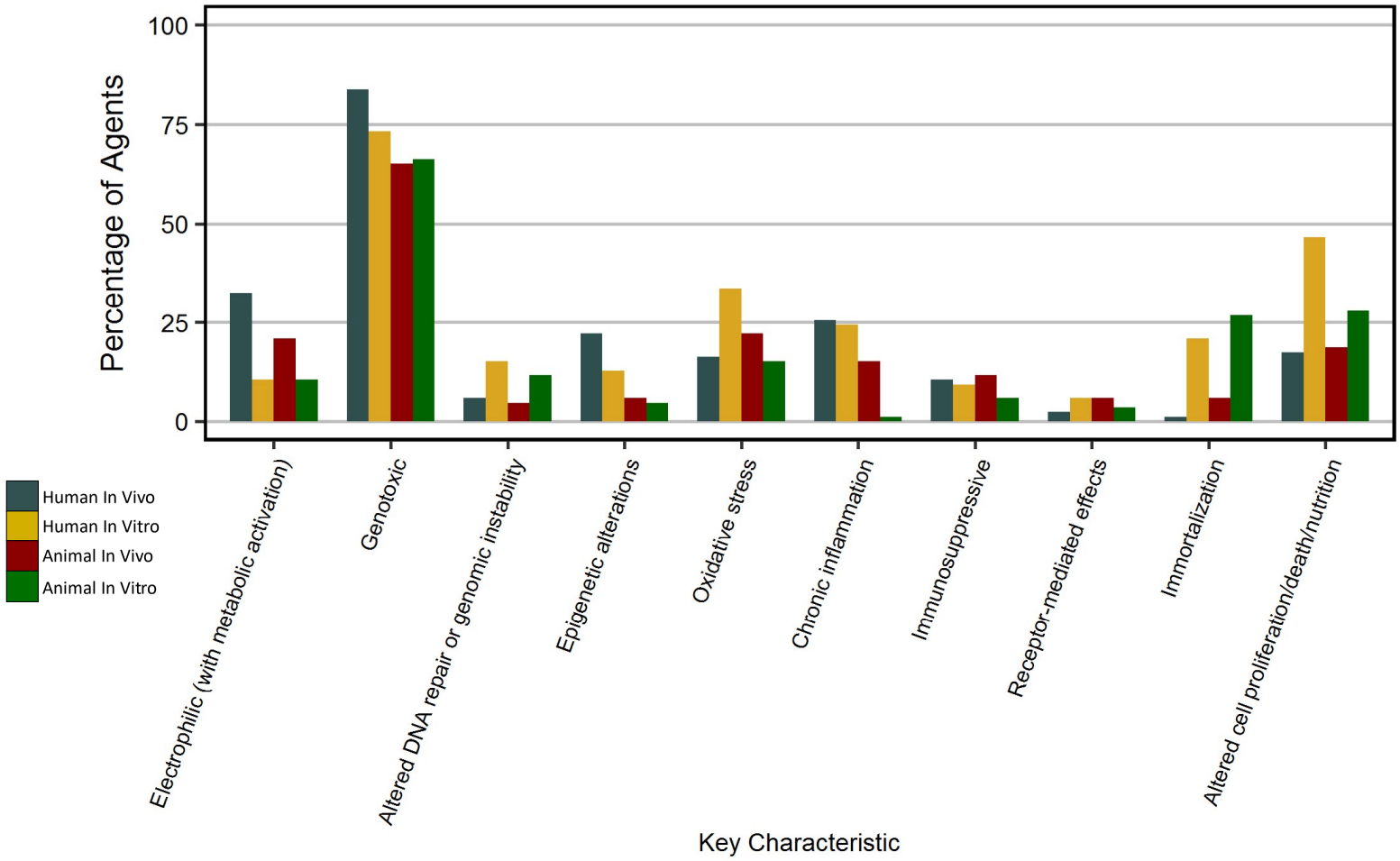
After the collection of information on the toxicological end-points identified by the Workshop participants during the April 2012 meeting, the database of key characteristics was then created by mapping the 24 toxicological end-points to the 10 characteristics as indicated in Table 22.2. As noted by Al-Zoughool et al. (2019), two of the toxicological end-points – susceptibility and changes in gene expression – did not link to any of the key characteristics, and thus were not included in the development of the database of key characteristics. Because the database includes information derived from human in vivo, human in vitro, animal in vivo and animal in vitro sources, it is possible to aggregate this information according to human and animal sources (by combining across in vivo and in vitro sources) or according to in vivo and in vitro sources (by combining across human and animal sources). Of primary interest here is aggregation across all four sources combined, to obtain an overall indicator of whether any of the key characteristics is associated with each of the 86 Group 1 agents of interest.

Statistical analysis

Descriptive statistical methods were used to explore the key characteristics associated with the 86 Group 1 agents, beginning with a tabulation of the number of agents demonstrating any of the 10 characteristics, both overall and stratified by the four sources of information noted above. To evaluate the extent to which the Group 1 agents demonstrated more than one key characteristic, the number of agents demonstrating multiple characteristics was also tabulated.

A heat map showing the number (0, 1, 2, 3, or 4) of sources of information (human in vivo, human in vitro, animal in vivo, and animal in vitro studies) supporting a given characteristic for a specified agent was prepared, to evaluate the consistency of information provided by different sources. A heat map showing the overlap between human and animal sources of information (after combining in vivo and in vitro sources in both cases) on the key characteristics was also prepared, to evaluate the extent to which there was overlap between these two sources.

Fig. 22.2. Sources of information on key characteristics of 86 Group 1 agents (sources are human in vivo, human in vitro, animal in vivo, and animal in vitro studies).



Overall mechanistic data were also tabulated by type of agent (pharmaceuticals; biological agents; arsenic, metals, fibres and dusts; radiation; personal habits and indoor combustions; and chemical agents and related occupations), to identify possible differences in mechanistic patterns by agent type.

Results

The key characteristics of the 86 Group 1 agents considered here are summarized in Fig. 22.1. The most prevalent mechanistic characteristic was "is genotoxic", followed by "alters cell proliferation, cell death, or nutrient supply", "induces oxidative stress", "is electrophilic or can be metabolically activated to electrophiles", and "induces chronic inflammation". Nearly all agents demonstrate genotoxicity as one of their mechanistic properties; a prominent exception is human immunodeficiency virus type 1 (HIV-1). Evidence of genotoxicity was provided by expression of the following toxicological end-points: DNA damage, gene mutations, and cytogenetic/clastogenic effects (including chromosomal aberrations, micronucleus formation, and aneuploidy).

Fig. 22.2 shows the key characteristics exhibited by the 86 agents classified according to the source of data (human in vivo, human in vitro, animal in vivo, and animal in vitro studies) on these characteristics. Information on all the mechanistic characteristics was available to different degrees from all four sources. Information on genotoxicity was available from each of the four sources for the majority of the agents. Human in vivo studies contribute the most evidence on four of the 10 key characteristics for these 86 agents, including "is genotoxic", "induces epigenetic alterations", and "induces chronic inflammation". Human in vitro studies provide the most information on an additional three key characteristics: "alters DNA repair or causes genomic instability", "induces oxidative stress", and "alters cell proliferation, cell death, or nutrient supply", and equivalent information to animal in vivo studies on "modulates receptor-mediated effects".

The prominence of human studies as sources of information on the key characteristics of human carcinogens may be attributed to the increasing use of molecular and genetic markers in human studies. Epidemiological studies conducted in the occupational or general environment often analyse biomarkers of DNA adduct formation, clastogenic effects, and gene mutations, all of which reflect DNA damage. Therefore, human in vivo studies are a major source of information on genotoxicity.

Fig. 22.3 shows the number of agents demonstrating multiple characteristics as evidenced from studies in animals and in humans. The 86 Group 1 agents considered here exhibit an average of approximately four key characteristics; the modal value is two characteristics, exhibited by 20 agents. All agents demonstrate at least one key characteristic, with two agents demonstrating nine characteristics and 14 agents showing six. No agent exhibited all 10 key characteristics.

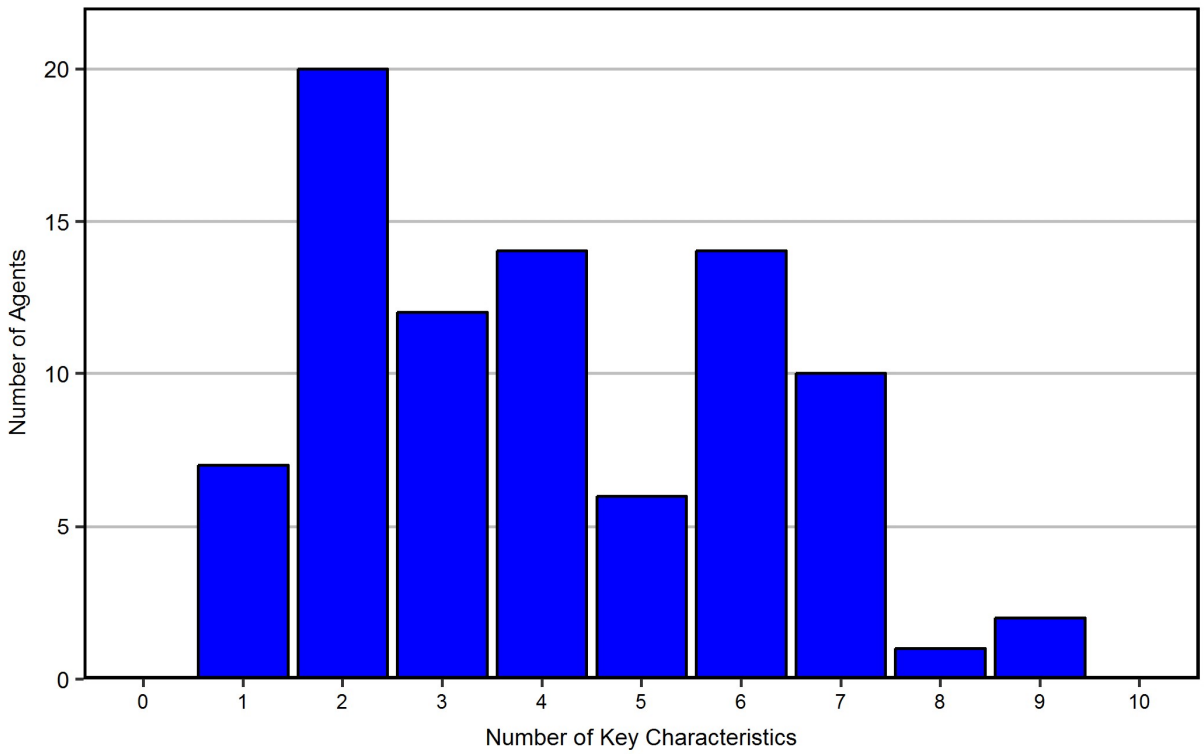
Fig. 22.4 presents a heat map indicating the strength of evidence of the different characteristics for the 86 individual Group 1 agents. The intensity of the colour reflects the number of sources of information (human in vivo, human in vitro, animal in vivo, and animal in vitro studies) on each key characteristic for each agent. As in Fig. 22.1, the single most prominent characteristic was genotoxicity: many agents (HIV-1 is a prominent exception) showed a positive response for genotoxicity in at least one of the four sources of information, and for many agents more than one source provided evidence of genotoxicity. For some agents (e.g. all radiation sources, some pharmaceutical agents, and some chemical agents), genotoxicity was demonstrated in all four test systems, confirming that genotoxicity is central to the carcinogenic pathways of these agents.

Fig. 22.4 also shows that most agents exhibit multiple key characteristics, with evidence drawn from more than one source of mechanistic information. Radiation sources and tobacco smoke are associated with many of the key characteristics, suggesting that these agents act by multiple pathways.

Several Group 1 agents, including several occupational exposures, are complex mixtures of chemicals and other substances. Coal-tar pitch, occupational exposure to soot, and coke production have similar characteristics, probably due to the strong presence in relevant workplaces of polycyclic aromatic hydrocarbons, although other factors such as the nature of inorganic substances and their chemical composition could also have a role. Other occupationally relevant agents (e.g. exposures during iron and steel founding and aluminium production) demonstrate only a single key characteristic, although this may reflect the difficulty of testing for other characteristics in these occupational exposure situations.

The degree of overlap between human and animal sources of information on the 10 key characteristics

Fig. 22.3. Number of Group 1 agents demonstrating one or more key characteristics.



of human carcinogens is shown in the heat map in Fig. 22.5. This heat map, prepared by combining the in vivo and in vitro sources of information on the key characteristics for humans and for animals, indicates whether information on the key characteristics for a given agent is derived from both human and animal sources (reflecting concordance between humans and animals), from human sources alone, from animal sources alone, or from neither of these. These results indicate overlap between human and animal sources of information for several agents. The concordance is particularly strong for genotoxicity: information from both human and animal sources is available for 63 of the 85 agents demonstrating evidence of genotoxicity. Ten agents – diethylstilbestrol, Kaposi sarcoma-associated herpesvirus, arsenic and inorganic arsenic compounds, cadmium and cadmium compounds, asbestos, crystalline silica, solar and ultraviolet radiation, sulfur mustard, diesel and gasoline engine exhausts, and trichloroethylene – demonstrate overlap between human and animal sources of information for at least five of the key characteristics.

Comparisons between the results in Fig. 22.4 and Fig. 22.5 can provide additional insights into the key characteristics of the Group 1 agents considered here. For example, in the case of diethylstilbestrol, Fig. 22.4 indicates that there is information from 1, 2, or 3 sources on nine key characteristics (all except "induces oxidative stress"), but Fig. 22.5 clarifies that there are both human and animal data for only five of these. For chlornaphazine, Fig. 22.4 shows two sources of information, for "is electrophilic or can be metabolically activated to electrophiles" and "is genotoxic", whereas the corresponding data in Fig. 22.5 show overlap between human and animal sources only for "is electrophilic or can be metabolically activated to electrophiles", with human but not animal data on "is genotoxic".

Fig. 22.6 shows the key characteristics of the six categories of Group 1 agents considered in Volume 100: pharmaceuticals; biological agents; arsenic, metals, fibres, and dusts; radiation; personal habits and indoor combustions; and chemical

Fig. 22.4. Heat map showing the strength of evidence for key characteristics of 86 Group 1 agents according to the number of information sources (sources are human in vivo, human in vitro, animal in vivo, and animal in vitro studies).

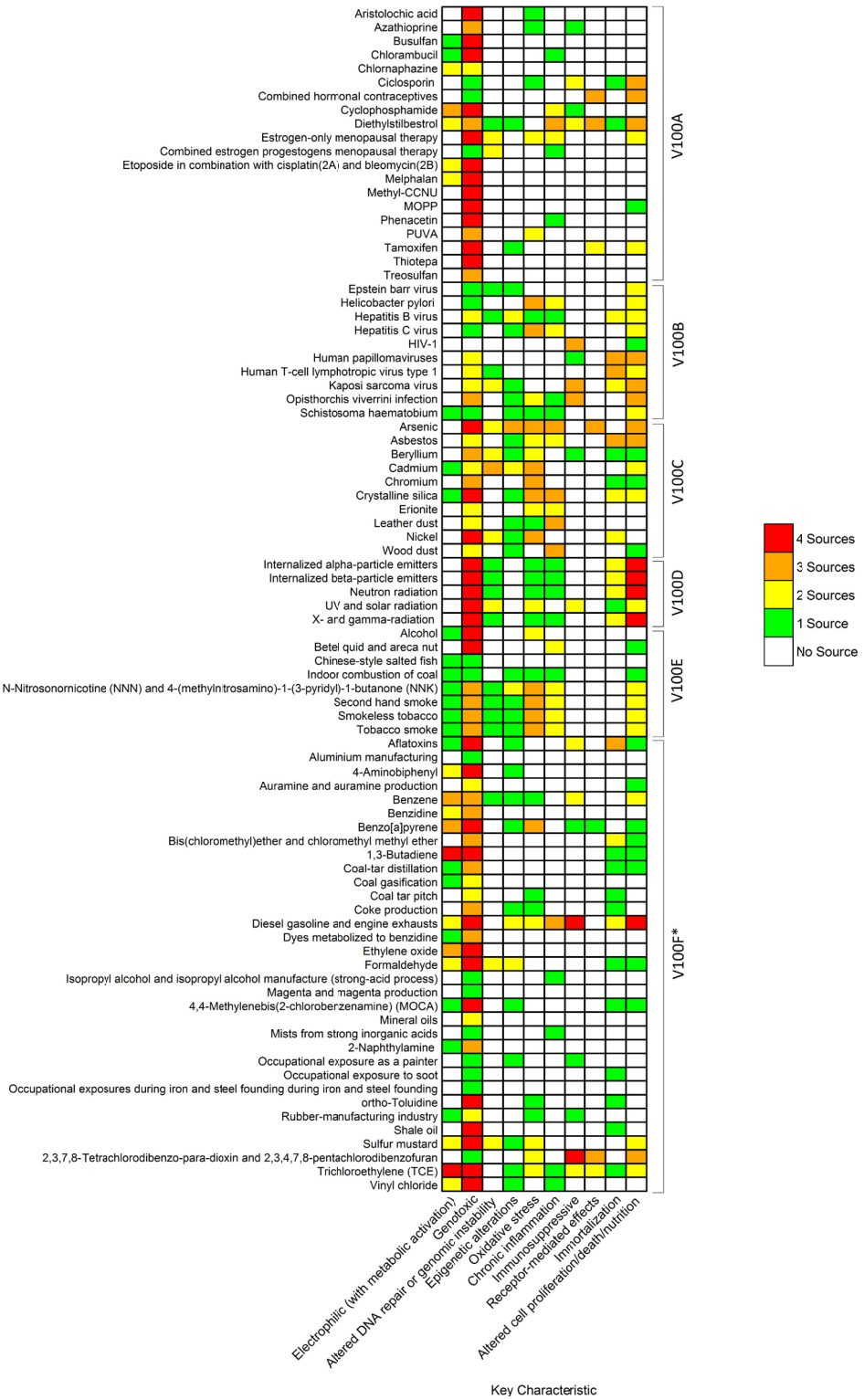


Fig. 22.5. Heat map showing the degree of concordance between human and animal sources of information on key characteristics of 86 Group 1 agents (after combining in vivo and in vitro sources of information for humans and for animals).

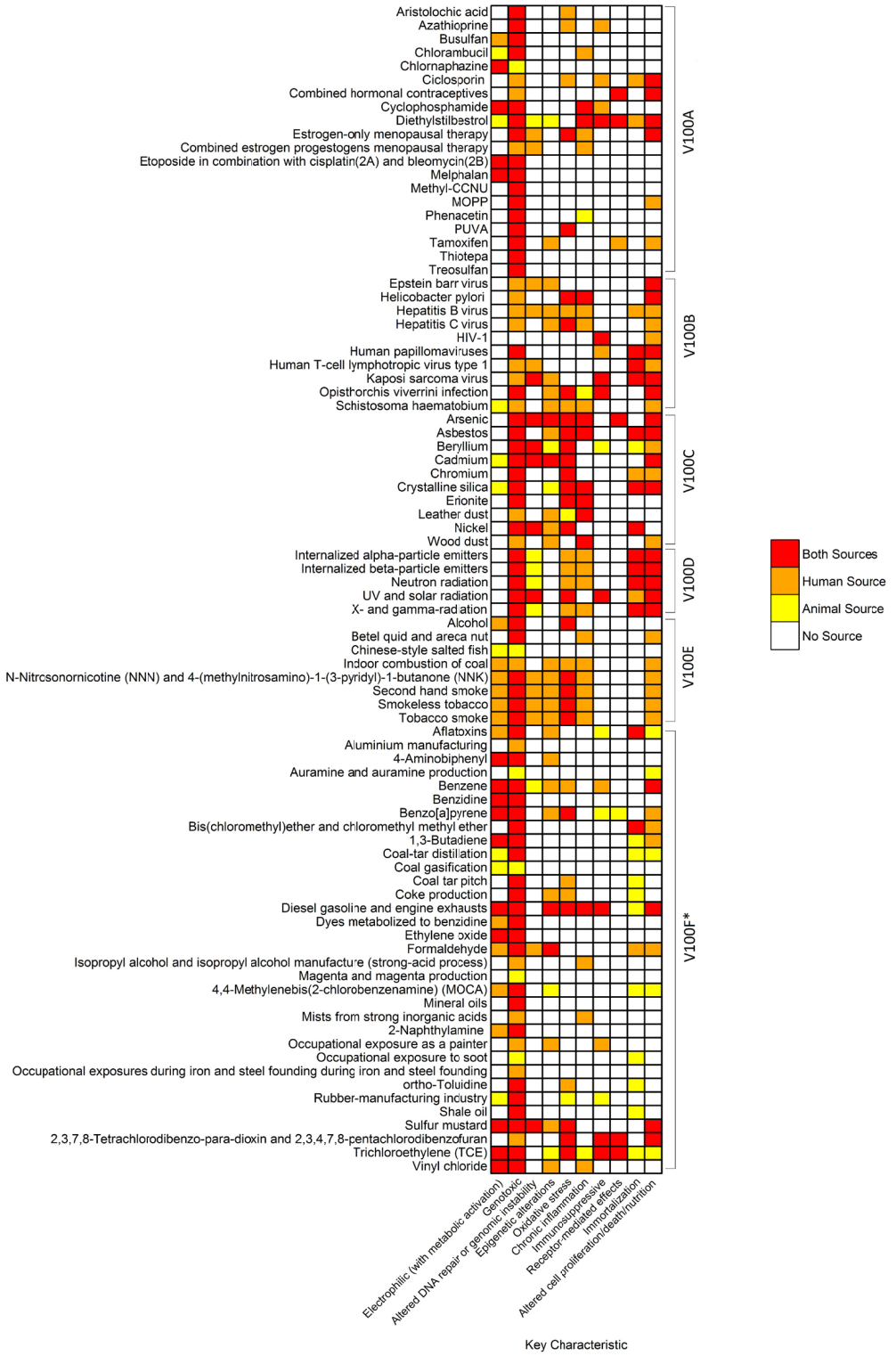


Fig. 22.6. Key characteristics of 86 Group 1 agents by type of agent (expressed as a percentage of the number of agents of each type demonstrating each of the 10 mechanistic characteristics): (a) pharmaceuticals; (b) biological agents; (c) arsenic, metals, fibres, and dusts; (d) radiation; (e) personal habits and indoor combustions; and (f) chemical agents and related occupations.

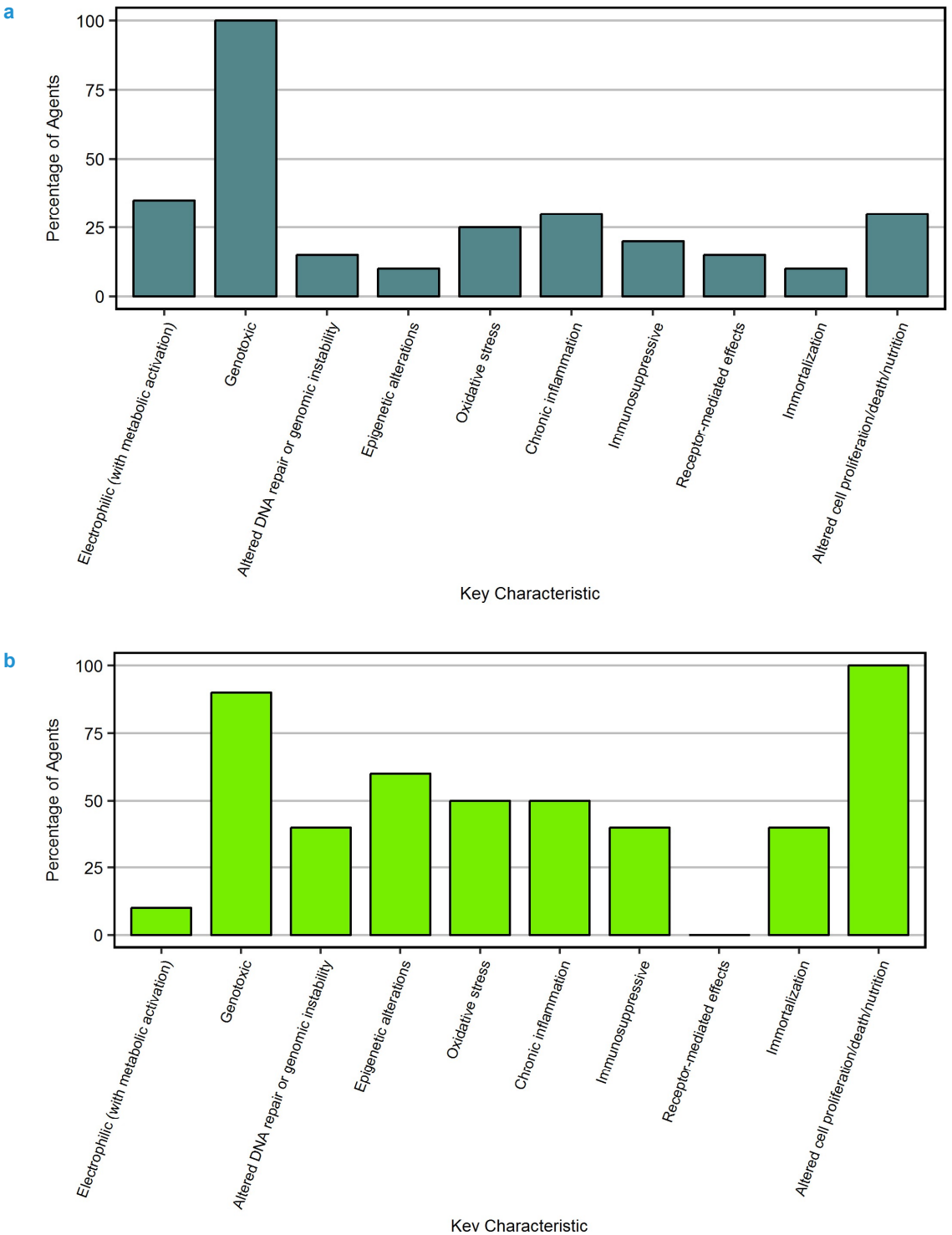
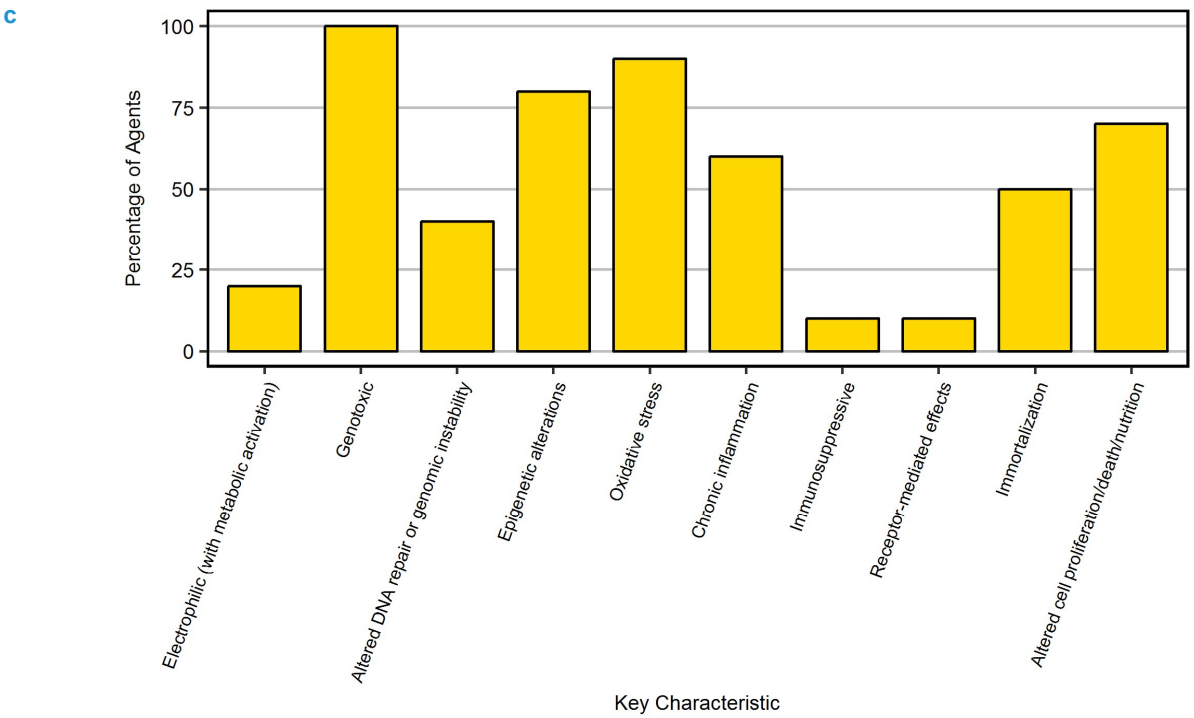


Fig. 22.6. Key characteristics of 86 Group 1 agents by type of agent (expressed as a percentage of the number of agents of each type demonstrating each of the 10 mechanistic characteristics): (a) pharmaceuticals; (b) biological agents; (c) arsenic, metals, fibres, and dusts; (d) radiation; (e) personal habits and indoor combustions; and (f) chemical agents and related occupations (continued).



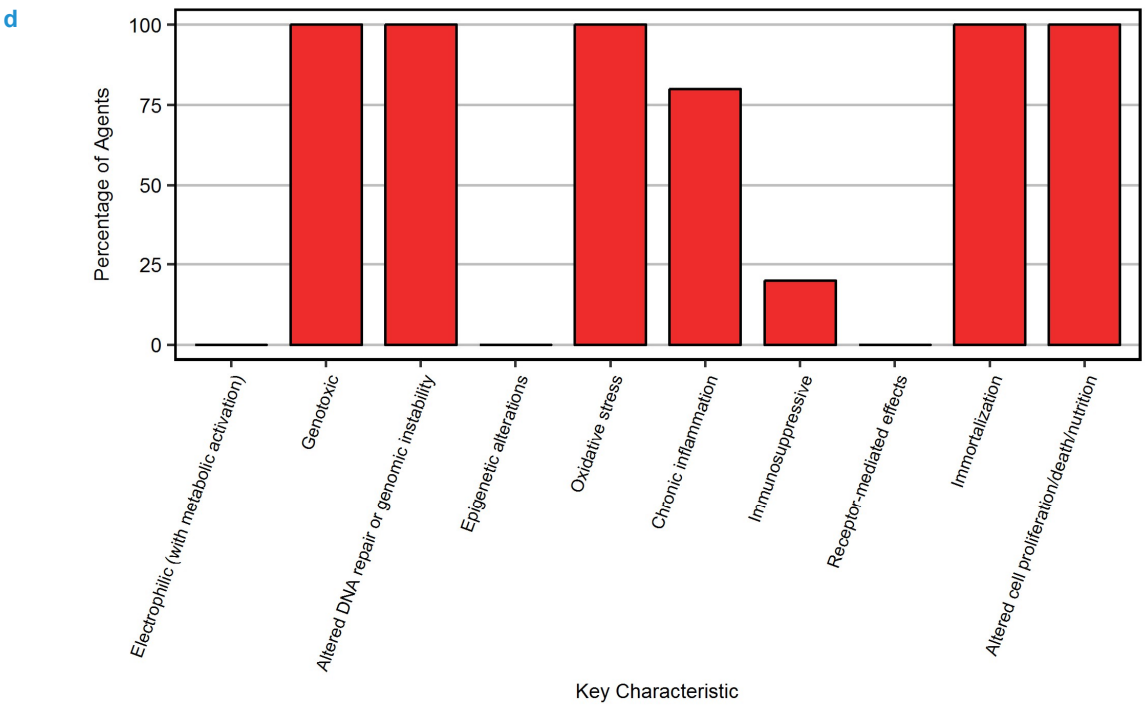
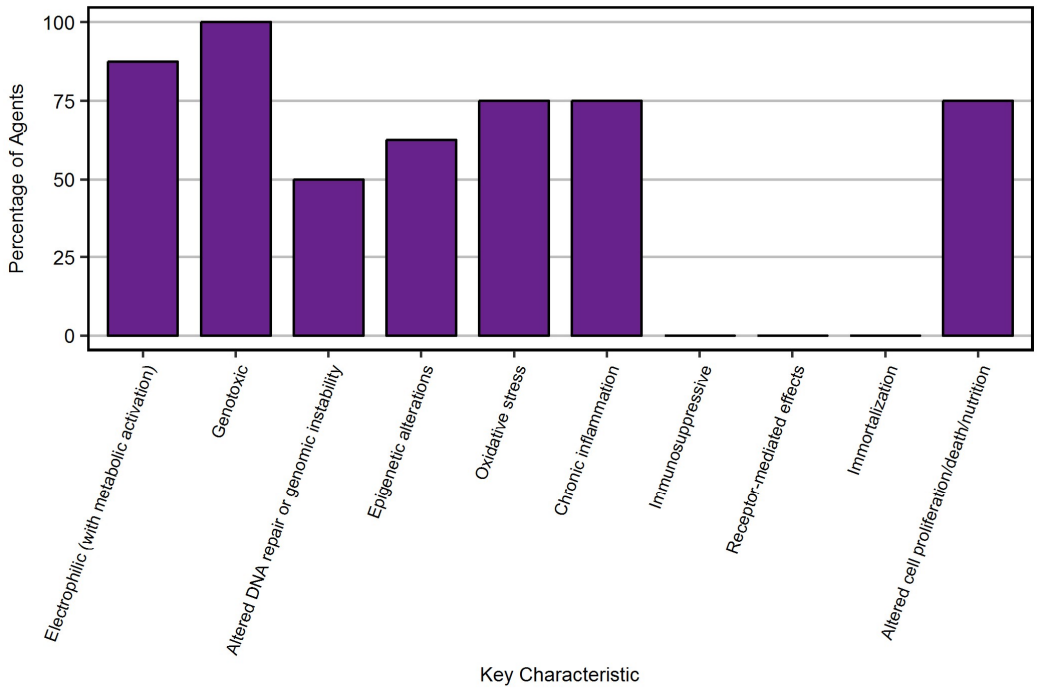


Fig. 22.6. Key characteristics of 86 Group 1 agents by type of agent (expressed as a percentage of the number of agents of each type demonstrating each of the 10 mechanistic characteristics): (a) pharmaceuticals; (b) biological agents; (c) arsenic, metals, fibres, and dusts; (d) radiation; (e) personal habits and indoor combustions; and (f) chemical agents and related occupations (continued).

e



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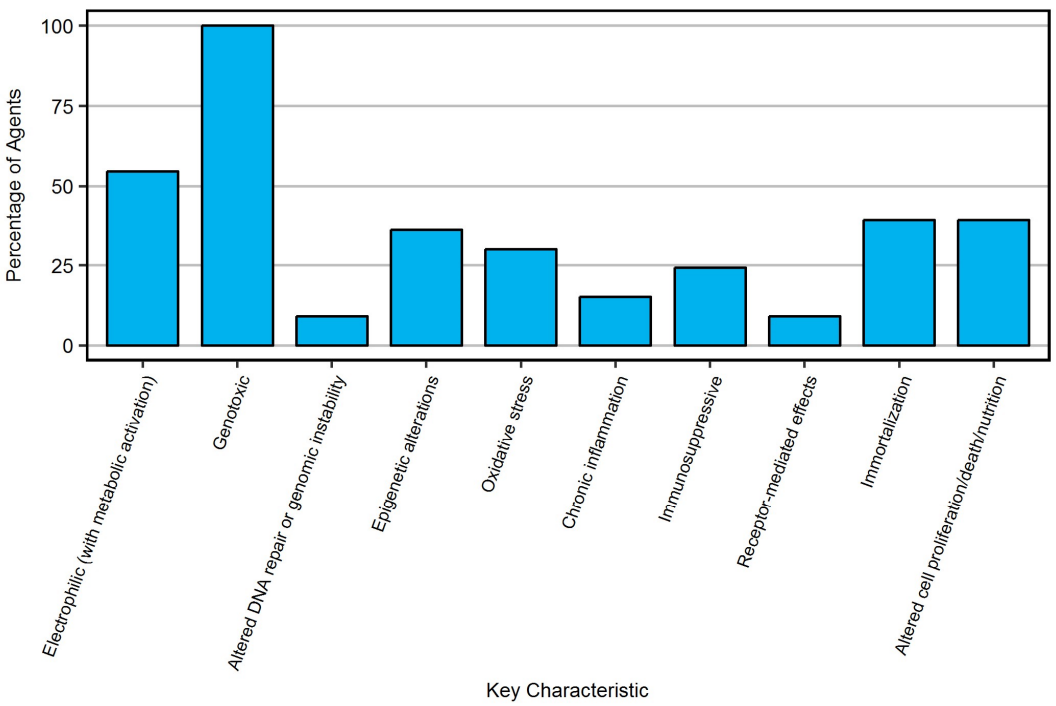

agents and related occupations. Genotoxicity was the most prevalent key characteristic demonstrated by agents in the categories of pharmaceuticals; arsenic, metals, fibres, and dusts; personal habits and indoor combustions; and chemical agents and related occupations, and genotoxicity was exhibited by all agents in the category of radiation. Immortalization, genotoxicity, and altered cell proliferation, cell death, or nutrient supply are prominent characteristics of the biological agents. None of the biological agents demonstrated modulation of receptor-mediated effects, and none of the agents in the category of personal habits and indoor combustions appeared to act through modulation of receptor-mediated effects, through immunosuppression or through immortalisation. There are five agents in the category of radiation, all of which demonstrate the following key characteristics: is genotoxic; alters DNA repair or causes genomic instability; induces oxidative stress; causes immortalization; and alters cell proliferation, cell death, or nutrient supply. The profiles of key characteristics for pharmaceutical agents and for chemical agents and related occupations are remarkably similar, possibly reflecting the fact that despite their different exposure circumstances, some of these chemical entities may act via similar mechanisms.

Discussion

The present analysis of key characteristics of 86 agents classified as *carcinogenic to humans* (Group 1) by the *IARC Monographs Programme* was based on mechanistic information retrieved from the *IARC Monographs* (see Al-Zoughool et al., 2019 and Birkett et al., 2019). The profiles of key characteristics of these agents show several interesting patterns. First, all but seven agents exhibited multiple characteristics, an observation consistent with previous findings on the complexity and heterogeneity of carcinogenic pathways (Hanahan and Weinberg, 2011; Floor et al., 2012; Baker, 2014; Pickup et al., 2014; Roessler et al., 2014). Agents in the categories of biological agents; arsenic, metals, fibres, and dusts; personal habits and indoor combustions; and radiation demonstrated a wide spectrum of biological activity. Radiation has been linked to many hallmarks of cancer (Boss et al., 2014): this mechanistic profile, with multiple pathways involved for most radiation agents, is consistent with the broad spectrum of tumours associated with exposure to ionizing radiation (Chapter 21, by Krewski et al.). Viral oncogenesis is also multifaceted, and the multistep nature of viral oncogenesis is thought to be influenced by host genetic variability (Mesri et al., 2014).

Genotoxicity was the most prevalent mechanistic characteristic, demonstrated by 85 of the 86 agents considered, possibly reflecting the fact that the process of carcinogenesis necessarily involves genomic changes. This finding is consistent with an earlier evaluation of 180 Group 1, Group 2A, and Group 2B agents conducted by Bartsch and Malaveille (1989), who reported that 80–90% of the agents in these three categories demonstrated genotoxic characteristics. In the present analyses, genotoxicity was considered to include the following end-points: DNA damage, cytogenetic effects (including chromosomal aberrations, micronucleus formation, and aneuploidy), and gene mutations. Information drawn from the *IARC Monographs* showed that the overwhelming majority of the agents examined here induce one or more of these end-points. Even biological agents such as viruses that act primarily through non-genotoxic mechanisms induce cytogenetic effects and gene mutations as secondary events through chronic inflammation and oxidative stress.

Another important observation is that information on the key characteristics of the 86 Group 1 agents considered here is often derived from multiple sources (human in vivo, human in vitro, animal in vivo, and animal in vitro studies); for many agents, evidence is available from more than one of these sources. Concordance between animal and human sources of information was seen for several agents, particularly with respect to genotoxicity, an observation that lends additional support to the relevance of animal data for human cancer risk assessment.

Some caution must be used in interpreting the distribution of key characteristics across the Group 1 agents considered here. It is possible that the near universality of genotoxicity as a carcinogenic mechanism may be related to the way in which the *IARC Monographs* were compiled, with emphasis on the reporting of genotoxicity data. This would have been partially mitigated by the inclusion of mechanistic information from outside the *IARC Monographs* in the preparation of the mechanistic database evaluated separately by Birkett et al. (2019). It should also be noted that the *IARC Monographs* have been published over a long time span, extending from the early 1970s to the present (Saracci and Wild, 2015). Studies of agents in earlier *Monographs* would

have focused on changes such as DNA damage that could have been detected by the techniques available at that time. These agents may not have been evaluated exhaustively for pathways that have been identified more recently, such as those involving the multifactorial nature of carcinogenesis, or for the multiplicity of pathways operating during the process of agent-induced carcinogenesis.

A related limitation of the present analysis is that it did not distinguish direct genotoxicity of the agent or its metabolites from genotoxicity that occurs as a result of other responses, because this information was not generally provided in the *IARC Monographs* from which the mechanistic data on the Group 1 agents were abstracted. It is recommended that the distinction between primary and secondary genotoxic effects be noted in future *Monographs*, whenever possible.

Another limitation of the present results is that they are based on the information on mechanisms in Section 4 ("Other relevant data") of the *IARC Monographs*, which focused primarily on "established" and "likely" mechanisms. A full systematic review of the entire literature on biological mechanisms for all Group 1 agents was not undertaken, so the database may not reflect all mechanistic characteristics of the different agents. As a sensitivity analysis to examine the extent to which the *Monographs* captured most of the relevant information in this regard, Birkett et al. (2019) conducted a supplementary PubMed search to identify additional information on key characteristics not cited in the *Monographs*, or published since 2009. Although this sensitivity analysis was not based on an exhaustive search, it did identify additional information sources, of which the most notable was the identification of evidence for six additional agents that demonstrate modulation of receptor-mediated effects, beyond the seven agents noted in Fig. 22.1. Nonetheless, the overall findings are largely comparable with those presented without the additional data search (for further details, see Birkett et al., 2019).

As the *IARC Monographs Programme* has evolved from its inception in the early 1970s until the present time, the guidelines for carcinogen identification as set out in the Preamble to the *IARC Monographs* (IARC, 2006) have been updated from time to time, with increasing emphasis on the use of mechanistic information in the overall evaluation in the most recent updates. Nonetheless, the identification of Group 1 agents continues to rest heavily on the availability of *sufficient evidence* of carcinogenicity in epidemiological or clinical studies. Of the 111 distinct Group 1 agents identified up to and including Volume 109, no less than 102 demonstrated *sufficient evidence* of carcinogenicity in humans, and the remaining nine agents were placed in Group 1 after reference to mechanistic data or other considerations (as "mechanistic upgrades" according to the evaluation criteria outlined in the Preamble to the *IARC Monographs*; see Table 21.4, in Chapter 21, by Krewski et al.). Despite the inherent reliance on human epidemiological data in identifying agents that may increase human cancer risk, Section 4 ("Other relevant data") of the *IARC Monographs* increasingly provides detailed descriptions of the mechanisms by which agents under review may act, including agents not assigned to Group 1.

The epigenetic characteristics of the 74 Group 1 agents considered in Volumes 100A–E were assessed by Herceg et al. (2013). As in the present analysis, those authors used DNA methylation, histone modification, and altered expression of miRNAs as indicators of epigenetic alterations. They considered information from both the *IARC Monographs* and the general scientific literature, and identified 22 of the 74 Group 1 agents (30%) as demonstrating epigenetic effects. The present analysis, which examined Group 1 agents in Volumes 100A–F as well as Volumes 105 and 106, identified 33 of the 86 Group 1 agents (38%) as mediating epigenetic change.

In an earlier evaluation, Hernández et al. (2009) reported that 45 of the 371 agents (12%) in Groups 1, 2A, and 2B at the time of their analysis were not genotoxic. In their study, an agent was considered non-genotoxic if it showed negative results in the *Salmonella* mutagenicity assay (the Ames test) as well as in the mouse lymphoma assay, the in vitro chromosomal aberration test, the in vitro micronucleus test, the in vivo micronucleus test, and the in vivo chromosomal aberration test in bone marrow in rodents. These results support the role of non-genotoxic pathways in carcinogenesis, an observation that is reinforced by the prevalence of multiple characteristics of human carcinogens not associated with genotoxicity in the present analysis.

To ensure that all relevant evidence on the 10 key characteristics of human carcinogens is taken into account in future *Monographs* evaluations of agents that may cause cancer in humans, a carefully designed systematic review of the scientific literature would be required in conjunction with each evaluation.

However, to conduct a series of comprehensive systematic reviews of the key characteristics of all 86 agents considered in the present analysis would require a considerable effort, and was not attempted as part of the present project. The expert opinion of future IARC Working Groups charged with evaluating the mechanistic data on new agents selected for evaluation by the *IARC Monographs* would be of considerable value in this regard, but would ideally be supported by a concomitant systematic review of the relevant scientific literature on the key characteristics to ensure that the analysis would be as complete as possible.

Another issue that arises when discussing key characteristics of human carcinogens is whether indirect effects should be considered. Many agents have a direct carcinogenic effect, but in other cases the carcinogenic characteristic is the result of a secondary event along the mechanistic pathway. For example, cell proliferation can arise either as a result of a direct action of the agent on the cell or indirectly, as a result of cytotoxicity that stimulates cell proliferation to replace cells, through alterations in cell signalling without cytotoxicity, or via inhibition of cell proliferation that then results in selection of an altered clone of cells with a high proliferation rate. Although the downstream effect is the same (increased cell proliferation), the pathway leading to that result can be different. A similar issue arises with genotoxicity: many agents are not directly genotoxic but cause DNA damage by stimulating a chain of molecular changes (e.g. chronic inflammation). The current database does not contain the information needed to address these issues and cannot be used to draw conclusions about the detailed mechanism of action of an agent.

The 10 key characteristics are features of carcinogens rather than mechanisms. The analysis presented here does not address the sequence of events involved in carcinogenesis. For example, if the carcinogenic mechanism of action is being investigated for a genotoxic agent that requires metabolic activation, the mechanism needs to consider the entire metabolic pathway. If the agent is not metabolized to produce an electrophile, DNA damage will not occur. In such a case, biological effects that occur after induction of DNA damage also would not be observed. This sequential relationship is also apparent for characteristics such as chronic inflammation, which acts through the production of oxidative stress, release of cytokines, and stimulation of cell proliferation, which ultimately produces DNA damage.

The results of the present analysis can provide a basis for future efforts to categorize mechanistic data for carcinogens through a systematic review process. A full systematic review of all agents and all potential carcinogenic mechanisms is an intimidating prospect. However, such a review would provide a more comprehensive examination of mechanisms, because it would include studies that failed to find effects. It might also support a process that involves a sequence of mechanistic steps and mechanistic characteristics relevant to the development of cancer in humans.

The importance of systematic review in assembling all relevant evidence on a particular issue has been emphasized in the recent review of the EPA's Integrated Risk Information System (IRIS) (National Research Council, 2014) and is currently being implemented within the IRIS programme as a way of summarizing all relevant data in a comprehensive and reproducible manner. The EPA is also currently supporting the development of software tools specifically designed for systematic review of toxicological and epidemiological data (ICF, 2017).

The strong evidence linking genotoxicity to carcinogenesis is consistent with epidemiological data and experimental research. Genotoxic effects include the formation of DNA adducts or induction of single- and double-strand DNA breaks. Several lines of evidence from epidemiological studies and in experimental animals and model systems have shown that DNA adducts are strongly associated with cancer (Kriek et al., 1998; Phillips et al., 2015). Some genotoxic effects can lead to gene mutation, an important event in the pathway towards carcinogenesis, especially if it involves oncogenes or tumour suppressor genes. Chromosomal aberrations are another type of genetic alteration that occurs frequently in many tumours, especially solid tumours. Most tumour cells display aneuploidy, and for some tumours, characteristic chromosomal abnormalities have been identified (e.g. the Philadelphia chromosome in chronic myeloid leukaemia).

The complexity of the pathways involved in carcinogenesis and the fact that the cellular response to carcinogen exposure is modulated by host cell physiology, genetics, and other variables have prompted the development and application of sensitive assays that measure toxicity pathways and perturbations in the molecular functioning of the cell. The newly proposed toxicological testing paradigm (Krewski et al., 2014) focuses on high-throughput screening to detect changes in the molecular pathways of the cell in response to chemical exposure. This new

paradigm would be useful in comprehensive cancer risk assessment and would be able to detect distinct key mechanistic pathways operating after carcinogen exposure. In a similar initiative, the Kyoto Encyclopedia of Genes and Genomes (KEGG) website has compiled a comprehensive list of pathways associated with specific diseases (see the KEGG pathway database at http://www.genome.jp/kegg/pathway.html). KEGG also identified major in vitro assays that can be used to detect targets of these pathways. This attempt to understand the biological mechanisms of carcinogenesis is consistent with current practice of using in vitro assays to detect changes in critical signalling and other molecular pathways in cancer development, as proposed by Krewski et al. (2014).

Further analyses

The extensive database on key characteristics of human carcinogens developed here offers considerable potential for further analysis. More in-depth analyses are under way to explore the level of agreement between mechanistic data derived from human sources on the one hand and from animal sources on the other, as well as from in vivo and in vitro sources, issues that have received only limited attention here. An analysis of the key characteristics demonstrated by Group 1 agents on a site-specific basis is also planned; if agents that cause tumours at a specific site (e.g. the lung or the liver) are shown to demonstrate similar characteristics, this could provide new insights into site-specific carcinogenesis.

Although the present analysis found that most Group 1 agents demonstrated multiple key characteristics, with an average of four characteristics per agent, no attempt was made to conduct a multivariate analysis of these characteristics to determine whether similar agents tended to express similar characteristics. Recalling that pharmaceuticals as a class demonstrated a mechanistic profile similar to that of chemical agents and related occupations, it is possible that the chemotherapeutic agents and some of the chemical agents act via the same carcinogenic mechanisms. Cyclophosphamide and benzene (once used as a chemotherapeutic agent) may have some commonality in this respect, as might treosulfan and butadiene through the formation of the same diepoxide. Further study of these two groups, in terms of both mechanism of action and tumour site concordance, may provide insight into tumours that result from long-term exposure to chemotherapeutic agents.

Searching for patterns within homogeneous classes of agents would also be of future research interest. For example, one could examine mechanistic patterns within subgroups of pharmaceuticals, including antineoplastic agents, hormonal products, immunosuppressants, and analgesic mixtures. In a similar vein, Shin et al. (2015) have recently used bioactivity profiles for 38 agents derived from high-throughput in vitro assays to investigate patterns of toxicity associated with different scenarios of use.

Exposure to a single agent may result in the development of more than one type of tumour, perhaps through different pathways that involve different mechanistic characteristics. It would be of interest to examine the key characteristics for agents associated with specific tumour types. This would extend the work of Krewski et al. (Chapter 21) that examined concordance between animals and humans for 39 tumour sites and 14 organ and tissue systems, based on the database on tumours and tumour sites in humans and experimental animals developed by Grosse et al. (Annex 1). The profiles of key characteristics of agents associated with specific tumour sites could be examined to obtain additional insights into the mechanisms by which specific tumours occur. It would be of particular interest to analyse whether certain tumour sites demonstrate signature profiles.

Extending the mechanistic database to include additional information such as structural alerts relevant to carcinogenesis could also be informative. Although the present version of the mechanisms database includes the International Union of Pure and Applied Chemistry (IUPAC) International Chemical Identifier (InChI) for key chemical coding (Stein et al., 2003; Heller et al., 2015), this information has not been taken into account in the analyses completed to date. One possible source of auxiliary information on toxicological end-points that may be related to the 10 key characteristics is EPA's Toxicity Forecaster (ToxCast) programme (Judson et al., 2014; Knudsen et al., 2015), which now includes in vitro, in vitro, and in silico data on diverse toxicological end-points for more than 10 000 chemical substances, some of which overlap with the set of Group 1 agents considered in this chapter. The ToxCast database also includes information on several hundred toxicological assays, which could enrich the database of key characteristics used in the present analysis.

With the elaboration of the 10 key characteristics articulated by

Smith (Chapter 10) and Smith et al. (2016), mechanistic evaluations of new agents undertaken within the *IARC Monographs Programme* are beginning to make use of these characteristics, including the use of formal methods of systematic review to identify relevant mechanistic information. This has been successfully attempted in recent evaluations of some organochlorine insecticides and some chlorophenoxy herbicides (Loomis et al., 2015; Volume 113) and of red meat and processed meat (Bouvard et al., 2015; Volume 114).

It is expected that the search strategies used in future mechanistic evaluations will be refined as experience with the key characteristics accumulates. In an earlier evaluation of evidence of epigenetic alterations for 28 Group 1 agents, Chappell et al. (2016) searched for evidence of DNA methylation, histone modification, and expression of non-coding miRNAs, as was done in the present analysis, but with the addition of several more detailed search terms, specifically long non-coding RNA (lncRNA), small RNA, chromatin, and promotor methylation. Chappell et al. (2016) noted that the great majority (89%) of the studies on lncRNAs included in their review reported alterations in miRNAs, leading to results largely consistent with the search terms used here: 43% (12 of 28) of the agents evaluated by these authors demonstrated evidence of epigenetic alterations, similar to the 38% (33 of 86) of agents included in the present analysis. Continued experience with the evaluation of the 10 key characteristics of human carcinogens can be expected to further refine the criteria used for their identification, including both the toxicological events associated with these key characteristics and the assays used as evidence of these events.

There could be value in revisiting the present retrospective analysis of the 86 Group 1 agents identified in the *IARC Monographs* up to and including Volume 106, with respect to the conduct of a series of comprehensive systematic reviews on the 10 key characteristics of these agents, followed by an in-depth evaluation of the findings of the systematic reviews by experts in relevant disciplines. The development of criteria for evaluating the weight of evidence for the key characteristics, similar to that included in the Preamble to the *IARC Monographs* for human and animal data (IARC, 2006), might be contemplated at that time. Group 1 agents identified beyond Volume 106 for which mechanistic information had become available could also be included in such an analysis.

Baker et al. (2016) have recently applied supervised machine learning techniques to classify PubMed literature according to the hallmarks of cancer (Hanahan and Weinberg, 2000, 2011). In a case study of basal cell carcinoma and melanoma, only 46,727 of 121,488 abstracts from their original systematic literature search were classified as relevant, reflecting the potential time savings that may be achieved through automatic classification. An approach to extracting information on the 10 key characteristics of human carcinogens would be to apply these machine learning techniques and biomedical text mining methods to identify, in an automated fashion, articles associating these key characteristics with specific Group 1 agents. Because of the sheer size of a full systematic review of mechanistic information on all Group 1 agents, the use of automated search algorithms of this type could offer considerable efficiency gains in identifying potentially relevant mechanistic information. Although this approach could expedite identification of relevant articles, expert opinion and application of weight-of-evidence criteria would still have value in reducing the errors in the assignment of key characteristics to specific agents.

Conclusions

In considering the results presented in this chapter, it is important to emphasize that these mechanistic analyses are a first step in understanding the biological mechanisms by which cancer may occur in humans. Although considerable effort was expended in developing the database of key characteristics and their analyses in this chapter, these results should be viewed as preliminary, to be refined through more exhaustive systematic reviews of the relevant scientific literature and/or through discussion with a broad panel of experts on the mechanisms of carcinogenesis. The 10 key characteristics were endorsed by the participants in the IARC Workshop on Tumour Site Concordance and Mechanisms of Carcinogenesis, which provided oversight for this project; additional experience with the exploration of these characteristics in cancer research will serve to define their utility more fully. Equally important is to consider the nature of the evidence needed to establish that specific mechanistic characteristics are associated with human carcinogens. The current database has relied on the demonstration of certain toxicological end-points as evidence of these mechanistic characteristics; further consideration of these and other possible markers of the key characteristics of human carcinogens is warranted.

Mechanistic considerations are becoming increasingly prominent in the *IARC Monographs*, thereby enriching the body of evidence on which future analyses of this type may be based. The authors are planning to update the mechanistic database to include *Monographs* published subsequent to Volume 106, a task that will be greatly facilitated by the documentation of key characteristics of agents evaluated in recent *Monographs*.

Summary

Since its inception in the early 1970s, the *IARC Monographs Programme* has evaluated more than 1000 agents with respect to their carcinogenic hazard; of these, up to and including Volume 119 of the *IARC Monographs*, 120 agents met the criteria for classification as *carcinogenic to humans* (Group 1). Volume 100 of the *IARC Monographs* provided a review and update of Group 1 carcinogens. These agents were divided into six broad categories: pharmaceuticals; biological agents; arsenic, metals, fibres, and dusts; radiation; personal habits and indoor combustions; and chemical agents and related occupations. Data on biological mechanisms of action were extracted from the *Monographs* to assemble a database on the basis of 10 key characteristics of human carcinogens. After some grouping of similar agents, the characteristic profiles were examined for 86 Group 1 agents for which mechanistic information was available in the *IARC Monographs* up to and including Volume 106, based on information derived from human in vivo, human in vitro, animal in vivo, and animal in vitro studies. The most prevalent key characteristic was "is genotoxic", followed by "alters cell proliferation, cell death, or nutrient supply" and "induces oxidative stress". Most agents exhibited several of the 10 key characteristics, with an average of four characteristics per agent, a finding consistent with the notion that cancer development in humans involves multiple pathways. Information on the key characteristics was often available from multiple sources, with many agents demonstrating concordance between human and animal sources, particularly with respect to genotoxicity. Although a detailed comparison of the characteristics of different types of agent was not attempted here, the overall characteristic profiles for pharmaceutical agents and for chemical agents and related occupations appeared similar. Further in-depth analyses of this rich database of characteristics of human carcinogens are expected to provide additional insights into the mechanisms of human carcinogenesis.

Acknowledgements

The authors are grateful to Kate Z. Guyton for many helpful comments, which resulted in both improvements in the presentation of this chapter and additional insights into the findings in this chapter, and to the editors for their thorough review of several preliminary drafts. Mélissa Billard was a Visiting Scientist at IARC during the summers of 2013 and 2014, working under the direction of Yann Grosse and Robert Baan on assembling the mechanistic database analysed in this chapter. Julian Little is the Canada Research Chair in Human Genome Epidemiology at the University of Ottawa. Daniel Krewski is the Natural Sciences and Engineering Research Council of Canada Chair in Risk Science at the University of Ottawa. This chapter is dedicated to the memory of Jan M. Zielinski, who succumbed to cancer in 2016; before becoming ill, Dr Zielinski led the analytical team that conducted the analyses reported here.

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ANNEX 1.

Development of a data set on tumours and tumour sites in humans and in experimental animals for Group 1 agents identified up to and including Volume 109 of the *IARC Monographs*

Yann Grosse, Pascale Lajoie, Mélissa Billard, Daniel Krewski, Jerry M. Rice, Vincent J. Cogliano, Michael Bird, and Jan M. Zielinski (deceased)

Introduction

Since its establishment in the early 1970s, the *IARC Monographs Programme* has conducted hazard evaluations of agents that may increase the risk of cancer in humans. The reviews of the relevant literature and the ensuing evaluations are conducted by international Working Groups of expert scientists according to a well-established and rigorous protocol that is described in the Preamble to the *IARC Monographs* (IARC, 2006). The reviews and evaluations are published as the *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*.

For Volume 100 of the *IARC Monographs*, a review was undertaken of relevant information on all the agents classified in Group 1 (*carcinogenic to humans*). For convenience, Volume 100 was organized in six parts (100A–100F), covering pharmaceuticals (IARC, 2012e); biological agents (IARC, 2012b);

arsenic, metals, fibres, and dusts (IARC, 2012a); radiation (IARC, 2012f); personal habits and indoor combustions (IARC, 2012d); and chemical agents and related occupations (IARC, 2012c).The reviews and analyses were discussed during a two-part Workshop on Tumour Site Concordance and Mechanisms of Carcinogenesis, which was convened by IARC on 16–18 April 2012 and 28–30 November 2012 in Lyon.

The data set described in this Annex also includes information on five additional human carcinogens that were added to Group 1 after completion of Volume 100, i.e. diesel engine exhaust (Volume 105; IARC, 2013), trichloroethylene (Volume 106; IARC, 2014), polychlorinated biphenyls (Volume 107; IARC, 2016b), and outdoor air pollution and particulate matter in outdoor air pollution (Volume 109; IARC, 2016a). For ease of reference, these five agents are included in an expanded group of chemical agents and related occupations denoted by Volume 100F*. Although additional Group 1 agents have been identified in subsequent volumes of the *Monographs*, the current data set extends only up to and including Volume 109, the last *Monograph* for which final results were available at the time this Annex was completed.

The reviews and updates in Volumes 100A–F specifically focused on identification of tumours, both in humans and in experimental animals, resulting from exposure to each of the Group 1 agents. In addition, the organs where the tumours were reported to arise were documented where possible. The availability of this information on the more than 100 human carcinogens in Group 1 prompted an investigation of what level of concordance may exist between humans and experimental animals with respect to tumours and tumour sites. To this end, the pertinent information in Volume 100 was captured in a comprehensive table (Table A1; online only; available from: http://publications.iarc.fr/578) that could then serve as a basis to develop a database on tumour sites in animals and humans. The creation of such a database – designed to be amenable to biostatistical analysis (see Chapter 21, by Krewski et al.) – was motivated by the interest in a statistical assessment of the degree of concordance between animal and human tumour sites. This important scientific question bears upon the extent to which the animal cancer data collected here may be extrapolated to humans. It is anticipated that the database will also find other applications, including in the development of human tumour profiles to assist in the identification of additional Group 1 agents.

It should be noted that for agents classified in Group 2A (*probably carcinogenic to humans*) or Group 2B (*possibly carcinogenic to humans*) the information on cancer in humans may often be lacking or may not be strong enough for a proper interspecies comparison to be made. For this reason, the concordance analysis (see Chapter 21, by Krewski et al.) is focused on agents in Group 1. In addition, it was decided that *sufficient evidence* of carcinogenicity in humans and *sufficient evidence* of carcinogenicity in experimental animals were required for an agent to be included in the statistical concordance analysis; with less than *sufficient evidence* of carcinogenicity, in humans or in animals, the definition of a tumour site in either species would become less reliable or impossible. Therefore, although the data set described in this Annex (Table A1; online only; available from: http://publications.iarc.fr/578) provides information on all the Group 1 agents, the actual database of human carcinogens eligible for the concordance analysis is appreciably smaller (see Chapter 21, by Krewski et al.).

Methods

In making an evaluation of the evidence of carcinogenicity to humans, an *IARC Monographs* Working Group is generally asked to identify organ sites in humans for which there is *sufficient evidence* of carcinogenicity of the agent under study. However, the Working Group is not required to identify organ sites for carcinogenicity in experimental animals at the time of the evaluation, but is required more simply to assess the overall weight of the evidence in experimental animals. Consequently, for the purpose of this IARC Scientific Publication, the species-specific tumour sites in experimental animals needed to be identified for each Group 1 agent before proceeding to explore concordance between animal and human cancers.

During the six meetings for Volumes 100A–F, the respective Working Groups identified studies in experimental animals that provided results on species-specific tumour sites. This was based on criteria adapted from the Preamble to the *IARC Monographs*. It was considered that there is *sufficient evidence* for identifying a species-specific tumour site in experimental animals under any one of the following three conditions:

- An increased incidence of malignant neoplasms or an appropriate

combination of benign and malignant neoplasms *originating from the same organ (or tissue)* is identified in 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 malignant neoplasms or an appropriate combination of benign and malignant neoplasms *originating from the same organ (or tissue)* is identified *in both sexes* of one species in one well-conducted study, ideally performed under good laboratory practice (GLP).
- A single study in one species and sex might be considered to provide *sufficient evidence* for a specific organ site when malignant neoplasms occur to an unusual degree with regard to incidence, type of tumour, or age at onset.

Confirmation of the tumours and tumour sites identified in Volume 100 was performed by one member each from the IARC secretariat and from the project team at the University of Ottawa, Canada, who systematically consulted the original publications describing the studies cited in the Volume 100 reviews. It was decided by the Workshop participants that extraction of the following information was required for each study: species, strain, sex, route of exposure, and tumour site including histology. Further information would be recorded as "study details", for example dose, number of test animals, number of control animals, age at start of exposure, duration of exposure, duration of follow-up, and statistical analyses. The two team members independently captured the information, and any disagreements were resolved in a group discussion. Tables summarizing this information were created to enable peer review by the Workshop participants to confirm the entries. Ultimately, more than 2000 studies were reviewed, and more than 1000 of these contributed to the identification of species-specific tumour sites in experimental animals. Studies were not considered if any one of the following exclusion descriptors was applicable:

- initiation–promotion studies;
- co-carcinogenicity studies;
- studies in genetically modified animals;
- studies with precancerous lesions as the outcome;
- studies on the carcinogenicity of metabolites and derivatives;
- studies with non-laboratory animals (livestock; companion animals);
- studies with analogous agents (similar chemical structure or similar virus type).

Results

Table A2 illustrates the format of the data set on tumours and tumour sites, with one agent from each of Volumes 100A–F. From epidemiological studies, human tumour sites with *sufficient evidence* and those with *limited evidence* are mentioned. For experimental animals, tumour sites are recorded only for agents that demonstrate *sufficient evidence* of carcinogenicity, as indicated above. Strain, sex, and route of exposure reported for each animal study are also captured. Comments are provided as appropriate. For example, no human tumour site is specified for aristolochic acid, because this agent was placed in Group 1 on the basis of the classification of plants containing aristolochic acid as a Group 1 agent, supported by mechanistic data on genotoxicity (IARC, 2012e). Together with other "mechanistic upgrades", this agent is listed in the complete data set (see Table A1; online only; available from: http://publications.iarc.fr/578) but is not included in the statistical analysis of concordance (see Chapter 21, by Krewski et al.).

All the information on tumours and tumour sites in humans and in experimental animals from *IARC Monographs* Volumes 100–109 is given in Table A1 (online only; available from: http://publications.iarc.fr/578).

Observations

For some Group 1 agents, there were only a few studies that contributed to the identification of a tumour site in experimental animals, and frequently the studies did not enable the definition of an organ site, as a result of inadequate reporting. There were many instances where the reported tumour incidences were uninformative, possibly as a result of the small number of animals tested. In other cases, studies reported an increased incidence of tumours but without mention of malignancy or proper description of histopathological details. Also, some reports did not specify the purity of the administered agent. In these cases the experts in the *Monographs* Working Groups and the two team members (one member each from the IARC secretariat and from the project team at the University of Ottawa, Canada) had to consider the possibility of confounding, because the existence of other agents in the administered sample could have contributed to the outcome. In some studies, animals were followed up for only short periods of time after treatment, especially in studies investigating acute adverse effects, which precluded observation of carcinogenic outcomes that may take longer to develop.

Table A2. Template for presentation of data on tumours and tumour sites in humans and in experimental animals from the *IARC Monographs*

Volume 100 part Agent number	Agent	Sites with *sufficient evidence* in humans	Sites with *limited evidence* in humans	Agent tested in experimental animals	Species Site	Histology	Study, sex, strain, exposure route	Comments
A 1	Aristolochic acid			Aristolochic acid	Rat Forestomach	Squamous cell carcinoma	Mengs et al. (1982) (Volume 82; Volume 100A), MF, Wistar, g.; Mengs (1983) (Volume 82; Volume 100A), M, Wistar, g.; Schmeiser et al. (1990) (Volume 100A), M, Wistar, d.w.; Hwang et al. (2006) (Volume 100A), M, Sprague-Dawley, g.	The experts consider concordance when an agent in humans such as *Plants containing aristolochic acid* is tested in animals by one of its main components such as *Aristolochic acid*.
A 1	Aristolochic acid			Aristolochic acid	Rat Renal pelvis	Transitional cell carcinoma	Mengs et al. (1982) (Volume 82; Volume 100A), M, Wistar, g.	The experts consider concordance when an agent in humans such as *Plants containing aristolochic acid* is tested in animals by one of its main components such as *Aristolochic acid*.
B 24	*Clonorchis sinensis* (infection with)	Cholangiocarcinoma						No data on animal experiments listed because of *limited evidence* of carcinogenicity.

Table A2. Template for presentation of data on tumours and tumour sites in humans and in experimental animals from the *IARC Monographs* (continued)

Volume 100 part Agent number	Agent	Sites with *sufficient evidence* in humans	Sites with *limited evidence* in humans	Agent tested in experimental animals	Species Site	Histology	Study, sex, strain, exposure route	Comments
C 35	Arsenic and inorganic arsenic compounds	Lung, urinary bladder, skin	Kidney, liver, prostate	Dimethylarsinic acid [DMA(V)], Monomethyl-arsonous acid [MMA(III)], Sodium arsenite	Mouse Lung	Bronchiolo-alveolar carcinoma	DMA(V): Tokar et al. (2012a), M, CD1, d.w.; Sodium arsenite: Waalkes et al. (2003), F, C3H/HeNCr, in utero; Waalkes et al. (2006a, b), M, CD1, in utero; Tokar et al. (2011), MF, CD1, in utero + p.o.; Tokar et al. (2012a), M, CD1, in utero; MMA(III): Tokar et al. (2012b), M, CD1, in utero	
C 35	Arsenic and inorganic arsenic compounds	Lung, urinary bladder, skin	Kidney, liver, prostate	Sodium arsenite, Monomethyl-arsonous acid [MMA(III)]	Mouse Liver	Hepatocellular carcinoma	Sodium arsenite: Waalkes et al. (2003), M, C3H/HeNCr, in utero; Waalkes et al. (2004a), M, C3H/HeNCr, in utero; Waalkes et al. (2006a, b), M, CD1, in utero; Tokar et al. (2011), MF, CD1, in utero + p.o.; Tokar et al. (2012a), M, CD1, in utero; MMAIII: Tokar et al. (2012b), M, CD1, in utero	
C 35	Arsenic and inorganic arsenic compounds	Lung, urinary bladder, skin	Kidney, liver, prostate	Dimethylarsinic acid [DMA(V)]	Rat Urinary bladder	Transitional cell carcinoma	Wei et al. (1999, 2002), M, F344/DuCrj, p.o.; Arnold et al. (2006), F, F344, p.o.	
D 45	Fission products including Sr-90	Solid cancers, leukaemia		Sr-90	Mouse Bone	Osteosarcoma	Nilsson (1970, 1971), M, CBA, i.p.; Nilsson et al. (1980), F, CBA, i.p.	

Table A2. Template for presentation of data on tumours and tumour sites in humans and in experimental animals from the *IARC Monographs* (continued)

Volume 100 part Agent number	Agent	Sites with *sufficient evidence* in humans	Sites with *limited evidence* in humans	Agent tested in experimental animals	Species Site	Histology	Study, sex, strain, exposure route	Comments
D 45	Fission products including Sr-90	Solid cancers, leukaemia		Sr-90	Dog Bone	Osteosarcoma	Gillett et al. (1992), MF, beagle, i.v.; White et al. (1993), MF, beagle, p.o.; Gillett et al. (1987), MF, beagle, inh.	
E 63	Acetaldehyde associated with consumption of alcoholic beverages	Oesophagus and upper aerodigestive tract combined						*Sufficient evidence* in experimental animals but no organ sites identified due to the absence of two (or more) studies of adequate design and quality pointing at the same organ site (with a similar histological origin) in the same species.
F 75	Acid mists, strong inorganic	Larynx	Lung					No animal data available.

b.pouch, buccal pouch; d.w., drinking water; F, positive female; g., gavage; i.col., intracolonic; i.f., intrafetal; i.m., intramuscular; i.mam., intramammary; inh., inhalation; i.p., intraperitoneal; i.pulmo., intrapulmonary; i.t., intratracheal; i.v., intravenous; M, positive male; MF, positive male and female; NR, not reported; per., perinatal; p.o., feeding; s.c., subcutaneous; skin, skin application.

Conclusions

The data set developed here to define tumour sites for carcinogenicity in humans and in experimental animals summarizes all available data on Group 1 agents identified in Volumes 100–109 of the *IARC Monographs*. At the time of completion of Volume 109, a total of 111 Group 1 agents had been identified, and these are included in the list presented in Table A1 (online only; available from: http://publications.iarc.fr/578). This comprehensive set of data constitutes a unique basis for addressing the important scientific question, i.e. to which extent these animal cancer data are comparable with human cancer data. The value of this data set is demonstrated by the initial concordance analyses that have been conducted with the database derived from it (see Chapter 21, by Krewski et al.).

Acknowledgements

Pascale Lajoie assembled the data set on tumours and tumour sites presented here while working as a Visiting Scientist under the direction of Yann Grosse at IARC during the summers of 2011 and 2012. Mélissa Billard also contributed to the development of the data set while working as a Visiting Scientist at IARC during the summers of 2013 and 2014 under the direction of Robert Baan and Yann Grosse. Daniel Krewski is the Natural Sciences and Engineering Research Council of Canada Chair in Risk Science at the University of Ottawa.

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Disclosures of interests

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 participant's organization and that do not support the participant'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 participant'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.

Mélissa Billard's travel expenses to attend the Workshop were paid by the R. Samuel McLaughlin Centre for Population Health Risk Assessment, where she was a master's student.

Michael Bird is retired from Exxon Mobil Biomedical Sciences Inc. and receives a pension and holds significant stock from the company. He has an affiliate appointment with the R. Samuel McLaughlin Centre for Population Health Risk Assessment, which also paid for his travel expenses to attend the Workshop.

Bernard D. Goldstein is a member of the Dow Chemical Sustainability External Advisory Committee.

Charles William Jameson serves as a consultant for the North American Insulation Manufacturers Association regarding the listing of glass wool fibres in the 12th Report on Carcinogens.

Agnes B. Kane received research support from the Gulf of Mexico Research Initiative (funded by BP) to investigate remediation of deep water oil spills, and from Unilever to Brown University to develop predictive toxicity testing for engineered nanomaterials.

Robert J. Kavlock's research unit receives research support from L'Oréal for the United States Environmental Protection Agency's Toxicity Forecaster (ToxCast).

Daniel Krewski holds a Natural Sciences and Engineering Research Council (NSERC) of Canada Industrial Research Chair in Risk Science at the University of Ottawa.

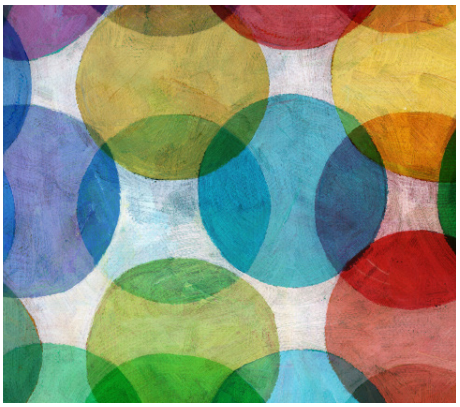
This Chair is funded under a competitive university–industry partnership programme administered by NSERC. He also serves as the Scientific Director for the R. Samuel McLaughlin Centre for Population Health Risk Assessment and receives salary support and has access to research funding from the R. Samuel McLaughlin Foundation. He has received an unrestricted grant from the ExxonMobil Research Foundation, administered through the NSERC Chair. He serves as Chief Risk Scientist and CEO for Risk Sciences International (RSI), a Canadian company established in partnership with the University of Ottawa. RSI undertakes consulting work with both public and private sector clients in Canada, the USA, and Europe.

Pascale Lajoie's travel expenses to attend the Workshop were paid by the R. Samuel McLaughlin Centre for Population Health Risk Assessment, where she was a master's student.

Ronald L. Melnick served as a consultant and expert witness for Bush Lewis, PLLC on health effects of 1,3-butadiene.

Jerry M. Rice served as a chair of the external Scientific Advisory Panel for the Shanghai Health Study on health risks from benzene exposure, funded by the American Petroleum Institute. He is also a partner in and holds stock of a small vineyard and winery corporation.

Martyn T. Smith received payment from various United States law firms for consulting and testimony in litigation involving benzene and other chemicals. He also received payment for travel and honorarium from the American Chemistry Council (ACC) and for an invited lecture from the International Life Sciences Institute (ILSI)-Health and Environmental Sciences Institute (HESI).



ISBN 978-92-832-2215-6