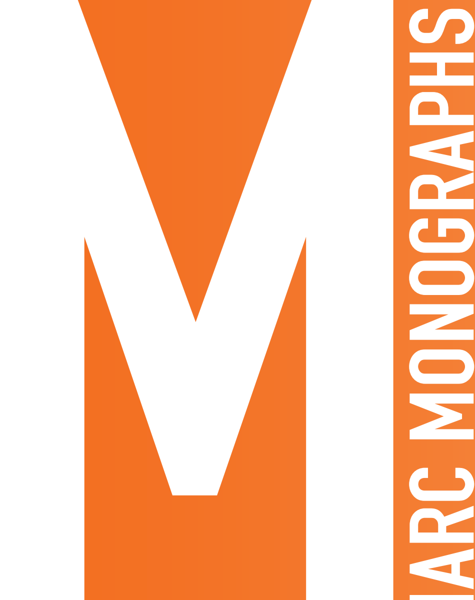


IARC MONOGRAPHS

STYRENE, STYRENE-7,8-OXIDE, AND QUINOLINE

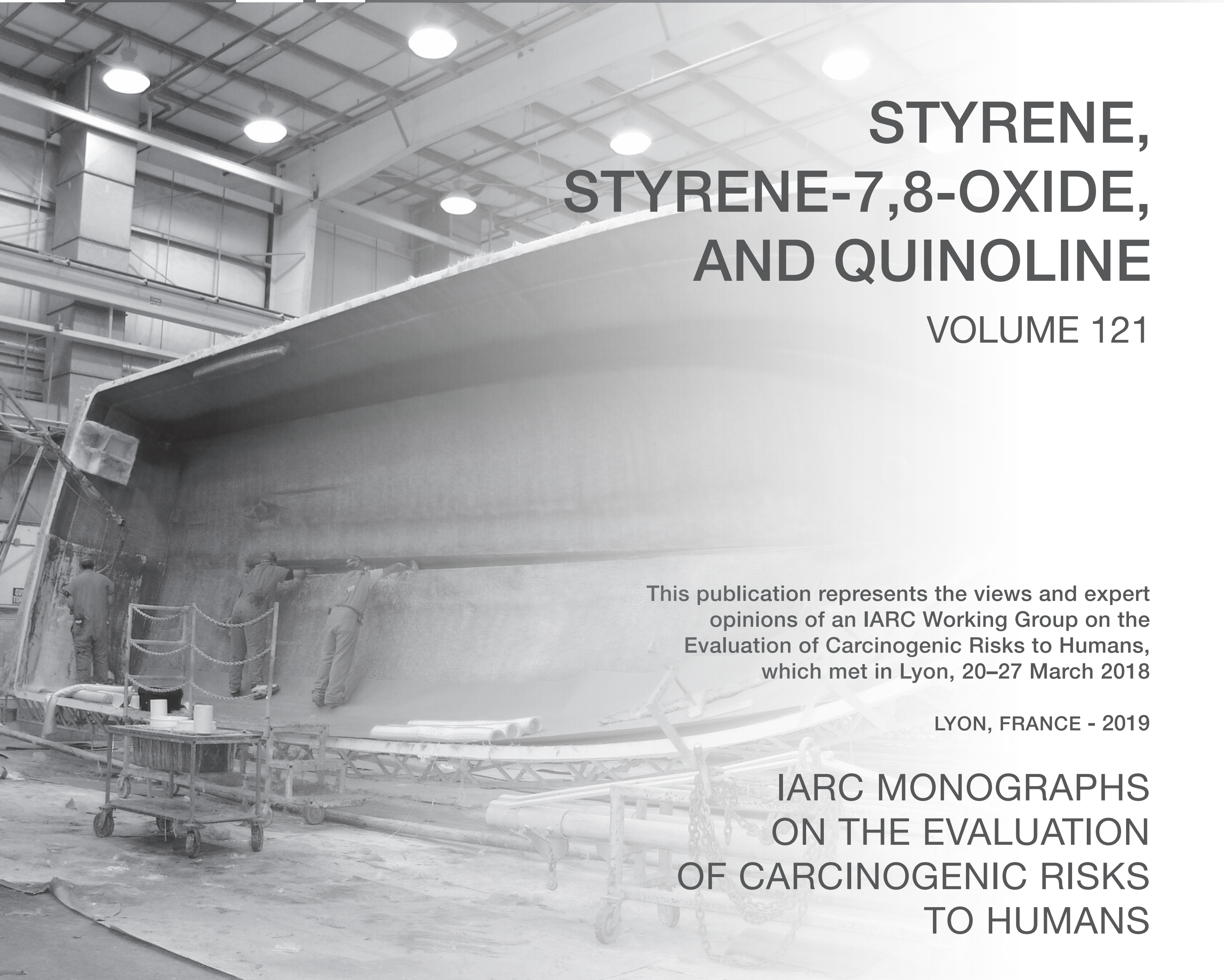
VOLUME 121

IARC MONOGRAPHS ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS

International Agency for Research on Cancer

World Health Organization

IARC MONOGRAPHS



STYRENE, STYRENE-7,8-OXIDE, AND QUINOLINE

VOLUME 121

This publication represents the views and expert opinions of an IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, which met in Lyon, 20–27 March 2018

LYON, FRANCE - 2019

IARC MONOGRAPHS ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS

International Agency for Research on Cancer

World Health Organization

IARC MONOGRAPHS

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

This programme has been supported since 1982 by Cooperative Agreement U01 CA33193 with the United States National Cancer Institute, Department of Health and Human Services. Additional support has been provided since 1986 by the European Commission Directorate-General for Employment, Social Affairs, and Inclusion, initially by the Unit of Health, Safety and Hygiene at Work, and since 2014 by the European Union Programme for Employment and Social Innovation "EaSI" (2014–2020) (for further information please consult: http://ec.europa.eu/social/easi). Support has also been provided since 1992 by the United States National Institute of Environmental Health Sciences, Department of Health and Human Services. The contents of this volume are solely the responsibility of the Working Group and do not necessarily represent the official views of the United States National Cancer Institute, the United States National Institute of Environmental Health Sciences, the United States Department of Health and Human Services, or the European Commission.

Published by the International Agency for Research on Cancer,
150 cours Albert Thomas, 69372 Lyon Cedex 08, France
©International Agency for Research on Cancer, 2019
On-line publication, September 2019

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).

Publications of the World Health Organization enjoy copyright protection in accordance with the provisions of Protocol 2 of the Universal Copyright Convention. All rights reserved.

Corrigenda to the *IARC Monographs* are published online at http://publications.iarc.fr
To report an error, please contact: editimo@iarc.fr

Co-funded by the European Union

The International Agency for Research on Cancer welcomes requests for permission to reproduce or translate its publications, in part or in full. Requests for permission to reproduce or translate IARC publications – whether for sale or for non-commercial distribution – should be addressed to the IARC Communications Group at: publications@iarc.fr.

The designations employed and the presentation of the material in this publication do not imply the expression of any opinion whatsoever on the part of the Secretariat of the World Health Organization concerning the legal status of any country, territory, city, or area or of its authorities, or concerning the delimitation of its frontiers or boundaries.

The mention of specific companies or of certain manufacturers' products does not imply that they are endorsed or recommended by the World Health Organization in preference to others of a similar nature that are not mentioned. Errors and omissions excepted, the names of proprietary products are distinguished by initial capital letters.

The *IARC Monographs* Working Group alone is responsible for the views expressed in this publication.

IARC Library Cataloguing in Publication Data

Styrene, Styrene-7,8-oxide, and Quinoline / IARC Working Group on the Evaluation of Carcinogenic Risks to Humans (2018: Lyon, France)

(IARC monographs on the evaluation of carcinogenic risks to humans ; volume 121)

1. Carcinogens 2. Styrene – adverse effects 3. Epoxy Compounds – adverse effects 4. Quinolines – adverse effects
5. Risk Factors

I. International Agency for Research on Cancer II. Series

ISBN 978-92-832-0188-5 (NLM Classification: W1)
ISSN 1017-1606

CONTENTS

NOTE TO THE READER . . . 1

LIST OF PARTICIPANTS . . . 3

PREAMBLE . . . 9
A. GENERAL PRINCIPLES AND PROCEDURES . . . 9
1. Background . . . 9
2. Objective and scope . . . 10
3. Selection of agents for review . . . 11
4. Data for the *Monographs* . . . 12
5. Meeting participants . . . 12
6. Working procedures . . . 13
B. SCIENTIFIC REVIEW AND EVALUATION . . . 14
1. Exposure data . . . 15
2. Studies of cancer in humans . . . 16
3. Studies of cancer in experimental animals . . . 20
4. Mechanistic and other relevant data . . . 23
5. Summary . . . 26
6. Evaluation and rationale . . . 27
References . . . 31

GENERAL REMARKS . . . 33

STYRENE AND STYRENE-7,8-OXIDE . . . 37
1. Exposure Data . . . 37
1.1 Styrene . . . 37
1.2 Styrene-7,8-oxide . . . 40
1.3 Measurement and analysis . . . 42
1.4 Occurrence and exposure . . . 53
1.5 Regulations and guidelines . . . 71
1.6 Exposure assessment of epidemiological studies . . . 71

2. Cancer in Humans . . . 83
2.1 Introduction . . . 83
2.2 Cohort studies . . . 86
2.3 Case–control studies . . . 135
2.4 Human cancer evidence synthesis . . . 150
3. Cancer in Experimental Animals . . . 156
3.1 Styrene . . . 156
3.2 Styrene-7,8-oxide . . . 175
4. Mechanistic and Other Relevant Data . . . 182
4.1 Absorption, distribution, metabolism, and excretion . . . 182
4.2 Mechanisms of carcinogenesis . . . 195
4.3 Other adverse effects . . . 254
4.4 Data relevant to comparisons across agents and end-points . . . 255
5. Summary of Data Reported . . . 260
5.1 Exposure data . . . 260
5.2 Cancer in humans . . . 261
5.3 Cancer in experimental animals . . . 261
5.4 Mechanistic and other relevant data . . . 263
6. Evaluation . . . 265
6.1 Cancer in humans . . . 265
6.2 Cancer in experimental animals . . . 265
6.3 Overall evaluation . . . 265
6.4 Rationale . . . 265
References . . . 266

QUINOLINE . . . 297
1. Exposure Data . . . 297
1.1 Identification of the agent . . . 297
1.2 Production and use . . . 298
1.3 Measurement and analysis . . . 299
1.4 Occurrence and exposure . . . 301
1.5 Regulations and guidelines . . . 305
2. Cancer in Humans . . . 305
3. Cancer in Experimental Animals . . . 305
3.1 Mouse . . . 305
3.2 Rat . . . 320
3.3 Syrian Golden hamster . . . 323
3.4 Guinea-pig . . . 323
4. Mechanistic and Other Relevant Data . . . 323
4.1 Toxicokinetic data . . . 323
4.2 Mechanisms of carcinogenesis . . . 326
4.3 Other adverse effects . . . 333

5. Summary of Data Reported 333
5.1 Exposure data 333
5.2 Cancer in humans 333
5.3 Cancer in experimental animals 333
5.4 Mechanistic and other relevant data 334
6. Evaluation 335
6.1 Cancer in humans 335
6.2 Cancer in experimental animals 335
6.3 Overall evaluation 335
References 335

LIST OF ABBREVIATIONS 341

ANNEX 1. SUPPLEMENTAL MATERIAL 345

NOTE TO THE READER

The term 'carcinogenic risk' in the *IARC Monographs* series is taken to mean that an agent is capable of causing cancer. The *Monographs* evaluate cancer hazards, despite the historical presence of the word 'risks' in the title.

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

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

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

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

LIST OF PARTICIPANTS

Members [1]

Allan Astrup Jensen

Nordic Institute of Product Sustainability, Environmental Chemistry and Toxicology (NIPSECT)
Frederiksberg C
Denmark

Stephen J. Bertke

Centers for Disease Control and Prevention
National Institute for Occupational Safety and Health
Cincinnati, OH
USA

Gloria M. Calaf

Instituto de Alta Investigacíón
Tarapaca University
Arica
Chile

Claudio Colosio

Department of Health Sciences
University of Milan
Milan
Italy

[1] Working Group Members and Invited Specialists serve in their individual capacities as scientists and not as representatives of their government or any organization with which they are affiliated. Affiliations are provided for identification purposes only. Invited Specialists do not serve as Meeting Chair or Subgroup Chair, draft text that pertains to the description or interpretation of cancer data, or participate in the evaluations.
Each participant was asked to disclose pertinent research, employment, and financial interests. Current financial interests and research and employment interests during the past 4 years or anticipated in the future are identified here. Minor pertinent interests are not listed and include stock valued at no more than US$ 1000 overall, grants that provide no more than 5% of the research budget of the expert's organization and that do not support the expert's research or position, and consulting or speaking on matters not before a court or government agency that does not exceed 2% of total professional time or compensation. All grants that support the expert's research or position and all consulting or speaking on behalf of an interested party on matters before a court or government agency are listed as significant pertinent interests.

Jason M. Fritz

National Center for Environmental Assessment
United States Environmental Protection Agency
Washington, DC
USA

Shoji Fukushima

Japan Bioassay Research Center
Japan Organization of Occupational Health and Safety
Hadano, Kanagawa
Japan

William M. Gwinn (Subgroup Chair, Cancer in Experimental Animals)

Predictive Toxicity and Screening Group
NTPL
Division of the National Toxicology Program
National Institute of Environmental Health Sciences
Research Triangle Park, NC
USA

Kari Hemminki

German Cancer Research Center (DKFZ)
Heidelberg
Germany

Manolis Kogevinas (Overall Chair)

Barcelona Institute for Global Health (ISGlobal)
Barcelona
Spain

Henrik Kolstad

Department of Occupational Medicine
Aarhus University Hospital
Aarhus C
Denmark

David Kriebel (Subgroup Chair, Exposure Data)

Department of Public Health
University of Massachusetts Lowell
Lowell, MA
USA

Jaroslav Mráz

Department for the Assessment of Exposure to Chemicals in the Workplace
National Institute of Public Health
Prague
Czechia

Stephen Nesnow

Stephen Nesnow, Consulting
Chapel Hill, NC
USA

Leena Nylander-French

Department of Environmental Sciences and Engineering
University of North Carolina at Chapel Hill
Chapel Hill, NC
USA

Marie-Elise Parent

Epidemiology and Biostatistics Unit
INRS–Institut Armand-Frappier
Laval, QC
Canada

David H. Phillips (Subgroup Chair, Mechanistic and Other Relevant Data)

Department of Analytical, Forensic and Environmental Sciences
School of Population Health and Environmental Sciences
King's College London
London
United Kingdom

Martha Sandy

Office of Environmental Health Hazard Assessment (OEHHA)
California Environmental Protection Agency
Oakland, CA
USA

Malcolm Sim (Subgroup Chair, Cancer in Humans)

Monash Centre for Occupational and Environmental Health (MonCOEH)
School of Public Health and Preventive Medicine
Faculty of Medicine, Nursing and Health Sciences
Monash University
Melbourne
Australia

Stephanie L. Smith-Roe

Biomolecular Screening Branch
National Toxicology Program
National Institute of Environmental Health Sciences
Research Triangle Park, NC
USA

Gary Stoner (unable to attend)

Department of Medicine
College of Medicine
Ohio State University
Columbus, OH
USA

Takayoshi Suzuki

Molecular Diagnosis Section
Division of Molecular Target and Gene Therapy Products
National Institute of Health Sciences
Kawasaki Kanagawa
Japan

João Paulo Teixeira

Environmental Health Department
National Institute of Health
Porto
Portugal

Pavel Vodicka

Institute of Experimental Medicine
The Czech Academy of Sciences
Prague
Czechia

Invited Specialist

Rogelio (“Mike”) Tornero-Velez [2]

Computational Exposure Division
National Exposure Research Laboratory
Office of Research and Development
United States Environmental Protection Agency
Research Triangle Park, NC
USA

Representative

Marcia Sarpa de Campos Mello (unable to attend) [3]

Occupational and Environmental Exposure and Cancer Unit
Prevention and Surveillance Coordination
National Cancer Institute (INCA)
Rio de Janeiro
Brazil

Observers [4]

Marcy Iva Banton [5]

LyondellBasell
Houston, TX
USA

Tamar Chachibaia (unable to attend) [6]

Savannah, GA
USA

Heinz-Peter Gelbke [7]

Nice
France

Ludmila Vodickova [8]

First Medical Faculty
Medical Faculty in Pilsen
Charles University
Prague
Czechia

[2] Rogelio Tornero-Velez was invited to a workshop with significant travel costs paid by the American Chemistry Council.
[3] Marcia Sarpa de Campos Mello was due to attend as a Representative of the Ministry of Health, Brazil.
[4] Each Observer agreed to respect the Guidelines for Observers at *IARC Monographs* meetings. Observers did not serve as Meeting Chair or Subgroup Chair, draft any part of a *Monograph*, or participate in the evaluations. They also agreed not to contact participants before the meeting, not to lobby them at any time, not to send them written materials, and not to offer them meals or other favours. IARC asked and reminded Working Group Members to report any contact or attempt to influence that they may have encountered, either before or during the meeting.
[5] Marcy Iva Banton attended as an Observer for the Styrene Information and Research Center (SIRC), USA.
[6] Tamar Chachibaia was due to attend as an Observer for the Ammonium Nitrate Safe Governance Initiative, a non-governmental organization in Georgia.
[7] Heinz-Peter Gelbke attended as an Observer for the Styrenics Steering Committee (SSC) as a consultant and has prepared a comment to the “Memorandum - risk management strategy for styrene” of the Danish Environmental Protection Agency (2015). He holds shares in BASF SE, a company that produces styrene. SSC paid for travel and an honorarium.
[8] Ludmila Vodickova attended as an Observer for Charles University, Czechia.

Michael Wilde [9]

Department of Philosophy
School of European Culture and Languages
University of Kent
Canterbury
United Kingdom

Jon Williamson [10]

Department of Philosophy
School of European Culture and Languages
University of Kent
Canterbury
United Kingdom

IARC/WHO Secretariat

Lamia Benbrahim-Tallaa (unable to attend)
Véronique Bouvard *(Rapporteur, Exposure Data)*
Tim Driscoll (*Visiting Scientist, University of Sydney, Australia*)
Fatiha El Ghissassi *(Rapporteur, Mechanistic and Other Relevant Data)*
Milena Foerster
Yann Grosse *(Rapporteur, Cancer in Experimental Animals)*
Neela Guha *(Rapporteur, Cancer in Humans)*
Kathryn Guyton *(Responsible Officer; Rapporteur, Mechanistic and Other Relevant Data*)
Amy Hall
Corentin Jaillet
Heidi Mattock *(Scientific Editor)*
Daniel Middleton
Kurt Straif *(Section Head)*
Madar Talibov
Nadia Vilahur

Administrative Assistance

Marieke Dusenberg
Sandrine Egraz
Michel Javin
Helene Lorenzen-Augros
Lucy Shedden

Production Team

Elisabeth Elbers
Fiona Gould
Solène Quennehen

Pre-Meeting Assistance

Nilmara de Oliveira Alves Brito

Post-Meeting Assistance

Karen Müller *(Scientific Editor)*
Elaine Rowan (*Technical Editor*)

Post-Meeting Scientific Assistance

Jennifer Girschik
Misty J. Hein
Mary Schubauer-Berigan
Eero Suonio

[9] Michael Wilde attended as an Observer for the University of Kent, United Kingdom.
[10] Jon Williamson attended as an Observer for the University of Kent, United Kingdom.

PREAMBLE

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

A. GENERAL PRINCIPLES AND PROCEDURES

1. Background

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

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

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

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

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

2. Objective and scope

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

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

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

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

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

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

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

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

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

3. Selection of agents for review

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

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

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

4. Data for the *Monographs*

Each *Monograph* reviews all pertinent epidemiological studies and cancer bioassays in experimental animals. Those judged inadequate or irrelevant to the evaluation may be cited but not summarized. If a group of similar studies is not reviewed, the reasons are indicated.

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

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

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

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

5. Meeting participants

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

(a) The Working Group

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

(b) Invited Specialists

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

or subgroup chair, draft text that pertains to the description or interpretation of cancer data, or participate in the evaluations.

(c) Representatives of national and international health agencies

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

(d) Observers with relevant scientific credentials

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

(e) The IARC Secretariat

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

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

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

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

6. Working procedures

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

preliminary working papers for specific sections are expected to supplement the IARC literature searches with their own searches.

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

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

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

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

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

B. SCIENTIFIC REVIEW AND EVALUATION

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

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

Exposure data

Studies of cancer in humans

Studies of cancer in experimental animals

Mechanistic and other relevant data

Summary

Evaluation and rationale

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

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

1. Exposure data

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

(a) General information on the agent

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

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

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

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

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

(b) Analysis and detection

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

(c) Production and use

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

which may give rise to different impurities, are described.

The countries where companies report production of the agent, and the number of companies in each country, are identified. Available data on production, international trade and uses are obtained for representative regions. It should not, however, be inferred that those areas or nations are necessarily the sole or major sources or users of the agent. Some identified uses may not be current or major applications, and the coverage is not necessarily comprehensive. In the case of drugs, mention of their therapeutic uses does not necessarily represent current practice nor does it imply judgement as to their therapeutic efficacy.

(d) Occurrence and exposure

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

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

(e) Regulations and guidelines

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

2. Studies of cancer in humans

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

(a) Types of study considered

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

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

In correlation studies, the units of investigation are usually whole populations (e.g. in

particular geographical areas or at particular times), and cancer frequency is related to a summary measure of the exposure of the population to the agent under study. In correlation studies, individual exposure is not documented, which renders this kind of study more prone to confounding. In some circumstances, however, correlation studies may be more informative than analytical study designs (see, for example, the *Monograph* on arsenic in drinking-water; IARC, 2004).

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

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

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

(b) Quality of studies considered

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

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

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

Second, the authors should have taken into account — in the study design and analysis — other variables that can influence the risk of disease and may have been related to the exposure of interest. Potential confounding by such variables should have been dealt with either in the design of the study, such as by matching, or in the analysis, by statistical adjustment. In cohort studies, comparisons with local rates of disease may or may not be more appropriate than

those with national rates. Internal comparisons of frequency of disease among individuals at different levels of exposure are also desirable in cohort studies, since they minimize the potential for confounding related to the difference in risk factors between an external reference group and the study population.

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

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

(c) Meta-analyses and pooled analyses

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

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

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

(d) Temporal effects

Detailed analyses of both relative and absolute risks in relation to temporal variables, such as age at first exposure, time since first exposure, duration of exposure, cumulative exposure, peak exposure (when appropriate) and

time since cessation of exposure, are reviewed and summarized when available. Analyses of temporal relationships may be useful in making causal inferences. In addition, such analyses may suggest whether a carcinogen acts early or late in the process of carcinogenesis, although, at best, they allow only indirect inferences about mechanisms of carcinogenesis.

(e) Use of biomarkers in epidemiological studies

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

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

(f) Criteria for causality

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

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

Several scenarios may increase confidence in a causal relationship. On the one hand, an agent may be specific in causing tumours at one site or of one morphological type. On the other, carcinogenicity may be evident through the causation of

multiple tumour types. Temporality, precision of estimates of effect, biological plausibility and coherence of the overall database are considered. Data on biomarkers may be employed in an assessment of the biological plausibility of epidemiological observations.

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

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

3. Studies of cancer in experimental animals

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

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

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

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

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

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

(a) Qualitative aspects

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

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

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

should nevertheless be suspected of being carcinogenic and requires further investigation.

(b) Quantitative aspects

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

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

(c) Statistical analyses

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

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

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

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

4. Mechanistic and other relevant data

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

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

(a) Toxicokinetic data

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

(b) Data on mechanisms of carcinogenesis

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

can be divided into three non-exclusive levels as described below.

(i) Changes in physiology

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

(ii) Functional changes at the cellular level

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

(iii) Changes at the molecular level

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

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

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

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

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

have been published (Montesano et al., 1986; McGregor et al., 1999).

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

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

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

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

(c) Other data relevant to mechanisms

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

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

plausible, but also have a consistent pattern of carcinogenic response across entire classes of related compounds.

(d) Susceptibility data

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

(e) Data on other adverse effects

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

5. Summary

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

(a) Exposure data

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

(b) Cancer in humans

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

(c) Cancer in experimental animals

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

(d) Mechanistic and other relevant data

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

6. Evaluation and rationale

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

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

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

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

(a) Carcinogenicity in humans

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

***Sufficient evidence of carcinogenicity*:**

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

***Limited evidence of carcinogenicity*:**

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

***Inadequate evidence of carcinogenicity*:**

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

***Evidence suggesting lack of carcinogenicity*:**

There are several adequate studies covering the full range of levels of exposure that humans are known to encounter, which are mutually consistent in not showing a positive association between exposure to the agent and any studied cancer at any observed level of exposure. The results from these studies alone or combined should have narrow confidence intervals with an upper limit close to the null value (e.g. a relative

risk of 1.0). Bias and confounding should be ruled out with reasonable confidence, and the studies should have an adequate length of follow-up. A conclusion of *evidence suggesting lack of carcinogenicity* is inevitably limited to the cancer sites, conditions and levels of exposure, and length of observation covered by the available studies. In addition, the possibility of a very small risk at the levels of exposure studied can never be excluded.

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

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

(b) Carcinogenicity in experimental animals

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

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

***Sufficient evidence of carcinogenicity*:**

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

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

***Limited evidence of carcinogenicity*:**

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

***Inadequate evidence of carcinogenicity*:**

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

***Evidence suggesting lack of carcinogenicity*:**

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

(c) Mechanistic and other relevant data

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

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

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

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

(d) Overall evaluation

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

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

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

Group 1: The agent is carcinogenic to humans.

This category is used when there is *sufficient evidence of carcinogenicity* in humans. Exceptionally, an agent may be placed in this category when evidence of carcinogenicity in humans is less than *sufficient* but there is *sufficient evidence of carcinogenicity* in experimental animals and strong evidence in exposed humans that the agent acts through a relevant mechanism of carcinogenicity.

Group 2.

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

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

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

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

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

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

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

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

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

An evaluation in Group 3 is not a determination of non-carcinogenicity or overall safety. It often means that further research is needed,

especially when exposures are widespread or the cancer data are consistent with differing interpretations.

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

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

(e) Rationale

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

References

Bieler GS, Williams RL (1993). Ratio estimates, the delta method, and quantal response tests for increased carcinogenicity. *Biometrics*, 49:793–801. doi:10.2307/2532200 PMID:8241374

Breslow NE, Day NE (1980). Statistical methods in cancer research. Volume I - The analysis of case-control studies. *IARC Sci Publ*, 32:5–338. PMID:7216345

Breslow NE, Day NE (1987). Statistical methods in cancer research. Volume II–The design and analysis of cohort studies. *IARC Sci Publ*, 82:1–406. PMID:3329634

Buffler P, Rice J, Baan R, et al. (2004). Workshop on mechanisms of carcinogenesis: contributions of molecular epidemiology. Lyon, 14–17 November 2001. Workshop report. *IARC Sci Publ*, 157:1–27. PMID:15055286

Capen CC, Dybing E, Rice JM, Wilbourn JD (1999). Species differences in thyroid, kidney and urinary bladder carcinogenesis. Proceedings of a consensus conference. Lyon, France, 3–7 November 1997. *IARC Sci Publ*, 147:1–225. PMID:10627184

Cogliano V, Baan R, Straif K, et al. (2005). Transparency in IARC Monographs. *Lancet Oncol*, 6:747. doi:10.1016/S1470-2045(05)70380-6

Cogliano VJ, Baan RA, Straif K, et al. (2004). The science and practice of carcinogen identification and evaluation. *Environ Health Perspect*, 112:1269–1274. doi:10.1289/ehp.6950 PMID:15345338

Dunson DB, Chen Z, Harry J (2003). A Bayesian approach for joint modeling of cluster size and subunit-specific outcomes. *Biometrics*, 59:521–530. doi:10.1111/1541-0420.00062 PMID:14601753

Fung KY, Krewski D, Smythe RT (1996). A comparison of tests for trend with historical controls in carcinogen bioassay. *Can J Stat*, 24:431–454. doi:10.2307/3315326

Gart JJ, Krewski D, Lee PN, et al. (1986). Statistical methods in cancer research. Volume III–The design and analysis of long-term animal experiments. *IARC Sci Publ*, 79:1–219. PMID:3301661

Greenland S (1998). Meta-analysis. In: Rothman KJ, Greenland S, editors. Modern epidemiology. Philadelphia: Lippincott Williams & Wilkins, pp. 643–673.

Greim H, Gelbke H-P, Reuter U, et al. (2003). Evaluation of historical control data in carcinogenicity studies. *Hum Exp Toxicol*, 22:541–549. doi:10.1191/0960327103ht394oa PMID:14655720

Haseman JK, Huff J, Boorman GA (1984). Use of historical control data in carcinogenicity studies in rodents. *Toxicol Pathol*, 12:126–135. doi:10.1177/019262338401200203 PMID:11478313

Hill AB (1965). The environment and disease: Association or causation? *Proc R Soc Med*, 58:295–300. PMID:14283879

Hoel DG, Kaplan NL, Anderson MW (1983). Implication of nonlinear kinetics on risk estimation in carcinogenesis. *Science*, 219:1032–1037. doi:10.1126/science.6823565 PMID:6823565

Huff JE, Eustis SL, Haseman JK (1989). Occurrence and relevance of chemically induced benign neoplasms in long-term carcinogenicity studies. *Cancer Metastasis Rev*, 8:1–22. doi:10.1007/BF00047055 PMID:2667783

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

IARC (1978). Chemicals with sufficient evidence of carcinogenicity in experimental animals – IARC Monographs Volumes 1–17 (IARC Intern Tech Rep No. 78/003).

IARC (1979). Criteria to select chemicals for IARC Monographs (IARC Intern Tech Rep No. 79/003).

IARC (1982). Chemicals, industrial processes and industries associated with cancer in humans (IARC Monographs, volumes 1 to 29). *IARC Monogr Eval Carcinog Risk Chem Hum Suppl*, 4:1–292.

IARC (1983). Approaches to classifying chemical carcinogens according to mechanism of action (IARC Intern Tech Rep No. 83/001).

IARC (1987). Overall evaluations of carcinogenicity: an updating of IARC Monographs volumes 1 to 42. *IARC Monogr Eval Carcinog Risks Hum Suppl*, 7:1–440. PMID:3482203

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

IARC (1991). A consensus report of an IARC Monographs Working Group on the Use of Mechanisms of Carcinogenesis in Risk Identification (IARC Intern Tech Rep No. 91/002).

IARC (2004). Some drinking-water disinfectants and contaminants, including arsenic. *IARC Monogr Eval Carcinog Risks Hum*, 84:1–477. PMID:15645577

IARC (2005). Report of the Advisory Group to Recommend Updates to the Preamble to the IARC Monographs (IARC Intern Rep No. 05/001).

IARC (2006). Report of the Advisory Group to Review the Amended Preamble to the IARC Monographs (IARC Intern Rep No. 06/001).

McGregor DB, Rice JM, Venitt S (1999). The use of short-and medium-term tests for carcinogens and data on genetic effects in carcinogenic hazard evaluation. Consensus report. *IARC Sci Publ*, 146:1–18. PMID:10353381

Montesano R, Bartsch H, Vainio H, et al., editors (1986). Long-term and short-term assays for carcinogenesis—a critical appraisal. *IARC Sci Publ*, 83:1–564. PMID:3623675

OECD (2002). Guidance notes for analysis and evaluation of chronic toxicity and carcinogenicity studies (Series on Testing and Assessment No. 35), Paris: OECD.

Peto R, Pike MC, Day NE, et al. (1980). Guidelines for simple, sensitive significance tests for carcinogenic effects in long-term animal experiments. *IARC Monogr Eval Carcinog Risk Chem Hum Suppl*, (2 Suppl):311–426. PMID:6935185

Portier CJ, Bailer AJ (1989). Testing for increased carcinogenicity using a survival-adjusted quantal response test. *Fundam Appl Toxicol*, 12:731–737. doi:10.1016/0272-0590(89)90004-3 PMID:2744275

Sherman CD, Portier CJ, Kopp-Schneider A (1994). Multistage models of carcinogenesis: an approximation for the size and number distribution of late-stage clones. *Risk Anal*, 14:1039–1048. doi:10.1111/j.1539-6924.1994.tb00074.x PMID:7846311

Stewart BW, Kleihues P, editors (2003). World cancer report, Lyon: IARC.

Tomatis L, Aitio A, Wilbourn J, Shuker L (1989). Human carcinogens so far identified. *Jpn J Cancer Res*, 80:795–807. doi:10.1111/j.1349-7006.1989.tb01717.x PMID:2513295

Toniolo P, Boffetta P, Shuker DEG, et al. (1997). Proceedings of the workshop on application of biomarkers to cancer epidemiology. Lyon, France, 20–23 February 1996. *IARC Sci Publ*, 142:1–318. PMID:9410826

Vainio H, Magee P, McGregor D, McMichael A (1992). Mechanisms of carcinogenesis in risk identification. IARC Working Group Meeting. Lyon, 11–18 June 1991. *IARC Sci Publ*, 116:1–608. PMID:1428077

Vainio H, Wilbourn JD, Sasco AJ, et al. (1995). [Identification of human carcinogenic risks in IARC monographs] *Bull Cancer*, 82:339–348. PMID:7626841

Vineis P, Malats N, Lang M, et al., editors (1999). Metabolic polymorphisms and susceptibility to cancer. *IARC Sci Publ*, 148:1–510. PMID:10493243

Wilbourn J, Haroun L, Heseltine E, et al. (1986). Response of experimental animals to human carcinogens: an analysis based upon the IARC Monographs programme. *Carcinogenesis*, 7:1853–1863. doi:10.1093/carcin/7.11.1853 PMID:3769134

GENERAL REMARKS

This one-hundred-and-twenty-first volume of the *IARC Monographs* contains evaluations of the carcinogenic hazard to humans of quinoline, styrene, and styrene-7,8-oxide.

Styrene was previously considered by *IARC Monographs* Working Groups in February 1978 (IARC, 1979), March 1987 (IARC, 1987), February 1994 (IARC, 1994), and February 2002 (IARC, 2002). Styrene-7,8-oxide was considered by *IARC Monographs* Working Groups in February 1976 (IARC, 1976), February 1978 (IARC, 1979), June 1984 (IARC, 1985), and February 1994 (IARC, 1994). New data have become available since these previous evaluations, and these have been included and considered in the present volume. Quinoline had not been previously evaluated by the *IARC Monographs* programme.

A summary of the findings of this volume appears in *The Lancet Oncology* (Kogevinas et al., 2018).

Quinoline

Quinoline is present in air pollution and in tobacco smoke. It is a high production volume chemical that is used to produce various drugs and dyes. Potential human exposures to quinoline in occupational settings are not well understood, with significant gaps in knowledge with respect to exposures occurring during the production and use of quinoline-derived drugs, the use of quinoline as an industrial solvent, and new uses of quinolinium as an ionic liquid crystal solvent. Other data relevant to the carcinogenicity of quinoline to humans are also sparse. No data were available on cancer in humans, or on absorption, distribution, metabolism, or excretion in humans. Furthermore, no data were available on carcinogen mechanisms in humans or in human cells. Quinoline is carcinogenic in mice and in rats, inducing rare tumours of various embryological origins. Malignant tumours were induced with a high incidence at the lowest dose tested, occurred with short latency, and caused early deaths. There was also strong evidence that quinoline is genotoxic in experimental systems, inducing mutations and chromosomal damage in rodents and in vitro upon metabolic activation.

Styrene-7,8-oxide and styrene

Styrene-7,8-oxide is primarily used to produce epoxy resins. Human exposure during the manufacture of styrene-7,8-oxide, or during the production or use of epoxy resins, is not well understood. Occupational exposure has been documented in the reinforced plastics industry, where styrene 7,8-oxide co-occurs with styrene, at concentrations that are typically 3 orders of magnitude lower than those of styrene. Styrene-7,8-oxide and its albumin and haemoglobin adducts have been detected in the blood in

occupationally exposed populations and the general population. In the reinforced plastics industry, styrene exposures are apparently decreasing, with likely concomitant reductions in styrene-7,8-oxide exposures.

Human exposure to styrene is better characterized, but breathing-zone air concentration data for occupational exposures throughout the production and use of styrene are sparse, particularly in low- and middle-income countries. The sources of styrene in indoor air have also not been quantitatively characterized. Population-based data are also not available, such as to inform the relative importance of different sources of styrene exposure (including occupational exposures, cigarette smoking, and indoor and outdoor air). Short-term, high-level exposure was the focus of many investigations of styrene in humans. A variety of exposure metrics are available, but the selection has not always been informed by consideration of the biological rationale.

In humans, there was *inadequate evidence* for the carcinogenicity of styrene-7,8-oxide. For styrene, the epidemiological studies provided *limited evidence* for carcinogenicity, based on positive associations with lymphohaematopoietic malignancies. For solid tumours, including lung cancer, the evidence was sparse or inconsistent. There was an increase in the incidence of sinonasal adenocarcinoma, a rare cancer, in one large cohort of workers in the reinforced plastics industry (Nissen et al., 2018), but cases were few and chance and confounding could not be discounted. In the studies of cancer in humans, inconsistency in the classification by haematopoietic subtypes was noted. Incidence-based studies, and pooling of data from large studies concerning rare cancers, may help to clarify gaps.

In experimental animals, there was *sufficient evidence* of carcinogenicity for styrene. There was also *sufficient evidence* of carcinogenicity for styrene-7,8-oxide. The overall evaluation of styrene-7,8-oxide as *probably carcinogenic to humans* (Group 2A) took into account the mechanistic and other relevant data pertinent to the key characteristics of carcinogens (Smith et al., 2016). Because styrene-7,8-oxide is the major metabolite of styrene, these mechanistic data also provided independent support of the classification of styrene as *probably carcinogenic to humans* (Group 2A). Styrene-7,8-oxide is an electrophile and reacts directly with DNA. Styrene-7,8-oxide and styrene are genotoxic. Styrene-7,8-oxide-derived DNA adducts were found in the blood (Rappaport et al., 1996) and urine of exposed workers. However, for other indicators of genotoxicity, the results were mixed. In human cells in vitro, styrene-7,8-oxide as well as styrene induced DNA damage, gene mutations, chromosomal aberrations, micronucleus formation, and sister-chromatid exchanges (Bastlová et al., 1995); similar findings were seen in various experimental systems. In rodents exposed to styrene-7,8-oxide or styrene, results were equivocal for cytogenetic effects, but positive for DNA damage in multiple tissues.

Other mechanistic data were also relevant to the evaluation of styrene. In particular, the human relevance of the styrene-induced lung tumours in mice was considered, and data pertinent to a proposed rodent-specific mechanism were reviewed. This proposed mechanism involves metabolism of styrene to 4-vinylphenol by CYP2F2, cytotoxicity in club (Clara) cells, and regenerative epithelial proliferation in the terminal bronchioles (Cruzan et al., 2012). Styrene induced cytotoxicity, lung cell proliferation, and bronchial hyperplasia in both CD-1 and C57BL/6 mice, but not in C57BL/6 *Cyp2f2*$^{(-/-)}$ mice or in a C57BL/6 *Cyp2f2*$^{(-/-)}$ humanized CYP strain (Cruzan et al., 2017). However, lung tumours developed only in CD-1, and not in C57BL/6, mice (Cruzan et al., 2017). Furthermore, no in vivo metabolism data were available in C57BL/6 strains, and the observed increases in lung cell proliferation did not persist beyond the short term, even with continuous

exposure. Overall, it was concluded that the mechanistic events for lung tumour induction by styrene in CD-1, B6C3F$_1$, and O20 mice have not been established.

The factors that may influence individual susceptibility to the carcinogenicity of styrene or styrene-7,8-oxide, including sex and life-stage differences, are not well understood.

Although high-throughput data were available for styrene, styrene-7,8-oxide, styrene glycol, and 2-phenylethanol, as well as quinoline, these data were not influential in the overall evaluations in this volume. High-throughput data streams have certain strengths, and may afford opportunities to fill gaps in evidence and to support mechanistic conclusions. However, there are also limitations to the applicability and utility of such data to *IARC Monographs* evaluations (see also Chiu et al., 2018; Guyton et al., 2018), because large-scale screening programmes were designed to aid prioritization of large chemical libraries for additional toxicity testing rather than to identify hazards of a specific chemical or chemical group.

References

Bastlová T, Vodicka P, Peterková K, Hemminki K, Lambert B (1995). Styrene oxide-induced HPRT mutations, DNA adducts and DNA strand breaks in cultured human lymphocytes. *Carcinogenesis*, 16(10):2357–62. doi:10.1093/carcin/16.10.2357 PMID:7586135

Chiu WA, Guyton KZ, Martin MT, Reif DM, Rusyn I (2018). Use of high-throughput in vitro toxicity screening data in cancer hazard evaluations by IARC Monograph Working Groups. *ALTEX*, 35(1):51–64. doi:10.14573/altex.1703231 PMID:28738424

Cruzan G, Bus J, Hotchkiss J, Harkema J, Banton M, Sarang S (2012). CYP2F2-generated metabolites, not styrene oxide, are a key event mediating the mode of action of styrene-induced mouse lung tumors. *Regul Toxicol Pharmacol*, 62(1):214–20. doi:10.1016/j.yrtph.2011.10.007 PMID:22041433

Cruzan G, Bus JS, Banton MI, Sarang SS, Waites R, Layko DB, et al. (2017). Editor's highlight: complete attenuation of mouse lung cell proliferation and tumorigenicity in CYP2F2 knockout and CYP2F1 humanized mice exposed to inhaled styrene for up to 2 years supports a lack of human relevance. *Toxicol Sci*, 159(2):413–21. doi:10.1093/toxsci/kfx141 PMID:28962520

Guyton KZ, Rusyn I, Chiu WA, Corpet DE, van den Berg M, Ross MK, et al. (2018). Application of the key characteristics of carcinogens in cancer hazard identification. *Carcinogenesis*, 39(4):614–22. doi:10.1093/carcin/bgy031 PMID:29562322

IARC (1976). Cadmium, nickel, some epoxides, miscellaneous industrial chemicals and general considerations on volatile anaesthetics. *IARC Monogr Eval Carcinog Risk Chem Man*, 11:1–306. Available from: http://publications.iarc.fr/29 PMID:992654

IARC (1979). Some monomers, plastics and synthetic elastomers, and acrolein. *IARC Monogr Eval Carcinog Risk Chem Hum*, 19:1–513. Available from: http://publications.iarc.fr/37 PMID:285915

IARC (1985). Allyl compounds, aldehydes, epoxides and peroxides. *IARC Monogr Eval Carcinog Risk Chem Hum*, 36:1–369. Available from: http://publications.iarc.fr/54.

IARC (1987). Silica and some silicates. *IARC Monogr Eval Carcinog Risk Chem Hum*, 42:1–239. Available from: http://publications.iarc.fr/60 PMID:2824337

IARC (1994). Some industrial chemicals. *IARC Monogr Eval Carcinog Risks Hum*, 60:1–560. Available from: http://publications.iarc.fr/78 PMID:7869568

IARC (2002). Some traditional herbal medicines, some mycotoxins, naphthalene and styrene. *IARC Monogr Eval Carcinog Risks Hum*, 82:1–556. Available from: http://publications.iarc.fr/100 PMID:12687954

Kogevinas M, Gwinn WM, Kriebel D, Phillips DH, Sim M, Bertke SJ, et al.; IARC Monographs Vol 121 Group (2018). Carcinogenicity of quinoline, styrene, and styrene-7,8-oxide. *Lancet Oncol*, 19(6):728–9. doi:10.1016/S1470-2045(18)30316-4 PMID:29680246

Nissen MS, Stokholm ZA, Christensen MS, Schlünssen V, Vestergaard JM, Iversen IB, et al. (2018). Sinonasal adenocarcinoma following styrene exposure in the reinforced plastics industry. *Occup Environ Med*, 75(6):412–4. doi:10.1136/oemed-2017-104974 PMID:29540567

Rappaport SM, Yeowell-O'Connell K, Bodell W, Yager JW, Symanski E (1996). An investigation of multiple biomarkers among workers exposed to styrene and styrene-7,8-oxide. *Cancer Res*, 56(23):5410–6. PMID:8968094

Smith MT, Guyton KZ, Gibbons CF, Fritz JM, Portier CJ, Rusyn I, et al. (2016). Key characteristics of carcinogens as a basis for organizing data on mechanisms of carcinogenesis. *Environ Health Perspect*, 124(6):713–21. doi:10.1289/ehp.1509912 PMID:26600562

STYRENE AND STYRENE-7,8-OXIDE

1. Exposure Data

1.1 Styrene

1.1.1 Identification of the agent

(a) Nomenclature

Chem. Abstr. Serv. Reg. No.: 100-42-5

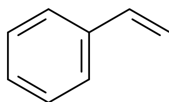
Previously used Chem. Abstr. Serv. Reg. No.: 79637-11-9

Chem. Abstr. Serv. name: Ethenylbenzene

IUPAC systematic name: Styrene

Synonyms: Cinnamene; cinnamenol; cinnamol; phenylethene; phenethylene; phenylethylene; styrol; styrole; styrolene; vinylbenzene; vinylbenzol

(b) Structural and molecular formulae, and relative molecular mass



Styrene

Molecular formula: C_8H_8

Relative molecular mass: 104.15

(c) Chemical and physical properties of the pure substance

Description: Colourless, viscous liquid with a pungent odour (WHO, 1983)

Melting/freezing point: −31 °C (IARC, 2002)

Boiling point: 145 °C at 101.3 kPa (IARC, 2002)

Density: 0.906 g/cm^3 at 25 °C (Merck, 2017a)

Relative density: $d_{20/4}$, 0.9060 (water, 1) (IARC, 2002)

Solubility in organic solvents: Miscible with acetone, benzene, carbon tetrachloride, (diethyl) ether, ethanol, heptane (Chevron Phillips Chemical Co., 2010)

Solubility in water: 0.31 g/L at 25 °C (NTP, 2016a)

Vapour pressure: 867 Pa at 25 °C (IARC, 2002)

Saturated vapour concentration: 8500 ppm at 25 °C (EPA, 1993)

Relative vapour density: 3.6 (air, 1) (IARC, 2002)

Evaporation rate: 1.92 (nBuAc, 1) (Chevron Phillips Chemical Co., 2010)

Odour threshold: Low: 0.20 mg/m^3 (pure) and 0.43 mg/m^3 (stabilized); high: 860 mg/m^3 (Ruth, 1986)

Reactivity: Polymerizes readily at room temperature in the presence of oxygen and oxidizes on exposure to light and air (WHO, 1983). Styrene is stabilized by a small amount

of a polymerization inhibitor; if this is not present in adequate concentration, styrene can polymerize and explode its container. Polymerization is also accelerated at temperatures above 66 °C (NIOSH, 1983); recommended storage temperature is 25 °C or less (Shell, 2017). The vapours are heavier than air and spread along the ground; distant ignition is possible (Shell, 2017).

Octanol/water partition coefficient (P): log K_{ow}, 2.95 (IARC, 2002)

Conversion factor: 1 ppm = 4.26 mg/m^3 at normal temperature (25 °C) and pressure (101 kPa) (IARC, 2002)

(d) Technical products and impurities

Styrene monomer is available as a commercial product with different levels of purity (i.e., 99.7% to >99.9%) The impurities present and their concentrations depend upon the manufacturing route, as well as plant performance characteristics. The typical inhibitor content of the standard grade is 10–15 ppm 4-*tert*-butylcatechol (CEFIC, 2007). The trace components in a commercial product of purity 99.93% are listed in Table 1.1 (Chevron Phillips Chemical Co., 2010).

Table 1.1 Trace components present in commercially produced styrene

Trace chemical	Concentration (ppm)
α-methylstyrene	175
Σ *m*- and *p*-xylene	120
o-xylene	125
Cumene	100
Propylbenzene	60
Ethylbenzene	50
Phenylacetylene	50
Σ *m*- and *p*-ethyltoluene	20
Aldehydes (as benzaldehyde)	15
4-*tert*-butylcatechol	12
Vinyltoluene	10
Σ *m*- and *p*-divinylbenzene	< 10
Peroxides (as benzoyl peroxides)	5
o-divinylbenzene	< 5
Polymerized styrene	1
Benzene	< 1
Chlorides (as Cl)	< 1
Sulfur	< 1
Toluene	< 1

ppm, parts per million.
Adapted from Chevron Phillips Chemical Co. (2010).

1.1.2 Production and use of styrene

Worldwide, styrene is one of the most important monomers for polymers and copolymers that are used in a wide range of applications. Globally, it is estimated that more than 15 000 industrial plants in many countries produce or use styrene in the manufacturing of polymeric products (The Styrene Forum, 2017).

(a) Production process

Styrene was first isolated in 1831 by distillation of storax, a natural balsam. Commercial production of styrene via dehydrogenation of ethylbenzene began in Germany in 1925 (Tossavainen, 1978).

There are two commercially viable methods of producing styrene. The most important, which accounts for over 90% of total world styrene production, is catalytic dehydrogenation in the vapour phase of high-purity ethylbenzene. Common catalysts are based on ferric oxide (Fe_2O_3) with chromia (Cr_2O_3) as a stabilizer and potassium oxide as a coke retardant (Behr, 2017). Typically, the crude product of the dehydrogenation process consists of 64% styrene, 32% ethylbenzene, 2% toluene, 1% benzene, and 1% other substances. The isolation of pure styrene from the mixture by distillations is difficult because of the similar boiling points of styrene and ethylbenzene (Behr, 2017).

The second process involves oxidation of ethylbenzene to its hydroperoxide and reaction with propylene to yield propylene oxide. The co-product α-methylphenyl carbinol is then dehydrated to styrene (Behr, 2017). A third

process, involving oxidative dehydrogenation of ethylbenzene to styrene with carbon dioxide, has been proposed (Chon, 2003).

(b) Production volume

Styrene is considered to be a high production volume chemical (USDOE, 2012). In 2004, the global styrene demand was reported to be over 24 million metric tonnes (CEFIC, 2007). In 2010, the global production of styrene was 27.5 million United States tons [25 million tonnes], of which approximately 4.4 million United States tons [4 million tonnes] originated in the USA. In 2012, the world production of styrene monomer exceeded 26.4 million metric tonnes (Aghayarzadeh et al., 2014).

China consumes far more styrene than other countries. In 2014, 30% of the world consumption of styrene was estimated to be by China (IHS Markit, 2017). Styrene consumption is expected to remain relatively constant, growing at an average rate of 1.6% per year during 2014–2019. Higher growth in the production of expandable polystyrene (EPS) is predicted, especially in construction where it is being increasingly used as concrete forms and as insulation, driven by demand for higher energy efficiency. Styrene consumption for EPS production is expected to grow at an average rate of 2.3% per year during 2019–2024. Styrene consumption for the production of acrylonitrile–butadiene–styrene (ABS) resins and styrene–butadiene rubber (SBR) is expected to see the highest annual growth rates of 3.6% and 4.1%, respectively (IHS Markit, 2017).

The production of styrene in the USA has risen steadily since 1960. Between 1960 and 2006, estimated production ranged from a low of 1740 million pounds [~7.9×10^5 tonnes] in 1960 to a high of 11 897 million pounds [~54×10^5 tonnes] in 2000. In 2006, eight United States manufacturers produced an estimated 11 387 million pounds [~51×10^5 tonnes] of styrene; the three largest producers accounted for 54% of production. United States consumption of styrene in 2006 was 9600 million pounds [~43×10^5 tonnes], more than 99% of which was in the production of polymers and copolymers (NTP, 2016a).

The USA is a producer and net exporter of styrene to the rest of the world. In 2014, the production capacity of styrene in Canada and the USA was 880 thousand tonnes and 4.8 million tonnes, respectively. The amount of styrene imported into the USA recently has been small, and only from Canada. In 2014, the USA exported 1.53 million tonnes of styrene (ICIS, 2014).

In 2016, 1 767 053 metric tons of styrene monomer was produced in Japan (Statista, 2017).

In 2016, China imported 3.5 million tonnes of styrene monomer; 1.23 million tonnes (35%) of this quantity came from the Republic of Korea, making it the largest supplier of styrene monomer to China. Meanwhile, China's total styrene monomer demand has gradually increased from 8.49 million tonnes in 2013 to 9.1 million tonnes in 2016, at an average annual growth rate of 2.38%. According to industry sources, China's annual domestic styrene monomer production was about 8.39 million tonnes in 2017, and was expected to rise by 2.3 million tonnes per year to 10.7 million tonnes by 2019 (Plastemart.com, 2017).

Styrene is presently manufactured in and/or imported into the European Economic Area at a rate of 1–10 million tonnes per year (ECHA, 2017).

(c) Uses

In the 1930s, styrene was used mainly in the production of synthetic rubber; the application of styrene as a solvent and cross-linking agent in the production of fibreglass-reinforced plastics started in the 1950s (Tossavainen, 1978). The resins generally contain between 30% and 50% styrene by weight (Haberlein, 1998). Today, styrene is primarily used as a monomer in the production of polystyrene plastics and resins.

Styrene producers sell styrene monomer to companies that use styrene to make various compounds and resins. Fabricators then process the resins into a wide variety of products (Cohen et al., 2002).

According to The Styrene Forum (2017), there are six major styrene resin families: (i) polystyrene (PS); (ii) SBR; (iii) styrene–butadiene latex (SBL); (iv) ABS; (v) styrene-based unsaturated polyester resins (UPR); and (vi) styrene–acrylonitrile (SAN). Industry estimates of the relative amounts of styrene consumed by these six resin families are as follows: PS, 50%; SBR, 15%; SBL, 12%; ABS, 11%; UPR, 11%; and SAN, 1% (The Styrene Forum, 2017). The uses, according to Chevron Phillips Chemical Co. (2017) are variable across these resin families. PS is primarily used in packaging, disposables, and low-cost consumer products. Improved grades of PS resins are used in higher-performance applications, such as home electronics and appliances. Uses of PS include as EPS beads in food and beverage packaging, building insulation, and cushion packaging. SBR, a thermoplastic synthetic elastomer, is used in the production of tyres. SBL is another thermoplastic synthetic elastomer used in carpet backing, for example. ABS and SAN have many uses in the consumer durables market. Styrene-based UPR are used in gel-coating and laminating operations in the production of fibreglass-reinforced plastic products such as boats, bathtubs, shower stalls, tanks, and drums, that is, items that provide a long service life in both indoor and outdoor applications (The Styrene Forum, 2017).

In addition, recycled polystyrene is used in packaging, construction materials, video cassettes, office supplies, and other products. A company in Oregon, USA, is developing a production plant that can convert scrap polystyrene into a liquid monomer (Association of Oregon Recyclers, 2017).

1.2 Styrene-7,8-oxide

1.2.1 Identification of the agent

(a) Nomenclature

Chem. Abstr. Serv. Reg. No.: 96-09-3; (*R*)-(+)-styrene-7,8-oxide: 20780-53-4; (*S*)-(-)-styrene-7,8-oxide: 20780-54-5; (±)-styrene-7,8-oxide: 67253-49-0

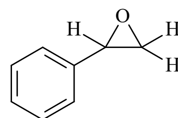
Previously used Chem. Abstr. Serv. Reg. No.: 62497-63-6

Chem. Abstr. Serv. name: 2-Phenyloxirane

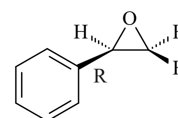
Synonyms: Epoxyethylbenzene; 1,2-(epoxyethyl)benzene; 1,2-epoxy-1-phenylethane; a,β-epoxystyrene; phenethylene oxide; 1-phenyl-1,2-epoxyethane; phenylethylene oxide; phenyloxirane; styrene epoxide; styrene oxide; styryl oxide

(b) Structural and molecular formulae, and relative molecular mass

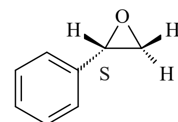
Styrene-7,8-oxide exists as two optical isomers and the commercial product is a racemic mixture.



(±)-styrene-7,8-oxide



(*R*)-(+)-styrene-7,8-oxide



(*S*)-(-)-styrene-7,8-oxide

Molecular formula: C_8H_8O

Relative molecular mass: 120.15

(c) Chemical and physical properties of the pure substance

Description: Clear, colourless to straw-coloured liquid with a sweet pleasant odour (PubChem, 2017)

Melting/freezing point: –37 °C (Merck, 2017b)

Boiling point: 193–195 °C at 101.3 kPa (Merck, 2017b)

Density: 1.05 g/cm³ at 20 °C (Merck, 2017b)

Relative density: $d_{20/4}$, 1.051–1.054 (water, 1) (Merck, 2017b)

Solubility in organic solvents: Soluble in acetone, benzene, carbon tetrachloride, heptane, and methanol (IARC, 1994)

Solubility in water: 3 g/L at 25 °C (Merck, 2017b)

Vapour pressure: 0.3 mm Hg = 40 Pa at 20 °C (Merck, 2017b)

Relative vapour density: 4.30 (air, 1) (HSDB, 2017b)

Odour threshold: Low: 0.3093 mg/m³; high: 1.9640 mg/m³ (Ruth, 1986)

Reactivity: Polymerizes exothermically and reacts violently with water in the presence of catalysts (acids, bases, certain salts). Should be stored at +15 to +25 °C. Decomposition temperature: > 250 °C (Merck, 2017b).

Octanol/water partition coefficient (P): log K_{ow}, 1.61 (IARC, 1994)

Conversion factor: 1 ppm = 4.91 mg/m³ at normal temperature (25 °C) and pressure (101.3 kPa) (IARC, 1994)

(d) Technical products and impurities

Typical product specification for styrene-7,8-oxide is 99% minimal purity and 0.1–0.2% maximal water content (IARC, 1994).

1.2.2 Production and use of styrene-7,8-oxide

(a) Production process

Styrene-7,8-oxide is produced commercially by the reaction of styrene with chlorine and water to form styrene chlorohydrin, followed by cyclization with aqueous base to produce styrene-7,8-oxide. It is also prepared by epoxidation of styrene with peroxyacetic acid (IARC, 1994). Styrene-7,8-oxide may be synthesized by air oxidation of styrene over cobalt-containing (Co_3O_4) catalysts (Lu et al., 2010).

Styrene monooxygenase produced by recombinant *Escherichia coli* can catalyse the enantiomeric oxidation of styrene to yield (*S*)-(-)-styrene-7,8-oxide (Panke et al., 2002).

(b) Production volume

Information available in 1991 indicated that styrene-7,8-oxide was produced by three companies in Japan and one company in the USA (IARC, 1994). In 2009, there was still only one United States manufacturer of styrene-7,8-oxide that had been identified (HSDB, 2017b).

Styrene-7,8-oxide is registered according to the European Union Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) regulation, and is manufactured in and/or imported into the European Economic Area in quantities of 100–1000 metric tonnes per year (ECHA, 2017).

(c) Uses

Styrene-7,8-oxide is used as a reactive plasticizer or diluent for epoxy resins and in the production of phenethyl alcohol (2-phenylethanol) and styrene glycol, and their derivatives (PubChem, 2017). It is used as a reactive plasticizer, in surface coatings, as a food-grade flavour, in styrene glycol, in cosmetics, and in perfumes (Bajpai & Mukherjee, 2017).

1.3 Measurement and analysis

1.3.1 Detection and quantification

(a) Air

Styrene and styrene-7,8-oxide concentrations in air during the lamination process in the reinforced plastics industry were measured by Pfäffli et al. (1979). The substances were sampled on charcoal tubes and desorbed with dichloromethane immediately after the sampling, and assayed by capillary column gas chromatography. The identification was performed by a mass spectrometer coupled with a gas chromatograph.

According to the National Institute for Occupational Safety and Health (NIOSH) Method 1501 for measurements of styrene in workplace air, the sample is adsorbed on charcoal and desorbed with carbon disulfide, and styrene determined by capillary column gas chromatography (GC) with a flame ionization detector (FID); the estimated limit of detection is 0.4 µg per sample (NIOSH, 1994).

Styrene in workplace air can also be sampled on a solid sorbent and desorbed with ethyl acetate; styrene is then determined by GC-FID (Tornero-Velez et al., 2000).

A thermal desorption GC mass spectrometry (MS) method to determine styrene in air and evaluate styrene levels in the workplace and its surrounding area was developed by Fernández-Villarrenaga Martín et al. (2000).

Styrene has been measured in ambient air and indoor air as part of exposure to volatile organic carbons (VOCs) of children (Adgate et al., 2004) and the general population (Rehwagen et al., 2003). Indoor air and ambient air were sampled by passive sampling followed by extraction with acetone and/or carbon disulfide (2:1 volume/volume) or carbon disulfide followed by GC-MS analysis. The quantitation limit for styrene was 0.6 µg/m^3 in the study on children (Adgate et al., 2004), and the detection limits in the general-population study were 0.01–0.05 µg/m^3 if converted to a sampling interval of 4 weeks (Rehwagen et al., 2003).

Styrene-7,8-oxide can be determined in air samples by GC-MS or GC-FID. The samples were collected on a solid sorbent and desorbed thermally with ethyl acetate or carbon disulfide (Pellizzari et al., 1976; Fjeldstad et al., 1979; Taylor, 1979; Stampfer & Hermes, 1981; Tornero-Velez et al., 2000). A detection limit as low as 2 ng/m^3 was reported (Krost et al., 1982).

(b) Water, soil, sediment, etc.

United States Environmental Protection Agency (EPA) Method 8260B can be used to determine the concentration of various VOCs, including styrene, by GC-MS in a variety of matrices such as groundwater, aqueous sludges, waste solvents, oily wastes, tars, soils, and sediments. Samples may be analysed using direct injection, purge-and-trap (PT), closed-system vacuum distillation, static headspace (solid samples), or desorption from trapping media (air samples) (EPA methods 5021, 5030, 5032, and 5041). The practical quantification limits are 5 µg/L for groundwater samples, 5 µg/kg (wet weight) for low-level soil and sediment samples, 250 µg/L for water-miscible liquid waste samples, 625 µg/kg for high-level soil and sludge samples, and 2500 µg/L for non-water-miscible waste samples (EPA, 1996).

Styrene-7,8-oxide and other electrophiles can be determined in environmental samples by reaction in aqueous solution with 4-nitrothiophenol to form thioethers; these have absorption maxima at about 445 nm and can be separated using high-performance liquid chromatography (HPLC). Styrene-7,8-oxide has two absorption maxima, at 339 nm and 340 nm. The level of detection was less than 1 part per billion (ppb) in water (Cheh & Carlson, 1981).

(c) Blood and urine

A rapid and simple PT-GC technique for the measurement of styrene in urine and blood samples was developed by Prieto et al. (2000, 2002).

Methods of isotope-dilution GC-MS have been described for determination of styrene and styrene-7,8-oxide in blood. Positive ion chemical ionization allowed the detection of styrene at concentrations greater than 2.5 µg/L blood and of styrene-7,8-oxide at concentrations greater than 0.05 µg/L blood (Tornero-Velez et al., 2001).

An alternative method for the measurement of styrene-7,8-oxide is reaction with valine, followed by derivatization with pentafluorophenyl isothiocyanate and analysis via negative ion chemical ionization GC and tandem mass spectrometry (MS/MS) (styrene-7,8-oxide detection limit, 0.025 µg/L blood). The detection limit for styrene-7,8-oxide by these two methods were 10–20-fold lower than those of the GC assays based upon either electron-impact MS or FID (Tornero-Velez et al., 2001).

Levels of unmetabolized styrene in urine can be determined by headspace solid-phase microextraction followed by GC-MS analysis, with a detection limit of 0.2 µg/L (Fustinoni et al., 2008).

Mandelic acid (MA) and phenylglyoxylic acid (PGA), urine metabolites of styrene and styrene-7,8-oxide, can be determined by HPLC (Ghittori et al., 1997; Marhuenda et al., 1997) or by liquid chromatography (LC) with MS/MS using negative ion mode and quantification by selected reaction monitoring (Manini et al., 2002). The detection limit of the HPLC method is 15 mg/L for MA and is 2 mg/L for PGA. The limit of detection for both MA and PGA is 0.1 mg/L using the LC-MS/MS method.

1.3.2 Styrene and styrene-7,8-oxide biomarkers in exposure assessment

Measurement of biological indicators of exposure incorporates the influence of multiple routes of absorption and the use of personal protective equipment within a comprehensive exposure assessment. The relationship between air concentrations and biological measures of exposure to styrene has been studied extensively. About 95% of the absorbed styrene is excreted in urine as MA and PGA. Reliable, sensitive, and specific analytical methods exist for monitoring occupational exposure to styrene and styrene-7,8-oxide. Among the biological monitoring methods available (Section 1.3.1), measurements of MA and PGA in urine are the most commonly used biological indices of exposure to styrene and styrene-7,8-oxide. Styrene itself can be measured in alveolar air, blood, and urine, and styrene-7,8-oxide and the haemoglobin adducts of styrene-7,8-oxide can be measured in blood. Table 1.2 and Table 1.3 provide summary data from occupational studies in which both personal inhalation exposure concentrations and biological indicators of styrene and styrene-7,8-oxide exposures in urine and blood, respectively, have been reported in reinforced plastics manufacturing workers.

(a) Mandelic acid and phenylglyoxylic acid in urine

Reliable, sensitive, and specific analytical methods exist for MA and PGA in urine (Marhuenda et al., 1997; Manini et al., 2002). MA and PGA are produced by sequential metabolism, MA appearing first in the urine (Guillemin & Bauer, 1979). The relationship between the excreted levels of MA and PGA varies according to factors such as the intensity of exposure and the sampling time. The biological half-life of PGA is longer than that of MA; accumulation of PGA can occur during a working week, whereas MA is mostly cleared within 24 hours (Perbellini

Table 1.2 Urine biomarker levels and their correlation coefficients (*r*) with styrene (and styrene-7,8-oxide) inhalation exposure concentrations in reinforced plastics manufacturing workers

Reference Location, collection date	Occupation description	No. of workers and/or samples	Sampling matrix and time	Mandelic acid + phenylglyoxylic acid		Mandelic acid		Phenylglyoxylic acid		Styrene	
				Mean exposure (range and/ or SD) (mg/g creatinine)[a]	*r*	Mean exposure (range and/ or SD) (mg/g creatinine)[a]	*r*	Mean exposure (range and/ or SD) (mg/g creatinine)[a]	*r*	Mean exposure (range and/or SD) (µg/L)[b]	*r*
Guillemin et al. (1982) Switzerland, 10 plants; NR	All workers	88	End of shift	NR	0.747	1003.7 (SD, 1206.8)	0.749	339.1 (SD, 360.2)	0.706		
	Hand lamination, containers of various sizes	4				1341.6 (SD, 756.2)		352.4 (SD, 98.1)			
	Hand and spray lamination of boats 13 m long and small objects	5				976.8 (SD, 485.4)		307.1 (SD, 163.3)			
	Hand, spray, and automatic system lamination of silos and small boats	8				655.1 (SD, 305.8)		240.2 (SD, 124.0)			
	Hand lamination of inside coating of oil tanks	6				1246.6 (SD, 1233.9)		255.8 (SD, 155.7)			
	Hand, spray, and artificial marble production and lamination of boats, showers, tables, sinters, etc.	12				715.6 (SD, 782)		187.6 (SD, 145.5)			

Table 1.2 (continued)

Reference Location, collection date	Occupation description	No. of workers and/or samples	Sampling matrix and time	Mandelic acid + phenylglyoxylic acid		Mandelic acid		Phenylglyoxylic acid		Styrene	
				Mean exposure (range and/or SD) (mg/g creatinine)[a]	*r*	Mean exposure (range and/or SD) (mg/g creatinine)[a]	*r*	Mean exposure (range and/or SD) (mg/g creatinine)[a]	*r*	Mean exposure (range and/or SD) (µg/L)[b]	*r*
Guillemin et al. (1982) (cont.)	Automatic system, pipes and others objects	3				551.7 (SD, 107.1)		230.8 (SD, 39.3)			
	Hand, spray, and vacuum lamination of basins, walls, baths, etc.	14				1179.5 (SD, 663.4)		465.0 (SD, 216.4)			
	Hand, spray, automatic system, UV-hardening, and silica reinforcement of large pipes	19				175.4 (SD, 127.7)		145.3 (SD, 102.4)			
	Hand, spray, and vacuum lamination of cabins, walls, etc.	15				2334.3 (SD, 1891.2)		763.4 (SD, 596.0)			
	Hand lamination of parts of car bodies	2				305.5 (SD, 68.6)		132.5 (SD, 118.1)			
Ikeda et al. (1982) Japan, five boat production plants; NR	Hand lamination	118	End of shift	NR	0.88	NR	0.86	NR	0.82		

Table 1.2 (continued)

Reference Location, collection date	Occupation description	No. of workers and/or samples	Sampling matrix and time	Mandelic acid + phenylglyoxylic acid		Mandelic acid		Phenylglyoxylic acid		Styrene	
				Mean exposure (range and/ or SD) (mg/g creatinine)[a]	*r*	Mean exposure (range and/ or SD) (mg/g creatinine)[a]	*r*	Mean exposure (range and/ or SD) (mg/g creatinine)[a]	*r*	Mean exposure (range and/or SD) (µg/L)[b]	*r*
Imbriani et al. (1986) Italy; NR	Boat manufacturing	121	End of shift							51 (median) (19.7–189.0)	0.89
Triebig et al. (1989) Germany, four plants; NR	Lamination of boats, pipes, or containers	36	End of shift	0.36 (0.02–4.29) g/L	NR	0.21 (median) (0.01–3.64) g/L	0.676	0.19 (median) (0.01–0.87) g/L	0.845		
Truchon et al. (1992) Canada (Quebec), three plants; NR	Chopper gun use	7	End of shift			0.73 (0.08–1.75) mmol/mmol cr					
	Painting (gel coat)	9				0.58 (0.11–0.94) mmol/mmol cr					
	Laminating (rollers)	18				1.25 (0.28–1.90) mmol/mmol cr					
	Foreman	8				0.28 (0.03–1.10) mmol/mmol cr					

Table 1.2 (continued)

Reference Location, collection date	Occupation description	No. of workers and/or samples	Sampling matrix and time	Mandelic acid + phenylglyoxylic acid		Mandelic acid		Phenylglyoxylic acid		Styrene	
				Mean exposure (range and/ or SD) (mg/g creatinine)[a]	*r*	Mean exposure (range and/ or SD) (mg/g creatinine)[a]	*r*	Mean exposure (range and/ or SD) (mg/g creatinine)[a]	*r*	Mean exposure (range and/or SD) (μg/L)[b]	*r*
Truchon et al. (1992) (cont.)	Cutter	11				0.24 (0.05–0.96) mmol/mmol cr					
	Warehouse work	19				0.05 (0.00–0.21) mmol/mmol cr					
	Finishing	31				0.08 (0.00–0.39) mmol/mmol cr					
	Mould repair	8				0.02 (0.00–0.08) mmol/mmol cr					
Gobba et al. (1993) Italy, 10 plants; NR	Hand lamination	65	End of shift	13.03 (0.31–53.22; SD, 12.76) mol/L	0.81	9.73 (0.24–40.23; SD, 9.94) mol/L	0.82	3.30 (0.07–12.99; SD, 2.82) mol/L	0.78	605.38 (38.40–2169.60; SD, 515.33) nmol/L	0.86
	Hand lamination	198		NR		NR		NR		556.13 (18.24–2015.04; SD, 461.76) nmol/L	0.88
Galassi et al. (1993) Italy, 87 plants; 1978–1990	Hand lamination	2386	End of shift			682 (GM, 450) (GSD, 2.75)					
	Spraying laminating	250				404 (GM, 211) (GSD, 3.3)					
	Rolling	63				327 (GM, 182) (GSD, 3.08)					
	Semi-automatic process	121				243 (GM, 154) (GSD, 2.59)					
	Non-process work	762				186 (GM, 94) (GSD, 3.27)					

Table 1.2 (continued)

Reference Location, collection date	Occupation description	No. of workers and/or samples	Sampling matrix and time	Mandelic acid + phenylglyoxylic acid		Mandelic acid		Phenylglyoxylic acid		Styrene	
				Mean exposure (range and/or SD) (mg/g creatinine)[a]	*r*	Mean exposure (range and/or SD) (mg/g creatinine)[a]	*r*	Mean exposure (range and/or SD) (mg/g creatinine)[a]	*r*	Mean exposure (range and/or SD) (μg/L)[b]	*r*
Ghittori et al. (1997) Italy; NR	Fibreglass-reinforced plastics	22	End of shift			580.0 (GM, 472.0) (GSD, 2.0)	0.86	174.0 (GM, 156.6) (GSD, 1.64)	0.82	25.6 (GM, 18.8) (GSD, 2.39)	0.83
Haufroid et al. (2001) Belgium; NR	Fibreglass-reinforced plastics	30	End of shift	434.9 (20.3–1757.8)	0.92	314.6 (15.5–1410.2)	0.90	120.3 (4.8–347.7)	0.93	18.2 ppm (0.9–68.9 ppm) [77.5 (3.8–293.5)]	
Prieto et al. (2002) Spain; NR	Boat construction	34	End of shift	GM 128	0.862	104.5 (GM, 91.1) (15–230; SD, 53.1)	0.834	42.6 (GM, 37.0) (10–83; 21.1)	0.841	5.7 (5.1 GM) (1.7–15.3; SD, 2.9)	0.788[c]
Teixeira et al. (2007) Portugal; NR	Fibreglass-reinforced plastics	72	Next morning	443 (23–1770; SD, 44)	0.85						
Fustinoni et al. (2008) Italy; NR	Fibreglass-reinforced plastics	8	End of shift; repeated (3–4×)	226.30 (51.73–779.61)	0.974 (0.787)[d]	148.13 (30.64–515.12)	0.975 (0.766)[d]	77.97 (20.89–248.99)	0.931 (0.818)[d]	7.5 (2.1–29.7)	0.810 (0.451)[d]
	Varnish workers	13		69.52 (16.11–178.31)		40.01 (7.95–130.71)		24.02 (8.13–56.71)		4.3 (1.8–53.6)	
Tranfo et al. (2012) Italy, two plants	Hand lay-up open-moulding process	7	End of shift	103 (76.7–132.3; SD, 21)	0.74						
	Compression closed moulding	12		85.9 (2.52–218.4; SD, 75.6)							

Table 1.2 (continued)

Reference Location, collection date	Occupation description	No. of workers and/or samples	Sampling matrix and time	Mandelic acid + phenylglyoxylic acid		Mandelic acid		Phenylglyoxylic acid		Styrene	
				Mean exposure (range and/ or SD) (mg/g creatinine)[a]	*r*	Mean exposure (range and/ or SD) (mg/g creatinine)[a]	*r*	Mean exposure (range and/ or SD) (mg/g creatinine)[a]	*r*	Mean exposure (range and/or SD) (µg/L)[b]	*r*
Bonanni et al. (2015) Italy, four plants; 2011–2013	Plant A: motorcycle helmets	20	End of shift	7.3 (median) (2.7–30.1) (25–75th percentile)	0.729						
	Plant B: fibreglass-reinforced plastic containers and pieces	12		76.7 (median) (20.9–117.1) (25–75th percentile)							
	Plant C: compression closed moulding	14		33.8 (median) (12.5–126.9) (25–75th percentile)							
	Plant D: fibreglass-reinforced plastic containers and pieces	12		331.1 (median) (214.1–400.1) (25–75th percentile)							
Carbonari et al. (2015) Italy, two plants	Fibreglass-reinforced plastics workers	30	End of shift	47.14 (SD, 427.26) (95th percentile)	0.895	NR	0.948	NR	0.821	43.92 (SD, 123.64 mg/g creatinine)	0.419

cr, creatinine; GM, geometric mean; GSD, geometric standard deviation; NR, not reported; SD, standard deviation.

[a] Concentrations given in mg/g creatinine unless indicated otherwise.

[b] Concentrations given in µg/L unless indicated otherwise.

[c] A high correlation was also reported between styrene in blood and styrene in urine: $r = 0.732$.

[d] Correlation coefficient for exposure to styrene-7,8-oxide (in brackets).

Table 1.3 Blood biomarker levels and their correlation coefficients (*r*) with styrene and styrene-7,8-oxide inhalation exposure levels in reinforced plastics manufacturing workers

Reference	Location; collection date	Occupation description	No. of workers and/or samples	Sampling matrix and time	Styrene			Styrene-7,8-oxide			Comments/ additional data
					Mean exposure (µg/L)[a]	Range and/ or SD (µg/L)[a]	*r*	Mean exposure (µg/L)[a]	Range and/or SD (µg/L)[a]	*r*	
Cherry et al. (1980)	United Kingdom, one plant; 1979	Boat industry	27	End of shift	6.9 µmol/L	2.0–20.9 µmol/L	0.44				
Triebig et al. (1989)	Germany, four plants; NR	Lamination of boats, pipes, or containers	36	End of shift	390	50–4820	0.823				
Gobba et al. (1993)	Italy, 10 plants; NR	Hand lamination	100	End of morning shift	5.3 µmol/L	0.16–20.80 µmol/L	0.86				Urine styrene and blood styrene *r* = 0.89
			36		5.65 µmol/L	0.48–17.93; 5.24 µmol/L	0.88				
Korn et al. (1994)	Germany, one plant; NR	Reinforced polyester resin production	13	Next morning	NR	78–836	0.87	NR	0.9–4.1	0.88	Blood styrene-styrene-7,8-oxide correlation *r* = 0.82
Somorovská et al. (1999)	Slovakia; NR	All workers	36	End of shift	601.2	1104.5					
		Hand laminators	9		2098.2	1330.8					
		Medium-exposed sprayers	12		81.3	80.3					
		Maintenance workers	15		84.5	81.9					
Prieto et al. (2002)	Spain; NR	Boat construction	34	End of shift	53.9	9.9–186.7	0.698				
Serdar et al. (2006)	USA, 17 plants; 1996–1999	All workers	295; 212	End of shift	83	< 1–2050	0.85	0.069	< 0.050–0.393	0.34	Blood styrene/ styrene-7,8-oxide correlation *r* = 0.49

NR, not reported; SD, standard deviation.

[a] Concentrations are given in µg/L unless indicated otherwise.

et al., 1988). The kinetics of MA and PGA formation and elimination can be influenced by exposure to other solvents, including ethylbenzene, phenylglycol, some pharmaceuticals, and alcoholic beverages, thus limiting the specificity of metabolite analyses as markers of styrene and styrene-7,8-oxide exposure. Prieto et al. (2002) observed that co-exposure to acetone reduced the concentrations of MA and PGA in urine. The sum of the two excreted metabolites (MA+PGA) is less affected by these confounding factors, and is therefore better suited for monitoring exposure to styrene and styrene-7,8-oxide (Ong et al., 1994). The reported correlation coefficients between personal inhalation exposures to styrene and styrene-7,8-oxide and creatinine-adjusted MA+PGA concentrations in urine are generally higher than those for the unadjusted values. Holz et al. (1995) observed urine MA+PGA concentrations of less than 10 mg/g creatinine in 25 control subjects who were not exposed to styrene, indicating that non-occupational exposure to styrene may occur but at a magnitude that will not likely influence biological monitoring results.

A strong correlation between measured styrene inhalation concentrations and MA+PGA concentrations in urine has been observed in multiple studies (see Table 1.2). These studies indicate a strong correlation (average, 0.84; range, 0.73–0.974) between the post-shift MA+PGA urine concentration and occupational inhalation exposure to styrene. Haufroid et al. (2001) measured a mean airborne styrene concentration of 18.2 ppm (range, 0.9–68.9 ppm) [77.5 (3.8–293.5) mg/m^3] in 30 workers in a fibreglass-reinforced plastics factory. The relationships observed between airborne styrene concentration and the concentrations of MA+PGA in post-shift urine samples were not different when considering MA+PGA (correlation coefficient (r) = 0.92, $P < 0.0001$) and MA ($r = 0.90$, $P < 0.0001$) or PGA ($r = 0.93$, $P < 0.0001$) alone. Urine concentrations of MA+PGA, MA, and PGA corresponding to inhalation exposure to 20 ppm [85 mg/m^3] of styrene were 420, 307, and 113 mg/g creatinine, respectively. Prieto et al. (2002) reported a mean air concentration of styrene of 70.5 mg/m^3 after 4 hours of exposure in 34 fibreglass boat construction workers. The geometric mean MA+PGA concentration in urine at the end of the 4-hour exposure was 128 mg/g creatinine with a correlation coefficient of 0.862 with the inhalation exposure. Using the regression equation provided by Prieto et al. (2002), inhalation exposure to 20 ppm [85 mg/m^3] of styrene is associated with a PGA+MA concentration of 170 mg/g creatinine in urine. Teixeira et al. (2007) reported a mean MA+PGA concentration of 443 mg/g creatinine in urine samples collected before the work shift in the morning, following personal inhalation exposure measurements of an 8-hour time-weighted average (TWA) air concentration of styrene of 30.4 ppm [129.5 mg/m^3] ($r = 0.85$), in 75 fibreglass-reinforced plastics workers.

(b) Styrene in urine

Reliable, sensitive, and specific analytical methods exist for styrene in urine (Imbriani et al., 1986; Ghittori et al., 1997; Fustinoni et al., 2008). Strong correlations between measured inhalation exposure to styrene and styrene concentrations in urine have been reported in six studies (Imbriani et al., 1986; Gobba et al., 1993; Ghittori et al., 1997; Prieto et al., 2002; Fustinoni et al., 2008; Carbonari et al., 2015) (see Table 1.2). Imbriani et al. (1986) reported a median breathing-zone air concentration of 109 mg/m^3 and a corresponding median concentration of 51 µg/L styrene in urine ($r = 0.89$) at the end of the 4-hour work shift in 121 workers in a plastic boat factory. A mean urine concentration of 43 µg/L was estimated to be associated with an inhalation exposure concentration of 20 ppm [85 mg/m^3] styrene. Gobba et al. (1993) reported a geometric mean breathing-zone air concentration of 87.9 mg/m^3 styrene and a mean styrene concentration of 556.13 nmol/L [57.9 µg/L] in urine ($r = 0.88$) at the

end of the 8-hour work shift in 198 fibreglass-reinforced plastics manufacturing workers. The authors calculated a urine styrene concentration of 38 μg/L to correspond to a styrene inhalation exposure of 20 ppm [85 mg/m³]. Ghittori et al. (1997) reported a mean breathing-zone air concentration of 112 mg/m³ styrene and a mean styrene concentration in urine of 25.6 μg/L at the end of an 8-hour work shift (r = 0.83) in 22 fibreglass-reinforced plastics workers. The urine concentration of styrene associated with an air concentration of 20 ppm [85 mg/m³] was 18 μg/L. Prieto et al. (2002) reported a mean styrene concentration of 5.7 μg/L in urine after 34 workers were exposed to a 4-hour mean styrene breathing-zone air concentration of 70.5 mg/m³ (r = 0.788) in a fibreglass boat manufacturing plant. The urine concentration of styrene associated with an inhalation exposure of 20 ppm of styrene was 6.6 μg/L. Fustinoni et al. (2008) reported mean styrene inhalation exposure concentrations of 3.4 mg/m³ for 13 varnish workers and 18.2 mg/m³ for 8 reinforced plastics workers exposed to styrene. The measured mean urine concentrations of styrene were 4.3 μg/L for varnish workers and 7.5 μg/L for plastics workers at the end of the 8-hour work shift, with a correlation of 0.810 between the air and urine styrene levels.

These studies indicate that styrene in urine can be used as a biomarker for styrene exposure. Styrene in urine does not appear to be affected by co-exposure to other solvents or alcohol (Prieto et al., 2002). However, monitoring styrene in urine can be limited by the fact that less than 1% of absorbed styrene is eliminated unchanged in urine (NTP, 2008). Precautions are necessary to avoid contamination of the sample from airborne styrene concentrations, because the monitored biomarker is the parent compound. In addition, the timing of sample collection is critical; because of the fast elimination kinetics, the sample should be obtained immediately after the cessation of exposure.

(c) Styrene and styrene-7,8-oxide in blood

The measurement of styrene and styrene-7,8-oxide in blood has only been published in a few studies to date (Somorovská et al., 1999; Serdar et al., 2006) (see Table 1.3). Somorovská et al. (1999) investigated styrene exposure and styrene levels in blood as well as other end-points including DNA strand breaks, chromosomal aberrations, immune parameters, and genotyping of polymorphic genes (see Section 4.2.1) in 44 workers exposed to styrene in a hand lamination plant in Europe. The mean breathing-zone air styrene concentration was 101 mg/m³ and the mean blood concentration of styrene was 601.2 μg/L. The mean air and blood styrene concentrations were 199 mg/m³ and 2098.2 μg/L for hand laminators (group exposed to high concentrations), 55 mg/m³ and 81.3 μg/L for sprayers (group exposed to medium concentrations), and 27 mg/m³ and 84.5 μg/L for maintenance workers (group exposed to low concentrations), respectively.

Serdar et al. (2006) measured styrene and styrene-7,8-oxide in personal breathing-zone air and blood samples collected repeatedly from 295 reinforced plastics workers in the USA. The median breathing-zone air styrene concentration was 9.14 ppm [85.19 mg/m³] and the median blood concentration of styrene was 0.083 mg/L (r = 0.85). The median breathing-zone air styrene-7,8-oxide concentration was 17.10 ppb [84.03 μg/L] and the median blood concentration of styrene-7,8-oxide was 0.069 μg/L (r = 0.34). Styrene inhalation exposure was 535-fold higher than exposure to styrene-7,8-oxide, and the blood concentrations of styrene were 1200-fold higher than those of styrene-7,8-oxide.

(d) Other styrene and styrene-7,8-oxide biomarkers

Styrene-7,8-oxide albumin and haemoglobin adducts are specific biomarkers for styrene and styrene-7,8-oxide exposure, and correlate well

with inhalation exposures to these compounds. However, the biomarker measurements have some disadvantages, including low concentrations and short half-life and/or lifespan in blood, and the analytical methods are time-consuming and not sufficiently sensitive for haemoglobin adducts (Teixeira et al., 2007; Fustinoni et al., 2008).

1.4 Occurrence and exposure

1.4.1 Environmental occurrence

Because of the extensive commercial use of styrene, people come into contact with styrene in air, food, water, consumer products, and the built environment. Styrene can be emitted to the air from industrial production and use of styrene and styrene-based polymers, motor-vehicle emissions and other combustion processes, off-gassing of building materials and consumer products, and cigarette smoking (IARC, 1994; ATSDR, 2010). Styrene has been measured in outdoor air (generally less than 1 ppb [4.3 μg/m^3]), but higher levels are generally found in indoor air, drinking-water, groundwater, surface water, soil, and food (Cohen et al., 2002; IARC, 2002). The presence of styrene in packaged foods is reported to be primarily due to monomer migrating from polystyrene containers (WHO, 1983). For the general population, daily styrene intake is expected to be orders of magnitude lower than daily intakes for workers in occupations with exposure to high concentrations of styrene (Cohen et al., 2002). Nevertheless, most of the general population has detectable levels of styrene in their biological fluids (e.g. blood and breast milk) (IARC, 2002; Blount et al., 2010; CDC, 2018). Although styrene exposure is common, arising from multiple sources, styrene is not a persistent chemical; it does not persist in air, water, or soil (Health Canada, 1993).

Styrene is not known to bioaccumulate in humans for extended periods of time (Bond & Bolt, 1989; Pekari et al., 1993; IARC, 2002; ATSDR, 2010).

The general public is indirectly exposed to styrene-7,8-oxide via metabolism of styrene. Styrene-7,8-oxide adducts of albumin and haemoglobin were detected in people who were not occupationally exposed to styrene or styrene-7,8-oxide (Yeowell-O'Connell et al., 1996).

(a) Ambient air

Styrene has been detected in the atmosphere in many locations. Its presence in air is principally due to emissions from industrial processes involving styrene and its polymers and copolymers. Other sources of styrene in the environment include vehicle exhaust, cigarette smoke, and other emissions from the combustion and incineration of styrene polymers (WHO, 1983).

In the USA, there was an overall decrease in styrene emissions from 2005 to 2015 driven mainly by decreases in styrene air releases from the plastics and rubber and transportation equipment industries. These reductions were substantial enough to effect a 46% decrease in the total air release of 180 United States Occupational Safety and Health Administration carcinogens reported to the Toxics Release Inventory (EPA, 2015).

Ambient air monitoring data from the USA include databases compiled and maintained by the California Air Resources Board. The 20 test stations are located in urban areas, representing the greatest proportion of the California population. Styrene is measured from a 24-hour sample collected once each month. Based on the measurements from each test station for each month from 1989 to 1995, the average reading for styrene was approximately 0.2 ppb [0.9 μg/m^3] over 6 years. The detection level for styrene was 0.1 ppb [0.4 μg/m^3] and the highest measurement was 2.9 ppb [12.4 μg/m^3] (Styrene Information & Research Center, 2001).

Styrene levels of 1.1–6.6 μg/m^3 were measured in air samples from the Pennsylvania Turnpike

Allegheny Mountain Tunnel in 1979. The mean concentration in the tunnel intake air was less than 0.1 μg/m³ (Hampton et al., 1983). Air concentrations of styrene in the vicinity of five rural hazardous waste sites in New Jersey, USA of up to 15.5 ppb [66.0 μg/m³] were measured (LaRegina et al., 1986).

As part of the Minnesota Children's Pesticide Exposure Study (MNCPES) survey of VOCs in households with children, styrene was quantified in 39% of outdoor samples (n = 100) with a mean of 0.5 μg/m³ (limit of quantitation, 0.6 μg/m³) in 1997 (Adgate et al., 2004).

In Canada, styrene levels in ambient air were determined in a survey of 18 sites (mostly urban) in 1988–1990. The mean concentrations in 586 24-hour samples ranged from 0.09 μg/m³ to 2.35 μg/m³ (Newhook & Caldwell, 1993). In a survey of VOCs in outdoor environments proximal to apartment dwellings in Leipzig, Germany in 2000–2001, Rehwagen et al. (2003) determined a mean styrene concentration of 0.09 μg/m³ (n = 222, 4-week samples). Median ambient styrene concentrations collected once a month at six sites in the Hyogo Prefecture, Japan during 2005–2009 were 0.25–0.73 μg/m³ (n = 60) (Okada et al., 2012).

Thermal degradation of styrene-containing polymers also releases styrene into ambient air (Hoff et al., 1982; Lai & Locke, 1983; Rutkowski & Levin, 1986). Styrene levels ranged from 0.003 ppm to 2.01 ppm [0.013–8.56 mg/m³] and, along with benzene, toluene, naphthalene, and 1,3-butadiene, accounted for 31% of the total concentration of VOCs found in smoke from municipal structural fires (Austin et al., 2001).

Although styrene-7,8-oxide was identified in air samples collected in the Los Angeles Basin and in other unidentified areas in the USA, measurements were not reported (IARC, 1985).

(b) Indoor air

For residential exposure, median concentrations obtained by personal air sampling are generally in the range of 1–4 μg/m³ (Wallace et al., 1985; Wallace, 1986). Off-gassing of styrene from some styrene-containing household products may contribute to indoor air levels (Knöppel & Schauenburg, 1989; Hodgson, 1999; Willem & Singer, 2010). Indoor air studies conducted in residential settings in Canada, Finland, France, Germany, and the USA have generally been consistent with the earlier findings of Wallace et al. (1985), and Wallace (1986). The MNCPES study determined an average indoor styrene air concentration of 1.4 μg/m³ (n = 101; 82% of samples above the limit of quantitation of 0.6 μg/m³) and average personal air concentrations of 1.2 μg/m³ (n = 73; 90% of samples above the limit of quantitation of 0.6 μg/m³) (Adgate et al., 2004). In a national survey of styrene levels in indoor air in 757 single-family dwellings and apartments, representative of the homes of the general population of Canada in 1991, the mean 24-hour concentration was 0.28 μg/m³, with values ranging from none detected (limit of detection, 0.48 μg/m³) up to 129 μg/m³ (Newhook & Caldwell, 1993). A national survey of indoor air VOCs was repeated in Canada during 2009–2011 for 3857 residences (Zhu et al., 2013). Although arithmetic means of VOCs declined 2–5-fold for 11 volatiles common to both Canadian studies, the exception was an increase in styrene from 0.30 μg/m³ in the 1992 study (n = 757) to 1.13 μg/m³ [geometric mean (GM), 0.72 μg/m³] in the 2009–2011 survey. The following indoor residential styrene concentrations were measured: 0.84 μg/m³ (GM, n = 201) for a 1996–1998 study conducted in Helsinki, Finland (Edwards et al., 2001); 1.47 μg/m³ (arithmetic mean, n = 1499) for a 1994–2001 study conducted in apartments in Leipzig, Germany (Rehwagen et al., 2003); and 1.0 μg/m³ (GM, n = 490) for a 2003–2005 study conducted in France (Billionnet et al., 2011).

(c) Tobacco

Styrene is one of the hundreds of individual components that may be quantified in tobacco smoke (IARC, 1986; Darrall et al., 1998; Health Canada, 1999; IARC, 2004). The styrene content of cigarette smoke has been reported to be 18–48 µg per cigarette (WHO, 1983) and, by a more recent assessment, 0.5–10.0 µg per cigarette (CDC, 2010). Exposure to styrene is approximately 6 times higher for smokers than for non-smokers, and tobacco smoke is the major source of styrene exposure for smokers (Wallace et al., 1987, 1989). Measurements in homes with and without smokers revealed that average styrene concentrations in the homes of smokers were approximately 0.5 µg/m^3 higher than those in the homes of non-smokers. The 2009–2011 indoor air study of Zhu et al. (2013) obtained a significantly higher estimate of styrene concentration in the houses of smokers relative to those of non-smokers by 0.44 µg/m^3 (P = 0.001), and in the apartments of smokers relative to those of non-smokers by 0.70 µg/m^3 (P = 0.001). Hodgson et al. (1996) also found that environmental tobacco smoke can contribute significantly to indoor airborne styrene concentrations. Environmental tobacco smoke was estimated to contribute 8% of the total styrene inhalation exposure of all non-smoking Californians (Miller et al., 1998). Chambers et al. (2011) assessed the impact of cigarette smoking on concentrations of VOCs in blood using 2003–2004 United States National Health and Nutrition Examination Survey (NHANES) data. The authors estimated a weighted median and 95th percentile styrene blood concentration of 0.072 ng/mL and 0.180 ng/mL, respectively, for daily smokers (n = 290). For less than daily smokers, the weighted median and 95th percentile were less than 0.03 ng/mL and 0.068 ng/mL, respectively (n = 864). Chambers et al. (2011) estimated a 0.010 ng/mL increase in blood styrene per cigarette per day.

(d) Water

Although styrene has been detected occasionally in estuaries, inland waters, and drinking-water, its presence is usually traceable to an industrial source or to improper disposal (WHO, 1983). In surveys of Canadian drinking-water supplies, the frequency of detection of styrene was low; when detected, it was generally at a concentration of less than 1 µg/L (Newhook & Caldwell, 1993). After accidental drinking-water contamination with styrene in Spain, transient levels of up to 900 µg/L were reported (Arnedo-Pena et al., 2003). Emissions of VOCs were studied from three heavily polluted rivers (Huijiang, Nancun, and Zengbian) in fast-developing Guangzhou, south China (Chen et al., 2013). Concentrations of styrene in water samples (n = 16) ranged from 14 300 µg/L to 36 500 µg/L (Chen et al., 2013).

Styrene-7,8-oxide released to the environment is not expected to persist in water. Hydrolytic half-lives of 0.17, 28, and 40.9 hours have been reported for styrene-7,8-oxide at pH values of 3, 7, and 9, respectively (Schmidt-Bleek et al., 1982). Although styrene-7,8-oxide may enter the environment through industrial discharges or spills in wastewater, it has rarely been detected in source drinking-water (IARC, 1994). In a survey of 4000 samples of wastewater taken from both industrial and publicly owned treatment centres in the USA, styrene-7,8-oxide was found at one site (IARC, 1994).

(e) Food and food packaging

Styrene has been detected in a wide range of foods and beverages (IARC, 1994). In a follow-up analysis of the United States Food and Drug Administration (FDA) Total Diet Study that focused on VOCs in 70 foods sampled between 1996 and 2000, the highest styrene concentrations were observed in strawberries (range, 12–350 µg/kg) and avocados (range, 3–550 µg/kg) [mean levels were not reported] (Fleming-Jones & Smith, 2003).

Analysis of styrene in 133 plastic food containers from retail food outlets in the United Kingdom showed concentrations ranging from 16 mg/kg to 1300 mg/kg; 73% of containers had styrene concentrations of 100–500 mg/kg, and only five containers had levels exceeding 1000 mg/kg. The food in the containers had levels of monomer ranging from less than 1 µg/kg to 200 µg/kg, although 77% of the foods had levels of less than 10 µg/kg and 26% had levels of less than 1 µg/kg (Gilbert & Startin, 1983). A more recent analysis conducted by the FDA, specific to polystyrene packaging, showed that styrene concentrations in foods had not significantly changed since the 1980s, ranging from 2.6 µg/kg to 163 µg/kg (Genualdi et al. 2014).

1.4.2 Occupational exposure

Workers can be exposed to both styrene and styrene-7,8-oxide in the industries and operations where they are produced and used, for example: in the production of styrene-containing polymer resins, plastics, and rubber products; in the fabrication of reinforced-polyester plastics composites; and in the use of products containing styrene, such as paints, adhesives, metal cleaners, and varnishes (US NLM, 2018). The most significant exposures to both styrene and styrene-7,8-oxide, measured in air and in biological media, occur in the manufacture of fibreglass-reinforced polyester products; exposures are generally lower in the production of styrene, polystyrene, and styrene-based plastics and rubbers. During lamination and curing, about 10% of the styrene may evaporate into the workplace air (NIOSH, 1983; Crandall & Hartle, 1985). Occupational exposure to styrene-7,8-oxide occurs in industries manufacturing or using styrene and styrene polyester resins due to its formation from styrene as an oxidation product. Styrene-7,8-oxide has been detected in association with styrene, but at much lower levels, in industries where unsaturated polyester resins are used (Pfäffli et al., 1979; Pfäffli & Säämänen, 1993; Rappaport et al., 1996; Nylander-French et al., 1999; Serdar et al., 2006). The ratio of styrene to styrene-7,8-oxide in the air in reinforced plastic manufacturing is approximately 1000:1 (see Section 1.4.2(a) and Table 1.4, last column).

(a) Production and processing of reinforced plastics

In terms of the numbers of workers exposed and exposure concentrations, occupational exposure to styrene is greatest in the fabrication of objects from fibreglass-reinforced polyester composite plastics, such as boats, tanks, wall panels, bath and shower units, and automotive parts. Exposure to styrene in this industry has been summarized in several publications (see Table 1.5). Styrene serves as a solvent and a reactant for the unsaturated polyester resin, in which it constitutes about 40% by weight. In the open-mould process, a releasing agent is usually applied to the mould, a first coat containing pigments (gel coat) is applied, then successive layers of chopped and/or woven fibreglass are deposited manually or with a chopper gun at the same time as the resin is sprayed or brushed on, and then the surface is rolled. During lamination and curing, about 10% of the styrene may evaporate into the workplace air (NIOSH, 1983; Crandall & Hartle, 1985).

Exposure to styrene-7,8-oxide has only been documented in a few reports (Table 1.4). In Finnish factories producing boats, car parts, and building materials from polyester-based reinforced plastics, both styrene and styrene-7,8-oxide were measured. The average styrene levels in personal breathing-zone samples were 132.9 ± 109.6 ppm [567 ± 467 mg/m^3] for hand lay-up and 130.2 ± 68.8 ppm [554 ± 293 mg/m^3] for spray application, and the average styrene-7,8-oxide concentration was 0.04 ± 0.02 ppm [0.20 ± 0.10 mg/m^3] for hand lay-up and 0.12 ± 0.07 ppm [0.59 ± 0.34 mg/m^3] for spray application (Pfäffli et al., 1979). In a Norwegian

Table 1.4 Personal styrene and styrene-7,8-oxide inhalation exposure levels in reinforced plastics manufacturing workers

Reference	Location; collection date	Occupation description	No. workers and/or samples	Sampling duration	Styrene		Styrene-7,8-oxide		Styrene-7,8-oxide concentration as a percentage of styrene concentration (%)
					Mean exposure (mg/m³)[a]	Range or SD (mg/m³)[a]	Mean exposure (µg/)[b]	Range or SD (µg/m³)[b]	
Fjeldstad et al. (1979)	Norway, one plant; NR	Mudguards	5	2 h	54 ppm (median) [230.02]	28–170 ppm [119.27–724.15]	38 ppb (median) [186.74]	28–86 ppb [137.6–422.61]	0.05–0.1 (median, 0.07)
		Car bodies	6		108 ppm [460.05]	65–278 ppm [276.88–1184.2]	48 ppb	12–71 ppb [58.97–348.9]	0.018–0.025 (median, 0.44)
		Other car parts	2		NR	31–51 ppm [132.05–217.25]	NR	29–64 ppb [142.51–314.5]	0.094–0.125
		Fibre machines	2		NR	17–46 ppm [72.42–195.95]	NR	< 3–4 ppb [< 14.74–19.66]	0.009–0.018
		Antenna elements	2		NR	89–102 ppm [379.11–434.49]	NR	41–65 ppb [201.48–319.42]	0.046–0.064
		Biological toilets	4		147 ppm [626.18]	83–289 ppm [353.56–1231.06]	19 ppb [93.37]	16–28 ppb [78.63–137.6]	0.008–0.034 (median, 0.011)
Pfäffli et al. (1979)	Finland, two plants; NR	Hand-lay-up application	29	20–60 min	132.9 ppm [566.12]	109.6 ppm [466.86]	0.04 ppm [196.4]	0.02 ppm [98.2]	0.03
		Spray application	8		130.2 ppm [554.61]	68.8 ppm [293.07]	0.12 ppm [589.2]	0.07 ppm [343.7]	0.09
Rappaport et al. (1996)	USA, one plant; 1987–1988	Laminators	16 (11)[c]	Full-shift; repeated (7×)	130	NR	182	NR	0.14
		Service	6 (2)[c]		27.9	NR	77.6	NR	0.28
		Mould repair	3		117	NR	198	NR	0.17
		Patcher	8 (2)[c]		13.6	NR	96	NR	0.71
		Painter	6 (1)[c]		27.7	NR	158	NR	0.57
		Spray operator	1 (1)[c]		141	NR	74.4	NR	0.05
Nylander-French et al. (1999)	Finland, 30 plants; 1988–1990	All workers	237	84	122	107	183	184	0.15
		Hand lamination of large objects	108		156	105	199	189	0.13
		Hand lamination of small objects	49		150	125	213	191	0.14

Table 1.4 (continued)

Reference	Location; collection date	Occupation description	No. workers and/or samples	Sampling duration	Styrene		Styrene-7,8-oxide		Styrene-7,8-oxide concentration as a percentage of styrene concentration (%)
					Mean exposure (mg/m³)[a]	Range or SD (mg/m³)[a]	Mean exposure (μg/)[b]	Range or SD (μg/m³)[b]	
Nylander-French et al. (1999) (cont.)		Spraying and gel-coating	11		130	53.7	382	276	0.29
		Automated lamination	23		55.8	32.3	95.3	64.2	0.17
		Assembly and mould preparation	28		49.7	40.3	140	139	0.28
		Foremen	18		42.6	92.5	71.2	77.7	0.17
		Boats	137		132	96.4	225	199	0.17
		Small and form parts	34		134	98.7	160	169	0.12
		Sheets/elements and car	39		90.3	127	126	130	0.14
		Containers and tubes	28		106	127	86	126	0.08
Serdar et al. (2006)	USA, 17 plants; 1996–1999	All workers	328	Full-shift; repeated (2–3×)	9.14 ppm (median) [38.93]	< 1–117 [< 4.26–498.39]	17.1 ppb [84.03]	< 1–138 ppb [< 4.91–678.15]	0.19
		Boat building	138		4.41 ppm [18.79]	< 1–68.6 ppm [< 4.26–292.22]	9.47 ppb [46.54]	< 1–51.1 ppb [< 4.91–251.11]	0.21
		Hot tub manufacture	13		6.85 ppm [29.18]	< 1–62.9 ppm [< 4.26–267.94]	16 ppb [78.63]	< 1–48.8 ppb [< 4.91–239.81]	0.23
		Pipe and tank	50		16 ppm [68.16]	1.67–79.0 ppm [7.11–336.52]	17.8 ppb [87.47]	2.02–138 ppb [9.93–678.15]	0.11
		Recreational vehicles	48		45.1 ppm [192.11]	6.74–117 ppm [28.71–498.39]	44 ppb [216.22]	10.4–109 ppb [51.11–535.64]	0.10
		Truck	76		4.22 ppm [17.98]	< 1–46.3 ppm [< 4.26–197.22]	22.2 ppb [109.09]	3.75–64.8 ppb [18.43–318.43]	0.53
		Laminators in boat building	70		14.4 ppm [61.34]	< 1–68.6 ppm [< 4.26–292.22]	13.3 ppb [65.36]	< 1–51.1 ppb [< 4.91–251.11]	0.09
		Laminators of hot tubs	7		9.84 ppm [41.92]	< 1–62.9 ppm [< 4.26–267.94]	36.4 ppb [178.87]	< 1–48.8 ppb [< 4.91–239.81]	0.37

Table 1.4 (continued)

Reference	Location; collection date	Occupation description	No. workers and/or samples	Sampling duration	Styrene		Styrene-7,8-oxide		Styrene-7,8-oxide concentration as a percentage of styrene concentration (%)
					Mean exposure (mg/m³)[a]	Range or SD (mg/m³)[a]	Mean exposure (µg/)[b]	Range or SD (µg/m³)[b]	
Serdar et al. (2006) (cont.)		Laminators of pipes and tanks	42		18.1 ppm [77.1]	2.57–79.0 ppm [10.95–336.52]	21.2 ppb [104.18]	2.56–138 ppb [12.58–678.15]	0.12
		Laminators of recreational vehicles	30		60.5 ppm [257.71]	14.4–117 ppm [61.34–498.39]	59 ppb [289.93]	10.4–109 ppb [51.11–535.64]	0.10
		Laminators of trucks	30		21.6 ppm [92.01]	3.84–46.3 ppm [16.36–197.22]	27.7 ppb [136.12]	15.9–64.8 ppb [78.13–318.43]	0.13
Fustinoni et al. (2008)	Italy; NR	Fibreglass-reinforced plastics workers	8	Full-shift; repeated (3–4×)	18.2 (median)	2.3–93.4	133.5 (median)	39.5–281.5	0.73
		Varnish workers	13		3.4	0.55–16.0	12.2	6.7–32.0	0. 36

h, hour(s); min, minute(s); NR, not reported; ppb, parts per billion; ppm, parts per million; SD, standard deviation.

[a] Concentrations are given in mg/m³ unless indicated otherwise.

[b] Concentrations are given in µg/m³ unless indicated otherwise.

[c] Number of workers with styrene-7,8-oxide measurements.

Table 1.5 Personal styrene inhalation exposure levels in reinforced plastics manufacturing workers

Reference	Location; collection date	Occupation description	No. workers and/or samples	Sampling duration	Mean exposure (mg/m^3)[a]	Exposure range (GSD) (mg/m^3)[a]
Guillemin et al. (1982)	Switzerland, 10 plants; NR	All workers	90	Full shift	47.2 ppm [201.06]	2–199 ppm (SD, 44.1) [201.06–847.68 (187.85)]
		Hand lamination, containers of various sizes	4		50.9 ppm [216.82]	27–64 ppm (SD, 16.3) [115.01–272.62 (69.43)]
		Hand and spray lamination; boats 13 m long, small objects	5		75.8 ppm [322.89]	41.12 ppm (SD, 29.30) [175.16 (124.81)]
		Hand, spray, automatic system lamination, silos and small boats	8		35.1 ppm [149.52]	17–50 ppm (SD, 11.6) [72.42–212.99 (49.41)]
		Hand lamination, inside coating of oil tanks	6		50.0 ppm [212.99]	11–109 ppm (SD, 33.6) [46.86–464.31 (143.13)]
		Hand, spray, and other	13		29.4 ppm [125.24]	3–94 ppm (SD, 33.4) [12.78–400.41 (142.27)]
		Automatic system, pipes and others objects	3		27.0 ppm [115.01]	17–39 ppm (SD, 11.1) [72.42–166.13 (47.28)]
		Hand, spray, and vacuum lamination, basins, walls, baths, etc.	15		52.7 ppm [224.49]	9–101 ppm (SD, 24.1) [224.49–430.23 (102.66)]
		Hand, spray, automatic system, UV-hardening, large pipes	19		11.3 ppm [48.13]	2–21 ppm (SD, 7.2) [8.52–89.45 (30.67)]
		Hand, spray, and vacuum lamination, cabins, walls, etc.	15		101.5 ppm [432.36]	9–199 ppm (SD, 64.6) [38.34–847.68 (275.18)]
		Hand lamination, parts of car bodies	2		23.0 ppm [97.97]	13–33 ppm (SD, 14.1) [55.38–140.57 (60.06)]
Ikeda et al. (1982)	Japan, five plants; NR	Hand lamination over boat shell mould	25	4 h	GM, 119 ppm [506.91]	34–256 ppm (1.6) [144.83–1090.49 (6.82)]
		Installation of ribs; laminators	3		GM, 17 ppm [72.42]	10–28 ppm (1.7) [42.6–119.27 (7.24)]
		Installation of ribs; laminator helpers	5		GM, < 1 ppm [< 4.26]	< 1–2 ppm (5.6) [< 4.26–8.52 (23.85)]
		Installation of division plates; laminators	5		GM, 22 ppm [93.71]	6–44 ppm (2.1) [25.56–187.43 (8.95)]

Table 1.5 (continued)

Reference	Location; collection date	Occupation description	No. workers and/or samples	Sampling duration	Mean exposure (mg/m^3)[a]	Exposure range (GSD) (mg/m^3)[a]
Ikeda et al. (1982) (cont.)		Installation of division plates; laminator helpers	5		GM, < 1 ppm [< 4.26]	ND
		Auxiliary lamination on deck	5		GM, 13 ppm [55.38]	2–43 ppm (4.0) [8.52–183.17 (183.17)]
		Lamination on hold walls	4		GM, 128 ppm [545.24]	104–211 ppm (1.4) [443.01–898.80 (5.96)]
		Equipment	8		GM, 2 ppm [8.52]	1–17 ppm (2.8) [4.26–72.42 (11.93)]
Crandall & Hartle (1985)	USA, seven plants; NR	Hull lamination	168	Full shift	77.7 ppm [330.98]	1.6–183 ppm (SD, 41.5) [6.82–779.53 (176.78)]
		Deck lamination	114		73.4 ppm [312.66]	12.3–160 ppm (SD, 38.1) [52.39–681.55 (162.3)]
		Small parts lamination	70		45.4 ppm [193.39]	9.3–130 ppm (SD, 22.0) [39.62–553.76 (93.71)]
		Gel coating	45		47.5 ppm [202.34]	5–103 ppm (SD, 27.3) [21.3–438.75 (116.29)]
Imbriani et al. (1986)	Italy; NR	Boat manufacturing	121	4 h	109 (median)	11.3–536.0 (2.332)
Sullivan & Sullivan (1986)	Canada, 10 plants; 1981	Boat lamination	59	Full shift	GM, 108 ppm [460.05]	327 ppm max (2.18) [1392.93 (9.29)]
		Non-boat lamination	23		GM, 93 ppm [396.15]	316 ppm max (1.72) [1346.07 (7.33)]
		Chopper gun use	8		GM, 115 ppm [489.87]	316 ppm max (2.35) [1346.07 (10.01)]
		Gel-coat spraying	6		GM, 58 ppm [247.06]	135 ppm max (2.93) [575.06 (12.48)]
		Filament winding	3	5–25 min	GM, 125 ppm [532.46]	181 ppm max (1.41) [771.01 (6.01)]
Triebig et al. (1989)	Germany, four plants; NR	Laminating boats, pipes, or containers	36	Full shift	18 ppm (median) [76.67]	3–251 ppm [12.78–1069.19]
		Plant A	12		136 ppm [579.32]	48–251 ppm [204.47–1069.19]
		Plant B	10		14 ppm [59.64]	10–40 ppm [42.60–170.39]
		Plant C	9		13 ppm [55.38]	3–32 ppm [12.78–136.31]
		Plant D	5		17 ppm [72.42]	14–29 ppm [59.64–123.53]

Table 1.5 (continued)

Reference	Location; collection date	Occupation description	No. workers and/or samples	Sampling duration	Mean exposure (mg/m³)[a]	Exposure range (GSD) (mg/m³)[a]
Jensen et al. (1990)	Denmark, 30 plants; 1955–1988	Fibreglass-reinforced plastics workers (1955–1970)	227	1–60 min	714	10–4700
		Fibreglass-reinforced plastics workers (1971–1980)	1117		274	4–1905
		Fibreglass-reinforced plastics workers (1981–1988)	1184		172	1–4020
		Hand lay-up (1955–1970)	94		542	24–2400
		Hand lay-up (1971–1980)	851		279	7–1905
		Hand lay-up (1981–1988)	84		142	1–961
		Spray-up (1955–1970)	77		1031	50–4700
		Spray-up (1971–1980)	125		288	21–1600
		Spray-up (1981–1988)	60		234	12–4018
Geuskens et al. (1992)	Netherlands, four plants; NR	Filament winding	18	Full shift	[GM, 314]	134–716
		Spraying	62		[GM, 227]	48–602
		Hand laminating	180		[GM, 148]	18–538
Truchon et al. (1992)	Canada, three plants; NR	Chopper gun use	7	Full-shift	564	307–938
		Painting (gel coat)	9		517	280–843
		Laminating (rollers)	18		502	292–865
		Foreman	8		97	18–279
		Cutter	11		75	16–234
		Warehouse work	19		35	9–187
		Finishing	31		34	8–110
		Mould repair	8		28	8–147
Galassi et al. (1993)	Italy, 87 plants; 1978–1990	Hand laminating	1028	NR	227 (GM, 165)	GSD, 2.48
		Spraying laminating	166		134 (GM, 82)	GSD, 3.18
		Rolling	40		163 (GM, 104)	GSD, 3.39
		Semi-automatic process	71		85 (GM, 48)	GSD, 3.03
		Non-process work	159		71 (GM, 38)	GSD, 3.8
Gobba et al. (1993)	Italy, 10 plants; NR	Hand lamination	64	Full shift	GM, 1090.4 µmol/m³ (113.6 mg/m³)	76.8–7396.8 µmol/m³ [8–770.4]
		Hand lamination	211		GM, 843.84 µmol/m³ [87.9]	23–7396.8 µmol/m³ [2.4–770.4]

Table 1.5 (continued)

Reference	Location; collection date	Occupation description	No. workers and/or samples	Sampling duration	Mean exposure (mg/m³)[a]	Exposure range (GSD) (mg/m³)[a]
Ghittori et al. (1997)	Italy; NR	Fibreglass-reinforced plastics workers	22	Full shift	112.7; GM, 98.6	44–228 (1.72)
Lenvik et al. (1999)	Norway, 234 plants; 1972–1996	Different lamination processes (1972–1996)	7011	Variable (min to h)	49.4 ppm (median, 33.0 ppm) [210.43 (140.57)]	
		Different lamination processes (1972–1976)	7011		62 ppm [264.1] (median)	
		Different lamination processes (1992–1996)	7011		7.1 ppm [30.24] (median)	
Somorovská et al. (1999)	Slovakia; NR	All workers	44	Full shift	101.2	SD, 102.4
		Hand laminators	17		199.1	SD, 101.6
		Medium-exposed sprayers	12		55	SD, 22.9
		Maintenance workers	15		27.3	SD, 25.1
Haufroid et al. (2001)	Belgium; NR	Fibreglass-reinforced plastics workers	30	Full shift	18.2 ppm [77.53]	0.9–68.9 ppm [3.83–297.75]
Prieto et al. (2002)	Spain; NR	Boat construction	34	Full shift	70.5	15–157
Teixeira et al. (2007)	Portugal; NR	Fibreglass-reinforced plastics workers	75	Full shift	30.4 ppm [129.5]	0.5–114 (SD, 3.7) ppm [2.13–485.61 (15.76)]
Tranfo et al. (2012)	Italy, two plants; NR	Hand lay-up open-mould process	7	Full shift	41.58	20.34–72.31 (SD, 18.73)
		Compression closed moulding	12		30.33	2.53–104.17 (SD, 26.34)
Bonanni et al. (2015)	Italy, four plants; 2011–2013	Plant A: motorcycle helmets	20	Full shift	43 (median)	5.0–157.5 [25–75 percentile]
		Plant B: fibreglass-reinforced plastic containers and pieces	12		31.5 (median)	4.9–47.4 [25–75 percentile]
		Plant C: compression closed moulding	14		24.1 (median)	7.2–28.3 [25–75 percentile]
		Plant D: fibreglass-reinforced plastic containers and pieces	12		94.0 (median)	27.9–129.3 [25–75 percentile]
		Plant A: moulders	7		139.7 (median)	58.6–178.3 [25–75 percentile]
		Plant D: moulders	9		114.9 (median)	89.3–143.3 [25–75 percentile]
Carbonari et al. (2015)	Italy, two plants; NR	Fibreglass-reinforced plastics workers	30	Full shift	28.75 (median)	142.37 [95th percentile]

GM, geometric mean; GSD, geometric standard deviation; h, hour(s); min, minute(s); ND, not determined; NR, not reported; ppm, parts per million; SD, standard deviation.

[a] Concentrations are given in mg/m³ unless indicated otherwise.

factory where similar processes were used, styrene levels ranged from 17 ppm to 289 ppm [72–1230 mg/m³] and styrene-7,8-oxide levels ranged from less than 0.003 ppm to 0.08 ppm [< 0.015–0.39 mg/m³] (Fjeldstad et al., 1979). Similarly, the measured mean styrene-7,8-oxide concentration was 0.16 mg/m³ for the 19 workers most heavily exposed to styrene in a boat manufacturing company in the USA (Rappaport et al., 1996). Säämänen et al. (1993) calculated a ratio of styrene-7,8-oxide to styrene of 1:1000 from measurements obtained in 32 Finnish plants. This ratio is fairly consistent across all the available studies in which both styrene and styrene-7,8-oxide were measured simultaneously (Table 1.4). Nylander-French et al. (1999) measured a mean 8-hour TWA concentration of 122 mg/m³ (range, 3.2–608 mg/m³; median, 103 mg/m³) for styrene and 183 μg/m³ (range, 0–883 μg/m³; median, 110 μg/m³) for styrene-7,8-oxide in 126 workers employed in 30 different reinforced plastics facilities (70% of which were boat manufacturing) in Finland. Exposure to styrene-7,8-oxide was positively correlated with exposure to styrene in selected job groups, and was greatest for hand lamination followed by assembly and mould preparation (Nylander-French et al., 1999). Serdar et al. (2006) reported on styrene and styrene-7,8-oxide exposure in 328 workers in the reinforced plastics industry in the USA. The median breathing-zone concentrations were 9.14 ppm (< 1–117 ppm) [39 (< 4.26–498 mg/m³)] for styrene and 17.1 ppb (< 1–138 ppb) [84 (< 4.91–678 μg/m³)] for styrene-7,8-oxide. The highest exposure levels were measured in the manufacture of recreational vehicles (median, 45.1 ppm [192.1 mg/m³] for styrene and 44 ppb [216 μg/m³] for styrene-7,8-oxide) in all workers. For laminators in different product groups, styrene and styrene-7,8-oxide exposure levels ranged from 9.84 ppm to 60.5 ppm [42–258 mg/m³] and 13.3 ppb to 59 ppb [65–290 μg/m³], respectively. Styrene exposure levels were typically 535-fold higher than for styrene-7,8-oxide. In general, the exposure levels were lower than those observed in previous studies (Fjeldstad et al., 1979; Pfäffli et al., 1979; Rappaport et al., 1996; Nylander-French et al., 1999).

Several factors influence the concentrations of styrene and styrene-7,8-oxide in workplace air. The manufacture of objects with a large surface area, such as boats, truck parts, baths, and showers, by the open-mould process results in the highest exposure. Tranfo et al. (2012) measured styrene exposure levels in two different production sites, one using an open moulding process and the other a closed compression moulding process. The median values for styrene exposure were 31.1 mg/m³ for open moulding (n = 10) and 24.4 mg/m³ for closed compression moulding (n = 14) (Tranfo et al., 2012). In a detailed survey of 12 plants making fibreglass in Washington State, USA, 40% of 8-hour samples contained styrene at concentrations of more than 100 ppm [430 mg/m³] (Schumacher et al., 1981); of these, chopper-gun operators were exposed to the highest concentrations, followed by laminators and gel-coat applicators. However, boat-building workers were exposed to the highest concentrations of all sectors. For 11 plants, a relationship was seen between level of exposure and the quantity of resin consumed per month per exposed employee (Schumacher et al., 1981). Similar results were reported by Sullivan and Sullivan in their survey of 10 plants in Ontario, Canada, who also noted that although dilution ventilation and often auxiliary fans were used in almost all plants, there was little use of local exhaust ventilation (Sullivan & Sullivan, 1986). Gel coaters are generally exposed to lower concentrations because they work in ventilated booths (Crandall & Hartle, 1985). The presence of flexible-exhaust ventilation hoses was reported to reduce styrene concentrations by a factor of 2 at a boat construction company in Japan (Ikeda et al., 1982). So-called low styrene emission (LSE) resins are thought to reduce styrene exposure (Säämänen et

al., 1991b; Säämänen, 1998). However, Nylander-French et al. (1999) did not observe a significant difference in styrene exposure between standard or LSE resin, and the use of LSE resins resulted in exposure to significantly greater concentrations of styrene-7,8-oxide ($P < 0.001$) than in the use of standard resins.

Jensen et al. (1990) analysed historical styrene air concentration measurement data from the archives of the Danish National Institute of Occupational Health in the selection of industrial cohorts for inclusion in epidemiological studies in Denmark. A mean styrene concentration of 265 mg/m³ was measured in a total of 2528 air samples collected from 256 workplaces during 1955–1988. The concentration of styrene decreased from 714 mg/m³ in the early period (1955–1970) to 172 mg/m³ in the later period (1981–1988). Spraying and unspecified lay-up and production of boats, carriages, and stationary containers were associated with the highest concentrations (Jensen et al., 1990).

Wearing a respirator appropriate for organic vapours reduces, but does not eliminate, styrene exposure (Brooks et al., 1980; Ikeda et al., 1982; Bowman et al., 1990; Truchon et al., 1992; Gobba et al., 2000). Truchon et al. (1992) reported that respirators were worn most often by gel-coat and chopper-gun operators but not by laminators, who consider that they hinder their work. Other investigators reported that single-use dust respirators, which provide no protection against styrene vapours, were often the only type of protection worn (Schumacher et al., 1981; Sullivan & Sullivan, 1986).

Despite early reports that percutaneous absorption of styrene was an important route of exposure, measurement of biological indicators of the exposure of workers who did and did not wear gloves and other forms of protective clothing indicated that absorption through the skin contributes negligibly to overall exposure in the manufacture of fibreglass-reinforced polyester products (Brooks et al., 1980; Bowman et al., 1990; Truchon et al., 1992). Limasset et al. (1999) compared the patterns of urinary excretion of styrene metabolites in four groups of workers who performed the same task at the same time in the same workshop, but wearing different protective equipment (total protection with an insulating suit and mask, respiratory equipment only, skin protection only, or no protection). The urinary excretion level of the group with total protection did not differ significantly from that of the group with respiratory protection only.

Other substances may be found in the workplace air during the production of unsaturated polyester-reinforced plastics, although at levels usually considerably lower than that of styrene. These compounds include: solvents mainly used to clean tools and equipment, such as ketones (e.g. acetone); chlorinated hydrocarbons (e.g. dichloromethane), aliphatic alcohols and esters, aromatic hydrocarbons, and organic peroxides used as initiators (e.g. methyl ethyl ketone peroxide and benzoyl peroxide); hydroquinone and analogues used as inhibitors (e.g. hydroquinone, quinone, and catechol); dusts and fibres originating mainly from filler and reinforcement materials (e.g. glass fibres, silica, and asbestos); foaming agents such as isocyanates; and cobalt salts and amines used as accelerators (Pfäffli et al., 1979; Högstedt et al., 1983; NIOSH, 1983; Coggon et al., 1987; Jensen et al., 1990; Bellander et al., 1994).

(b) *Production of styrene-butadiene rubber*

Workers are also exposed to styrene during the production of styrene-butadiene rubber (SBR) (Table 1.6). In two adjacent SBR production plants in the USA, the measured TWA concentrations of styrene were 0.94 ppm and 1.99 ppm [4.00 mg/m³ and 8.50 mg/m³], with an overall range of 0.03–12.30 ppm [0.13–52.40 mg/m³] (Meinhardt et al., 1982). The mean concentrations in 159 personal air samples collected in various departments at another SBR production plant in the USA were usually below 1 ppm

Table 1.6 Personal styrene inhalation exposure levels in styrene–butadiene rubber manufacturing workers

Reference	Location; collection date	Occupation description	No. workers and/or samples	Sampling duration	Styrene (ppm [mg/m³])		Butadiene (ppm [mg/m³])		Benzene (ppm [mg/m³])	
					Mean exposure	Exposure range (GSD)	Mean exposure	Exposure range (GSD)	Mean exposure	Exposure range (GSD)
Checkoway & Williams (1982)	USA, one plant; 1979	Tank farm	8	Full shift	13.67 [58.23]	0.51–65.16 [2.17–277.56]	20.03 [44.31]	0.14–53.37 [0.31–118.07]	0.03 [0.096]	0–0.08 [0–0.26]
		Reactor and recovery	28		0.93 [3.96]	0–2.32 [0–9.88]	0.77 [1.70]	0–3.11 [0–6.88]	0.02 [0.064]	0–0.11 [0–0.35]
		Solution	12		0.99 [4.22]	0.27–1.68 [1.15–7.16]	0.59 [1.31]	0–2.18 [0–4.82]	0.10 [0.32]	0–0.24 [0–0.77]
		Shipping and receiving	2		0.13 [0.55]	0.08–0.18 [0.34–5.03]	0.08 [0.18]	0.03–0.12 [0.07–0.27]	0 [0]	
		Storeroom	1		0.48 [2.04]		0.08 [0.18]		0.10 [0.32]	
		Factory service	56		1.69 [7.20]	0–17.48 [0–74.46]	0.37 [0.82]	0–1.46 [0–3.23]	0.04 [0.13]	0–0.93 [0–2.97]
		Maintenance	52		0.57 [2.43]	0–2.35 [0–10.01]	0.97 [2.15]	0–20.70 [0–45.79]	0.07 [0.22]	0–0.24 [0–0.77]
Meinhardt et al. (1982)	USA, two plants; 1943–1976	Plant A	55 [area samples]	TWA	0.94 [4.0]	0.03–6.46 (SD, 1.23) [0.13–27.52 (5.24)]	1.24 [2.74] (*n* = 41)	0.11–4.17 (SD, 1.2) [0.24–9.23 (2.65)]	0.10 [0.32] (*n* = 3)	0.08–0.14 (SD, 0.04) [0.26–0.45 (0.11)]
		Plant B	35 [area samples]		1.99 [8.48]	0.05–12.30 (SD, 3.0) [0.21–52.39 (12.78)]	13.5 [29.87] (*n* = 47)	0.34–174.0 (SD, 29.9) [0.75–384.93 (66.15)]		

GSD, geometric standard deviation; SD, standard deviation; TWA, time-weighted average.

[4.3 mg/m³], except for factory service and tank farm workers who were exposed to mean concentrations of 1.69 ppm and 13.7 ppm [7.20 mg/m³ and 58.4 mg/m³], respectively (Checkoway & Williams, 1982). An average styrene concentration of 3.53 ppm [15.0 mg/m³], with a standard deviation of 14.3 ppm [60.9 mg/m³], was measured in 3649 samples collected in five SBR plants in the USA during 1978–1983 (Matanoski et al., 1993). Macaluso et al. (1996) used industrial hygiene data obtained from the same facilities as Matanoski et al. (1993) and, together with a series of air dispersion models, estimated how TWA styrene exposure levels in the SBR industry may have changed since the 1940s (Macaluso et al., 1996). Their calculations suggest that TWA exposures declined from an average of 1.8 ppm [7.7 mg/m³] during the 1940s to 0.1 ppm [0.4 mg/m³] in the 1990s.

No data were available for measurements of styrene-7,8-oxide exposures in the production of SBR and other styrene-based polymers.

(c) *Production of styrene monomer and polymers*

Average exposure to styrene in styrene production and polymerization factories has been reported as rarely exceeding 85 mg/m³ and is usually a result of occasional bursts and leakages of reactors, tubing, and other equipment (Tossavainen, 1978; Pfäffli et al., 1979; Samimi & Falbo, 1982; Pfäffli & Säämänen, 1993; Rappaport et al., 1996; Nylander-French et al., 1999). Surveys conducted in plants in the USA developing or manufacturing styrene-based products during 1962–1976 showed that the average exposure of employees in all jobs was less than 10 ppm [42.6 mg/m³] (NIOSH, 1983). Peak concentrations of up to 50 ppm [213 mg/m³] were measured during the drumming of styrene (Ott et al., 1980). Wolff et al. (1978) measured the highest levels of styrene in polymerization, manufacturing, and purification areas (mean, 8–35 ppm [34–149 mg/m³]), whereas levels of less than 5 ppm [21 mg/m³] were measured in maintenance, laboratory, and packaging operations. In a German styrene production, polymerization, and processing plant, samples acquired during 1975–1976 in various areas of the plant contained up to 6.8 ppm [29.0 mg/m³] of styrene; most values were less than 1 ppm [4.3 mg/m³] (Thiess & Friedheim, 1978). Air samples in a polystyrene manufacturing area contained up to 47 ppm [200 mg/m³] of styrene; most values were less than 1 ppm [4.3 mg/m³] (Thiess & Friedheim, 1978).

In an acrylic ester–styrene copolymer [incorrectly called polystyrene by Samimi & Falbo (1982)] production plant in the USA, breathing-zone concentrations ranged from none detected (< 1 ppb [4.3 μg/m³]) to 19.8 ppm [84.3 mg/m³] (n = 50), with an average of about 0.6 ppm [2.5 mg/m³]; the highest concentrations occurred during styrene unloading operations (Samimi & Falbo, 1982).

Styrene was measured as a thermal degradation product in the air of a Finnish factory during the processing of polystyrene, impact polystyrene, and ABS resins (Hoff et al., 1982); the 6-hour mean concentrations were 0.4, 0.1, and 0.06 mg/m³, respectively. Styrene levels measured in personal 8-hour samples collected during polystyrene and ABS moulding in three different companies in the USA during 1978–1980 varied considerably: 8–67 ppm [34–285 mg/m³] (Burroughs, 1979); 1.4–3.8 mg/m³ (Belanger & Elesh, 1980); and less than 0.01 mg/m³ [below the limit of detection] (Ruhe & Jannerfeldt, 1980).

Styrene-7,8-oxide exposure was reported in only one study conducted in the styrene and polystyrene production industry. Korn et al. (1994) measured styrene and styrene-7,8-oxide concentrations of 78–836 μg/L and 0.9–4.1 μg/L, respectively, in the blood of 13 workers exposed to styrene concentrations of 10–73 ppm in the production of reinforced polyester resins. Styrene inhalation exposure was strongly correlated with styrene blood levels (r = 0.87). A strong linear

relationship was also observed between styrene-7,8-oxide in blood and styrene in ambient air (r = 0.88), between styrene-7,8-oxide in blood and styrene in blood(r = 0.82), and a steady-state level of styrene-7,8-oxide of about 1 μg/L blood at a styrene inhalation exposure of 20 ppm [85.19 mg/m^3] was calculated.

(d) Miscellaneous operations

In a study of the styrene concentrations that firefighters are exposed to, air samples taken during the knockdown phase of a fire contained styrene at a mean concentration of 1.3 ppm [5.5 mg/m^3]; none was detected during the overhaul phase (Jankovic et al., 1991). During working operations at a hazardous waste site in the USA in 1983, a mean styrene concentration of 235 μg/m^3 (maximum, 678 μg/m^3) was measured in air for a group of workers nearest the areas where chemically contaminated materials were handled (Costello, 1983). During the manufacture of polyester paints, lacquers, and putties in Finland, occasional exposure to high concentrations of styrene was recorded, with 5% of measurements above 20 ppm [85 mg/m^3]; use of the same products resulted in exposure of less than 1 ppm [4.3 mg/m^3] (Säämänen et al., 1991a). Application of polyester putty during cable splicing operations for a telephone company in the USA resulted in short-term levels (3–16 minutes) ranging over 2–16 ppm [8.5–68 mg/m^3] in four samples (Kingsley, 1976).

In four 100-minute area air samples taken in 1982 at a college in the USA during a sculpture class in which polyester resins were used, styrene concentrations of 0.8–1.2 ppm [3.4–5.1 mg/m^3] were measured; two personal breathing-zone air samples contained styrene at 2.8 ppm and 3.0 ppm [11.9 mg/m^3 and 12.8 mg/m^3] (Reed, 1983). Taxidermists who used polyester resins during specimen preparation were shown to be exposed for short periods (2–34 minutes) to concentrations of styrene ranging from 5 ppm to less than 50 ppm [21 mg/m^3 to < 213 mg/m^3] (Kronoveter & Boiano, 1984a).

Styrene exposure has also been reported in photocopy operations. Stefaniak et al. (2000) reported that the personal exposure of photocopier operators to styrene varied from 0.1 ppb to 0.7 ppb [0.43–2.98 μg/m^3] in three photocopy centres. Stefaniak et al. (2017) also investigated the emissions of airborne VOCs, including styrene, during desktop fused deposition modelling 3-dimensional and laser printing. The average of styrene emissions varied from 100.5 ± 11.7 μg/m^3 (black colour) to 252.1 ± 128.7 μg/m^3 (natural colour) for fused deposition modelling 3-dimensional printing and from 4.4 μg/m^3 to 8.1 μg/m^3 for 80 pages of laser printing.

No data were available for the measurement of styrene-7,8-oxide exposures in these miscellaneous operations.

1.4.3 Exposure of the general population

(a) Biomonitoring data

The United States Centers for Disease Control and Prevention *National Report on Human Exposure to Environmental Chemicals* provides an ongoing assessment of the exposure of the United States population to styrene and other environmental chemicals by biomonitoring, conducted on random samples of NHANES participants. The median blood styrene concentration declined in the United States population from 0.041 ng/mL in 1988–1994 to less than 0.030 ng/mL in 2001–2008, and the 95th percentile declined from 0.177 ng/mL in 1988–1994 to 0.130 ng/mL in 2007–2008 (Su et al., 2011; CDC, 2018; Table 1.7).

Styrene exposures vary by region and season. Children enrolled in the School Health Initiative: Environment, Learning, Disease study in Minneapolis, Minnesota displayed seasonal variation in blood styrene with a range of median concentrations of 0.07–0.11 ng/mL, exceeding the national median (Sexton et al., 2005). The

Table 1.7 Concentrations of styrene in blood of United States and Italian general populations

Population	Age (years)	Sample size	Blood styrene percentiles (ng/mL)				Survey years	Source
			50th	75th	90th	95th		
GuLF Study, USA (smokers)	< 30 (17.6%), 30–45 (35.1%), > 45 (47.2%), NR (0.1%)	234	NR	NR	NR	1.110	2011–2013	Doherty et al. (2017)
GuLF Study, USA (non-smokers)	< 30 (17.6%), 30–45 (35.1%), > 45 (47.2%), NR (0.1%)	141	NR	NR	NR	0.882	2011–2013	Doherty et al. (2017)
USA	12–60+	2719	< 0.03	0.045	0.096	0.130	2007–2008	NHANES IV (CDC, 2018)
USA	12–60+	2808	< 0.03	0.047	0.099	0.135	2005–2006	NHANES IV (CDC, 2018)
USA	20–59	1245	< 0.03	0.050	0.089	0.120	2003–2004	NHANES IV (CDC, 2018)
USA	20–59	950	< 0.03	0.080	0.130	0.200	2001–2002	NHANES IV (CDC, 2018)
Louisiana, USA[a]	15–60+	297	0.021	0.036	0.049	0.056	2002	Uddin et al. (2014)
Minnesota, USA[b]	6–10	103	0.07	0.18	0.74	0.85	2000 (Feb)	Sexton et al. (2005)
Minnesota, USA[b]	6–10	108	0.09	0.18	0.54	0.68	2000 (May)	Sexton et al. (2005)
Minnesota, USA[b]	6–10	54	0.09	0.10	0.11	0.11	2001(Feb)	Sexton et al. (2005)
Minnesota, USA[b]	6–10	88	0.11	0.12	0.17	0.21	2001 (May)	Sexton et al. (2005)
USA	20–59	2476	0.021	NR	0.110	0.158	1999–2004	NHANES 1999–2004; Su et al. (2011)
USA	20–59	624	0.041	NR	0.129	0.177	1988–1994	NHANES III: 1988–1994; Su et al. (2011)
Italy	20–59	81	0.17	NR	NR	0.51	Prior to 1993	Brugnone et al. (1993)

GuLF, Gulf Long-term Follow-up; NHANES, United States National Health and Nutrition Examination Survey; NR, not reported.
[a] Population of Calcasieu and Lafayette parishes, Louisiana.
[b] Children enrolled in School Health Initiative: Environment, Learning, Disease study, Minneapolis.
Compiled by the Working Group.

95th percentile concentration of styrene in blood of participants ranged from 0.11 ng/mL to 0.85 ng/mL (Sexton et al., 2005), comparable to that of an Italian population of 0.51 ng/mL, assessed before 1993 (Brugnone et al., 1993). Sexton et al. (2005) noted that high concentrations of styrene in blood among children are unlikely to be explained by tobacco use, and suggest an unknown source of styrene.

After the 2010 *Deepwater Horizon* incident, the Gulf Long-term Follow-up study evaluated benzene, toluene, ethylbenzene, xylenes, and styrene concentrations in blood of study participants approximately 3 years after the spill (Doherty et al., 2017; Kwok et al., 2017; Table 1.7). Most participants (95%) lived in a county adjacent to the Gulf coast, and most (89%) had worked on the oil spill. For non-smokers (n = 141), the GM exposure was 0.052 ng/mL [consistent with the range of 50th percentile values reported in Table 1.7 of < 0.03–0.17] and the 95th percentile exposure was 0.882 ng/mL (Doherty et al., 2017). For smokers (n = 234), the GM exposure was 0.098 ng/mL and the 95th percentile exposure was 1.110 ng/mL (Doherty et al., 2017). [According to Chambers et al. (2011), a cigarette smoking habit

at a rate of 1 pack per day is estimated to increase blood styrene concentration by 0.2 ng/mL (assuming 20 cigarettes per pack), which may partially account for the 0.228 ng/mL difference in the 95th percentiles between non-smokers and smokers observed by Doherty et al. (2017).]

Styrene was detected in manually expressed human breast milk from a convenience sample of 12 healthy women from the Baltimore, Maryland metropolitan area who were breast-feeding, at least 30 days postpartum. The average styrene concentration in the breast milk was 0.219 ng/mL, with a range of 0.055–0.710 ng/mL and median of 0.129 ng/mL (Blount et al., 2010).

Pierce & Tozer (1992) reported a styrene concentration of 1.12 ± 1.06 ppm (mean ± standard deviation) in the adipose tissue samples of non-occupationally exposed individuals; samples were obtained from three elective surgery patients and eight postmortem donors (styrene detected in 7 samples).

(b) Total intake estimates

Total intake of styrene is estimated on the basis of two studies; although in general agreement with each other, the studies differ in the estimated contribution of styrene from food and inhaled air.

Tang et al. (2000) estimated that styrene exposure for the general population is in the range of 18.2–55.2 µg per person per day (0.3–0.8 µg/kg body weight (bw)), mainly from inhaled styrene (> 90% of the total intake), as well as form food intake. For smokers, intake of styrene due to 20 cigarettes was estimated to exceed the total daily intake from food and air in a single day.

For the Canadian general population, daily total styrene intake was estimated to range from <0.19 µg/kg bw to > 0.85 µg/kg bw. Intakes from ambient air ranged from 0.004 µg/kg bw per day up to 0.17 µg/kg bw per day, and those from indoor air from 0.07 µg/kg bw per day up to 0.10 µg/kg bw per day. Intake from food was calculated to range from less than 0.11 µg/kg bw per day to more than 0.58 µg/kg bw per day. The estimated intakes from drinking-water and soil were negligible. Potential exposure from cigarette smoke was estimated to be 2.86 µg/kg bw per day for adults. The Canadian study estimated that styrene in food may represent a major exposure source for the general population (Health Canada, 1993; Newhook & Caldwell, 1993).

(c) Food intake estimates

The general-population exposure to styrene from food ingestion has been estimated in several studies, most often using predictive models that consider styrene migration from food-contact materials (FCMs) and dietary consumption factors. Using this methodology, Lickly et al. (1995) estimated dietary styrene exposure at 9 µg per day from the use of polystyrene in FCMs in the USA. A prior study of residents of the United Kingdom in 1983 estimated a styrene intake of 1–4 µg per day (MAFF, 1989). Holmes et al. (2005) employed a probabilistic modelling approach, considering the variation in consumption among individuals in the United Kingdom, and estimated a median daily intake of styrene from food contaminated with FCMs to be 0.039 µg/kg bw per day for adults (2.3 µg per day per 60 kg bw), 0.048 µg/kg bw per day for youths, and 0.035 µg/kg bw per day for seniors.

Studies with dairy products have consistently shown the importance of fat content in the solvation and mass transfer of styrene from polystyrene and styrene–acrylonitrile copolymer food packaging (Ehret-Henry et al., 1994; López et al., 2008; Tawfik & BaAbdullah, 2014). Vitrac & Leblanc (2007) estimated a styrene intake of 1–35 µg per day (5th and 95th percentiles) from consumption of yogurt packaged in polystyrene cups based on migration modelling and yearly purchase data from 5400 households in France. El-Ziney & Tawfik (2016) assessed styrene monomer migration levels in dairy products packaged in polystyrene containers, and found that styrene levels

increased with dairy fat content and storage temperature. Styrene intakes for nine packaged dairy products were calculated as 1.1–10.2 µg per day per person, assuming a modest 100 g intake of dairy product. Consumption of all tested dairy products (900 g) amounted to 50.6 µg per day per person (El-Ziney & Tawfik, 2016).

Philo et al. (1997) measured styrene-7,8-oxide in polystyrene FCMs under the premise that it may form from oxidation of residual styrene monomer. Styrene-7,8-oxide was detected in 11 of 16 food packaging articles at concentrations of up to 2.9 mg/kg. Investigating the potential for food contamination, the same pattern of migration as that for styrene monomer was assumed. The concentration of styrene-7,8-oxide in food was estimated to be 0.002–0.150 µg/kg based on a packaging content of 0.5–3.0 mg/kg.

1.5 Regulations and guidelines

1.5.1 Exposure limits and guidelines

Occupational exposure limits and guidelines for styrene are presented in Table 1.8. Deutsche Forschungsgemeinschaft has classified styrene as Category 5, "Substances that cause cancer in humans or animals or that are considered to be carcinogenic for humans and for which a MAK value [maximum permissible concentration at the workplace] can be derived" (DFG, 2017a). No regulations or guidelines have been established for occupational exposure to styrene-7,8-oxide.

A tolerable daily intake of 7.7 µg/kg bw for styrene has been established by the World Health Organization, with a guideline value of 20 µg/L in drinking-water (WHO, 2004). The EPA has set a maximum contaminant level for styrene in public water systems in the USA at 0.1 mg/L (EPA, 2001).

The FDA has established regulations for the use of polymers and copolymers of styrene in products in contact with food in the USA (FDA, 2017). For styrene and methyl methacrylate copolymers as components of paper and paperboard in contact with fatty foods, the monomer content in the copolymer is limited to 0.5%. For styrene–acrylic copolymers, the level of residual styrene monomer in the polymer should not exceed 0.1% by weight. In the European Union, Commission Regulation No. 10/2011 on plastic materials and articles intended to come into contact with food was published on 14 January 2011 (EU, 2011).

1.5.2 Reference values for biological monitoring of exposure

Biological monitoring reference values for exposure to styrene, based on styrene metabolite levels in urine, are given in Table 1.9. No regulations or guidelines have been established for occupational exposure to styrene-7,8-oxide.

1.6 Exposure assessment of epidemiological studies

The exposure assessments in key epidemiological studies investigating lymphohaematopoietic cancers discussed in Section 2 are summarized and evaluated in this section (see also Table 1.10 and Table 1.11). The studies are divided in the same way here as they are presented in Section 2. Cohort studies have been conducted in three broad categories: the reinforced plastics industry, the synthetic rubber industry, and the styrene monomer and polymers industries. There are also relevant epidemiological case–control studies conducted in samples of the general population. Methods for assessing the two fundamental dimensions of exposure – duration and intensity – will be described, followed by a description of the exposure metrics used in epidemiological models. An evaluation of the quality of the exposure assessments follows these summaries (Section 1.6.3).

Table 1.8 Occupational exposure limits and guidelines for styrene

Country or region	8-hour limit (ppm)	8-hour limit (mg/m³)	Short-term limit (ppm)[a]	Short-term limit (mg/m³)[a]
Australia	50	213	100	426
Austria	20	85	80	340
Belgium	50	216	100	432
Canada, Ontario	35		100	
Canada, Quebec	50	213	100	426
China		50		100
Czechia		200		1000
Denmark	25	105	25[b]	105[b]
Finland	20	86	100	430
France	23.3[c]	100[c]	46.6[c]	200[c]
Germany (DFG)[d]	20	86	40[e]	172[e]
Hungary[f]		50		
Ireland	20	85	40	170
Israel	20	85	40	170
Italy	50	100	215	430
Japan (JSOH)[g]	20	85		
Latvia		10		30
Mexico	50	215	100	425
Netherlands	25	106	50	213
New Zealand	50	215	100	425
Norway	25	106	37.5	160
Philippines	100	425		
Poland[g]	24	50	72	200
Republic of Korea	20	85	40	170
Singapore	50	213	100	426
Spain	20	86	40	172
Sweden	10	43	20	86
Switzerland	20	85	40	170
Thailand	100	426	200	852
Turkey	100	426		
United Kingdom[h]	100	430	250[i]	1065[i]
USA (ACGIH)[j]	20	85	40	170
USA (NIOSH)	50	215	100	425
USA (OSHA)	100	420	200	852

ACGIH, American Conference of Governmental Industrial Hygienists; DFG, Deutsche Forschungsgemeinschaft; JSOH, Japan Society for Occupational Health; NIOSH, National Institute for Occupational Safety and Health; OSHA, Occupational Safety and Health Administration; ppm, parts per million.

[a] 15-minute average value.
[b] Ceiling value.
[c] Indicative statutory limit value.
[d] Category 5, "Substances that cause cancer in humans or animals or that are considered to be carcinogenic for humans and for which a MAK [maximum concentration at the workplace] value can be derived".
[e] 30-minute average value.
[f] Suspected of having carcinogenic potential.
[g] Absorption through the skin may be a significant source of exposure.
[h] Maximum exposure limit, obligation to reduce as much as possible.
[i] 10-minute average value.
[j] A4, not classifiable as a human carcinogen.

Sources: HSE (2011); ILO (2011); Finnish Ministry of Social Affairs and Health (2016); ACGIH (2017); DFG (2017b); IFA (2017); OSHA (2018).

Table 1.9 Reference values for biological monitoring of exposure to styrene

Determinant	Sampling time	Biological exposure index[a]	BAT[b]	Finland[c]
Mandelic acid plus phenylglyoxylic acid in urine	End of shift	400 mg/g creatinine[d]	600 mg/g creatinine	1.2 mmol/L[e]
Styrene in urine	End of shift	40 µg/L	NA	NA

BAT, Biologischer Arbeitsstoff-Toleranzwert (biological tolerance value); NA, not applicable.
[a] American Conference of Governmental Industrial Hygienists (ACGIH, 2017).
[b] Deutsche Forschungsgemeinschaft (Schaller & Triebig, 2012).
[c] Finnish Ministry of Social Affairs and Health (2016).
[d] Non-specific, as it is also observed after exposure to other chemicals such as ethylbenzene.
[e] Next morning sample.
Compiled by the Working Group.

1.6.1 Occupational cohort studies

(a) Reinforced plastics industry

Analyses of the risk of cancer have been conducted for several cohorts of workers exposed to styrene in the reinforced plastics industry: a European industry-wide study (Kogevinas et al., 1994; Loomis et al., 2019); a Danish industry-wide study (Christensen et al., 2018); a United Kingdom industry-wide study (Coggon et al., 2015); a United States industry-wide study (Collins et al., 2013); and a small study of two boatbuilding facilities in Washington State, USA (Bertke et al., 2018). There were significant overlaps between some of these cohorts; these are discussed in more detail in Section 2.2.

(i) European industry-wide study

Approximately 41 000 workers in plants in six European countries were studied by Kogevinas and colleagues (Kogevinas et al., 1994; Loomis et al., 2019). These workers were employed in 287 plants where the main product was reinforced plastic, and more than 50% of these workers were estimated to have been exposed to styrene. Employment records were used to determine duration of exposure and to assign workers to common-exposure job groups. Intensity of exposure to styrene was estimated using 16 500 personal air samples covering the period 1955–1990, although data on exposures before 1970 were only available from Denmark. Production records and detailed job-task information were used to estimate styrene intensities specific to country, time period, and job. Workers were assigned an exposure intensity based on the job held for the longest time. Summary measures of exposure included time since first exposure, average exposure, and cumulative exposure.

(ii) Danish industry-wide study

Workers in 443 small- to medium-sized factories making reinforced plastics during 1964–2007 were identified and followed through national registers, including the cancer registry, to identify incident cases as opposed to mortality (mortality was recorded by all the other cohort studies) (Christensen et al., 2017, 2018). There were 72 292 such workers (1.7 million person-years), making this by far the largest published study. Data were available on dates of hiring and leaving employment, but not job titles or tasks. The investigators took advantage of the variability in working conditions among the large number of small shops with a few employees in each to estimate exposure intensity and probability for each cohort member over their working life, using information about the company's products and work practices, in an analysis conducted for cancers of the lymphoid and haematopoietic system (Christensen et al., 2018).

Styrene exposure intensity was estimated by building a statistical model of the determinants of 1122 personal measurements of styrene airborne

Table 1.10 Comparison of exposures and exposure metrics: key epidemiological (occupational cohort) studies of styrene

Principal reference	Exposure assessment reference	Study location	Exposure period	Mean exposed years	Mean intensity (ppm)	Mean cumulative exposure (ppm-years)	Important co-exposures	Exposure intensity estimation	Exposure metrics	Exposure assessment limitations
Reinforced plastics										
Collins et al. (2013)	Wong (1990)	USA	1948–1977	4.3[a]	28[a]	120[b]	NA	IH surveys	Duration of exposure, cumulative and average exposure, cumulative number of peak exposure periods	27% of cohort missing exposure information for recent years
Coggon et al. (2015)	Coggon et al. (1987)	United Kingdom	1947–1984	3.5[c]	50[d]	175[b]	NA	Expert judgment	High-exposure job for at least 1 year	10% of cohort missing exposure information for recent years
Bertke et al. (2018)	Crandall & Hartle (1985), Ruder et al. (2016)	USA	1959–1978	0.4 (median)[e]	23[f]	9[b]	NA	IH surveys	High versus low exposure, duration	33% of cohort missing exposure data for recent years
Christensen et al. (2018)	Kolstad et al. (2005)	Denmark	1964–2007	3.5[g]	23[h]	81[b]	NA	Predictive model	Cumulative exposure, average intensity, average probability, duration	Exposure information missing for early and recent years
Loomis et al. (2019)	Kogevinas et al. (1994)	6 European countries	1945–1991	2.2[i]	63[i]	158[i]	NA	IH surveys	Cumulative exposure, average intensity, duration	Exposure data missing for early years
Synthetic rubber										
Sathiakumar et al. (2015)	Macaluso et al. (2004), Macaluso et al (1996)	USA and Canada	1943–1991	11[j]	1.5[k]	17[l]	Butadiene	Expert judgment	Cumulative exposure	21% of cohort missing exposure information for recent years
Production of styrene monomer and polymers										
Hodgson & Jones (1985)		United Kingdom	1945–1974	7[m]	50[n]	350[b]	Benzene, butadiene	None	Exposed or unexposed	Exposure defined by employment

Table 1.10 (continued)

Principal reference	Exposure assessment reference	Study location	Exposure period	Mean exposed years	Mean intensity (ppm)	Mean cumulative exposure (ppm-years)	Important co-exposures	Exposure intensity estimation	Exposure metrics	Exposure assessment limitations
Bond et al. (1992)	Ott et al. (1980)	USA	1937–1977	7[o]	5[p]	35[b]	Benzene, butadiene	IH surveys	Intensity or duration categories	No individual quantitative exposure estimates

IH, industrial hygiene; ppm, parts per million; TWA, time-weighted average.

Notes on calculating mean exposure duration, intensity and cumulative exposure:

[a] From Collins et al. (2013), p. 196.

[b] Product of mean duration and mean intensity.

[c] From Coggon et al. (1987), Table 2: weighted average “length of potential exposure to styrene”.

[d] From Coggon et al. (1987), Table 2: weighted average “grade”: high = 70 ppm, moderate = 25, low = 5, based on p. 95, statement that “...high exposure category corresponds to ... 40–100 ppm”.

[e] From Bertke et al. (2018), Table I.

[f] From Ruder et al. (2016), p. 98: mean high exposure in plant A = 42.5 ppm/day; plant B = 71.7 ppm/day; low exposure at plants A and B = 5 ppm/day; weighted mean using population data from table 1.

[g] From Christensen et al. (2018), Table 2: person-years weighted mean duration of employment.

[h] From Kolstad et al. (2005), p. 159: geometric mean of long-term samples 98.5 mg/m^3 = 23 ppm.

[i] From Loomis et al. (2019), Table 1.

[j] From Macaluso et al. (2004), Table III: all employees mean cumulative exposure = 17 ppm-years, divided by mean intensity.

[k] From Macaluso et al. (2004), Fig. 2.

[l] From Macaluso et al. (2004), Table 3: all employees mean cumulative exposure = 17 ppm-years.

[m] From Hodgson & Jones (1985), Table 6, footnote a: mean years exposed for referents = 6.6.

[n] From Hodgson & Jones (1985), p. 350: “It is believed that the exposures of the exposed workers would in general have been well below the hygienic standard of 100 ppm (420 mg/m^3) with isolated excursions to higher levels associated with certain faults and some maintenance work”.

[o] From Ott et al. (1980), Table 11: weighted mean exposure duration for groups exposed to styrene.

[p] From Ott et al. (1980), pp. 448–450: mean TWAs were well below 10 ppm in all processes, generally less than 5 ppm.

Table 1.11 Comparison of exposures and exposure metrics: key epidemiological (case–control) studies of styrene in the general population

Principal reference	Exposure period	Percentage of study controls ever exposed	Mean exposed years	Mean intensity (ppm)	Mean cumulative exposure (ppm-years)	Exposure intensity method	Exposure metrics	Exposure assessment limitations
Gérin et al. (1998)	Pre-1950–1980s	1.78[a]	NA	NA	NA	Expert judgment	Categorical based on estimated duration, concentration, and frequency of exposure	Exposure metrics only semiquantitative
Scélo et al. (2004)	Pre-1950s–2002	1.51	< 1[b]	< 1[c]	< 1[b]	Expert judgment	Cumulative exposure, duration	Exposure metrics only semiquantitative
Miligi et al. (2006)	Pre-1940s–1990s	2.25	NA	NA	NA	Expert judgment	Categorical probability and exposure intensity	Exposure metrics only semiquantitative
Seidler et al. (2007)	1940s–2000	23.8	NA	NA	~5[d]	Expert judgment	Categorical intensity	Exposure metrics only semiquantitative
Cocco et al. (2010)	1940s–2000	2.36	NA	1.5[e]	NA	Expert judgment	Cumulative exposure categories (based on duration, intensity, and probability scores), duration	Exposure metrics only semiquantitative
Karami et al. (2011)	1940s–2000s	1.18	NA	NA	NA	Expert judgment	Probability, cumulative exposure, average exposure, and duration	Exposure metrics only semiquantitative

NA, not available; ppm, parts per million.
[a] Lymphomas as end-point.
[b] Calculated using data from Scélo et al. (2004), Table 5.
[c] Calculated using data from Scélo et al. (2004), Table 2.
[d] Calculated using data from Seidler et al. (2007), Table 2.
[e] Calculated using data from Cocco et al. (2010), Supplementary Table F.
Compiled by the Working Group.

concentrations gathered in 133 companies between 1970 and 2011. The production process, products manufactured, and the decade in which this took place were important predictors, allowing the investigators to produce estimates of styrene concentrations in all participating factories using these determinants (Christensen et al., 2018).

Exposure probability was also estimated for each company and assigned to each worker. A mailed survey in 2013 (11 493 responses, 76% response rate) asked a stratified random sample of workers about the work practices and products in their plant (Christensen et al., 2017, 2018). From these data, a model was built to predict the probability of an individual worker being exposed to styrene. Important determinants were calendar year, production process (lamination or other processes), products (boats, wind turbine wings, or other products), sex, occupation, and company size (Christensen et al., 2018).

Summary measures of exposure included: duration of exposure, mean exposure probability, and (for the analyses of cancers of the lymphoid and haematopoietic system; Christensen et al., 2018) mean exposure intensity and cumulative exposure score (the product of exposure intensity and probability, summed over all exposed years). The cumulative exposure score was analysed in tertiles based on the person-year exposure distribution. Time windows of exposure were also investigated with a priori division of time before onset into three windows: less than 15 years, 15–29 years, and 30 years or more.

(iii) United Kingdom industry-wide study

Eight of the United Kingdom factories in the European-wide cohort study (Kogevinas et al., 1994) were also studied separately with follow-up extended through 2012 (Coggon et al., 2015). The cohort included 7970 workers who began work in reinforced plastics factories between the 1940s and 1970s (start dates varied by plant). Cohort entry ended in the early 1980s (again, varying by plant). Work records were used to identify start and end dates of exposed jobs. No air monitoring data were available, and an expert industrial hygiene survey was not performed. Managers and staff assisted in classifying jobs into four categories of potential styrene exposure: high (hand laminators); moderate (frequently near styrene operations); low (occasionally near styrene operations); and background (all other). Summary measures of exposure were assessed by the highest category of exposure held by each participant: high for 1 year or longer, high for less than 1 year, low and/or moderate, and background.

(iv) United States industry-wide study

Potential exposure to styrene during 1948–1977 of a cohort of approximately 16 000 workers from 30 plants in the USA producing reinforced plastics was first studied by Wong (1990) and then by Collins et al. (2013). Work records were used to estimate duration of styrene exposure and to assign workers to work areas and jobs. An industrial hygiene survey was conducted at each plant, including detailed analyses of plant records and air monitoring data [whether these were personal breathing-zone or area samples was not indicated]. Airborne styrene exposure intensity was estimated for each job and work area over the period 1948–1977. Summary measures of exposure used by Collins et al. in the 2013 updated mortality analyses were duration of exposure, average exposure, cumulative exposure, and the cumulative number of peak exposure periods, defined as 15-minute periods with mean exposure of more than 100 ppm (considered the threshold for acute irritation). A total of 27% of the cohort members worked past 1977, the end of the period for which exposures were estimated, and therefore had truncated individual exposure estimates. [Although this unmeasured exposure was likely to have been low (average exposure intensities in the industry fell substantially during the follow-up period),

this missing information for more than one quarter of the cohort may still have introduced important exposure misclassification for some of the long-term workers.]

(v) Boatbuilding facilities in Washington State, USA

NIOSH studied two boatbuilding facilities in Washington State that used styrene in the construction of fibreglass-reinforced plastic boats (Crandall & Hartle, 1985; Ruder et al., 2016; Bertke et al., 2018). All 1678 employees who worked at one of the two facilities for at least 1 year between 1959 and 1978 were followed until 2011. Work records were used to determine start and end dates of employment, but job title information was incomplete and not used to estimate the exposure to styrene of workers. Industrial hygiene surveys collecting both personal and area styrene air concentrations were conducted in 1978 and used to classify work areas as exposed to either high or low concentrations. In company A, mean styrene exposure in the areas of high concentration was 42.5 ppm (range, 12–85 ppm), and in company B the mean styrene exposure in the areas of high concentration was 71.7 ppm (range, 10–183 ppm). There was considerable heterogeneity of personal breathing-zone exposure concentrations within the areas of high concentration, but all workers were assigned a mean exposure because of the lack of individual job or task information. For the 555 workers still employed in 1978, there was no information on exposure subsequent to that date. Both plants ceased production in about 1990, meaning that all exposure estimates in later years were incomplete for one third of the cohort. The summary measure of exposure was "high" versus "low" styrene exposure.

(b) Synthetic rubber industry

A large and long-running study of workers at six North American factories producing SBR developed quantitative exposure estimates and used these data to quantify risk of mortality from cancer (Macaluso et al., 1996, 2004; Graff et al., 2005; Sathiakumar et al., 2015). Duration of exposure was determined from work histories, which were available for 97% of the cohort. Combinations of department and job title (n = 8281) were grouped by occupational hygienists into 308 homogeneous exposure groups based on similar products, processes, and environmental conditions. The intensities of exposures to styrene, 1,3-butadiene, and benzene were estimated for each homogeneous exposure group in different time periods covering the study period 1943–1991. The process for estimating these styrene and other chemical intensities used technical specifications for jobs, tasks, materials, and work areas supplemented by interviews with managers and workers, quantitative models based on assumptions about ventilation and other determinants of exposure, as well as expert judgment. Job-exposure matrices were then constructed for each combination of work area, job group, and year. When combined with individual work histories, this yielded individual exposure intensity estimates for each worker in each year. The primary summary measure of exposure was cumulative exposure, used in several ways in statistical models including as a continuous variable, in deciles, and with varying time lags. Exposure intensity estimates and work history records ended in 1991; for the 21% of workers still employed after that date, no exposure estimates were available for that period.

(c) Styrene monomer and polymers industry

Studies of workers in petrochemical plants where styrene was manufactured were conducted in the 1990s and earlier. These used very limited exposure classification methods, usually simply comparing the mortality of employees in a factory with that of the general population. Hodgson & Jones (1985) studied mortality in a cohort of styrene production workers in the United Kingdom. Exposed versus non-exposed

workers were distinguished based on the production process area in which they worked, and their mortality was studied separately. No other exposure assessment was conducted to inform the epidemiological analyses. A styrene-based production cohort of chemical employees in the USA was studied first by Ott et al. (1980) and then by Bond et al. (1992). Work records were available to calculate the period of employment; this was used to estimate duration of exposure to a wide range of different chemicals, including styrene. An exposure assessment was conducted using industrial hygiene surveys (with both area and personal monitoring data), historical data on production and plant layout, work records, and other relevant data. The investigators estimated exposure intensities for job and area combinations and then used these to assign exposures to individual cohort members. There were other carcinogenic exposures, including benzene and butadiene. Analyses of mortality were presented for categories of duration of exposure and for groupings of different chemical exposures, including styrene and other potentially important co-exposures. No quantitative exposure–response modelling for styrene and cancer mortality was presented.

1.6.2 General-population studies

From the 1990s onwards, at least six case–control studies of cancer investigating occupational chemical exposures including styrene used variations of a common approach, starting from a self-reported job history collected on each subject (Gérin et al., 1998; Scélo et al., 2004; Miligi et al., 2006; Seidler et al., 2007; Cocco et al., 2010; Karami et al., 2011). Experts then evaluated job information and assigned exposure scores to each job, combining these to yield individual exposure estimates. Exposure assignments based on job, industry, and tasks were often supplemented with detailed follow-up questions when a reported job was likely to have incurred exposure to chemicals of interest. For example, there might be specific questions for painters, pesticide applicators, and machinists.

The highest-quality exposure assessment methods were observed in the studies by Scélo et al. (2004), Seidler et al. (2007), and Cocco et al. (2010), which used similar exposure assessment methods. Face-to-face interviews with cases and controls were used to gather information on all jobs held for at least 1 year. Specialized questionnaires were used for jobs judged a priori to involve exposure to chemicals of interest (18 supplementary questionnaires in the study by Scélo et al., 2004; 14 supplementary questions in each of the studies by Seidler et al., 2007 and Cocco et al., 2010). Experts evaluated each job for exposure to a list of chemicals of interest, including styrene. Because jobs were evaluated and not individuals, the raters were necessarily blind to case or control status. In the study by Scélo et al. (2004) this work was performed by experts in industrial hygiene; these individuals were selected in each of the 15 centres from which cases and controls were obtained, and attended training workshops during which the exposure assessment protocol was developed and validation exercises were regularly conducted. A single industrial physician evaluated the jobs in the study by Seidler et al. (2007), and trained industrial hygienists from each of the participating centres across the six European countries performed the evaluations in the study by Cocco et al. (2010).

The three studies used essentially the same scoring system. Each job was scored for exposure to each chemical on three dimensions: intensity, the relative exposure level (reported as an approximate air concentration) of the chemical for workers engaged in that job; frequency, the typical proportion of work time involving contact with the chemical; and confidence, the experts' degree of certainty that a worker in the job was truly exposed to the chemical. Each of these three dimensions was scored on a semiquantitative three- or four-point scale (scales varied

slightly among the three studies). The duration of exposure was also calculated for each worker in Scélo et al. (2004) and Cocco et al. (2010) from the self-reported start and stop dates of each job held.

Scélo et al. (2004) and Seidler et al. (2007) trained their experts to estimate styrene exposure intensity on a three-point scale using the following air concentration ranges: low intensity, 0.5–5.0 ppm; medium intensity, more than 5 ppm to 50 ppm; and high intensity, more than 50 ppm. Using the midpoints of these three categories (2.5, 25, and 100 ppm), cumulative exposures could be calculated for each participant.

The other three key case–control studies (Gérin et al., 1998; Miligi et al., 2006; Karami et al., 2011) used similar methods to those described above with slight variations; however, they did not attempt to relate their semiquantitative exposure intensity estimates to air concentrations. In other ways, they were quite similar in terms of strengths and weaknesses to the studies of Scélo et al. (2004), Seidler et al. (2007), and Cocco et al. (2010).

1.6.3 Evaluation of exposure assessment quality of epidemiological studies

The exposure assessments of the key epidemiological studies cited in this *Monograph* are evaluated according to five principal considerations: exposure opportunity, carcinogenic co-exposures, completeness of exposure history data, accuracy of exposure intensity measurement, and appropriateness of exposure metrics used in the epidemiological models of risk of cancer.

(a) Exposure opportunity

An ideal epidemiological study for investigating the carcinogenicity of styrene would evaluate a population exposed to a high concentration over a long period of time. The Working Group evaluated each study against this ideal under the heading of exposure opportunity. Ideally, this cohort would not have other carcinogenic exposures, or at least these other exposures would not be correlated with styrene exposures. This first consideration of quality is not strictly about the exposure assessment, but concerns the exposure to the chemical of interest and its distribution across the population and over time. A rough indicator of exposure opportunity can be calculated as the product of the mean intensity of styrene exposure (ppm) over the entire cohort and the mean exposure duration (years). This product is the mean cumulative exposure (ppm-years) for the entire cohort; it was calculated for both cohort and case–control studies (Table 1.10 and Table 1.11), with several assumptions made due to different amounts and quality of information provided in the publications.

Not surprisingly, the occupational cohort studies report much higher mean cumulative exposures than the general-population case–control studies. Within the cohort studies, there was also a fairly wide range depending on the average air concentration and the duration of employment (including turnover) of the exposed cohort. Three of the cohorts – Bond et al. (1992), Sathiakumar et al. (2015), and Bertke et al. (2018) – experienced relatively low mean cumulative exposures, indicating lower exposure opportunity. In the studies by Bond et al. (1992) and Bertke et al. (2018), the average duration of exposure (mean exposed years) was short (~7 years and < 1 year, respectively), and in the study by Sathiakumar et al. (2015) the average intensity of styrene exposure was reportedly low, with mean exposure of about 1.5 ppm-years. The other five studies (Hodgson & Jones, 1985; Collins et al., 2013; Coggon et al., 2015; Christensen et al., 2018; Loomis et al., 2019) reported relatively high exposure opportunity as estimated by the mean cumulative exposure.

Where this type of evaluation was possible with the data available, the key case–control studies had much lower mean cumulative

exposures, as expected. The statistical power of a study to detect an elevated risk of cancer is a function of both the exposure opportunity and the sample size, particularly the number of cases. A study with low mean cumulative exposure may still have sufficient power to detect an effect if the effect is very large. The statistical power of the case–control studies is discussed further in Section 2.3.

(b) Carcinogenic co-exposures

There were important co-exposures to chemicals established as human carcinogens by the International Agency for Research on Cancer (IARC) in three of the cohort studies – Hodgson & Jones (1985) (benzene, butadiene), Bond et al. (1992) (benzene and butadiene), and Sathiakumar et al. (2015) (butadiene) – limiting the value of these studies in evaluating the carcinogenicity of styrene. Sufficient evidence exists for the carcinogenicity of benzene in humans for the end-point of acute non-lymphocytic leukaemia, including acute myeloid leukaemia. Limited evidence exists in humans for cancer of the lung, non-Hodgkin lymphoma, chronic lymphoid leukaemia, multiple myeloma, and chronic myeloid leukaemia, and for acute myeloid leukaemia in children (IARC, 2018). Sufficient evidence also exists for the carcinogenicity of 1,3-butadiene in humans for the end-points of leukaemia and/or lymphoma (IARC, 2012b). The issue of co-exposure with other human carcinogens is considered again in Section 2.3 as a problem of potential confounding. Here we note that in these cohort studies the exposure assessments did not convincingly provide independent estimates of styrene intensity or duration that would confidently allow the effects of styrene to be separated from those of the other potential carcinogens using conventional statistical methods. The absence of co-exposures of other known carcinogens is a strength of the epidemiological studies in the reinforced plastics industry (see Section 2.2.1).

(c) Completeness of exposure histories

All of the cohort studies used work records to document exposure duration, time since first exposure, and other temporal variables. Many also estimated intensities of exposure (see the following section) and then used the work histories to assign time-varying exposure intensities to cohort members. In four of the cohort studies (Collins et al., 2013; Coggon et al., 2015; Sathiakumar et al., 2015; Bertke et al., 2018), the periods for which exposure information were available ended before the end of follow-up, meaning that a fraction of cohort members had truncated exposure histories. These truncations were common in Collins et al. (2013), Sathiakumar et al. (2015), and Bertke et al. (2018) (occurring for 27%, 21%, and 33% of the cohort members, respectively), and could have had a significant impact on the accuracy of the exposure assessments and therefore the fit of the exposure–risk models. Completeness of exposure histories was not an important concern in any of the case–control studies.

(d) Accuracy of exposure intensity measurement

The principal route of styrene exposure for the epidemiological studies discussed here is via inhalation, and the breathing-zone air concentration in ppm or mg/m^3 (1 ppm = 4.26 mg/m^3) is the primary measure of exposure intensity. A wide variety of methods were used to measure exposure intensity in the eight key cohort studies (Table 1.10); the case–control studies of higher quality all used similar semiquantitative methods based on expert judgement (Table 1.11).

Hodgson & Jones (1985) and Coggon et al. (1987, 2015) used only the judgement of investigators, informed by input from company records or plant personnel. Based on limited measurements in some plants in later years, Coggon et al. (1987, 2015) estimated that the high-concentration

exposure category corresponded to an average intensity of 40–100 ppm.

The SBR cohort study by Sathiakumar et al. (2015) used an extensive industrial hygiene analysis to develop time- and job-specific estimates of styrene and butadiene exposure intensities for individual cohort members (Macaluso et al., 2004). The exposure assessors based estimates of exposure intensity on mathematical models predicting exposure levels from production process and environmental conditions, and not on air measurements. However, NIOSH conducted styrene air monitoring in two facilities that employed cohort members during 1976–1977, and the NIOSH data were used as a comparison or partial validation of the exposure estimates of the teams.

The remaining cohort studies – Bond et al. (1992), Collins et al. (2013), Bertke et al. (2018), Christensen et al. (2018), and Loomis et al. (2019) – all used styrene air concentration measurements in some way to estimate the styrene exposure intensities of cohort members, but there were important differences in methods used and the likely accuracy of assignments. In the epidemiological analyses of the styrene-based production cohort in the USA (Bond et al., 1992), the primary epidemiological analyses used simple qualitative measures, such as work area and exposure grouping; however, this did not permit separate quantitative estimates of styrene and other potential carcinogens, notably butadiene. Similarly, the NIOSH cohort of boat builders did not assign individual exposure intensity estimates to each worker; rather, each worker was assigned to either potentially high or low concentrations of exposure (Bertke et al., 2018).

The United States industry-wide reinforced plastics cohort study (Collins et al., 2013) relied on an extensive industrial hygiene survey to estimate styrene concentrations in air for particular areas and/or job titles. These estimates were then used as inputs to individual exposure histories with annual intensity estimates for each cohort member. There were parallel estimates of the number of peak exposures for each work area and/or job title. Peaks were defined as 15-minute intervals when exposure exceeded 100 ppm. Despite these strengths, there are important gaps in the documentation of the methods used by Collins et al. (2013), described in earlier papers by Wong (1990) and Wong et al. (1994), that limit confidence in their exposure estimates. For example, the industrial hygiene survey data are not available, even in summary form.

The European industry-wide study was informed by an extensive industrial hygiene survey and assigned exposure intensities according to job title based on air monitoring data and, in the case of incomplete data, modelling (Loomis et al., 2019).

Finally, the Danish reinforced plastics industry study (Christensen et al., 2018) used a detailed exposure assessment method, as summarized in Section 1.6.1(a)(ii), to estimate both intensity and probability of styrene exposure for each cohort member over their working lifetime.

The better-quality case–control exposure assessments – that is, the studies of Scélo et al. (2004), Seidler et al. (2007), and Cocco et al. (2010) – were semiquantitative, using expert judgements of exposure intensity that were dependent upon explicit air concentration ranges. However, these were probably not as accurate as the exposure intensity estimates in the cohort studies of Bond et al. (1992), Collins et al. (2013), Sathiakumar et al. (2015), Bertke et al. (2018), Christensen et al. (2018), or Loomis et al. (2019). In addition to the lower exposure opportunity, the case–control studies probably had a greater degree of non-differential exposure misclassification than the more informative cohort studies.

In summary, the estimation of styrene exposure intensity appears to have been most reliably estimated by Christensen et al. (2018) and Loomis et al. (2019).

(e) Appropriateness of exposure metrics

Identifying the so-called ideal exposure metric (or summary measure of exposure) for carcinogenicity studies of styrene requires knowledge, or a strong assumption, of the pathophysiological mechanism (Kriebel et al., 2007). The best metric might even differ from one cancer site to another because of internal dose dynamics. Despite this uncertainty about the ideal form, occupational cancer epidemiology has often used cumulative exposure and its components (average exposure intensity and duration) as exposure metrics. There is good empirical evidence that these are often proportional to risk of cancer when applied to the data for known carcinogens such as asbestos and silica (IARC, 2012a). It is important to stress that no one standard summary measure of exposure can be said a priori to be closer to the so-called ideal metric for a particular chemical and target organ; it is therefore reasonable to fit models with several of these metrics.

Another well-recognized consideration in evaluating summary measures of exposure is that cancers typically develop after long periods of latency; it is therefore important to evaluate associations between exposures and their effect by evaluating exposures occurring at different time periods before the onset of disease. The only key epidemiological study to investigate multiple quantitative summary measures of exposure (cumulative exposure, average intensity, average probability, and duration) in time windows of exposure was that of Christensen et al. (2018). Several others (Collins et al., 2013; Sathiakumar et al., 2015; Loomis et al., 2019) used a simpler approach, such as setting a minimum latency that each exposed worker had to achieve before at-risk follow-up time began accruing, or the use of exposure lagging.

1.6.4 Overall summary of exposure assessment in key epidemiological studies

Analyses of the exposure assessments for the key epidemiological studies indicate that two of the cohort studies (Christensen et al., 2018; Loomis et al., 2019) are likely to be more informative than others for two reasons: the substantial exposure experience of cohort members (duration and intensity of exposure to styrene) and the use of high-quality, well-documented exposure assessment methods. Other good-quality exposure data are found in Collins et al. (2013) and Coggon et al. (2015). Several case–control studies (Scélo et al., 2004; Seidler et al., 2007; Cocco et al., 2010) were of relatively high quality in terms of assessment methods; however, the prevalence of styrene exposure and estimated average levels of exposure were considerably lower in these general-population studies than for the cohort studies, limiting their informativeness.

2. Cancer in Humans

2.1 Introduction

Styrene is an important industrial chemical and a major intermediate in the manufacture of both synthetic rubber and certain plastics. Epidemiological studies covering the working populations in all major industries using styrene have been conducted. Industry-based cohorts have evaluated the exposure to styrene of workers in the reinforced plastics, synthetic rubber, and styrene monomer and polymers industries. General-population studies include case–control studies in adults and children.

Early research in occupational cohorts focused mainly on the potential associations between exposure to styrene and leukaemia and lymphomas, whereas more recent analyses have also evaluated several other outcomes,

including cancer of the lung, kidney, breast, and oesophagus. Available studies involved mostly men, examined incidence and/or mortality, and were conducted in North America and western Europe even though styrene is produced and used in many more countries. The Working Group excluded studies without an assessment of styrene exposure. No attempt was made by the Working Group to take account of co-exposures to styrene-7,8-oxide. Such exposures were considered likely, but to be at very low concentrations relative to that of styrene.

In examining the epidemiological evidence, several factors need to be considered (see Section 1.6): the size of the study and, in particular, the number of subjects exposed; the potential for confounding by other chemicals in the work environment and also by lifestyle or social factors; uncertainty in exposure levels and exposure contrasts, exposure misclassification and, related to this, the exposure metric(s) used; outcome misclassification; and, finally, selection bias, which may be of importance in some studies due to the high turnover of the workforce.

The accuracy and precision of the effect estimates are dependent upon the size of the study, the proportion of exposed subjects, and also the frequency of the outcome of interest. There are several large industry-based studies of tens of thousands of workers and, for most outcomes of interest, the size of the study is therefore not a major issue. However, for general-population studies (mainly case–control studies) size may be a serious issue because exposure to styrene is not common; only about 1–2% of the population may have been exposed to styrene and, if exposed, usually not to high concentrations. This is not a problem which is specific to styrene exposure, but is present in many general-population studies regarding exposure to occupational carcinogens.

Confounding has been discussed extensively in some of the industry-based studies concerning co-exposure to other chemicals in the workplace. This is particularly an issue in the synthetic rubber industry, mainly due to the high correlation (correlation coefficient, ~0.7) between exposure to styrene and exposure to 1,3-butadiene, which is an identified human carcinogen (IARC Group 1). Although the main study in synthetic rubber production provides effect estimates adjusted for this co-exposure, this still remains an issue of concern because of the high exposure correlation and the likely misclassification of styrene for 1,3-butadiene, and vice versa. Exposure to benzene and other chemicals also occurs in styrene production and polymerization and, as a consequence, potential confounding by these chemicals may have an impact on effect estimates in those studies.

Studies in the reinforced plastics industry do not appear to be particularly affected by potential confounding due to other co-exposures. Conversely, confounding from lifestyle and socioeconomic factors may be an issue in the reinforced plastics industry because of the high proportion of short-term workers who may have different lifestyle patterns and exposures from other jobs than workers with more stable work histories. However, nearly all major studies in this industry have conducted internal comparisons that, to some extent, take into account confounding by lifestyle factors. Although this selection out of employment may be adjusted for through appropriate statistical analysis, it may still complicate interpretation and affect exposure indices based on duration of exposure.

The concentrations of styrene exposure were up to 1–2 orders of magnitude higher in studies in the reinforced plastics industry compared with those in the synthetic rubber industry, in the styrene monomer and polymers industries, and in the general-population studies. As a consequence, the studies in reinforced plastics are the most informative for hazard identification purposes. Exposure misclassification is probably a more important problem in general-population studies, although several of these have

applied elaborate exposure assessment protocols including job-exposure matrices (JEMs) and expert assessment.

In the cohort studies, workplace styrene exposure was measured extensively in the later periods of work history coverage, and less exposure information was available for earlier periods of employment, potentially affecting the validity of exposure assessment estimates. However, as described in Section 1.6, exposure and work history information was not collected in recent years and was assumed to be constant. However, many of the large cohorts have extensive data on workplace exposures; although exposure misclassification is certainly an issue, it is very unlikely that this has had a considerable effect on the main exposure subgroups evaluated, particularly in the reinforced plastics industry. The exposure metric used varied between studies, for example, peaks of exposure, average exposure levels (intensity), and cumulative exposure (which combines average intensity with duration). Results from some of the larger studies appear to vary depending on the exposure metric used; there is no way through statistical analysis to identify which metric is the most appropriate, since this is essentially an issue related to biological mechanisms.

Outcome misclassification is a less important issue for most cancers but may have affected some analyses, particularly of leukaemia and lymphomas. Studies conducted in earlier periods (corresponding roughly to before the year 2000) examined phenotypes of leukaemia and lymphomas that were shown later to be heterogeneous concerning etiology and prognosis. This may have resulted in an underestimation of exposure–disease associations. In addition, changes in disease definitions for leukaemia and lymphomas over time complicate the comparison of results between studies conducted over different periods. Another concern regarding outcome definition is that most of the cohort studies have used cancer mortality, rather than cancer incidence, data for neoplasms that have relatively good prognosis, such as cancer of the prostate or chronic lymphocytic leukaemia (CLL). In some circumstances, this may result in bias due to different characteristics of incidence versus mortality data, as the mortality data may include a lower proportion of cases with a good prognosis. This factor mostly results in a loss of precision, because not all subjects with a diagnosis of disease will be identified in mortality studies. Poorer cancer prognosis may also be related to reduced access to health care and lower socioeconomic status, so that deaths in mortality studies may overrepresent workers with lower socioeconomic status compared with cancer cases in incidence studies.

Finally, selection bias in the context of industry-based studies has usually been discussed in relation to the healthy worker effect. This is undoubtedly an issue in the styrene cohorts, and would probably tend to underestimate exposure–disease associations when mortality is compared with that of the general population. Many of the larger cohorts have conducted internal analyses that would minimize the potential problem of the healthy worker effect, a type of confounding, and have also incorporated time-related variables in the analysis. As mentioned earlier in this introduction, the relatively high proportion of short-term workers in the reinforced plastics industry is of particular concern.

Two reviews of the epidemiology of styrene exposure and cancer have been published in the last decade (Boffetta et al., 2009; Collins & Delzell, 2018). The Working Group noted that several human studies considered in this *Monograph* were published after the more recent of the two reviews (Bertke et al., 2018; Christensen et al., 2018; Loomis et al., 2019; Nissen et al., 2018).

Overall, the available epidemiological studies have many strengths and, notwithstanding the potential limitations present in industrial cohort and population-based studies, provide a solid base for the evaluation of the association

between exposure to styrene and risk of cancer in human populations. A careful evaluation of the strengths and limitations of the different study designs and the industries examined is included to summarize the evidence in an informative way.

2.2 Cohort studies

2.2.1 Occupational cohort studies

(a) Reinforced plastics industry

See Table 2.1.

In the reinforced plastics industry, boats, tanks, containers, car parts, and other goods are produced from unsaturated polyester resin by hand and spray lamination in open moulds, by vacuum moulding, or by other closed or semi-closed processes (IARC, 2002). The *IARC Monographs* Volume 82 (IARC, 2002) included results on cancer incidence or mortality from five reinforced plastics industry cohorts from Europe (Kogevinas et al., 1993, 1994), Denmark (Kolstad et al., 1993, 1994, 1995), the United Kingdom (Coggon et al., 1987), the USA (Wong, 1990; Wong et al., 1994), and Washington State (Okun et al., 1985). Since then, the Danish (Christensen et al., 2017, 2018; Nissen et al., 2018), United Kingdom (Coggon et al., 2015), United States (Collins et al., 2013), and Washington State (Ruder et al., 2016; Ruder & Bertke, 2017; Bertke et al., 2018) cohorts have all been updated with extended follow-up and the European cohort reanalysed (Loomis et al., 2019). No additional reinforced plastics industry cohorts are included in the current *Monograph*. A succinct summary of the evaluated cohorts is provided in Supplemental Table S1.

Styrene is the dominant exposure; average workplace air concentrations of 100–200 ppm were measured in the 1960s and 1970s, and have significantly declined since then. According to the information provided in the publications, workers may also have been exposed to fibreglass and acetone, and, in some jobs, to glycols, anhydrides, methyl ethyl ketone peroxide, benzoyl peroxide, paints, or wood dust, but encountered no or minimal exposure to benzene or 1,3-butadiene.

Collins et al. (2013) reported cancer mortality for 1948–2008 among 15 826 workers employed at 30 United States reinforced plastics plants during 1948–1977. Wong (1990) and Wong et al. (1994) had previously followed this population from 1948 to 1977 and from 1948 to 1989, respectively. The study population comprised workers who had worked for at least 6 months in an area with potential exposure to styrene.

Styrene exposure estimates were constructed from production characteristics obtained from all companies about 1980 and routine exposure monitoring. A total of 43% of the study population had been directly exposed to styrene (Wong, 1990). The average styrene exposure level was 35 ppm during the 1960s and 25 ppm in 1977 (Collins et al., 2013).

Standard SMR analyses and internal Cox regression models analysed hazard ratios (HRs) by cumulative styrene exposure. Wong (1990) also estimated the association between exposure to styrene and mortality from cancer of the respiratory system among 40 cases and 102 controls nested within the study population; 83% of 63 controls reported ever smoking (Wong, 1990).

For all workers, increased standardized mortality ratios for all cancers (SMR, 1.12; 95% confidence interval (CI), 1.05–1.18) and cancer of the lung (SMR, 1.34; 95% CI, 1.23–1.46) were observed. Standardized mortality ratios greater than 1.10 were observed for cancer of the buccal cavity and pharynx (SMR, 1.16; 95% CI, 0.78–1.66), kidney (SMR, 1.18; 95% CI, 0.83–1.62), and urinary bladder (SMR, 1.25; 95% CI, 0.87–1.74), chronic myeloid leukaemia (CML) (SMR, 1.17; 95% CI, 0.43–2.55), all other myeloid leukaemia (SMR, 1.50; 95% CI, 0.18–5.41), all other cancers of the lymphopoietic tissue

Table 2.1 Occupational cohort studies of exposure to styrene in the reinforced plastics industry

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Collins et al. (2013) USA 1948–2008 Cohort	15 826 (11 958 men and 3868 women) at 30 United States plants; previous update of this study followed vital status to 31 December 1989 and reduced the number of study participants to 15 826 workers by eliminating 30 duplicate records and removing 52 workers exposed to styrene for < 6 mo Exposure assessment method: expert judgement; employment records available at each plant combined with industrial hygiene assessment of styrene levels about 1980	Lymphatic and haematopoietic (all)	Cumulative exposure to styrene (ppm-mo)			Sex, age, year of hire	Strengths: long follow-up and high number of cancer cases; exposure to styrene at high concentrations; limited competing risk factors in the reinforced plastics industry Limitations: limited information on the quantitative exposure assessment
			0.0–149.9	26	0.85 (0.56–1.25)		
			150.0–399.9	23	0.80 (0.51–1.21)		
			400.0–1199.9	29	0.90 (0.60–1.29)		
			≥ 1200	28	0.80 (0.53–1.16)		
			Trend test *P* value, 0.819				
		HL	Cumulative exposure to styrene (ppm-mo)				
			0.0–149.9	2	1.07 (0.13–3.88)		
			150.0–399.9	1	0.60 (0.02–3.33)		
			400.0–1199.9	1	0.59 (0.02–3.27)		
			≥ 1200	1	0.65 (0.02–3.60)		
			Trend test *P* value, 0.827				
		NHL	Cumulative exposure to styrene (ppm-mo)				
			0.0–149.9	13	1.08 (0.58–1.85)		
			150.0–399.9	2	0.17 (0.02–0.64)		
			400.0–1199.9	12	0.94 (0.49–1.64)		
			≥ 1200	9	0.65 (0.30–1.23)		
			Trend test *P* value, 0.766				
		Leukaemia	Cumulative exposure to styrene (ppm-mo)				
			0.0–149.9	7	0.61 (0.25–1.26)		
			150.0–399.9	14	1.30 (0.71–2.18)		
			400.0–1199.9	8	0.66 (0.28–1.30)		
			≥ 1200	11	0.83 (0.42–1.49)		
			Trend test *P* value, 0.908				
		Leukaemia (lymphoid)	Cumulative exposure to styrene (ppm-mo)				
			0.0–149.9	1	0.35 (0.09–1.97)		
			150.0–399.9	3	1.14 (0.23–3.32)		
			400.0–1199.9	1	0.33 (0.08–1.83)		
			≥ 1200	3	0.87 (0.18–2.54)		
			Trend test *P* value, 0.681				

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Collins et al. (2013) (cont.)		Leukaemia (myeloid)	Cumulative exposure to styrene (ppm-mo)				
			0.0–149.9	5	0.90 (0.29–2.10)		
			150.0–399.9	5	0.96 (0.31–2.23)		
			400.0–1199.9	4	0.69 (0.19–1.77)		
			≥ 1200	8	1.27 (0.55–2.50)		
			Trend test *P* value, 0.432				
		All other lymphopoietic tissue (including multiple myeloma)	Cumulative exposure to styrene (ppm-mo)				
			0.0–149.9	4	0.79 (0.22–2.02)		
			150.0–399.9	6	1.24 (0.46–2.70)		
			400.0–1199.9	8	1.43 (0.62–2.81)		
			≥ 1200	7	1.11 (0.45–2.29)		
			Trend test *P* value, 0.912				
		Pancreas	Cumulative exposure to styrene (ppm-mo)				
			0.0–149.9	14	0.90 (0.49–1.51)		
			150.0–399.9	17	1.15 (0.67–1.84)		
			400.0–1199.9	9	0.53 (0.24–1.01)		
			≥ 1200	23	1.24 (0.78–1.86)		
			Trend test *P* value, 0.274				
		Lung	Cumulative exposure to styrene (ppm-mo)				
			0.0–149.9	157	1.60 (1.36–1.87)		
			150.0–399.9	131	1.41 (1.18–1.67)		
			400.0–1199.9	138	1.31 (1.10–1.55)		
			≥ 1200	130	1.10 (0.92–1.31)		
			Trend test *P* value, 0.003				
		Kidney	Cumulative exposure to styrene (ppm-mo)				
			0.0–149.9	6	0.76 (0.28–1.66)		
			150.0–399.9	8	1.09 (0.47–2.15)		
			400.0–1199.9	8	0.98 (0.42–1.94)		
			≥ 1200	16	1.79 (1.02–2.91)		
			Trend test *P* value, 0.045				

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Collins et al. (2013) (cont.)		Urinary bladder	Cumulative exposure to styrene (ppm-mo)				
			0.0–149.9	7	1.09 (0.44–2.25)		
			150.0–399.9	11	1.82 (0.91–3.26)		
			400.0–1199.9	11	1.53 (0.77–2.74)		
			≥ 1200	6	0.72 (0.26–1.57)		
			Trend test *P* value, 0.137				
		Lymphatic and haematopoietic (all combined)	No. days at peak exposure				
			0	57	0.79 (0.59–1.02)		
			1–179	30	0.93 (0.63–1.33)		
			720–1799	9	0.81 (0.37–1.54)		
			≥ 1800	10	0.97 (0.47–1.78)		
			Trend test *P* value, 0.601				
		HL	No. days at peak exposure				
			0	4	1.06 (0.29–2.71)		
			1–179	1	0.51 (0.01–2.81)		
			720–1799	0	0 (0–0)		
			≥ 1800	0	0 (0–0)		
			Trend test *P* value, 0.157				
		NHL	No. days at peak exposure				
			0	20	0.70 (0.43–1.08)		
			1–179	9	0.70 (0.32–1.33)		
			720–1799	5	1.12 (0.37–2.63)		
			≥ 1800	2	0.49 (0.06–1.76)		
			Trend test *P* value, 0.868				
		Leukaemia	No. days at peak exposure				
			0	21	0.76 (0.47–1.17)		
			1–179	12	0.99 (0.51–1.74)		
			720–1799	3	0.72 (0.15–2.09)		
			≥ 1800	4	1.03 (0.28–2.63)		
			Trend test *P* value, 0.691				

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Collins et al. (2013) (cont.)		Leukaemia (lymphoid)	No. days at peak exposure				
			0	4	0.57 (0.16–1.47)		
			1–179	2	0.68 (0.08–2.46)		
			720–1799	0	0 (0–3.52)		
			≥ 1800	2	1.95 (0.24–7.03)		
			Trend test *P* value, 0.177				
		Leukaemia (myeloid)	No. days at peak exposure				
			0	11	0.86 (0.43–1.53)		
			1–179	7	1.15 (0.46–2.38)		
			720–1799	2	0.96 (0.12–3.48)		
			≥ 1800	2	1.07 (0.13–3.86)		
			Trend test *P* value, 0.835				
		All other lymphopoietic tissue (including MM)	No. days at peak exposure				
			0	4	0.79 (0.22–2.02)		
			1–179	6	1.24 (0.46–2.70)		
			720–1799	8	1.43 (0.62–2.81)		
			≥ 1800	7	1.11 (0.45–2.29)		
			Trend test *P* value, 0.835				
		Pancreas	No. days at peak exposure				
			0	32	0.84 (0.58–1.19)		
			1–179	20	1.21 (0.74–1.87)		
			720–1799	3	0.52 (0.11–1.51)		
			≥ 1800	8	1.45 (0.63–2.85)		
			Trend test *P* value, 0.337				
		Lung	No. days at peak exposure				
			0	314	1.32 (1.18–1.47)		
			1–179	154	1.50 (1.28–1.76)		
			720–1799	49	1.34 (1.00–1.77)		
			≥ 1800	39	1.06 (0.76–1.46)		
			Trend test *P* value, 0.201				

Table 2.1 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Collins et al. (2013) (cont.)		Kidney	No. days at peak exposure				
			0	16	0.88 (0.50–1.42)		
			1–179	9	1.08 (0.49–2.04)		
			720–1799	8	2.73 (1.17–5.38)		
			≥ 1800	5	1.82 (0.59–4.24)		
			Trend test *P* value, 0.054				
		Urinary bladder	No. days at peak exposure				
			0	22	1.31 (0.82–1.98)		
			1–179	7	1.12 (0.45–2.31)		
			720–1799	0	0 (0–1.54)		
			≥ 1800	6	2.35 (0.87–5.13)		
			Trend test *P* value, 0.337				
		Lymphatic and haematopoietic (all)	Cumulative exposure to styrene (ppm-mo)			Sex, year of hire, year of birth, age	
			Continuous	NR	0.994 (0.983–1.006)		
		HL	Cumulative exposure to styrene (ppm-mo)				
			Continuous	NR	0.957 (0.843–1.086)		
		NHL	Cumulative exposure to styrene (ppm-mo)				
			Continuous	NR	0.994 (0.976–1.013)		
		Leukaemia	Cumulative exposure to styrene (ppm-mo)				
			Continuous	NR	0.996 (0.979–1.014)		
		Leukaemia (lymphoid)	All workers: continuous	NR	1.010 (0.994–1.027)		
		Leukaemia (myeloid)	All workers: continuous	NR	0.991 (0.962–1.019)		
		Other: all other leukaemia	All workers: continuous	NR	0.900 (0.767–1.056)		
		Lymphatic and haematopoietic: all other including MM	Cumulative exposure to styrene (ppm-mo)				
			Continuous	NR	0.994 (0.972–1.017)		

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Collins et al. (2013) (cont.)		Pancreas	Cumulative exposure to styrene (ppm-mo)				
			Continuous	NR	1.008 (1.002–1.015)		
		Lung	Cumulative exposure to styrene (ppm-mo)				
			Continuous	NR	0.997 (0.993–1.002)		
		Kidney	Cumulative exposure to styrene (ppm-mo)				
			Continuous	NR	1.009 (1.000–1.017)		
		Urinary bladder	Cumulative exposure to styrene (ppm-mo)				
			Continuous	NR	1.004 (0.992–1.016)		
		All cancers combined	All workers: reinforced plastic	1431	1.12 (1.05–1.18)	Sex, age, calendar period	
		Lymphatic and haematopoietic (all combined)	All workers: reinforced plastic	106	0.84 (0.69–1.02)		
		Leukaemia (ALL)	All workers: reinforced plastic	2	0.75 (0.09–2.71)		
		NHL (CLL)	All workers: reinforced plastic	6	0.71 (0.26–1.55)		
		Leukaemia	All workers: reinforced plastic	40	0.84 (0.60–1.14)		
		Leukaemia (lymphoid)	All workers: reinforced plastic	8	0.67 (0.29–1.32)		
		Leukaemia (myeloid)	All workers: reinforced plastic	22	0.96 (0.60–1.46)		
		Leukaemia (AML)	All workers: reinforced plastic	14	0.85 (0.47–1.43)		

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Collins et al. (2013) (cont.)		NHL	All workers: reinforced plastic	36	0.72 (0.50–1.00)		
		HL	All workers: reinforced plastic	5	0.74 (0.24–1.72)		
		Leukaemia (CML)	All workers: reinforced plastic	6	1.17 (0.43–2.55)		
		MM: all and other lymphopoietic tissue (except HL, NHL, leukaemia)	All workers: reinforced plastic	25	1.15 (0.74–1.69)		
		All other leukaemia	All workers: reinforced plastic	9	0.79 (0.36–1.49)		
		All other myeloid leukaemia	All workers: reinforced plastic	2	1.50 (0.18–5.41)		
		Pancreas	All workers: reinforced plastic	63	0.96 (0.73–1.22)		
		Lung	All workers: reinforced plastic	556	1.34 (1.23–1.46)		
		Breast	All workers: reinforced plastic	55	0.88 (0.66–1.15)		
		Prostate	All workers: reinforced plastic	68	1.03 (0.8–1.31)		

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Collins et al. (2013) (cont.)		Kidney	All workers: reinforced plastic	38	1.18 (0.83–1.62)		
		Urinary bladder	All workers: reinforced plastic	35	1.25 (0.87–1.74)		
		Buccal cavity and larynx	All workers: reinforced plastic	29	1.16 (0.78–1.66)		
Kogevinas et al. (1994) Denmark (1970–1990); Finland (1958–1989); Italy (Liguria, 1969–1991; Emilia Romagna, 1956–1989); Norway (1956–1991); Sweden (1955–1987); United Kingdom (1, 1945–1990; 2, 1961–1988) Cohort	40 688 (34 560 men and 6128 women); ~60% of the total population had been employed in the industry for < 2 yr; proportion of short-term workers varied among countries from 9% (Finland) to 81% (Denmark); ~50% of the cohort was first employed at age < 25 yr, similar proportion was first employed after 1975 Exposure assessment method: records; employment histories combined with an exposure matrix constructed from 16 500 personal workroom air styrene measurements and 18 500 urinary styrene metabolites	Lymphatic and haematopoietic neoplasms: ICD-8 (code 200–208)	Cumulative exposure to styrene (ppm-yr)			Age, sex, country, calendar period, time since first exposure	Strengths: large international study population characterized by quantitative measures of styrene exposure Limitations: short duration of follow-up (average 13 yr); no smoking information
			< 75	20	1		
			75–199	8	0.98 (0.43–2.26)		
			200–499	10	1.24 (0.57–2.72)		
			≥ 500	9	0.84 (0.35–2.02)		
			Trend test *P* value, 0.65				
		Leukaemia: ICD-8 (code 204–208)	Cumulative exposure to styrene (ppm-yr)				
			< 75	11	1		
			75–199	2	0.46 (0.10–2.09)		
			200–499	3	0.69 (0.19–2.53)		
			≥ 500	5	0.86 (0.26–2.83)		
			Trend test *P* value, > 0.52				
		Malignant lymphomas: ICD-8 (code 200–202); lymphomas and HL	Cumulative exposure to styrene (ppm-yr)				
			< 75	5	1		
			75–199	5	2.63 (0.74–9.32)		
			200–499	5	2.99 (0.82–10.91)		
			≥ 500	3	1.64 (0.34–7.82)		
			Trend test *P* value, 0.52				

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Kogevinas et al. (1994) (cont.)		Oesophagus	Cumulative exposure to styrene (ppm-yr)				
			< 75	5	1		
			75–199	2	1.01 (0.20–5.23)		
			200–499	3	1.67 (0.39–7.18)		
			≥ 500	4	1.76 (0.42–7.30)		
			Trend test *P* value, 0.31				
		Pancreas	Cumulative exposure to styrene (ppm-yr)				
			< 75	9	1		
			75–199	5	1.44 (0.48–4.34)		
			200–499	6	1.90 (0.65–5.53)		
			≥ 500	10	2.56 (0.90–7.31)		
			Trend test *P* value, 0.068				
		Lung	Cumulative exposure to styrene (ppm-yr)				
			< 75	73	1		
			75–199	25	0.75 (0.47–1.19)		
			200–499	26	0.74 (0.47–1.16)		
			≥ 500	37	0.90 (0.58–1.38)		
			Trend test *P* value, < 0.43				
		Kidney	Cumulative exposure to styrene (ppm-yr)				
			< 75	2	1		
			75–199	3	4.40 (0.71–27.15)		
			200–499	2	3.30 (0.42–25.60)		
			≥ 500	3	6.04 (0.74–49.45)		
			Trend test *P* value, 0.12				
		All cancers combined	All workers: reinforced plastic	686	0.87 (0.81–0.94)		
		All lympho-haematopoietic	All workers: reinforced plastic	60	0.93 (0.71–1.20)		

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Kogevinas et al. (1994) (cont.)		Leukaemia (myeloid)	All workers: reinforced plastic	16	1.10 (0.63–1.79)		
		Leukaemia	All workers: reinforced plastic	28	1.04 (0.69–1.50)		
		NHL	All workers: reinforced plastic	15	0.77 (0.43–1.28)		
		HL	All workers: reinforced plastic	7	0.90 (0.36–1.84)		
		MM	All workers: reinforced plastic	10	0.99 (0.48–1.83)		
		Buccal cavity and pharynx	All workers: reinforced plastic	5	0.33 (0.11–0.77)		
		Oesophagus	All workers: reinforced plastic	17	0.82 (0.47–1.31)		
		Rectum	All workers: reinforced plastic	21	0.62 (0.38–0.95)		
		Pancreas	All workers: reinforced plastic	37	1.00 (0.71–1.38)		
		Larynx	All workers: reinforced plastic	10	1.11 (0.53–2.05)		
		Lung	All workers: reinforced plastic	235	0.99 (0.87–1.13)		

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Kogevinas et al. (1994) (cont.)		Lung	Laminators: reinforced plastic	60	1.06 (0.81–1.36)		
		Breast	All workers: reinforced plastic	13	0.52 (0.28–0.89)		
		Prostate	All workers: reinforced plastic	41	1.02 (0.74–1.39)		
		Urinary bladder	All workers: reinforced plastic	25	0.95 (0.61–1.40)		
		Brain	All workers: reinforced plastic	18	0.62 (0.37–0.98)		
Coggon et al. (2015) England 1946–1984, followed up until 2012 Cohort	7970 (6650 men and 1320 women); all employed during specified periods at eight reinforced plastics companies in England using styrene Exposure assessment method: records; from employment histories, participants were classified into four levels of potential for styrene exposure; exposure to styrene at 40–100 ppm was estimated for the high-exposure category between 1975 and 1984	Leukaemia	Background	10	1.15 (0.55–2.12)	Age, sex, calendar period	Strengths: expected exposure to styrene at high concentrations; limited exposure to other suspected occupational carcinogens; long follow-up Limitations: no styrene exposure information since 1984; no smoking information; 11.5% lost from follow-up
			Low/moderate	6	0.86 (0.31–1.87)		
			High for < 1 yr	4	0.72 (0.20–1.84)		
			High for ≥ 1 yr	3	0.76 (0.16–2.22)		
		NHL (including CLL)	Background	10	1.20 (0.58–2.21)		
			Low/moderate	3	0.44 (0.09–1.28)		
			High for < 1 yr	6	1.04 (0.38–2.26)		
			High for ≥ 1 yr	5	1.22 (0.40–2.85)		
		Lymphatic and haematopoietic (all combined)	Background	43	1	Age, sex, calendar period, factory	
			Low/moderate	28	0.73 (0.40–1.33)		
			High for < 1 yr	31	0.81 (0.47–1.41)		
			High for ≥ 1 yr	20	0.76 (0.40–1.44)		

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Coggon et al. (2015) (cont.)		NHL (including CLL)	Background	26	1		
			Low/moderate	14	0.53 (0.24–1.15)		
			High for < 1 yr	18	0.61 (0.30–1.25)		
			High for ≥ 1 yr	11	0.54 (0.23–1.27)		
		HL	Background	3	1		
			Low/moderate	2	1.05 (0.17–13.56)		
			High for < 1 yr	1	0.39 (0.03–5.09)		
			High for ≥ 1 yr	1	0.74 (0.06–9.94)		
		MM	Background	6	1		
			Low/moderate	7	2.15 (0.51–9.12)		
			High for < 1 yr	6	2.66 (0.67–10.64)		
			High for ≥ 1 yr	5	2.66 (0.62–11.35)		
		Leukaemia: all other	Background	8	1		
			Low/moderate	5	0.60 (0.13–2.79)		
			High for < 1 yr	6	0.84 (0.23–3.02)		
			High for ≥ 1 yr	3	0.62 (0.13–3.03)		
		Oesophagus	Background	12	0.86 (0.45–1.51)	Age, sex, calendar period	
			Low/moderate	13	1.02 (0.54–1.74)		
			High for < 1 yr	12	1.18 (0.61–2.06)		
			High for ≥ 1 yr	10	1.41 (0.68–2.60)		
		Pancreas	Background	21	1.41 (0.87–2.15)		
			Low/moderate	11	0.95 (0.47–1.70)		
			High for < 1 yr	10	1.11 (0.53–2.03)		
			High for ≥ 1 yr	6	0.89 (0.33–1.95)		
		Lung	Background	100	1.07 (0.87–1.30)		
			Low/moderate	98	1.20 (0.98–1.47)		
			High for < 1 yr	68	1.22 (0.95–1.55)		
			High for ≥ 1 yr	60	1.44 (1.10–1.86)		

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Coggon et al. (2015) (cont.)		Prostate	Background	19	0.85 (0.51–1.33)		
			Low/moderate	20	0.79 (0.48–1.22)		
			High for < 1 yr	15	1.03 (0.58–1.70)		
			High for ≥ 1 yr	9	0.86 (0.39–1.63)		
		Kidney	Background	10	1.49 (0.72–2.75)		
			Low/moderate	7	1.18 (0.47–2.43)		
			High for < 1 yr	7	1.43 (0.58–2.95)		
			High for ≥ 1 yr	4	1.17 (0.32–30.0)		
		Urinary bladder	Background	10	0.90 (0.43–1.65)		
			Low/moderate	16	1.58 (0.90–2.57)		
			High for < 1 yr	8	1.23 (0.53–2.43)		
			High for ≥ 1 yr	3	0.62 (0.13–1.81)		
		Leukaemia	All workers: reinforced plastic	23	0.91 (0.58–1.36)	Age, sex, calendar period	
		NHL	All workers: reinforced plastic	24	0.95 (0.61–1.42)		
		HL	All workers: reinforced plastic	2	0.49 (0.06–1.77)		
		MM	All workers: reinforced plastic	13	0.94 (0.50–1.60)		
		Lymphatic and haematopoietic	All workers: reinforced plastic	62	0.89 (0.68–1.14)		
		Pharynx	All workers: reinforced plastic	9	1.34 (0.61–2.54)		

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Coggon et al. (2015) (cont.)		Oesophagus	All workers: reinforced plastic	47	1.06 (0.78–1.41)		
		Pancreas	All workers: reinforced plastic	48	1.13 (0.83–1.50)		
		Larynx	All workers: reinforced plastic	13	1.70 (1.91–2.91)		
		Lung	All workers: reinforced plastic	329	1.20 (1.08–1.34)		
		Breast	All workers: reinforced plastic	24	0.77 (0.49–1.15)		
		Prostate	All workers: reinforced plastic	63	0.86 (0.66–1.10)		
		Kidney	All workers: reinforced plastic	28	1.33 (0.88–1.92)		
		Urinary bladder	All workers: reinforced plastic	38	1.16 (0.82–1.59)		

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Christensen et al. (2017) Denmark Enrolment 1964–2007/ follow-up 1968–2012 Cohort	72 292 (60 478 men and 11 774 women) workers employed in 443 small- and medium-sized companies producing reinforced plastics Exposure assessment method: records; annual employment information from national register; expert assessment; worker survey	Leukaemia (lymphoid)	All workers: reinforced plastic	123	0.96 (0.79–1.14)	Age, sex, calendar period	Strengths: large population of workers of small- and medium-sized companies with expected homogeneous and high-concentration styrene exposure and a long and almost complete follow-up Limitations: no use of quantitative estimates of styrene exposure or smoking information
		Lymphatic and haematopoietic	All workers: reinforced plastic	661	0.97 (0.90–1.04)		
		Leukaemia (myeloid)	All workers: reinforced plastic	101	1.06 (0.86–1.28)		
		MM (Multiple myeloma)	All workers: reinforced plastic	90	[0.79 (0.64–0.97)]		
		NHL	All workers: reinforced plastic	270	0.97 (0.86–1.10)		
		HL	All workers: reinforced plastic	64	1.21 (0.93–1.54)		
		Monocytic leukaemia	All workers: reinforced plastic	< 4	0.77 (0.15–2.25)		
		Other and unspecified leukaemia	All workers: reinforced plastic	10	1.05 (0.50–1.94)		
		Pharynx	All workers: reinforced plastic	170	1.21 (1.03–1.40)		
		Buccal activity and pharynx	All workers: reinforced plastic	398	1.20 (1.08–1.32)		
		Oesophagus	All workers: reinforced plastic	160	1.05 (0.89–1.22)		

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Christensen et al. (2017) (cont.)		Stomach/gastric cancer	All workers: reinforced plastic	237	1.11 (0.97–1.26)		
		Pancreas	All workers: reinforced plastic	247	1.04 (0.91–1.18)		
		Nasal cavity and sinuses	All workers: reinforced plastic	40	1.62 (1.16–2.21)		
		Larynx	All workers: reinforced plastic	176	1.34 (1.15–1.55)		
		Lung	All workers: reinforced plastic	1638	1.28 (1.22–1.34)		
		Breast	All workers: reinforced plastic	432	0.95 (0.86–1.05)		
		Prostate	All workers: reinforced plastic	1025	0.88 (0.83–0.94)		
		Kidney	All workers: reinforced plastic	247	1.12 (0.98–1.27)		
		Urinary bladder	All workers: reinforced plastic	675	1.06 (0.98–1.14)		

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Kolstad et al. (1994) Denmark 1964–1988 Cohort	53 720 workers (36 525 male employees of 386 companies producing reinforced plastics and 14 254 employees not exposed to styrene of similar industries) included in historical cohort study; observed numbers of newly diagnosed cases of lymphohaematopoietic malignancies in the study population compared with expected numbers based on the national rates; study conducted in the Danish reinforced plastics industry, in which exposure to high concentrations of styrene occurs frequently in an environment free of most other suspected carcinogens Exposure assessment method: semiquantitative: cumulated styrene exposure scores modelled from job title, styrene exposure probability, styrene exposure levels since the early 1970s, and duration of employment	Leukaemia	Exposed jobs vs unexposed jobs: time since first employment; time window (1964–1970)			Age, sex, year of diagnosis	Strengths: large study population of workers exposed to high concentration of styrene; semiquantitative exposure characterization; long follow-up; high number of incident and specific lymphohaematopoietic malignancies; analyses of exposure time windows Limitations: lack of individual work histories
			< 10 yr since first employment	5	1.06 (0.35–2.48)		
			≥ 10 yr since first employment	25	1.69 (1.09–2.49)		
			Total	30	1.54 (1.04–2.19)		

Table 2.1 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Kolstad et al. (1996) Denmark 1970–1990 Nested case–control	12 cases; 19 identified with myeloid leukaemia in the Danish cohort (see Christensen et al. 2017, 2018) diagnosed between 1970 and 1991, 12 of which showed clonal chromosome aberrations Controls: 57 Exposure assessment method: semiquantitative: cumulated styrene exposure scores modelled from job title, styrene exposure probability, styrene exposure levels since the early 1970s, and duration of employment	Leukaemia (AML)	Cumulative exposure to styrene ((mg/m^3)-yr)			Calendar year, time since first employment, year of first employment, age	Strengths: may indicate that chromosome aberrations might be part of the disease process in relation to styrene exposure and myeloid carcinoma Limitations: results are only preliminary because of the few observations, the lack of specific exposure data, and the incomplete case ascertainment
			Any exposed vs unexposed	11	2.5 (0.2–25.0)		
			Cumulative exposure to styrene ((mg/m^3)-yr)				
			Low	8	3.0 (0.3–32.2)		
			High	3	1.6 (0.1–22.0)		
			Cumulative exposure to styrene ((mg/m^3)-yr): year of first employment				
			Later than 1970	4	2.3 (0.2–26.2)		
			Before 1970	7	5.9 (0.6–57.8)		
			Cumulative exposure to styrene ((mg/m^3)-yr): length of exposed employment				
			< 1 yr	8	5.9 (0.5–74.3)		
			≥ 1 yr	3	1.1 (0.1–15.3)		
Wong (1990) USA 1948–1977 Nested case–control	Cases: 40 deaths from respiratory cancer Controls: 102; for each case, a maximum of 3 controls were selected from deceased members of the cohort, matched with respect to plant, age at death (within 5 yr), year of death (within 5 yr), sex, and race (from death certificates) Exposure assessment method: other	Respiratory tract	Direct exposure to styrene			Calendar year, time since first employment, year of first employment, age	Cohort nested in the population described in Collins et al. (2013)
			Exposed	15	0.63		
			Trend test *P* value, 0.29				

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Ruder & Bertke (2017) Washington, USA 1991–2007 Cohort	3704 employees of two reinforced plastics facilities during1959–1978 and living in Washington State by 1991 Exposure assessment method: records; exposure assessment identical to that of the Ruder et al. (2016) study	Lymphatic and haematopoietic	All workers	47	1.03 (0.77–1.35)	Age, sex, calendar period, race	Strengths: incidence data Limitations: only 71.2% of the original study population of 5203 workers included
			Low exposure	35	1.05 (0.73–1.46)		
			High exposure	18	0.99 (0.59–1.57)		
		Lung	All workers	87	1.11 (0.89–1.37)		
			Low exposure	50	0.96 (0.71–1.27)		
			High exposure	37	1.42 (1.00–1.95)		
		Breast	All workers	21	0.81 (0.50–1.23)		
			Low exposure	6	0.67 (0.25–1.46)		
			High exposure	15	0.88 (0.49–1.45)		
		Prostate	All workers	140	0.82 (0.69–0.97)		
			Low exposure	89	0.74 (0.60–0.91)		
			High exposure	51	1.02 (0.76–1.34)		
		Urinary organ	All workers	51	1.00 (0.75–1.32)		
			Low exposure	32	0.93 (0.63–1.31)		
			High exposure	19	1.17 (0.70–1.82)		

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Christensen et al. (2018) Denmark 1968–2011 Cohort	73 036 male and female workers of 456 small- and medium-sized companies producing reinforced plastics Exposure assessment method: semiquantitative: cumulated styrene exposure scores modelled from job title, styrene exposure probability, styrene exposure levels since the early 1970s, and duration of employment	Leukaemia (AML)	Cumulative exposure score: complete work history ((mg/m³)-yr)			Age, sex, calendar period	Results for additional subcategories of lymphohaematopoietic cancers are reported Strengths: large study population of workers exposed to high concentrations of styrene; semiquantitative exposure characterization; long and almost complete follow-up; high number of incident and specific lymphohaematopoietic malignancies; and analyses of exposure time windows Limitations: exposure characterization included an element of probability
			1–17	12	1		
			18–70	12	0.77 (0.34–1.74)		
			> 70	26	1.35 (0.65–2.80)		
			Trend test *P* value, 0.28				
			Cumulative exposure score: previous < 15 yr ((mg/m³)-yr)				
			0	28	1		
			1–28	10	1.01 (0.46–2.20)		
			> 28	12	0.81 (0.38–1.73)		
			Trend test *P* value, 0.60				
			Cumulative exposure score: previous 15–29 yr ((mg/m³)-yr)				
			0	18	1		
			1–45	10	1.34 (0.60–2.97)		
			> 45	22	2.35 (1.21–4.57)		
			Trend test *P* value, 0.01				
			Cumulative exposure score: previous ≥ 30 yr ((mg/m³)-yr)				
			0	37	1		
			1–45	7	2.12 (0.82–5.48)		
			> 45	6	1.55 (0.57–4.26)		
			Trend test *P* value, 0.28				
		HL	Cumulative exposure score: complete work history ((mg/m³)-yr)				
			1–17	16	1		
			18–70	16	1.04 (0.51–2.13)		
			> 70	25	1.60 (0.81–2.16)		
			Trend test *P* value, 0.15				

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Christensen et al. (2018) (cont.)		HL	Cumulative exposure score: previous < 15 yr ((mg/m³)-yr)				
			0	20	1		
			1–28	16	1.17 (0.55–2.48)		
			> 28	21	1.72 (0.79–3.75)		
			Trend test *P* value, 0.17				
			Cumulative exposure score: previous 15–29 yr ((mg/m³)-yr)				
			0	38	1		
			1–45	12	1.17 (0.59–2.34)		
			> 45	7	0.61 (0.26–1.43)		
			Trend test *P* value, 0.36				
			Cumulative exposure score: previous ≥ 30 yr ((mg/m³)-yr)				
			0	44	1		
			1–45	7	2.25 (0.86–5.88)		
			> 45	6	1.71 (0.62–4.74)		
			Trend test *P* value, 0.21				
		Lymphatic and haematopoietic (all combined)	Cumulative exposure score: complete work history ((mg/m³)-yr)				
			1–17	182	1		
			18–70	220	0.92 (0.76–1.13)		
			> 70	263	0.92 (0.76–1.13)		
			Trend test *P* value, 0.042				
		Leukaemia (CML)	Cumulative exposure score: complete work history ((mg/m³)-yr)				
			1–17	6	1		
			18–70	7	0.74 (0.24–2.22)		
			> 70	11	0.94 (0.34–2.63)		
			Trend test *P* value, 0.99				

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Christensen et al. (2018) (cont.)		NHL (all combined)	Cumulative exposure score: complete work history ((mg/m³)-yr)				
			1–17	67	1		
			18–70	80	0.94 (0.68–1.32)		
			> 70	89	0.91 (0.65–1.27)		
			Trend test *P* value, 0.58				
		NHL (B-cell lymphoma)	Cumulative exposure score: complete work history ((mg/m³)-yr)				
			1–17	49	1		
			18–70	62	1.00 (0.68–1.46)		
			> 70	63	0.87 (0.59–1.29)		
			Trend test *P* value, 0.46				
		MM	Cumulative exposure score: complete work history ((mg/m³)-yr)				
			1–17	19	1		
			18–70	20	0.77 (0.40–1.46)		
			> 70	30	0.93 (0.51–1.70)		
			Trend test *P* value, 0.91				
		Leukaemia (lymphoid) (all combined)	Cumulative exposure score: complete work history ((mg/m³)-yr)				
			1–17	29	1		
			18–70	40	0.94 (0.58–1.53)		
			> 70	33	0.60 (0.36–1.02)		
			Trend test *P* value, 0.04				
		NHL (T-cell lymphoma)	Cumulative exposure score: complete work history ((mg/m³)-yr)				
			1–17	NR	1		
			18–70	NR	1.14 (0.25–5.15)		
			> 70	NR	3.21 (0.87–11.77)		
			Trend test *P* value, 0.04				

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Christensen et al. (2018) (cont.)			Cumulative exposure score: previous < 15 yr ((mg/m³)-yr)				
			0	NR	1		
			1–28	NR	0.56 (0.14–2.28)		
			> 28	NR	0.18 (0.02–1.52)		
			Trend test *P* value, 0.09				
			Cumulative exposure score: previous 15–29 yr ((mg/m³)-yr)				
			0	NR	1		
			1–45	NR	0.85 (0.22–3.28)		
			> 45	NR	2.04 (0.75–5.52)		
			Trend test *P* value, 0.17				
			Cumulative exposure score: previous ≥ 30 yr ((mg/m³)-yr)				
			0	NR	1		
			1–45	NR	2.78 (0.80–9.60)		
			> 45	NR	2.40 (0.68–8.46)		
			Trend test *P* value, 0.15				

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Nissen et al. (2018) Denmark 1968–2011 Nested case–control	Cases: 9 adenocarcinomas, 15 squamous cell carcinomas, 13 other subtypes, 73 092 reinforced plastics workers Controls: 90, 150, 130, none Exposure assessment method: semiquantitative: cumulated styrene exposure scores modelled from job title, styrene exposure probability, styrene exposure levels since the early 1970s, and duration of employment	Nasal cavity and sinuses: adenocarcinoma	Complete work history: ≥ 37 (mg/m³)-yr	9	5.11 (0.58–45.12)	Age, sex, employment in a reinforced plastics company producing boats or in the wood industry	Strengths: large study population of workers exposed to high concentrations of styrene; semiquantitative exposure characterization; long follow-up; information on specific and incident histological subtypes Limitations: exposure characterization included an element of probability
			Per 100 (mg/m³)-yr	9	1.08 (0.96–1.21)		
		Nasal cavity and sinuses: squamous cell carcinoma	Complete work history: ≥ 37 (mg/m³)-yr	15	1.15 (0.34–3.89)		
			Per 100 (mg/m³)-yr	15	1.02 (0.83–1.25)		
		Nasal cavity and sinuses: other histological subtypes	Complete work history: ≥ 37 (mg/m³)-yr	13	0.74 (0.22–2.42)		
			Per 100 mg/m³-yr	13	0.75 (0.46–1.23)		
Loomis et al. (2019) International cohort 1945–1991 Cohort	40 668 (34 560 men and 6128 women) workers enrolled from eight centres from more than 600 plants Exposure assessment method: quantitative measurements	NHL	Mean styrene exposure (ppm)			Sex, age, and calendar decade	Strengths: large international study population characterized by quantitative measures of styrene exposure; internal analysis Limitations: short duration of follow-up (average 13 yr); no sampling information
			0 yr lag	NR	2.31 (1.29–4.12)		
			5 yr lag	NR	2.29 (1.33–3.93)		
			10 yr lag	NR	1.78 (1.05–3.02)		
		MM	Mean styrene exposure (ppm)				
			0 yr lag	NR	1.86 (0.71–4.86)		
			5 yr lag	NR	2.25 (0.92–5.48)		
			10 yr lag	NR	1.31 (0.48–3.58)		
		Leukaemia (myeloid, acute and chronic combined)	Mean styrene exposure (ppm)				
			0 yr lag	NR	0.92 (0.37–2.32)		
			5 yr lag	NR	1.34 (0.61–2.94)		
			10 yr lag	NR	1.50 (0.71–3.17)		

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Loomis et al. (2019) (cont.)		Oesophagus	Mean styrene exposure (ppm)			Age	
			0 yr lag	NR	2.44 (1.11–5.36)		
			10 yr lag	NR	2.53 (1.30–4.90)		
			20 yr lag	NR	3.36 (1.74–6.49)		
		Pancreas	Mean styrene exposure (ppm)				
			0 yr lag	NR	1.89 (1.17–3.06)		
			10 yr lag	NR	1.31 (0.82–2.10)		
			20 yr lag	NR	1.14 (0.57–2.28)		
		Lung	Mean styrene exposure (ppm)			Sex, age, country	
			0 yr lag	NR	0.98 (0.79–1.22)		
			10 yr lag	NR	0.97 (0.80–1.18)		
			20 yr lag	NR	0.81 (0.59–1.12)		
		Prostate	Mean styrene exposure (ppm)			Age, country	
			0 yr lag	NR	1.26 (0.76–2.11)		
			10 yr lag	NR	1.03 (0.64–1.68)		
			20 yr lag	NR	1.07 (0.55–2.10)		
		NHL	Exposed jobs vs unexposed jobs			Age, country, calendar decade, sex	
			All exposed	22	1.01 (0.37–2.74)		
		MM	Exposed jobs vs unexposed jobs				
			All exposed	8	1.05 (0.20–5.37)		
		Leukaemia (myeloid, acute and chronic combined)	Exposed jobs vs unexposed jobs				
			All exposed	12	0.57 (0.09–3.49)		
		Oesophagus	Exposed jobs vs unexposed jobs			Age	
			All exposed	15	3.50 (0.46–26.82)		
		Pancreas	Exposed jobs vs unexposed jobs				
			All exposed	27	1.06 (0.46–2.46)		
		Lung	Exposed jobs vs unexposed jobs			Age, country, calendar decade, sex	
			All exposed	159	0.85 (0.57–1.19)		

Table 2.1 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Loomis et al. (2019) (cont.)		Prostate	Exposed jobs vs unexposed jobs			Age, country	
			All exposed	27	1.85 (0.64–5.36)		
		Kidney	Exposed jobs vs unexposed jobs			Age, country, calendar decade, sex	
			All exposed	9	0.83 (0.18–3.99)		
		Urinary bladder	Exposed jobs vs unexposed jobs				
			All exposed	21	0.92 (0.22–3.80)		
Bertke et al. (2018) Washington, USA 1959–1978/1 January 1960–31 December 2016 Cohort	5201 workers employed in two Washington boatbuilding facilities Exposure assessment method: records; work history information was used to construct an exposure index based on exposure duration and exposure potential (from industrial hygiene surveys conducted at each plant)	NHL	Person-time employed: external comparison (yr)			Regressions matched on attained age, sex, race, calendar period	Supersedes Ruder et al. (2016) Strengths: exposure to high concentrations of styrene; few competing risk factors; long follow-up Limitations: the lack of quantitative styrene exposure and smoking information
			≥ 1	5	0.60 (0.19–1.40)		
		Leukaemia	Person-time employed: external comparison (yr)				
			≥ 1	7	0.88 (0.35–1.81)		
		MM	Person-time employed: external comparison (yr)				
			≥ 1	6	1.50 (0.55–3.25)		
		Lymphatic and haematopoietic (all combined)	Person-time employed: external comparison (yr)				
			≥ 1	18	0.85 (0.51–1.35)		
		Buccal cavity and pharynx	Person-time employed: external comparison (yr)				
			≥ 1	< 5	0.49 (0.06–1.78)		
		Oesophagus	Person-time employed: external comparison (yr)				
			≥ 1	7	1.06 (0.43–2.19)		
		Pancreas	Person-time employed: external comparison (yr)				
			≥ 1	13	1.11 (0.59–1.90)		
		Larynx	Person-time employed: external comparison (yr)				
			≥ 1	< 5	0.61 (0.02–3.39)		
		Lung: trachea, bronchus, and lung (162)	Person-time employed: external comparison (yr)				
			≥ 1	76	1.20 (0.95–1.51)		
		Breast	Person-time employed: external comparison (yr)				
			≥ 1	5	0.97 (0.32–2.26)		
		Prostate	Person-time employed: external comparison (yr)				
			≥ 1	23	1.38 (0.87–2.07)		

Table 2.1 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Bertke et al. (2018) (cont.)		Kidney	Person-time employed: external comparison (yr)				
			≥ 1	9	1.66 (0.76–3.16)		
		Urinary bladder	Person-time employed: external comparison (yr)				
			≥ 1	8	1.34 (0.58–2.65)		
		Lymphatic and haematopoietic	Duration employed as continuous			Regressions matched on attained age, sex, race, calendar period, employment duration of < or > 1 yr	
			Log-linear per 1 yr employment	49	1.2 (1.0–1.3)		
		NHL	Duration employed as continuous				
			Log-linear per 1 yr employment	18	0.9 (0.2–1.4)		
		MM	Duration employed as continuous				
			Log-linear per 1 yr employment	11	1.1 (0.6–1.5)		
		Leukaemia	Duration employed as continuous				
			Log-linear per 1 yr employment	18	1.3 (1.0–1.5)		
		Oesophagus	Duration employed as continuous				
			Log-linear per 1 yr employment	21	1.2 (0.8–1.6)		
		Pancreas	Duration employed as continuous				
			Log-linear per 1 yr employment	38	1.0 (0.6–1.2)		
		Respiratory tract: trachea, bronchus, and lung (162)	Duration employed as continuous				
			Log-linear per 1 yr employment	204	0.9 (0.7–1.0)		

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Bertke et al. (2018) (cont.)		Breast	Duration employed as continuous				
			Log-linear per 1 yr employment	6	0.3 (0.0–0.69)		
		Prostate	Duration employed as continuous				
			Log-linear per 1 yr employment	44	1.2 (1.0–1.4)		
		Kidney	Duration employed as continuous				
			Log-linear per 1 yr employment	15	1.1 (0.7–1.3)		
		Urinary bladder	Duration employed as continuous				
			Log-linear per 1 yr employment	15	1.2 (0.8–1.5)		

ALL, acute lymphoblastic/lymphocytic leukaemia; AML, acute myeloid leukaemia; CI, confidence interval; CLL, chronic lymphocytic leukaemia; CML, chronic myeloid leukaemia; HL, Hodgkin lymphoma; ICD, International Classification of Diseases; MM, multiple myeloma; mo, month(s); NHL, non-Hodgkin lymphoma; NR, not reported; ppm, parts per million; RR, relative risk; vs, versus; yr, year(s).

including multiple myeloma (SMR, 1.15; 95% CI, 0.74–1.69), and myelodysplasia (SMR, 1.73; 95% CI, 0.70–3.57), but not for cancer of the prostate, pancreas, or breast or for Hodgkin lymphoma (HL), non-Hodgkin lymphoma (NHL), acute lymphoblastic/lymphocytic leukaemia (ALL), CLL, or acute myeloid leukaemia (AML).

Internal Cox analyses showed increasing trends by cumulative styrene exposure of statistical significance for cancer of the pancreas (HR, 1.008; 95% CI, 1.002–1.015) and kidney (HR, 1.009; 95% CI, 1.000–1.017) per 100 ppm-months. A decreasing trend of borderline significance was seen for cancer of the lung (HR, 0.997; 95% CI, 0.993–1.002). No significant trends were apparent for cancer of the bladder, all cancers of the lymphoid and haematopoietic tissues, NHL, HL, lymphoid or myeloid leukaemia, or all other cancers of the lymphopoietic tissue including multiple myeloma (MM). Increased standardized mortality ratios were seen for myeloid leukaemia (SMR, 1.27; 95% CI, 0.55–2.50) and cancer of the kidney (SMR, 1.79; 95% CI, 1.02–2.91) in the category of highest exposure (≥ 1200 ppm-months).

Analyses of days with peak exposure (> 100 ppm for 15 minutes of the working day) showed increased point estimates in the category of highest exposure (≥ 1800 days with peaks) for lymphoid leukaemia (SMR, 1.95; 95% CI, 0.24–7.03), other lymphatic cancers (SMR, 2.13; 95% CI, 0.58–5.45), and cancer of the pancreas (SMR, 1.45; 95% CI, 0.63–2.85), kidney (SMR, 1.82; 95% CI, 0.59–4.24), and bladder (SMR, 2.35; 95% CI, 0.87–5.13). The nested case–control study within this cohort showed no association between styrene exposure and cancer of the respiratory system (Wong, 1990). [The Working Group noted that the strengths of this study were the long follow-up, the high number of cases, the high concentrations of styrene exposure, and the lack of known carcinogenic occupational co-exposures within the industry. Quantitative styrene exposure metrics were applied but information on the exposure assessment was sparse; no styrene intensity information was apparently available for a substantial part of the exposure period, namely between 1948 and 1976, and 27% of the cohort was missing exposure data after 1977.]

Bertke et al. (2018) reported cancer mortality from 1959 to 2016 for 5201 workers employed in two reinforced plastics boatbuilding facilities in Washington State between 1959 and 1978. This was an update of the same population or subsets thereof previously followed up until 1978 (Okun et al., 1985), 1998 (Ruder et al., 2004), and 2011 (Ruder et al., 2016). Full-shift average concentrations of styrene exposure of 42.5 ppm and 71.7 ppm were reported for exposed workers between 1978 and 1979. In the most recent follow-up (Bertke et al., 2018), for those with more than 1 year of employment, many cancer sites showed increased standardized mortality ratios when compared with Washington State expected values, including cancer of the lung (SMR, 1.20; 95% CI, 0.95–1.51) but not cancer of the buccal cavity and pharynx (SMR, 0.49; 95% CI, 0.06–1.78) or breast (SMR, 0.97; 95% CI, 0.32–2.26), lymphatic and haematopoietic malignancies (SMR, 0.85; 95% CI, 0.51–1.35), NHL (SMR, 0.60; 95% CI, 0.19–1.40), or leukaemia (SMR, 0.88; 95% CI, 0.35–1.81).

In internal Cox regression analyses, leukaemia showed increasing mortality with duration of employment in an employment category of high concentration of exposure with a relative risk (RR) estimate of 1.3 (95% CI, 1.0–1.5) per year. Results were also provided for cancers of the lymphoid and haemopoietic tissues (RR, 1.2; 95% CI, 1.0–1.3), NHL (RR, 0.9; 95% CI, 0.2–1.4), and MM (RR, 1.1; 95% CI, 0.6–1.5). Decreasing mortality with duration was observed for cancer of the lung (RR, 0.9; 95% CI, 0.7–1.0), but not for cancer of the kidney (RR, 1.1; 95% CI, 0.7–1.3). [The strengths of this study were the high concentrations of styrene exposure, the few competing risk factors, and the long follow-up. Limitations

were the lack of quantitative styrene exposure and information on smoking.]

Ruder & Bertke (2017) studied the incidence of cancer during 1991–2007 among 3704 workers of the Washington cohort who were living in Washington State by 1991, with no restriction on duration of employment, using data from the Washington State cancer registry and applying statistical methods as for the mortality analyses (Ruder et al., 2016). Elevated standardized incidence ratios (SIRs) for lung cancer were observed for the total population (SIR, 1.11; 95% CI, 0.89–1.37) and for workers potentially exposed to high concentrations of styrene (SIR, 1.42; 95% CI, 1.00–1.95). For all workers and for those exposed to styrene at high concentrations, standardized incidence ratios for cancers of the lymphoid and haematopoietic tissues were 1.03 (95% CI, 0.77–1.35) and 0.99 (95% CI, 0.59–1.57); for cancer of the urinary organ, 1.00 (95% CI, 0.75–1.32) and 1.17 (95% CI, 0.70–1.82); and for cancer of the breast, 0.81 (95% CI, 0.50–1.23) and 0.88 (95% CI, 0.49–1.45), respectively. [The Working Group noted that the cancer incidence data were a strength, and the limited time period coverage of this outcome information was a limitation. Other quality aspects were as for Bertke et al. (2018).]

Kogevinas et al. (1994) reported cancer mortality among 40 688 employees of 660 plants in Denmark (15 867), Finland (2085), Italy (7256), Norway (2035), Sweden (3667), and the United Kingdom (9778). Cancer mortality of the United Kingdom subset followed up until 2012 was also reported by Coggon et al. (2015), and cancer incidence for the Danish subset until 2012 was also reported by Christensen et al. (2017, 2018) and Nissen et al. (2018). The follow-up periods started between 1945 (UK) and 1970 (Denmark) and ended between 1987 (Sweden) and 1991 (Norway).

From job titles recorded on individual payroll records, the pooled population was categorized as laminators (n = 10 629), workers with unspecified tasks (n = 19 408), other exposed workers with bystander exposure (n = 5406), workers not exposed to styrene (n = 4044), and workers with unknown job titles (n = 1201) (Kogevinas et al., 1994). An exposure matrix was constructed from 16 500 personal styrene measurements obtained between 1955 and 1990, and from 18 500 measurements of styrene metabolites in urine sampled in the 1980s. Styrene exposure levels recorded among laminators declined from about 200 ppm before 1965 to less than 80 ppm in the 1980s.

Among all workers, the overall cancer mortality (SMR, 0.87; 95% CI, 0.81–0.94) was lower than that for the European reference population. Standardized mortality ratios of 1.0 or more were observed for cancer of the larynx (SMR, 1.11; 95% CI, 0.53–2.05) and myeloid leukaemia (SMR, 1.10; 95% CI, 0.63–1.79). No increased mortality was observed for other cancer sites of primary interest.

In internal analyses, no increasing mortality with increasing cumulative styrene exposure was observed for cancer of the lung, all lymphohaematopoietic malignancies, or leukaemia. Relative risks of between 1.64 (95% CI, 0.34–7.82) and 2.99 (95% CI, 0.82–10.91) were seen for HL and NHL combined, but there was no linear trend (P value, 0.52). Mortality increased with increasing latency; for 20 years or more since first exposure, the relative risk for lymphohaematopoietic malignancies was 3.97 (95% CI, 1.30–12.13; P value for trend, 0.012), for leukaemia was 3.71 (95% CI, 0.70–20.59; P value for trend, 0.094), and for HL and NHL combined was 5.16 (95% CI, 0.90–29.47; P value for trend, 0.072). Mortality increased statistically significantly with increasing average styrene exposure for lymphohaematopoietic malignancies (P value for trend, 0.019) and for HL and NHL combined (P value for trend, 0.52), but not for leukaemia (P value for trend, 0.47). For average styrene exposure at 200 ppm or more, the relative risk for all lymphohaematopoietic malignancies was 3.59 (95% CI, 0.98–13.14), for HL and NHL combined

was 4.40 (95% CI, 0.42–45.99), and for leukaemia was 2.16 (95% CI, 0.29–16.24; *P* value for trend, 0.47). Increasing mortality by cumulative styrene exposure was suggested for cumulative exposure of 500 ppm-years or more for cancer of the pancreas (RR, 2.56; 95% CI, 0.90–7.31; *P* value for trend, 0.068) and kidney (RR, 6.04; 95% CI, 0.74–49.45; *P* value for trend, 0.12). No significant trend was seen for cancer of the oesophagus or lung. [The Working Group noted that the large European study population, characterized by quantitative measures of styrene exposure, was a strength of this study. The study was limited by the short follow-up (average, 13 years) and lack of smoking information.]

Loomis et al. (2019) reanalysed the European cohort (Kogevinas et al., 1994) with no additional follow-up, while also excluding data from Norway due to new national privacy protection legislation. Lymphomas and leukaemias were regrouped to approximate current World Health Organization classification. Lymphosarcoma and reticulosarcoma (ICD-8, ICD-9 codes 200), other malignant neoplasms of lymphoid and histiocytic tissue (202), CLL (204.1), and MM (203) were aggregated under the heading of NHL. Internal adjusted analyses showed a relative risk of 2.31 (95% CI, 1.29–4.12) per 100 ppm mean styrene exposure for NHL with a zero lag, but not with cumulative exposure. For AML and CML combined, the relative risk was 1.50 (95% CI, 0.71–3.17) when a 10-year lag was applied. An association between mean styrene exposure as well as cumulative exposure, lagged by 20 years, and cancer of the oesophagus was reported. [The Working Group noted that a strength of this study was the internal analyses by quantitative measures of styrene exposure. Other quality aspects were as for Kogevinas et al. (1994).]

Coggon et al. (2015) studied cancer mortality of 7970 workers employed in eight reinforced plastics companies in the United Kingdom between 1946 and 1984, and followed up until 2012. This was an update of an original study of 7949 workers employed in the same companies during 1947–1984 and followed up until 1984 (Coggon et al., 1987). Follow-up of this population until 1990 was included in the study by Kogevinas et al. (1994).

From personnel records, workers were classified into four concentrations of styrene exposure: high (laminators, 44%); moderate (regular bystander exposure, 7%); low (occasional bystander exposure, 17%); or background exposure (all other jobs, including employment for which job title was missing, 32%) (Coggon et al., 1987, 2015). Based on measurements conducted at five of the companies during 1975–1984, the authors estimated that hand laminators were exposed to styrene at 8-hour time-weighted average concentrations of 40–100 ppm, but this information was not used to classify workers in the statistical analyses.

Among all workers, increased standardized mortality ratios of note were reported for cancer of the lung (SMR, 1.20; 95% CI, 1.08–1.34), pharynx (SMR, 1.34; 95% CI, 0.61–2.54), larynx (SMR, 1.70; 95% CI, 0.91–2.91), bladder (SMR, 1.16; 95% CI, 0.82–1.59), and kidney (SMR, 1.33; 95% CI, 0.88–1.92). Subgroup analyses for cancer of the lung yielded a standardized mortality ratio of 1.44 (95% CI, 1.10–1.86) for a category defined by 1 year or more of employment with exposure to a high concentration of styrene.

Internal analyses of a case–control study nested within the study population defining cases by underlying and contributing causes of death and cancer registrations showed doubled odds ratios (OR) for MM, including one of 2.66 (95% CI, 0.62–11.35) for a category defined by 1 year or more of employment with exposure to a high concentration of styrene. No increased occurrence was indicated for HL, NHL including CLL, or leukaemia in the total population or the internal analyses. [The Working Group noted that exposure to a high concentration of styrene, limited exposure to other suspected carcinogens, and the long follow-up were the strengths of this

study. However, the study was limited by a lack of styrene exposure information since 1984, quantitative styrene exposure, or smoking information, and by the high loss to follow-up (11.5%).]

Christensen et al. (2017) studied cancer incidence in 72 292 workers employed during 1964–2007 in 443 small- and medium-sized companies producing reinforced plastics, followed up until 1968–2012. Kolstad et al. (1993) previously studied the incidence of cancer during 1970–1990 for about 64 000 workers of 552 companies with an assumed relevant production and 36 500 male workers of 386 companies with confirmed relevant production (Kolstad et al., 1994, 1995). The workers ever employed in these companies during 1964–2007 were identified in a national pension fund register. Based on a survey conducted in 2013 of current and former employees, the proportion of workers exposed to styrene in each company was computed and workers were classified into four categories of probability of styrene exposure. Sixteen per cent of all person-years at risk was observed in workers employed in companies with a probability of exposure to styrene of 75–100%. A smoking survey showed a slightly lower ever-smoking prevalence with longer duration of employment. The concentrations of exposure to styrene, measured mainly during lamination work, were 180 ppm during 1964–1970, 88 ppm during 1971–1975, and 43 ppm during 1976–1988, corresponding to an annual decline of 7% (Kolstad et al., 1994).

Among all workers in the study by Christensen et al. (2017), estimates of standardized incidence ratios of greater than 1.1 were observed for cancer of the pharynx (SIR, 1.21; 95% CI, 1.03–1.40), oesophagus (SIR, 1.05; 95% CI, 0.89–1.22), nasal cavities (SIR, 1.62; 95% CI, 1.16–2.21), lung (SIR, 1.28; 95% CI, 1.22–1.34), and kidney (SIR, 1.12; 95% CI, 0.98–1.27), and for HL (SIR, 1.21; 95% CI, 0.93–1.54). A decreased risk was observed for cancer of the prostate (SIR, 0.88; 95% CI, 0.83–0.94). The standardized incidence ratios for the other cancer types of priority were not increased.

Subgroup analyses showed lower standardized incidence ratios for cancer of the lung with a longer duration of employment. Standardized incidence ratios for HL and cancer of the sinonasal cavities were higher with one or more of the following factors: longer duration of employment, earlier year of first employment, and higher probability of exposure to styrene. The risks of myeloid leukaemia and cancer of the kidney were higher with longer duration of employment and higher probability of exposure to styrene. A standardized incidence ratio of 1.69 (95% CI, 1.09–2.49) for all leukaemia was observed for those first employed during early years; workers were followed up until 1989 (Kolstad et al., 1994). [The Working Group noted that the strengths of this study were the large study population of workers of small- and medium-sized companies, with expected homogeneous and high-concentration exposure to styrene, and a long and almost complete follow-up. The limitations were the lack of quantitative estimates of exposure to styrene or any information on the prevalence of smoking.]

In a case–control study nested within the cohort, Kolstad et al. (1996) studied the association between exposure to styrene and myeloid leukaemia with clonal chromosome aberrations. The study was based on 12 cases with an identifiable chromosomal analysis (out of 34 cases of myeloid leukaemia in the total study population) and 57 incidence density sampled controls. The classification of exposure was based upon information in Kolstad et al. (1994, 1995). An increased risk (OR, 2.5; 95% CI, 0.2–25.0) was observed for any employment with exposure to styrene; however, the association was stronger for workers employed for less than 1 year (OR, 5.9; 95% CI, 0.5–74.3) than for workers employed for 1 year or longer (OR, 1.1; 95% CI, 0.1–15.3). [The Working Group noted the very small numbers of cases and controls but, considered with the

positive findings for AML in other analyses, this study was found to be relevant for the current evaluation.]

Christensen et al. (2018) analysed the exposure–response relation between cumulative styrene exposure scores and the incidence of 21 different lymphohaematopoietic malignancies and their combinations in an internal analysis of the Danish reinforced plastics industry during 1968–2011. The study population was principally the same as for Christensen et al. (2017), but included an additional 744 workers from 13 companies. Cumulative styrene exposure scores were modelled from 1122 historical measurements of personal styrene exposure intensity, job title, survey data of 11 264 current and former workers, and duration of employment during styrene production. Data were analysed using a discrete time hazard model and spline regression.

The authors observed 50 cases of AML and an increasing risk with increasing cumulative exposure to styrene experienced during the prior 15–29 years (*P* value for trend, 0.01); the adjusted incidence rate ratio was 2.35 (95% CI, 1.21–4.57) for the highest compared with the lowest exposure tertile. Increased incidence was also suggested after styrene exposure experienced at least 30 years earlier, whereas no increased incidence was observed after exposure within the previous 15 years. Increasing incidence with increasing cumulative styrene exposure accrued during the full work history were seen for T-cell lymphoma (*P* value for trend, 0.04); the adjusted incidence rate ratio was 3.21 (95% CI, 0.87–11.77) for the highest compared with the lowest exposure tertile. Increasing incidence with increasing cumulative styrene exposure was also indicated for HL (*P* value for trend, 0.15); the adjusted incidence rate ratio was 1.60 (95% CI, 0.81–2.16) for the highest compared with the lowest exposure tertile. However, the incidence patterns for T-cell lymphoma and HL were inconsistent across the previous 1–14 years, 15–29 years, and 30 years or more exposure windows. No increasing incidences with cumulative exposure were seen for other lymphohaematopoietic malignancies. [The strengths of this study were the large study population of workers from small- and medium-sized companies exposed to high concentrations of styrene, the semiquantitative measures of styrene exposure, the long and almost complete follow-up, the high number of incident cases of 21 different lymphohaematopoietic malignancies, and the analyses of exposure time windows.]

Nissen et al. (2018) analysed the association between exposure to styrene and adenocarcinoma, squamous cell carcinoma, and a category of other histological subtypes of sinonasal cancers in a case–control study nested within the Danish reinforced plastics industry cohort. The study population and assessment of exposure corresponded to that of Christensen et al. (2018). The authors observed 9 cases of sinonasal adenocarcinoma, corresponding to a 5-fold increase in odds ratio, adjusted for age, sex, and whether employed in the wood industry, for high versus low cumulative styrene exposure (OR, 5.11; 95% CI, 0.58–45.12). The increased incidence was confined to exposure during the previous 15 years. No association was seen for the other histological subtypes. [The main strength of this analysis was the specific histological information; however, the study was limited by the small number of cases due to the rarity of the disease and possible residual confounding from exposure to wood dust.]

(b) Synthetic rubber industry

See Table 2.2.

All of the information on workers in the synthetic rubber industry is from cohort studies of the mortality of North American workers in the styrene–butadiene rubber (SBR) industry. According to Matanoski et al. (1990), there were initially 15 plants built in the USA and 1 in Canada in the early 1940s; an additional plant was built in the USA in the 1950s. As of 1977, there were only 10 of these 17 plants still in

Table 2.2 Occupational cohort studies on exposure to styrene in the synthetic rubber industry

Reference, location, enrolment/ follow-up period	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Sathiakumar et al. (2005) North America 1944–1998	17 924 men who worked for at least 1 yr before 1 January 1992 in one of eight synthetic rubber plants. Exposure assessment method: records; job/ employment histories	Leukaemia	Employment type			Age, race, calendar period	Strengths: large cohort with long follow-up Limitations: 21% of the cohort were actively employed in 1991, and their exposure estimates would therefore be incomplete after the date; styrene exposure strongly correlated with 1,3-butadiene exposure; no control for smoking Results by job title are also reported for HL, MM, and NHL
			Hourly	63	1.23 (0.94–1.57)		
		NHL	Employment type				
			Hourly	49	1.11 (0.82–1.47)		
		MM	Employment type				
			Hourly	20	0.86 (0.53–1.33)		
		HL	Employment type				
			Hourly	7	0.77 (0.31–1.58)		
		Leukaemia (ALL)	Job title				
			Coagulation	10	2.31 (1.11–4.25)		
			Job title				
			Polymerization	18	2.04 (1.21–3.22)		
			Job title				
			Laboratory jobs	14	3.26 (1.78–5.46)		
			Job title				
			Maintenance labour	15	2.03 (1.14–3.35)		
		All cancers combined	Full cohort	1608	0.92 (0.88–0.97)		
		Lymphatic and haematopoietic	Full cohort	162	1.06 (0.90–1.23)		
		Leukaemia	Full cohort	71	1.16 (0.91–1.47)		
		Pharynx	Full cohort	22	0.47 (0.29–0.71)		
		Oesophagus	Full cohort	44	0.94 (0.68–1.26)		
		Pancreas	Full cohort	76	0.87 (0.68–1.08)		
		Stomach	Full cohort	64	0.85 (0.65–1.08)		
		Larynx	Full cohort	17	0.71 (0.41–1.13)		
		Lung	Full cohort	563	0.91 (0.84–0.99)		
		Prostate	Full cohort	154	1.04 (0.88–1.21)		
		Kidney	Full cohort	39	0.96 (0.68–1.31)		
		Urinary bladder	Full cohort	37	0.90 (0.64–1.25)		

Table 2.2 (continued)

Reference, location, enrolment/ follow-up period	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Graff et al. (2005) North America 1943–1998	16 579 men who had worked at any of the six study plants for at least 1 yr by the end of 1991 and who were actively working as of a calendar year that varied by plant from 1943 to 1950 Exposure assessment method: expert judgement; quantitative estimates of exposure to styrene, 1,3-butadiene, and DMDTC were developed by identifying, for each work area/job group at each plant, its component tasks and historical changes in tasks; mathematical models were used to calculate job- and time-period-specific exposure estimates to create a JEM that was linked to subjects' work history	NHL (CLL)	Cumulative exposure (ppm-yr)			Age, years since hire	Strengths: large cohort with long follow-up; internal comparisons by exposure level; results attempted to control for confounding by 1,3-butadiene and DMDTC Limitations: styrene exposure strongly correlated with 1,3-butadiene exposure (Spearman rank correlation of 0.79)
			0 to < 8.3	7	1		
			8.3 to < 61.1	11	1.7 (0.7–4.4)		
			≥ 61.1	7	2.6 (0.9–7.3)		
			Cumulative exposure (ppm-yr)			Age, years since hire, 1,3-butadiene, DMDTC	
			0 to < 8.3	7	1		
			8.3 to < 61.1	11	1.2 (0.4–3.7)		
			≥ 61.1	7	0.9 (0.2–3.7)		
		Leukaemia (CML)	Cumulative exposure (ppm-yr)			Age, years since hire	
			0 to < 8.3	4	1		
			8.3 to < 61.1	8	2.1 (0.6–7.1)		
			≥ 61.1	4	2.7 (0.7–10.9)		
			Cumulative exposure (ppm-yr)			Age, years since hire, 1,3-butadiene, DMDTC	
			0 to < 8.3	4	1		
			8.3 to < 61.1	8	1.0 (0.2–4.1)		
			≥ 61.1	4	0.6 (0.1–3.5)		
		Leukaemia (AML)	Cumulative exposure (ppm-yr)			Age, years since hire	
			0 to < 8.3	9	1		
			8.3 to < 61.1	14	1.9 (0.8–4.4)		
			≥ 61.1	3	1.0 (0.3–3.9)		
			Cumulative exposure (ppm-yr)			Age, years since hire, 1,3-butadiene, DMDTC	
			0 to < 8.3	9	1		
			8.3 to < 61.1	14	2.1 (0.8–5.8)		
			≥ 61.1	3	1.1 (0.2–5.6)		
		NHL	Cumulative exposure (ppm-yr)			Age, years since hire	
			0	6	1		
			> 0 to < 8.3	16	1.4 (0.5–3.6)		
			8.3 to < 31.8	11	1.1 (0.4–2.9)		
			31.8 to < 61.1	9	1.5 (0.5–4.2)		
			≥ 61.1	16	2.3 (0.9–5.9)		

Table 2.2 (continued)

Reference, location, enrolment/ follow-up period	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Graff et al. (2005) (cont.)		NHL	Cumulative exposure (ppm-yr)			Age, years since hire, DMDTC	
			0	6	1		
			> 0 to < 8.3	16	1.4 (0.4–4.9)		
			8.3 to < 31.8	11	1.3 (0.3–5.2)		
			31.8 to < 61.1	9	1.7 (0.4–7.0)		
			≥ 61.1	16	2.3 (0.6–9.2)		
		MM	Cumulative exposure (ppm-yr)			Age, years since hire	
			0	4	1		
			> 0 to < 8.3	10	1.4 (0.4–4.4)		
			8.3 to < 31.8	3	0.5 (0.1–2.1)		
			31.8 to < 61.1	2	0.6 (0.1–3.3)		
			≥ 61.1	8	2.0 (0.6–6.6)		
		MM	Cumulative exposure (ppm-yr)			Age, years since hire, 1,3-butadiene, DMDTC	
			0	4	1		
			> 0 to < 8.3	10	0.8 (0.1–4.6)		
			8.3 to < 31.8	3	0.2 (0.0–1.7)		
			31.8 to < 61.1	2	0.3 (0.0–2.5)		
			≥ 61.1	8	0.8 (0.1–5.7)		
		Leukaemia	Cumulative exposure (ppm-yr)			Age, years since hire	
			0	7	1		
			> 0 to < 8.3	18	1.3 (0.6–3.2)		
			8.3 to < 31.8	19	1.6 (0.7–3.9)		
			31.8 to < 61.1	18	3.0 (1.2–7.1)		
			≥ 61.1	19	2.7 (1.1–6.4)		
			Cumulative exposure (ppm-yr)			Age, years since hire, 1,3-butadiene	
			0	7	1		
			> 0 to < 8.3	18	1.2 (0.4–3.7)		
			8.3 to < 31.8	19	1.4 (0.4–4.5)		
			31.8 to < 61.1	18	1.9 (0.6–6.5)		
			≥ 61.1	19	1.3 (0.4–4.3)		

Table 2.2 (continued)

Reference, location, enrolment/ follow-up period	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Graff et al. (2005) (cont.)			Cumulative exposure (ppm-yr)			Age, years since hire, 1,3-butadiene, DMDTC	
			0	7	1		
			> 0 to < 8.3	18	0.6 (0.2–2.2)		
			8.3 to < 31.8	19	0.7 (0.2–2.5)		
			31.8 to < 61.1	18	0.8 (0.2–3.1)		
			≥ 61.1	19	0.5 (0.1–2.0)		
Sathiakumar & Delzell (2009) USA and Canada 1943–2002	4863 women who were employed for at least 1 d before the close of cohort ascertainment for the study of male synthetic rubber workers at the same plants (31 December 1991), who had been at work during the period when her plant systematically retained the personnel records of former workers and who had personnel records providing identifying and work history information Exposure assessment method: as for Graff et al. (2005)	All cancers combined	All workers: synthetic rubber	374	0.92 (0.83–1.02)	Race, age, calendar period	Limitations: few exposed women (31%) with lower levels of exposure compared with men (exposed men had 7.6× higher median styrene exposure); styrene exposure strongly correlated with 1,3-butadine exposure; no smoking information
			Ever hourly: synthetic rubber	139	1.01 (0.85–1.19)	As above	
		MM	All workers: synthetic rubber	7	0.89 (0.36–1.83)	As above	
			Ever hourly: synthetic rubber	3	0.91 (0.19–2.67)	As above	
		NHL	All workers: synthetic rubber	15	1.05 (0.59–1.73)	As above	
			Ever hourly: synthetic rubber	7	1.54 (0.62–3.17)	As above	
		Leukaemia	All workers: synthetic rubber	10	0.78 (0.38–1.44)	As above	
			Ever hourly: synthetic rubber	2	0.46 (0.06–1.64)	As above	

Table 2.2 (continued)

Reference, location, enrolment/ follow-up period	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Sathiakumar & Delzell (2009) (cont.)		HL	All workers: synthetic rubber	1	0.63 (0.02–3.49)	As above	
			Ever hourly: synthetic rubber	0	0 (0–0)	Not applicable	
		Lymphatic and haematopoietic (all combined)	All workers: synthetic rubber	34	0.95 (0.66–1.33)	Race, age, calendar period	
			Ever hourly: synthetic rubber	12	0.99 (0.51–1.74)	As above	
		Oesophagus	All workers: synthetic rubber	3	0.70 (0.14–2.04)	As above	
			Ever hourly: synthetic rubber	0	0 (0–0)	Not applicable	
		Pancreas	All workers: synthetic rubber	14	0.69 (0.38–1.15)	Race, age, calendar period	
			Ever hourly: synthetic rubber	6	0.80 (0.29–1.73)	As above	
		Larynx	All workers: synthetic rubber	0	0 (0–0)	Not applicable	
			Ever hourly: synthetic rubber	0	0 (0–0)	Not applicable	
		Lung	All workers: synthetic rubber	106	1.14 (0.93–1.38)	Race, age, calendar period	
			Ever hourly: synthetic rubber	47	1.59 (1.17–2.11)	As above	

Table 2.2 (continued)

Reference, location, enrolment/ follow-up period	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Sathiakumar & Delzell (2009) (cont.)		Breast	All workers: synthetic rubber	72	0.97 (0.76–1.22)	As above	
			Ever hourly: synthetic rubber	18	0.76 (0.45–1.21)	As above	
		Kidney	All workers: synthetic rubber	2	0.29 (0.04–1.05)	As above	
			Ever hourly: synthetic rubber	0	0 (0–0)	Not applicable	
		Urinary bladder	All workers: synthetic rubber	8	1.74 (0.75–3.43)	Race, age, calendar period	
			Ever hourly: synthetic rubber	6	3.32 (1.22–7.23)	As above	
Sathiakumar et al. (2009) Canada and USA 1943–2002 (women); 1944–1998 (men)	4101 (women) and 15 958 (men); exclusions from the original cohorts were (i) 352 women and 1345 men who worked at two of the eight plants that originally were studied; and (ii) 410 women and 621 men who dropped out of follow-up at ages younger than the youngest lung cancer decedent Exposure assessment method: as for Graff et al. (2005)	Lung	Men ever exposed styrene: synthetic rubber	NR	1.0 (0.76–1.24)	Age, year of birth, race, years since hire, plant, and pay status	Strengths: large cohort and long follow-up; internal comparisons by exposure metrics Limitations: 21% of the cohort were actively employed in 1991 and their exposure estimates would therefore be incomplete after this date; no smoking information; styrene exposure strongly correlated with 1,3-butadiene exposure
		Lung	Women ever exposed styrene: synthetic rubber	NR	1.64 (1.02–2.65)		

Table 2.2 (continued)

Reference, location, enrolment/ follow-up period	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Sathiakumar et al. (2015) North America 1944–2009	16 579 male workers employed before 1 January 1992 for at least 1 yr at any of the six synthetic rubber plants located in Texas, Louisiana, Kentucky, and Canada, and for whom detailed work histories and historical exposure information were available Exposure assessment method: as for Graff et al. (2005)	Leukaemia	0 yr lagged: cumulative exposure to styrene (RR at 25 ppm-yr)			Age, race, year of birth, plant	Strengths: large cohort with long follow-up Limitations: 21% of the cohort were actively employed in 1991, and their exposure estimates would therefore be incomplete after the date; styrene exposure strongly correlated with 1,3-butadiene exposure; no control for smoking
			Log-log at 25 ppm-yr	114	[2.99 (1.46–6.12)]		
			Trend test *P* value, < 0.01				
		NHL	0 yr lagged: cumulative exposure to styrene (RR at 25 ppm-yr)				
			Log-log at 25 yr	89	[1.51 (0.93–2.45)]		
			Trend test *P* value, 0.10				
		MM	0 yr lagged: cumulative exposure to styrene (RR at 25 ppm-yr)				
			at 25 ppm-yr	48	[0.84 (0.66–1.06)]		
			Trend test *P* value, 0.14				

ALL, acute lymphoblastic/lymphocytic leukaemia; AML, acute myeloid leukaemia; CI, confidence interval; CLL, chronic lymphocytic leukaemia; CML, chronic myeloid leukaemia; d, day(s); DMDTC, dimethyldithiocarbamate; HL, Hodgkin lymphoma; JEM, job-exposure matrix; MM, multiple myeloma; NHL, non-Hodgkin lymphoma; NR, not reported; ppm, parts per million; RR, relative risk; yr, year(s).

operation. Researchers from the Johns Hopkins School of Hygiene and Public Health established a cohort from eight of these plants (Matanoski & Schwartz, 1987; Matanoski et al., 1990, 1993, 1997; Santos-Burgoa et al., 1992), and researchers from the National Institute for Occupational Safety and Health (NIOSH) established a cohort from the remaining two plants (Meinhardt et al., 1982). In the mid-1990s, researchers from the University of Alabama combined and analysed workers from seven of the eight plants from the Johns Hopkins cohort studies and the two plants from the NIOSH cohort studies (Delzell et al., 1996, 2001; Macaluso et al., 1996; Sathiakumar et al., 1998). The cohort size analysed in these initial studies varied between 13 000 and 17 000, depending on the various inclusion criteria, but all studies were restricted to males employed for at least 1 year in the North American SBR industry between 1943 and 1991, with mortality updated until 1991. More recent studies of this pooled cohort have updated mortality until as late as 2009 (e.g. Sathiakumar et al., 2015), and have begun considering the approximately 5000 women employed for at least 1 day between 1943 and 1991. These more recent studies will be the focus of the remainder of this section.

Macaluso et al. (2004) assigned quantitative estimates of exposure to styrene, as well as the confounder 1,3-butadiene and the stopping agent dimethyldithiocarbamate (DMDTC), by identifying the component tasks, and historical changes in tasks, for each work area and/or job group at each plant. This exposure assessment was restricted to only six of the eight plants since two plants did not contain sufficiently specific work histories; as a result, exposure–response analyses were limited to 16 579 of the original 17 964 men. The results of this exposure assessment estimated that approximately 84% of the male cohort were exposed to styrene with a median exposure of 13 ppm-years and that approximately 77% of the male cohort were exposed to 1,3-butadiene with a median exposure of 54 ppm-years. The correlation coefficient for these two exposures was 0.79 (Graff et al., 2005).

The most recent overall standardized mortality ratio analysis for men (Sathiakumar et al., 2005) contains mortality data until 1998. Although the cohort has been updated until 2009 (Sathiakumar et al., 2015), this later study does not report overall external comparisons (i.e. SMRs) for the full cohort. In the 1998 follow-up of the cohort of 17 924 men, vital status was established for 97% of the cohort and all cancer mortality was lower than expected with 1608 cancer deaths (SMR, 0.92; 95% CI, 0.88–0.97) (Sathiakumar et al., 2005). However, mortality from cancer of the lymphoid and haematopoietic tissues was slightly elevated with 162 deaths (SMR, 1.06; 95% CI, 0.90–1.23), which included 71 leukaemia deaths (SMR, 1.16; 95% CI, 0.91–1.47). Mortality from all leukaemias was significantly elevated among workers who were employed in polymerization (18 deaths; SMR, 2.04; 95% CI, 1.21–3.22), coagulation (10 deaths; SMR, 2.31; 95% CI, 1.11–4.25), maintenance labour (15 deaths; SMR, 2.03; 95% CI, 1.14–3.35), and laboratory jobs (14 deaths; SMR, 3.26; 95% CI, 1.78–5.46), which are jobs the authors note have a high potential for exposure to styrene as well as to 1,3-butadiene. In addition to leukaemia, a slight excess of HL (12 deaths; SMR, 1.11; 95% CI, 0.58–1.95) was observed, whereas mortality from NHL, MM, and cancers of the larynx, oesophagus, stomach, kidney, prostate, pancreas, and bladder was not elevated, with standardized mortality ratios ranging from 0.8 to 1.1. There were significantly fewer deaths from cancer of the lung than expected (563 deaths; SMR, 0.91; 95% CI, 0.84–0.99).

Sathiakumar et al. (2015) updated the mortality of this cohort until 2009 and performed a Cox regression with age as the time scale, and further controlled for race, year of birth, and plant to assess the exposure–response relation between styrene exposure as well as 1,3-butadiene exposure, both as continuous variables, and mortality from leukaemia, NHL, and MM. This study was

restricted to the 16 579 men with calculated individual exposure metrics, and found a significant association between unlagged styrene exposure and risk of mortality from leukaemia (114 cases). [The Working Group used the β coefficients and standard errors reported in the manuscript to calculate a relative risk of 2.99 (95% CI, 1.46–6.12) from exposure to 25 ppm-years compared with 0 ppm-years from the best-fitting model.] A positive relationship was observed between exposure to styrene and risk of mortality from NHL (89 cases). [The Working Group calculated a relative risk of 1.51 (95% CI, 0.93–2.45) from exposure to 25 ppm-years compared with 0 ppm-years from the best-fitting model.] There did not appear to be a relationship between exposure to styrene and risk of mortality from MM (48 cases). [The Working Group calculated a relative risk of 0.84 (95% CI, 0.66–1.06) from exposure to 25 ppm-years compared with 0 ppm-years from the best-fitting model.] [The Working Group noted that no model considered exposure to both styrene and 1,3-butadiene; the study therefore provides no insight into the effect of exposure to styrene independently of exposure to 1,3-butadiene.]

In an earlier publication on this cohort, Graff et al. (2005) modelled exposure to styrene while also controlling for exposure to 1,3-butadiene as well as to DMDTC. This study additionally considered mortality from various subtypes of leukaemia. This earlier report followed up workers until 1998 and performed a Poisson regression, controlling for age and number of years since hire date. This study showed a positive relationship between unlagged styrene exposure and mortality from leukaemia (81 cases); however, this trend decreased when 1,3-butadiene was also added to the model. The increased relative risks for leukaemia without controlling for 1,3-butadiene were 1.0 (referent), 1.3 (95% CI, 0.6–3.2), 1.6 (95% CI, 0.7–3.9), 3.0 (95% CI, 1.2–7.1), and 2.7 (95% CI, 1.1–6.4) at cumulative styrene exposure levels of 0, more than 0 to less than 8.3, 8.3 to less than 31.8, 31.8 to less than 61.1, and 61.1 ppm-years or more, respectively. These relative risks decreased to 1.0 (referent), 1.2 (95% CI, 0.4–3.7), 1.4 (95% CI, 0.4–4.5), 1.9 (95% CI, 0.6–6.5), and 1.3 (95% CI, 0.4–4.3) when cumulative 1,3-butadiene exposure was included in the model. The association between exposure and response effectively disappeared when exposure to DMDTC was also included in the model. The subtypes of leukaemia considered in this study were CLL, AML, and CML. For cumulative styrene exposures of less than 8.3 ppm-years, 8.3 to less than 61.1 ppm-years, and 61.1 ppm-years or more, the relative risks for these analyses, without controlling for 1,3-butadiene or DMDTC, were: 1.0 (reference), 1.7 (95% CI, 0.7–4.4), and 2.6 (95% CI, 0.9–7.3) for CLL; 1.0 (referent), 1.9 (95% CI, 0.8–4.4), and 1.0 (95% CI, 0.3–3.9) for AML; and 1.0 (referent), 2.1 (95% CI, 0.6–7.1), and 2.7 (95% CI, 0.7–10.9) for CML, respectively. Again, with the exception of AML, the relative risks decreased dramatically when models controlled for 1,3-butadiene and DMDTC; the results for AML were largely unchanged after this adjustment, yielding relative risks of 1.0 (referent), 2.1 (95% CI, 0.8–5.8), and 1.1 (95% CI, 0.2–5.6). [For the leukaemia subtype analyses, the only adjusted models presented controlled for DMDTC. The Working Group put more emphasis on the results adjusted for 1,3-butadiene rather than those adjusted for DMDTC. The models that controlled for DMDTC, hypothesized to affect metabolism, may have been overadjusted due to a lack of evidence of the carcinogenicity of DMDTC.]

Sathiakumar & Delzell (2009) were the first to investigate women from this cohort, and considered 4863 women who had worked at a synthetic rubber plant for at least 1 day between 1943 and 1991. Mortality was updated for these women until 2002. Women in this cohort tended to be exposed to styrene at lower concentrations than men, with a median cumulative exposure of 1.9 ppm-years (Sathiakumar et al., 2009). Deaths

from any cancer were lower than expected, with 374 deaths (SMR, 0.92; 95% CI, 0.83–1.02). There was no elevation in overall cancer of the lymphoid and haematopoietic tissues, or for NHL, HL, leukaemia, or MM. The authors noted that those workers ever employed with an hourly payroll designation were most likely to have been exposed to higher concentrations of styrene; among this subcohort, there was a non-significant elevation of NHL with 7 deaths (SMR, 1.54; 95% CI, 0.62–3.17), a significant elevation of cancer of the lung with 47 deaths (SMR, 1.59; 95% CI, 1.17–2.11), and a significant elevation of cancer of the bladder with 6 deaths (SMR, 3.32; 95% CI, 1.22–7.23).

Sathiakumar et al. (2009) further investigated the elevation of risk of cancer of the lung found among women by fitting an exposure–response Cox regression model. The study also extended the analysis of mortality from cancer of the lung to men. Men did not show evidence of an association between exposure to styrene and mortality from cancer of the lung; comparing person-time ever exposed to styrene versus never exposed to styrene provided a relative risk of 1.0 (95% CI, 0.76–1.24). In contrast, women did show an increased risk of cancer of the lung associated with styrene exposure; a comparison of person-time ever exposed to styrene versus never exposed to styrene yielded a relative risk of 1.64 (95% CI, 1.02–2.65). [A limitation of this analysis was the lack of information on smoking as a possible confounder. In addition, the Working Group noted that the different inclusion criteria could have explained the discrepancy in results between men and women. Overall, the strengths of the analyses of this cohort were its size and long follow-up time. A major limitation was the high correlation of exposure to styrene with other confounding exposures, namely 1,3-butadiene, which is classified as Group 1 (*carcinogenic to humans*) as a risk factor for cancers of the haematolymphatic organs. Further, 21% of the cohort were employed at the time of records collection in 1991, with incomplete information on exposure after this date.]

(c) Styrene monomer and polymers industry

See Table 2.3.

Four cohort studies of workers in the styrene monomer and polymers industry were identified. This industry is known to incur exposures to lower concentrations of styrene and has a more stable workforce than for the reinforced plastics industry, which has a high proportion of short-term workers. Three of these studies only resulted in a single publication each with no follow-up papers; the fourth study resulted in an initial paper (Ott et al., 1980) and a later follow-up (Bond et al., 1992). The most recent paper was published 25 years ago, and no new papers have been published since styrene was first considered by an IARC Working Group. All of the papers, except for the Nicholson et al. (1978) paper, were included in the previous *IARC Monograph* published in 2002.

The most informative cohort study was the largest, which comprised 2904 male workers who were employed for at least 1 year at one of four plants in the USA where styrene-based products were being developed and produced. The cohort members were identified from census lists of employees starting work from 1937, and mortality was followed up from 1940 to 1 January 1976 (Ott et al., 1980). Bond et al. (1992) extended the follow-up of this cohort by 11 years to the end of 1986, by which time the average follow-up was 31 years. The level of exposure to styrene varied by process; an industrial hygienist assigned all manufacturing jobs an exposure intensity with respect to five chemical exposures (e.g. styrene, 1–4 ppm or ≥ 5 pm). Other chemicals that workers were exposed to at the plants included benzene, acrylonitrile, 1,3-butadiene, ethylbenzene, dyes, and pigments.

Bond et al. (1992) found that overall mortality from cancer for the whole cohort was significantly reduced (162 observed deaths; SMR, 0.81;

Table 2.3 Occupational cohort studies on exposure to styrene in the styrene monomer and polymers industries

Reference, location, enrolment/ follow-up period	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Bond et al. (1992) USA 1937–1986	2904 male workers who were potentially exposed to styrene and related materials for 1 yr or more during 1937–1971 Exposure assessment method: records	Leukaemia	All workers: styrene production	9	1.18 (0.54–2.24)	Age, calendar year	Strengths: long follow-up Limitations: small number of deaths; multiple exposures
		Lymphatic and haematopoietic (all)	All workers: styrene production	28	1.44 (0.95–2.08)		
		HL	All workers: styrene production	5	2.22 (0.71–5.18)		
		MM	All workers: styrene production	7	1.84 (0.74–3.80)		
		NHL	All workers: styrene production	7	1.17 (0.47–2.40)		
		Lymphoma (type not specified)	All workers: styrene monomer and finishing	5	1.28		
			Polymerization, colouring, and extrusion	16	1.72		
		Oesophagus	All workers: styrene production	3	0.63 (0.13–1.85)		
		Stomach	All workers: styrene production	11	1.27 (0.64–2.28)		
		Pancreas	All workers: styrene production	5	0.49 (0.16–1.13)		
		Larynx	All workers: styrene production	1	NR		
		Lung	All workers: styrene production	56	0.81 (0.61–1.05)		
		Prostate	All workers: styrene production	10	0.85 (0.41–1.57)		
		Kidney	All workers: styrene production	5	0.98 (0.32–2.30)		

Table 2.3 (continued)

Reference, location, enrolment/follow-up period	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Bond et al. (1992) (cont.)		Urinary bladder	All workers: styrene production	2	NR		
		Lymphatic and haematopoietic (all)	Relative to unexposed workers from non-styrene plant: styrene production	28	1.39 (0.92–2.08)	Age, interval since entry, pay status	
		HL	Relative to unexposed workers from non-styrene plant: styrene production	5	2.43 (0.94–6.28)		
		NHL	Relative to unexposed workers from non-styrene plant: styrene production	7	1.09 (0.48–2.49)		
		MM	Relative to unexposed workers from non-styrene plant: styrene production	7	2.45 (1.07–5.65)		
		Leukaemia	Relative to unexposed workers from non-styrene plant: styrene production	9	1.18 (0.58–2.39)		

Table 2.3 (continued)

Reference, location, enrolment/ follow-up period	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Hodgson & Jones (1985) England 1945–1974	622 men; 131 exposed to styrene among others chemicals in laboratories and 491 with mixed chemical exposures and a specific potential exposure to styrene in the production, polymerization, and manufacture of products Exposure assessment method: records	All cancers combined	All workers: styrene production	10	0.9	Age, calendar year, duration of exposure	No relationship between length of employment in jobs with exposure to styrene and risk of cancer Limitations: small cohort; mixed exposures; no information on smoking status
		Lymphoma (type not specified)	All workers: styrene production	3	[5.40 (1.10–16.0)]	Age, time since first exposure, duration of exposure	
		Lymphohaematopoietic	All workers: styrene production	4	[2.50 (0.67–6.40)]		
		Larynx	All workers: styrene production	3	[6.0 (1.20–1.80)]		
		Lung	All workers: styrene production	5	[1.20 (0.39–2.80)]		
Nicholson et al. (1978) USA 1960–1975	560 male workers employed for > 5 yr were considered for inclusion in a cohort in which each individual would be followed prospectively from 1 May 1960 or upon attaining his 10th anniversary of employment Exposure assessment method: records	Leukaemia	All workers: styrene production	1	[1.26 (0.03–7.05)]	Age, years since hire	Limitations: small cohort with short follow-up; complex mix of exposures; potential for confounding from co-exposures
		Lung	All workers: styrene production	6	[0.86 (0.32–1.87)]		

Table 2.3 (continued)

Reference, location, enrolment/ follow-up period	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Frentzel-Beyme et al. (1978) Germany 1931–1976	1960 workers in styrene and polystyrene manufacture facility; employed for > 1 mo during 1931–1976 Exposure assessment method: records	All cancers combined	All workers: styrene production	12	–	Age, duration of exposure	Strengths: long follow-up Limitations: follow-up incomplete for non-German workers; low statistical power; no individual exposure data; potential for confounding by co-exposures to known carcinogens
		Pancreas	All workers: styrene production	2	–		
		Lung	All workers: styrene production	3	–		

CI, confidence interval; HL, Hodgkin lymphoma; MM, multiple myeloma; mo, month(s); NHL, non-Hodgkin lymphoma; NR, not reported; yr, year(s).

95% CI, 0.69–0.95). An increased, but not statistically significant, standardized mortality ratio was observed for all deaths from cancer of the lymphoid and haematopoietic tissues (28 deaths; SMR, 1.44; 95% CI, 0.95–2.08). This was contributed to by small to moderate non-significant excesses across all types of cancer of the lymphoid and haematopoietic tissues, with the highest for HL (SMR, 2.22; 95% CI, 0.71–5.18), but this was based on only 5 deaths. There were 7 deaths from NHL (SMR, 1.17; 95% CI, 0.47–2.40), 7 deaths from MM (SMR, 1.84; 95% CI, 0.74–3.80), and 9 deaths from leukaemia and aleukaemia (SMR, 1.18; 95% CI, 0.54–2.24). A similar pattern of small to moderate non-statistically significant excesses of deaths from cancer of the lymphoid and haematopoietic tissues was seen for workers with the job titles of "polymerization, colouring, and extrusion" and "styrene monomer and finishing". Further analyses of the deaths from cancer of the lymphoid and haematopoietic tissues by duration of exposure, exposure intensity, and lag periods revealed no clear patterns. [These analyses were limited by the small numbers.]

When unexposed workers from another non-styrene plant were used as the reference group, a statistically significant increase in risk for MM (RR, 2.45; 95% CI, 1.07–5.65) was observed, as well as a small non-significant increase in risk of death from cancer of the lymphoid and haematopoietic tissues (RR, 1.39; 95% CI, 0.92–2.08) and a moderate non-significant increase in risk of death from HL (RR, 2.43; 95% CI, 0.94–6.28). [No analyses were adjusted for other known lymphohaematopoietic carcinogens at these plants, implying that confounding cannot be ruled out as an explanation for the mortality results from cancer of the lymphoid and haematopoietic tissues.]

Cancer of the stomach was the only other cancer type to have an excess of deaths (11) with a standardized mortality ratio of 1.27 (95% CI, 0.64–2.28). No elevated risk was observed for cancer of the lung (56 deaths; SMR, 0.81; 95% CI, 0.61–1.05), kidney (5 deaths; SMR, 0.98; 95% CI, 0.32–2.30), oesophagus (3 deaths; SMR, 0.63; 95% CI, 0.13–1.85), prostate (10 deaths; SMR, 0.85; 95% CI, 0.41–1.57), and pancreas (5 deaths; SMR, 0.49; 95% CI, 0.16–1.13). There were only 2 deaths from cancer of the bladder (no SMR was estimated).

Nicholson et al. (1978) conducted a mortality study of 560 workers who had been employed at a styrene monomer and polymerization plant in the USA for at least 5 years. Workers were followed from 1 May 1960 or from the 10th anniversary of their employment to the end of December 1975. NIOSH measurements in 1974 showed exposure to styrene at concentrations of less than 1 ppm in low-concentration areas and at 5–20 ppm in the high-concentration areas. Workers were also exposed to other chemicals at the plant, including ethylbenzene, toluene, xylene, and benzene; benzene was produced at the plant from 1943 to 1962 to form ethylbenzene, resulting in potentially significant exposure to benzene for longer-term workers in the cohort.

Seventeen deaths from cancer were observed in the cohort during the follow-up period, less than the 21 expected. The numbers of observed (expected) deaths were 6 (6.99) from cancer of the lung, 1 (0.79) from leukaemia, and 1 (1.25) from lymphoma. An analysis by type of employment found that the number of observed cancer deaths was half that of the expected number (4 vs 8.17) for production and polymerization workers in the plant. No analyses were conducted for specific types of cancer death, as numbers were too small. [The Working Group noted that this study had too few cancer deaths to be informative for overall cancer deaths or for deaths from any specific cancer type. There was also potential for confounding by co-exposures to known carcinogens in the plants.]

Frentzel-Beyme et al. (1978) studied 1960 workers engaged in the manufacture of styrene and polystyrene polymers in Germany for longer

than 1 month between 1931 and the end of 1975. The percentage of those workers who were followed up was much lower for non-German workers (29%) than for German workers (93%); however, non-German workers tended to be shorter-term workers, with almost half of them exposed for less than 6 months. Workers were exposed to styrene at concentrations that were generally less than 1 ppm, according to measurements in 1975 and 1976. However, higher concentrations were occasionally recorded and no information was available on concentrations of exposure in earlier years when open systems were used (Thiess & Friedheim, 1978).

A total of 12 deaths from cancer were observed. A higher than expected number of deaths from cancer of the pancreas was observed, but the numbers were small (2 observed vs 0.72 expected) and the excess was not statistically significant. Deaths from cancer of the lung were lower than expected (3 observed vs 5.4 expected). Other analyses by exposure duration and age groups involved too few numbers to be informative. [This study had too few cancer deaths to be informative for overall cancer deaths or for deaths from any specific cancer type. There was also a potential for confounding by co-exposures to known carcinogens in the plants.]

Hodgson & Jones (1985) reported on the mortality of a cohort of 622 men who had worked for at least 1 year in the production, polymerization, and processing of styrene at a chemical site in England between 1945 and 1974. The workers were followed up until the end of 1978. A cohort of 3072 male workers who worked at the same site but were not exposed to styrene was used as a reference group. No concentrations of styrene exposure were available, but the authors stated that the styrene process was enclosed and they believed that the concentrations of exposure were well below 100 ppm, the hygiene standard at the time. Other chemicals that the workers were possibly exposed to at the plant included 1,3-butadiene, acrylonitrile, benzene, dyes, and ethylene oxide.

Ten cancer deaths were observed (10.9 expected) among the workers exposed to styrene (SMR, 0.90; no 95% CI provided). A statistically significant excess of deaths from lymphoma was observed (3 vs 0.56 expected). [Although the standardized mortality ratio was not provided in the Hodgson & Jones (1985) paper, this was calculated for the previous *IARC Monograph* in 2002 as 5.40 (95% CI, 1.10–16.0).]

An analysis of cancer registrations for this cohort until the end of 1981 showed no overall excess of the incidence of cancer, with 22 observed versus 23.7 expected. A total of 4 incident cases of cancer of the lymphoid and haematopoietic tissues was found, with a standardized incidence ratio (calculated for the 2002 *IARC Monograph*) of 2.50 (95% CI, 0.67–6.40). Three of these cases were from lymphoma, which was significantly more than expected (SIR, 3.75; *P* value for trend, 0.047). In addition, 3 incident cases of cancer of the larynx were observed, which was significantly higher than the number expected (SIR, 6.0; 95% CI, 1.20–18.0) (calculated for the 2002 *IARC Monograph*). [This study had too few cancer deaths and cases to be informative for overall cancer or any specific cancer site. There was also a potential for confounding by co-exposures to known carcinogens in the plants.]

2.2.2 General-population cohort studies

There were no population-based cohort studies with information on styrene exposure available to the Working Group.

2.3 Case–control studies

See Table 2.4.

Several case–control studies have investigated the association between workplace exposure to styrene and the risk of various cancers. Cancers of the lymphoid and haematopoietic

Table 2.4 General-population case–control studies on exposure to styrene

Reference, location, enrolment/follow-up period	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Flodin et al. (1986) Sweden 1977–1982	Cases: 59 incident from medical clinics and a hospital cytology department Controls: 354 from general-population register, matched by sex, age, and parish (n = 236) or randomly selected (n = 118) Exposure assessment method: expert judgement; mailed questionnaire eliciting leisure and occupational activities (17 questions); quantitative assessment (five categories) based on judgement; nine exposures evaluated	Leukaemia (AML, ICD 1965 205.00)	Ever exposed	3	18.9 (1.9–357.0)	Time of diagnosis or selection	Men and women aged 20–70 yr; 0.3% of controls exposed to styrene Limitations: few exposed subjects; low response rate among cases (~50%); eligible patients too ill to participate or deceased excluded (no proxies); self-reported nature of exposure circumstances
Cantor et al. (1995) USA (24 states) 1984–1989	Cases: 29 397 White women and 4112 Black women; from death certificates Controls: 102 955 White women and 14 839 Black women; from death certificates (excluding cancers) Exposure assessment method: records; usual occupation and industry from death certificate; population JEM to assign probability (0–4) and level (0–3) of exposure; JEM constructed from professional judgment based on literature (NIOSH JEM, IMIS-OSHA)	Breast (ICD 174)	White women: probability of exposure			Age, SES (based on occupation on death certificate)	Similar findings for level of exposure after excluding subjects with low probability of exposure; among controls, 4.9% of White women and 2.6% of Black women were exposed to styrene Strengths: large sample Limitations: no adjustment for known breast cancer risk factors; use of death certificates as primary source of information; use of usual occupation; later jobs held may be in higher SES levels; no information on duration and latency
			1	804	1.13 (1.00–1.20)		
			2	527	1.18 (1.10–1.30)		
			3	64	1.38 (1.00–1.90)		
			4	4	NR		
			White women: level of exposure				
			1	807	1.16 (1.10–1.30)		
			2	522	1.13 (1.00–1.30)		
			3	70	1.19 (0.90–1.60)		
			Black women: probability of exposure				
			1	80	1.49 (1.10–2.00)		
			2	61	1.52 (1.10–2.10)		
			3	7	1.32 (0.50–3.30)		
			4	2	NR		
			Black women: level of exposure				
			1	87	1.59 (1.20–2.10)		
			2	63	1.41 (1.00–1.90)		

Table 2.4 (continued)

Reference, location, enrolment/ follow-up period	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Gérin et al. (1998) Montreal, Canada 1979–1986	Cases: 3730 incident cases (15 types), ascertained across hospitals Controls: 1066: 533 population controls from electoral lists and 533 other cancers (excluding lung) Exposure assessment method: expert judgement; lifetime job history from interview; expert-based assessment assigning frequency and intensity of exposure categorized as low, medium, or high exposure (converted to 1, 4, 9 scores); cumulative exposure (product of intensity, frequency, and duration) was expressed as low, medium, and high, defined by cut-points at the 70th and 90th percentile of the distribution among exposed; 294 agents evaluated	NHL (ICD9 200, 202)	Ever exposed	8	2.0 (0.8–4.8)	Age, family income, ethnic group, cigarette smoking, proxy status	Men aged 35–70 yr; other cancers included oesophagus, stomach, pancreas, kidney, melanoma, HL, and lung oat-cell and adenocarcinoma subtypes (< 5 exposed cases); lifetime prevalence of exposure to styrene was 2% with 45% of exposures in the high confidence level Strengths: expert-based assessment; adjustment for several potential confounders Limitations: few exposed subjects
		Colon (ICD9 153)	Ever exposed	11	1.2 (0.6–2.5)	Age, family income, ethnic group, cigarette smoking, proxy status	
		Rectum (ICD9 154)	Cumulative exposure				
			Low	4	1.0 (0.3–2.9)		
			Medium/high	5	5.1 (1.4–19.4)		
		Lung (ICD9 162)	Cumulative exposure				
			Low	5	0.3 (0.1–0.9)		
			Medium/high	5	0.9 (0.2–3.3)		
		Lung (SCC)	Ever exposed	6	0.7 (0.3–1.9)	Age, family income, ethnic group, cigarette smoking, proxy status, exposure to arsenic, asbestos, chromium VI, nickel, crystalline silica, beryllium, cadmium, and PAHs	
		Prostate (ICD9 185)	Cumulative exposure			Age, family income, ethnic group, cigarette smoking, proxy status	
			Low	5	1.0 (0.4–2.9)		
			Medium/high	7	5.5 (1.4–21.8)		

Table 2.4 (continued)

Reference, location, enrolment/ follow-up period	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Gérin et al. (1998) (cont.)		Urinary bladder (ICD9 188)	Cumulative exposure			Age, family income, ethnic group, cigarette smoking, proxy status, exposure to aromatic amines	
			Low	9	1.0 (0.4–2.4)		
			Medium/high	3	0.7 (0.2–2.6)		
Dumas et al. (2000) Montreal, Canada 1979–1986	Cases: 257 incident cases ascertained across hospitals Controls: 1295 other cancers, excluding lung and adjacent intestinal sites Exposure assessment method: expert judgement; interview with lifetime job history; exposure assessment assigning frequency and intensity of exposure categorized as low, medium, or high exposure; substantial cumulative exposure defined as > 5 yr exposure at medium or high concentration and frequency; 294 agents evaluated	Rectum (ICD9 154)	Cumulative exposure			Age, education, respondent status, cigarette smoking, beer consumption, BMI	Expansion of Gérin et al. (1998) and Siemiatycki (1991); men aged 35–70 yr; results shown are based on cancer controls; analyses were also conducted using a population control series (*n* = 533); unexposed subjects included possible exposures and exposures only in recent 5 yr; 5-yr lag applied Strengths: expert-based assessment; adjustment for several potential confounders Limitations: few exposed subjects; no information on diet
			Any level	6	1.7 (0.7–4.5)		
			Substantial level	5	3.9 (1.2–12.9)		

Table 2.4 (continued)

Reference, location, enrolment/ follow-up period	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Scélo et al. (2004) Europe (Czechia, Hungary, Poland, Romania, Russian Federation, Slovakia, United Kingdom) 1998–2002	Cases: 2861 incident from hospitals and a dispensary Controls: 3118 from hospitals (excluding cancers and tobacco-related diseases) and from population registers (2 centres) Exposure assessment method: expert judgement; lifetime job history (≥1 yr) from interview; expert-based exposure assessment to assign confidence (low, medium, high), intensity (< 5 ppm, 5–50 ppm, > 50 ppm for styrene), and frequency (1–5%, > 5–30%, > 30%); 70 agents evaluated	Lung (NR)	Ever exposed	51	0.70 (0.42–1.18)	Centre, sex, age, tobacco consumption, vinyl chloride, acrylonitrile, formaldehyde, inorganic pigments in dust	Men (75%) and women (25%); the proportion of ever-exposed controls was 1.5% Strengths: expert-based assessment; high response rates; ability to control for smoking and other potential confounders; several sensitivity analyses (20-yr lag, restricting to exposures with high confidence level) Limitations: few exposed subjects
			Duration (yr)				
			1–6	13	0.98 (0.37–2.61)		
			7–14	19	0.72 (0.33–1.59)		
			> 14	19	0.59 (0.26–1.34)		
			Weighted duration (yr)				
			0.01–0.50	13	0.67 (0.28–1.56)		
			0.51–3.00	21	1.19 (0.52–2.73)		
			> 3.00	17	0.38 (0.13–1.03)		
			Cumulative exposure (ppm-yr)				
			0.01–2.75	22	1.15 (0.55–2.41)		
			2.76–12.50	9	0.37 (0.13–1.08)		
			> 12.50	20	0.53 (0.20–1.43)		

Table 2.4 (continued)

Reference, location, enrolment/ follow-up period	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Miligi et al. (2006) Italy 1991–1993	Cases: 1135 incident from hospitals or cancer registry Controls: 1246 from general-population demographic or health services files, frequency matched by sex, age group, and area Exposure assessment method: expert judgement; detailed occupational history; assigned probability (low, medium, high) and intensity (very low, low, medium, high) of exposure	NHL (NR)	Intensity			Sex, age, area, education	Men (52%) and women (48%) aged 20–74 yr; subjects with low probability of exposure were excluded; reference category were unexposed to any solvent; duration of exposure also analysed; DLBCL also included (3 exposed cases); associations between styrene exposure and Hodgkin disease not reported; the prevalence of exposure to styrene among controls (NHL series) was 2.2% Strengths: expert-based assessment; relatively high response rates; use of pathologic classification; 20% of cases and uncertain cases reviewed by pathologists for consistency Limitations: few exposed subjects
			Very low/low	9	0.7 (0.3–1.6)		
			Medium/high	14	1.3 (0.6–2.9)		
		NHL (NR)	Duration at medium/high level (yr)				
			≤ 15	9	1.3 (0.5–3.7)		
			> 15	4	NR		
		NHL (SLL/CLL)	Intensity				
			Medium/high	5	1.6 (0.5–5.1)		

Table 2.4 (continued)

Reference, location, enrolment/follow-up period	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Cocco et al. (2010) Europe (Czech Republic, France, Germany, Ireland, Italy, Spain) 1998–2004	Cases: 2348 incident recruited from hospitals Controls: 2462 from hospitals (excluding cancers and infectious or immunodeficient diseases) except in two countries in which general-population controls used (matched by sex, age group, residence area) Exposure assessment method: expert judgement; lifetime job history (≥1 yr) from interview; expert-based exposure assessment assigning confidence (based on probability of exposure and proportion of exposed workers), intensity (low, medium, high), and frequency (1–5%, > 5–30%, > 30%); 43 agents evaluated	NHL (B-cell lymphoma)	Ever exposed	66	1.6 (1.1–2.3)	Age, sex, education, centre	Epilymph study; men (55%) and women (45%); reference category were unexposed to any solvent; Bonferroni adjustment of estimates for cumulative exposure; duration also analysed; NHL (T-cell) also ascertained (2 exposed cases); prevalence of exposure to styrene in the study population: 2–3% Strengths: expert-based assessment; up-to-date pathological definitions; 20% of cases per centre reviewed by panel of pathologists for consistency; consideration of multiple comparisons Limitations: few exposed subjects; lower response rate (52%) among population controls; Bonferroni adjustment probably too conservative
			Cumulative exposure				
			Low	19	1.3 (0.7–2.4)		
			Medium	30	3.1 (1.6–5.9)		
			High	17	1.0 (0.5–1.9)		
			Trend test P value, 0.04				
		HL (NR)	Ever exposed	10	1.1 (0.5–2.3)		
			Cumulative exposure				
			Low	5	0.9 (0.3–2.6)		
			Medium	4	2.1 (0.6–7.0)		
			High	1	0.6 (0.1–4.4)		
			Trend test P value, 0.90				
		NHL (DLBCL)	Ever exposed	20	1.5 (0.9–2.5)		
			Cumulative exposure				
			Low	2	0.4 (0.1–1.6)		
			Medium	11	3.5 (1.5–7.8)		
			High	7	1.4 (0.6–3.4)		
			Trend test P value, 0.06				
		NHL (follicular)	Ever exposed	11	2.6 (1.3–5.2)		
			Cumulative exposure				
			Low	4	2.5 (0.8–7.7)		
			Medium	5	4.8 (1.7–13.9)		
			High	2	1.2 (0.3–5.3)		
			Trend test P value, 0.04				
		NHL (CLL)	Ever exposed	10	1.2 (0.6–2.5)		
			Cumulative exposure				
			Low	2	0.8 (0.2–3.3)		
			Medium	5	2.9 (1.0–8.5)		
			High	3	0.8 (0.2–2.7)		
			Trend test P value, 0.65				
		MM (NR)	Ever exposed	6	0.9 (0.4–2.2)		

Table 2.4 (continued)

Reference, location, enrolment/ follow-up period	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Karami et al. (2011) Central and eastern Europe (Czechia, Poland, Romania, Russian Federation) 1999–2003	Cases: 1097 incident from hospitals Controls: 1476 hospital controls (excluding conditions associated with smoking or genitourinary disorders) Exposure assessment method: expert judgement; lifetime job history (≥1 yr) from interview; expert-based assessment assigning confidence (< 40%, 40–90%, > 90% probability), intensity (low, medium, high), and frequency (1–5%, 5–30%, > 30%); 72 agents evaluated	Kidney (RCC, ICD C64)	Ever exposed	17	1.7 (0.8–3.6)	Age, sex, study centre, BMI, self-reported hypertension, smoking status, family history of cancer	Central and eastern European RCC study; men (62%) and women (38%) aged 20–88 yr; no association with duration or average exposure (not shown) or modification by genetic polymorphisms; 1.2% of controls were ever exposed to styrene Strengths: expert-based assessment; high response rates; ability to control for several potential confounders; several sensitivity analyses including 20-yr lag and restricting to exposures assessed with high confidence Limitations: few exposed subjects; hospital controls may be less representative of the general population
			Cumulative exposure				
			< median	NR	0.6 (0.2–1.7)		
			≥ median	NR	6.7 (1.8–24.3)		

Table 2.4 (continued)

Reference, location, enrolment/ follow-up period	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Heck et al. (2013) California, USA 1990–2007	Cases: 75 incident, children aged < 6 yr from cancer registry Controls: 14 602 population controls from birth records, frequency matched to cases by birth year Exposure assessment method: quantitative measurements; ambient air monitoring stations (n = 39); average exposure levels during pregnancy assigned to subjects living within a 5 km radius of home address or zip code listed on birth certificates; mean styrene exposure, 0.16 (SD, 0.12) ppbV; interquartile range, 0.14 ppbV	Brain: neuroblastoma (ICCC-3 041)	Per interquartile range increase			Birth year, mother's age and race/ethnicity, payment method for prenatal care (as proxy for SES and family income)	Air Pollution and Childhood Cancer study; matching rate of cases to birth registry to obtain address and other variables: 89%; analyses also considered other radii; maternal education and neighbourhood socioeconomic index had little influence on risk estimates Strengths: use of measurements and registry; no recall or selection bias; ability to study associations at several time points Limitations: limited number of cases; no individual exposure estimates; no information on other sources of exposure or on smoking; using home address does not take into account other locations; did not consider moves; use of zip code centroid for some subjects
			Entire pregnancy	48	1.22 (0.84–1.78)		
			First trimester	48	1.05 (0.76–1.43)		
			Second trimester	48	1.10 (0.83–1.47)		
			Third trimester	48	1.10 (0.96–1.26)		

Table 2.4 (continued)

Reference, location, enrolment/ follow-up period	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Heck et al. (2014) California, USA 1990–2007	Cases: 69 ALL and 46 AML incident children aged < 6 yr from cancer registry Controls: 2994 and 19 209 population controls from birth records, frequency matched to cases by birth year Exposure assessment method: quantitative measurements as in Heck et al. (2013); estimates assigned to subjects living within a 2 km (for ALL) or 6 km (for AML) radius from station	Leukaemia (ALL)	Per interquartile range increase			Birth year; maternal race/ ethnicity, birthplace, and parity; neighbourhood socioeconomic index	Matching rate of cases to birth registry to obtain address and other variables: 89%; sex, urban/ rural area of residence, maternal age, education, and payment method for prenatal care had little influence on risk estimates Strengths: as for Heck et al. (2013) Limitations: as for Heck et al. (2013)
			Entire pregnancy	46	0.87 (0.58–1.32)		
			First trimester	46	0.85 (0.60–1.19)		
			Second trimester	46	0.89 (0.65–1.22)		
			Third trimester	46	1.01 (0.83–1.23)		
			First year of life	36	0.97 (0.57–1.66)		
		Leukaemia (AML)	Per interquartile range increase				
			Entire pregnancy	36	1.38 (0.94–2.03)		
			First trimester	36	1.27 (0.92–1.75)		
			Second trimester	36	1.20 (0.86–1.68)		
			Third trimester	36	1.08 (0.92–1.27)		
			First year of life	21	1.63 (0.93–2.83)		
Heck et al. (2015) California, USA 1990–2007	Cases: 103 incident; children aged < 6 yr from cancer registry Controls: 30 601 population controls from birth records, frequency matched to cases by birth year Exposure assessment method: quantitative measurements as in Heck et al. (2013); estimates assigned to subjects living within a 5-mile radius from station	Eye: retinoblastoma (ICCC3 050)	Per interquartile range increase			Birth year, paternal age, maternal race and birthplace, payment method for prenatal care (as proxy for SES and family income)	Matching rate of cases to birth registry: 89% Strengths: as for Heck et al. (2013) Limitations: as for Heck et al. (2013)
			Entire pregnancy: all tumours	69	1.28 (0.96–1.69)		
			Unilateral tumours	51	1.35 (0.98–1.85)		
			Bilateral tumours	18	1.07 (0.59–1.93)		
			First year of life: all tumours	31	1.64 (1.12–2.39)		

Table 2.4 (continued)

Reference, location, enrolment/ follow-up period	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
von Ehrenstein et al. (2016) California, USA 1990–2007	Cases: 34 medulloblastoma, 43 PNET, 106 astrocytoma; incident; children aged < 6 yr from cancer registry Controls: 30 569 population controls from birth records, frequency matched to cases by birth year Exposure assessment method: quantitative measurements as in Heck et al. (2013); estimates assigned to subjects living within a 5-mile radius from station	Brain (childhood cancer: PNET, ICD-O 9473)	Per interquartile range increase			Birth year, maternal age, race/ethnicity, education, and birthplace	Matching rate of cases to birth registry: 89%; type of insurance (proxy for SES), rural/urban residence, sex, parity and pre-term birth had little influence on risk estimates Strengths: as for Heck et al. (2013) Limitations: as for Heck et al. (2013)
			Entire pregnancy	29	1.31 (0.88–1.94)		
			First trimester	29	1.31 (0.99–1.73)		
			Second trimester	29	1.24 (0.94–1.64)		
			Third trimester	29	0.99 (0.69–1.43)		
			First year of life	21	1.27 (0.72–2.25)		
		Brain (childhood cancer: medulloblastoma, ICD-O 9470)	Entire pregnancy	25	0.95 (0.56–1.62)		
			First year of life	14	0.96 (0.43–2.14)		
		Brain (childhood cancer: astrocytoma, ICCC3 032)	Entire pregnancy	67	0.73 (0.51–1.04)		
			First year of life	47	0.70 (0.42–1.17)		

ALL, acute lymphoblastic/lymphocytic leukaemia; AML, acute myeloid leukaemia; BMI, body mass index; CI, confidence interval; CLL, chronic lymphocytic leukaemia; DLBCL, diffuse large B-cell lymphoma; HL, Hodgkin lymphoma; ICCC, International Classification of Childhood Cancer; ICD, International Classification of Diseases; ICD-O, International Classification of Diseases for Oncology; IMIS, Integrated Management Information System; JEM, job-exposure matrix; MM, multiple myeloma; NHL, non-Hodgkin lymphoma; NIOSH, National Institute for Occupational Safety and Health; NR, not reported; OSHA, Occupational Safety and Health Administration; PAH, polycyclic aromatic hydrocarbon; PNET, primitive neuroectodermal tumour; ppbV, parts per billion by volume; ppm, parts per million; RCC, renal cell carcinoma; SCC, squamous cell carcinoma; SD, standard deviation; SES, socioeconomic status; SLL, small lymphocytic lymphoma; yr, year(s).

tissues, as well as renal cell carcinoma and cancer of the lung, have received particular attention. The Epilymph study, a multicentre case–control study of lymphoma conducted in six European countries (Czechia, France, Germany, Ireland, Italy, and Spain), included 2348 men and women diagnosed during 1998–2004 (Cocco et al., 2010). Controls (n = 2462) were recruited from hospitals (excluding other cancers, infectious diseases, and immune-deficient diseases), except for in Germany and Italy where general-population controls were used, and frequency matched to cases by age, sex, and residence area. Lifetime occupational histories covering all jobs held for at least 1 year were obtained by interviews and from standardized questionnaires. Experts then coded semiquantitative levels of confidence, intensity, and frequency of exposure to 43 agents for each job. Several meetings were held to evaluate and standardize the exposure assessment between centres. A total of 78 cases (3.3%) and 58 controls (2.4%) were considered ever exposed to styrene. Statistical models did not adjust for occupational co-exposures. However, the potential for confounding by other solvents was limited; for instance, only 12% of subjects exposed to styrene were also exposed to benzene, toluene, or xylene. Increased risk of B-cell NHL (OR, 1.6; 95% CI, 1.1–2.3; 66 exposed cases) and follicular lymphoma (OR, 2.6; 95% CI, 1.3–5.2; 11 exposed cases) was observed for ever exposure to styrene. Analyses by categorical cumulative exposure, which applied a Bonferroni adjustment, found increased risks for exposure to medium concentrations for B-cell (OR, 3.1; 95% CI, 1.6–5.9; 30 exposed cases), diffuse large B-cell (OR, 3.5; 95% CI, 1.5–7.8; 11 exposed cases) and follicular (OR, 4.8; 95% CI, 1.7–13.9; 5 exposed cases) lymphomas. Associations between exposure to styrene and T-cell lymphoma were presented, but based on only 2 exposed cases. The German sample of the Epilymph study was the object of an earlier analysis (Seidler et al., 2007), and is reported here as part of the study by Cocco et al. (2010). [The Working Group noted that the strengths of the study included the expert exposure assessment and the attention to the pathological classification of cases. However, the study was limited by the lower participation rate among controls, the high proportion of hospital controls (42%), and the few exposed cases, especially at high concentrations. Co-exposures such as benzene were not adjusted for, although the overlap in exposure with styrene was small. Applying the Bonferroni corrections was probably too stringent. Information provided on occupations exposed to styrene or on benchmark occupations used to assign intensity of exposure was sparse. Finally, the Working Group noted the unusually high prevalence of exposure (24%) among controls in the German study (Seidler et al., 2007).]

The associations between occupational exposure to solvents and lymphohaematopoietic malignancies have been investigated in a multicentre study conducted in Italy during 1991–1993 (Seniori Costantini et al., 2001, 2008; Miligi et al., 2006). Expert judgement was used to assign the probability and intensity of exposure. Among controls for the NHL analysis, the prevalence of exposure was 2.2%. In the analysis of NHL risk (Miligi et al., 2006) based on 1135 cases and 1246 population controls, the odds ratio for very low or low intensity was 0.7 (95% CI, 0.3–1.6; 9 exposed cases) and for medium or high intensity was 1.3 (95% CI, 0.6–2.9; 14 exposed cases). Among subjects exposed for 15 years or less at medium or high intensity, the odds ratio was 1.3 (95% CI, 0.5–3.7; 9 exposed cases). Associations were also reported for small lymphocytic lymphoma and diffuse NHL, but these were based on only 5 and 3 exposed cases, respectively. Because of the small numbers, no associations were reported between exposure to styrene and risk of HL (Miligi et al., 2006) or risk of leukaemia or MM in a related publication (Seniori Costantini et al., 2008). [The Working Group noted that, although exposure assessment was based on a strong protocol and

the cancer outcomes on a detailed pathological evaluation, only a few subjects of the study were exposed to styrene.]

Flodin et al. (1986) conducted a case–control study including 59 cases of AML and 354 controls in Sweden to assess potential risk factors including radiation, medications, and eight occupational exposures. Cases aged 20–70 years were identified at hospitals in Sweden between 1977 and 1982. Two series of controls were drawn from a population register: one was matched to cases for sex, age (within 5 years), and location, and the other was a random population sample. Information on exposure was obtained from a mailed questionnaire. Exposure status was assigned by judgement. An elevated risk was observed from the 3 cases and 1 control who were exposed to styrene (OR, 18.9; 95% CI, 1.9–357.0). [The Working Group noted the small numbers, the low response rates among cases, and the lack of detail on exposure assessment. The prevalence of exposure to styrene among controls (0.3%) was low. There were no cases and only 3 controls exposed to benzene.]

A population-based case–control study including 3730 histologically confirmed cases of cancer at 15 major sites (excluding leukaemia) in men was conducted in Montreal, Canada. Cases were newly diagnosed in 19 major hospitals between 1979 and 1986, and aged 35–70 years. General-population controls (n = 533) were obtained from electoral lists (Siemiatycki, 1991; Gérin et al., 1998). Exposure to 294 occupational agents was assessed by chemists/hygienists based on history of jobs held, and cases of cancer at each site were compared with those in the rest of the study population. Two percent of subjects were classified as having been ever exposed to styrene. Although they did not necessarily entail exposure to styrene at the highest concentrations, the most common occupations assigned exposure to styrene were firefighters, mechanics and repairmen, and painters not working in construction. Cumulative exposures at low, medium, or high concentrations were computed based on the product of duration, frequency, and concentration of exposure. In single-exposure models, elevated odds ratios for exposure to styrene at medium or high concentrations were found for cancers of the prostate (n = 449) (OR, 5.5; 95% CI, 1.4–21.8; 7 exposed cases) and rectum (n = 257) (OR, 5.1; 95% CI, 1.4–19.4; 5 exposed cases). No statistically significant increase in risk emerged for cancer of the lung (n = 857) (OR, 0.3; 95% CI, 0.1–0.9; 5 cases exposed to low concentration; OR, 0.9; 95% CI, 0.2–3.3; 5 cases exposed to medium or high concentration), NHL (n = 215) (OR, 2.0; 95% CI, 0.8–4.8; 8 ever exposed cases), or HL (n = 54) (OR, 2.4; 95% CI, 0.5–11.6; 2 ever exposed cases) (Gérin et al., 1998). Expanded analyses of cancer of the rectum were conducted by Dumas et al. (2000), yielding an odds ratio for ever exposure to styrene of 1.7 (95% CI, 0.7–4.5; 6 exposed cases). Substantial cumulative exposure was defined as exposure to medium or high concentration, at a medium or high frequency, for more than 5 years. For men exposed to styrene at concentrations referred to as "substantial", the odds ratio for cancer of the rectum was 3.9 (95% CI, 1.2–12.9; 5 exposed cases). Other cancers analysed in the study but not presented here, as there were too few exposed cases or too few other studies reporting on these sites, include those of the colon (n = 497), bladder (n = 484), stomach (n = 251), kidney (n = 177), pancreas (n = 116), and oesophagus (n = 99), as well as melanoma (n = 103). [The Working Group noted that the concentrations of exposure among subjects exposed to styrene were probably much lower than those in the cohort studies. The strengths of the study included the exposure assessment and the adjustment for potential confounders, including occupational co-exposures, for some cancer sites. However, analyses were based on only a few subjects exposed to styrene.]

A multicentre case–control study of renal cell carcinoma was conducted in central and eastern Europe (Czechia, Poland, Romania, and the

Russian Federation), including 1097 confirmed incident cases (648 men and 449 women) aged 20–88 years (Karami et al., 2011). Controls (*n* = 1476) were recruited from the same participating hospitals as cases, and excluded urological diseases and diseases related to smoking. Lifetime occupational history covering each job held for at least 12 months was collected. Experts assessed exposure to 72 agents for each job using semiquantitative ratings of frequency, intensity, and level of confidence. The prevalence of exposure to styrene was 1.2% among controls. Occupational exposure to styrene was assigned primarily to styrene manufacture operators, tank cleaners and tank operators of copolymer manufacturers, auto body repair workers who used polyester resins, and plastic boat manufacturers who processed unsaturated polyesters. Overall, 31 subjects (17 cases and 14 controls) were ever exposed with an odds ratio of 1.7 (95% CI, 0.8–3.6) for the association with renal cell carcinoma. Relative to a reference group who were not exposed to styrene, a positive association was observed for cumulative exposure at or above the median concentration (OR, 6.7; 95% CI, 1.8–24.3) and a reduced risk (OR, 0.6; 95% CI, 0.2–1.7) was reported for a cumulative exposure lower than the median concentration. No association was found for duration or average exposure. [The Working Group noted that the strengths of this study were the exposure assessment, the adjustment for non-occupational confounders, the exceptionally high response rates, and several sensitivity analyses. However, the study included only a few exposed cases and relied on hospital controls, which may be less representative of the general population. In addition, models were not adjusted for potential occupational confounders such as exposure to trichloroethylene, although a low prevalence would have been expected at the population level.]

Scélo et al. (2004) reported on a case–control study of cancer of the lung covering 16 centres in seven countries (Czechia, Hungary, Poland, Romania, the Russian Federation, Slovakia, and the United Kingdom) and including 2861 incident cases (2205 men and 656 women). Controls (*n* = 3118) were selected from hospitals (excluding cancers and tobacco-related diseases), except for at two centres where population controls were recruited instead. Lifetime occupational histories including every job held for at least 1 year were collected using questionnaires and interviews. Expert assessment was used to assign semiquantitative indices of frequency, intensity (exposure to styrene at < 5 ppm, 5–50 ppm, > 50 ppm), and confidence of exposure to 70 agents in each job held. The proportion of ever-exposed controls was 1.5%. The odds ratio for the association between ever exposure to styrene and risk of cancer of the lung was 0.70 (95% CI, 0.42–1.18). For tertiles of cumulative exposure to styrene (0.01–2.75 ppm-years, 2.76–12.50 ppm-years, and > 12.50 ppm-years), odds ratios were 1.15 (95% CI, 0.55–2.41), 0.37 (95% CI, 0.13–1.08), and 0.53 (95% CI, 0.20–1.43), respectively. After excluding exposures of low confidence, no associations were observed using lifetime duration and frequency-weighted duration of exposure across several sensitivity analyses. [Although this study benefited from the detailed exposure assessment and the ability to adjust for smoking and other occupational exposures, the Working Group noted that risk estimates were imprecise because of the limited number of exposed cases.]

A case–control study of mortality from cancer of the breast was conducted by Cantor et al. (1995) based on information from a database of death certificates covering 24 states across the USA for the period 1984–1989, and for which occupation and industry codes were assigned to the usual occupation. Cases (*n* = 33 509) were women with cancer of the breast as the underlying cause of death, and 4 controls per case (*n* = 117 794) were randomly selected from all non-cancer deaths, frequency matched to cases by age and race. Subjects with a usual occupation

of homemaker (i.e. not in paid employment) were excluded. Semiquantitative indices for the probability and level of exposure to 31 agents were assigned using a JEM based on occupation and industry. Among controls, 4.9% of White women and 2.6% of Black women were assigned an exposure to styrene. Odds ratios for the risk of mortality from cancer of the breast by probability and level of exposure were computed separately by race. Increases in mortality from cancer of the breast were observed for all probability categories and all levels of exposure to styrene for both racial groups. For instance, odds ratios for increasing levels of exposure to styrene were 1.16 (95% CI, 1.10–1.30), 1.13 (95% CI, 1.00–1.30), and 1.19 (95% CI, 0.90–1.60) among White women, and 1.59 (95% CI, 1.20–2.10) and 1.41 (95% CI, 1.00–1.90) among Black women. Risk estimates were higher when women with a low probability of exposure were excluded. [A strength of this study was that it was based on a large sample. However, the use of a population-based JEM likely resulted in substantial misclassification of exposure. Further, the limited information available from death certificates did not allow for other known risk factors for cancer of the breast, the duration of employment, or other jobs held during the lifetime to be taken into account. The Working Group could not exclude potential confounding from well-identified risk factors for cancer of the breast.]

Four case–control studies have also been conducted among children from the general population using the same database, focusing on prenatal and infant exposure to styrene in ambient air. Potential associations between prenatal or early exposure to ambient levels of environmental contaminants and the incidence of several types of cancer among children younger than 6 years were investigated in a registry-based study in California (Heck et al., 2013, 2014, 2015; von Ehrenstein et al., 2016). The types of cancer evaluated included neuroblastoma (n = 75) (Heck et al., 2013), ALL (n = 69) and AML (n = 46) (Heck et al., 2014), retinoblastoma (n = 103) (Heck et al., 2015), and medulloblastoma (n = 34), primitive neuroectodermal tumour (n = 43), and astrocytoma (n = 106) (von Ehrenstein et al., 2016). Cases were identified through the California Cancer Registry for the period 1990–2007 and linked to birth certificate records; population controls (between 2994 and 30 569 depending on the cancer site) were selected from birth certificates and frequency matched to cases by year of birth. Exposure to concentrations of styrene from ambient air recorded by California's network of air monitoring stations were assigned to subjects based on the nearest station and averaged by trimester of pregnancy, total pregnancy period, and first year of life. The average styrene concentration measured by the stations over the period 1990–2007 was 0.159 ppb (Heck et al., 2013). Analyses were restricted to subjects living (at birth) within a set radius from a monitoring station, with a distance varying between 2 km for ALL (Heck et al., 2014) and 5 miles for retinoblastoma (Heck et al., 2015). Exposure to styrene (expressed per increase of one interquartile range of 0.137 ppb) during pregnancy was positively associated with risk of AML (OR, 1.38; 95% CI, 0.94–2.03), as was exposure in the first year of life (OR, 1.63; 95% CI, 0.93–2.83). Corresponding figures for retinoblastoma were 1.28 (95% CI, 0.96–1.69) and 1.64 (95% CI, 1.12–2.39), respectively. For primitive neuroectodermal tumour, elevated risks were observed for an exposure during the first (OR, 1.31; 95% CI, 0.99–1.73) and second (OR, 1.24; 95% CI, 0.94–1.64) trimesters (von Ehrenstein et al., 2016). [The Working Group noted that the strengths of the study included the use of registry information and of measurements made at several time-points, although exposures were not measured at the individual level. However, the study was weakened by the small number of subjects, and assessment of the cancer hazard posed by a single agent was limited by the relatively high correlations of exposure between the

agents. There was no available information on other sources of exposure or on indoor exposure (see Section 1.4.1); indoor exposure could have been higher than outdoor exposure, especially in households with smokers (Adgate et al., 2004). No information was available on maternal smoking. Finally, the use of home addresses only did not consider other locations and moves were not taken into account. Zip code centroids, rather than exact addresses, were used for some subjects.]

2.4 Human cancer evidence synthesis

Several epidemiological studies have reported on styrene exposure and cancer outcomes. Cohort studies have been conducted in three main industries: reinforced plastics, synthetic rubber, and styrene monomer and polymers. Most of the industry studies have studied cancer mortality rather than incidence of cancer; this has implications for the strength of the studies as well as a potential bias towards more aggressive tumours or factors related to access to health care. Several population-based case–control studies are also available; most of these assessed occupational styrene exposure for adult cancers, and a small number assessed the association between styrene in ambient air and childhood cancers.

In assessing the human carcinogenicity of styrene, cohort studies in the reinforced plastics industry were considered to be the most informative. There were indications of a higher prevalence of smoking among short-term workers compared with the general population (Wong, 1990; Boffetta et al., 1998; Christensen et al., 2017; Bertke et al., 2018); for that reason, most emphasis was placed on internal analyses. Workers were exposed to the highest concentrations of styrene in this industry compared with the other industries studied. Other suspected workplace carcinogens, if present, were usually measured at low concentrations. Cancer risk was assessed in five cohorts of workers exposed to styrene in the reinforced plastics industry, including industry-wide studies in Europe (Kogevinas et al., 1994; Loomis et al., 2019), Denmark (Christensen et al., 2017), the United Kingdom (Coggon et al., 2015), and the USA (Collins et al., 2013; Wong, 1990), and a small study of two boatbuilding facilities in Washington State, USA (Ruder et al., 2016; Bertke et al., 2018). There were large partial overlaps with respect to study participants and follow-up periods between the European (Kogevinas et al., 1994; Loomis et al., 2019), Danish (Christensen et al., 2017), and United Kingdom cohorts (Coggon et al., 2015). In contrast to most other studies that only included mortality data, the Danish study (Christensen et al., 2017) (the largest of the industry-wide cohort studies) provided data on the incidence of cancer.

Workers in the synthetic rubber industry were exposed to styrene at average concentrations of about 10–50 times lower than those in the reinforced plastics industry; however, because employment duration was longer in the synthetic rubber industry than in the reinforced plastics industry, cumulative exposure was similar. All of the relevant findings from the synthetic rubber industry come from a large and long-running study of the mortality experience of workers at eight North American factories producing styrene–butadiene rubber (Macaluso et al., 1996; Graff et al., 2005; Sathiakumar et al., 2015). Exposure to styrene was observed to be highly correlated with exposure to butadiene, a known human carcinogen, in the styrene–butadiene rubber cohort.

Exposure to styrene in the styrene monomer and polymer industry was found to be comparable to that in the synthetic rubber industry, but there was also potential for the co-exposure of workers to benzene and butadiene, known human carcinogens. Four mortality cohort studies of workers have been published from this

industry (Frentzel-Beyme et al., 1978; Nicholson et al., 1978; Hodgson & Jones, 1985; Bond et al., 1992), but only the United States mortality study (Bond et al., 1992) of workers in four plants developing and producing styrene-based products was considered to be informative, as the other studies had very few cancer deaths.

General-population studies included seven case–control studies of occupational exposure to styrene and four case–control studies of prenatal and infant exposure to styrene in ambient air. All but one of these studies evaluated the incidence of cancer as opposed to mortality from cancer. Based on their exposure assessment methods, overall quality, and size, the more informative of these studies assessing occupational exposure to styrene were the Epilymph study of lymphoma (Cocco et al., 2010), a study of cancer of the lung (Scélo et al., 2004), a study of renal cell carcinoma (Karami et al., 2011), and a study of 15 different cancer types in Canada (Gérin et al., 1998).

2.4.1 All lymphohaematopoietic malignancies

The classification of lymphohaematopoietic malignancies has changed over time, which makes the comparison of results for subtypes at different time periods difficult to interpret. The overall category of non-Hodgkin lymphoma (NHL) used in older studies corresponds mostly to the category B-cell lymphoma in more recent publications; in addition, chronic lymphocytic leukaemia (CLL) and multiple myeloma (MM) are currently classified as lymphomas. Wherever feasible, the findings discussed in the following sections are based on the current classification.

(a) Non-Hodgkin lymphoma (all types combined)

In the reinforced plastics industry, the risk of mortality from NHL was observed to increase with average concentration of exposure, but not cumulative exposure, to styrene in the large European industry-wide cohort (Loomis et al., 2019). The four other cohort studies within this industry showed no association between exposure to styrene and NHL (Collins et al., 2013; Coggon et al., 2015; Bertke et al., 2018; Christensen et al., 2018).

For workers in the synthetic rubber industry, a positive association between cumulative exposure to styrene and NHL was observed (Graff et al., 2005), with the risk estimated to be 2.3 times higher in the groups exposed to the highest concentrations compared with the groups exposed to the lowest concentrations. The only informative styrene monomer and polymers production industry study from the USA (Bond et al., 1992) showed some evidence of excess mortality from NHL, but this was based on only 7 deaths.

Two population-based studies considered the risk of NHL. An Italian case–control study observed a marginal increase in risk of NHL with exposure to medium and/or high concentrations of styrene (Miligi et al., 2006). In the Canadian case–control study, ever exposure compared with no exposure was associated with a 2-fold increase in risk of NHL, but there was poor precision in this estimate (Gérin et al., 1998).

(b) T-cell lymphoma

In the Danish reinforced plastics industry-wide cohort study, the incidence of T-cell lymphoma was strongly associated with cumulative exposure to styrene; the risk of incidence was more than 3 times higher for workers with a high cumulative exposure compared with those with a low cumulative exposure to styrene, but there was low precision in the risk estimate (Christensen et al., 2018). The Epilymph case–control study reported no association between exposure to styrene and T-cell lymphoma, based on only 2 exposed cases (Cocco et al., 2010).

(c) B-cell lymphoma

In the Danish reinforced plastics industry-wide cohort study, there was no evidence of any association between exposure to styrene and incidence of B-cell, follicular B-cell, or diffuse B-cell lymphoma (Christensen et al., 2018). A decreased incidence of chronic B-cell leukaemia was observed with increasing cumulative exposure to styrene. In the synthetic rubber industry, mortality from CLL increased with increasing cumulative exposure to styrene; the risk was 2.6 times higher for workers with the highest cumulative exposure compared with those with the lowest cumulative exposure.

The Epilymph case–control study reported a strong association between B-cell lymphoma in 66 cases ever exposed to styrene compared with unexposed controls, with additional evidence of a positive exposure–response relationship (Cocco et al., 2010). A positive relationship with cumulative exposure was also suggested for diffuse B-cell and follicular B-cell lymphomas, and for CLL. Compared with unexposed subjects, a marginal increase in small lymphocytic NHL with exposure to styrene at medium and/or high concentrations was observed in an Italian case–control study (Miligi et al., 2006).

The United Kingdom reinforced plastics industry-wide cohort study reported a doubled risk of MM for the group exposed to the highest concentration of styrene versus the lowest, but this was based on only 5 exposed cases (Coggon et al., 2015). A reanalysis of the European cohort suggested an association between mean exposure lagged for 5 years and MM (Loomis et al., 2019). The other cohort studies in this industry showed little evidence of an association between exposure to styrene and MM (Collins et al., 2013; Bertke et al., 2018; Christensen et al., 2018). In the synthetic rubber industry cohort study, no positive association between exposure to styrene and risk of mortality from MM was found (Sathiakumar et al., 2015). The most informative study in the styrene monomer and polymers production industry reported an excess of MM mortality, based on 7 deaths (Bond et al., 1992). The Epilymph study reported no association between exposure to exposure and MM, based on 6 exposed cases (Cocco et al., 2010).

(d) Hodgkin lymphoma

The Danish reinforced plastics industry-wide cohort study showed an elevated incidence of Hodgkin lymphoma (HL) with cumulative exposure to styrene, with an increased risk by a factor of 1.6 for high versus low concentration of exposure (Christensen et al., 2018). The four other cohort studies in this industry showed little or no evidence of an association between exposure to styrene and HL (Kogevinas et al., 1994; Collins et al., 2013; Coggon et al., 2015; Bertke et al., 2018). In the synthetic rubber industry cohort study there were 12 deaths from HL, but no risk estimates were reported (Graff et al., 2005). The only informative cohort study in the styrene monomer and polymers production industry reported some evidence of excess deaths from HL, based on only 5 deaths (Bond et al., 1992). The risk of HL was not elevated with ever exposure to styrene compared with no exposure in the Epilymph study (Cocco et al., 2010).

(e) All leukaemias

The European reinforced plastics industry-wide cohort study found increasing risk of mortality from all leukaemias with time since first exposure, largely based on the Danish data (Kogevinas et al., 1994). The Danish cohort study found an increased incidence during the early years of first exposure, which was considered to be indicative of a high concentration of exposure, but not with duration of employment (Kolstad et al., 1994). The United States boatbuilding cohort study showed a strong association between increasing mortality from all leukaemias and duration of employment in jobs involving exposure to styrene at high concentrations (Bertke et

al., 2018). The remaining two cohort studies in this industry (one based in the United Kingdom, the other in the USA) showed no increasing mortality from all leukaemias with higher cumulative exposure or a longer duration of exposure to styrene, but the findings in the United Kingdom study were based on small numbers and there were limitations in the exposure assessment in the United States industry-wide study (Collins et al., 2013; Coggon et al., 2015).

In the synthetic rubber industry, mortality from all leukaemias was strongly elevated among male workers exposed to styrene at high concentrations compared with the general population and compared with increasing cumulative exposure to styrene within the industry (Graff et al., 2005; Sathiakumar et al., 2005, 2015). The only informative study in the styrene monomer and polymers production industry showed a slightly increased mortality from all leukaemias, but this was based on only 9 deaths (Bond et al., 1992). It should be noted that these analyses of all leukaemias included both lymphoid and myeloid leukaemias. There were no informative findings from the case–control studies for all leukaemias or their subtypes.

(f) Myeloid leukaemias

In the reinforced plastics industry, the most informative study (Danish) showed that the incidence of acute myeloid leukaemia (AML) increased strongly with increasing cumulative exposure to styrene when accounting for a latency period of 15 years (Christensen et al., 2018). This study also found that the incidence of AML for high cumulative exposure to styrene was twice that for low cumulative exposure in the previous 15–29 years. Increased mortality from myeloid (not specifying whether acute or chronic) leukaemia was reported for the highest cumulative exposure to styrene category in the United States industry-wide cohort study when compared with the general population (Collins et al., 2013). There was no overall increased mortality from AML and chronic myeloid leukaemia (CML) combined observed in the reanalysis of the European reinforced plastics industry cohort study, but an increase was observed with mean intensity of exposure in a 10-year lag analysis (Loomis et al., 2019). In the Danish study, no increased incidence of CML was found after exposure to styrene (Christensen et al., 2018). The remaining reinforced plastics industry cohort studies and the only informative study of the styrene monomer and polymers production industry did not report on myeloid leukaemia subtypes (Bond et al., 1992; Kolstad et al., 1994; Coggon et al., 2015; Bertke et al., 2018). There was no clear association between mortality from AML and cumulative exposure to styrene observed in the synthetic rubber industry study (Graff et al., 2005).

Because of some commonalities in cell lineages of origin and in risk factors, the Working Group assessed the pattern of the findings for lymphohaematopoietic malignancies as a whole; it was considered that there was not enough information on specific lymphohaematopoietic malignancies to permit separate conclusions. While noting inconsistent findings across the lymphohaematopoietic malignancies as a whole, the Working Group considered that there was more consistency within specific subtypes, especially for leukaemias and in particular myeloid leukaemia. The Working Group also placed greater weight on the findings of the Danish reinforced plastics industry-wide cohort study because of its large size, the fact that it reported incidence rather than mortality, and the lack of confounding by other occupational carcinogens. However, effect estimates were often small with low precision and many different analyses were undertaken using several different exposure metrics, so chance findings could not be discounted.

2.4.2 Lung

In external analyses of mortality from or incidence of cancer of the lung in the reinforced plastics industry, modest increases were observed in four of the five cohort studies (Collins et al., 2013; Coggon et al., 2015; Christensen et al., 2017; Bertke et al., 2018) (not in the European cohort study) (Kogevinas et al., 1994; Loomis et al., 2019). In internal analyses, mortality from cancer of the lung showed a decreasing trend in two United States studies of boat builders (Collins et al., 2013; Bertke et al., 2018), and a lower incidence in long-term workers than in short-term workers or in the general population in the Danish study (Christensen et al., 2017). No positive trend was reported in the reanalysis of the European study with higher cumulative exposure to styrene or longer duration of employment (Loomis et al., 2019). In the synthetic rubber industry cohort study, results for cancer of the lung were not consistent between workers of different sex in a single study. Confounding by cigarette smoking could not be ruled out. In the styrene monomer and polymers production industry, the only informative cohort study found fewer than expected deaths from cancer of the lung (Bond et al., 1992).

Two case–control studies of cancer of the lung reported no association with exposure to styrene according to various exposure metrics (Scélo et al., 2004; Gérin et al., 1998). Smoking and exposure to many known workplace carcinogens were adjusted for in both studies.

In conclusion, the Working Group attributed more weight to the null findings from the internal analyses that were less likely to be influenced by confounding and lack of direct adjustment for cigarette smoking. The panel also attributed weight to the null findings from the case–control studies in which adjustment for smoking and work co-exposures was performed. The Working Group concluded that these findings do not support an association between incidence of or mortality from cancer of the lung and exposure to styrene.

2.4.3 Sinonasal cavity

The Danish reinforced plastics cohort study, which was the only informative study on cancer of the sinonasal cavity, found an elevated incidence of unspecified cancer of the sinonasal cavity compared with the general population (Christensen et al., 2017). In a follow-up study of the same cohort, a 5-fold increased incidence of adenocarcinoma, adjusted for age, sex, and whether employed in the wood industry, was observed for high versus low cumulative styrene exposure (Nissen et al., 2018); however, this result was based on only 9 cases with resulting low precision. The Working Group noted the high concentration of exposure to styrene in this single study for a very rare cancer, as well as adjustment for other potential confounding factors, and considered this to be a noteworthy result that requires further investigation; however, the possible explanations of chance or confounding by wood dust could not be confidently ruled out.

2.4.4 Kidney

There was a modest increase in mortality from or incidence of cancer of the kidney reported from three (Danish, United Kingdom, and USA) of the five reinforced plastics industry cohort studies (Collins et al., 2013; Coggon et al., 2015; Christensen et al., 2017). One of the cohort studies reported a positive exposure–response relationship (Collins et al., 2013). The initial analyses of the European cohort study showed a positive exposure–response relationship (Kogevinas et al., 1994), but a recent reanalysis using different exposure metrics found no exposure–response relationship (Loomis et al., 2019). The cohort study of United States boat builders (Bertke et al., 2018), the single informative study in the styrene monomer and polymers

production industry (Bond et al., 1992), and the synthetic rubber industry cohort study found no association between exposure to styrene and the incidence of cancer of the kidney (Sathiakumar et al., 2005; Sathiakumar & Delzell, 2009). Based on 17 exposed cases, a European case–control study of renal cell carcinoma found an elevated risk for higher cumulative exposure to styrene, but not with increased duration or average exposure.

The Working Group noted some isolated modest associations and some exposure–response relationships for some exposure metrics for cancer of the kidney; however, considering the lack of consistency in the findings and lack of adjustment in the cohort studies for lifestyle factors (e.g. cigarette smoking) or possible work co-exposures, the Working Group concluded that there was no convincing evidence for an association between exposure to styrene and cancer of the kidney.

2.4.5 Bladder

The United Kingdom reinforced plastics industry-wide cohort study (Coggon et al., 2015) and the study of the two United States boatbuilding facilities reported modest excesses of deaths from cancer of the bladder (Bertke et al., 2018). The other three reinforced plastics industry-wide cohort studies showed no consistent associations (Kogevinas et al., 1994; Collins et al., 2013; Christensen et al., 2017). A synthetic rubber industry study reported a higher than expected number of deaths from cancer of the bladder in women exposed to styrene but not in men (Sathiakumar et al., 2005; Sathiakumar & Delzell, 2009). There was no association between exposure to styrene and cancer of the bladder in the Canadian case–control study, in which risk estimates were adjusted for smoking, and exposure to several non-occupational factors and aromatic amines (Gérin et al., 1998). The Working Group concluded that there was no convincing evidence of an association from these studies; confounding by cigarette smoking in the cohort studies could not be ruled out.

2.4.6 Breast

None of the five reinforced plastics industry cohort studies or the synthetic rubber industry cohort study found an association between exposure to styrene and cancer of the breast (Kogevinas et al., 1994; Sathiakumar & Delzell, 2009; Collins et al., 2013; Coggon et al., 2015; Christensen et al., 2017; Ruder & Bertke, 2017; Bertke et al., 2018). A case–control study of cancer of the breast using death certificates reported elevated risks of cancer of the breast with exposure to styrene among White and Black women (Cantor et al., 1995). However, the study only considered usual job and did not consider duration of exposure or any known risk factors for cancer of the breast. The Working Group therefore concluded that there was no convincing evidence of an association from these studies.

2.4.7 Oesophagus

A reanalysis of the European reinforced plastics industry cohort study found an association between mean styrene exposure, as well as cumulative exposure lagged by 20 years, and cancer of the oesophagus (Loomis et al., 2019). Further, the United States boatbuilding facility study found an excess risk of cancer of the oesophagus with increasing duration of high concentrations of styrene exposure (Bertke et al., 2018). The United Kingdom study also found an excess of cancer of the oesophagus with exposure to styrene (Coggon et al., 2015) but the larger Danish study, based on incidence and not mortality, did not find an excess in the incidence of cancer of the oesophagus (Christensen et al., 2017). In the synthetic rubber industry cohort study there were fewer than expected deaths compared with the reference population (Sathiakumar et al.,

2005). The Working Group concluded that there was no convincing evidence of an association.

2.4.8 Prostate

Two of the smaller studies – the case–control study reporting incidence of cancer of the prostate (Gérin et al., 1998) and the United States boatbuilding facility study assessing mortality from cancer of the prostate (Bertke et al., 2018) – showed positive associations, but the larger Danish and European studies of reinforced plastics workers found no positive association with incidence or mortality (Kogevinas et al., 1994; Christensen et al., 2017; Loomis et al., 2019).

2.4.9 Other cancers

Several other cancers were investigated in relation to styrene exposure, including cancers of the colon, rectum, stomach, pancreas, larynx, pharynx, brain, and central nervous system (including childhood cancers), and melanoma. However, the Working Group concluded that no reliable conclusions could be made either because of the small number of studies reporting results, inconsistencies in the findings, or the use of weak method(s) for assessing exposure to styrene.

3. Cancer in Experimental Animals

3.1 Styrene

3.1.1 Mouse

See Table 3.1.

(a) Transplacental exposure and oral administration (by gavage)

Female O20 or C57BL mice were exposed by gavage to a single dose of styrene (purity, 99%) at 0 (vehicle), 300 (C57BL), or 1350 (O20) mg/kg body weight (bw) in olive oil on gestational day 17 (Ponomarkov & Tomatis, 1978). Male and female progeny were then similarly exposed once per week from weaning for 16 weeks for O20 mice (dosing was stopped at 16 weeks due to toxicity, instead of occurring over the lifespan of the progeny as originally intended) or 120 weeks for C57BL mice. All surviving mice were killed at experimental week 120, although very few O20 mice survived to the end of study. For O20 mice, 6/47 female untreated controls, 0/9 female dams treated with olive oil only, 0/22 female progeny treated with olive oil only, 0/29 female dams exposed to styrene, 0/39 female progeny exposed to styrene, 7/54 male untreated controls, 0/20 male progeny treated with olive oil only, and 0/45 male progeny exposed to styrene survived until week 120. The effective number of animals used for tumour evaluation was the number of survivors in all groups at the time of the first tumour. In male O20 mice progeny, there was a significantly increased incidence of adenoma or adenocarcinoma (combined) of the lung after exposure to styrene compared with male mice progeny given olive oil only ($P < 0.01$). In female O20 mice progeny, there was a significantly increased incidence of adenoma or adenocarcinoma (combined) of the lung after exposure to styrene compared with female mice progeny given olive oil only ($P < 0.01$) and compared with untreated female controls ($P < 0.001$). There was also a significantly increased incidence of adenocarcinoma of the lung in female mice progeny after exposure to styrene compared with female mice progeny given olive oil only [$P < 0.01$]. Tumours appeared at or before week 57 in male O20 mice or week 65 in female O20 mice. For C57BL mice, 19/49 female untreated controls, 3/5 female dams given olive oil only, 4/13 female progeny given olive oil only, 4/15 female dams exposed to styrene, 12/27 female progeny exposed to styrene, 33/51 male untreated controls, 7/12 male progeny given olive oil alone, and 15/27 male progeny exposed to styrene survived until week 120. There were no significant differences in the incidence of tumours of the lung in

Table 3.1 Studies of carcinogenicity in mice exposed to styrene

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence and/or multiplicity of tumours	Significance	Comments
Full carcinogenicity Mouse, O20 (M) GND 17 120 wk Ponomarkov & Tomatis (1978)	Transplacental exposure/gavage Styrene, 99% Olive oil (0.1 mL) 0 (untreated), 0 (vehicle, progeny), 1350 (progeny) mg/kg bw 1× on GND 17 (to dams) then 1×/wk to progeny beginning at weaning for 16 wk 54, 20, 45 7, 0, 0	*Lung* Adenoma or adenocarcinoma (combined) 34/53 (64.2%), 8/19 (42.1%), 20/23 (87.0%)* Adenocarcinoma 12/53 (22.6%), 4/19 (21.1%), 8/23 (34.8%) Adenoma 22/53 (41.5%), 4/19 (21.1%), 12/23 (52.2%)	 **P* < 0.01 vs vehicle control [NS] [NS]	Principal limitations: not chronic exposure (16 wk only); poor survival; high/toxic dose (thus stopped at 16 wk) The effective number of animals (denominator) is the number of survivors at the time the first tumour was observed; the first lung tumour appeared at or before week 57 for all groups; tumours appeared earlier in styrene-treated male progeny (average age, 48.8 wk) compared with other groups
Full carcinogenicity Mouse, O20 (F) GND 17 120 wk Ponomarkov & Tomatis (1978)	Transplacental exposure/gavage Styrene, 99% Olive oil (0.1 mL) 0 (untreated), 0 (vehicle, dams), 0 (vehicle, progeny), 1350 (dams), 1350 (progeny) mg/kg bw 1× on GND 17 (to dams) then 1×/wk to progeny beginning at weaning for 16 wk 47, 9, 22, 29, 39 6, 0, 0, 0, 0	*Lung* Adenoma or adenocarcinoma (combined) 25/47 (53.2%), 5/8 (62.5%), 14/21 (66.7%), 11/20 (55%), 32/32 (100%)* Adenocarcinoma 14/47 (29.8%), 4/8 (50.0%), 4/21 (19.0%), 7/20 (35.0%), 18/32 (56.2%)* Adenoma 11/47 (23.4%), 1/8 (12.5%), 10/21 (47.6%), 4/20 (20.0%), 14/32 (43.8%)	 **P* < 0.01 vs vehicle control (progeny), *P* < 0.001 vs untreated control *[*P* < 0.01 vs vehicle control (progeny)] [NS]	Principal imitations: not chronic exposure (16 wk only); compared progeny with untreated (adult) controls; poor survival; high/toxic dose (thus stopped at 16 wk); low number of animals for vehicle control female dams The effective number of animals (denominator) is the number of survivors at the time the first tumour was observed; the first lung tumour appeared at or before week 65 for all groups; tumours appeared earlier in styrene-treated female progeny (average age, 57.8 wk) compared with the other groups

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence and/or multiplicity of tumours	Significance	Comments
Full carcinogenicity Mouse, C57BL (M) GND 17 120 wk Ponomarkov & Tomatis (1978)	Transplacental exposure/gavage Styrene, 99% Olive oil (0.1 mL) 0 (untreated), 0 (vehicle, progeny), 300 (progeny) mg/kg bw 1× on GND 17 (to dams) then 1×/wk to progeny beginning at weaning for life 51, 12, 27 33, 7, 15	*Lung*: tumour (unspecified) 5/47 (10.6%), 3/12 (25.0%), 1/24 (4.2%)	NS	Principal limitations: poor survival (but better than O20 strain); low starting number of animals for all treated-groups The effective number of animals (denominator) is the number of survivors at the time the first tumour was observed
Full carcinogenicity Mouse, C57BL (F) GND 17 120 wk Ponomarkov & Tomatis (1978)	Transplacental exposure/gavage Styrene, 99% Olive oil (0.1 mL) 0 (untreated), 0 (vehicle, dams), 0 (vehicle, progeny), 300 (dams), 300 (progeny) mg/kg bw Once on GND 17 (to dams) then once weekly to progeny beginning at weaning for life 49, 5, 13, 15, 27 19, 3, 4, 4, 12	*Lung*: tumour (unspecified) 1/47 (2.1%), 0/5, 1/13 (7.7%), 1/12 (8.3%), 1/24 (4.2%)	NS	Principal limitations: compared progeny with untreated (adult) controls; low number of animals for vehicle control female dams; poor survival (but better than O20 strain); low starting number of animals for all treated-groups The effective number of animals (denominator) is the number of survivors at the time the first tumour was observed
Full carcinogenicity Mouse, $B6C3F_1$ (M) 6 wk 91 wk NTP (1979a)	Gavage Styrene, > 99% Corn oil 0 (vehicle control), 150, 300 mg/kg bw, 5 d/wk for 78 wk plus 13-wk observation phase (no exposure) 20, 50, 50 20, 46, 39	*Lung* Bronchioloalveolar adenoma or carcinoma (combined) 0/20*, 6/44, 9/43**	*$P = 0.023$ (Cochran–Armitage trend test) **$P = 0.024$ (vs control, Fisher exact test)	Principal limitations: smaller number of controls compared with treated groups
		Bronchioloalveolar carcinoma 0/20, 3/44, 5/43	NS	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence and/or multiplicity of tumours	Significance	Comments
Full carcinogenicity Mouse, B6C3F$_1$ (F) 6 wk 91 wk NTP (1979a)	Gavage Styrene, > 99% Corn oil 0 (vehicle control), 150, 300 mg/kg bw, 5 d/wk for 78 wk plus 13-wk observation phase (no exposure) 20, 50, 50 18, 40, 38	*Liver*: hepatocellular adenoma		Principal limitations: smaller number of controls compared with treated groups
		0/20*, 1/44, 5/43	*P = 0.034 (Cochran–Armitage trend test)	
Full carcinogenicity Mouse, B6C3F$_1$ (M) 6 wk 92 wk NTP (1979b)	Gavage Styrene/β-nitrostyrene solution, NR Corn oil 0 (vehicle control), 87.5, 175 mg/kg bw, 3 d/wk for 78 wk plus 14-wk observation phase (no exposure) 20, 50, 50 18, 43, 33	*Lung*		Principal limitations: no dose–response in males (low dose effect only); purity of styrene in mixture not reported; smaller number of controls compared with treated groups; solution contained ~70% styrene and ~30% β-nitrostyrene; doses were based on β-nitrostyrene concentration; unusual exposure schedule of 3 d/wk
		Bronchioloalveolar adenoma or carcinoma (combined)		
		0/20, 11/49*, 2/36	*P = 0.016 (Fisher exact test)	
		Bronchioloalveolar carcinoma		
		0/20, 3/49, 1/36	NS	
Full carcinogenicity Mouse, B6C3F$_1$ (F) 6 wk 92 wk NTP (1979b)	Gavage Styrene/β-nitrostyrene solution, NR Corn oil 0 (vehicle control), 87.5, 175 mg/kg bw, 3 d/wk for 78 wk plus 14-wk observation phase (no exposure) 20, 50, 50 17, 47, 38	Any tumour type: no significant increase		Principal limitations: purity of styrene in mixture not reported; smaller number of controls compared with treated groups; solution contained ~70% styrene and ~30% β-nitrostyrene; doses were based on β-nitrostyrene concentration; unusual exposure schedule of 3 d/wk

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence and/or multiplicity of tumours	Significance	Comments
Full carcinogenicity Mouse, CD-1 (M) 4 wk 104 wk Cruzan et al. (2001), OEHHA (2010)	Inhalation (whole-body exposure) Styrene, > 99.5% Air 0, 20, 40, 80, 160 ppm, 6 h/d, 5 d/wk 50, 50, 50, 50, 50 36, 27, 27, 37, 33	*Lung* Bronchioloalveolar adenoma		Principal strengths: GLP study Male mice exposed to styrene at 80 and 160 ppm had decreased body weights over the course of the study
		15/50*, 21/50, 35/50**, 30/50***, 33/50**	*[$P < 0.001$ (Cochran–Armitage trend-test)], **[$P < 0.001$ (Fisher exact test)], ***[$P = 0.005$ (Fisher exact test)]	
		Bronchioloalveolar carcinoma		
		4/50, 5/50, 3/50, 6/50, 7/50	NS	
		Bronchioloalveolar adenoma or carcinoma (combined)		
		17/50*, 24/50, 36/50**, 30/50**, 36/50**	*$P < 0.001$ (Cochran–Armitage trend test), **$P < 0.01$ (Fisher exact test)	
		All tumours		
		Total tumours: 24, 32, 62, 62, 68	NR	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence and/or multiplicity of tumours	Significance	Comments
Full carcinogenicity Mouse, CD-1 (F) 4 wk 98 wk Cruzan et al. (2001), OEHHA (2010)	Inhalation (whole-body exposure) Styrene, > 99.5% Air 0, 20, 40, 80, 160 ppm, 6 h/d, 5 d/wk 50, 50, 50, 50, 50 27, 32, 33, 34, 35	*Lung* Bronchioloalveolar adenoma 6/50*, 16/50**, 16/50**, 11/50, 24/50***	*[$P = 0.001$ (Cochran–Armitage trend test)], **[$P = 0.028$ (Fisher exact test)], ***[$P < 0.001$ (Fisher exact test)]	Principal strengths: GLP study Principal limitations: lower survival of controls vs treated Due to mortality in control females (23/50 mice), the surviving females were killed 6 wk earlier than originally scheduled; all four treated groups had greater survival than the controls; female mice exposed to styrene at 160 ppm had decreased body weights over the course of the study
		Bronchioloalveolar carcinoma 0/50*, 0/50, 2/50, 0/50, 7/50**	*[$P < 0.001$ (Cochran–Armitage trend test)], **[$P = 0.012$, Fisher exact test]	
		Bronchioloalveolar adenoma or carcinoma (combined) 6/50*, 16/50**, 17/50**, 11/50, 27/50***	*$P < 0.001$ (Cochran–Armitage trend test), **$P < 0.05$ (Fisher exact test, ***$P < 0.01$ (Fisher exact test)	
		All tumours Total tumours: 7, 22, 22, 14, 47	NR	
Full carcinogenicity Mouse, CD-1 (M) 6–7 wk 104 wk Cruzan et al. (2017)	Inhalation (whole-body exposure) Styrene, 99.95% Clean air 0, 120 ppm, 6 h/d, 5 d/wk 75, 75 7, 12	*Lung* Bronchioloalveolar hyperplasia 0/67, 50/67*	*[$P < 0.0001$]	Principal limitations: single dose; one sex only; lack of statistical analysis in the article Five animals killed per group after 1, 26, 26, 52, and 78 wk for histopathology and cell proliferation assessment; initial number of animals for survival analyses, 50
		Bronchioloalveolar adenoma 15/67, 14/67	[NS]	
		Bronchioloalveolar carcinoma 7/67, 17/67*	*[$P < 0.05$]	
		Bronchioloalveolar adenoma or carcinoma (combined) 21/67, 31/67	[NS]	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence and/or multiplicity of tumours	Significance	Comments
Full carcinogenicity Mouse, C57BL/6 (wildtype) (M) 6–7 wk 104 wk Cruzan et al. (2017)	Inhalation (whole-body exposure) Styrene, 99.95% Clean air 0, 120 ppm, 6 h/d, 5 d/wk 75, 75 4, 18	*Lung* Bronchioloalveolar hyperplasia 0/69, 55/70* Bronchioloalveolar adenoma 3/69, 1/70 Bronchioloalveolar carcinoma 0/69, 0/70	 *[$P < 0.0001$] [NS] [NS]	Principal limitations: single dose; one sex only; lack of statistical analysis in the article Five animals killed per group after 1, 26, 26, 52, and 78 wk for histopathology and cell proliferation assessment; initial number of animals for survival analyses, 50; treated animals had a significantly higher survival
Full carcinogenicity Mouse, *Cyp2f2*$^{(-/-)}$ (KO) (M) 6–7 wk 104 wk Cruzan et al. (2017)	Inhalation (whole-body exposure) Styrene, 99.95% Clean air 0, 120 ppm, 6 h/d, 5 d/wk 75, 75 21, 23	*Lung* Bronchioloalveolar hyperplasia 0/69, 0/69 Bronchioloalveolar adenoma 0/69, 0/69 Bronchioloalveolar carcinoma 2/69, 0/69	 [NS] [NS] [NS]	Principal limitations: single dose; one sex only; lack of statistical analysis in the article Five animals killed per group after 1, 26, 26, 52, and 78 wk for histopathology and cell proliferation assessment; initial number of animals for survival analyses, 50; KO mice with C57BL/6 background
Full carcinogenicity Mouse, *Cyp2f2*KO-*2f1*-transgenic (M) 6–7 wk 104 wk Cruzan et al. (2017)	Inhalation (whole-body exposure) Styrene, 99.95% Clean air 0, 120 ppm, 6 h/d, 5 d/wk 75, 75 9, 14	*Lung* Bronchioloalveolar hyperplasia 0/69, 0/68 Bronchioloalveolar adenoma 2/69, 1/68 Bronchioloalveolar carcinoma 1/69, 0/68 Bronchioloalveolar adenoma or carcinoma (combined) 3/69, 1/68	 [NS] [NS] [NS] [NS]	Principal limitations: single dose; one sex only; lack of statistical analysis in the article Five animals killed per group after 1, 26, 26, 52, and 78 wk for histopathology and cell proliferation assessment; initial number of animals for survival analyses, 50; transgenic mice with C57BL/6 background

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence and/or multiplicity of tumours	Significance	Comments
Full carcinogenicity Mouse, A/J (F) 6–8 wk ~27 wk (20 wk after final injection) Brunnemann et al. (1992)	Intraperitoneal injection Styrene, > 99% Olive oil (0.1 mL) 0 (vehicle control), 10 µmol per mouse, 3×/wk (20 injections total; total dose, 200 µmol per mouse) 25, 25 NR	*Lung*: adenoma 1/25 (4%), 3/25 (12%) Tumour multiplicity: 0.04 ± 0.20, 0.80 ± 3.52	 NS NR	Principal limitations: not chronic exposure; non-physiological exposure route; no survival or body-weight data

bw, body weight; d, day(s); F, female; GND, gestation day; GLP, good laboratory practice; h, hour(s); KO, knockout; M, male; NR, not reported; NS, not significant; ppm, parts per million; vs, versus; wk, week(s).

C57BL mice. [The Working Group noted that the study was limited by the non-chronic exposure (16 weeks only), the poor survival, the high/toxic dose used for O20 mice (resulting in exposure stopping at week 16), the comparison of progeny with untreated (adult) controls, the low number of vehicle-control female dams, and the low starting numbers for all treated groups of C57BL mice.]

(b) Oral administration

Groups of male and female $B6C3F_1$ mice were exposed by gavage to styrene (purity, > 99%) at 0 (vehicle controls) (n = 20 per sex), 150 mg/kg bw (n = 50 per sex), or 300 mg/kg bw (n = 50 per sex) in corn oil (NTP, 1979a). Mice were treated 5 days per week for 78 weeks and then observed (no treatment) for 13 weeks (91 weeks in total). For male mice, 20/20 (100%) of the vehicle controls, 46/50 (92%) of the group given the low dose, and 39/50 (78%) of the group given the high dose survived until the end of study. For female mice, 18/20 (90%) of the vehicle controls, 40/50 (80%) of the group given the low dose, and 38/50 (76%) of the group given the high dose survived until the end of study. In males, the incidence of bronchioloalveolar adenoma or carcinoma (combined) of the lung at week 91 was significantly increased (vs vehicle control) at the highest dose (P = 0.024), and there was a significantly increased positive trend (P = 0.023). In females, there was a significantly increased positive trend (P = 0.034) for hepatocellular adenoma at week 91. [The Working Group noted the smaller number of controls compared with the numbers of treated mice.]

Groups of male and female $B6C3F_1$ mice were given a solution of styrene [purity unspecified] (~70%) and β-nitrostyrene (~30%) at a dose of 0 (vehicle controls) (n = 20 per sex), 87.5 mg/kg bw (n = 50 per sex), or 175 mg/kg bw (n = 50 per sex) by gavage in corn oil (NTP, 1979b). Doses were selected based on the concentration of β-nitrostyrene in the mixture. Mice were treated 3 days per week for 78 weeks and then observed (no treatment) for 14 weeks (92 weeks in total). For male mice, 18/20 (90%) of the vehicle controls, 43/50 (86%) of the group given the low dose, and 33/50 (66%) of the group given the high dose survived until the end of study. For female mice, 17/20 (85%) of the vehicle controls, 47/50 (94%) of the group given the low dose, and 38/50 (76%) of the group given the high dose survived until the end of study. Decreased mean body weight (compared with controls) was only observed in the female mice given the high dose. In males, the incidence of bronchioloalveolar adenoma or carcinoma (combined) at week 92 was significantly increased (vs vehicle control) for the group exposed to the lowest dose (P = 0.016). There was no significantly increased incidence of tumours in female mice. [The Working Group noted the smaller number of controls compared with the numbers of treated mice. The study was also limited by the test agent being a mixture of styrene and β-nitrostyrene, the purity of styrene in the mixture not being reported, and the unusual exposure schedule of 3 days per week. The Working Group also noted that there was no significant dose–response in males and no significant increase in males given the high dose.]

(c) Inhalation

In a good laboratory practice (GLP) study, groups of 50 male and 50 female CD-1 mice were exposed to styrene (purity, > 99.5%) via inhalation (whole-body exposure) at 0 (air control), 20, 40, 80, or 160 ppm for 6 hours per day, 5 days per week, for 104 weeks (males) or 98 weeks (females) (Cruzan et al., 2001). The percentages of male mice surviving at week 104 were 72% (control), 54% (20 ppm), 54% (40 ppm), 74% (80 ppm), and 66% (160 ppm). The corresponding percentages of female mice surviving at week 98 were 54%, 64%, 66%, 68%, and 70%. Males exposed at 80 ppm and 160 ppm exhibited significant weight loss (23% and 31%, respectively, compared with controls) at week 104. Females exposed at

160 ppm exhibited significant weight loss (15% compared with controls) at week 98. In males, the incidence of bronchioloalveolar adenoma of the lung was significantly increased (compared with controls) at week 104 with exposure to styrene at 40 ppm [$P < 0.001$], 80 ppm [$P = 0.005$], and 160 ppm [$P < 0.001$], and there was a significant positive trend [$P < 0.001$]. There was no significant increase in the incidence of bronchioloalveolar carcinoma of the lung in males, but the incidence of bronchioloalveolar adenoma or carcinoma (combined) was significantly increased with exposure at 40, 80, and 160 ppm, with a significant positive trend (OEHHA, 2010). In females, the incidence of bronchioloalveolar adenoma of the lung at week 98 was significantly increased (compared with controls) with exposure to styrene at 20 ppm [$P = 0.028$], 40 ppm [$P = 0.028$], and 160 ppm [$P < 0.001$], with a significant positive trend [$P = 0.001$]. The incidence of bronchioloalveolar carcinoma of the lung was significantly increased (compared with controls) with exposure to the highest dose in females [$P = 0.012$], with a significant positive trend [$P < 0.001$] (Cruzan et al., 2001). The incidence of bronchioloalveolar adenoma or carcinoma (combined) was significantly increased with exposure at 20, 40, and 160 ppm, with a significant positive trend in females (OEHHA, 2010). [The Working Group noted that the lower survival of female controls compared with treated females was a weakness of this study. Further, there were no historical control data from inhalation studies at the testing laboratory for bronchioloalveolar adenoma and carcinoma in CD-1 mice.]

Groups of 75 male CD-1, C57BL/6 wildtype, *Cyp2f2*$^{(-/-)}$ knockout, and *Cyp2f2*KO-*Cyp2f1* transgenic mice were exposed to styrene (purity, 99.95%) via inhalation (whole-body exposure) at 0 ppm (air control) or 120 ppm for 6 hours per day, 5 days per week, for 104 weeks. Five mice per group were killed after 1, 26, 26, 52, and 78 weeks for histopathological examination and cell proliferation assessment. Treated wildtype mice had a significantly higher survival compared with controls. The incidences of bronchioloalveolar hyperplasia were significantly increased in CD-1 mice [$P < 0.0001$] and wildtype mice [$P < 0.0001$] exposed to styrene (50/67 and 55/70, respectively) compared with control mice (0/67 and 0/69, respectively). Bronchioloalveolar hyperplasia was not observed in any of the knockout or transgenic mice (whether controls or mice exposed to styrene). The incidence of bronchioloalveolar adenoma or carcinoma (combined) was increased in CD-1 mice exposed to styrene (31/67) compared with control mice (22/67), but this increase was not statistically significant. There was no significant increase for the three groups of animals with a C57BL/6 background: the incidence of bronchioloalveolar adenoma or carcinoma (combined) was 1/70 (wildtype), 0/69 (knockout), and 1/68 (transgenic) for mice exposed to styrene and 3/69 (wildtype), 2/69 (knockout), and 3/69 (transgenic) for control mice. The incidence of bronchioloalveolar carcinoma was significantly increased in CD-1 mice [$P < 0.05$] exposed to styrene (17/67) compared with control mice (7/67). For the other groups, the incidences of bronchioloalveolar carcinoma were 0/70 (wildtype), 0/69 (knockout), and 0/68 (transgenic) for mice exposed to styrene and 0/69 (wildtype), 2/69 (knockout), and 1/69 (transgenic) for control mice (Cruzan et al., 2017). [The Working Group noted the use of a single dose and of males only, as well as the lack of statistical analysis in the article. There were no historical control data for bronchioloalveolar adenoma and bronchioloalveolar carcinoma in CD-1 mice.]

(d) *Intraperitoneal injection*

Two groups of 25 female A/J mice were given styrene (purity, > 99%) by intraperitoneal injection at doses of 0 (vehicle control) or 10 µmol per mouse in olive oil 3 times per week for a total of 20 injections (total dose, 200 µmol per mouse) (Brunnemann et al., 1992). Although there was

an increase in the percentage of mice exposed to styrene with adenomas of the lung (compared with the vehicle controls) at 20 weeks after the last injection (duration of the experiment, ~27 weeks), the difference was not statistically significant. [The Working Group noted that the study was limited by the lack of survival or body-weight data, the non-chronic exposure, and the non-physiological exposure route.]

3.1.2 Rat

See Table 3.2.

(a) Oral administration

(i) Gavage

Groups of 50 male and 50 female Fischer 344 rats were given styrene (purity, 99.7%) in corn oil by gavage at doses of 0, 1000 (medium dose), and 2000 (high dose) mg/kg bw, 5 days a week for 78 weeks, followed by a 27-week observation period. Groups of 20 male and 20 female rats received the corn oil vehicle alone (medium- and high-dose vehicle control) (NTP, 1979a). Mortality among male and female rats given the high dose was significantly higher than their respective vehicle controls. In response to this elevated and early mortality, a group given a low dose of styrene was included for each sex at experimental week 23. These dosed rats were intubated with styrene at a dose of 500 mg/kg bw for 103 weeks, followed by a 1-week observation period; a separate vehicle control group was also started for each sex for this low-dose group. Males surviving at the end of the experiment were 17/20 (low-dose vehicle control), 18/20 (medium- and high-dose vehicle control), 44/50 (low dose), 47/50 (medium dose), and 6/50 (high dose, at week 53). Corresponding numbers of surviving females were 15/20, 18/20, 46/50, 46/50, and 7/50 (at week 53). A dose-related mean body-weight decrease compared with their controls was observed in male rats, but there was no significant mean body-weight decrease in female rats when compared with their controls. [Since there was a significant accelerated mortality among rats exposed at the high dose, it is possible that the medium dose may have exceeded the maximum tolerated dose.] There were inadequate numbers of rats that survived the high dose of styrene to determine the risk of late-developing tumours; these groups were therefore excluded from the statistical analyses. There was no significant increase in the incidence of any tumour type in treated males or females. [The Working Group noted the smaller number of controls.]

Groups of 50 male and 50 female Fischer 344 rats were given a solution of styrene [purity unspecified] and β-nitrostyrene (70% and 30%, respectively) in corn oil by gavage at doses of 150 or 300 mg/kg bw for males and 75 or 150 mg/kg bw for females, 3 days per week for 79 weeks, followed by an additional observation period of 29 weeks. Groups of 20 male and 20 female rats received the corn oil vehicle alone (NTP, 1979b). Doses were selected based on the concentration of β-nitrostyrene in the mixture. There was no significant difference between the survival of male and female rats given the test solution and that of their controls. The numbers of surviving males at the end of the experiment were 16/20 (controls), 34/50 (low dose), and 31/50 (high dose); the corresponding numbers of surviving females were 12/20, 33/50, and 31/50. A decrease in mean body weight of the male rats given the high dose, compared with their controls, was observed, indicating that the doses may have approximated the maximum tolerated dose. There was no significant increase in the incidence of any tumour type in treated males or females. [The Working Group noted the smaller number of controls compared with the treated groups. The study was also limited by the test agent being a mixture of styrene and β-nitrostyrene, the lack of information about the purity of styrene in the mixture, and the unusual exposure schedule of 3 days per week.]

Table 3.2 Studies of carcinogenicity in rats exposed to styrene

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, Fischer 344 (M) 6 wk 104–105 wk NTP (1979a)	Gavage Styrene, 99.7% Corn oil 0 (low-dose vehicle control), 0 (medium- and high-dose vehicle control), 500 mg/kg bw (low-dose) for 103 wk, 1000 mg/kg bw (medium-dose group) for 78 wk, and 2000 mg/kg bw (high-dose group) for 78 wk, 5 d/wk, then observation at wk 104–105 20, 20, 50, 50, 50 17, 18, 44, 47, 6	*Skin and subcutaneous tissue*: fibroma		Principal limitations: inadequate numbers of surviving high-dose (2000 mg/kg bw) rats, meaning that these were excluded from the statistical analyses (only 6/50 male rats survived past week 53 to the end of the study); smaller number of controls compared with treated groups
		0/20, 0/20, 3/50, 0/50	NS	
		Adrenal gland: pheochromocytoma		
		2/20, 1/19, 1/48, 4/49	NS	
		Testis: interstitial cell tumours		
		15/20, 19/20, 42/47, 48/50	NS	
Full carcinogenicity Rat, Fischer 344 (F) 6 wk 104–105 wk NTP (1979a)	Gavage Styrene, 99.7% Corn oil 0 (low-dose vehicle control), 0 (medium-dose vehicle control), 500 mg/kg bw (low-dose) for 103 wk, 1000 mg/kg bw (medium-dose group) for 78 wk, and 2000 mg/kg bw (high-dose group) for 78 wk, 5 d/wk, then observation at wk 104–105 20, 20, 50, 50, 50 15, 18, 46, 46, 7	*Uterus*: endometrial stromal polyp		Principal limitations: inadequate numbers of surviving high-dose (2000 mg/kg bw) rats, meaning that these were excluded from the statistical analyses (only 7/50 female rats survived past week 53 to the end of the study); smaller number of controls compared with treated groups
		4/18, 3/20, 9/48, 5/50	NS	
Full carcinogenicity Rat, F344 (M) 6 wk 108 wk NTP (1979b)	Gavage 70% styrene, 30% β-nitrostyrene, NR Corn oil 0, 150, 300 mg/kg bw, 3 d/wk for 79 wk, 29 wk observation 20, 50, 50 16, 34, 31	*Pituitary gland*: chromophobe adenoma		Principal limitations: purity of styrene in mixture not reported; smaller number of controls compared with treated groups; solution contained ~70% styrene and ~30% β-nitrostyrene; doses were based on β-nitrostyrene concentration; unusual exposure schedule of 3 d/wk
		4/17*, 4/42, 1/44**	*$P = 0.010$ (trend, decrease), **$P = 0.019$ (decrease)	
		Pancreas: islet cell adenoma		
		2/18*, 1/42, 0/42	*$P = 0.039$ (trend, decrease)	
		Adrenal gland: pheochromocytoma		

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, F344 (M) 6 wk 108 wk NTP (1979b) (cont.)		1/19, 3/48, 1/46	NS	
		Thyroid: C-cell adenoma or carcinoma (combined)		
		0/18, 1/47, 3/41	NS	
		Testis: interstitial cell tumours		
		15/19, 38/47, 39/46	NS	
Full carcinogenicity Rat, F344 (F) 6 wk 108 wk NTP (1979b)	Gavage 70% styrene, 30% β-nitrostyrene, NR Corn oil 0, 75, 150 mg/kg bw, 3 d/wk for 79 wk, 29 wk observation 20, 50, 50 12, 33, 31	*Pituitary gland*: chromophobe adenoma		Principal limitations: purity of styrene in mixture not reported; smaller number of controls compared with treated groups; solution contained ~70% styrene and ~30% β-nitrostyrene; doses were based on β-nitrostyrene concentration; unusual exposure schedule of 3 d/wk
		5/18, 15/49, 18/44	NS	
		Mammary gland: fibroadenoma		
		2/20, 5/50, 7/50	NS	
		Uterus		
		Adenocarcinoma, NOS		
		1/20, 3/48, 0/45	NS	
		Endometrial stromal polyp		
		1/20, 9/48, 8/45	NS	
Full carcinogenicity Rat, Sprague-Dawley (M) 13 wk Lifetime Conti et al. (1988)	Gavage Styrene, 99.8% Olive oil 0, 50, 250 mg/kg bw, 1×/d, 4–5×/wk, 52 wk 40, 40, 40 NR	*Mammary gland*		Principal strengths: adequate number of animals used, randomly allocated in groups Principal limitations: no mortality data were included in this study; statistical analysis was not conducted by the authors; limited reporting The authors reported no significant difference in survival related to treatment; Experiment BT102
		"Benign tumours"		
		4/40, 3/40, 4/40	[NS]	
		"Malignant tumours"		
		0/40, 0/40, 0/40	[NS]	
		Haematopoietic and lymphoid tissues: leukaemia		
		0/40, 0/40, 2/40	[NS]	

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, Sprague-Dawley (F) 13 wk old Lifetime Conti et al. (1988)	Gavage Styrene, 99.8% Olive oil 0, 50, 250 mg/kg bw, 1×/d, 4–5×/wk, 52 wk 40, 40, 40 NR	*Mammary gland* "Benign and malignant tumours" 24/40, 30/40, 15/40 "Malignant tumours" 5/40, 6/40, 5/40 *Haematopoietic and lymphoid tissues*: leukaemia 1/40, 3/40, 0/40	 [NS] [NS] [NS]	Principal strengths: adequate number of animals used, randomly allocated in groups Principal limitations: no mortality data were included in this study; statistical analysis was not conducted by the authors; limited reporting The authors reported a higher mortality rate in high-dose females; Experiment BT102
Full carcinogenicity Rat, BDIV (F) GND 17 120 wk Ponomarkov & Tomatis (1978)	Gavage (of pregnant females) Styrene, 99% Olive oil 0, 1350 mg/kg bw, 1×/wk 10, 21 8, 10	*Stomach*: tumours 0/10, 1/20 *Mammary gland*: tumours 3/10, 6/20 *Uterus*: carcinoma	 NS NS	Principal strengths: the duration of observation was adequate Animals were dams of male and female offspring of transplacental exposure/gavage experiment (see experiment below)
Full carcinogenicity Rat, BDIV (F) GND 17 120 wk Ponomarkov & Tomatis (1978) (cont.)		0/10, 3/20 *Pituitary gland*: adenoma 0/10, 3/20 *Ovary*: tumours 0/10, 1/20	NS NS NS	
Full carcinogenicity Rat, BDIV (M) GND 17 120 wk Ponomarkov & Tomatis (1978)	Transplacental exposure/gavage Styrene, 99% Olive oil 0, 500 mg/kg bw, 1×/wk beginning at weaning for life 36, 73 14, 8	*Nerve trigeminus*: neurinoma 0/32, 1/54 *Heart*: neurinoma 0/32, 1/54	 NS NS	Principal strengths: adequate number of animals used; duration of exposure and observation was adequate Animals studied were offspring from dams treated with 0 or 1350 mg/kg bw of styrene dissolved in olive oil by gavage 1× on day 17 of gestation (see experiment above)

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, BDIV (F) GND 17 120 wk Ponomarkov &Tomatis (1978)	Transplacental exposure/gavage Styrene, 99% Olive oil 0, 500 mg/kg bw, 1×/wk beginning at weaning for life 39, 71 18, 20	*Stomach*: tumours 1/35, 2/68 *Liver*: tumours 0/35, 1/68	 NS NS	Principal strengths: adequate number of animals used; duration of exposure and observation was adequate Animals studied were offspring from dams treated with 0 or 1350 mg/kg bw of styrene dissolved in olive oil by gavage 1× on day 17 of gestation (see experiment above)
Full carcinogenicity Rat, Charles River COBS (SD) BR (M) 50 d 2 yr Beliles et al. (1985)	Drinking-water Styrene, 98.9% Drinking-water 0, 125, 250 ppm [mg/L] ad libitum 76, 50, 50 42, 27, 31	*Mammary gland*: tumours 1/65, 0/23, 0/40	 NS	Principal strengths: adequate number of animals used; adequate duration of exposure and observation Principal limitations: dosing was limited by the solubility of styrene in water; styrene intake was calculated from the concentration, water consumption, and body weight as being 7.7 and 14 mg/kg bw per day in males
Full carcinogenicity Rat, Charles River COBS (SD) BR (F) 50 d 2 yr Beliles et al. (1985)	Drinking-water Styrene, 98.9% Drinking-water 0, 125, 250 ppm [mg/L] ad libitum 106, 70, 70 60, 40, 44	*Mammary gland*: tumours 54/96, 20/30, 45/60	 NS	Principal strengths: adequate number of animals used; adequate duration of exposure and observation Principal limitations: dosing was limited by the solubility of styrene in water; styrene intake was calculated from the concentration, water consumption, and body weight as being 12 and 21 mg/kg bw per day in females
Full carcinogenicity Rat, Sprague-Dawley (M) 13 wk Lifetime Conti et al. (1988)	Inhalation (whole-body exposure) Styrene, 99.8% Air flow 0, 25, 50, 100, 200, 300 ppm, 4 h/d, 5 d/wk for 52 wk 60, 30, 30, 30, 30, 30 NR	*Mammary gland* "Benign and malignant tumours (combined)" 8/60, 6/30, 3/30, 6/30, 4/30, 5/30 "Malignant tumours" 1/60, 1/30, 1/30, 0/30, 1/30, 0/30	 [NS] [NS]	Principal limitations: no mortality data were included in this study; statistical analysis was not conducted by the authors; limited reporting The authors reported no significant difference in survival related to treatment; Experiment BT101

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, Sprague-Dawley (F) 13 wk Lifetime Conti et al. (1988)	Inhalation (whole-body exposure) Styrene, 99.8% Air flow 0, 25, 50, 100, 200, 300 ppm, 4 h/d, 5 d/wk for 52 wk 60, 30, 30, 30, 30, 30 NR	*Mammary gland* "Benign and malignant tumours (combined)" 34/60*, 24/30**, 21/30, 23/30, 24/30**, 25/30**	*[$P < 0.05$, Cochran–Armitage trend test], **[$P < 0.05$, Fisher exact test]	Principal limitations: no mortality data were included in this study; statistical analysis was not conducted by the authors; limited reporting The authors reported no significant difference in survival related to treatment; Experiment BT101
		"Malignant tumours" 6/60*, 6/30, 4/30, 9/30**, 12/30**, 9/30**	*[$P \leq 0.013$, Cochran–Armitage trend-test], **[$P < 0.05$, Fisher exact test]	
Full carcinogenicity Rat, CD (Sprague-Dawley) (M) NR (received at age 4 wk) 104 wk Cruzan et al. (1998)	Inhalation (whole-body exposure) Styrene, > 99.5% Vapour (air flow) 0, 50, 200, 500, 1000 ppm, 6 h/d for 5 d/wk 60, 60, 60, 60, 60 NR	*Mammary gland*: adenocarcinoma 0/60, 0/60, 0/60, 1/60, 0/60	NS	Principal strengths: adequate number of animals used; adequate duration of exposure and observation; adequate schedule of exposure; GLP study

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, CD (Sprague-Dawley) (F) NR (received at age 4 wk) 104 wk Cruzan et al. (1998)	Inhalation (whole-body exposure) Styrene, > 99.5% Vapour (air flow) 0, 50, 200, 500, 1000 ppm, 6 h/d for 5 d/wk 60, 60, 60, 60, 60 29, 28, 29, 40, 49	*Mammary gland* Adenocarcinoma 20/60*, 13/60, 9/60**, 5/60***, 2/60****	 *[$P < 0.001$ (trend) (decrease)] **[$P = 0.032$ (decrease)] ***[$P = 0.001$ (decrease)] ****[$P < 0.001$ (decrease)]	Principal strengths: adequate number of animals used; adequate duration of exposure and observation; adequate schedule of exposure; GLP study
		Fibroadenoma 21/60, 16/60, 13/60, 18/60, 17/60	 NS	
Full carcinogenicity Rat, Sprague-Dawley (M) 13 wk Lifetime Conti et al. (1988)	Intraperitoneal injection Styrene, 99.8% Olive oil 0, 50 mg, 4× (with 2-mo interval) over 8 mo 40, 40 NR	*Mammary gland* "Benign tumours" 1/40, 6/40 "Malignant tumours" 0/40, 0/40	 [NS] [NS]	Principal strengths: adequate number of animals used Principal limitations: no mortality data were included in this study; statistical analysis was not conducted by the authors; non-physiological exposure route; short duration of treatment and low dose; limited reporting The authors reported no significant difference in survival related to treatment; Experiment BT103
Full carcinogenicity Rat, Sprague-Dawley (F) 13 wk Lifetime Conti et al. (1988)	Intraperitoneal injection Styrene, 99.8% Olive oil 0, 50 mg, 4× (with 2-mo interval) over 8 mo 40, 40 NR	*Mammary gland* "Benign and malignant tumours (combined)" 24/40, 25/40 "Malignant tumours" 7/40, 6/40	 [NS] [NS]	Principal strengths: adequate number of animals used Principal limitations: no mortality data were included in this study; statistical analysis was not conducted by the authors; non-physiological exposure route; short duration of treatment and low dose; limited reporting The authors reported no significant difference in survival related to treatment; Experiment BT103

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, Sprague-Dawley (M) 13 wk Lifetime Conti et al. (1988)	Subcutaneous injection Styrene, 99.8% Olive oil 0, 50 mg (1×) 40, 40 NR	*Mammary gland* "Benign and malignant tumours (combined)" 8/40, 3/40 "Malignant tumours" 1/40, 0/40 *Adrenal gland*: pheochromocytoma 0/40, 1/40	 [NS] [NS] [NS]	Principal strengths: adequate number of animals used Principal limitations: no mortality data were included in this study; statistical analysis was not conducted by the authors; non-physiological exposure route; short duration of treatment; limited reporting The authors reported no significant difference in survival related to treatment; Experiment BT104
Full carcinogenicity Rat, Sprague-Dawley (F) 13 wk Lifetime Conti et al. (1988)	Subcutaneous injection Styrene, 99.8% Olive oil 0, 50 mg (1×) 40, 40 NR	*Mammary gland* "Benign and malignant tumours (combined)" 24/40, 25/40 "Malignant tumours" 7/40, 6/40 *Adrenal gland*: pheochromocytoma 0/40, 3/40	 [NS] [NS] [NS]	Principal strengths: adequate number of animals used Principal limitations: no mortality data were included in this study; statistical analysis was not conducted by the authors; non-physiological exposure route; short duration of treatment; limited reporting The authors reported no significant difference in survival related to treatment; Experiment BT104

bw, body weight; d, day(s); F, female; GLP, good laboratory practice; GND, gestation day; M, male; mo, month(s); NOS, not otherwise specified; NR, not reported; NS, not significant; ppm, parts per million; wk, week(s).

Groups of 40 male and 40 female Sprague-Dawley rats were exposed by gavage to styrene (purity, 99.8%) at 0, 50, or 250 mg/kg bw in olive oil for 4–5 days per week for 52 weeks, and then observed until death. There was no significant increase in the incidence of any tumour type in treated males or females (Conti et al., 1988). [The Working Group noted the limited reporting of this study and the 1-year duration of treatment.]

(ii) Transplacental exposure and oral administration (by gavage)

Ponomarkov & Tomatis (1978) reported on a group of 21 pregnant BDIV rats that were given styrene (purity, 99%) at a dose of 1350 mg/kg bw in olive oil by a single gastric intubation on day 17 of gestation. A control group of 10 pregnant rats were given olive oil alone. There was a slight treatment-related increase in neonatal mortality. Groups of 73 male and 71 female progeny of dams that were given styrene were also exposed to styrene at 500 mg/kg bw in olive oil by gastric intubation once per week from weaning for up to 120 weeks, at which point the experiment was terminated. Control groups of 36 male and 39 female offspring rats were given olive oil alone. There was no treatment-related effect on body weight or survival. At the time of observation of the first tumour, 10 control and 20 treated dams, 32 male control and 54 treated male offspring, and 35 female control and 68 treated female offspring were still alive. Non-neoplastic stomach lesions [morphology and incidence unspecified, probably glandular] were reported in rats who were exposed to styrene. There was no significant treatment-related increase in tumour incidence at any site.

(iii) Drinking-water

In a chronic toxicity and three-generation reproduction study, two groups of 50 male and two groups of 70 female Charles River COBS (SD) BR rats (F_0 generation rats) were continuously exposed to styrene (purity, 98.9%) in drinking-water at nominal doses of 125 or 250 ppm for 2 years (chronic study). Groups of 76 male and 106 female rats were observed as vehicle controls. Males (10–15) and females (20–30) from each group in the chronic study were mated to produce F_1 pups (reproductive toxicity study) 90 days after the start of the experiment. At weaning of the litters, the F_0 parents were returned to the chronic toxicity study. At 52 weeks, 10 F_0 rats per sex and group were killed. There was no significant difference between the survival and food consumption of rats exposed to styrene in drinking-water and those of their controls. The numbers of surviving males at the end of the experiment were 42/76 (control), 27/50 (low dose), and 31/50 (high dose); the corresponding numbers of surviving females were 60/106, 40/70, and 44/70. There was a decrease in mean body weight in female rats given the high dose. No treatment-related increase in the incidence of any type of tumour was observed (Beliles et al., 1985). [The Working Group noted the adequate number of animals used and the adequate duration of exposure and observation.]

(b) Inhalation

Conti et al. (1988) reported on groups of 30 male and 30 female Sprague-Dawley rats that were exposed to styrene (purity, 99.8%) by whole-body inhalation at doses of 25, 50, 100, 200, or 300 ppm for 4 hours per day, 5 days a week, for 52 weeks followed by lifetime observation; groups of 60 male and 60 female rats served as control groups. There was no significant difference in survival between the groups exposed to styrene by inhalation and controls. There was no relevant body weight difference between exposed groups and controls. The incidence of benign and malignant (combined) mammary tumours was significantly higher in exposed female rats than in controls: 34/60 (controls), 24/30 (25 ppm), 21/30 (50 ppm), 23/30 (100 ppm), 24/30 (200 ppm), and 25/30 (300 ppm) [$P < 0.05$, trend test; $P < 0.05$ for groups exposed at 25, 200, and 300 ppm vs

controls]. The incidence of malignant mammary tumours was significantly increased in treated females than in controls: 6/60 (controls), 6/30 (25 ppm), 4/30 (50 ppm), 9/30 (100 ppm), 12/30 (200 ppm), and 9/30 (300 ppm) [$P \leq 0.013$, trend test; $P < 0.05$ for groups exposed at 100, 200, and 300 ppm vs controls]. There was no significant increase in the incidence of benign and/or malignant mammary tumours in males. [The Working Group noted the 1-year duration of treatment and incomplete reporting of the study.]

In a GLP study, Cruzan et al. (1998) reported on groups of 60 male and 60 female CD (Sprague-Dawley) rats that were exposed to air containing styrene vapour (purity, > 99.5%) at doses of 0 (control), 50, 200, 500, and 1000 ppm by whole-body inhalation, 6 hours per day, 5 days a week, for 104 weeks. During week 61, a technical problem which resulted in liquid styrene dripping into the exposure chambers at a discrete location meant that eight males in the group exposed at 1000 ppm and six males in the group exposed at 500 ppm experienced important dermal exposure of styrene; all the rats died or were killed within the 2 weeks that followed, and these were excluded from the mortality or tumour incidence analysis. Styrene had no effect on survival in males, but a dose-related increase in survival of females exposed at 500 or 1000 ppm was observed. Males exposed at 500 or 1000 ppm gained less weight than the controls during the first year, and maintained the difference during the second year. Females exposed at 200, 500, or 1000 ppm gained less weight during the first year, and those exposed at 500 or 1000 ppm continued to gain less weight during months 13–18. From week 91 to termination of the study, females exposed at 500 ppm weighed less than those exposed at 1000 ppm. Exposure to styrene did not cause treatment-related increases in the incidence of any tumour type in males or females. A significant dose-dependent decrease in the incidence of mammary gland adenocarcinoma was reported in females. [The Working Group noted that the number of animals used, the duration of exposure and observation, and the schedule of exposure were all adequate.]

(c) Intraperitoneal injection

Groups of 40 male and 40 female Sprague-Dawley rats were given styrene (purity, 99.8%) by intraperitoneal injection at 50 mg per animal in olive oil, 4 times, at 2-month intervals (total dose, 200 mg). Control groups of 40 male and 40 female rats were given olive oil alone. The study was terminated when the last rat died. There was no significant increase in the incidence of any tumour type (Conti et al., 1988). [The Working Group noted the incomplete reporting of the data, short duration of treatment, and low total dose.]

(d) Subcutaneous injection

Groups of 40 male and 40 female Sprague-Dawley rats were given a single subcutaneous injection of 50 mg styrene (purity, 99.8%) per animal in olive oil. Control groups of 40 male and 40 female rats were given olive oil alone. The study was terminated when the last rat died. There was no significant increase in the incidence of any tumour type (Conti et al., 1988). [The Working Group noted the limited reporting of the study and the short duration of the treatment.]

3.2 Styrene-7,8-oxide

See Table 3.3.

3.2.1 Mouse

(a) Oral administration

Groups of 52 male and 52 female B6C3F$_1$ mice were given styrene-7,8-oxide (purity, 96.6%; two of the three impurities were unspecified amounts of benzaldehyde and benzene) at a dose of 0 (control), 375, or 750 mg/kg bw in corn oil by gavage 3 times per week, for 104 weeks. All

Table 3.3 Studies of carcinogenicity in experimental animals exposed to styrene-7,8-oxide

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Mouse, B6C3F$_1$ (M) 7 wk 107–108 wk Lijinsky (1986)	Gavage Styrene-7,8-oxide, 96.6% Corn oil 0, 375, 750 mg/kg bw, 3×/wk for 104 wk 52, 52, 52 42, 34, 6	*Forestomach* Squamous cell papilloma 2/51*, 22/51**, 8/52***	*[$P < 0.001$ (trend)], **[$P < 0.001$], ***[$P < 0.05$]	Principal strengths: adequate number of animals used Survival read from figure, marked reduction at high dose; body weight and body-weight gain reduction in treated males
		Squamous cell carcinoma 0/51*, 16/51**, 15/52**	*[$P < 0.001$ (trend)], **[$P < 0.001$]	
		Squamous cell papilloma or carcinoma (combined) 2/51*, 37/51**, 21/52**	*[$P < 0.001$ (trend)], **$P < 0.001$	
		Liver: hepatocellular adenoma or carcinoma (combined) 12/51*, 28/51**, 15/52	*[$P = 0.002$ (trend)], **$P < 0.001$	
Full carcinogenicity Mouse, B6C3F$_1$ (F) 7 wk 107–108 wk Lijinsky (1986)	Gavage Styrene-7,8-oxide, 96.6% Corn oil 0, 375, 750 mg/kg bw, 3×/wk for 104 wk 52, 52, 52 35, 33, 18	*Forestomach* Squamous cell papilloma 0/51*, 14/50**, 17/51**	*[$P < 0.001$ (trend)], **[$P < 0.001$]	Principal strengths: adequate number of animals used Survival read from figure, marked reduction at high dose; body-weight gain reduction in treated females
		Squamous cell carcinoma 0/51*, 10/50**, 3/51	*[$P < 0.001$ (trend)], **[$P < 0.001$]	
		Squamous cell papilloma or carcinoma (combined) 0/51*, 24/50**, 20/51**	*[$P < 0.001$ (trend)], **$P < 0.001$	
		Liver: hepatocellular adenoma or carcinoma (combined) 7/51, 4/50, 9/51	NS	

Table 3.3 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, Sprague-Dawley (M) 13 wk Lifetime Conti et al. (1988)	Gavage Styrene-7,8-oxide, NR Olive oil 0, 50, 250 mg/kg bw, 4–5×/wk for 52 wk 40, 40, 40 NR	*Forestomach*		Principal strengths: adequate number of animals used Principal limitations: limited reporting Slight increase in mortality rate was observed in treated males; Experiment BT105
		Squamous cell papilloma and acanthoma		
		0/40*, 3/40, 9/40**	*[$P = 0.002$ (trend)], **[$P < 0.05$]	
		Squamous cell carcinoma		
		0/40*, 11/40**, 30/40**	*[$P < 0.001$ (trend)], **[$P < 0.01$]	
		Squamous cell carcinoma (in situ)		
		0/40*, 6/40**, 18/40***	*[$P < 0.001$ (trend)], **[$P < 0.05$], ***[$P < 0.01$]	
		Squamous cell carcinoma (invasive)		
		0/40*, 5/40**, 12/40***	*[$P < 0.001$ (trend)], **[$P < 0.05$], ***[$P < 0.01$]	
		Adrenal gland: pheochromocytoma		
		2/40, 4/40, 6/40	[NS]	
		Mammary gland		
		Benign tumours		
		1/40*, 0/40, 10/40**	*[$P < 0.001$ (trend)], **[$P < 0.01$]	
		Malignant tumours		
		0/40, 0/40, 0/40	[NS]	

Table 3.3 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, Sprague-Dawley (F) 13 wk Lifetime Conti et al. (1988)	Gavage Styrene-7,8-oxide, NR Olive oil 0, 50, 250 mg/kg bw, 4–5×/wk for 52 wk 40, 40, 40 NR	*Forestomach* Squamous cell papilloma and acanthoma 0/40, 3/40, 5/40*	*[$P < 0.05$]	Principal strengths: adequate number of animals used Principal limitations: limited reporting Experiment BT105
		Squamous cell carcinoma 0/40*, 8/40**, 33/40**	*[$P < 0.001$ (trend)], **[$P < 0.01$]	
		Squamous cell carcinoma (in situ) 0/40*, 7/40**, 19/40**	*[$P < 0.001$ (trend)], **[$P < 0.01$]	
		Squamous cell carcinoma (invasive) 0/40*, 1/40, 14/40**	*[$P < 0.001$ (trend)], **[$P < 0.01$]	
		Adrenal gland: pheochromocytoma 1/40, 2/40, 0/40	[NS]	
		Mammary gland Benign and malignant tumours 4/40, 7/40, 9/40	[NS]	
		Malignant tumours 1/40, 0/40, 1/40	[NS]	
Full carcinogenicity Rat, F344/N (M) 9 wk 107–108 wk Lijinsky (1986)	Gavage Styrene-7,8-oxide, 96.6% Corn oil 0, 275, 550 mg/kg bw, 3×/wk for 104 wk 52, 52, 52 29, 33, 12	*Forestomach* Squamous cell papilloma 1/52*, 23/52**, 18/51**	*[$P < 0.001$ (trend)], **[$P < 0.001$]	Principal strengths: adequate number of animals used Survival read from figure; marked reduction of survival and body-weight gain at high dose
		Squamous cell carcinoma 0/52*, 35/52**, 43/51**	*[$P < 0.001$ (trend)], **[$P < 0.001$]	

Table 3.3 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, F344/N (M) 9 wk 107–108 wk Lijinsky (1986) (cont.)		Squamous cell papilloma or carcinoma (combined) 1/52*, 50/52**, 50/51**	 *[$P < 0.001$ (trend)], **[$P < 0.001$]	
Full carcinogenicity Rat, F344/N (F) 9 wk 107–108 wk Lijinsky (1986)	Gavage Styrene-7,8-oxide, 96.6% Corn oil 0, 275, 550 mg/kg bw, 3×/wk for 104 wk 52, 52, 52 38, 39, 17	*Forestomach* Squamous cell papilloma 0/52*, 21/52**, 24/52** Squamous cell carcinoma 0/52*, 32/52**, 36/52** Squamous cell papilloma or carcinoma (combined) 0/52*, 46/52**, 50/52**	 *[$P < 0.001$ (trend)], **[$P < 0.001$] *[$P < 0.001$ (trend)], **[$P < 0.001$] *[$P < 0.001$ (trend)], **[$P < 0.001$]	Principal strengths: adequate number of animals used Survival read from figure; marked reduction of survival and body-weight gain at high dose
Full carcinogenicity Rat, BDIV (M) GND 17 120 wk Ponomarkov et al. (1984)	Transplacental exposure and oral (gavage) administration Styrene-7,8-oxide, 97% Olive oil 0, 100–150 mg/kg bw, 1×/wk at age 4 wk until experimental wk 120 49, 43 49 (when first tumour observed), 42 (when first tumour observed)	*Forestomach* Papilloma 0/49, 7/42* Carcinoma (in situ) 0/49, 4/42* Carcinoma 0/49, 10/42*	 *$P < 0.003$ *$P < 0.04$ *$P < 0.0002$	Principal strengths: adequate number of animals used Principal limitations: age of dams NR Dams were given a single dose of olive oil (control) or 200 mg/kg bw styrene-7,8-oxide on day 17 of gestation

Table 3.3 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, BDIV (F) GND 17 120 wk Ponomarkov et al. (1984)	Transplacental exposure and oral (gavage) administration Styrene-7,8-oxide, 97% Olive oil 0, 100–150 mg/kg bw, 1×/wk at age 4 wk until experimental wk 120 55, 62 55 (when first tumour observed), 60 (when first tumour observed)	*Forestomach* Papilloma 2/55, 2/60 Carcinoma (in situ) 0/55, 6/60* Carcinoma 1/55, 16/60*	 NS *$P < 0.02$ *$P < 0.0001$	Principal strengths: adequate number of animals used Principal limitations: age of dams NR Dams were given a single dose of olive oil (control) or 200 mg/kg bw styrene-7,8-oxide on day 17 of gestation

bw, body weight; F, female; GND, gestation day; M, male; NR, not reported; NS, not significant; wk, week(s).

surviving animals were killed 3–4 weeks after the last dose. There was a marked reduction in the survival of male and female mice given the high dose, and body-weight gains were reduced in all mice exposed to styrene-7,8-oxide. Exposure resulted in a significant increase (with a significant positive trend) in the incidence of squamous cell papilloma of the forestomach at both low and high doses in males and females, and a significant increase (with a significant positive trend) in the incidence of squamous cell carcinoma of the forestomach in males at both low and high doses and in females at the low dose. The incidences of squamous cell papilloma or carcinoma (combined) of the forestomach were significantly increased (with a significant positive trend) at both low and high doses in males and females. A significant increase in the incidence (with a significant positive trend) of hepatocellular adenoma or carcinoma (combined) in males given the low dose was observed (Lijinsky, 1986).

(b) Skin application

Two groups of 40 C3H mice [sex unspecified] were given styrene-7,8-oxide [purity unspecified] by skin application of a dose of 5% or 10% in acetone [volume unspecified] on the clipped dorsal skin 3 times per week for life. Of the mice given the low dose, 17 survived over 24 months. Of the mice given the high dose, 18 survived to 12 months but only 2 of these mice survived to 17 months. No skin tumours were observed (Weil et al., 1963). [The Working Group noted the limited reporting of study details and the lack of controls, and concluded that the study was inadequate for the evaluation of the carcinogenicity of styrene-7,8-oxide.]

A group of 30 male Swiss ICR/Ha mice was given three applications per week of styrene-7,8-oxide [purified, but purity unspecified] for life on the clipped dorsal skin at a dose of 100 mg per application of a 10% solution in benzene. The median survival time was 431 days. Three of the 30 mice developed skin tumours (papillomas or cancers), one of which was a cancer that was probably a squamous cell carcinoma. Of the 150 controls treated with benzene only, 11 developed skin tumours (papillomas or cancers), one of which was a cancer that was probably a squamous cell carcinoma (Van Duuren et al., 1963). [The Working Group noted the carcinogenicity of the vehicle in experimental animals by other routes of exposure, and concluded that the study was inadequate for the evaluation of the carcinogenicity of styrene-7,8-oxide.]

3.2.2 Rat

(a) Oral administration

Groups of 40 male and 40 female Sprague-Dawley rats were given styrene-7,8-oxide [purity unspecified] at a dose of 0 (control), 50, or 250 mg/kg bw in olive oil by gavage for 4–5 days per week for 52 weeks, and then observed until they died. A slight increase in mortality rate was observed in treated males but not in treated females, and the last death occurred 156 weeks after the start of the experiment. The exposure was not observed to have any effect on body weight in both sexes. The treatment resulted in significantly increased incidences of squamous cell papilloma and acanthoma of the forestomach in males given the high dose and in females given the high dose, of squamous cell carcinoma of the forestomach (with a significant positive trend) in both groups of treated males and females, and of benign mammary tumours in males given the high dose. The incidences of acanthosis and dysplasia of the forestomach epithelium in males and females were related to treatment. No significant increase in the incidence of tumours at other sites was observed (Maltoni et al., 1979; Conti et al., 1988). [The Working Group noted the 1-year duration of treatment and the limited reporting of study details.]

Groups of 52 male and 52 female Fischer 344/N rats were given styrene-7,8-oxide (purity, 96.6%; two of the three impurities were unspecified

amounts of benzaldehyde and benzene) at a dose of 0 (control), 275, or 550 mg/kg bw in corn oil by gavage 3 times per week for 104 weeks. The experiment was terminated at 107–108 weeks. Survival and body-weight gain were reduced in males and females given the high dose. Treatment resulted in a significant increase (with a significant positive trend) in the incidence of squamous cell papilloma of the forestomach in treated males and females and in the incidence (with a significant positive trend) of squamous cell carcinoma of the forestomach in treated males and females. The incidences of squamous cell papilloma or carcinoma (combined) of the forestomach were also significantly increased in males and females, with a significant positive trend. There was a significant increase in the incidence of forestomach hyperplasia in treated males and females. No significant increase in the incidence of tumours at other sites was found (Lijinsky, 1986).

(b) Transplacental exposure and oral administration

A group of 14 pregnant BDIV inbred rats [age, unspecified] were given a single dose of styrene-7,8-oxide (purity, 97%) at 200 mg/kg bw in olive oil by gavage on day 17 of gestation. At 4 weeks of age, their offspring (43 males and 62 females) were given styrene-7,8-oxide (purity, 97%) at a dose of 100–150 mg/kg bw in olive oil by gavage once a week. The study was terminated at 120 weeks, to give estimated total doses of 5.0 g for males and 2.5 g for females. Control groups of 49 male and 55 female rats with no prenatal exposure were given olive oil only. At the time of appearance of the first tumour, 42 male and 60 female progeny that had been treated with styrene-7,8-oxide were still alive. In treated male progeny, the incidences of forestomach papilloma and forestomach carcinoma were significantly increased; in female progeny, the incidence of forestomach carcinoma was significantly increased. Hyperplasia, dysplasia, and hyperkeratosis of the forestomach were also reported in treated rats. There was no significant increase in the incidence of tumours at other sites in treated males and females (Ponomarkov et al., 1984).

4. Mechanistic and Other Relevant Data

4.1 Absorption, distribution, metabolism, and excretion

Styrene is extensively metabolized to styrene-7,8-oxide in humans and in experimental systems. As a result, external exposures to styrene engender internal exposures to both styrene and styrene-7,8-oxide. The absorption, distribution, metabolism, and excretion of styrene and styrene-7,8-oxide in humans have been previously reviewed (IARC, 1994, 2002; NTP, 2016a, b).

4.1.1 Absorption

(a) Humans

(i) Styrene

Styrene is absorbed by inhalation, dermal contact, or ingestion through consumption of food (Cohen et al., 2002). The predominant route in occupational settings is inhalation. A substantial number of studies in humans exposed to styrene have been conducted using occupational cohorts or volunteers exposed in inhalation chambers or by mask exposures. The results of these previously reviewed studies (IARC, 1994, 2002; NTP, 2016a, b) demonstrate that styrene is found in the blood of those exposed. The average pulmonary uptake of styrene under experimental conditions ranged from 63% to 68% (Wigaeus et al., 1984; Löf et al., 1986). An average concentration of styrene in blood of 15.3 μM has been reported in workers exposed to styrene in a

reinforced plastics factory (Brugnone et al., 1993). The clearance of styrene from blood was biphasic in human volunteers (Ramsey et al., 1980).

The concentrations of styrene in blood were determined in 86 reinforced plastics workers exposed to styrene and 42 control subjects. The average styrene concentration in blood was 5.4 µM in the exposed group and 0.67 µM in the control group (Vodička et al., 2004). Blood styrene levels and other exposure markers were measured in 58 workers exposed to styrene in four fibreglass-reinforced plastics industries. A log-linear correlation (r = 0.746) was found between blood and salivary levels of styrene in exposed subjects (Bonanni et al., 2015).

Blood levels of the primary metabolite of styrene, styrene-7,8-oxide, and its metabolite styrene glycol, were measured in exposed workers (Wigaeus et al., 1983; Tornero-Velez et al., 2001). The concentrations of styrene-7,8-oxide in blood were found to be variable, dependent on exposure conditions, and generally at nanomolar levels, and the concentrations of styrene glycol in blood were generally found at low micromolar levels (Wigaeus et al., 1983).

In humans, dermal absorption was reported to be very low in occupational exposures (Limasset et al., 1999), and up to 4% using urinary styrene metabolites and exhaled styrene as markers in experimental studies (Berode et al., 1985). It was concluded that inhalation exposure leading to pulmonary absorption is the major route of absorption of styrene in exposed workers (Berode et al., 1985).

(ii) Styrene-7,8-oxide

No studies on the exclusive absorption of styrene-7,8-oxide in humans were available to the Working Group. However, in workers exposed to both styrene and styrene-7,8-oxide in the air, concentrations of styrene-7,8-oxide–albumin adducts in blood correlated with styrene-7,8-oxide exposure concentrations, indicating styrene-7,8-oxide absorption into the blood (Rappaport et al., 1996).

(b) Experimental systems

(i) Styrene

Absorption studies of styrene have been conducted in Sprague-Dawley rats (inhalation and oral route), Wistar rats (inhalation and intravenous injection), Fischer 344 rats (inhalation and dermal contact), B6C3F$_1$ mice (inhalation), and CD2F1 and NMRI mice (intraperitoneal injection) (IARC, 1994, 2002). Rodents exposed to styrene by inhalation experienced pulmonary absorption, resulting in rapid blood uptake. The uptake efficiencies of styrene in the upper respiratory tract of male CD-1 mice and male Sprague-Dawley rats were inversely related to the exposure concentration (Morris, 2000). A maximum blood concentration of 10 µg/mL (96 µM) was observed after male Fischer 344 rats were exposed to gaseous styrene at 3000 ppm by dermal absorption for 4 hours (McDougal et al., 1990). In male B6C3F$_1$ mice, the concentration of styrene in blood was 21.8 µg/mL (200 µM) and of styrene-7,8-oxide in blood was 2.25 µg/mL (20 µM) after exposure by inhalation to 500 ppm styrene for 6 hours per day, for 14 days (Mahler et al., 1999).

The concentrations of styrene-7,8-oxide in the lungs of male Sprague-Dawley rats exposed to styrene vapour using an isolated lung perfusion system, and in the lungs of male B6C3F$_1$ mice exposed to styrene vapour using an in vivo lung perfusion system, were measured over a range of exposure concentrations. After adjusting the experimental data to the species-specific in vivo conditions of lung perfusion and ventilation, the mean styrene-7,8-oxide concentrations in mouse lungs were about twice as high as those in rat lungs at equal styrene exposure concentrations of up to 410 ppm (Hofmann et al., 2006).

(ii) Styrene-7,8-oxide

Absorption studies of styrene-7,8-oxide have been conducted in Fischer 334 rats (oral route), Sprague-Dawley rats (oral route and intraperitoneal injection), B6C3F$_1$ mice (oral route and intraperitoneal injection), and CD2F1 mice (intraperitoneal injection). In CD2F1 mice given a single intraperitoneal injection of 200 mg/kg body weight (bw) of styrene-7,8-oxide, peak plasma levels were attained within 7 minutes and styrene-7,8-oxide was not detectable at 60 minutes (Bidoli et al., 1980). Oral exposure yielded poor bioavailability and variable absorption in both rats and mice, probably because of the acid-catalysed degradation of styrene-7,8-oxide (IARC, 1994). In male Fischer 344 rats exposed by inhalation to styrene (1000 ppm) or styrene-7,8-oxide (25 ppm and 50 ppm) for 6 hours per day, 5 days per week for 4 weeks, concentrations of styrene-7,8-oxide in blood of 0.37 ± 0.08 μg/g were measured. This concentration was numerically between the concentrations of styrene-7,8-oxide in the blood of rats exposed to styrene-7,8-oxide at 25 ppm and 50 ppm under the same experimental conditions (Gaté et al., 2012).

4.1.2 Distribution

(a) Humans

(i) Styrene

After exposure by inhalation, styrene is rapidly absorbed into the blood and is distributed throughout the body. Industrial workers and volunteers had styrene in their adipose tissues, and subcutaneous adipose tissue contained a higher concentration of styrene compared with blood (Wigaeus et al., 1983). It was estimated that about 8% of the styrene was retained in adipose tissues, and the half-life of styrene in adipose tissues was approximately 2.8–5.2 days (Engström et al., 1978a, b). No constant increase was observed in the mean values of urinary styrene metabolites in workers exposed over a 4-day period, suggesting that styrene does not continuously accumulate in the body (Pekari et al., 1993).

(ii) Styrene-7,8-oxide

No data on the distribution of styrene-7,8-oxide in humans were available to the Working Group.

(b) Experimental systems

(i) Styrene

The tissue distribution of styrene was determined in male Sprague-Dawley rats and CD-1 mice exposed to radiolabelled styrene at 160 ppm for 6 hours using a nose-only exposure system. Urinary excretion was the primary route of excretion in both mice and rats. Radioactivity levels were observed in many organs of both species, with nasal mucosa in both rats and mice having the highest levels. A significantly higher level of radioactivity was measured in mouse lung and nasal passages compared with rat lung and nasal passages (Boogaard et al., 2000a). The tissue distribution and time-course of styrene accumulation and loss in tissues of male CD2F1 mice given a single intraperitoneal injection of styrene at 200 mg/kg were determined. Styrene levels peaked in brain, heart, lungs, liver, kidneys, and spleen within 5–30 minutes and then declined rapidly. In perirenal fat, in which the highest concentration of styrene was measured, styrene levels peaked later (Pantarotto et al., 1980).

(ii) Styrene-7,8-oxide

The tissue distribution of styrene-7,8-oxide was determined in one study in male Sprague-Dawley rats given a single intraperitoneal injection of radiolabelled styrene-7,8-oxide at 460 μmol. The radioactivity levels were higher in liver, brain, kidney, and duodenal contents than in blood, lungs, and spinal cord (Savolainen & Vainio, 1977).

(c) Toxicokinetic and pharmacokinetic models

Multicompartment physiological-based pharmacokinetic models and toxicokinetic models have been previously described for styrene exposure (inclusive of styrene-7,8-oxide formation), and estimate styrene distribution and metabolism in humans, rats, and mice (Csanády et al., 1994; Wang et al., 1996; Tornero-Velez & Rappaport, 2001; Cohen et al., 2002; Filser et al., 2002; Sarangapani et al., 2002). Pharmacokinetic modelling of styrene-7,8-oxide concentrations in the terminal bronchioles of humans, rats, and mice predicted tissue concentrations 100-fold higher in mouse compared with human and 10-fold higher in mouse compared with rat from exposure to styrene at 0.01–10.0 ppm by inhalation (Sarangapani et al., 2002).

The physiological-based pharmacokinetic model of Csanády et al. (1994) that estimated the distribution and metabolism of styrene and styrene-7,8-oxide in humans, rats, and mice after exposure by inhalation, intravenous injection, intraperitoneal injection, and by the oral route was modified to include the parameters of enzymatic formation of styrene-7,8-oxide and styrene glycol, and of consumption of glutathione (GSH). The model simulated the distribution of styrene-7,8-oxide in both liver and lung, and the subsequent metabolism of styrene-7,8-oxide by epoxide hydrolase and glutathione-*S*-transferase (GST). The formation of styrene-7,8-oxide–haemoglobin adducts and styrene-7,8-oxide–DNA adducts in the lymphocytes of humans, rats, and mice exposed to styrene was also simulated (Csanády et al., 2003).

A population- and physiological-based pharmacokinetic model for styrene was developed using Bayesian methods to reduce the uncertainty of the partition coefficients and metabolic parameters (Jonsson & Johanson, 2002).

The physiological toxicokinetic model of Csanády et al. (1994) was used to predict the concentrations of styrene-7,8-oxide in the blood of humans and rats after oral exposure to styrene to predict genotoxic potential (Filser & Gelbke, 2016).

4.1.3 Metabolism

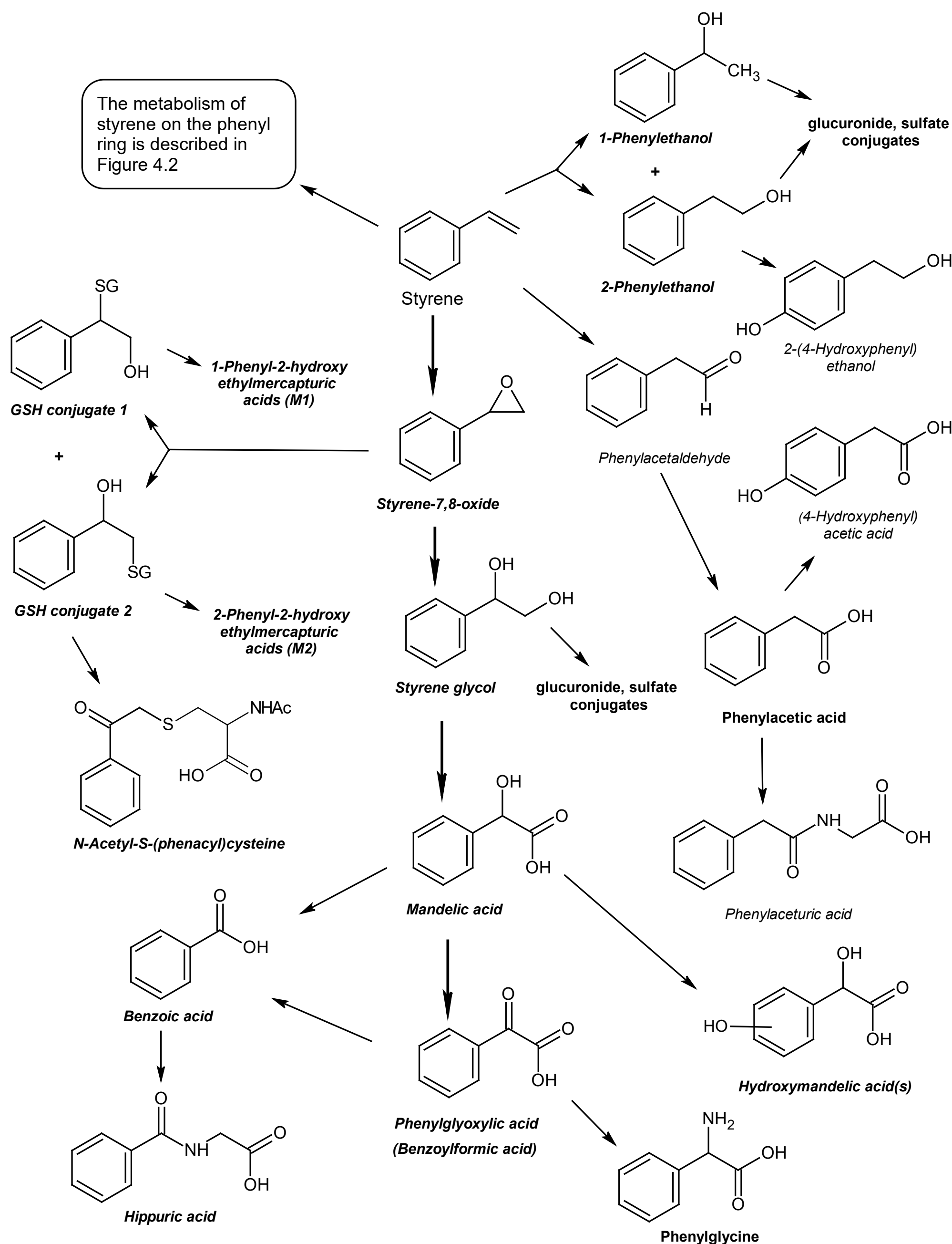
(a) Humans

See Fig. 4.1 and Fig. 4.2.

In humans, styrene is initially oxidized by cytochrome P450s (CYPs) through three distinct pathways: (i) epoxidation of the vinyl double bond, the major metabolic pathway; (ii) oxidation on the vinyl group; and (iii) oxidation on the phenyl ring. Metabolites from all three pathways have been detected in humans exposed to styrene and in experimental studies.

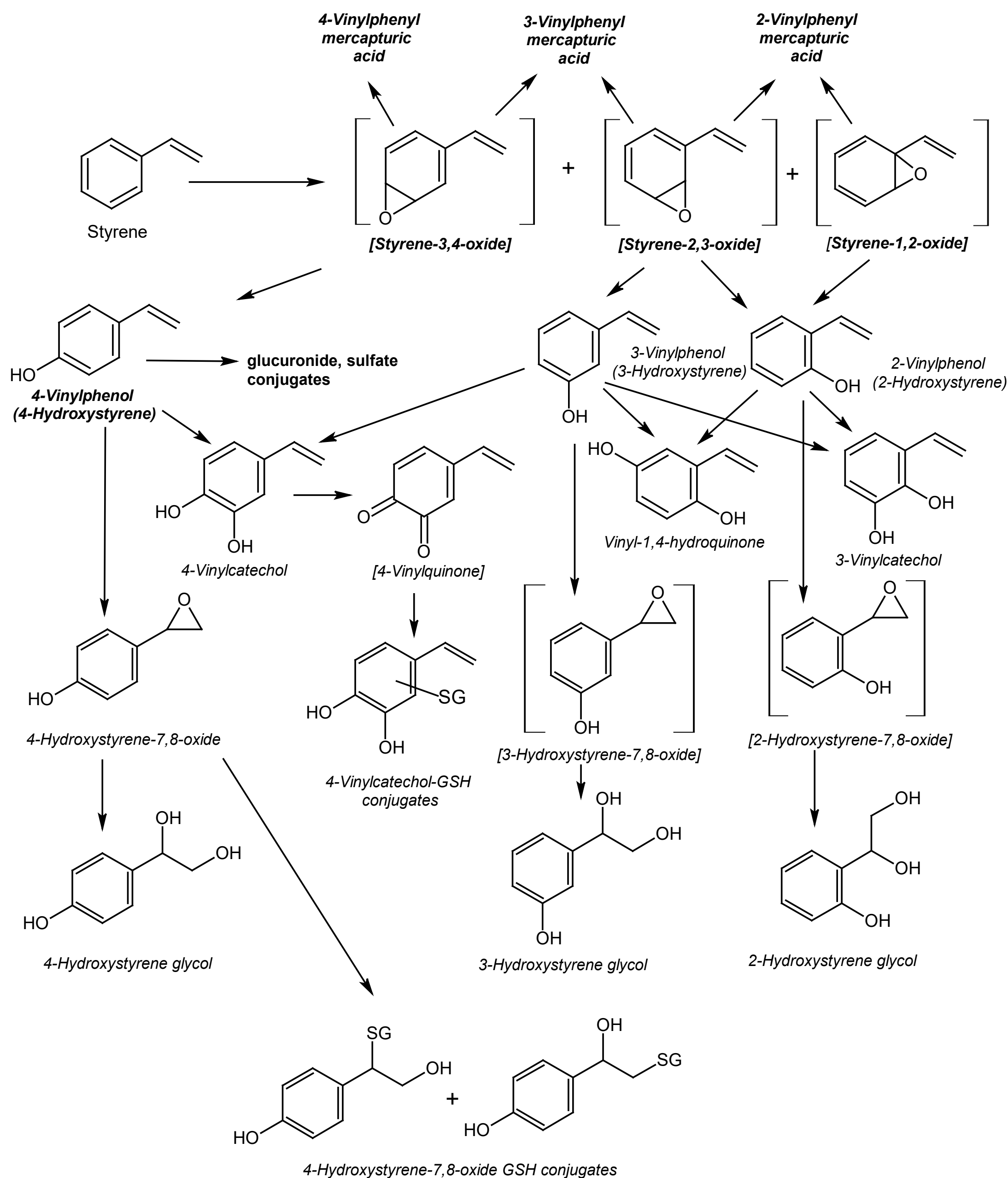
Based on in vitro studies, styrene is metabolized on the vinyl double bond to styrene-7,8-oxide by a group of human CYPs: CYP1A2, CYP2B6, CYP2C8, CYP2E1, CYP2F1, CYP3A3/3A4/3A5, and CYP4B1. CYP2B6 and CYP2E1 are found in human liver and/or lungs and CYP2F1 is found in human lungs (Nakajima et al., 1994a; Carlson, 2008). Human CYP2A13, which is preferentially expressed in the nasal mucosa, lung, and trachea (Su et al., 2000), also metabolized styrene to styrene-7,8-oxide (Fukami et al., 2008). CYP2E1 was found to play a primary role in styrene metabolism in human liver samples (Kim et al., 1997; Wenker et al., 2001a). Styrene-7,8-oxide underwent enzymatic hydration by human liver microsomal epoxide hydrolases to produce styrene glycol (phenylethylene glycol) (Oesch et al., 1974). This enzymatic process was not inhibited by the product styrene glycol (Oesch et al., 1974); however, in a racemic mixture, *R*-styrene-7,8-oxide inhibited the hydration of *S*-styrene-7,8-oxide (Wenker et al., 2000). Human liver cytosolic epoxide hydrolase can also hydrate styrene-7,8-oxide to styrene glycol, but the V_{max} was 7-fold lower compared with the microsomal form (Schladt et al., 1988). Styrene glycol was also formed by human lung microsomes (Nakajima

Fig. 4.1 Metabolism of styrene based on human and experimental studies



Metabolites in bold were found in human studies, metabolites in italics were found in experimental studies, and metabolites in both bold and italics were found in both human and experimental studies. Main pathways are indicated by thick arrows. GSH, reduced glutathione.
Adapted from Review of the Metabolic Fate of Styrene, Sumner & Fennell (1994), *Critical Reviews in Toxicology*, Taylor & Francis, reprinted by permission of the publisher (Taylor & Francis Ltd, http://www.tandfonline.com), and Boyd et al. (1990).

Fig. 4.2 Metabolism of styrene on the phenyl ring based on human and experimental studies



Metabolites in bold were found in human studies, metabolites in italics were found in experimental studies, and metabolites in both bold and italics were found in both humans and in experimental studies. Metabolites in brackets are putative.
GS, glutathione; GSH, reduced glutathione.
Figure compiled using information from Shen et al. (2010), Zhang et al. (2011), and Linhart et al. (2012).

et al., 1994a). Styrene glycol was enzymatically conjugated to glucuronic acid by human uridine 5′-diphospho (UDP)-glucuronosyltransferases and to sulfate by human sulfotransferases, forming glucuronide and sulfate conjugates (Korn et al., 1985). Styrene-7,8-oxide can also be conjugated to GSH by GSTs to yield GSH conjugate 1 (*S*-(1-phenyl-2-hydroxyethyl)glutathione) and GSH conjugate 2 (*S*-(2-phenyl-2-hydroxyethyl) glutathione) (Pachecka et al., 1979); the human GSTs involved are GSTM1, GSTP1, and GSTT1 (Ollikainen et al., 1998; De Palma et al., 2001). These styrene-7,8-oxide–glutathione conjugates are catabolized to isomeric phenylhydroxyethylmercapturic acids (PHEMAs), *N*-acetyl-*S*-(1-phenyl-2-hydroxyethyl)-L-cysteine (M1, also known as *N*-acetyl-*S*-(2-hydroxy-1-phenylethyl) cysteine) and *N*-acetyl-*S*-(2-phenyl-2-hydroxyethyl)-L-cysteine (M2, also known as *N*-acetyl-*S*-(2-hydroxy-2-phenylethyl)cysteine), which have been identified in the urine of exposed humans (see Section 4.1.5(a)(i)). The GSH conjugation process can also occur without enzyme involvement (Yagen et al., 1981). *N*-acetyl-*S*-(phenylacyl) cysteine is a catabolite of M2 (Manini et al., 2002). In addition to the formation of conjugates with glucuronic and sulfuric acids, styrene glycol is also metabolized to mandelic acid (MA). Depending on the sequence of oxidation (or reduction) of the α- and β-carbons of the glycol, there are several possible metabolic pathways from styrene glycol to MA. In a genetic polymorphism study of aldehyde dehydrogenase, Weng et al. (2016) proposed that styrene glycol is first metabolized by alcohol dehydrogenase to styrene glycolaldehyde (2-phenyl-2-hydroxyacetaldehyde), which is then metabolized to MA by aldehyde dehydrogenase. In humans, MA is metabolized to phenylglyoxylic acid (PGA, also known as benzoylformic acid) (Nagwekar & Kostenbauder, 1970) by alcohol dehydrogenase (Gao et al., 2009) and to *p*-hydroxymandelic acid (Pekari et al., 1993). Benzoic acid was detected in human urine after exposure to MA (Nagwekar & Kostenbauder, 1970), and is formed through a series of uncharacterized sequential oxidations. Hippuric acid (benzoylglycine), detected in human urine (Johanson et al., 2000), results from the conjugation of benzoic acid to glycine by the human glycine *N*-acyltransferases (Lino Cardenas et al., 2010). Phenylglycine was also detected in workers exposed to styrene (Manini et al., 2002; Fustinoni et al., 2008). It has been proposed that phenylglycine is formed from MA and/or PGA (Haufroid et al., 2002; Manini et al., 2002).

Another route of metabolism of styrene involves initial oxidation on the vinyl group by CYPs and then further metabolism of the observed metabolites, although the precise mechanisms of these processes are unknown. It has been proposed that the vinyl group initially undergoes both α- and β-oxidation, eventually leading to 1- and 2-phenylethanol (Cosnier et al., 2012). Racemic 1- and 2-phenylethanol were detected in the urine of workers exposed to styrene in both unconjugated forms and as glucuronide and sulfate conjugates (Korn et al., 1985, 1987). Phenylacetic acid is the oxidation product of phenylacetaldehyde (Wang et al., 2009).

In human studies, oxidation of the phenyl ring forms the putative arene oxides styrene-1,2-oxide, styrene-2,3-oxide, and styrene-3,4-oxide. Styrene-1,2-oxide and styrene-3,4-oxide can rearrange to form 2-vinylphenol (2-hydroxystyrene) and 4-vinylphenol (4-hydroxystyrene), respectively (Watabe et al., 1982). 4-Vinylphenol is conjugated to glucuronic acid and sulfate in humans, and these 4-vinylphenol conjugates represent about 0.5–1.0% of the total excretion of styrene metabolites (Manini et al., 2003). 4-Vinylphenyl mercapturic acid and traces of 2- and 3-vinylphenyl mercapturic acids were also detected in human urine, implying the formation of styrene-1,2-oxide, styrene-2,3-oxide, and styrene-3,4-oxide (Linhart et al., 2012).

Epoxidation of the vinyl group of styrene results in the formation of optically active

S- and *R*-styrene-7,8-oxides. With respect to enantiomeric selectivity, human liver microsomal epoxide hydrolase hydrated *S*-styrene-7,8-oxide 5 times faster than *R*-styrene-7,8-oxide. *R*-styrene-7,8-oxide was hydrated mainly to *R*-styrene glycol and *S*-styrene-7,8-oxide was hydrated mainly to *S*-styrene glycol. The overall activities of human styrene-7,8-oxide epoxide hydrolase varied between individuals by about 3–5-fold (Wenker et al., 2000). *R*-styrene glycol and *S*-styrene glycol were found in human blood (unconjugated and conjugated) and urine (conjugated). The maximum concentration of unconjugated *R*-styrene glycol in blood was greater than that of unconjugated *S*-styrene glycol, and the half-life of *R*-styrene glycol was longer (Wenker et al., 2001b). The ratio of the urinary stereoisomers favoured the *S*-form (or L-form as reported) (Korn et al., 1985, 1987). Both *R*- and *S*-enantiomers of MA were found in human urine at a ratio of 1:1.6 (Wenker et al., 2001b). Three mercapturic acids, degradation products of styrene-7,8-oxide–glutathione conjugates, were detected in human urine: *N*-acetyl-*S*-(1-phenyl-2-hydroxyethyl)-L-cysteine (M1-*R*, M1-*S*) and *N*-acetyl-*S*-(2-phenyl-2-hydroxyethyl)-L-cysteine (M2). M2 concentrations were higher than M1-*S* concentrations, and both were considerably greater than M1-*R* concentrations (Ghittori et al., 1997). In another study, two pairs of mercapturic acid diastereomers were identified in human urine with concentrations of M1-*R* and M2-*S* exceeding those of M1-*S* and M2-*R* (De Palma et al., 2001).

Genetic polymorphisms in styrene-metabolizing enzymes can play a role in the metabolism of styrene in humans, and urinary concentrations of the corresponding metabolites have been associated with these genetic polymorphisms. Decreased excretion levels of urinary MA and PGA, as well as decreased expression of *CYP2E1* mRNA levels, were noted in 49 subjects exposed to styrene carrying the heterozygous allele of *CYP2E1*5B* (c1/c2) when compared with subjects carrying the homozygote (c1/c1) (Prieto-Castelló et al., 2010). Of 30 workers from two fibreglass-reinforced plastics manufacturing plants, those carrying both the heterozygous alleles *CYP2E1*5B* and *CYP2E1*6* excreted lower levels of MA and PGA in their urine compared with homozygous subjects (Carbonari et al., 2015). Genotype (*CYP2E1*, *CYP2B6*, *EPHX1*, *GSTM1*, *GSTT1*, and *GSTP1* genetic polymorphisms) and lifestyle (smoking, alcohol consumption) did not significantly affect the levels of urinary MA and PGA in 73 reinforced plastics workers exposed to styrene. However, urinary MA and PGA levels were significantly decreased in a subgroup of non-smokers with the c1/c1 alleles of *CYP2E1*5B1* compared with those with the c1/c2 genotype (Ma et al., 2005). No relationships were found between genetic polymorphisms in *CYP2E1*, *EPHX1*, *GSTM1*, *GSTT1*, and *GSTP1* genes and levels of urinary MA and PGA in 75 workers in the fibreglass-reinforced plastics industry (Costa et al., 2012). In 56 workers exposed to styrene in the fibreglass-reinforced plastics manufacturing industry, the urinary concentrations of MA and PGA were significantly higher in the individuals carrying high-activity genotypes of epoxide hydrolase (*EPHX1*) compared with those carrying low-activity genotypes (Zhang et al., 2013). In 30 workers from two fibreglass-reinforced plastics manufacturing plants, individuals carrying the low-activity *EPHX1* codon 113 polymorphism exhibited reduced excretion of urinary MA and PGA compared with individuals carrying the high-activity genotype (Carbonari et al., 2015). Other studies have shown that the *EPHX1* genetic polymorphisms have no effect on urinary levels of MA and PGA in human studies (Ma et al., 2005; Costa et al., 2012). Genetic polymorphisms have been found in aldehyde dehydrogenase, and lower levels of urinary MA and PGA were observed in a group of 329 workers exposed to styrene carrying the variant inactive *ALDH2*2* allele (Weng et al., 2016). In 26 subjects experimentally exposed in a chamber to

styrene, the urinary levels of the diastereomeric PHEMAs M1 (*R*,*R*; *S*,*R*) and M2 (*R*,*R*; *S*,*R*) were significantly higher in *GSTM1*-positive subjects compared with *GSTM1*-null subjects (Haufroid et al., 2002). GSTM1-1 is the major isoenzyme catalysing GSH conjugation of styrene-7,8-oxide in humans (De Palma et al., 2001). A study of genetic polymorphisms in *EPHX1*, *GSTT1*, *GSTM1*, *GSTP1*, and *NAT2* in 95 fibreglass-reinforced plastics or polyester resins workers exposed to styrene found that *GSTM1*-null individuals produced a significantly lower level of PHEMAs compared with *GSTM1*-positive subjects (Migliore et al., 2006). In another study of 28 reinforced plastics workers exposed to low concentrations of styrene, *GSTM1*-null subjects had higher urinary concentrations of MA and PGA compared with those carrying the non-null genotype (Teixeira et al., 2004).

(b) Experimental systems

The metabolism of styrene in experimental systems is qualitatively similar to that described for humans (Section 4.1.3(a)(i)) with some quantitative differences.

In previous reviews (IARC, 1994, 2002) it was reported that, in experimental systems, the enzymatic action of Cyp catalyses the epoxidation of styrene on its vinyl group to enantiomers of styrene-7,8-oxide. Styrene-7,8-oxide can be hydrolysed by epoxide hydrolases to enantiomers of styrene glycol, or enzymatically conjugated with GSH by the GSTs to form isomeric GSH conjugates. These styrene-7,8-oxide–glutathione conjugates are further catabolized to the corresponding PHEMAs. M2 is catabolized to *N*-acetyl-*S*-(phenacyl)cysteine (Manini et al., 2002). Styrene glycol is further metabolized to MA enantiomers, and these are oxidized to PGA. Further catabolic action on MA and/or PGA eventually leads to benzoic acid and its glycine conjugate, hippuric acid. The ring oxidation of styrene by CYP forms styrene-3,4-oxide which yields 4-vinylphenol. CYP-mediated oxidation of the vinyl side chain of styrene eventually leads to 1-phenylethanol, 2-phenylethanol, and phenylacetaldehyde. Phenylacetaldehyde is a direct rat liver microsomal metabolite of styrene (Mansuy et al., 1984), and is further oxidized by aldehyde dehydrogenase to phenylacetic acid (Wang et al., 2009). The glycine conjugate of phenylacetic acid, phenylaceturic acid, was also detected in rat urine (Delbressine et al., 1980).

The Cyps involved in styrene metabolism in rat liver include Cyp2c11/6, Cyp2b1/2, Cyp1a1/2, and Cyp2e1, although in rat lung only Cyp2b1/2 is active (Nakajima et al., 1994b). Cyp2f2 and Cyp2e1 were associated with styrene metabolism in mouse lung and Cyp2e1 in mouse liver (Carlson, 1997a; Green et al., 2001a). The rate of styrene metabolism to styrene-7,8-oxide was greater in mouse lung club (Clara) cells compared with mouse type-II pneumocytes, and greater in mouse club cells compared with rat club cells. *R*-styrene-7,8-oxide was preferentially formed in mouse lung microsomes and club cells, although *S*-styrene-7,8-oxide was preferentially formed in rat lung microsomes and rat type-II pneumocytes (Hynes et al., 1999). A comparison of the rate of microsomal metabolism of styrene to styrene-7,8-oxide between species demonstrated that it was highest in mouse liver, followed by rat liver, followed by human liver (Nakajima et al., 1994a).

Male B6C3F$_1$ mice given a single intraperitoneal dose of styrene at 400 mg/kg bw metabolized the styrene to urinary 1-phenylethane diol (styrene glycol), MA, two isomeric hydroxymandelic acids (2-(4-hydroxyphenyl) ethanol and (4-hydroxyphenyl)acetic acid), and the mercapturic acids *N*-acetyl-*S*-(2-hydroxy-2-phenylethyl)cysteine and *N*-acetyl-*S*-(2-hydroxy-1-phenylethyl)cysteine, which represented 10–15% of the given dose. PGA was a minor metabolite. Male B6C3F$_1$ mice given a single intraperitoneal injection of racemic styrene-7,8-oxide, *R*-styrene-7,8-oxide, or *S*-styrene-7,8-oxide at 150 mg/kg bw produced many of

the same metabolites as styrene. Styrene was predominantly metabolized to *S*-mandelic acid, *R*-styrene-7,8-oxide was predominantly metabolized to *R*-mandelic acid, and *S*-styrene-7,8-oxide was predominantly metabolized to *S*-mandelic acid (Linhart et al., 2000).

[The Working Group noted that there were no in vivo studies available in knockout mice or transgenic humanized mice exposed to styrene that measured the levels of styrene metabolites in the blood or in the urine. Moreover, the hepatic Cyp reductase knockout mice, epoxide hydrolase knockout mice, and transgenic humanized mice were derived from C57BL/6 mice, a strain that is insensitive to the tumorigenic effects of styrene.]

A series of in vitro metabolic studies, using liver and lung tissues taken from genetically altered mice that had metabolic enzymes related to styrene metabolism removed from their genomes, have been reported. These mice were deficient in Cyps (*Cyp2e1* knockout, *Cyp2f2* knockout), epoxide hydrolase (epoxide hydrolase knockout mice), or a hepatic Cyp reductase (Cyp reductase knockout mice). The rates of the microsomal metabolism of styrene to each enantiomer of styrene-7,8-oxide were similar using liver microsomes from *Cyp2e1* knockout mice and from wildtype controls; the rate of formation of styrene-7,8-oxide by lung microsomes from *Cyp2e1* knockout mice was lower compared with microsomes from wildtype mice. These results indicated that other Cyps in the knockout mice were capable of metabolizing styrene to styrene-7,8-oxide. Using specific inhibitors, the Cyp2e1 inhibitor diethyldithiocarbamate was the most potent towards inhibiting the formation of both enantiomers of styrene-7,8-oxide in lung and liver microsomes from both *Cyp2e1* knockout and wildtype mice, indicating that other unknown Cyps in the knockout mice were sensitive to this inhibitor (Carlson, 2003). The rates of metabolism of styrene to *R*-styrene-7,8-oxide was slightly reduced in liver microsomes from *Cyp2f2* knockout mice compared with wildtype controls, although the metabolic rates of *S*-styrene-7,8-oxide formation were similar in both *Cyp2f2* knockout mice and wildtype mice. There was a large decrease in the metabolic rate of *R*-styrene-7,8-oxide formation in the *Cyp2f2* knockout mice compared with wildtype controls, and a smaller decrease in the rate of formation of *S*-styrene-7,8-oxide in lung microsomes from both mouse strains (Carlson, 2012). It has been reported that liver and lung microsomal protein levels of Cyp1a, Cyp2a, Cyp2b, Cyp2e, and Cyp3a were similar in *Cyp2f2* knockout and wildtype mice (Li et al., 2011). The rates of liver microsomal metabolism of styrene to each enantiomer of styrene-7,8-oxide, and the enantiomeric ratios of styrene-7,8-oxides between wildtype mice and mice deficient in epoxide hydrolase, were not different. The rates of liver microsomal metabolism of styrene-7,8-oxide to both enantiomers of styrene glycol were lower in mice deficient in epoxide hydrolase than in wildtype mice (Carlson, 2010a). The liver and lung microsomal metabolism of styrene to enantiomers of styrene-7,8-oxide were compared between wildtype and hepatic Cyp reductase knockout mice. The rates of formation of each of the *R*- and *S*-enantiomers in liver microsomes decreased to greater than 96% in the hepatic Cyp reductase knockout mice. However, in lung microsomes there was a higher rate of formation of the *R*-enantiomer in hepatic Cyp reductase knockout mice compared with wildtype mice, but no difference in the formation of the *S*-enantiomer (Carlson, 2012).

In studies of male CD-1 mice, 2-vinylphenol, 3-vinylphenol (3-hydroxystyrene), 4-vinylphenol, and styrene glycol were detected in both liver and lung microsomal incubations of styrene, with the liver microsomes producing a higher rate of formation of each metabolite compared with the lung incubations. Liver microsomal incubations of styrene also produced 2-hydroxystyrene glycol, 4-hydroxystyrene glycol, and vinyl-1,4-hydroquinone. Liver microsomal incubations of the individual vinylphenols produced

the following results: 2-vinylphenol was metabolized to 2-hydroxystyrene glycol, 3-vinylcatechol, and vinyl-1,4-hydroquinone; 3-vinylphenol was metabolized to 3-hydroxystyrene glycol, 3-vinylcatechol, vinyl-1,4-hydroquinone, and 4-vinylcatechol; and 4-vinylphenol was metabolized to 4-hydroxystyrene glycol and 4-vinylcatechol. These glycols are hydration products of the intermediary hydroxystyrene-7,8-oxides. Using inhibitors, it appeared that Cyp2f2 may be more important in the metabolism of styrene to vinylphenols than Cyp2e1 in the mouse lung (Shen et al., 2010).

The rates of overall metabolism (loss of substrate) of 4-vinylphenol in lung and liver microsomes in male wildtype and *Cyp2e1* knockout mice were not different (Vogie et al., 2004).

The lung and liver microsomal metabolism of styrene to styrene glycol and 2-, 3-, and 4-vinylphenol was compared between wildtype mice and *Cyp2e1* knockout mice, and between wildtype mice and *Cyp2f2* knockout mice. *Cyp2e1* knockout mouse liver microsomes had reduced metabolic rates compared with liver microsomes from wildtype mice in producing 2-vinylphenol, 4-vinylphenol, and styrene glycol, although only the metabolic rate of 2-vinylphenol formation was reduced in the lung microsomal incubations. The order of the rates of vinylphenol metabolites formed were 2-vinylphenol > 4-vinylphenol > 3-vinylphenol in liver and lung microsomes from both *Cyp2e1* knockout and wildtype mouse strains. *Cyp2f2* knockout mouse liver microsomes produced no detectable levels of 3-vinylphenol and 4-vinylphenol, and reduced rates of formation of 2-vinylphenol and styrene glycol compared with wildtype mouse liver microsomes. *Cyp2f2* knockout mouse lung microsomes produced no detectable levels of 2-, 3-, or 4-vinylphenol, and a reduced rate of formation of styrene glycol compared with wildtype mouse lung microsomes (Shen et al., 2014).

Male CD-1 mouse lung microsomes metabolized 4-vinylphenol to 4-hydroxystyrene-7,8-oxide (4-(2-oxiranyl)-phenol) and 4-vinylcatechol, with 4-vinylcatechol predominant. Co-incubation of 4-vinylphenol with GSH and microsomes from male CD-1 mouse liver, mouse lung, male Sprague-Dawley rat lung, or human lung produced a group of 4-hydroxystyrene-7,8-oxide–glutathione conjugates. Mouse lung microsomes metabolized 4-vinylphenol at greater rates compared with rat or human lung microsomes. Co-incubation of 4-vinylphenol with GSH and mouse lung microsomes also produced 4-vinylcatechol–glutathione conjugates formed via a 4-vinylcatechol metabolite [possibly a quinone, although this metabolite has not been isolated]. 4-Hydroxystyrene-7,8-oxide–glutathione conjugates and 2-hydroxystyrene-7,8-oxide–glutathione conjugates were detected in incubations of mouse liver microsomes with *R*-styrene-7,8-oxide in the presence of GSH, suggesting an alternate metabolic pathway to vinylphenol metabolites. Using specific inhibitors, both Cyp2f2 and Cyp2e1 were implicated in the metabolism of 4-vinylphenol to 4-hydroxystyrene-7,8-oxide–glutathione conjugates (Zhang et al., 2011).

The metabolic parameters of the metabolism of styrene in isolated mitochondrial and microsomal (endoplasmic reticulum) fractions from livers of female Sprague-Dawley rats were applied to a kinetic model that incorporated Cyp2e1 levels. At low styrene concentrations (10 µM), 67% of the styrene metabolism occurred in mitochondria and 33% in the endoplasmic reticulum. At higher styrene concentrations (500 µM), the estimated styrene metabolism was 85% in the endoplasmic reticulum and 15% in the mitochondria. These results are explained by the increased metabolic efficiency of Cyp2e1 in the endoplasmic reticulum (Hartman et al., 2015).

4.1.4 Modulation of metabolic enzymes

(a) Styrene

Inhalation pre-exposure to styrene had no effect on the metabolism of styrene after subsequent styrene exposure (Wang et al., 1996). In 58 moulders and finishers exposed to styrene and acetone at four fibreglass-reinforced plastics manufacturing sites, simultaneous exposure to styrene and acetone modified styrene metabolism as evidenced by a reduction in the combined levels of urinary MA and PGA (Bonanni et al., 2015). However, the metabolism of styrene was not affected in workers exposed to styrene or human subjects experimentally co-exposed to styrene and acetone (Wigaeus et al., 1984; De Rosa et al., 1993; Apostoli et al., 1998). Multiple oral doses of ethanol temporally lowered urinary levels of MA and PGA in subjects exposed to styrene by inhalation (Cerný et al., 1990). Ethanol is a known inducer of CYP2E1 (IARC, 2012). Both single and multiple doses of ethanol shifted the excretion level versus time relationship of the urinary excretion of MA and PGA in subjects exposed to styrene by inhalation (Wilson et al., 1983; Cerný et al., 1990). Styrene metabolism in workers was not affected by occupational co-exposure to methanol and methyl acetate (Kawai et al., 1995).

(b) Styrene-7,8-oxide

No data on the modulation of metabolic enzymes by styrene-7,8-oxide in humans were available to the Working Group.

4.1.5 Excretion

(a) Humans

(i) Styrene

Micromolar levels of unmetabolized styrene were found in the urine of occupational (Ghittori et al., 1997) and experimental subjects (Johanson et al., 2000). Chamber exposure studies demonstrated that about 92% of the total absorbed dose of styrene was metabolized. Of the amount absorbed, 37% was eliminated in the urine as MA and 54% as PGA after 8 hours (Caperos et al., 1979). In another chamber exposure study, the cumulative percentage of MA and PGA excreted was 58% after 28 hours of exposure (Wigaeus et al., 1983). Several other previously discussed studies have confirmed the presence of urinary MA (Vodička et al., 1999). PGA (Wieczorek & Piotrowski, 1985), phenylethylene glycol (styrene glycol) (Korn et al., 1987), hippuric acid (Johanson et al., 2000), *p*-hydroxymandelic acid (Pekari et al., 1993), and 4-vinylphenol were excreted as a conjugate (Pfäffli et al., 1981). Urinary PHEMAs were found in workers exposed to styrene, and represent about 1% of styrene uptake in humans (De Palma et al., 2001). MA, PGA, 4-vinylphenol conjugates, and regioisomeric PHEMA levels were determined at the end of the shift in the urine of 86 reinforced plastics workers (employed in three plants) exposed to styrene, in 16 controls (maintenance workers from one of the plants), and in 26 unexposed individuals (external controls). The mean concentrations of total 4-vinylphenol conjugates were 5.64 ± 4.82 mg/g creatinine in exposed workers, with concentrations of 0.39 ± 0.39 mg/g creatinine in the plant controls and none detectable in the external controls (Vodička et al., 2004).

Phenylacetic acid and/or phenylaceturic acid accounted for less than 5% of the total excreted metabolites (Johanson et al., 2000). 1-Phenylethanol and 2-phenylethanol in both conjugated and unconjugated forms were found in human urine (Korn et al., 1985, 1987).

The urine of 10 workers exposed to styrene, sampled at the end of shift and the next morning, contained MA, PGA, phenylglycine, *N*-acetyl-*S*-(phenacyl)cysteine, the glucuronide and sulfate conjugates of 4-vinylphenol and of styrene glycol, and PHEMAs. The median concentrations at the end of shift of 4-vinylphenol glucuronide and 4-vinylphenol sulfate were 7.5 mg/g creatinine

and 6.5 mg/g creatinine, respectively (Manini et al., 2002). [The Working Group noted that no exposure data were provided.] The urine of 174 workers exposed to styrene, 26 volunteers exposed to styrene at 11.8 ppm for 8 hours, and 99 subjects not occupationally exposed to styrene was examined for levels of MA, PGA, 4-vinylphenol glucuronide, and 4-vinylphenol sulfate. The total 4-vinylphenol conjugate concentrations at the end of the shift were 1.2–3.97 mg/g creatinine (geometric mean). These metabolites comprised about 0.5–1.0% of the total excreted styrene metabolites and were eliminated following monophasic kinetics. In unexposed subjects, the background levels of total 4-vinylphenol conjugates were 0.22 mg/g creatinine (geometric mean). Smoking was related to the excretion of total 4-vinylphenol conjugates, but did not appear to be related to the excretion of MA and PGA in exposed workers (Manini et al., 2003). In a companion study, the distribution of the background levels of MA and PGA in the urine of 129 workers not exposed to styrene was log-normal across the population. No significant correlation was found between metabolite levels and sex, age, smoking, or alcohol consumption (Manini et al., 2004).

Urinary concentrations of styrene, MA, PGA, phenylglycine, 4-vinylphenol glucuronide and sulfate conjugates, and the PHEMAs M1 and M2 were determined in workers (before and after the shift) exposed to styrene and styrene-7,8-oxide (Fustinoni et al., 2008). Median exposure concentrations of styrene and styrene-7,8-oxide were 18.2 mg/m^3 and 133 μg/m^3 for reinforced plastics workers, 3.4 mg/m^3 and 12 μg/m^3 for varnish workers, and less than 0.3 mg/m^3 and less than 5 μg/m^3 for controls, respectively. The study group included 13 varnish workers and 8 reinforced plastics workers, and 22 automobile mechanics as controls. The median concentrations of urinary metabolites in the samples taken at the end of the shift were generally greater in the reinforced plastics workers compared with the varnish workers. The median concentrations of the total of MA and PGA at the end of the shift in workers exposed to styrene were 69.5–226.3 mg/g creatinine, compared with 1.17 mg/g creatinine in control subjects. The median concentrations of total 4-vinylphenol conjugates at the end of the shift in workers exposed to styrene were 1.72–3.69 mg/g creatinine, compared with 0.245 mg/g creatinine in control subjects (Fustinoni et al., 2008). Analysis of repeated measurements (four measurements per worker over 6 weeks) of the concentrations of urinary styrene and styrene metabolites in these workers indicated that within-worker variability was typically much smaller than between-worker variability for the majority of exposure metrics examined (Fustinoni et al., 2010).

The total concentrations of MA and PGA in the urine of 10 male hand-lamination workers in a reinforced plastics plant occupationally exposed to styrene were 141–1466 mg/g creatinine in the samples from the end of shift and 41.5–784.0 mg/g creatinine in the samples from the next morning. The urine samples also contained 4-vinylphenyl mercapturic acid, the degradation product of the styrene-3,4-oxide–glutathione adduct. The mean concentration of 4-vinylphenyl mercapturic acid in the samples from the end of shift was 4.59 ± 3.64 ng/mL, although in the samples from the next morning it was 2.14 ± 2.07 ng/mL. Total urinary 4-vinylphenyl mercapturic acid accounted for about 3.4×10^{-4}% of the absorbed dose of styrene (Linhart et al., 2012).

(ii) Styrene-7,8-oxide

No data on the excretion of styrene-7,8-oxide in humans were available to the Working Group.

(b) Experimental systems

(i) Styrene

The primary route of excretion in male F344 rats, male CD-1 mice, and male B6C3F$_1$ mice using nose-only exposure to radiolabelled styrene was in urine; faecal excretion was a

minor route (Sumner et al., 1997). The overall quantitative excretion of styrene and its metabolites was similar in male CD-1 mice and male Sprague-Dawley rats exposed to radiolabelled styrene at 160 ppm for 6 hours by nose-only inhalation (Boogaard et al., 2000a). Male Fischer 344 or Sprague-Dawley rats exposed to styrene by inhalation at 75 ppm and 250 ppm for 4 days excreted increased levels of MA, PGA, and hippuric acid in their urine compared with controls. After 1 day of exposure, the urinary MA and PGA concentrations of rats exposed to styrene at 250 ppm were 256 ± 55 mg/g creatinine and 672 ± 258 mg/g creatinine, respectively (Cosnier et al., 2012). Male Sprague-Dawley rats exposed by inhalation to styrene at 25–200 ppm, 6 hours per day, 5 days per week for 4 weeks, excreted MA, PGA, *N*-acetyl-*S*-(1-phenyl-2-hydroxyethyl)-L-cysteine (M1) and *N*-acetyl-*S*-(2-phenyl-2-hydroxyethyl)-L-cysteine (M2) (Truchon et al., 1990). The urine of male Sprague-Dawley rats given styrene at 1.1 mmol by intraperitoneal injection contained MA, PGA, and PHEMA (the major metabolites), and phenylglycine, *N*-acetyl-*S*-(phenacyl)cysteine, glucuronide, and sulfate conjugates of 4-vinylphenol and of styrene glycol (Manini et al., 2002). Although Bakke & Scheline (1970) found 1-phenylethanol and traces of 2-phenylethanol in the urine of rats given styrene by gavage, Manini et al. (2002) did not report the detection of the conjugates or the free forms of 1- and 2-phenylethanol.

Male B6C3F$_1$ mice given a single intraperitoneal injection of styrene at 400 mg/kg bw excreted MA, *N*-acetyl-*S*-(2-hydroxy-2-phenylethyl)cysteine, *N*-acetyl-*S*-(2-hydroxy-1-phenyl ethyl)cysteine, 1-phenylethane-1,2-diol (styrene glycol), 2-(4-hydroxyphenyl)ethanol, (4-hydroxyphenyl)acetic acid, 2-(methylthio)-2-phenylethanol, 2-(methylthio)-1-phenylethanol, and two unidentified isomeric hydroxymandelic acids (Linhart et al., 2000). The mercapturic acids were the major metabolites, followed by MA and 1-phenylethane-1,2-diol (styrene glycol) (Linhart et al., 2000). 2-Vinylphenol, 3-vinylphenol, and 4-vinylphenol were measured in the urine of male NMRI mice exposed to styrene at 600 ppm and 1200 ppm for 6 hours. Mercapturic acids related to styrene-2,3-oxide–glutathione and styrene-3,4-oxide–glutathione conjugates, that is, 2-, 3-, and 4-vinylphenylmercapturic acids, were also found in the urine at a ratio of 2:1:6. The urinary concentrations of 4-vinylphenyl mercapturic acid after exposure at 600 ppm and 1200 ppm were 0.75 ± 0.1 mg/L and 1.09 ± 0.07 mg/L, which represented 0.047% and 0.043%, respectively, of the adsorbed dose of styrene (Linhart et al., 2010).

(ii) Styrene-7,8-oxide

The urinary concentrations of MA, PGA, and hippuric acid were determined in male Fischer 344 rats exposed by inhalation to styrene-7,8-oxide at 25 ppm and 75 ppm for 4 days. After 4 days of exposure at 75 ppm, the concentrations of MA, PGA, and hippuric acid were 279 ± 92, 294 ± 118, and 1619 ± 525 mg/g creatinine, respectively (Cosnier et al., 2012). Male B6C3F$_1$ mice given a single intraperitoneal injection of styrene-7,8-oxide at 150 mg/kg bw excreted MA, *N*-acetyl-*S*-(2-hydroxy-2-phenylethyl)cysteine, *N*-acetyl-*S*-(2-hydroxy-1-phenylethyl)cysteine, 1-phenylethane-1,2-diol (styrene glycol), 2-(4-hydroxyphenyl)ethanol, (4-hydroxyphenyl) acetic acid, and two partially identified isomeric hydroxymandelic acids. Based on excretion, the mercapturic acids were the major metabolites followed by MA (Linhart et al., 2000).

4.2 Mechanisms of carcinogenesis

4.2.1 Protein adducts

Because of its electrophilicity, styrene-7,8-oxide produces stable covalent adducts not only with DNA (see Section 4.2.2) but also at the nucleophilic sites in proteins. Most relevant from this point of view are the adducts with blood protein globin because: (i) globin is easily

accessible in large amounts; (ii) a range of procedures for analysis of globin adducts is available; and (iii) globin adducts accumulate in the body during prolonged exposure in a predictable way, reflecting the lifespan of erythrocytes (~120 days in humans). The adducts with albumin have also been studied, but to a lesser extent. The three major areas of styrene-7,8-oxide–protein adducts described in this section are studies in exposed humans, in adduct characterization in human cells in vitro, and in experimental animals.

(a) Exposed humans

A limited number of studies have been undertaken to assess the styrene-7,8-oxide adduct levels in the blood proteins of reinforced plastics manufacturing workers occupationally exposed to styrene or to both styrene and styrene-7,8-oxide (Table 4.1).

The concentrations of styrene-7,8-oxide–valine in exposed workers, measured in all studies using the modified Edman degradation procedure, were mostly of the order of picomoles per gram of globin, close to the limits of detection of the gas chromatography (GC) -MS methods used. Regarding control globins from unexposed subjects, the concentrations of styrene-7,8-oxide–valine in some studies were below the limit of detection or not distinctly lower than those of exposed workers (Brenner et al., 1991; Severi et al., 1994). In other studies, the reported concentrations of 3.08 ± 3.30 pmol/g globin (Godderis et al., 2004) and 2.59 ± 0.25 pmol/g globin (Teixeira et al., 2007) were remarkably higher than that of less than 0.1 pmol/g globin reported by Vodička et al. (1999). [The Working Group noted the low variability in background levels reported by Teixeira et al. (2007).] With the exception of the study by Christakopoulos et al. (1993), no clear relationship between external exposure to styrene and the adduct levels could be established among the studies in exposed workers.

In addition, in a single relevant study on volunteers, Johanson et al. (2000) exposed four men to [$^{13}C_8$]styrene vapour at 50 ppm (213 mg/m^3) for 2 hours to attain a concentration of styrene-7,8-oxide–valine of 0.3 pmol/g globin, that is, 0.003 pmol/g per parts per million by weight (ppmh). A comparable value derived from the study of Christakopoulos et al. (1993) was 0.001 pmol/g per ppmh.

In several studies, styrene-7,8-oxide–cysteine concentrations in globin and albumin of workers exposed to known levels of styrene and styrene-7,8-oxide simultaneously were assessed using the procedure of reductive cleavage by Raney nickel followed by GC–electrochemical detector or GS-MS. No evidence was found of any exposure-related increase in globin adducts. In contrast, albumin adducts were observed to increase with exposure to either styrene or styrene-7,8-oxide. There was a stronger association with exposure to styrene-7,8-oxide than to styrene. Of the two isomeric styrene-7,8-oxide–cysteine adducts, better correlation with the concentrations of the parent compounds was observed for *S*-(2-hydroxy-1-phenylethyl)cysteine adduct, that is, the adduct in which styrene-7,8-oxide was bound to cysteine via α-carbon, and was measured as 2-phenylethanol (Yeowell-O'Connell et al., 1996). Only non-significant differences in styrene-7,8-oxide–cysteine adduct concentrations among exposed workers and controls were found by Fustinoni et al. (1998, 2008).

(b) Experimental systems in vivo

In studies in mice, the binding of intraperitoneally injected styrene or styrene-7,8-oxide to globin and to plasma proteins has been demonstrated. A linear dose–response relationship was demonstrated at all but the highest dose of styrene (0.28–4.35 mmol/kg bw) for the concentrations of styrene-7,8-oxide–valine in globin collected 3 hours later (range, 15–305 pmol/g globin) (Pauwels et al., 1996). In a separate study, styrene-7,8-oxide given at up to

Table 4.1 Styrene and styrene-7,8-oxide adducts in blood proteins of exposed workers in reinforced plastics manufacturing

Styrene or styrene-7,8-oxide concentration (mg/m³)[a]	Exposed workers			Controls		Reference
	No.	Adduct level[a]	Correlation with exposure (if $P \leq 0.05$)	No.	Adduct concentration[a]	
N-terminal valine (adduct concentration in pmol/g globin)						
Styrene: GM, 47 (3–189)	13	GM, 5.5 (2.0–15.3)		8	GM, 2.2 (0–24)	Brenner et al. (1991)
Styrene: ~300	7	28 (15–52)		3	< 13 (LOD not specified))	Christakopoulos et al. (1993)
Styrene: 31	52	< 10 (LOD)		24	< 10 (LOD)	Severi et al. (1994)
Styrene: 68 ± 49	13	1.7 ± 1.1	Styrene-7,8-oxide–valine vs styrene ($P = 0.001$) Styrene-7,8-oxide–valine vs MA ($P = 0.002$)	8	< 0.1 (LOD)	Vodička et al. (1999)
Styrene: 41 ± 41 (0–157)	44	5.23 ± 3.49 (< 1–25.52)	Styrene-7,8-oxide–valine vs styrene ($P = 0.017$)	44	3.08 ± 3.30 (< 1–13.06) (LOD, 1)	Godderis et al. (2004)
Styrene: 131 ± 16 (2–490)	57	5.98 ± 0.41	Styrene-7,8-oxide–valine vs MA+PGA ($P < 0.02$)	71	2.59 ± 0.25 (LOD, 1)	Teixeira et al. (2007)
Cysteine (adduct concentration in nmol/g protein)						
Styrene: 64.3 (0.9–235) Styrene-7,8-oxide: 0.159 (0.013–0.525)	48	1-PE-Hb: 0.084 ± 0.014 (0.02–0.45) 2-PE-Hb: 0.078 ± 0.003 (0.03–0.16) 1-PE-Alb: 0.29 ± 0.04 (0.02–1.8) 2-PE-Alb: 1.68 ± 0.12 (0.24–3.7)	2-PE-Alb vs styrene ($P = 0.017$) 2-PE-Alb vs styrene-7,8-oxide (0.010)	NR	NR	Yeowell–O'Connell et al. (1996)
Styrene: ~100	22	1-PE-Hb: 0.43 ± 0.10 (0.23–0.63) 2-PE-Hb: 5.44 ± 1.10 (3.71–7.97) 1-PE-Alb: 0.60 ± 0.49 (0.28–2.36) 2-PE-Alb: 2.84 ± 1.31 (1.02–6.79)	2-PE-Hb vs MA ($P = 0.053$) 2-PE-Alb vs MA ($P = 0.001$)	15	1-PE-Hb: 0.39 ± 0.17 (0.19–0.73) 2-PE-Hb: 5.27 ± 1.65 (1.93–8.57) 1-PE-Alb: 0.50 ± 0.23 (0.24–1.06) 2-PE-Alb: 2.74 ± 1.01 (1.17–4.91)	Fustinoni et al. (1998)

Table 4.1 (continued)

Styrene or styrene-7,8-oxide concentration (mg/m^{3})[a]	Exposed workers			Controls		Reference
	No.	Adduct level[a]	Correlation with exposure (if $P \leq 0.05$)	No.	Adduct concentration[a]	
Styrene: Median, 18.2 (2.3–93.4) Styrene-7,8-oxide: 0.134 (0.040–0.282) Styrene: 3.4 (0.55–16.0) Styrene-7,8-oxide: 0.012 (0.007–0.032)	8 13	1-PE-Hb: median, 0.20 (< 0.03–0.74) 2-PE-Hb: 2.31 (2.18–5.12) 1-PE-Alb: 0.23 (< 0.03–1.22) 2-PE-Alb: 5.91 (4.40–8.14) 1-PE-Hb: 0.11 (< 0.03–0.55) 2-PE-Hb: 2.80 (< 0.60–4.48) 1-PE-Alb: 0.48 (0.21–0.75) 2-PE-Alb: 6.18 (2.66–9.53)		22	1-PE-Hb: median, 0.22 (< 0.03–0.99) 2-PE-Hb: 1.96 (1.01–3.33) 1-PE-Alb: 0.19 (< 0.03–0.53) 2-PE-Alb: 3.57 (< 0.90–5.18)	Fustinoni et al. (2008)
Carboxylic acids (adduct concentration in nmol/g protein)						
Styrene: 64.3 (0.9–235) Styrene-7,8-oxide: 0.159 (0.013–0.525)	41 48	SG-Hb: 0.481 ± 0.132 (0.09–4.8) SG-Alb: 1.80 ± 0.19 (0.1–6.3)				Yeowell–O'Connell et al. (1996)

1-PE, 1-phenylethanol releasing moiety (i.e. 2-hydroxy-2-phenylethyl); 2-PE, 2-phenylethanol releasing moiety (i.e. 2-hydroxy-1-phenylethyl); Alb, albumin; GM, geometric mean; Hb, haemoglobin; LOD, limit of detection; MA, mandelic acid; NR, not reported; PGA, phenylglyoxylic acid; SG, styrene glycol releasing moiety (i.e. 2-hydroxy-2-phenylethyloxy or 2-hydroxy-1-phenylethyloxy); vs, versus.

[a] Mean ± SD (minimum to maximum) unless stated otherwise. The values represent pooled values from both non-smokers and smokers.

1.1 mmol/kg bw was bound to both globin and plasma proteins at a higher-than-proportional extent, although styrene-7,8-oxide given at up to 4.9 mmol/kg bw was bound to globin at a higher- and to plasma proteins at a lower-than-proportional extent (Byfält Nordqvist et al., 1985). In the linear components of dosage–binding relationships in both types of blood proteins, similar levels of radioactivity were found for both styrene and styrene-7,8-oxide. In other comparable studies (see paragraph below), the extent of styrene binding was much lower than that of styrene-7,8-oxide.

In a study by Rappaport et al. (1993), the concentrations of styrene-7,8-oxide–cysteine adducts in globin and albumin of rats given equimolar levels of styrene and styrene-7,8-oxide by intraperitoneal injection were about 50 times lower in both proteins after dosing with styrene. In the same paper, the in vitro reactivity of styrene-7,8-oxide with rat and human blood proteins was assessed. The values of second-order rate constants (expressed in (L/mol protein) per hour) of styrene-7,8-oxide binding (i.e. rat globin, 72; rat albumin, 63; human globin, 2.4; human albumin, 32) indicate that human globin was the least reactive among the proteins tested.

In a similar study, in both mice and rats given non-labelled and labelled styrene or styrene-7,8-oxide at up to 2.5 mmol/kg bw (Osterman-Golkar et al., 1995) by intraperitoneal injection, relationships between styrene or styrene-7,8-oxide exposure and styrene-7,8-oxide–valine adduct levels in globin were faster than linear, reflecting overload of the detoxification pathways. At low doses, styrene-7,8-oxide exposure generated 4–7 times higher concentrations of styrene-7,8-oxide–valine adduct than that for styrene exposure; at higher doses the difference was even greater. The elimination rate of total radioactivity and styrene-7,8-oxide–valine adducts from globin corresponded to the lifespan of mice erythrocytes (40 days) although carboxylic acid adducts were eliminated faster, perhaps because of hydrolysis of the ester bonds. In plasmatic proteins, initial bound radioactivity was about 25-fold higher compared with the binding to globin but its elimination rate was much faster, corresponding to the half-life of mice serum albumin (2 days).

In male Wistar rats given a single intraperitoneal injection of styrene-7,8-oxide at 0.83 mmol/kg bw, the total styrene-7,8-oxide–valine concentration in globin 24 hours after exposure was estimated as 0.72 nmol/g globin (Mráz et al., 2016b). For comparison, giving Sprague-Dawley rats an intraperitoneal injection of styrene-7,8-oxide at 1 mmol/kg bw resulted in a styrene-7,8-oxide–valine concentration of about 0.4 nmol/g globin. However, in the study by Osterman-Golkar et al. (1995), only a single diastereomer of styrene-7,8-oxide–valine was determined. In two recent studies in rats, the fate of several types of styrene-7,8-oxide–globin adducts following physiological removal of erythrocytes was investigated. In the urine of rats dosed intravenously with erythrocytes modified by styrene-7,8-oxide, styrene-7,8-oxide–valine and styrene-7,8-oxide–valine-leucine (N-terminal dipeptide of α-globin adducted by styrene-7,8-oxide) were identified as ultimate products of styrene-7,8-oxide-adducted N-terminal Val in globin, whereas the adducts with cysteine, histidine, and lysine were excreted in the form of corresponding N_α-acetyl derivatives (Mráz et al., 2016a). The same urinary products were also found in the urine of rats given styrene-7,8-oxide by intraperitoneal injection (Mráz et al., 2016b).

(c) Studies in vitro

In contrast to the binding of styrene-7,8-oxide to proteins, which is a straightforward chemical reaction, adduct formation from styrene is only possible in systems containing enzymes that oxidize styrene to styrene-7,8-oxide, that is, in vivo, in some cellular in vitro systems, and in subcellular microsomal fractions.

In the incubations in vitro of styrene-7,8-oxide with human or rat blood, erythrocytes,

haemoglobin, or globin, the formation of adducts was reported with cysteine, histidine, N-terminal valine, and lysine based on mass spectrometric confirmation (Hemminki, 1986; Kaur et al., 1989; Basile et al., 2002; Jágr et al., 2007). In addition, adducts derived from styrene-7,8-oxide with carboxylic acids in globin (phenylhydroxyethyl esters of aspartic and glutamic acids) were indirectly identified following mild base hydrolysis of these ester adducts to styrene glycol (Sepai et al., 1993). In some of the above studies, the concentrations of styrene-7,8-oxide adducts with particular amino acids in globin have been quantified. However, the relative proportions of all adduct types produced in globin under physiological conditions within a single experiment have not been reported. Nevertheless, by combining results from various studies, the adduct concentrations appear to decrease in the order cysteine > histidine > valine > lysine. In a study where human blood was incubated with styrene-7,8-oxide, and serum and haemoglobin were separated, digested with proteinase K, and analysed by HPLC, cysteine adducts greatly predominated both in haemoglobin and serum proteins (Hemminki, 1986). In a similar type of experiment using digestion with pronase, Jágr et al. (2007) reported histidine adducts in haemoglobin at concentrations approximately 10-fold higher than those of cysteine adducts. However, it later became obvious that the pronase digestion cleaves cysteine adducts of styrene-7,8-oxide incompletely, underestimating true concentrations by a factor of 20–30 (Mráz et al., 2016a). Histidines were found to be a dominant alkylated site in human haemoglobin when incubation was carried out with a high excess of styrene-7,8-oxide, perhaps because of the increasing number of alkylated histidine units after saturation of cysteine (Kaur et al., 1989). Detailed structural analysis of human globin adducted by styrene-7,8-oxide, based on tryptic digestion followed by HPLC-MS, revealed valine-1, histidine-20, histidine-45, histidine-50, histidine-72, and cyseine-104 as major alkylating sites in the globin α-chain, and valine-1, histidine-77, cysteine-93, histidine-97, cysteine-112, and histidine-143 as major alkylating sites in the globin β-chain (Kaur et al., 1989; Basile et al., 2002).

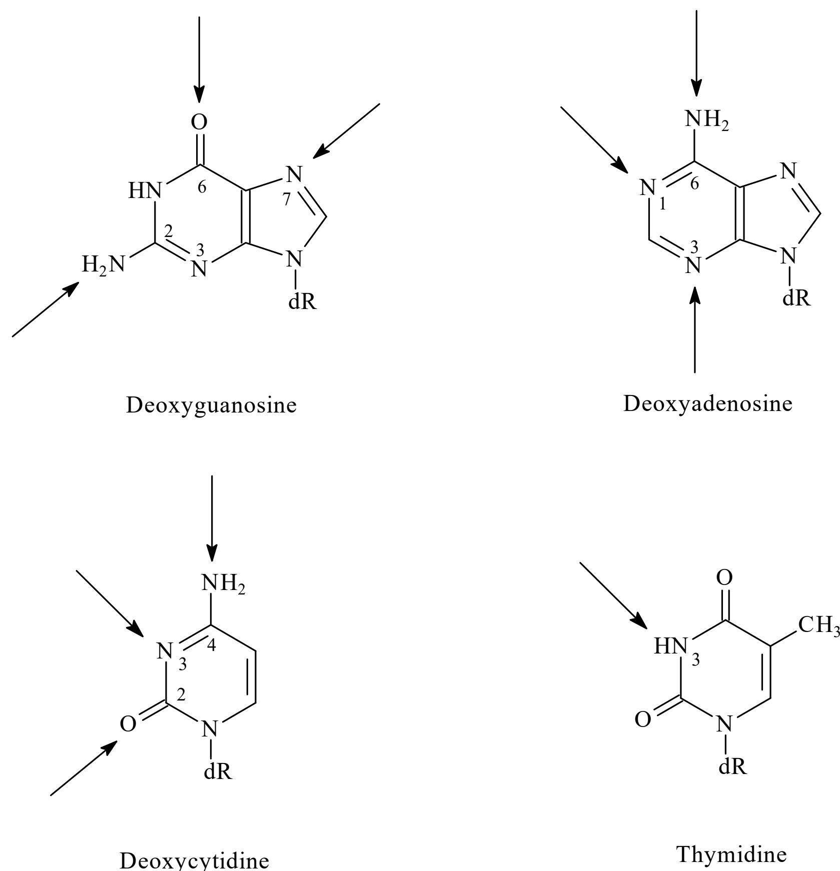
Cysteine was also found to be a dominant binding site for styrene-7,8-oxide in human albumin (Rappaport et al., 1993).

4.2.2 DNA adducts

(a) Properties

Styrene requires metabolic activation to be effectively eliminated from the body and to become genotoxic. Its principal metabolite in vivo, styrene-7,8-oxide, contributes quantitatively by far the most (> 95% in humans) to the genotoxicity of styrene; minor ring oxidation products are also shown to contribute to local toxicities, especially in the respiratory system (Vodička et al., 2006a). Styrene-7,8-oxide interacts covalently with biological macromolecules; its two electrophilic carbons, the α- and β-carbons of the epoxide moiety, attack nucleophilic sites in DNA (Fig. 4.3). Modification of the ring-nitrogens of purines and pyrimidines through the β-carbon follows the SN2 (nucleophilic substitution 2) reaction kinetics. In contrast, the epoxides that have a substituent capable of stabilizing positive charge can also react at the exocyclic amino groups through the α-carbon, the reaction following the SN1 type of mechanism (Koskinen & Plná, 2000). Table 4.2 summarizes covalent binding sites of styrene-7,8-oxide and their proportions, stabilities, and expected role in mutagenesis. *N*7-guanine is the primary target of styrene-7,8-oxide alkylation in DNA, followed by the N^2 and O^6 positions of guanine; *N*7-alkylation occurs predominantly through the β-carbon, whereas the other sites in guanine are primarily reacted through the α-carbon. As reported by Moschel et al. (1986), in O^6-guanine substitution the α-isomer can isomerize to that of β. Adenine residues are alkylated at the *N*3,

Fig. 4.3 Nucleophilic sites in DNA bases found to be covalently modified by styrene-7,8-oxide



dR, 2′-deoxyribose; SN1, nucleophilic substitution 1.
Compiled by the Working Group.

*N*1, and N^6 positions. Both α- and β-isomers of *N*1-adenine adducts are formed, the β-isomer in moderate excess (Barlow et al., 1997; Barlow & Dipple, 1998). The N^6 position is initially reacted only through the α-carbon of styrene-7,8-oxide; however, a considerable proportion of the βN^6 product arises, probably because of the Dimroth rearrangement of the β*N*1-adenine (Qian & Dipple, 1995; Barlow & Dipple, 1998). In addition, *N*1-adenine adducts undergo hydrolytic deamination under neutral conditions; the α-isomer adduct deaminates fairly readily (Barlow & Dipple, 1998). In vitro mechanisms for the formation of 2′-deoxyadenosine adducts with styrene-7,8-oxide have been summarized and compared with polycyclic aromatic hydrocarbon dihydrodiol epoxides (Kim et al., 2000). Styrene-7,8-oxide alkylates cytosine, mainly at the *N*3 position through the β-carbon and at the N^4 position through the α-carbon (Barlow & Dipple, 1999). In addition, the α-isomers of *N*3- and O^2-cytosine adducts have been identified. Interestingly, the *N*3-substituted deoxycytidine undergoes rapid hydrolytic deamination to the corresponding deoxyuridine adduct (the half-lives of the α- and β-isomer are 6 minutes and 160 minutes, respectively). O^2-cytosine adducts are also unstable, being prone to depyrimidation and interconversion between the α- and β-isomers (Koskinen et al., 2000b). Thymidine is a poor nucleophile towards styrene-7,8-oxide, and only minor alkylation at the *N*3 position has been

Table 4.2 Spectrum of styrene-7,8-oxide DNA adducts: stabilities, proportions, and expected role in mutagenesis

Base	Position	Chemical stability	Proportion and half-life in DNA[a]	Expected mutation	References
Guanine	*N*7	Depurination, ring-opening	α*N*7 29%, 51 h β*N*7 45%, 51 h	GC → TA	Hemminki & Hesso (1984); Moschel et al. (1986); Vodička et al. (1994, 2002); Koskinen et al. (2000a, 2001a)
	N[2]	Stable	α*N*[2] 3%	GC → TA	
	O[6]	Unstable, α to β isomerization	~1%, 1320 h	GC → AT	
Adenine	*N*[6]	Stable	α*N*[6] 6% β*N*[6] 1%	AT → GC	Savela et al. (1986); Qian & Dipple (1995); Koskinen et al. (2000a, 2001a, b); Vodička et al. (2002)
	*N*3	Depurination	α*N*3 6%, 10 h β*N*3 3%, 20 h	AT → TA	
	*N*1	Dimroth rearrangement (→ *N*[6] adenine), hydrolytic deamination (→ *N*1 hypoxanthine)	α*N*1 < 1%, 94 h β*N*1 2%, 450 h α*N*1-hypoxic 2%	AT → GC	
Cytosine	*N*3	Hydrolytic deamination (→ *N*3 uracil)	β*N*3-uracil 2%	CG → AT, GC → TA	Barlow & Dipple (1999); Koskinen et al. (2000b, 2001a)
	N[4]	Stable	α*N*[4] 1%	–	
	O[2]	Depyrimidation, α/β interconversion	ND	–	
Thymidine	*N*3		ND	–	Savela et al. (1986); Koskinen et al. (2000a)
Guanine/ deoxyguanosine	8-OHdG	Stable	ND	GC → TA	Vettori et al. (2005)

8-OHdG, 8-hydroxy-2′-deoxyguanosine (determined in human SK-N-MC neuroblastoma cell lines); h, hour(s); ND, not determined.

[a] Relative proportions of various adducts in double-stranded DNA treated for 32 h with styrene-7,8-oxide in a buffered solution at pH 7.4, including depurinated fraction.

Source: Styrene metabolism, genotoxicity, and potential carcinogenicity. Vodička et al. (2006a), *Drug Metabolism Reviews*, reprinted by permission of the publisher (Taylor & Francis Ltd, http://www.tandfonline.com) and of Dr Vodička.

observed (Koskinen & Plná, 2000; Koskinen et al., 2000a). The phosphate group was also identified as a target of alkylation in nucleotides; however, in the dinucleotide dGpdT no styrene-7,8-oxide alkylation of the intervening phosphodiester was observed (Koskinen & Hemminki, 1999). As apparent from Table 4.2, the relative proportions of the DNA adducts depend on the time of exposure to the alkylating agent. Adducts induced by styrene-7,8-oxide at *N*7-guanine [7-(hydroxyphenylethyl)guanines] and *N*3-adenine [3-(hydroxyphenylethyl)adenines] readily depurinate and reach an apparent saturation level considerably faster than adducts formed at positions involved in base-pairing. Half-lives of depurination from double-stranded DNA are listed in Table 4.2. *N*1-adenine adducts are partially unstable in DNA, undergoing Dimroth rearrangement or hydrolytic deamination; the corresponding half-lives are listed in Table 4.2. Chemically stable styrene-7,8-oxide–DNA adducts are α*N*[6]-adenine, α*N*[2]-guanine, and β*N*3-uracil adducts. *O*[6]-guanine adducts account for about 1%; the respective half-life for the β-isomer form of this adduct in double-stranded DNA has been estimated as 1320 hours (Vodička et al., 1994, 2002). The chemical reactivity of styrene-7,8-oxide with 4-(*p*-nitrobenzyl)pyridine (with similar nucleophilic character as DNA bases) was recently investigated; the reactivity is strongly affected by temperature and pH, suggesting careful

consideration of these parameters in physiological models (González-Pérez et al., 2014).

(b) Mutagenic potential

Table 4.2 illustrates the mutagenic potential of the different styrene-7,8-oxide–DNA adducts. Mutations in human cells and in experimental systems, including the Ames assay, are addressed in Sections 4.2.3(b) and 4.2.3(c), respectively; studies of mutations in exposed humans are addressed in Section 4.2.3(a).

*N*7-guanine adducts are expected to result in GC → TA and *N*3-adenine adducts in AT → TA transversions (Vodička et al., 2002, 2006a), since DNA polymerases preferentially insert an adenine opposite an apurinic site (Loeb et al., 1986). In assays for mutagenicity in cultured human T-lymphocytes treated in vitro with styrene-7,8-oxide, these mutations were found more frequently in hypoxanthine-guanine phosphoribosyl transferase (*HPRT*) mutant clones than in controls, although the predominant mutation type was AT → GC transition (Bastlová & Podlutsky, 1996). AT → TA mutations were also observed in a site-specific mutation study (carried out on oligodeoxynucleotides) in which a styrene-7,8-oxide adduct at the N^6 position of adenine was inserted in the N-*RAS* gene at codon 61 (Latham et al., 1993; Latham & Lloyd, 1994). However, the N^6-adenine adduct showed only minor miscoding potential, as it still has the ability to base-pair with thymine. Possibilities of by-passing of the N^6-adenine adduct in experimental systems were further discussed by Latham et al. (1996), but no major impact on DNA polymerization was reported (Latham et al., 2000; Hennard et al., 2001). Additional evidence suggests that major groove N^6-adenine adducts of styrene and butadiene oxides do not strongly perturb DNA structure and are not particularly mutagenic (Simeonov et al., 2000; Scholdberg et al., 2004). The *N*1-adenine or the corresponding *N*1-hypoxanthine adducts may contribute more to mutagenesis at AT-base pairs, since these adducts block a central hydrogen bonding site of adenine residues. The role of *N*3-cytosine adducts in mutagenesis may be deduced on the basis of experiments with protein extracts from mammalian cells that enzymatically repair *N*3-(2-hydroxypropyl)-dCyd, but not the corresponding uracil adduct (Plna et al., 1999). *N*3-(2-hydroxyethyl)-2′-deoxyuridine represents a mutagenic lesion, resulting in a GC → AT transition and, to a minor extent, GC → TA transversion mutations (Zhang et al., 1995). Since the β-isomer of the *N*3-uracil adduct was found in DNA treated in vitro with styrene-7,8-oxide , *N*3-uracil adducts may contribute to the GC → TA mutations, detected in the *HPRT* gene (Bastlová & Podlutsky, 1996). Alkylation at the O^6 position of guanine is also considered as a pro-mutagenic lesion, resulting in GC → AT transitions (Jansen et al., 1995); however, molecular analysis of styrene-induced mutations at the *HPRT* locus suggested that O^6-guanine adducts do not represent a major mutagenic lesion (Bastlová & Podlutsky, 1996). In an in vitro study of oligonucleotides containing N^2-guanine adducts derived from butadiene, acrolein, crotonaldehyde, and styrene, all the adducts blocked deoxycytidine triphosphate incorporation opposite them; adenine was preferentially incorporated opposite the acrolein- and crotonaldehyde-formed adducts, although thymine incorporation was preferred at the butadiene- and styrene-derived adduct sites (Zang et al., 2005).

No studies on the DNA binding potential of styrene-2,3-oxide and styrene-3,4-oxide were available to the Working Group.

(c) Exposed humans

See data reported in Table 4.3.

There were no data available to the Working Group on DNA adducts from exposure to styrene-7,8-oxide only.

In an early study, two DNA adducts, chromatographically similar to products of styrene-7,8-oxide, reacted with DNA and

Table 4.3 DNA damage and gene mutation in humans exposed to styrene

End-point	Location, date	Setting	Sampling matrix	No. of subjects	Mean (range)	Covariates controlled	Result (significance)[a]	Reference
DNA adducts	NR	Reinforced plastics	Peripheral blood	1 exposed, 1 control	96 ppm (8 h TWA)		+	Liu et al. (1988)
DNA adducts (^{32}P-postlabelling)	NR	Hand-lamination workers (two workplaces)	Peripheral blood	10 exposed, 8 controls 13 exposed, 10 controls	370 (300–700) mg/m^3 210 mg/m^3		+	Vodička et al. (1993) See also Vodička & Hemminki (1993), Hemminki & Vodička (1995)
DNA adducts (^{32}P-postlabelling)	NR	Boat manufacturing	Peripheral blood	47 exposed	65.6 (1–235) mg/m^3		+ (one-sided $P = 0.049$ for adduct 1, one-sided $P = 0.012$ for adduct 2)	Horvath et al. (1994)
DNA adducts	Czech Republic, 1993	Hand-lamination workers	Lymphocytes Granulocytes	9 exposed, 7 controls	122 (40–225) mg/m^3	Age, smoking	+ ($P < 0.01$) – ($P > 0.05$)	Vodička et al. (1994); see also Hemminki & Vodička (1995)
• DNA damage/ SSBs • DNA adducts	Germany, 1991	Styrene production plant	Peripheral blood	25 exposed, 25 controls	0.31 (SD, 0.88) ppm (8 h TWA)	Smoking, age, sex	– ($P > 0.05$) – ($P > 0.05$)	Holz et al. (1995)
DNA adducts	NR	Reinforced plastics workers	Urine	61 exposed, 22 controls	NR (up to 286) mg/m^3	Smoking	+ ($P < 0.05$)	Mikes et al. (2010)
DNA damage/ SSBs	USA, 1988	Reinforced plastics workers	Peripheral blood	14 exposed, 9 controls	GM, 11.2 (1–44) ppm (8 h TWA)		+ ($P < 0.003$)	Brenner et al. (1991)
DNA adducts	Czech Republic, NR	Hand-lamination workers	Peripheral blood	9 exposed, 11 controls	76.2 (10–115) mg/m^3	Sex, age, smoking, alcohol, medication	+	Koskinen et al. (2001b)
DNA damage/ SSBs (comet assay)	Italy, 2003	Reinforced plastics and polyester resins workers	Peripheral blood	48 exposed, 14 controls	GM, 36.8 (1.2–115.7) ppm (8 h TWA)		+ ($P < 0.001$)	Buschini et al. (2003)

Table 4.3 (continued)

End-point	Location, date	Setting	Sampling matrix	No. of subjects	Mean (range)	Covariates controlled	Result (significance)[a]	Reference
• DNA damage/ SSBs (comet assay) • Oxidative damage to DNA/ comet assay version (Fpg and EndoIII) • Chromosomal aberrations	Czech Republic, 1999	Reinforced plastics workers	Peripheral blood	17 high concentration, 12 medium concentration, 19 controls 44 exposed, 19 controls	199.1 (SD, 101.6) mg/m^3 55.0 (SD, 22.9) mg/m^3 199.1 (SD, 101.6) mg/m^3 55.0 (SD, 22.9) mg/m^3 101.2 (SD, 102.4) mg/m^3	Smoking	(+) ($P < 0.001$) – ($P > 0.05$) + ($P < 0.001$)	Somorovská et al. (1999); see also Vodička et al. (2001b)
DNA damage/ SSBs	Egypt, 2002	Reinforced plastics workers	Peripheral blood	26 exposed, 26 controls	Median, 130 (90–170) mg/g creatinine MA in urine Median, 110 (88–150) mg/g creatinine PGA in urine	Smoking, sex	+ ($P < 0.01$)	Shamy et al. (2002)
DNA damage/ SSBs (comet assay)	Spain, 2002	Reinforced plastics workers	Peripheral blood	14 exposed, 30 controls	< 20 ppm (8 h TWA; converted from MA in urine)		+ ($P < 0.01$)	Laffon et al. (2002a)
• DNA damage/ SSBs (comet assay) • Oxidative damage to DNA (8-OHdG)	Thailand, 2011	Reinforced plastics workers	Peripheral blood	50 exposed, 40 controls	17.0 (0.3–66.53) ppm (8 h TWA)	Smoking, alcohol	+ ($P < 0.05$) + ($P < 0.05$)	Wongvijitsuk et al. (2011)
DNA damage/ SSBs	Sweden, 1993	Plastics factory	Peripheral blood	17 exposed	7.0 (0.04–20.0) ppm (8 h TWA)		+ ($P = 0.03$)	Walles et al. (1993)
DNA damage/ SSBs (comet assay)	Portugal, 2012	Reinforced plastics workers	Peripheral blood	67 exposed, 68 controls	30.4 (0.5–114.0) ppm (8 h TWA)	Smoking, age, sex	– ($P = 0.058$)	Costa et al. (2012)

Table 4.3 (continued)

End-point	Location, date	Setting	Sampling matrix	No. of subjects	Mean (range)	Covariates controlled	Result (significance)[a]	Reference
• DNA damage/ SSBs (comet assay) • Chromosomal aberrations • Base excision repair capacity of oxidative damage to DNA • Oxidative DNA damage/comet assay version (EndoIII) • Micronuclei	Czech Republic, 2004	Reinforced plastics workers	Peripheral blood	86 exposed, 42 controls	81.3 (SD, 56.3) mg/m^3		– ($P > 0.05$) – + ($P < 0.001$) – ($P > 0.05$) (+) ($P = 0.002$)	Vodička et al. (2004)
• DNA damage/ SSBs (comet assay) • Oxidative damage to DNA/ comet assay version (Endo III) • DNA repair capacity • Micronuclei	Czech Republic, 2010	Reinforced plastics workers	Peripheral blood	60 exposed, 37 controls 60 exposed, 37 controls 60 exposed, 37 controls 62 exposed, 50 controls	50.3 (0–238) mg/m^3	Smoking, age, sex Smoking, age, sex Smoking, age, sex Smoking	– ($P < 0.001$) – ($P > 0.05$) + ($P = 0.005$) – ($P > 0.05$)	Hanova et al. (2010)
• DNA damage/ SSBs (comet assay) • Micronuclei in blood and nasal mucosa	Belgium, 2000–2001	Reinforced plastics industries	Peripheral blood Peripheral blood, nasal mucosa	37 exposed, 44 controls 38 exposed, 41 controls (blood); 23 exposed, 17 controls (nasal mucosa)	9.5 (SD, 9.6) ppm (converted from urine)	Smoking, age Smoking, alcohol consumption, age	– ($P = 0.878$) + ($P < 0.05$)	Godderis et al. (2004)
DNA damage/ SSBs (comet assay)	Italy, 2002	Reinforced plastics workers	Sperm	46 exposed, 27 controls	Median, 173.6 (5.8–1428.7) mg/g creatinine MA in urine	Age, smoking	+ ($P < 0.001$)	Migliore et al. (2002)

Table 4.3 (continued)

End-point	Location, date	Setting	Sampling matrix	No. of subjects	Mean (range)	Covariates controlled	Result (significance)[a]	Reference
• DNA damage/ SSBs, DSBs (comet assay) • Oxidative damage to DNA/ comet assay (version Fpg and EndoIII)	Italy, 2009	Reinforced plastics workers	Peripheral blood	34 exposed, 29 controls	46.74 (9.86–106.10) ppm (8 h TWA)	Smoking, sex, age	+ ($P < 0.0001$) – ($P > 0.05$)	Fracasso et al. (2009)
Oxidative damage to DNA, 8-OHdG	Germany, 1997	Boat builders	Peripheral blood	17 exposed, 67 controls	NR	Smoking, age	+ ($P < 0.001$)	Marczynski et al. (1997a)
• Oxidative damage to DNA, 8-oxo-dGuo • Oxidative damage to DNA: U-8-oxodGuo, U-8-oxoGuo, U-8-oxoGua	Italy, 2009	Reinforced plastics workers	Peripheral blood Urine	60 exposed, 50 controls	107.4 mg/m^3 66.7 mg/m^3	Smoking, age	– ($P = 0.002$) +/– ($P = 0.24$) ($P = 0.008$) ($P = 0.74$)	Manini et al. (2009)
Gene mutation/ GPA in vivo somatic cell mutation assay	Berkeley, USA, 1993	Boat manufacturing and maintenance workers	Peripheral blood	15 high concentration 22 low concentration	32 ppm 1.2 ppm (8 h TWA)		– ($P = 0.028$)	Compton-Quintana et al. (1993)
Gene mutation/ GPA in vivo somatic cell mutation assay	Finland, 1996	Reinforced plastics workers	Peripheral blood	47 exposed, 47 controls	37 (6–114) ppm (8 h TWA)	Age, smoking, sex	– ($P = 0.058$)	Bigbee et al. (1996)
Gene mutation/ *HPRT* MF	Czechia, 1999	Reinforced plastics workers	Peripheral blood	19 exposed, 19 controls	101.2 (SD, 102.4) mg/m^3		+/– ($P > 0.05$)	Vodička et al. (2001b)

Table 4.3 (continued)

End-point	Location, date	Setting	Sampling matrix	No. of subjects	Mean (range)	Covariates controlled	Result (significance)[a]	Reference
• DNA damage/ SSBs (comet assay) • O^6 guanine adducts • N-terminal valine of haemoglobin • Gene mutation/ *HPRT* MF	Czechia, 1995	Reinforced plastics workers	Peripheral blood	13 exposed, 13 controls	68.0 (15–156) mg/m³	Smoking	(+) ($P < 0.001$) (+) ($P < 0.001$) (+) ($P < 0.001$) (+) ($P = 0.039$)	Vodička et al. (1999)
Gene mutation/ *HPRT* MF	Czechia, 1993–1994	Hand-lamination workers	Peripheral blood	9 exposed, 15 controls	91 (25–250) mg/m³	Smoking	– ($P = 0.021$)	Vodička et al. (1995)

8-OHdG, 8-hydroxy-2′-deoxyguanosine; 8-oxodGuo, 8-oxo-7,8-dihydro-2′-deoxyguanosine; 8-oxoGua, 8-oxo-7,8-dihydroguanine; 8-oxoGuo, 8-oxo-7,8-dihydroguanosine; DSB, double-strand break; EndoIII, endonuclease III; Fpg, formamido pyrimidine glycosylase; GM, geometric mean; GPA, glycophorin A; h, hour(s); *HPRT*, hypoxanthine-guanine phosphoribosyl transferase; MA, mandelic acid; MF, mutation frequency; NR, not reported; PGA, phenylglyoxylic acid; ppm, parts per million; SD, standard deviation; SSB, single-strand break; TWA, time-weighted average; U-, urinary.

[a] +, positive; –, negative; +/–, equivocal (variable response in several experiments within an adequate study); (+), positive result in a study of limited quality.

deoxyguanosine monophosphate in vitro were detected by ^{32}P-postlabelling analysis in DNA isolated from lymphocytes of a worker exposed to styrene (Liu et al., 1988). There followed reports of styrene–DNA adducts in the leukocytes of 23 lamination workers, characterized as O^6-guanine adducts at a level of about 5 adducts per 10^8 nucleotides (Vodička & Hemminki, 1993; Vodička et al., 1993). Background levels in unexposed controls (n = 8) were at less than 1 adduct per 10^8 nucleotides. [The Working Group noted that these may not have been styrene-derived adducts because they were distinct from the standard (Hemminki & Vodička, 1995).]

In a study of 47 boat makers exposed to styrene, 2 DNA adducts were detected in blood mononuclear cells by ^{32}P-postlabelling analysis (Horvath et al., 1994). Adduct 1 was identified as N^2-(2-hydroxy-1-phenylethyl)-2′-deoxyguanosine-3′,5′-bisphosphate, for which a standard was available, although adduct 2 was not characterized. Adduct 1 was detected at a level of 0.6–102.1 per 10^8 nucleotides, and adduct 2 at 0.1–70.9 adducts per 10^8 nucleotides.

In a study of the same population, involving multiple sampling of 48 healthy workers at a boat manufacturing factory exposed to both styrene and styrene-7,8-oxide, adducts 1 and 2 were again detected; the correlation between the concentrations of the adducts and of the styrene exposure was observed to be at or near the 0.05 level of statistical significance (Rappaport et al., 1996). Adduct concentrations were more highly correlated with styrene exposure among non-smokers (n = 22) than among smokers (n = 26).

In a study of 9 lamination workers, ^{32}P-postlabelling analysis was used to detect styrene–DNA adducts formed at the O^6 position of guanine in blood cells (Vodička et al., 1994). Adduct levels in lymphocytes, but not granulocytes, were significantly higher in workers than in 7 unexposed controls (clerical workers at the same factory): 5.4 adducts per 10^8 nucleotides versus 1.0 adducts per 10^8 nucleotides. After 2 weeks of vacation, adduct levels in the same workers were not significantly diminished. After an additional 1 month of work, adduct concentrations remained similar.

The results of these three sampling times, plus a fourth sampling about 6 months later, were reported in a subsequent study (Vodička et al., 1995). Adduct concentrations in lymphocytes were very similar at all four time points, and significantly higher in exposed workers than in controls.

Similar concentrations of styrene–O^6-guanine adducts were again reported in exposed workers, higher than in controls in all consecutive samplings, in a subsequent 3-year study of lamination workers (Vodička et al., 1999),

The concentrations of several uncharacterized DNA adducts were not elevated in the peripheral leukocytes of 25 healthy workers at a styrene production plant, reportedly exposed to low concentrations of styrene (73–3540 μg/m³), relative to those in 25 healthy unexposed controls matched for age and sex (Holz et al., 1995). [The Working Group noted that no authentic standards were used, in direct contrast to other studies.]

In a study of 61 hand-lamination workers at four reinforced plastics plants, urine samples were collected from 58 men and 3 women at the end of the work shift (Mikes et al., 2010). Ten workers also provided a sample the next morning. Using MA measurements as an indicator of exposure concentrations, 28 samples at the end of the shift (68–1145 mg/g creatinine) and all 10 of the samples from the next morning were analysed for $N3$-adenine adducts of styrene [3-(2-hydroxy-1-phenylethyl)adenine ($N3\alpha$A) and 3-(2-hydroxy-2-phenylethyl)adenine ($N3\beta$A)] by mass spectrometry. As controls, samples from non-smoking volunteers (17 men and 5 women) were analysed. The adducts were detected in 7 out of 9 samples with MA concentrations greater than 400 mg/g creatinine, and in 6 out of 19 samples with MA concentrations less

than 400 mg/g creatinine. *N*3αA and *N*3βA were also detected in 9 and 3 samples, respectively, of the 22 control samples. The concentrations of the two adducts at the end of the shift were 2.8 ± 1.6 pg/mL in the group exposed to a high concentration of styrene ($n = 9$), 1.8 ± 1.3 pg/mL in the group exposed to a low concentration of styrene ($n = 19$), and 1.5 ± 1.3 pg/mL in the unexposed controls ($n = 22$).

A method combining high-performance liquid chromatography (HPLC) and ^{32}P-postlabelling that was developed to detect *N*1-adenine DNA adducts in workers exposed to styrene had a detection limit of 0.4 adducts per 10^8 nucleotides (Koskinen et al., 2001b). Using this method, adducts were detected in 3 out of 9 exposed workers (mean level, 0.79 ± 0.14 adducts per 10^9 nucleotides), but not in any of the unexposed control subjects ($n = 11$).

(d) Human cells in vitro

See also data reported below, in Table 4.4.

A dose-dependent increase in both *N*7-guanine and alkali-labile lesions in human embryonic lung cells treated with styrene-7,8-oxide was demonstrated. The level of *N*7-guanine adducts was 3-fold higher after treatment with styrene-7,8-oxide at 100 μM for 3 hours compared with the treatment for 18 hours, whereas alkali-labile lesions continued to increase in a concentration-dependent manner. The differences in *N*7-guanine adduct levels following 3-hour and 18-hour treatments could be ascribed to the conversion of *N*7-guanines into abasic sites, either spontaneously or as a result of DNA repair processes (Vodička et al., 1996).

Treatment of whole blood with styrene-7,8-oxide at a concentration of 40 μM, followed by DNA isolation and analysis by HPLC–mass spectrometry (MS) by electrospray ionization, led to the detection of adducts formed at *N*7 of guanine (Pauwels & Veulemans, 1998). Similarly, styrene-derived *N*7-guanine adducts were detected by ^{32}P-postlabelling analysis in human embryonal lung cells treated in vitro with styrene-7,8-oxide (Vodička et al., 1996).

In peripheral blood lymphocytes, styrene-7,8-oxide treatment resulted in a dose-dependent decrease in cell survival and an increase in O^6-guanine adducts in DNA, alkali-labile lesions, and *HPRT* mutations, whereas higher concentrations induced pronounced cytotoxic effects. The levels of O^6-guanine [O^6-(2-hydroxy-1-phenylethyl)guanine] adducts in DNA in treated cells correlated with styrene-7,8-oxide concentrations (4 adducts per 10^8 nucleotides were detected at the highest concentration of styrene-7,8-oxide). O^6-guanine adducts in DNA were still detectable in peripheral blood lymphocytes cultured for 6–8 days after treatment, suggesting slow removal of these adducts. Although O^6-guanine adducts in DNA correlated strongly with alkali-labile lesions in DNA comet assay, no correlation was found between DNA adducts and *HPRT* mutant frequencies (Bastlová et al., 1995). A concentration-related increase of diastereomeric *N*7β-guanine adducts was observed in peripheral blood lymphocytes by using an optimized technique including HPLC and ^{32}P-postlabelling. *N*7-guanine adducts were found at a 150-fold excess compared with O^6-guanine adducts; N^2-guanine adducts have also been detected (Vodička et al., 2002).

(e) Experimental systems

The potential of styrene and styrene-7,8-oxide to induce DNA adducts in experimental animals was initially studied using radiolabelled styrene or styrene-7,8-oxide (Cantoreggi & Lutz, 1992, 1993; Lutz et al., 1993), and reviewed by Phillips & Farmer (1994). Comparisons between animal studies are difficult because of varying experimental designs (rats and mice, various routes of exposure, and doses varying over several orders of magnitude). As described in Table 4.5 the following DNA adducts induced by styrene have been identified in experimental animals: *N*7-guanine, O^6-guanine, and *N*1-adenine

Table 4.4 Genetic and related effects of styrene and styrene-7,8-oxide in human cells in vitro

End-point	Tissue, cell line (if specified)	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Styrene						
DNA strand breaks (comet assay)	Blood mononuclear leukocytes	+	NT	5 mM [521 µg/mL]		Laffon et al. (2003b)
Other (comet assay)	Human skin in vitro	+	NT	10 000 ppm	Positive dose–response relationship	Costa et al. (2006)
Chromosomal aberrations, micronuclei	Human lymphocytes (whole-blood lymphocytes)	+	NT	0.03% (v/v)	Single dose	Linnainmaa et al. (1978a)
Chromosomal aberrations	Human lymphocytes (whole-blood lymphocytes)	+	NT	0.5 mM [52 µg/mL]		Pohlová et al. (1984)
Chromosomal aberrations	Human lymphocytes (whole-blood lymphocytes and isolated lymphocytes)	+	NT	1 mM [104 µg/mL]		Jantunen et al. (1986)
Sister-chromatid exchange	Human lymphocytes (whole-blood lymphocytes)	+	NT	2 mM [208 µg/mL]	Positive dose–response relationship	Norppa et al. (1983)
Sister-chromatid exchange	Human lymphocytes (whole-blood lymphocytes)	+	NT	10 µM [1 µg/mL]	Positive dose–response relationship	Chakrabarti et al. (1993)
Sister-chromatid exchange	Human lymphocytes (whole-blood lymphocytes)	+	NT	0.5 mM [52 µg/mL]		Lee & Norppa (1995)
Sister-chromatid exchange	Human lymphocytes (whole-blood lymphocytes)	+	+	0.5 mM [52 µg/mL]	Concurrent lack of GSTM1/GSTT1 increase in genotoxicity of styrene; dose–response with two doses	Bernardini et al. (2002)
Styrene-7,8-oxide						
DNA adducts (*N*7-guanine adducts, ^{32}P-postlabelling); DNA strand breaks (comet assay)	Human embryonal lung fibroblasts	+		10 µM [1.2 µg/mL]		Vodička et al. (1996)

Table 4.4 (continued)

End-point	Tissue, cell line (if specified)	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
DNA adducts (*N*7-guanine adducts, HPLC EMS)	Human whole blood (lymphocytes)	+		40 µM [5 µg/mL]		Pauwels & Veulemans (1998)
DNA adducts (*O*[6]-guanine adducts, ^{32}P-postlabelling); *HPRT* locus mutations	Human lymphocytes, peripheral blood mononuclear cells, and T-lymphocytes	+		0.2 mM [24 µg/mL]		Bastlová et al. (1995), Bastlová & Podlutsky (1996)
DNA strand breaks (comet assay)	Human lymphocytes, peripheral blood mononuclear cells, and T-lymphocytes	+		50 µM [6 µg/mL]		Bastlová et al. (1995)
DNA strand breaks (comet assay)	Isolated human lymphocytes	+		50 µM [6 µg/mL]	Endonuclease III sensitive sites were induced at 100 µM	Köhlerová & Stětina (2003)
DNA strand breaks (comet assay)	Human lymphocytes (whole-blood lymphocytes)	+		0.025 mM [3 µg/mL]		Speit et al. (2012)
DNA strand breaks (comet assay)	Human lymphocytes, peripheral blood mononuclear cells, and T-lymphocytes	+		0.01 mM [1.2 µg/mL]		Bausinger & Speit (2014)
DNA strand breaks (comet assay)	Human lymphocytes (isolated lymphocytes)	+		0.05 mM [6 µg/mL]		Cemeli et al. (2009)
DNA strand breaks (comet assay)	Human blood mononuclear leukocytes	+		0.1 mM [12 µg/mL]		Laffon et al. (2002b)
DNA strand breaks (comet assay)	Human peripheral blood mononuclear cells	+		0.1 mM [12 µg/mL]		Godderis et al. (2004)
DNA strand breaks (comet assay)	Human peripheral blood mononuclear cells	+		0.05 mM [6 µg/mL]		Fabiani et al. (2012)
DNA strand breaks (comet assay)	Human testicular cells	+		0.1 mM [12 µg/mL]		Bjørge et al. (1996)
DNA strand breaks (DNA fragmentation PFGE)	Whole-blood cells	+		0.06 µmole, 7 mL of blood [8.6 µM, 1 µg/mL]		Marczynski et al. (1997b)

Table 4.4 (continued)

End-point	Tissue, cell line (if specified)	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
HPRT locus	Human lymphoblastoid cell lines	+		0.6 mM [72 μg/mL]	GSTM1 reduces mutagenicity and toxicity of styrene-7,8-oxide	Shield & Sanderson (2004)
Chromosomal aberrations	Human lymphocytes (whole-blood lymphocytes)	+		0.1 mM [12 μg/mL]	Dose–response with two doses, including gaps	Fabry et al. (1978)
Chromosomal aberrations, micronuclei	Human lymphocytes (whole-blood lymphocytes)	+		0.008% (v/v)	Single dose	Linnainmaa et al. (1978a)
Chromosomal aberrations	Human lymphocytes (whole-blood lymphocytes)	+		0.05 mM [6 μg/mL]		Pohlová et al. (1984)
Micronuclei	Human lymphocytes (whole-blood lymphocytes)	+		0.6 mM [72 μg/mL]		Speit et al. (2012)
Micronuclei	Human peripheral blood lymphocytes	+		100 μM [12 μg/mL]		Laffon et al. (2001b)
Micronuclei	Human lymphocytes (isolated from whole blood)	+		50 μM [6 μg/mL]		Laffon et al. (2003a)
Micronuclei	Human peripheral blood mononuclear cells	+		0.1 mM [12 μg/mL]		Godderis et al. (2006)
Sister-chromatid exchange	Human lymphocytes (whole-blood lymphocytes)	+		0.15 mM [18 μg/mL]		Norppa et al. (1983)
Sister-chromatid exchange	Human lymphocytes (whole-blood lymphocytes)	+		0.005 mM [0.6 μg/mL]		Pohlová et al. (1984)
Sister-chromatid exchange	Human lymphocytes (whole-blood lymphocytes)	+	NT	100 μM [12 μg/mL]		Chakrabarti et al. (1997)
Sister-chromatid exchange	Human lymphocytes (whole-blood lymphocytes)	+	NT	50 μM [6 μg/mL]		Ollikainen et al. (1998)

Table 4.4 (continued)

End-point	Tissue, cell line (if specified)	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Sister-chromatid exchange	Human lymphocytes (whole-blood lymphocytes)	+	NT	0.1 mM [12 µg/mL]	Single dose	Zhang et al. (1993)
Sister-chromatid exchange	Human lymphocytes (whole-blood lymphocytes)	+	NT	0.05 mM [6 µg/mL]	Dose–response with two doses	Lee & Norppa (1995)
Sister-chromatid exchange	Human lymphocytes (whole-blood lymphocytes)	+	NT	0.05 mM [0.6 µg/mL]	Dose–response with two doses; no change by *GSTM1* null genotype	Uusküla et al. (1995)
Sister-chromatid exchange	Human peripheral blood lymphocytes	+	NT	50 µM [6 µg/mL]		Laffon et al. (2001b)

EMS, electrospray mass spectrometry; GSTM1, glutathione *S*-transferase mu1; GSTT1, glutathione *S*-transferase θ1; HIC, highest ineffective concentration; HPLC, high-performance liquid chromatography; *HPRT*, hypoxanthine-guanine phosphoribosyl transferase; LEC, lowest effective concentration; NT, not tested; PFGE, pulsed-field gel electrophoresis; v/v, volume/volume.

[a] +, positive; the level of significance was set at $P < 0.05$ in all cases.

Table 4.5 Studies of DNA adducts in experimental animals exposed to styrene

End-point	Species, strain (sex)	Tissue	Result (number of adducts/dNp)[a]	Route, dosing regimen	Reference
*N*7-(2-hydroxy-1-phenylethyl and 2-hydroxy-2-phenylethyl)guanine O^6-(2-hydroxy-1-phenylethyl)guanine	Mouse, NMRI (M)	Liver, spleen, lungs	+ (up to 6.3/10^7 dNp) + (up to 3.8/10^7 dNp)	Intraperitoneal injection, 0–4.35 mmol/kg bw [457 mg/kg bw], 3 h	Pauwels et al. (1996)
O^6-(2-hydroxy-1-phenylethyl and 2-hydroxy-2-phenylethyl)guanine	Rat, NR	Liver	± (7/10^7 dNp)	Inhalation, 1000 ppm [4300 mg/m^3], 2 yr	Otteneder et al. (1999)
7-(2-hydroxy-1-phenylethyl and 2-hydroxy-2-phenylethyl)guanine Unidentified adducts	Rat, Sprague-Dawley Mouse, CD-1	Lungs, liver	+ (1–3/10^8 dNp) + (up to 9/10^8 dNp)	Inhalation, 160 ppm [700 mg/m^3], 6 h	Boogaard et al. (2000a,b)
7-(2-hydroxy-2-phenylethyl)guanine 1-(2-hydroxy-2-phenylethyl)adenine	Mouse, NMRI (M)	Lungs	+ (up to 23/10^8 dNp) + (up to 0.6/10^8 dNp)	Inhalation, 750 and 1500 mg/m^3 [176 and 352 ppm] for 1, 3, 7, and 21 d for 6 h, 5 d/wk	Vodička et al. (2001a)
O^6-(2-hydroxy-1-phenylethyl and 2-hydroxy-2-phenylethyl)guanine	Mouse, CD-1	Lungs	– (1/10^7 dNp, i.e. detection limit)	Inhalation, 40 and 60 ppm [172 and 688 mg/m^3], 6 h/d, 5 d/wk, 2 wk	Otteneder et al. (2002)
3-(2-hydroxy-1-phenylethyl)adenine (*N*3αA), 3-(2-hydroxy-2- phenylethyl) adenine (*N*3βA) 7-(2-hydroxy-1-phenylethyl)guanine (*N*7αG), 7-(2-hydroxy-2-phenylethyl) guanine (*N*7βG)	Mouse, NMRI (M)	Urine	+ Dose-dependent urinary excretion, *N*3αA and *N*3βA up to 0.8 × 10^{-5}% of the absorbed dose + *N*7αG and *N*7βG amounted to 1.4 × 10^{-5}% of the absorbed dose	Inhalation, 0, 600, and 1200 mg/m^3 [0, 141, and 282 ppm], 6 h/d, 10 d	Mikes et al. (2009)

bw, body weight; d, day(s); dNp, deoxynucleotide; h, hour(s); M, male; NR, not reported; ppm, parts per million; wk, week(s); yr, year(s).

[a] +, positive result; –, negative result.

adducts (Pauwels et al., 1996; Otteneder et al., 1999, 2002; Boogaard et al., 2000b; Vodička et al., 2001a). More recently, *N*3αA and *N*3βA have been detected in the urine of NMRI mice after exposure to styrene by inhalation (Mikes et al., 2009). However, some unidentified styrene-related alkylation products were also reported (Boogaard et al., 2000b). The main stable adducts identified in vitro (αN^2-guanine, αN^6-adenine, and β*N*3-uracil) have not been demonstrated to be formed in experimental animals. A single intraperitoneal injection of styrene to NMRI mice resulted in a dose-related increase in both *N*7-guanine and O^6-guanine adducts. The highest concentrations of DNA adducts were found in the lungs, followed by liver and spleen (Pauwels et al., 1996; reviewed by Vodička et al., 2006a). By using a modified ^{32}P-postlabelling method, O^6-guanine adducts were detected at the concentration of 7 adducts per 10^7 nucleotides in the liver of rats exposed by inhalation to styrene at 1000 ppm (4300 mg/m^3) for 2 years (Otteneder et al., 1999). The formation of the O^6-guanine adduct in the lung DNA of CD-1 mice exposed by inhalation to styrene at 172 mg/m^3 and 688 mg/m^3 for 6 hours per day, 5 days per week for 2 weeks, was studied by the same authors; the adduct concentration was below the limit of detection of 1 adduct per 10^7 nucleotides (Otteneder et al., 2002). *N*7-guanine (β-isomers) and *N*1-adenine adducts in lung and liver DNA of mice exposed by inhalation to styrene at concentrations of 750 mg/m^3 and 1500 mg/m^3 for 1, 3, 7, and 21 days (Table 4.5) were investigated (Vodička et al., 2001a). In the lungs, β*N*7-guanine adducts were 40-fold more abundant than β*N*1-adenine adducts. Both adducts correlated strongly with exposure parameters, particularly with styrene concentration in blood. The N^2-guanine and β*N*3-uracil adducts were below the limit of detection (Vodička et al., 2002). A quantitative comparison of *N*7-guanine adducts excreted in urine with those in lungs (after correction for depurination) revealed that persisting *N*7-guanine DNA adducts in lungs amounted to about 0.5% of the *N*7-guanine adducts in urine. The total styrene-specific *N*7-guanine alkylation accounted for about 1.0×10^{-5}% of the total styrene uptake, whereas *N*1-adenine alkylation was still considerably lower (Vodička et al., 2006b). Boogaard et al. (2000a, b) found higher alkylation levels in the livers than in the lungs of rats and mice after inhalation of ^{14}C-styrene at 160 ppm [700 mg/m^3] for 6 hours (Boogaard et al., 2000a, b); the quantitative data on the two isomeric *N*7-styrene-7,8-oxide–guanine adducts in mouse lungs were in concordance with contemporary studies (reviewed by Vodička et al., 2006a, b). In mice exposed to styrene by inhalation, excretion of *N*3-adenine adducts (*N*3αA and *N*3βA) in the urine amounted to nearly 0.8×10^{-5}% of the absorbed dose, and urinary *N*7-guanine adducts 7-(2-hydroxy-1-phenylethyl)guanine (*N*7αG) and 7-(2-hydroxy-2-phenylethyl)guanine (*N*7βG) amounted to nearly 1.4×10^{-5}% of the dose. The excretion of both *N*3-adenine and *N*7-guanine adducts ceased shortly after the end of the exposure period as a result of their rapid depurination from the DNA (Mikes et al., 2009).

4.2.3 Other genetic effects

(a) Exposed humans

See Table 4.3 and Table 4.6.

(i) DNA damage (comet assay)

Most of the available epidemiological studies detected DNA damage by alkaline comet assay.

Buschini et al. (2003) evaluated DNA damage by comet assay in 48 workers exposed to styrene at 36.8 ± 0.7 ppm and in 14 unexposed controls. The levels of DNA damage were significantly higher ($P < 0.001$) in the groups exposed to styrene compared with the controls. [The Working Group noted the low number of individuals in the control group.] In another study, lymphocytes from 29 hand-lamination workers and 19

Table 4.6 Cytogenetic effects in humans exposed to styrene

End-point	Location, date	Setting	Sampling matrix	No. of subjects	Mean (range)	Covariates controlled	Result (significance)[a]	Reference
Micronuclei	Italy, NR	Reinforced plastics production	Peripheral blood	92 exposed, 98 controls	37.1 (2–535) mg/m³; 300.0 (10.2–1856) mg/g creatinine MA+PGA in urine	Smoking, sex, age	+ ($P < 0.001$)	Migliore et al. (2006)
• Micronuclei • Sister-chromatid exchanges	USA, before 1993	Reinforced plastic boat manufacturing facility	Peripheral blood	48 exposed	64.2 (0.88–235.35) mg/m³ (8 h TWA)	Smoking, sex, age	– ($P > 0.05$) + ($P < 0.05$)	Yager et al. (1993)
• Chromosomal aberrations • Micronuclei • Sister-chromatid exchanges	China, 1992	Fibreglass-reinforced plastics factories	Peripheral blood	83	129.3 (48.3–223.9) mg/m³		+ + –	Huang (1992)
Chromosomal aberrations	Milan, Italy, 1985–1986	Factory A, reinforced plastic laminates and insulating polymers; Factory B, small plastic boats manufacture	Peripheral blood	Factory A, 32 exposed; Factory B, 8 exposed, 40 controls	A, 123–249 (up to 1978), 1.7–17.0 (after 1978) mg/m³; B, 41–198 (after 1978) mg/m³	Smoking, age, other exposures to mutagenic chemicals	+/– (Factory A, $P < 0.001$; Factory B, $P < 0.05$)	Forni et al. (1988)
Micronuclei	Sweden, 1983	Reinforced plastics and polyester resins workers	Peripheral blood	38 exposed, 20 controls	13 (1–40) ppm (8 h TWA)	Sex	+ ($P = 0.005$)	Högstedt (1984)
Chromosomal aberrations	Egypt (El Oboor City), before 2013	Reinforced plastics production	Peripheral blood	40 exposed, 50 controls	1117 (SD, 64.52) µg/L in blood 246 (SD, 21.60) µmol/L MA in urine	Smoking, sex, socioeconomic status, age	+ ($P < 0.001$)	Helal & Elshafy (2013)
• Chromosomal aberrations • Sister-chromatid exchanges	Italy, 1983	Reinforced plastics industries (six plants)	Peripheral blood	25 exposed, 22 controls	NR (30–400) mg/m³	Age, sex, smoking	+ ($P < 0.005$ for all 6 plants) + ($P < 0.005$ for 2 plants, $P < 0.05$ for 2 plants, $P > 0.05$ for 2 plants)	Camurri et al. (1983)

Table 4.6 (continued)

End-point	Location, date	Setting	Sampling matrix	No. of subjects	Mean (range)	Covariates controlled	Result (significance)[a]	Reference
• Chromosomal aberrations • Sister-chromatid exchanges	Sweden, 1978	Factory making boats from fibreglass-reinforced plastics	Peripheral blood	36 exposed, 37 controls 20 exposed, 21 controls	Low concentration, 137 (6–283) mg/m^3; high concentration, 1204 (710–1589) mg/m^3	Age, sex	+ (P < 0.001) +/– (P < 0.05)	Andersson et al. (1980)
• Chromosomal aberrations • Micronuclei	Italy, 1990	Reinforced plastics production factories (Group 1, fibreglass tanks; Group 2, small boat production)	Peripheral blood	Group 1, 7 exposed, 7 controls; Group 2, 12 exposed, 12 controls	Group 1, NR (21–100); Group 2, NR (112–435) mg/m^3 Group 1, 186 (46–345); Group 2 725 (423–1325) mg/g creatinine MA in urine	Smoking, age, sex	+ (P < 0.05) – (P > 0.05)	Tomanin et al. (1992)
• Chromosomal aberrations • Micronuclei	Sweden, 1980	Fibreglass-reinforced polyester factory	Peripheral blood	15 exposed, 13 controls 12 exposed, 12 controls	24 ppm NR (< 2) mmol/L MA in urine	Sex, smoking	– (P > 0.05) + (one-sided P = 0.00017)	Nordenson & Beckman (1984)
Chromosomal aberrations	Germany, NR	Boat manufacturing	Peripheral blood	14 exposed, 7 controls	< 100 mg/m^3 35 (1.5–211) µg/L styrene in blood	Smoking	+/–	Oberheitmann et al. (2001)
Chromosomal aberrations	Czechia, NR	Laminators of various kinds of sport utensils, boats, and containers	Peripheral blood	11 exposed, 11 controls	253 (118–582) mg/m^3 NR (214–711) µL/mmol creatinine MA NR (50–175) µL/mmol creatinine PGA	Smoking, sex, alcohol consumption, drug intake, X-ray examination, rtg. therapy	–	Jablonická et al. (1988)
• Chromosomal aberrations • Sister-chromatid exchanges • Micronuclei	Finland, before 1991	Reinforced plastics production	Peripheral blood	109 exposed, 54 controls 70 exposed, 31 controls 50 exposed, 37 controls	Laminators, 43 (5–182) ppm Others, 11 (1–133) ppm (8 h TWA); laminators, 2.2 (SD, 2.4) nmol/L MA+PGA in urine	Age, smoking	– (P > 0.05) – –	Sorsa et al. (1991)
• Chromosomal aberrations • Micronuclei	Sweden, 1985–1986	Reinforced plastics production	Peripheral blood	11 exposed, 14 controls 20 exposed, 22 controls	43–221 mg/m^3, 4–551 mg/m^3 (1974–1986); 128 (< 6–317) mmol/mol creatinine, MA+PGA in urine (in 1985)	Smoking, age	– (P > 0.5) – (P > 0.5)	Hagmar et al. (1989)

Table 4.6 (continued)

End-point	Location, date	Setting	Sampling matrix	No. of subjects	Mean (range)	Covariates controlled	Result (significance)[a]	Reference
• Chromosomal aberrations • Micronuclei • Sister-chromatid exchanges	Finland, 1987	Reinforced plastics workers	Peripheral blood	21 exposed, 21 controls	98 (34–263) mg/m^3; 1.6 (< LOD–7) mmol/L MA in urine	Smoking, sex	– – –	Mäki-Paakkanen (1987)
• Chromosomal aberrations • Gaps	Czechia, before 1985	Two polystyrene plants: A, food vessel manufacturing; B, boat manufacturing	Peripheral blood	A, 36 exposed, 19 controls; B, 22 exposed, 22 controls	A, 70–150 (5.6–982.8) mg/m^3; B, ~200 (39–548) mg/m^3	Smoking, acute viral diseases, sex, drug intake The above plus X-ray examinations, alcohol	– +	Pohlová & Srám (1985)
• Chromosomal aberrations • Gaps • Sister-chromatid exchanges	Norway, before 1984	Reinforced plastics production	Peripheral blood	(i) 11 exposed, (ii) 7 exposed; 9 controls	(i) 7.5 (2–13) ppm; (ii) 22.3 (14–44) ppm	Smoking, sex, age	– ($P > 0.1$) + ($P = 0.0002$) –	Hansteen et al. (1984)
Chromosomal aberrations	Germany, 1975	Polystyrene production plant	Peripheral blood	12 exposed, 12 controls	GM, 0.23 (0.02–46.92) ppm; NR (< 10–100) mg/L MA in urine	Age, sex, smoking, drug intake, acute viral diseases, X-ray examinations, vaccinations	–	Thiess & Fleig (1978)
• Chromosomal aberrations • Sister-chromatid exchanges	Italy, Viareggio, 1988–1990	Fibre-reinforced plastic boat factory	Peripheral blood	(i) 23 low concentration; (ii) 23 high concentration, 51 controls	(i) NR, 2–120; (ii) NR, 86–1389 mg/m^3	NR	(+) ($P < 0.01$) +/– ($P < 0.05$)	Artuso et al. (1995)

Table 4.6 (continued)

End-point	Location, date	Setting	Sampling matrix	No. of subjects	Mean (range)	Covariates controlled	Result (significance)[a]	Reference
• Chromosomal aberrations • Micronuclei • Sister-chromatid exchanges	Finland, before 1991	Reinforced plastics production in a plant manufacturing containers	Peripheral blood	17 exposed, 17 controls	300 (NR) mg/m³ (based on ACGIH conversion); 9.4 (< 1–21.5) mmol/L MA in urine	Age, sex, smoking, viral infections, vaccinations, other exposures to mutagenic chemicals, alcohol consumption, drug intake	+/– (one-sided *P* < 0.02) – –	Mäki-Paakkanen et al. (1991)
Chromosomal aberrations	Finland, 1977	Plants manufacturing polyester plastic products	Peripheral blood	10 exposed, 5 controls	NR	Sex	(+)	Meretoja et al. (1977)
Sister-chromatid exchanges	NR, before 1990	Fibre-reinforced plastic boat factories	Peripheral blood	20 exposed, 20 controls	Smokers, 209.8 (7.5–570.8) mg/m³; non-smokers, 230.1 (25.3–564.1) mg/m³ Smokers, 275 (SD, 241) mg/g creatinine MA in urine; non-smokers: 323 (SD, 224) mg/g creatinine MA in urine	Smoking, alcohol, age, coffee	(–)	Kelsey et al. (1990)
Chromosomal aberrations	Poland, before 1983	Laminated styrene plates production	Peripheral blood	37 exposed, 2 controls	NR (< 100) mg/m³		+/–	Dolmierski et al. (1983)
• Chromosomal aberrations • Sister-chromatid exchanges	Finland, 1976–1977	Reinforced plastics production, two plants	Peripheral blood	16 exposed, 6 controls	569.8 (55–3257) mg/g creatinine MA in urine in 1976 329.3 (53–1646) mg/g creatinine MA in urine in 1977	Smoking	+ (*P* < 0.001) – (*P* > 0.05)	Meretoja et al. (1978)

Table 4.6 (continued)

End-point	Location, date	Setting	Sampling matrix	No. of subjects	Mean (range)	Covariates controlled	Result (significance)[a]	Reference
Chromosomal aberrations	Finland, NR	Three plants: (i) styrene manufacturing; (ii) polystyrene production; (iii) unsaturated polyester resins processing	Peripheral blood	(i) 5 exposed; (ii) 12 exposed; (iii) 14 exposed, 20 controls	(i) NR (19–40) mg/L MA in urine; (ii) NR (< 5–100) mg/L MA in urine; (iii) NR (102 to > 1500) mg/L MA in urine		+/–	Fleig & Thiess (1978)
Chromosomal aberrations	Lithuania, before 1993	Chemical plant	Capillary blood	109 exposed, 64 controls	NR (< 1.9 ppm) in year before sampling	Sex, smoking	(+) ($P < 0.01$)	Mierauskiene et al. (1993)
Chromosomal aberrations	Lithuania, (i) 1983–1984; (ii) 1985–1986	Two plants: (i) carpet production; (ii) plastics production	Peripheral blood	(i) 79 exposed; (ii) 97 exposed, 90 controls	(i) NR (0.13–1.4) mg/m³; (ii) NR (4.4–6.2) mg/m³	Smoking, age	(+) ($P < 0.0001$)	Lazutka et al. (1999)
Aneuploidy and diploidy	Italy, Tuscany, before 2002	Reinforced plastics production	Semen	18 out of 46 exposed, 13 out of 27 controls	292.5 (20.8–947.8) mg/g creatinine MA in urine	Smoking, age, alcohol consumption	+/– ($P > 0.05$)	Naccarati et al. (2003)
• Chromosomal aberrations • Sister-chromatid exchanges	Japan, before 1983	Boat manufacturing	Peripheral blood	18 exposed, 6 controls	40–50 (NR) ppm	Smoking, age, sex	+/– +/–	Watanabe et al. (1983)
Chromosomal aberrations (four basic classes)	Czech Republic, 1989?	Workers occupationally exposed to styrene	Peripheral blood	13 women exposed, 6 women controls	225 (83–366) mg/m³	Sex	+	Smejkalová et al. (1989)
Chromosomal aberrations	Sweden, 1977	Plant manufacturing polyester resin boats	Peripheral blood	6 exposed, 6 controls	115 (50–400) mg/m³	Sex, age, smoking	(+) ($P = 0.001$)	Högstedt et al. (1979)

Table 4.6 (continued)

End-point	Location, date	Setting	Sampling matrix	No. of subjects	Mean (range)	Covariates controlled	Result (significance)[a]	Reference
• Chromosomal aberrations • Micronuclei • Sister-chromatid exchanges • Gene mutation/ *HPRT* MF	Germany, 1990	Container and board manufacturing (plus dichloromethane exposure)	Peripheral blood	46 exposed, 23 controls 46 exposed, 22 controls 46 exposed, 23 controls 45 exposed, 5 of 23 controls	70 (0–598) mg/m^3 (8 h TWA)	Smoking, age, sex	(+) ($P < 0.0001$) (+) ($P < 0.0001$) (+) ($P < 0.0001$) (–)	Tates et. al (1994)

ACGIH, American Conference of Governmental Industrial Hygienists; *HPRT*, hypoxanthine-guanine phosphoribosyl transferase; LOD, limit of detection; MA, mandelic acid; MF, mutation frequency; NR, not reported; PGA, phenylglyoxylic acid; SD, standard deviation; TWA, time-weighted average

[a] +, positive; –, negative; +/–, equivocal (variable response in several experiments within an adequate study); (+)/(–), positive/negative result in a study of limited quality.

unexposed controls were assayed for alkali-labile lesions (Somorovská et al., 1999). DNA damage was measured by comet assay, and was observed to be increased in workers exposed to styrene compared with controls ($P < 0.001$); the increase was also correlated with years of exposure. Shamy et al. (2002) similarly reported an increase in DNA damage in 26 reinforced plastics workers compared with 26 controls. Laffon et al. (2002a) reported a significant increase in DNA damage in a group of 14 workers exposed to styrene at less than 20 ppm [< 84.4 mg/m^3] compared with 30 controls ($P < 0.01$). Wongvijitsuk et al. (2011) studied 50 workers exposed to styrene in fibreglass-reinforced plastics production at levels below the American Conference of Governmental Industrial Hygienists limit of 20 ppm and 40 unexposed control subjects. Workers were stratified into three exposure groups: group I workers were exposed at less than 10 ppm (< 42.20 mg/m^3); group II at 10–20 ppm (42.20–84.40 mg/m^3); and group III at more than 20 ppm (> 84.4 mg/m^3). Urinary MA and PGA concentrations were observed to increase in relation to increasing levels of environmental styrene exposure. DNA strand breaks in peripheral leukocytes were higher in the exposed workers ($P < 0.05$). Walles et al. (1993) carried out a study on male workers exposed to styrene at 0.04–20.0 ppm [0.17–85.0 mg/m^3] in a Swedish plastics factory. The single-strand breaks in leukocytes were observed to be increased at the end of the shift, but not before a shift or the next morning. No control subjects were studied. Brenner et al. (1991) reported a significant increase of DNA damage in 14 subjects exposed to styrene at 11.2 ± 0.9 ppm compared with 9 controls ($P < 0.003$); however, the study did not control for smoking habits [and the number of subjects studied was small].

However, these positive results were not confirmed in other publications. Costa et al. (2012) reported no effect of exposure to styrene at the high concentration of 30.4 ppm [~130 mg/m^3] (8-hour time-weighted average, TWA) on DNA damage in a group of 67 reinforced plastics workers when compared with 68 controls subjects. Teixeira et al. (2010) reported the same result in a population of 46 workers exposed to styrene at 29.9 ppm [127 mg/m^3] compared with 47 controls. Similarly, other studies of exposure at different concentrations did not support these findings. Hanova et al. (2010) did not find significant differences in the levels of DNA damage measured by comet assay in a group of 60 workers exposed to styrene at 50.3 mg/m^3 compared with that of 37 control individuals. Vodička et al. (2004) did not find significant differences in the levels of DNA damage between 86 reinforced plastics workers exposed at 81.3 mg/m^3 compared with 42 controls. Similarly, Godderis et al. (2004) reported no differences in DNA damage between 37 workers exposed to styrene at 9.5 ppm [40 mg/m^3] and 44 controls.

In another study, DNA damage was evaluated in germ cells (sperm) in 46 reinforced plastics workers exposed to styrene (median value of concentration of MA at the end of the work shift: 173.6 mg/g creatinine); the study reported a significant increase in sperm DNA damage when compared with 27 unexposed individuals (Migliore et al., 2002).

(ii) Oxidative damage to DNA

Fracasso et al. (2009) evaluated oxidative damage to DNA in the enzyme-modified version of the comet assay (sites sensitive to formamido pyrimidine glycosylase (Fpg) and endonuclease III) in lymphocytes from 34 reinforced plastics workers exposed at 46.74 ppm and 29 unexposed controls, and Somorovská et al. (1999) evaluated 29 exposed workers and 19 controls. Neither study found any effect of occupational exposure in reinforced plastics workers. Using only endonuclease III in the comet assay, other studies (e.g. Hanova et al., 2010) reported no significant differences in the levels of oxidative damage to

DNA in workers exposed to styrene compared with control subjects.

8-Hydroxy-2′-deoxyguanosine (8-OHdG) was increased in leukocyte DNA in 17 boat builders exposed to styrene compared with 67 age-matched controls with no prior occupational exposure to styrene (Marczynski et al., 1997a). Among the exposed workers, the 11 workers with more than 10 years of exposure had a higher level of 8-OHdG than the 6 workers with less than 10 years of exposure, although the difference was not statistically significant ($P = 0.05$). [The Working Group noted the small number of subjects exposed to styrene in the study.]

In the study of Wongvijitsuk et al. (2011) mentioned in the previous section, 8-OHdG levels in peripheral leukocytes were higher in the exposed workers compared with controls ($P < 0.05$).

In a study of a group of 60 reinforced plastics workers and 50 controls (Manini et al., 2009), lower concentrations of leukocyte 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodGuo) were observed in exposed workers compared with controls; however, significantly higher levels of urinary 8-oxo-7,8-dihydroguanosine (8-oxoGuo) were observed in exposed workers compared with controls.

(iii) Gene mutations

Since the previous IARC evaluation of styrene (IARC, 1994), no further data have become available on glycophorin A variant frequency in erythrocytes (Compton-Quintana et al., 1993; Bigbee et al., 1996). Concerning the induction of mutation in the *HPRT* locus in lymphocytes of reinforced plastics workers exposed to styrene at 101.2 mg/m^3, Vodička et al. (2001b) reported a slight increase of *HPRT* mutant frequencies in exposed workers compared with controls, but the observed difference was not significant. Other studies were not informative because of their small number of subjects or other limitations (Tates et al., 1994; Vodička et al., 1995, 1999).

(iv) Chromosomal damage

More than 30 studies investigated whether exposure to styrene had effects on chromosomal damage in humans (see Table 4.6). No studies were found that addressed the main styrene metabolite, styrene-7,8-oxide. In most of the studies considered, environmental and/or biological monitoring exposure data are available. In the discussion that follows, the studies are briefly described in terms of study size, design, the presence of controls and the criteria for their selection, the presence of and adjustment for confounders, the concordance of different end-points tested, and the exposure levels at which the effects were observed. The statistical analyses of the data and the levels of significance of the results are also considered.

Several studies of adequate size and design reported positive effects on chromosomal end-points. Several of these encompassed relatively low concentrations of exposure (< 100 ppm). A significantly higher frequency of micronucleated binucleated cells (13.8 ± 0.5% vs 9.2 ± 0.4%; $P < 0.001$) was observed in peripheral blood lymphocytes of 92 reinforced plastics production workers exposed to styrene compared with 98 controls (Migliore et al., 2006). Workers were exposed to styrene at 37.1 ± 3.9 mg/m^3 [9.0 ± 0.9 ppm], producing levels of excretion of 300 ± 338.2 mg/g creatinine of MA and PGA in urine. In *GSTM1*-null workers exposed to styrene, a significantly lower concentration of PHEMAs in urine and a higher frequency of micronucleated lymphocytes were observed. In another study, the lymphocytes from 44 hand-lamination workers exposed to styrene, discussed in Section 4.2.3(a)(i) and (ii) above (Somorovská et al., 1999; see Table 4.3), showed a significantly increased frequency of chromosomal aberrations. Yager et al. (1993) published the results of a study of 48 reinforced plastics production workers (approximately 50% men and 50% women); micronucleated cells and

sister-chromatid exchanges were determined in peripheral lymphocytes 4 times at intervals of 3 months for a 1-year period. Individual levels of exposure ranged from 0.88 to 235.35 mg/m^3 of styrene (mean, 64.2 ± 71.5 mg/m^3) and exhaled styrene concentrations ranged from 0 to 7.16 mg/m^3 (mean, 1.65 ± 1.82 mg/m^3). Individual mean sister-chromatid exchanges increased with exposure to styrene and with cigarette smoking. Multivariate analysis showed that smoking contributed about 62% and exposure to styrene contributed 25% to variability, although no variation was observed in micronucleus frequencies. The study also showed significant inter-individual differences in micronucleus frequencies associated with sex, with the highest levels observed in women. Huang (1992) studied a group of 83 Chinese workers exposed to styrene in reinforced plastics production, showing that exposure to styrene at a concentration of 129.3 mg/m^3 (range, 48.3–223.9 mg/m^3) resulted in an increase in the frequencies of chromosomal aberrations and micronuclei, but had little influence on the frequency of sister-chromatid exchange. Forni et al. (1988) studied a group of 40 workers at a medium-sized reinforced plastics manufacturing plant, with a history of exposure to high concentrations but with low concentrations of exposure at the time of the study (1978, 32 subjects, plant A), and a modern boat manufacturing plant with good environmental hygiene conditions (8 subjects, plant B). The study included 40 unexposed controls matched for age, sex, and smoking. Plant A workers had a significantly higher rate of total abnormal metaphases excluding gaps and chromosome-type unstable aberrations, with a significantly different distribution of chromosome-type unstable aberrations and asymmetric chromosome exchanges. Plant B workers showed a significantly higher frequency of chromatid-type aberrations, but did not have significantly increased gaps. The study failed to show any correlation between exposure indices and chromosomal aberration rates. However, in the subjects who had been exposed to the highest cumulative concentrations of styrene, higher rates of chromosome-type aberrations or dicentric and ring chromosomes were observed. Högstedt (1984) carried out a study to determine a new method for the assessment of cytogenetic damage in humans; frequencies of micronuclei and chromosomal aberrations were measured in 38 male reinforced plastics production workers exposed to styrene at 13 ppm (range, 1–40 ppm) and in 20 unexposed controls. The study revealed a statistically significant effect of styrene ($P = 0.005$) and of smoking ($P = 0.014$) on micronuclei in lymphocytes. Age and smoking was also correlated with these changes, but all these factors combined explained only 12–24% of the total variance.

Three studies involving exposure to styrene at higher concentrations also reported positive results, with weaker evidence. In particular, a statistically significant difference in gap, iso-gap, break, iso-break, centromere separation, deletion, and total aberrations was reported in an Egyptian study of 40 male reinforced plastics production workers exposed to styrene (blood styrene concentration, 1117 ± 64.52 µg/L; MA in urine, 246 ± 21.60 µmol/L) and 50 unexposed healthy controls (blood styrene level, 0.24 ± 0.15 µg/L; MA in urine, 4.20 µmol/L) matched with the exposed group for sex, socio-economic status, and smoking habits (Helal & Elshafy, 2013). Camurri et al. (1983) studied a group of 25 workers exposed to styrene in six reinforced plastics production industries characterized by different concentrations of exposure (30–400 mg/m^3). A control group was selected for each plant, up to a total of 22 subjects. Comparisons were made between exposed workers and controls within each plant and for the entire group of workers. Styrene airborne concentrations were measured during winter and summer and individual doses were measured by the determination of the concentrations of MA and PGA in the urine of the

workers. Evaluation of chromosomal aberrations and sister-chromatid exchanges revealed an increased frequency of chromosomal aberrations in the exposed workers, mainly of the chromatid type. In addition, sister-chromatid exchanges were higher in the workers at four out of the six plants, at which airborne styrene concentrations of 30–250 mg/m^3 were measured. Sister-chromatid exchanges were only observed in groups exposed to styrene at more than 200 mg/m^3, but no linear regression between sister-chromatid exchanges and urinary levels of styrene metabolites was observed, and there was no significant correlation between either smoking habit or years of exposure and chromosomal aberrations or sister-chromatid exchanges in the exposed workers. During 1973–1978, Andersson et al. (1980) studied a group of 36 reinforced plastics production workers exposed to styrene and unexposed controls employed in the same factory matched for age and sex. Environmental styrene concentrations measured over a 3-year period allowed identification of two exposure groups: a low-concentration group with mean levels of exposure of 137 mg/m^3, and a high-concentration group with mean levels of exposure of 1204 mg/m^3. The study revealed a statistically significant increase in chromosomal aberrations ($P < 0.001$) compared with controls ($n = 37$). A slight increase in sister-chromatid exchange was also observed in the exposed group ($n = 20$) compared with controls ($n = 21$), with no difference between the groups exposed to the high and low concentrations.

Similar but less clear results were reported in three smaller studies. In the study on reinforced plastics production workers by Tomanin et al. (1992), two groups were studied. Group 1 comprised 7 subjects exposed to styrene at 21–100 mg/m^3 (urinary MA, 46–345 mg/g creatinine) while engaged in the production of fibreglass tanks over a period of 9.0 ± 7.3 years (range, 1–19 years); Group 2 comprised 12 subjects exposed to styrene at 112–435 mg/m^3 (urinary MA, 423–1325 mg/g creatine) while engaged in the production of fibreglass boats over a period of 7.2 ± 4.7 years (range, 1.5–15.0 years). Control groups ($n = 7$ and $n = 12$, respectively) were not occupationally exposed to genotoxicants and were matched for sex, age, and smoking habits. A significant increase in the percentage of aberrant cells and total aberrations was only observed in Group B, although micronuclei frequencies were not significantly increased in either group. No correlation between length of exposure and frequency of chromosomal aberrations or micronuclei in exposed subjects were found. In the Nordenson & Beckman (1984) study, micronuclei in lymphocytes were significantly increased in 12 men exposed to styrene in a factory where fibreglass-reinforced polyester was used, compared with 12 controls. The average styrene concentration was 24 ppm, and the levels of urinary MA were less than 2 mmol/L. No significant increase in the rates of chromosomal aberrations (gaps and breaks) was found in peripheral lymphocytes, but the rate of micronucleus formation was significantly increased in the exposed group. An earlier study by Watanabe et al. (1983) investigated 18 workers exposed to styrene at concentrations of 40–50 ppm in reinforced plastics production (boat manufacturing) and 6 unexposed controls. The study showed only a marginal increase in chromosomal aberrations in the exposed workers compared with controls. A slight but statistically significant correlation between the rate of sister-chromatid exchange and the concentration of exposure was reported.

In 10 studies of adequate size and design, no effects of styrene on chromosomal end-points were observed, including in subjects exposed at more than 100 ppm. In the study by Hanova et al. (2010; see Table 4.3), micronuclei were assessed in 62 subjects exposed to styrene and 50 control individuals, but were not observed to increase with styrene exposure. In the study by Vodička et al. (2004) (see Table 4.3), cytogenetic markers were investigated in 86 lamination workers

exposed to styrene and 42 control individuals, but did not increase with styrene exposure. In a study of a group of 14 workers exposed to styrene at less than 100 mg/m³ while engaged in the production of fibreglass boats, Oberheitmann et al. (2001) did not observe any statistically significant difference in the rate of exchange-type aberrations between the exposed workers and the 7 unexposed controls. No differences in chromosomal aberrations in the peripheral lymphocytes of exposed workers were observed in 11 women exposed to styrene at 253 mg/m³ (mean) while performing various kinds of laminating (sport utensils, boats, and containers) compared with 11 unexposed controls matched for age, social habits, and living and working environment (Jablonická et al., 1988). Sorsa et al. (1991) did not find an increase in the frequency of sister-chromatid exchanges, chromosomal aberrations, or micronuclei in peripheral lymphocytes in subjects in 32 productive settlements exposed to styrene compared with unexposed controls. Similarly, no increase in micronuclei frequencies or size ratios, irrespective of proliferative stimulation with phytohaemagglutinin or pokeweed mitogen, or in chromosomal aberrations, was reported in a study of workers engaged in the production of reinforced plastics in Sweden (Hagmar et al., 1989). Micronuclei were assessed in 18 operators and 2 supervisors exposed to styrene and 22 unexposed controls, and chromosomal aberrations were assessed in 11 exposed and 14 control subjects. The mean levels of styrene exposure for the period 1974–1986 were 43–221 mg/m³ (range, 4–551 mg/m³) and the concentration of urinary MA and PGA in 1985 was 128 mmol/mol creatinine (range, < 6–317 mmol/mol creatinine). No increase was detected in the frequency of any of the cytogenetic end-points studied (sister-chromatid exchange, micronuclei, and other chromosomal aberrations) in a group of 21 workers exposed to styrene at 98 mg/m³ (range, 34–263 mg/m³) in reinforced plastics production compared with 21 subjects working mainly in office jobs, matched according to sex and smoking habits (Mäki-Paakkanen, 1987). Further, no correlations between the number of aberrations, micronuclei, or sister-chromatid exchanges and the extent or duration of exposure to styrene could be detected. Pohlová & Srám (1985) did not report an effect on chromosomal aberrations in exposed workers, but an increase was found in the rate of gaps in two groups of exposed workers in two production plants (A and B). In plant A, at which workers were engaged in polystyrene production, there were 36 exposed workers (27 men and 9 women) and 19 unexposed controls (15 men and 4 women). In plant B, at which workers were engaged in reinforced plastics production, there were 22 exposed workers (7 men and 15 women) and 22 unexposed controls (13 men and 9 women). Airborne concentrations of styrene were 5.6–982.8 mg/m³ (plant A) and 39–548 mg/m³ (plant B). Hansteen et al. (1984) reported no increase in chromosomal aberrations or sister-chromatid exchange, but did find a significant (P = 0.0002) increase in gaps in two groups of subjects (n = 11 and 7) exposed to styrene in the production of reinforced plastics compared with unexposed office employees (n = 9) matched for sex, age group, and smoking habits. Thiess et al. (1980) failed to identify a statistically significant increase in chromosomal aberration rates in a study of 24 workers exposed to styrene at concentrations of 6 ppm (in an annexed laboratory) to 58.1 ppm (in a pilot polystyrene processing plant) for 4–27 years compared with 24 unexposed controls [number not clearly reported]. Likewise, in a previous study Thiess & Fleig (1978) considered a group of 12 subjects extracted from a larger group of 93 subjects engaged in the synthesis of styrene from ethylbenzene, 10 with 19–39 years of possible exposure and 2 with high concentrations of MA in urine, without finding any increase in chromosomal aberrations. Levels of exposure were of the order of fractions of parts per million for most of

the workers under study. Different levels of exposure were considered, but no control group was included in the study.

Other available studies were limited either by their small sample size or by other critical factors. Artuso et al. (1995) carried out a study on two groups each of 23 workers exposed to styrene in fibreglass-reinforced plastic boat production, one group at a low concentration of 2–120 mg/m^3 and the other at a high concentration of 86–1389 mg/m^3, and 51 matched controls living in the same area but not exposed to styrene. Through statistical multivariate analysis, an increase in aberrations were found in the low-concentration group and in the high-concentration group. The incidence of sister-chromatid exchanges showed a dose–response association. However, X-ray irradiations were more frequent in controls, smokers more frequent in the high-concentration group, and chromosome aberrations were evaluated after 72 instead of 48 hours. [The Working Group noted the limitations of the study, although the consistency among indicators and the evidence of an exposure–response suggest that the changes observed were at least partially attributed to styrene exposure.] Mäki-Paakkanen et al. (1991) studied the frequency of chromosomal aberrations, micronuclei, and sister-chromatid exchanges in 17 workers exposed to styrene in the production of plastic containers and 17 unexposed controls. The concentrations of exposure, estimated through the determination of MA in urine, were of the order of 300 mg/m^3. Chromosomal aberrations were significantly higher in the exposed workers, without a significant correlation with levels of exposure, although the frequencies of micronuclei and sister-chromatid exchanges did not differ between the two groups. [The Working Group noted the inconsistency of the results obtained by different end-points, and the lack of a clear dose–response correlation.] Meretoja et al. (1977) studied 10 male workers exposed to styrene in three plastics manufacturers and 5 unexposed controls; frequency of chromosomal aberrations of 11–26% (vs 3% in controls), and a higher frequency of micronuclei and cells connected with nuclear bridges, were measured in the peripheral lymphocytes of the exposed workers. However, no correlation between styrene exposure, estimated through the determination of MA concentration in urine, and the incidence of chromosomal aberrations was found. [The Working Group noted the small number of subjects and the absence of a dose–response relationship.] Kelsey et al. (1990) studied a group of 20 workers exposed to styrene in the construction of boats in two factories and a control group comprising 20 unexposed workers from the same small companies. Exposure was measured through determination of styrene concentration in environmental and exhaled air, and by urinalysis of styrene metabolites. Mean airborne styrene concentrations of 209.8 mg/m^3 and 230.1 mg/m^3 and mean MA concentrations of 275 mg/g creatinine and 323 mg/g creatinine were measured for smokers and non-smokers, respectively. The frequency of sister-chromatid exchange did not differ between exposed and unexposed workers, although smoking significantly induced sister-chromatid exchange in these workers. [The Working Group noted that the main limitation of this study was that controls might have experienced exposure to styrene previously.] Smejkalová et al. (1989) evaluated the number of chromosomal aberrations in peripheral lymphocytes of 13 women occupationally exposed to styrene and 6 paired controls. The average styrene airborne concentration was 225 ± 89 mg/m^3. The number of chromosomal aberrations in the exposed group was significantly higher than in controls, but the study groups were small. Dolmierski et al. (1983) conducted a study on 37 workers engaged in the production of laminated styrene plates and 2 controls. They found chromosomal aberrations in 6.8% of cells in the exposed subjects. [The Working Group noted that the main limitations of the study were the small control group

(n = 2), the apparently younger age of controls compared with exposed subjects (22–28 years), and the apparent lack of control for confounders.] Meretoja et al. (1978) published the results of a study conducted during 1976–1977 on a group of 16 workers exposed to styrene in two reinforced plastics production plants and a control group of 6 unexposed subjects. Occupational histories and smoking habits were recorded for both exposed and unexposed subjects. Workers were exposed to styrene at 200–300 ppm (occasionally), and the concentrations of MA in urine were measured as 55–3257 mg/g creatinine in 1976 and 53–1646 mg/g creatinine in 1977. There was a significant increase in chromosomal aberrations confirmed 1 year later, although the frequency of sister-chromatid exchange was not increased. In a study conducted in Sweden, Högstedt et al. (1979) considered a group of 6 reinforced plastics production workers (3 smokers) exposed to styrene at 50–400 mg/m^3 and 6 unexposed controls (3 smokers) matched for sex, age, and smoking. The results showed a significant increase in the frequency of chromosomal aberrations in the lymphocytes of exposed workers compared with controls (5.2 per 100 cells). [The Working Group noted that the study was limited by the small number of subjects in both groups.] Fleig & Thiess (1978) studied three groups of workers engaged in styrene manufacturing (5 men), polystyrene production (12 men), and unsaturated polyester resins production (14 workers from three different plants, 6 + 3 +5), where each group was matched with 20 controls, and found an increase in chromosomal aberrations only in the polyester resins production group. [The Working Group noted that the limitations of the study were the small sizes of the study groups, the lack of control for confounders, and unclear criteria for the selection of controls; however, the evidence of effects only in the group of workers exposed to the highest concentration, together with the absence of effects in the group exposed to the lowest concentration, may suggest the existence of a dose–response relationship.]

Three other studies were limited by co-exposure to other genotoxicants. The study of Tates et al. (1994; see Table 4.6) investigated a group of 46 workers exposed to styrene and dichloromethane (used to clean machines) engaged in the production of containers and boards and 23 paired unexposed controls. Workers were exposed to styrene at 17 ppm [70 mg/m^3] and dichloromethane at 31 ppm (TWA values). The study showed statistically significant increases in the frequencies of chromosomal aberrations with or without gaps ($P < 0.0001$), of aberrant cells (gaps always included), micronuclei, and sister-chromatid exchange ($P < 0.0001$; one-sided). Both duration and intensity of exposure were not correlated with genetic effects, but the TWA value for dichloromethane was positively correlated with the frequencies of chromosomal aberrations (with gaps) and aberrant cells. [The Working Group noted the contemporary presence of two genotoxicants; moreover, a correlation between dose and effect was observed only for dichloromethane and not for styrene.] In a study based in Lithuania, Mierauskiene et al. (1993) studied a group of 109 workers (68 women and 41 men; 38 smokers) exposed to styrene, formaldehyde, and phenol (concentrations not measured) for a duration of 1–25 years and 64 unexposed controls (25 women and 39 men; 16 smokers), mostly students and clerks (mean age, 37.4 years; range, 18–60 years). The frequency of aberrant cells in occupationally exposed workers was significantly higher than in controls. In addition, an increased frequency of breaks (both chromosome and chromatid) was observed, but not exchanges. Also in Lithuania, Lazutka et al. (1999) conducted a study on several environmental risk factors of different groups, including 38 men and 41 women engaged in the production of carpets, and 34 men and 63 women employed at a plastics production plant. Each group was compared with 90 unexposed controls (26 women and 64 men;

27 smokers and 63 non-smokers) approximately matched by age. Exposure to other chemical substances was observed in both groups (phenol and formaldehyde). An increased frequency of chromosomal aberrations was observed in the lymphocytes of exposed workers in both groups.

One study reported on chromosomal end-points in semen samples. Naccarati et al. (2003) studied chromosomal spermatozoa aberrations in 18 workers exposed to styrene in the production of fibreglass-reinforced plastics and in 13 paired controls. The median value of MA urine concentration in these workers was 292.5 mg/g creatinine (range, 20.8–947.8 mg/g creatinine). A cytogenetic analysis by fluorescence in situ hybridization conducted on semen samples did not show a statistically significant difference in the incidence of aneuploidy and diploidy between the group of 18 exposed workers and the 13 unexposed controls. The only statistically significant finding was an excess of nullisomy in the exposed non-smokers (Naccarati et al., 2003).

(v) DNA repair

Using the ^{32}P-postlabelling technique, Vodička et al. (1994) assessed the persistence of O^6-guanine DNA adducts in lamination workers exposed to styrene. Although lymphocyte adduct concentrations were higher in laminators compared with controls (see Section 4.2.2(c)), no decrease was noted between concentrations before and after a 2-week vacation, indicating that the repair process was slow. Oberheitmann et al. (2001) used the challenge assay, a cytogenetic approach to measure the repair competence, to assess DNA repair in lamination workers exposed to styrene. Exchange-type aberrations per 100 metaphases after X-ray challenge were 13.26 in 2 historical controls and 16.19 in 14 exposed laminators ($P < 0.038$). Among the exposed group, the challenge response was significantly correlated with the cumulative lifetime exposure to styrene ($P < 0.015$) but not with the current exposure as measured in blood, which was suggested to indicate the interference of long-term exposure with DNA repair.

Vodička et al. (2004) investigated cytogenetic markers, DNA single-strand breaks, urinary metabolites, and DNA repair rates in 86 lamination workers exposed to styrene and in 42 control individuals. A negative correlation between all exposure parameters and single-strand breaks (see Section 4.2.3(a)(i)), and a positive correlation between exposure parameters and DNA repair rates, were reported. Occupational exposure to styrene was studied in 34 workers employed in the production of fibreglass-reinforced plastic sheets compared with 29 unexposed healthy controls (Fracasso et al., 2009). A decrease in DNA repair activity compared with controls was observed.

Repair polymorphisms and gene expression in workers exposed to styrene

The Working Group noted that the results described in the paragraphs that follow were from a small number of individuals and that some results are not consistent. Godderis et al. (2004) studied 44 workers exposed to styrene (calculated average concentration, 9.5 ppm) and 44 matched controls to examine the influence of polymorphisms on genes encoding biotransformation, and of DNA repair enzymes on the levels of N-terminal haemoglobin adducts and on genotoxicity biomarkers. In the group of 88 individuals in total, higher frequencies of micronucleated mononucleated lymphocytes were found in individuals possessing the *XRCC3* Met^{241} allele. Individuals with the *XRCC1* Gln^{399} allele showed higher frequencies of micronucleated mononucleated lymphocytes and micronucleated binucleated lymphocytes. The same study population was used to investigate more polymorphisms, but no new associations were reported for subjects exposed to styrene (Mateuca et al., 2008). Kuricova et al. (2005) analysed 1-(2-hydroxy-phenylethyl)adenine

(1-styrene-7,8-oxide–adenine) DNA adducts, DNA single-strand breaks, chromosomal aberrations, mutant frequency at the *HPRT* gene, and immune parameters (relating to haematological and humoral immunity) in human subjects exposed to styrene (n = 48) and controls (n = 24). Results were correlated with genetic polymorphisms in DNA repair genes (xeroderma pigmentosum *XPD*, exon 23; *XPG*, exon 15; *XPC*, exon 15; *XRCC1*, exon 10; and *XRCC3*, exon 7) and cell cycle gene cyclin D1. The polymorphism in exon 23 of the *XPD* gene modulated levels of chromosomal and DNA damage and *HPRT* mutation frequency, and moderately affected DNA adduct levels. The highest levels of biomarkers were associated with the wildtype homozygous AA genotype. The exposed individuals with the wildtype GG genotype for the *XRCC1* gene exhibited the lowest frequencies of chromosomal aberrations compared with those with an A allele ($P < 0.05$). The same team also showed that, in 24 lamination workers occupationally exposed to styrene and 15 unexposed controls, DNA repair capacity was significantly lower in individuals with variant *Gln/Gln* genotype in *XRCC1 Arg399Gln* than in those with heterozygous *Arg/Gln* and wildtype *Arg/Arg* genotypes (Slyskova et al., 2007). Significantly lower repair capacity was also found in individuals with the wildtype *Lys/Lys* genotype in *XPC* at locus *Lys939Gln* compared with those homozygous for the *Gln/Gln* variant genotype.

Vodička and colleagues investigated 60 workers exposed to styrene and 50 unexposed clerks for mRNA expression levels of the human 8-oxoguanine DNA *N*-glycosylase (*hOGG1*) gene, and the role of the *hOGG1* polymorphism *Ser326Cys* (Manini et al., 2009). Subjects bearing the (*hOGG1*) *Ser/Ser* genotype showed lower levels of 8-oxodGuo in leukocytes than those with at least one variant *Cys* allele. Workers showed higher levels of *hOGG1* expression compared with controls ($P < 0.0005$).

In subsequent studies, Vodička and colleagues studied the modulation of DNA repair capacity and mRNA expression levels of *XRCC1*, *hOGG1*, and *XPC* genes in workers exposed to styrene (Hanova et al., 2010). The study assessed the associations between DNA strand breaks, micronuclei, DNA repair capacity, and mRNA expression in *XRCC1*, *hOGG1*, and *XPC* genes in 71 workers exposed to styrene and in 51 control individuals. The mRNA expression levels of *XRCC1*, *hOGG1*, and *XPC* were negatively correlated with styrene concentrations in the blood and in workplace air ($P < 0.001$), and positively correlated with strand breaks ($P < 0.001$). In a related study on the same worker population, the team studied the relationship between DNA damage, DNA repair rates, and mRNA expression levels of cell cycle genes *TP53*, *p21*^{CDKN1A}, *BCL2*, and *BAX* (Hanova et al., 2011). The results showed negative correlations between mRNA expression of *TP53*, *BCL2*, and *BAX* ($P < 0.001$ for all parameters) and styrene exposure, although a positive correlation with *p21*^{CDKN1A} expression and exposure was recorded ($P = 0.001$). DNA strand breaks and sites sensitive to endonuclease III increased with increasing mRNA levels of *TP53* ($P < 0.001$ for both) and *BCL2* ($P = 0.038$ and $P = 0.002$, respectively), and decreased with increasing mRNA levels of *p21*^{CDKN1A} ($P < 0.001$ and $P = 0.007$, respectively). In a study of 50 fibreglass-reinforced plastics workers exposed to styrene at less than 10 ppm, 10–20 ppm, and more than 20 ppm, and 40 control subjects (Wongvijitsuk et al., 2011), the expression of *CYP2E1*, *hOGG1*, and *XRCC1* in all groups exposed to styrene was higher than that of the control group ($P < 0.05$).

(b) Human cells in vitro

See Table 4.4.

(i) Styrene

Styrene-induced DNA damage was detected by comet assay in isolated human leukocytes treated in vitro (Laffon et al., 2003b) and in

human skin treated in vitro (Costa et al., 2006) without metabolic activations. Cytogenetic effects were analysed in human whole-blood lymphocytes treated in vitro without metabolic activations, including chromosomal aberrations (Linnainmaa et al., 1978a, b; Pohlová et al., 1984; Jantunen et al., 1986), micronucleus formation (Linnainmaa et al., 1978a), and sister-chromatid exchange (Norppa & Vainio, 1983a, b; Norppa et al., 1983; Chakrabarti et al., 1993; Lee & Norppa, 1995; Bernardini et al., 2002). Chromosomal aberration was also analysed in human isolated lymphocytes treated in vitro without metabolic activation systems (Jantunen et al., 1986). These various reports showed positive results without exogenous metabolic activation systems. Activation, probably resulting from the conversion of styrene to styrene-7,8-oxide, was attributed to erythrocytes present in the cultures (Norppa & Vainio, 1983a; Norppa et al., 1983).

(ii) Styrene-7,8-oxide

Using the comet assay, DNA damage induced by styrene-7,8-oxide was detected in isolated human lymphocytes (Köhlerová & Stětina, 2003; Speit et al., 2012; Bausinger & Speit, 2014), in whole-blood lymphocytes (Cemeli et al., 2009; see also Section 4.2.6(b)), and in human peripheral blood mononuclear cells (mononuclear leukocytes) (Bastlová et al., 1995; Laffon et al., 2002b;Godderis et al., 2004; Fabiani et al., 2012; Bausinger & Speit, 2014) treated in vitro. The sites sensitive to endonuclease III, corresponding to apurinic sites, were also shown to be induced (Köhlerová & Stětina, 2003). Induction of DNA strand breaks in human testicular germ cells treated with styrene-7,8-oxide in vitro was reported using the alkaline elution assay (Bjørge et al., 1996). Induction of DNA strand breaks was also reported in whole-blood cells in vitro using pulsed-field gel electrophoresis (Marczynski et al., 1997b). 7-Alkylguanine adducts of styrene-7,8-oxide and DNA single-strand breaks were determined by ^{32}P-postlabelling and DNA alkaline unwinding assay in human embryonal lung fibroblasts, respectively (Vodička et al., 1996). There was a concentration-dependent increase of both 7-alkylguanine adducts and DNA strand breaks.

As noted above in Section 4.2.2(b), the induction of gene mutations in human lymphocytes in vitro was reported at the *HPRT* locus (Bastlová et al., 1995; Bastlová & Podlutsky, 1996). A *GSTM1*-positive human recombinant cell line (FB7) showed lower mutation frequency at the *HPRT* locus than its parent *GSTM1*-negative cell line (WIL2NS) after treatment with styrene-7,8-oxide (Shield & Sanderson, 2004).

Chromosomal damage was analysed in human whole-blood lymphocyte cultures treated in vitro for chromosomal aberrations (Fabry et al., 1978; Linnainmaa et al., 1978a; Pohlová et al., 1984), micronuclei (Linnainmaa et al., 1978a; Speit et al., 2012), and sister-chromatid exchanges (Norppa & Vainio, 1983b; Norppa et al., 1983; Pohlová et al., 1984; Zhang et al., 1993; Lee & Norppa, 1995; Uusküla et al., 1995; Chakrabarti et al., 1997; Ollikainen et al., 1998). The frequency of micronucleus formation and sister-chromatid exchange was also analysed in isolated human mononuclear leukocytes (Laffon et al., 2001b, 2003a). Micronucleus induction was also analysed in human peripheral blood mononuclear cells (Godderis et al., 2006); results were consistently positive.

(c) Experimental systems

(i) Styrene: in vivo

See Table 4.7.

Rats

The comet assay was negative in leukocytes of male Fischer 344 rats (Gaté et al., 2012). However, a significant increase in DNA damage was observed on the 3rd, but not the 20th, day of treatment in the presence of Fpg, an enzyme able to recognize and excise DNA at the level of some oxidized DNA bases.

Table 4.7 Genetic and related effects of styrene and styrene-7,8-oxide in non-human mammals in vivo

End-point	Species, strain, (sex)	Tissue	Results[a]	Concentration (LEC or HIC) or dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Styrene							
Oxidative damage to DNA (comet assay with Fpg sensitive sites)	Rat, F344 (M)	Leukocytes	+/–	75 ppm	Inhalation, 6 h/d, 5 d/wk, 4 wk	Comet assay assessed at 3rd and 20th days; in the presence of Fpg, positive result was observed at 3rd but not at 20th day	Gaté et al. (2012)
DNA strand breaks, micronuclei	Rat, F344 (M)	Leukocytes. peripheral blood reticulocytes	–	1000 ppm	Inhalation, 6 h/d, 5 d/wk, 4 wk	Comet assay and micronuclei evaluated at the 3rd and 20th day of exposure	Gaté et al. (2012)
DNA strand breaks (comet assay), chromosomal aberrations, micronuclei	Rat, F344 (F)	Lymphocytes	–	500 ppm	Inhalation, 6 h/d, 14 d		Kligerman et al. (1993)
Chromosomal aberrations	Rat, Sprague-Dawley (M, F)	Bone marrow	–	1000 ppm	Inhalation, 6 h/d, 5 d/wk, 1 yr		Sinha et al. (1983)
Chromosomal aberrations, sister-chromatid exchange	Rat, F344 (M)	Peripheral blood lymphocytes	–	1000 ppm	Inhalation, 6 h/d, 5 d/wk, 4 wk		Preston & Abernethy (1993)
Micronuclei	Rat, Porton (M)	Bone marrow (PCE)	–	3000 mg/kg	Intraperitoneal injection, 48 h after treatment		Simula & Priestly (1992)
Sister-chromatid exchange	Rat, F344 (F)	Lymphocytes	(+)	125 ppm	Inhalation, 6 h/d, 14 d	Small increase, although statistically significant	Kligerman et al. (1993)
Unscheduled DNA synthesis	Mouse CD-1 (F)	Liver	–	250 ppm	Inhalation, 6 hr		Clay (2004)
DNA strand breaks (comet assay)	Mouse, C57BL/6 (M)	Lymphocytes, liver, kidney, bone marrow	+	250 mg/kg	Intraperitoneal injection, 4 h after treatment	LED is 350 mg/kg bw for bone marrow	Vaghef & Hellman (1998)
DNA strand breaks (DNA unwinding)	Mouse NMRI (M)	Kidney, liver, lung, testis, brain	+	8.3 mmol/kg bw	Intraperitoneal injection, NR		Solveig Walles & Orsén (1983)
DNA adducts (^{32}P-postlabelling)	Mouse, NMRI (M)	Lung	+	750 mg/m^3	Inhalation, 5 h/d, 7 d/wk, 1–21 d	*N*7-guanine and *N*1-adenine adducts were analysed	Vodička et al. (2001a)

Table 4.7 (continued)

End-point	Species, strain, (sex)	Tissue	Results[a]	Concentration (LEC or HIC) or dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
DNA strand breaks (comet assay without EndoIII) and oxidative damage to DNA (comet with EndoIII)	Mouse, NMRI (M)	Lymphocytes, bone marrow	+/–	750 mg/m^3	Inhalation, 5 h/d, 7 d/wk, 1–21 d	Positive in bone marrow for EndoIII-sensitive sites after 21 d	Vodička et al. (2001a)
DNA strand breaks (comet assay without EndoIII) and oxidative damage to DNA (comet with EndoIII)	Mouse, NMRI (M)	Liver	–	1500 mg/m^3	Inhalation, 5 h/d, 7 d/wk, 1–21 d	Negative results with and without EndoIII	Vodička et al. (2001a)
Chromosomal aberrations, micronuclei	Mouse B6C3F$_1$ (F)	Lung, spleen	–	500 ppm	Inhalation, 6 h/d, 14 d		Kligerman et al. (1993)
Chromosomal aberrations	Mouse, CD1 (M, F)	Bone marrow	–	1000 mg/kg	Gavage, single dose (1×), 24 h after treatment		Loprieno et al. (1978)
Chromosomal aberrations	Mouse, CD-1 (M)	Bone marrow	–	200 × 70, 500 × 4 mg/kg	Oral, 4 or 70 mg/kg per day		Sbrana et al. (1983)
Chromosomal aberrations, sister-chromatid exchange	Mouse, C57BL/6 (M)	Bone marrow	–	1000 mg/kg bw	Intraperitoneal injection, BrdU-labelled M1 cells 16 h after BrdU implantation		Sharief et al. (1986)
Micronuclei	Mouse, NMRI (M)	Bone marrow	+/–	1500 mg/m^3	Inhalation, 5 h/d, 7 d/wk, 1–21 d	2-fold higher than in control after 7 d (but not 21 d) of exposure	Vodička et al. (2001a)
Micronuclei	Mouse, NMRI (NR)	Bone marrow (PCE)	–	1500 mg/m^3	Inhalation, 6 h/d, 1–21 d		Engelhardt et al. (2003)
Micronuclei	Mouse, LACA Swiss (M)	Bone marrow (PCE)	+	600 mg/kg	Intraperitoneal injection, 48 h after treatment		Simula & Priestly (1992)
Micronuclei	Mouse, C57BL/6 (M)	Bone marrow (PCE)	+	250 mg/kg bw	Intraperitoneal injection, 30 h after treatment		Norppa (1981)

Table 4.7 (continued)

End-point	Species, strain, (sex)	Tissue	Results[a]	Concentration (LEC or HIC) or dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Sister-chromatid exchange	Mouse, BDF1 (M)	Bone marrow, liver, alveolar macrophages	+	387 ppm	Inhalation, 6 h/d, 4 d	Partial hepatectomy for liver regeneration	Conner et al. (1980)
Sister-chromatid exchange	Mouse, $B6C3F_1$ (F)	Lung, spleen, lymphocytes	(+)	125 ppm	Inhalation, 6 h/d, 14 d	Small increase, although statistically significant	Kligerman et al. (1993)
Sister-chromatid exchange	Mouse, LACA Swiss (M)	Splenocytes	(+)	45 mg/kg	Intraperitoneal injection, 24 h after treatment +43 h culture	Small increase, although statistically significant	Simula & Priestly (1992)
Chromosomal aberrations	Hamster, Chinese (M)	Bone marrow	–	300 ppm	Inhalation, 6 h/d, 5 d/wk, 4 d or 3 wk		Norppa et al. (1980)
Micronuclei	Hamster, Chinese (M)	Bone marrow	–	1000 mg/kg bw	Intraperitoneal injection, 30 h after treatment		Penttilä et al. (1980)
Styrene-7,8-oxide							
DNA strand breaks (comet assay), micronuclei	Rat, F344 (M)	Leukocytes	–	75 ppm	Inhalation, 6 h/d, 5 d/wk, 4 wk	Evaluated at 3rd and 20th day; comet assay results negative with and without Fpg	Gaté et al. (2012)
DNA adducts (covalent binding to DNA (HPLC))	Rat, CD (M)	Forestomach	+	1.3 mg/kg bw	Gavage, 4 h after treatment		Lutz et al. (1993)
DNA strand breaks (comet assay)	Mouse, ddY (M)	Stomach, colon, liver, kidney, bladder, lung, brain, bone marrow	+	400 mg/kg bw	Intraperitoneal injection, 3 h after treatment		Tsuda et al. (2000)
DNA strand breaks (comet assay)	Mouse, CD-1 (M)	Liver, kidney, lung, spleen, bone marrow	+	400 mg/kg bw	Intraperitoneal injection, 3 h and 24 h after treatment		Sasaki et al. (1997)
DNA strand breaks (comet assay)	Mouse, C57BL/6 (M)	Lymphocytes, liver, kidney, bone marrow	+	100 mg/kg bw	Intraperitoneal injection, 4 h after treatment	LED is 150 mg/kg bw for bone marrow	Vaghef & Hellman (1998)

Table 4.7 (continued)

End-point	Species, strain, (sex)	Tissue	Results[a]	Concentration (LEC or HIC) or dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
DNA strand breaks (DNA unwinding)	Mouse, NMRI (M)	Kidney, liver, lung, testis, brain	+	5.3 mmol/kg bw [637 mg/kg bw]	Intraperitoneal injection, 5×/wk, 4 wk		Solveig Walles & Orsén (1983)
Dominant lethal test (dominant lethal)	Mouse, BALB/c (M)	Fetus	–	250 mg/kg bw	Intraperitoneal injection, mate after 1–3 wk	No positive control	Fabry et al. (1978)
Chromosomal aberrations	Mouse, BALB/c (M)	Spermatocytes	–	250 mg/kg bw	Intraperitoneal injection, 2 mo after treatment	No positive control	Fabry et al. (1978)
Chromosomal aberrations, micronuclei	Mouse, BALB/c (M)	Bone marrow	–	250 mg/kg bw	Intraperitoneal injection, 1–13 d (chromosomal aberration) or 30 d (micronuclei) after treatment	No positive control	Fabry et al. (1978)
Chromosomal aberrations	Mouse, CD1 (M, F)	Bone marrow	+	50 mg/kg bw	Gavage, 1×, 24 h after treatment		Loprieno et al. (1978)
Chromosomal aberrations, sister-chromatid exchanges	Mouse, CD-1 (M)	Bone marrow	+	Enantiomer (*S*- or *R*-) 100 mg/kg bw	Intraperitoneal injection, 24 h after treatment (chromosomal aberration)	Positive only for *S*-enantiomer	Sinsheimer et al. (1993)
Chromosomal aberrations, sister-chromatid exchanges	Hamster, Chinese (M)	Bone marrow	–	100 ppm	Inhalation, 9 h		Norppa et al. (1979)
Chromosomal aberrations, sister-chromatid exchanges	Hamster, Chinese (M)	Bone marrow	(+)	500 mg/kg bw	Intraperitoneal injection, 24 h after treatment	Single dose, positive only for dead animals	Norppa et al. (1979)
Micronuclei	Hamster, Chinese (M)	Bone marrow	–	250 mg/kg bw	Intraperitoneal injection, 30 h after treatment		Penttilä et al. (1980)

BrdU, bromodeoxyuridine; bw, body weight; d, day(s); EndoIII, endonuclease III; F, female; h, hour(s); HIC, highest ineffective concentration; HPLC, high-performance liquid chromatography; LEC, lowest effective concentration; LED, lowest effective dose; M, male; mo, month(s); NR, not reported; NT, not tested; PCE, polychromatic erythrocytes; ppm, parts per million; wk, week(s); yr, year(s).

[a] +, positive; –, negative; +/–, equivocal (variable response in several experiments within an adequate study); (+)/(–), positive/negative result in a study of limited quality; the level of significance was set at $P < 0.05$ in all cases.

Styrene did not induce DNA damage as determined by the comet assay, chromosomal aberrations, or sister-chromatid exchanges in female Fischer 344 rat lymphocytes (Kligerman et al., 1993).

Negative results for chromosomal aberrations were also reported in male Fischer 344 rats (Preston & Abernethy, 1993) and in bone marrow of male and female Sprague-Dawley rats (Sinha et al., 1983).

Micronucleus assay results were negative in the 3-week inhalation study in bone marrow of female Fischer 344 rats (Kligerman et al., 1993) and in the 4-week inhalation study in peripheral blood reticulocytes of male Fischer 344 rats (Gaté et al., 2012).

A negative result for sister-chromatid exchange was obtained in lymphocytes of male rats (Preston & Abernethy, 1993). A positive result was obtained for sister-chromatid exchange in splenocytes of the male Porton rat (Simula & Priestly, 1992).

Overall, results were negative or weakly positive for chromosomal damage in rats after exposure to styrene.

Mice

No data were available to the Working Group in transgenic models.

Styrene did not induce unscheduled DNA synthesis in female CD-1 mouse liver (Clay, 2004).

DNA damage induced by styrene was detected by comet assay in female C57BL/6 mouse lymphocytes, liver, kidney, and bone marrow (Vaghef & Hellman, 1998). DNA strand breaks in kidney, liver, lung, testis, and brain of male NMRI mice were detected by the DNA unwinding assay (Solveig Walles & Orsén, 1983).

DNA damage induced by styrene was detected by the comet assay in bone marrow of male NMRI mice (with an increase in sites sensitive to endonuclease III), although no induction was observed in liver (Vodička et al., 2001a). DNA adducts were detected in lung.

Negative results for chromosomal aberrations were reported in the lung and spleen of female B6C3F$_1$ mice (Kligerman et al., 1993). Negative results for chromosomal aberrations were reported in male and in female CD-1 mouse bone marrow (Loprieno et al., 1978; Sbrana et al., 1983).

Results for micronucleus induction were negative in the bone marrow of male NMRI mice (Engelhardt et al., 2003), and in the spleen and peripheral blood of female B6C3F$_1$ mice (Kligerman et al., 1993). An equivocal result for micronucleus induction was reported in male NMRI mouse bone marrow (Vodička et al., 2001a). A weak micronucleus induction was reported in bone marrow of male LACA Swiss mice (Simula & Priestly, 1992) and C57BL/6 mice (Norppa, 1981).

In the assay for sister-chromatid exchange, positive results were obtained in bone marrow, liver, and alveolar macrophages of male BDF1 mice (Conner et al., 1980). Equivocal results were obtained in the lung, spleen, and lymphocytes of female B6C3F$_1$ mice (Kligerman et al., 1993). In the assay for sister-chromatid exchange, an equivocal result was reported in male LACA Swiss mouse splenocytes (Simula & Priestly, 1992) and a negative result was obtained in male C57BL/6 mouse bone marrow (Sharief et al., 1986).

Overall, results for chromosomal damage induced by styrene were negative or weak in mouse cells in vivo.

Hamsters

After exposure by inhalation (Norppa et al., 1980) or intraperitoneal injection (Penttilä et al., 1980), negative results for cytogenetic changes were reported in the bone marrow of male Chinese hamster.

All results were negative for chromosomal damage induced by styrene in hamster.

(ii) Styrene-7,8-oxide: in vivo

Rats

Results were negative in leukocytes as measured by comet assay, with and without Fpg, and were negative for micronucleus induction in peripheral blood reticulocytes when analysed during a 4-week inhalation study in male Fischer 344 rat (Gaté et al., 2012).

Mice

DNA strand breaks were detected by the DNA unwinding assay in kidney, liver, lung, testis, and brain of male NMRI mice (Solveig Walles & Orsén, 1983). DNA damage induced by styrene-7,8-oxide was detected by comet assay in all organs tested in male CD-1 mice (liver, kidney, lung, spleen, and bone marrow) (Sasaki et al., 1997) and in male ddY mice (stomach, colon, liver, kidney, urinary bladder, lung, brain, and bone marrow) (Tsuda et al., 2000). DNA damage was detectable in multiple organs after exposure to styrene-7,8-oxide by intraperitoneal injection.

A positive result for chromosomal aberration was reported in male and female CD-1 mouse bone marrow (Loprieno et al., 1978). A negative or equivocal result for chromosomal aberration was reported in the bone marrow and spermatocytes of BALB/c mice (Fabry et al., 1978). The bone marrow micronucleus test was negative in BALB/c mice (Fabry et al., 1978). A negative result for the mouse dominant lethal test was also reported (Fabry et al., 1978).

Enantiomers of styrene-7,8-oxide were tested by an assay for sister-chromatid exchange in male CD-1 mouse bone marrow; only the *S*-enantiomer gave a positive result without including gaps (Sinsheimer et al., 1993).

[The Working Group noted the possibility that only the *S*-enantiomer has the potential to induce chromosomal damages, but there was only one study.]

Hamsters

In male Chinese hamsters exposed to styrene-7,8-oxide by inhalation, the results of assays for sister-chromatid exchange and chromosomal aberration were negative; however, in male Chinese hamsters exposed by intraperitoneal injection, the results were equivocal for both cytogenetic tests (Norppa et al., 1979).

The bone marrow micronucleus test was negative in Chinese hamster after intraperitoneal injection (Penttilä et al., 1980).

Overall, no positive result was obtained in hamsters.

(iii) Styrene: in vitro

The majority of genotoxicity testing for styrene in non-human mammalian in vitro systems and in non-mammalian systems is reported in *IARC Monographs* Volume 60 (IARC, 1994). Styrene has been studied for its effects on DNA strand breaks, mutagenesis and chromosomal aberrations, and sister-chromatid exchanges, primarily in rodent cell lines, *Drosophila melanogaster*, yeast, and *Escherichia coli*. These results are summarized below and in Supplemental Table S2 and Supplemental Table S3.

In a study in non-human mammalian cells using the comet assay, exposure to styrene at 2.5 mM for 2 hours significantly increased DNA damage in isolated hepatocytes obtained from male Swiss albino mice (Fontaine et al., 2004). This effect was attenuated when cells were pre-incubated with SKF-525A, a broad inhibitor of CYP enzymes.

In earlier studies, styrene induced DNA strand breaks in rat primary hepatocytes in an alkaline elution assay (Sina et al., 1983). Styrene induced mutations at the *Hprt* locus in Chinese hamster lung V79 cells with, but not without, exogenous metabolic activation system (Loprieno et al., 1976; Beije & Jenssen, 1982). Two studies on the induction of chromosomal aberrations in Chinese hamster lung cells reported negative results without exogenous metabolic

activation and weakly positive results with exogenous metabolic activation (Matsuoka et al., 1979; Ishidate & Yoshikawa, 1980). Sister-chromatid exchanges were induced in Chinese hamster ovary cells with, but not without, exogenous metabolic activation system (de Raat, 1978), whereas sister-chromatid exchanges were induced in rat lymphocytes without exogenous metabolic activation (Norppa et al., 1985).

In non-mammalian systems, styrene (0.2 mg/L in seawater) significantly increased DNA damage in cells from the peripheral blood of fish (*Symphodus melops*) and the haemolymph of mussels (*Mytilus edulis*) after 7 days of continuous exposure (Mamaca et al., 2005).

Styrene was positive for sex-linked recessive lethal mutations and negative for aneuploidy in *D. melanogaster* (Donner et al., 1979), whereas Penttilä et al. (1980) reported negative results for aneuploidy. Rodriguez-Arnaiz (1998) reported negative results for induction of somatic mutations by styrene in *D. melanogaster*; however, in the same study styrene was positive in two strains of flies that had higher levels of CYP activity.

Styrene induced chromosomal aberrations in the root tip cells of *Allium cepa* (Linnainmaa et al., 1978a,b).

Styrene was genotoxic for several end-points in yeast, including gene conversion and reverse mutation (Del Carratore et al., 1983). It was also shown that enhancing the metabolic activity of mouse liver S9 by treating animals twice (4–5 weeks between injections) with phenobarbital and β-naphthoflavone increased the genotoxicity of styrene in *Saccharomyces cerevisiae* D7 in assays for mitotic gene conversion, mitotic crossing-over, and point reverse mutation (Paolini et al., 1988).

Styrene was tested in various *Salmonella typhimurium* strains (TA98, TA100, TA1530, TA1535, TA1537, and TA1538) by several research groups. Styrene was reported negative in the absence of exogenous metabolic activation in eight tests for TA100, and negative in the presence of exogenous metabolic activation in seven studies and positive in one (de Meester et al., 1981). In a ninth test, styrene was reported as weakly mutagenic in the presence and absence of exogenous metabolic activation; however, concern was expressed about the results because of toxicity (Vainio et al., 1976). In one study, positive results were reported for TA1530 in the presence and absence of exogenous metabolic activation (de Meester et al., 1981). [The Working Group noted that the authors reported that the result in the absence of metabolic activation is a false-positive result because of contamination with a volatile genotoxicant, possibly styrene-7,8-oxide, from neighbouring plates that had been incubated with styrene and S9 (de Meester et al., 1981).] Of nine tests reported for TA1535, all were negative in the absence of exogenous metabolic activation and four were positive with exogenous metabolic activation (Vainio et al., 1976; de Meester et al., 1977, 1981; Poncelet et al., 1980). Styrene was negative in TA98, TA1537, and TA1538 with or without exogenous metabolic activation (Vainio et al., 1976; de Meester et al., 1977, 1981; Stoltz & Whitey, 1977; Watabe et al., 1978; Busk, 1979; Florin et al., 1980). In studies conducted by the National Toxicology Program, styrene was tested in TA97, TA98, TA100, TA1535, and TA1537 with and without exogenous metabolic activation; negative results were found (Zeiger et al., 1988). Styrene was also negative in an *E. coli* SOS chromotest for DNA damage in the absence of exogenous metabolic activation (Brams et al., 1987). [The Working Group noted that, overall, styrene was mutagenic with exogenous metabolic activation in strains that detect base-pair substitutions (TA100, TA1530, and TA1535), but not in strains that detect frameshift mutations (TA98, TA1537, and TA1538). Overall, styrene is not mutagenic in the Ames assay in the absence of exogenous metabolic activation.]

(iv) Styrene-7,8-oxide: in vitro

The majority of genotoxicity testing for styrene-7,8-oxide in non-human mammalian in vitro systems and in non-mammalian systems was reported in *IARC Monographs* Volume 60 (IARC, 1994). Similar to styrene, styrene-7,8-oxide has been studied for effects on DNA strand breaks, mutagenesis, chromosomal aberrations, and sister-chromatid exchanges primarily in rodent cell lines, *D. melanogaster*, and yeast. These results are included in Supplemental Table S2 and Supplemental Table S3.

Styrene-7,8-oxide induced sister-chromatid exchanges, micronuclei, and mutations at the *Hprt* locus in Chinese hamster lung V79 cells (Loprieno et al., 1976, 1978; Beije & Jenssen, 1982; Nishi et al., 1984), and induced sister-chromatid exchanges in Chinese hamster ovary cells, in the absence of exogenous metabolic activation (de Raat, 1978). DNA damage was also induced by 50 µM of styrene-7,8-oxide in Chinese hamster lung V79 cells as measured by alkaline elution assay (Oesch et al., 2000); however, it was shown that 200 µM of styrene-7,8-oxide was required to produce the same amount of DNA damage in Chinese hamster lung V79 cells engineered to express human microsomal epoxide hydrolase at levels comparable to that of human liver cells (Oesch et al., 2000). Styrene-7,8-oxide also induced mutations at the *Tk* locus in mouse lymphoma L5178Y cells without exogenous metabolic activation; however, the result was negative when styrene-7,8-oxide was tested with uninduced rat liver S9 (Amacher & Turner, 1982). Styrene-7,8-oxide induced DNA strand breaks in rat primary hepatocytes (Sina et al., 1983) and rat pheochromocytoma PC12 cells (without exogenous metabolic activation) (Dypbukt et al., 1992) in alkaline elution assays.

Styrene-7,8-oxide was positive for sex-linked recessive lethal mutations in *D. melanogaster* (Donner et al., 1979).

Styrene-7,8-oxide induced chromosomal aberrations and micronuclei in the root tip cells of *Allium cepa* (Linnainmaa et al., 1978a,b).

Styrene-7,8-oxide was tested in various *S. typhimurium* strains (TA97, TA98, TA100, TA104, TA1530, TA1535, TA1537, TA1538, TA4001, and TA4006) and in *E. coli* WP2 *uvrA* by several research groups. Positive results were obtained without the need for exogenous metabolic activation in TA97, TA100, TA104, TA1530, TA1535, TA4001, TA4006, and *E. coli* WP2 *uvrA*, all of which detect base substitution mutations. Styrene-7,8-oxide was also mutagenic in all of these strains with exogenous metabolic activation except for TA104, TA4001, TA4006, and *E. coli* WP2 *uvrA*, which were not tested under these conditions (Sugiura et al., 1978; Sugiura & Goto, 1981; Einistö et al., 1993). Nearly all testing performed with styrene-7,8-oxide in TA98, TA1537, and TA1538, which detects frameshift mutations, produced negative results in the absence or presence of metabolic activation (e.g. Wade et al., 1978; Watabe et al., 1978; de Meester et al., 1981); however, a single study that used TA97, which also detects frameshift mutations, reported a positive result without metabolic activation (Brams et al., 1987). Styrene-7,8-oxide was positive in TA100 and negative in TA98 with or without exogenous metabolic activation in studies conducted by the National Toxicology Program (Zeiger et al., 1992). Styrene-7,8-oxide was also positive in *S. typhimurium* and *E. coli* SOS chromotests for DNA damage without exogenous metabolic activation (Głośnicka & Dziadziuszko, 1986; Nakamura et al., 1987; von der Hude et al., 1990). More recently, the mutagenic potency of styrene-7,8-oxide was reduced in TA100 by using S9 prepared from rats treated with organic sulfur compounds obtained from garlic and onions to enhance epoxide hydrolase activity (Guyonnet et al., 2001). [The Working Group noted that, overall, the Ames assay results for styrene-7,8-oxide are largely in agreement with the observation that styrene was mutagenic

in strains that detect base substitutions when an exogenous metabolic activation system was used.]

4.2.4 Alteration of cell proliferation or cell death

(a) Exposed humans

(i) Styrene

Exposure to styrene among 18 fibreglass-reinforced plastics workers was associated with significantly reduced rates of cell proliferation (bromodeoxyuridine (BrdU) incorporation) in their cultured lymphocytes compared with 6 unexposed control subjects (Watanabe et al., 1983). Exposure to styrene in two groups of hand laminators (27 workers) suppressed the proliferative responses of isolated cultured lymphocytes stimulated by the mitogen concanavalin A compared with 19 control workers (Somorovská et al., 1999) (see Section 4.2.7(a)).

(ii) Styrene-7,8-oxide

No data on the alteration of cell proliferation or cell death in humans by styrene-7,8-oxide were available to the Working Group.

(b) Human cells in vitro

(i) Styrene

The treatment of whole-blood cultures from 12 donors with 10–200 μM of styrene increased the length of the cell cycle in a dose-related manner (Chakrabarti et al., 1993).

(ii) Styrene-7,8-oxide

[The Working Group noted the small number of donors as a limitation of the available studies.] In whole-blood cultures from four donors treated with styrene-7,8-oxide at 50 μM and 200 μM, there was large inter-individual variation in the expression of *p53*, *p21*, *bcl-2*, and *bax* genes in lymphocytes. Apoptosis was increased in cells from two of the donors at either 50 μM and/or 200 μM of styrene-7,8-oxide. The cytokinesis block proliferation index decreased in lymphocytes from all four donors at 200 μM of styrene-7,8-oxide, suggesting a delay in the cell cycle (Laffon et al., 2001a).

Styrene-7,8-oxide (10–200 μM) reduced the proliferative rate index (BrdU incorporation) in isolated cultured human peripheral leukocytes from four donors; the extent of the reductions were dependent on the donor. Similar results with styrene-7,8-oxide were found using cytokinesis block proliferation indices of binucleated cells with micronuclei (Laffon et al., 2001b).

Styrene-7,8-oxide (100 μM) significantly reduced the replication index (BrdU incorporation) in human whole-blood lymphocyte cultures from two donors; however, there was no linear relationship between the replication index and the duration of exposure (Chakrabarti et al., 1997).

In another study, styrene-7,8-oxide (100 μM) significantly reduced the replication index (BrdU incorporation) in human whole-blood lymphocyte cultures from two donors from 1.65 to 1.30, with a simultaneous 20% reduction in cell viability (Zhang et al., 1993).

(c) Experimental systems

(i) Styrene, styrene-7,8-oxide, and other styrene metabolites: in vivo

After exposure by inhalation of male and female CD-1 mice to styrene at 150–200 ppm for 6 hours per day, 5 days per week, cell proliferation (BrdU incorporation) in lung Clara cells was increased after 2 weeks (by up to 3.6-fold) and 5 weeks (by 1.3-fold) of exposure. No changes in cell proliferation in Clara cells were observed after 13 weeks of exposure, and no increases in cell proliferation were noted in type-II pneumocytes or in hepatocytes.

A dose-related increase in cell proliferation (BrdU incorporation) was seen in the terminal and large bronchioles of male and female CD-1 mice exposed by inhalation to styrene at 40 ppm

or 160 ppm for 6 hours per day for 5 days. The increased cell replication was not present after a 2-day break in exposure, but reoccurred in the large bronchioles when the mice were exposed for a further 5 days for 6 hours per day. Similar effects were seen in mice given styrene at 10, 100, or 200 mg/kg by oral gavage every day for 5 days. Increases in cell proliferation were seen in the terminal bronchioles in mice given styrene at 100 mg/kg and 200 mg/kg, but not at 10 mg/kg (Green et al., 2001b).

Cell proliferation (Ki-67 staining) was increased compared with controls in the terminal bronchioles of male CD-1 mice (3.9-fold) and male C57BL/6 wildtype mice (5.7-fold) exposed by inhalation to styrene at 120 ppm for 6 hours per day after 1 week, but not after 26, 52, or 72 weeks, of treatment. No increases in cell proliferation were detected in the terminal bronchioles of male *Cyp2f2*$^{(-/-)}$ mice or male *Cyp2f2*$^{(-/-)}$ mice containing a human *CYP2F1*/*CYP2A13*/*CYP2B6* transgene under the same experimental conditions. The cell proliferation results in these strains of mice mirror the results of the chronic bioassays of styrene under the same experimental conditions (Cruzan et al., 2017). Compared with their respective vehicle controls, significantly increased hyperplasia of the terminal bronchioles was seen in male CD-1 and male C57BL/6 wildtype mice given styrene between weeks 78 and 104, although epithelial degeneration was also induced in C57BL/6 mice for 26 weeks but largely resolved within 52 weeks of exposure. No responses were observed in the vehicle control or treated male *Cyp2f2*$^{(-/-)}$ mice or male *Cyp2f2*$^{(-/-)}$ mice containing a human *CYP2F1*/*CYP2A13*/*CYP2B6* transgene (Cruzan et al., 2017). Cell proliferation (BrdU incorporation) was not altered in the terminal bronchioles of male *Cyp2f2*$^{(-/-)}$ mice given styrene at 400 mg/kg bw once per day for 5 days by gavage, although cell proliferation in the terminal bronchioles of male C57BL/6 wildtype control mice dosed under the same conditions increased 14.2-fold. Intraperitoneal injection of *R*-styrene-7,8-oxide or *S*-styrene-7,8-oxide at 200 mg/kg per day for 5 days in male and female *Cyp2f2*$^{(-/-)}$ mice and C57BL/6 wildtype control mice elicited similar responses as observed in studies where styrene was given by intraperitoneal injection. The observation that the substantial cell proliferation responses in each sex of wildtype mice treated with either enantiomer of styrene-7,8-oxide were not present in *Cyp2f2*$^{(-/-)}$ mice of the corresponding sex treated with the same agents indicates that styrene-7,8-oxide requires CYP2F2 to further metabolize it to a metabolite or to metabolites that induce cell proliferation (Cruzan et al., 2012).

Exposure of male B6C3F$_1$ mice to styrene by inhalation at 500 ppm for 6 hours per day produced large increases in hepatic cell proliferation (BrdU incorporation) after 1, 6, and 14 days of exposure (Mahler et al., 1999). In male or female CD rats exposed by inhalation to styrene at 500–1500 ppm for 6 hours per day, 5 days per week, no increase in cell proliferation was observed in hepatocytes in the liver or in alveolar and bronchiolar cells in the lungs after 2, 5, or 13 weeks of exposure (Cruzan et al., 1997). In male Sprague-Dawley CD rats exposed to styrene by inhalation at 500 ppm for 6 hours per day for up to 5 days, there was no increase in cell proliferation in any region of the lungs at any time point (Green et al., 2001b).

Cell proliferation was increased in the saccus caecus, midregion, and prefundic regions of the forestomach of male Fischer 344 rats given styene-7,8-oxide at 137, 275, and 550 mg/kg bw by gavage 3 times per week for 4 weeks. No dose–response was evident (Cantoreggi et al., 1993). Increases in cell proliferation (^{3}H-thymidine incorporation) in the forestomach of male F344 rats were detected after both a single dose of styrene-7,8-oxide at 800 mg/kg bw by gavage and after nine doses. Increases in dose-related cell proliferation were detected after a single dose of styrene-7,8-oxide at 50–250 mg/kg bw, with

the cell proliferation response plateauing at doses of less than 800 mg/kg bw (Dalbey et al., 1996). Some of these doses were used in previously reported cancer studies of styrene-7,8-oxide using the same route of administration.

Styrene, *R*-styrene-7,8-oxide, or *S*-styrene-7,8-oxide given to C57BL/6 control mice of both sexes at 200 mg/kg bw per day for 5 days by intraperitoneal injection increased cell proliferation in the terminal bronchioles (Cruzan et al., 2012, 2013). Comparable exposure did not increase cell proliferation in male and female *Cyp2f2*$^{(-/-)}$ mice (Cruzan et al., 2012) or in male and female *Cyp2f2*$^{(-/-)}$ mice containing a human *CYP2F1*/*CYP2A13*/*CYP2B6* transgene (verified for functional CYP2A13 and CYP2F1 activities by in vitro studies) (Cruzan et al., 2013). In male and female CD-1 mice given 4-vinylphenol at 60 mg/kg bw and 105 mg/kg bw per day for 5 days by intraperitoneal injection, a 3.8-fold to 7.6-fold (higher dose) dose-dependent increased cell proliferation in the terminal bronchioles was observed. The equivalent treatment of C57BL/6 wildtype mice yielded a 8.9-fold to 9.4-fold (higher dose) dose-dependent increased cell proliferation, but not for *Cyp2f2*$^{(-/-)}$ mice. Treatment with 4-vinylphenol at 105 mg/kg bw per day for 5 days by intraperitoneal injection increased cell proliferation in the terminal bronchioles of male and female *Cyp2f2*$^{(-/-)}$ mice containing a human *CYP2F1*/*CYP2A13*/*CYP2B6* transgene by 2.4- to 3.1-fold compared with transgenic mice used as vehicle controls, and in male and female C57BL/6 wildtype transgenic control mice by 4.8- to 6.6-fold compared with vehicle controls. [The Working Group noted that 4-vinylphenol, but not styrene or *R*- or *S*-styrene-7,8-oxide, induced cell proliferation in humanized transgenic mice.]

In 3-day studies of female Crl Icr: CD1 mice given styrene or styrene-7,8-oxide at 100 mg/kg bw per day by intraperitoneal injection, increased cell proliferation in the large and/or medium bronchi, terminal bronchioles, and alveoli was observed. Treatment with 4-vinylphenol at 20 mg/kg bw or 35 mg/kg bw per day increased cell proliferation in the large and/or medium bronchi and terminal bronchioles, whereas phenylacetaldehyde at 100 mg/kg bw per day decreased cell proliferation in the large and/or medium bronchi and terminal bronchioles and increased cell proliferation in the alveoli. Phenylacetic acid at 100 mg/kg bw per day decreased cell proliferation in terminal bronchioles and increased cell proliferation in the alveoli. At lower doses of 35 mg/kg bw per day, phenylacetaldehyde had no effect and phenylacetic acid decreased cell proliferation in the large and/or medium bronchi and terminal bronchioles. Treatment with 1-phenylethanol, 2-phenylethanol, or acetophenone at 100 mg/kg bw per day produced either no effects or marginal effects on cell proliferation. Styrene-7,8-oxide, phenylacetaldehyde, or phenylacetic acid at 100 mg/kg bw per day produced some increases in apoptosis that were not statistically significant (Kaufmann et al., 2005).

A single intraperitoneal injection of styrene at 600 mg/kg bw or *R*-styrene-7,8-oxide at 300 mg/kg bw significantly increased the ratio of bax/bcl-2 based on RNA and protein expression in Clara cells isolated from male CD-1 mice at various time points. Treatment with either racemic styrene-7,8-oxide, *S*-styrene-7,8-oxide, or 4-vinylphenol at 300 mg/kg bw was without effect. A small increase in caspase 3 activity was measured in Clara cells from male CD-1 mice exposed to *R*-styrene-7,8-oxide at 300 mg/kg bw by intraperitoneal injection, although no changes in caspase 8 activity was detected (Harvilchuck et al., 2009).

(ii) Styrene, styrene-7,8-oxide, and other styrene metabolites: in vitro

Styrene-7,8-oxide (0.8–1.0 mM) induced significant apoptosis, mediated by caspase 3 activation, in Norway rat adrenal pheochromocytoma PC12 cells in culture. Styrene-7,8-oxide also reduced Bcl-2 protein levels and increased

Bax protein levels, decreasing the Bcl-2/Bax ratio (Boccellino et al., 2003).

Styrene (1 nM to 1 mM) had no effect on C57BL/6 female mouse splenic T-lymphocyte or B-lymphocyte proliferation induced by the mitogens concanavalin A or lipopolysaccharide (LPS) (Poirier et al., 2002).

An increase in apoptosis as measured by caspase 3 activity was induced by styrene-7,8-oxide (75 µM) in C3H/An mouse fibroblast L929 cells in culture (Brockmann et al., 2006).

4.2.5 Receptor-mediated effects

Multiple studies in workers exposed to styrene have reported increases in serum prolactin levels. Serum prolactin levels were increased in 53 glass-reinforced plastics workers (33 men, $P < 0.01$; 20 women, $P < 0.05$) compared with 60 unexposed industrial workers comparable in age, sex, and smoking and drinking habits (Bergamaschi et al., 1996), as well as in 30 female glass-reinforced plastics workers compared with 30 age-matched female factory workers living in the same area, but not exposed to styrene or other industrial chemicals (Mutti et al., 1984). Serum prolactin levels were positively correlated with urinary styrene metabolite levels (MA and PGA) (Mutti et al., 1984). Plasma prolactin levels were increased in 46 male glass-reinforced plastics workers ($P < 0.001$) compared with 30 male blue-collar workers in local industries with no history of chemical exposures (Bergamaschi et al., 1997). In a study with repeated measurements of serum prolactin taken about 1 year apart over the course of 2–3 years in a cohort of glass-reinforced plastics workers from several different facilities (173 men, 33 women), a 2-fold increase in serum prolactin was associated with every 10-fold increase in blood styrene concentration (Luderer et al., 2004). Prolactin release from the anterior pituitary gland can be inhibited by dopamine secreted by the tuberoinfundibular dopaminergic system in the hypothalamus, although prolactin release can be stimulated by thyrotrophin-releasing hormone (TRH). A challenge dose of TRH was given to 16 female workers exposed to styrene and 16 sex- and age-matched controls; 15 of the 16 women exposed to styrene responded with abnormally high serum prolactin levels, compared with an abnormal serum prolactin response in only 1 of 16 controls (Arfini et al., 1987). When two of the women in the group exposed to styrene with abnormally high serum prolactin responses were removed from styrene exposure for 2 months, serum prolactin responses were within the normal range after TRH challenge (Arfini et al., 1987). [The Working Group noted that this is consistent with styrene inhibiting dopamine release by tuberoinfundibular dopaminergic system neurons, as suggested by Mutti et al. (1984).]

Workers exposed to styrene also had higher serum levels of growth hormone and lower levels of thyroid-stimulating hormone (TSH) than the unexposed controls, although no differences were seen for follicle-stimulating hormone or luteinizing hormone (Mutti et al., 1984). In 38 male glass-reinforced plastics workers exposed to styrene, no significant differences were observed in thyroid volume, serum levels of TSH, free thyroxine (FT4), or free triiodothyronine (FT3) compared with 123 unexposed male workers. Among workers exposed to styrene, urinary styrene metabolite levels were correlated positively with serum FT4 levels, as well as with the ratio of serum FT4 to serum FT3, although a negative correlation was observed between urinary styrene metabolite levels and serum FT3 levels; no correlation was observed between urinary styrene metabolite levels and serum TSH levels (Santini et al., 2008). Among workers exposed to styrene, increasing duration (years) of styrene exposure was correlated positively with thyroid volume; no correlation was observed between duration of styrene exposure and urinary styrene metabolite levels. [The Working Group noted the

small number of workers exposed to styrene in this study.]

No relevant data from human in vitro studies, or from experimental animal studies, were available to the Working Group.

4.2.6 Oxidative stress

Studies on oxidative damage to DNA are discussed in Section 4.2.3(a).

(a) Exposed humans

No additional studies were available to the Working Group.

(b) Human cells in vitro

(i) Styrene

Chakrabarti et al. (1993) incubated human whole-blood lymphocytes and isolated lymphocytes obtained from 10 healthy male non-smokers and non-consumers of alcohol with no recent exposure to radiation or pharmaceuticals with styrene (0–200 µM) for 72 hours. At non-cytotoxic concentrations of styrene, dose-dependent depletion of GSH (measured as total non-protein sulfhydryls) and concentration-dependent increases in malondialdehyde (MDA, measured as thiobarbituric acid-reactive substances), a marker of lipid peroxidation, occurred in both the whole-blood lymphocyte and isolated lymphocyte cultures.

In vitro exposure of human abdominal skin to styrene vapour for 8 hours decreased the concentration of GSH as well as GST, superoxide dismutase (SOD), and catalase activities in a dose-dependent manner, while increasing MDA and carbonyl compounds derived from protein peroxidation, markers of oxidative damage (Costa et al., 2006); as noted in Section 4.2.3(b)(i), this study also reported DNA damage (as measured by the comet assay).

In a series of studies with human A549 lung bronchioloalveolar epithelial carcinoma cells exposed to styrene vapour in a multicell chamber culture system, non-cytotoxic concentrations of styrene were found to increase concentrations of intracellular reactive oxygen species (ROS, as measured by 2,7′-dichlorofluorescein diacetate), induce several responses associated with oxidative stress, and activate the redox-sensitive transcription factors NF-κB and p38 MAPK (Röder-Stolinski et al., 2008; Mörbt et al., 2009; Mögel et al., 2011). Specific responses indicative of oxidative stress included an initial increase in GSH concentrations after 1 hour of exposure followed by depletion of GSH with longer exposure, and increases in *GSTP1* mRNA expression and GSTP1 and hemoxygenase-1 protein concentrations. These effects were abrogated in the presence of *N*-acetylcysteine (NAC). Additional responses indicative of oxidative stress included increases in concentrations of SOD1, biliverdin reductase A, DJ-1, Clic1, transaldolase 1 (TALDO1), 6-phosphogluconate dehydrogenase, COX2, prostaglandin E_2 (PGE_2), and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), and a decrease in peroxiredoxin 4 protein. NAC was shown to abrogate increased concentrations of COX2, PGE_2, and $PGF_{2\alpha}$ induced by styrene, although increasing NF-κB gene expression and NF-κB phosphorylation.

(ii) Styrene-7,8-oxide

Treatment of blood samples from nine healthy unexposed individuals with styrene-7,8-oxide reduced high-molecular-weight DNA fragments and increased low-molecular-weight DNA fragments in leukocytes from seven of the individuals (Marczynski et al., 2000). Reductions in leukocyte high-molecular-weight DNA fragments were also observed in these samples after treatment with hydrogen peroxide. [Although DNA fragmentation may result from oxidative DNA damage, the Working Group noted that increased DNA fragmentation is not a specific marker of oxidative stress.] In the study by Cemeli et al. (2009) (see Section 4.2.3(b)(ii)), the addition of catalase during treatment with

styrene-7,8-oxide did not significantly affect the level of DNA damage induced.

Treatment of human neuroblastoma 3K-H-MC cells with styrene-7,8-oxide increased lipid peroxidation (MDA, measured as thiobarbituric acid-reactive substances) after 12 hours, concomitant with an increase in mitochondrial dysfunction, measured as a decrease in mitochondrial Ca^{2+} capacity (Daré et al., 2004). The addition of the superoxide scavenger manganese(III) tetrakis(4-benzoic acid) porphyrin reduced the degree of mitochondrial dysfunction and cell death induced by styrene-7,8-oxide in these cells at 12, 14, and 16 hours; MDA levels were not assessed.

(c) Experimental systems

(i) Styrene: in vivo

Subchronic exposure to styrene via inhalation decreased the levels of reduced or total GSH (including all non-protein sulfhydryls) in rat liver, lung, and brain when evaluated within a few hours after the last exposure (reviewed in Vainio et al., 1979; Elovaara et al., 1990; Coccini et al., 1996; Coccini et al., 1997; NTP, 2008; NRC, 2014), similar to observations after acute exposures via intraperitoneal injection (Srivastava et al., 1983; Coccini et al., 1997); however, no effects on tissue GSH levels were reported when evaluated 24 hours or more after the last exposure (Katoh et al., 1989; Coccini et al., 1997). In terms of duration of effect, the concentrations of GSH in liver and lung of rats exposed to styrene decreased the most severely in the first 4 weeks of an 11-week exposure (Vainio et al., 1979). Decreased GSH has also been reported in fetal livers isolated from Wistar dams given styrene orally (Srivastava et al., 1992). In a detailed evaluation of the depletion of tissue GSH with time in male Wistar rats, liver concentrations of both GSH and oxidized glutathione (GSSG) decreased rapidly after exposure by repeated intraperitoneal injection with styrene, remained depleted until 12 hours after exposure, recovered to overshoot control levels after 24 hours, and finally decreased back to control levels after 40 hours (Katoh et al., 1989).

Although tissue GSH concentrations were affected in a time- and dose-dependent manner, lipid peroxidation (LPO) was not increased in the brain, liver, or lung from male Sprague-Dawley rats after subchronic inhalation exposure to styrene (Coccini et al., 1996, 1997). A dose-responsive increase in markers of oxidative stress (decreased ratio of ferric:ferrous iron, total thiol molecules, and increased protein carbonyls) was observed in the plasma and livers of male Wistar rats subchronically exposed via gavage, with increased concentrations of ROS and LPO products observed at sequentially higher doses (Niaz et al., 2017a, b). [The Working Group noted that all the identified studies evaluated ROS concentrations using dichlorofluorescein, and recognized the significant limitations of using this compound as a measure of oxidative stress (Rota et al., 1999; Bonini et al., 2006).] In a separate study, LPO concentrations were elevated in serum from both male and female Wistar rats subchronically exposed to styrene via oral gavage (El-Ziney et al., 2016). [The Working Group noted the 1000-fold disparity between the dose reported by El-Ziney et al. (2016) and other studies evaluating LPO levels in rats.] However, LPO was not elevated in the brain or lung of male Sprague-Dawley or Wistar rats after acute to subchronic exposure by intraperitoneal injection (Srivastava et al., 1983; Katoh et al., 1989; Coccini et al., 1997). Other studies reported LPO induction in the liver of male rats after styrene exposure via intraperitoneal injection in the absence of decreased concentrations of tissue GSH (Katoh et al., 1989; Hirasawa et al., 2007). [The Working Group noted the tissues in these studies were harvested more than 24 hours after the last dose, a time point at which tissue GSH concentrations would have normalized.] In male rat livers evaluated within 3 hours of the last

intraperitoneal dose, increased LPO concentrations were only observed after styrene exposures that decreased tissue GSH concentrations by more than 50% (Srivastava et al., 1983).

Consistent with this relationship between sufficient depletion of tissue GSH and increased measures of lipid oxidation, daily supplementation with NAC almost abrogated the ototoxicity observed after subchronic oral gavage exposure to styrene, as determined by histological evaluation of cochlear cells and hearing loss in Long-Evans rats (Yang et al., 2009). Hepatotoxicity induced by acute exposure could not be abrogated by NAC pre-treatment in C57BL/6 mice (Morgan et al., 1997), although GSH depletion induced by buthionine sulfoximine (BSO) greatly enhanced hepatotoxicity after a single oral dose in dYY mice (Mizutani et al., 1994) or intraperitoneal injection in non-Swiss albino (NSA) mice, but elicited a protective effect on pneumotoxicity (Gadberry et al., 1996).

Dose-responsive decreases in total GSH concentrations were also observed in the livers of mice repeatedly exposed to styrene via inhalation; the degree of GSH depletion was correlated with concentrations of styrene-7,8-oxide in blood, and differences with sex (greater correlation for females than males) and strain sensitivity (in order of greatest correlation, $B6C3F_1 \geq DBA/2 > Swiss$) have been reported (Morgan et al., 1993, 1995). GSH, GSSG, and/or total GSH concentrations were decreased in the lungs of female CD-1 mice after inhalation exposure (Gamer et al., 2004), as well as in the lungs, livers, and plasma of male CD-1 and NSA mice after acute exposure via intraperitoneal injection, with a time-course describing depletion, recovery, and overshoot in liver concentrations similar to that described at the beginning of this section in rats (Turner et al., 2005; Carlson et al., 2006; Carlson, 2010b). [The Working Group noted that the concentrations of GSH and GSSG followed a similar time-course of decrease and resurgence in rats and mice, indicating that this effect is primarily due to conjugation to styrene or metabolites, and not oxidation of GSH to GSSG.] Acute exposure to 4-vinylphenol by intraperitoneal injection also decreased GSH or total GSH concentrations in the liver and lung of male or female CD-1 mice, albeit to a lesser extent than styrene, and was followed by a more rapid recovery (Kaufmann et al., 2005; Turner et al., 2005). In club (Clara) cells isolated from male CD-1 mice exposed via intraperitoneal injection, 4-vinylphenol increased total GSH concentrations after 3 hours (unlike styrene), which rebounded in a manner similar to styrene after 12 hours (Harvilchuck & Carlson, 2006).

LPO levels fluctuated in the lungs of female CD-1 mice exposed via inhalation for up to 4 weeks (Gamer et al., 2004), and increased transiently in the livers but not lungs of male CD-1 mice exposed to styrene via intraperitoneal injection, but no effects were observed in either tissue after exposure to 4-vinylphenol (Carlson et al., 2006). Although production of ROS in lung homogenates of female CD-1 mice did not clearly increase after inhalation exposure for up to 4 weeks (Gamer et al., 2004), ROS concentrations increased in club cells isolated from male CD-1 mice exposed to styrene via intraperitoneal injection, but not for 4-vinylphenol exposure (Harvilchuck et al., 2009). Lung activity of cellular antioxidant enzymes such as SOD were generally unaffected (Gamer et al., 2004; Harvilchuck et al., 2009), although lung catalase activity decreased after 4 weeks of inhalation exposure (Gamer et al., 2004). Lung mRNA and concentrations of club cell secretory protein associated with inflammatory lung disease (CC10 or uteroglobin) fluctuated over time, although concentrations of another club cell product (surfactant protein A) were unaffected (Harvilchuck et al., 2008).

(ii) Styrene-7,8-oxide: in vivo

As for styrene, styrene-7,8-oxide (racemic mixture) given by repeated intraperitoneal injection rapidly decreased levels of both GSH and GSSG in male Wistar rat liver. GSH and GSSG remained depleted for up to 12 hours, recovered to overshoot control levels after 24 hours, and finally decreased back to control levels after 40 hours (Katoh et al., 1989). Styrene-7,8-oxide induced a greater effect than styrene on liver GSH and GSSG, in addition to depleting brain GSH (Katoh et al., 1989), and dramatically decreased GSH concentrations in the lungs and livers of male Sprague-Dawley rats when evaluated within a few hours of exposure via intraperitoneal injection (Coccini et al., 1997). LPO induction has been reported in the absence of decreased tissue GSH concentrations in the liver of male Wistar rats after subchronic exposure to styrene-7,8-oxide via intraperitoneal injection (Katoh et al., 1989). [The Working Group noted that these tissues were harvested more than 24 hours after the last dose, when tissue GSH concentrations would have normalized (see discussion for styrene in Section 4.2.6(c)(i) above).] No change in LPO levels were observed in the brain.

As summarized in the National Research Council review (National Research Council, 2014), racemic styrene-7,8-oxide and *R*-styrene-7,8-oxide decreased GSH or total GSH concentrations in the liver and lung of male and female CD-1 mice to a greater extent than styrene after acute exposure via intraperitoneal injection, but followed a more abbreviated time-course (i.e. recovery by 6–12 hours; Turner et al., 2005; Carlson et al., 2006). *R*-styrene-7,8-oxide but not *S*-styrene-7,8-oxide depleted total GSH from the bronchioloalveolar lavage fluid (BALF) and plasma in male NSA mice (Carlson, 2010b). Comparable results were reported in club cells isolated from CD-1 mice similarly exposed, although *S*-styrene-7,8-oxide treatment induced a greater recovery in total GSH concentrations after 12 hours compared with *R*-styrene-7,8-oxide (Harvilchuck & Carlson, 2006). No changes in LPO levels were observed in the livers or lungs of male CD-1 mice acutely exposed to *R*-styrene-7,8-oxide or *S*-styrene-7,8-oxide via intraperitoneal injection (Carlson et al., 2006). ROS production increased in club cells isolated from male CD-1 mice exposed to *R*-styrene-7,8-oxide, *S*-styrene-7,8-oxide, and racemic styrene-7,8-oxide via intraperitoneal injection when isolated within 3 hours of treatment (but not at later time-points), although SOD activity was only induced by *R*-styrene-7,8-oxide (Harvilchuck et al., 2009). Lung mRNA and concentrations of club cell secretory protein associated with inflammatory lung disease (CC10, or uteroglobin) fluctuated in a time-dependent manner after exposure to *R*-styrene-7,8-oxide and racemic styrene-7,8-oxide, but not *S*-styrene-7,8-oxide, although levels of surfactant protein A were unaffected by any isomer (Harvilchuck et al., 2008).

Although pre-treatment of male NSA mice with either NAC or GSH was unable to protect against most of the lung toxicity resulting from acute exposure to *R*-styrene-7,8-oxide via intraperitoneal injection, liver toxicity was significantly attenuated (Meszka-Jordan et al., 2009). BSO-induced GSH depletion elicited a protective effect on pneumotoxicity after a single intraperitoneal injection of styrene-7,8-oxide without any induction of hepatotoxicity in NSA mice (Gadberry et al., 1996); however, BSO pre-treatment potentiated hepatotoxicity after a single oral dose of styrene-7,8-oxide dose in dYY mice (Mizutani et al., 1994).

(iii) Styrene: in vitro

LPO decreased when exogenous GSH was added to rat liver homogenates treated with styrene (Srivastava et al., 1983). Increased production of ROS was observed in primary mouse club cells after treatment with styrene but not after treatment with 4-vinylphenol (Harvilchuck et

al., 2009), despite the greater depletion of total GSH after 4-vinylphenol exposure compared with styrene exposure (Harvilchuck & Carlson, 2006).

(iv) Styrene-7,8-oxide: in vitro

Murine embryonic fibroblasts (MEFs) from *Nrf2*$^{(-/-)}$ mice, which contained 20% of the GSH content and lower Gclc, Gclm, and GST subunit protein levels compared with MEFs from *Nrf2*$^{(+/+)}$ mice, were more sensitive to cytotoxicity induced by styrene-7,8-oxide compared with MEFs from *Nrf2*$^{(+/+)}$ mice (Higgins & Hayes, 2011). Furthermore, *Nrf2*$^{(-/-)}$ MEFs were not protected by pre-treatment with sulforaphane, which induced *Gcl*, *Gst*, and *Nqo1* mRNA expression, increased cellular GSH levels by more than 50% in *Nrf2*$^{(+/+)}$ MEFs, and attenuated cytotoxicity induced by styrene-7,8-oxide (Higgins & Hayes, 2011). As observed with styrene, increased levels of ROS were reported in primary mouse club cells after treatment with several styrene-7,8-oxide isoforms (*R*-, *S*-, and racemic styrene-7,8-oxide; Harvilchuck et al., 2009), although *S*-styrene-7,8-oxide was the least effective in decreasing total GSH (Harvilchuck & Carlson, 2006).

4.2.7 Immunosuppression

(a) Exposed humans

No studies on functional changes in the immune system of exposed humans were available to the Working Group.

Multiple studies of workers exposed to styrene have reported changes in peripheral blood leukocytes. [The Working Group noted that different panels of markers were evaluated across studies; three showed an increase in peripheral blood monocytes, but a fourth did not.] Hagmar et al. (1989) reported a 30% increase in the number of peripheral blood monocytes in 20 glass-reinforced plastics workers compared with controls. Increases in peripheral blood monocytes were observed in two other studies: a study of 221 glass-reinforced plastics workers by Stengel et al. (1990), in which the increase remained significant after adjusting for age, sex, tobacco use, and alcohol consumption; and a study of glass-reinforced plastics workers in a hand-lamination plant (Somorovská et al., 1999; Tulinská et al., 2000), in which the increase remained significant after adjusting for age, sex, and smoking status. In a study of 22 male glass-reinforced plastics workers and 27 healthy age-matched unexposed males, no difference in the percentage of peripheral blood CD14+ monocytes was observed; however, lower plasma levels of soluble human leukocyte antigen G (soluble HLA-G or sHLA-G), an anti-inflammatory substance secreted by CD14+ monocytes, and plasma interleukin (IL) 10, the primary inducer of sHLA-G production by monocytes, were present in workers exposed to styrene compared with unexposed controls (Rizzo et al., 2009). Reduced production of IL-10 and sHLA-G was also observed in peripheral blood monocytes isolated from workers exposed to styrene and stimulated with LPS compared with LPS-stimulated isolated monocytes from unexposed controls (Rizzo et al., 2009).

In the study by Mutti et al. (1992) of 32 glass-reinforced plastics workers exposed to styrene and 19 unexposed controls, a decrease in T helper (CD4+) lymphocytes and an increase in T CD8+ (suppressor or cytotoxic) lymphocytes were reported in workers exposed to styrene compared with controls, and a decreased CD4+:CD8+ ratio (0.92) was reported in workers exposed to higher concentrations of styrene (> 50 ppm; 8-hour TWA) compared with workers exposed to lower concentrations (1.37) and unexposed controls (1.43). In a group of 71 glass-reinforced plastics workers, decreases in total T (CD3+) lymphocytes, T helper (CD4+) lymphocytes, T (CD4+45+) cells with suppressor and/or inducer function, and the T helper (CD4+):T suppressor or cytotoxic (CD8+) lymphocyte ratio were observed compared with 65 blue-collar worker controls (Bergamaschi et al., 1995),

with dose-dependent decreases observed with increasing levels of urinary styrene metabolites.

Tulinská et al. (2000) observed a dose-dependent decrease in the percentage of large granular lymphocytes with increasing styrene exposure, measured as either blood styrene or exhaled styrene. In the study by Mutti et al. (1992), an increase in natural killer (NK) T-cells in workers exposed to styrene compared with controls, dose-dependent with increasing levels of urinary styrene metabolites, was observed. Similarly, Bergamaschi et al. (1995) observed a dose-dependent increase in NK T-cell phenotypes (CD56+, CD56+16+, CD56+16−) with increasing urinary styrene metabolites. Exposure to styrene was associated with reduced lytic activity of NK T-cells isolated from a subset of 14 workers compared with unexposed controls (Bergamaschi et al., 1995). Reduced NK cell lytic activity is a sign of immunosuppression.

An increase in B (CD19+) lymphocytes in workers exposed to styrene compared with controls was observed in the study by Bergamaschi et al. (1995), but not in the study by Mutti et al. (1992).

Bergamaschi et al. (1995) observed a dose-dependent increase in lymphocytes expressing the activation markers DR+ and CD25+ (the IL-2 receptor) with increasing urinary styrene metabolites in workers exposed to styrene. In the study of glass-reinforced plastics workers exposed to styrene in a hand-lamination plant, Somorovská et al. (1999) observed increased expression of several adhesion molecules, which are often associated with activation, on lymphocytes (CD54, CD49d, CD62L, CD18, and CD11b) and monocytes (CD54, CD49d, and CD11a) compared with controls. Further analyses of this study, focusing on workers exposed to higher concentrations of styrene, were reported by Tulinská et al. (2000) and Jahnová et al. (2002). Similar increases in adhesion molecule expression were evident in the lymphocytes (CD54, CD49d, CD62L, CD18, and CD11b), monocytes (CD54, CD49d, CD62L, CD18, CD11a, and CD11b), and granulocytes (CD54, CD49d, CD62L, CD11a, and CD11b) of the workers exposed to the highest concentrations (Jahnová et al., 2002). CD54, also known as intercellular adhesion molecule 1, is responsible for cell-to-cell contact and adhesion to endothelial cells and to fibrinogen, and is expressed at higher concentrations on activated cells. A decrease in soluble CD54 was observed in the workers exposed to the highest concentrations compared with controls. CD49d, or VLA-4, is a very late activation antigen that mediates the adhesion of lymphocytes, monocytes, and eosinophils, and is a ligand of vascular cell adhesion molecule-1 expressed on endothelial cells. The adhesion molecules CD62L and CD11b are known as L-selectin and the C3bi fragment of complement, respectively. Tulinská et al. (2000) found higher levels of both the C3 and the C4 components of complement, which are acute phase reactants involved in inflammatory responses, in workers exposed to styrene compared with controls. The levels of the C3 component were positively correlated with duration of styrene exposure. [The Working Group noted that expression of some of these activation markers is associated with immunosuppression or anti-inflammatory actions; others are associated with inflammation, and some have been associated with both immunosuppression and inflammation.]

As noted above (see Section 4.2.4(a)), in a small study of 18 glass-reinforced plastics workers and 6 unexposed controls, it was reported that the proliferation of cultured lymphocytes isolated from workers exposed to styrene was significantly reduced compared with controls (Watanabe et al., 1983). In the larger study by Somorovská et al. (1999), a dose-dependent decrease in the proliferative response of T lymphocytes stimulated by the mitogen concanavalin A was observed in cells isolated from workers exposed to styrene compared with those of unexposed controls.

Governa et al. (1994) reported that chemotaxis was impaired in polymorphonuclear leukocytes of workers exposed to styrene (n = 21), as measured ex vivo following a chemotactic stimulus, and that the chemotaxic indices of the workers improved after a 3-week period with no styrene exposure.

(b) Human cells in vitro

Governa et al. (1994) reported that in vitro styrene treatment of polymorphonuclear leukocytes isolated from healthy unexposed controls decreased chemotaxis in a dose-dependent manner following a chemotactic stimulus.

As noted in Section 4.2.4(b), styrene-7,8-oxide treatment of mitogen-stimulated lymphocytes cultured from healthy non-smokers decreased indices of cell proliferation (Laffon et al., 2001b).

(c) Experimental systems

(i) Styrene: in vivo

As reviewed in NRC (2014), styrene appears to suppress several components of innate immunity (e.g. decreased monocytic and NK cell activity) as well as stimulate some elements of adaptive immunity (e.g. enhancing cytokine production and delayed-type hypersensitivity; see Section 4.2.8(c)). Male UF rats subchronically exposed to styrene via oral gavage failed to effectively resolve subsequent infection by a hookworm parasite compared with controls who successfully resolved the infection, providing evidence of functional immunosuppression (Dogra et al., 1992). In male Sprague-Dawley rats, subchronic exposure via inhalation was associated with effects on bone marrow progenitor cells, including an accumulation of immature erythroblasts, along with decreases in more mature erythroblasts, promyelocytes, and myelocytes (reviewed in Nano et al., 2000; NTP, 2008). Alterations to bone marrow erythropoietic cell populations were also observed after repeated exposure via intraperitoneal injection (Nano et al., 2000). After subchronic inhalation exposure, peripheral blood neutrophil populations appeared immature and diminished in number, coinciding with lymphocyte numbers, before increasing after 3 weeks of recovery (Nano et al., 2000). In another study, acute exposure by inhalation decreased total leukocyte counts (Brondeau et al., 1990), consistent with transient leukocytopenia (NRC, 2014).

As for rats, the immune system of male Swiss mice was less effective in responding to infection; increased mortality from both viral and malarial parasite infection after subchronic exposure to styrene via oral gavage (Dogra et al., 1992), at doses associated with decreased spleen cellularity, was reported (reviewed in Dogra et al., 1989; NRC, 2014). Of note, peritoneal exudate macrophage cellular attachment (rosette formation) and phagocytosis was inhibited (Dogra et al., 1989). Subchronic inhalation exposure has also been reported to selectively suppress bone marrow haematopoiesis in female C57BL/6 and DBA/2 F1 hybrids; both early and late bone marrow erythroid progenitor populations decreased in a dose-responsive manner and, although pluripotent stem cell populations were unaffected, blood lymphocyte numbers also decreased (Seidel et al., 1990).

(ii) Styrene-7,8-oxide: in vivo

No in vivo data in experimental systems were available to the Working Group.

(iii) Styrene: in vitro

Inhibition of NK cell-mediated destruction of allogenic tumour cells was observed at 500 µM or more (Grayson & Gill, 1986). A few studies evaluated the effect of direct styrene addition to murine splenocyte suspensions ex vivo; no significant cytotoxicity, proliferation, or impact on phytomitogen-induced blast formation at doses of up to 1000 µM for 1–3 hours, or 250 µM for up to a few days, have been reported (Sharma

et al., 1981; Grayson & Gill, 1986; Poirier et al., 2002).

(iv) Styrene-7,8-oxide: in vitro

Treatment with styrene-7,8-oxide inhibited NK cell activity at lower doses than with styrene (e.g. ≥ 200 μM) with no effect on cytotoxic T lymphocyte activity, although the inhibition was transient and was abrogated by the addition of exogenous GSH (reviewed in Grayson & Gill, 1986; NRC, 2014). However, low concentrations of styrene-7,8-oxide (0.05 μM) decreased the production of type-I interferons by 90% in MEFs after inoculation with Newcastle disease virus (Barnes et al., 1981), consistent with studies reporting decreased clearing responses in rodent infection models after in vivo exposure to styrene. Low concentrations of styrene-7,8-oxide have also been reported to inhibit phytomitogen-induced blast formation in both rat (Snyder & Valle, 1991) and mouse splenocytes (Tomar et al., 1991), although another study reported similar inhibition only at high concentrations (500 μM; Grayson & Gill, 1986). Of note, two studies reported the stimulation of splenocyte blast formation at low micromolar concentrations (Sharma et al., 1981; Tomar et al., 1991), suggesting a biphasic response to treatment with styrene-7,8-oxide.

4.2.8 Chronic inflammation

(a) Exposed humans

As described in Section 4.2.7 on immunosuppression, studies of workers exposed to styrene have reported changes in immune cells that are consistent with a proinflammatory response, such as changes in the balance of peripheral blood leukocyte subsets (Hagmar et al., 1989; Stengel et al., 1990; Mutti et al., 1992; Bergamaschi et al., 1995; Somorovská et al., 1999; Tulinská et al., 2000), increased expression of activation markers on lymphocytes (Bergamaschi et al., 1995; Somorovská et al., 1999; Tulinská et al., 2000; Jahnová et al., 2002), lower serum levels of anti-inflammatory molecules such as soluble HLA-G and IL-10, and lower production of soluble HLA-G and IL-10 in LPS-stimulated monocytes isolated from workers exposed to styrene (Rizzo et al., 2009).

(b) Human cells in vitro

(i) Styrene

Increases in the release of the proinflammatory chemotactic monocyte chemoattractant protein 1, which activates monocytes, lymphocytes, mast cells, eosinophils, and basophils, and the proinflammatory molecules IL-6 and IL-8, were observed in human A549 lung bronchioloalveolar epithelial carcinoma cells exposed to non-cytotoxic concentrations of styrene vapour (Fischäder et al., 2008). As described in Section 4.2.6 on oxidative stress, other studies conducted in this in vitro lung cell model suggest that styrene is able to induce inflammation in the lung airways though a mechanism that involves the generation of ROS, oxidative stress, and activation of the NF-κB pathway (Röder-Stolinski et al., 2008; Mörbt et al., 2009; Mögel et al., 2011). Findings supportive of the inflammatory effect of styrene in A549 cells include increased phosphorylation of NF-κB associated with increased expression of monocyte chemoattractant protein 1 (Röder-Stolinski et al., 2008), upregulation of moesin and annexin A1 and downregulation of heat shock protein B1 (Mörbt et al., 2009), increased levels of COX-2 protein and activity, and increased release of PGE_2 and $PGF_{2\alpha}$ (Mögel et al., 2011).

(ii) Styrene-7,8-oxide

Treatment of polymorphonuclear leukocytes isolated from six individuals with either the *R*- or *S*-enantiomers of styrene-7,8-oxide or the racemic mixture stimulated the release of the TH1 cytokines interferon-γ (IFN-γ) and IL-12, which can lead to increased inflammation (Merker et al., 2006). Variability in the response

to styrene-7,8-oxide was observed between the six individuals.

(c) Experimental systems

(i) Styrene

As discussed in NRC (2014), styrene may affect the polarization or recruitment of leukocytes and thereby stimulate some elements of the adaptive immune system, including allergic sensitization and type-IV hypersensitivity. Subchronic exposure via inhalation increased total lung protein concentrations associated with leukocytic infiltration and airway obturation in guinea-pigs (Petrova et al., 1992). Exposure by subchronic oral gavage induced moderate parenchymal congestion and scattered islet degeneration in the pancreas in guinea-pigs, associated with a dose-responsive decrease in serum insulin levels, as well as moderate pancreatic inflammation in male albino mice; responses in male albino rats were limited (reviewed in Khanna et al., 1994; NTP, 2008). In an assay designed to evaluate acute inflammatory responses in the dermis of male Wistar rats after dermal exposure to a styrene solution (unoccluded), mast cell degranulation and microvascular leakage were increased, and determined to be partially operating via a neurogenic (e.g. tachykinin NK1 receptor) mechanism (Futamura et al., 2009). Severe liver degeneration and/or hepatocellular necrosis was observed in female $B6C3F_1$ mice after subchronic exposure to styrene via inhalation; although this hepatocellular injury resolved within 10 days of exposure, residual chronic inflammation remained. Liver pathology and residual inflammation were largely absent in male $B6C3F_1$ mice and in both sexes of Swiss mice (Morgan et al., 1995), consistent with liver GSH depletion sensitive to mouse sex and strain (described in Section 4.2.6(c)(i) above).

Styrene also exacerbated ovalbumin-induced allergic asthma in female BALB/c mice after acute inhalation exposure, augmenting BALF levels of the TH2 cytokines IL-4, IL-5, and IL-13, and increasing both ovalbumin-specific and total serum IgE levels, as well as BALF eosinophilia, lung inflammation, and goblet cell hyperplasia (reviewed in Ban et al., 2006; NRC, 2014). A modifying (or adjuvant) effect on the immune response was also observed on the plaque-forming cell (PFC) response of female BALB/c mouse lung-associated lymph node (LALN) cells and splenocytes ex vivo, after acute styrene exposure via inhalation and sensitization to sheep red blood cells in vivo. A dose-dependent increase in IFN-γ production was observed in splenocytes without any effect on PFC response, whereas LALN lymphocyte IFN-γ production followed an inverse dose–response (i.e. highest IFN-γ production at exposure to the lowest concentrations of styrene), and the LALN PFC response increased after exposure to styrene at the highest concentration (300 ppm; Ban et al., 2003). Together, this suggests that styrene exposure may differentially impact antigen-presenting cell and lymphocyte interactions at the portal of entry (LANL) versus systemically (spleen) (NRC, 2014). In male Swiss mice acutely exposed to styrene by oral gavage, the splenocyte PFC response was inhibited in a dose-responsive manner and the total serum Ig titre decreased (Dogra et al., 1989). Low doses of styrene stimulated male CD-1 or Swiss mouse basal splenocyte mitogenesis ex vivo after acute to subchronic exposure via oral gavage in vivo, and also increased the proliferation induced by a variety of phytomitogens; however, the highest dose evaluated (50 mg/kg per day) appeared to have a more variable effect, consistent with the inhibition of the PFC response noted above (reviewed in Sharma et al., 1981; Dogra et al., 1989; NRC, 2014). The severity of type-IV hypersensitivity was also enhanced, with a diffuse and marked infiltration of mononuclear cells, suggesting that cellular immunity was stimulated along with lymphocyte proliferation (Dogra et al., 1989).

No in vitro data in experimental systems on styrene were available to the Working Group.

(ii) Styrene-7,8-oxide

No in vivo or in vitro data in experimental systems were available to the Working Group.

4.3 Other adverse effects

4.3.1 Styrene

Respiratory effects in humans resulting from occupational exposure to styrene at concentrations of more than 20 ppm include chronic bronchitis, asthma, and pneumonia, and are associated with alterations in hepatic clearance of bilirubin and in serum alanine and aspartate transaminase activities, suggesting altered liver function in humans (reviewed in NTP, 2008). The effects of acute exposure in both humans and experimental systems include irritation of the eyes, skin, and respiratory tract; longer exposures in experimental systems are also associated with toxicity in the liver, kidney, and pancreas (NTP, 2008). Effects on the haematopoietic system in workers exposed to styrene have also been described (NTP, 2008), including an increase in mean corpuscular volume and a decrease in mean corpuscular haemoglobin concentrations, associated with increasing levels of urinary styrene metabolites (Stengel et al., 1990). Although neurological effects in humans have been reported at inhalation exposures of 100 ppm or more, specific effects on memory, colour vision, reaction time, and postural stability have consistently been reported as a result of long-term occupational exposures as low as 20 ppm, although the association between occupational styrene exposure and ototoxicity is still uncertain (for further discussion, see Lawton et al. (2006) and NTP (2008)). Notably, hearing loss and impaired learning have also been observed in rats (Hoet & Lison, 2008; Yang et al., 2009).

No studies have evaluated the long-term effects of styrene exposure on children, despite the potential for exposure during the early life stage via breast milk (ATSDR, 2010).

Pneumotoxicity in experimental systems was commonly evaluated by measuring BALF cellularity, protein levels, and lactate dehydrogenase activity, with histological tissue evaluation reported less frequently. In mice, pneumotoxicity involving the upper and lower respiratory tracts was reported after short-term to subchronic exposures via inhalation, oral gavage, and/or intraperitoneal injection at 50 ppm or more, with the club cell as the main site of bioactivation, toxicity, and proliferation in the lung (reviewed in NTP, 2008). Nasal toxicity (extending down to the trachea) has also been consistently observed in rats after subchronic exposures via inhalation (Cruzan et al., 1997; reviewed in NTP, 2008), although reports of diffuse pneumotoxicity involving the tracheal, bronchiolar, and/or alveolar epithelium have been less consistent (Coccini et al., 1997; Green et al., 2001b).

Hepatotoxicity in experimental systems was commonly evaluated by measuring serum sorbitol dehydrogenase activity, with histological tissue evaluation reported less frequently. In both mice and rats, short-term to subchronic exposure was also associated with hepatotoxicity including degeneration, hepatocellular necrosis, and steatosis, although liver toxicity was observed less frequently after subchronic or longer exposures (Mahler et al., 1999; reviewed in NTP, 2008; ATSDR, 2010). Acute inhalation or intraperitoneal injection of styrene induced hepatotoxicity preceding pneumotoxicity in NSA mice (Gadberry et al., 1996), and CD-1 mice were more resistant to these effects than NSA or C57BL/6 mice (Carlson, 1997b; Sumner et al., 1997). Hepatotoxicity was decreased in *Cyp2e1*$^{(-/-)}$ or *Cyp2f2*$^{(-/-)}$ mice exposed via a single intraperitoneal injection and, although pneumotoxicity was similar between the wild-type and knockout mice (Carlson, 2004), pneumotoxicity

was not observed in *Cyp2f2*$^{(-/-)}$ mice (Carlson, 2012). Of note, 4-vinylphenol causes pneumotoxicity in mice expressing *Cyp2f2* and in *Cyp2f2*$^{(-/-)}$ knockout mice expressing human *CYP2F1/CYP2B6/CYP2A13*, induces hepatotoxicity in both mice and rats, and has been reported as a more potent toxicant than either styrene or styrene-7,8-oxide in some studies (reviewed in NTP, 2008; Cruzan et al., 2013) or similarly potent in others (Carlson, 2011).

4.3.2 Styrene-7,8-oxide

Although fewer data on exposure to styrene-7,8-oxide are available, the effects reported are qualitatively similar to those observed after exposure to styrene. Chronic oral exposure via gavage increased the incidence of basal cell hyperplasia and/or hyperkeratosis in the forestomach of both sexes of mice and rats (Lijinsky, 1986; Conti et al., 1988).

Pneumotoxicity, involving both the upper and lower respiratory tract, as well as hepatotoxicity, has been observed in mice after acute exposure (Carlson, 2011). Although the *R*-isomer was observed to be more potent than the *S*-isomer in inducing hepatotoxicity, the *R*- and *S*-enantiomers generally elicited comparable pneumotoxicity in mice expressing *Cyp2f2* (reviewed in Gadberry et al., 1996; NTP, 2008; Cruzan et al., 2012). Hepatotoxicity and pneumotoxicity in C57BL/6 mice exposed to styrene-7,8-oxide were similar to that observed in *Cyp2e1*$^{(-/-)}$ or *Cyp2f2*$^{(-/-)}$ mice as described in the previous section for exposure to styrene (Carlson, 2004, 2012).

4.4 Data relevant to comparisons across agents and end-points

4.4.1 High-content gene expression studies

(a) Humans

No data in exposed humans were available to the Working Group.

In human TK6 lymphoblastoid cells in vitro, styrene (5 mM) in the presence of S9 and styrene-7,8-oxide (0.5 mM) without S9 resulted in 297 differentially expressed genes (DEGs) in common (no significant effects were observed at lower doses), involving gene ontology categories describing purine and pyrimidine transport, and protein complex assembly (Godderis et al., 2012). The addition of S9 fraction alone resulted in 885 DEGs (Godderis et al., 2012). The styrene concentrations investigated (≤ 5.0 mM) did not decrease the viability of human TK6 cells (Godderis et al., 2012), although 0.8 mM styrene was previously reported to induce a small but significant increase in necrotic cell death in human primary cord blood mononucleated cells (Diodovich et al., 2004; NTP, 2008).

An increase in the expression ratio of Bcl-2 family members (e.g. $BclX_{S/L}$) to Bax, as well as fluctuations in c-Myc, c-Fos, and/or c-Jun expression levels, was observed in human primary cord blood mononucleated cells after exposure to styrene at 0.8 mM (Diodovich et al., 2004), and in human HepG2 liver carcinoma cells after exposure to styrene-7,8-oxide at 0.2 mM, but not to styrene at 1 mM (Diodovich et al., 2006). Interestingly, TGFβ2 and/or TGFβR3 concentrations increased after exposure to styrene and styrene-7,8-oxide in HepG2 cells, but decreased in primary human hepatocytes. Of note, neither styrene nor styrene-7,8-oxide induced CYP1A2 or CYP2E1 expression in HepG2 cells (Diodovich et al., 2006).

(b) Experimental systems

(i) Styrene

Andersen et al. (2017) conducted gene expression studies on mice previously reported by Cruzan et al. (2017), exposed via inhalation to styrene for up to 26 weeks. After 1 day of exposure, DEGs were identified using whole-genome gene expression profiling in the lungs of male C57BL/6 (parental strain) mice exposed to styrene at 5 ppm or more; up to 155 DEGs were reported in *Cyp2f2*$^{(-/-)}$ mice or in *Cyp2f2*$^{(-/-)}$ mice containing a human *CYP2F1*/*CYP2A13*/*CYP2B6* transgene, consistent with no effect on lung histopathology in the knockout and transgenic mice. After 1–5 days of exposure, pathways induced in the lungs of the parental strain mice included cell cycle and mitotic regulation, DNA repair including the ATM/ATR pathway, circadian gene expression, and cholesterol and fatty acid biosynthesis; TGFβ signalling and various cytokine and immune response pathways were induced only after 1 day, although insulin and insulin-like growth factor receptors were downregulated. Few significantly affected DEGs persisted at 4 weeks, and no differences were seen at 26 weeks. As noted above in Section 4.2.4, proliferation (determined by Ki-67 and/or BrdU labelling indices) was transiently increased in the bronchiolar epithelium, whereas hyperplasia remained elevated in the terminal bronchioles after 26 weeks of exposure. Using information-dependent enrichment analysis rank-ordering (a less restrictive analysis than the combination of magnitude of effect and statistical false discovery rate, used to identify DEGs) after 4 weeks (but not 26 weeks), expression of cellular energetic pathways and circadian genes increased, although decreases were observed in peroxisome proliferator-activated receptor α gene expression, immune system, and heat stress pathways. Various transcription factors were up- or downregulated in the parental strain mice, and some dose–response trends in transcription factor expression were also observed in the knockout and transgenic mice. Andersen et al. (2017) reported 50–1000% more DEGs after the 1-day exposures performed in 2013 compared with those performed under similar exposure conditions in 2016; furthermore, the authors observed unusually large numbers of genes with sufficient magnitude of effect that failed to satisfy their statistical criteria. [The Working Group noted the high variability in gene expression levels across groups of similarly exposed mice. The Working Group further noted the questionable relevance of the study to the carcinogenic process because of the absence of styrene-induced lung tumorigenesis in parental C57BL/6 mice (Cruzan et al., 2017), a strain resistant to chemically induced lung tumorigenesis (Malkinson, 1989).]

In Sprague-Dawley rats orally exposed to styrene for 5 days, of the 120 genes evaluated in testes only 5 genes, including peroxiredoxin-1, were upregulated, although clusterin expression was decreased in a dose- and tissue-specific manner; clusterin expression was unchanged in heart, liver, lung, or kidneys (Han et al., 2007).

No data from in vitro studies in experimental systems were available to the Working Group.

(ii) Styrene-7,8-oxide

No data from studies on styrene-7,8-oxide exposure in experimental systems were available to the Working Group.

4.4.2 High-throughput screening studies

High-throughput screening data from the Toxicity Testing in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast) research programs of United States science agencies (Kavlock et al., 2012; Tice et al., 2013) were considered in the assessment of the chemicals in Volume 121, similarly to past *IARC Monographs*, as described by Chiu et al. (2018). To date, a systematic analysis of responses from over 9000 chemicals have been

evaluated as part of the ToxCast and Tox21 efforts, across a series of 1192 assay end-points measured using seven high-throughput assay technology platforms (Judson et al., 2016; EPA, 2017a). The resulting concentration–response models and bioactivity determinations have been publicly released via the Interactive Chemical Safety for Sustainability (iCSS) ToxCast Dashboard (EPA, 2017a). Summary matrix files, the ToxCast data analysis pipeline R package, and connected database (invitrodb_v2) are also available (EPA, 2017b; Filer et al., 2017). The ToxCast data analysis pipeline R package and associated database enables access to all of the concentration–response data, the underlying automated decision logic and methods, concentration–response model outputs, bioactivity determinations, and bioactivity caution flags (Filer et al., 2017). Although styrene was only evaluated in the Tox21 assays, styrene-7,8-oxide and quinoline were evaluated in both ToxCast and Tox21 assays. Among other styrene metabolites of interest, styrene glycol and 2-phenylethanol, but not 4-vinylphenol, were evaluated in Tox21 assays along with a limited subset of ToxCast assays.

One limitation of the high-throughput evaluation of these chemicals is that small-molecular-weight compounds (approximately < 150 Da), such as styrene, styrene-7,8-oxide, and quinoline, may have low affinity for biomolecular interactions because of limited free energy for binding (Hopkins et al., 2004). In vitro screening at the concentrations used in the ToxCast and Tox21 test batteries (generally ≤ 200 µM and ≤ 100 µM, respectively) may therefore be insufficient to detect molecular receptor-type interactions, which are commonly evaluated in these test systems. As a volatile organic compound, styrene has a fairly high vapour pressure (867 Pa at 25 °C; see Section 1) that could lead to the loss of sample during storage and/or testing and therefore failure to reach effective active concentrations. Other compounds considered in Volume 121 have lower vapour pressures (styrene-7,8-oxide, 40 Pa at 20 °C; quinolone, 11 Pa at 25 °C; see Section 1, Monograph 2). In addition, styrene may polymerize in solution, which would decrease the effective concentration of bioactive monomeric agent available; however, this can be prevented by the inclusion of a polymerization inhibitor (see Section 1). Solubility may limit the maximum testable dose and, of the Volume 121 compounds, quinoline is the most soluble in water (predicted value, 0.047 mol/L; EPA, 2017c), with styrene-7,8-oxide (0.025 mol/L) and styrene (0.0030 mol/L) less soluble (EPA, 2017c). In addition, compounds with a shorter half-life because of chemical or biological reactivity, such as styrene-7,8-oxide, may degrade substantially within the exposure window, increasing uncertainty when interpreting null or negative results. Finally, some of the in vitro assays either fully lacked (e.g. biochemical NovaScreen or NVS assays) or had uncharacterized xenobiotic metabolism capacity, which may have limited the evaluation of effects to those elicited by the compound itself.

The Tox21 and ToxCast in vitro assays were selected to cover a broad range of potential toxicity mechanisms and are not specifically focused on carcinogenesis; the Working Groups of *IARC Monographs* Volumes 112 and 113 therefore mapped the 821 assay end-points available at that time to the key characteristics of known human carcinogens (Smith et al., 2016), resulting in 265 assay end-points mapped to 6 out of the 10 key characteristics as described by Chiu et al. (2018). Considering only these 6 key characteristics, different assay sets were probed for each of the Volume 121 agents: the assay end-points evaluated and bioactivity determinations or "hit calls" relating to the 6 key characteristics for the compounds of interest are included as supplemental material to the present volume (Annex 1) and are summarized in Table 4.8. A brief description of the assay coverage pertaining specifically to all Volume 121 agents is provided below (for more discussion, see Chiu et al., 2018).

Table 4.8 Summary of evidence of key characteristics of carcinogens from high-throughput testing

Key characteristic	Styrene	Styrene-7,8-oxide	Styrene glycol	2-Phenylethanol	Quinoline
1. Is electrophilic or can be metabolically activated	0 out of 1 assay[a]	0 out of 11 assays	0 out of 2 assays	0 out of 2 assays	0 out of 11 assays
2. Is genotoxic	NA	NA	NA	NA	NA
3. Alters DNA repair or causes genomic instability	NA	NA	NA	NA	NA
4. Induces epigenetic alterations	NT	0 out of 10 assays	0 out of 4 assays	0 out of 4 assays	0 out of 10 assays
5. Induces oxidative stress	0 out of 3 assays	0 out of 16 assays	0 out of 7 assays	0 out of 7 assays	1[b] (0) out of 16 assays
6. Induces chronic inflammation	0 out of 1 assay	0 out of 45 assays	0 out of 2 assays	0 out of 2 assays	0 out of 45 assays
7. Is immunosuppressive	NA	NA	NA	NA	NA
8. Modulates receptor-mediated effects	1 out of 20 assays	4 out of 92 assays	0 out of 89 assays	1 out of 89 assays	1 out of 92 assays
9. Causes immortalization	NA	NA	NA	NA	NA
10. Alters cell proliferation/death or nutrient supply	1[b] (0) out of 19 assays	0 out of 62 assays	0 out of 25 assays	0 out of 25 assays	1[b] (0) out of 60 assays
Total number of assays mapped to key characteristics	44	236	129	129	234

NA, no assays in ToxCast and/or Tox21 were determined applicable to the evaluation of the indicated key characteristic; NT, not tested.

[a] Indicates the number of positive results out of the number of assays mapped to key characteristics of carcinogens, as described by Chiu et al. (2018).

[b] Indicates an active call in an assay (i.e. "hit") which was determined to be most likely a false-positive artefact upon review of the assay parameters and dose–response data by the Working Group [the number following in "()" reflects the true number of biological hits in the opinion of the Working Group].

[The Working Group noted that these assays are not considered to comprehensively cover the full spectrum of relevant biological activity for any of the key characteristics.]

1. *Is electrophilic or can be metabolically activated*: 10 cell-free CYP biochemical activity assays, and 1 assay end-point evaluating CYP19A1 activation in MCF-7 cells; no assay end-point for electrophilicity.
2. *Induces epigenetic alterations*: 11 primarily biochemical assays targeting histone deacetylases, sirtuins, and other enzymes modifying chromatin, as well as cellular transcription factor assays.
3. *Induces oxidative stress*: 17 cellular assays targeting nuclear factor-like 2 and/or the antioxidant responsive element, other stress-related transcription factors, and upregulation of matrix-metalloproteinases.
4. *Induces chronic inflammation*: 45 assays in co-cultured primary human cells evaluating expression of cellular adhesion molecules, cytokines, and NF-κB.
5. *Modulates receptor-mediated effects*: 92 nuclear receptor assays, for example for transactivation, receptor dimerization, and nuclear translocation, in cells or cell-free systems.
6. *Alters cell proliferation, cell death, or nutrient supply*: 65 assays evaluating various measures of cytotoxicity or proliferation in human cells and/or developing zebrafish larvae.

Brief summaries follow for each chemical evaluated in Volume 121, or identified metabolites evaluated in these technology platforms.

Styrene (Chem. Abstr. Serv. Reg. No. 100-42-5): Styrene was inactive in all but 2 of the 44 Tox21 assay end-points mapped to the key characteristics of carcinogens (styrene was not evaluated in the ToxCast assay battery). It displayed borderline activity in a single estrogen receptor (ER) α agonist assay (TOX21_ERa_LUC_BG1_Agonist) with a calculated concentration for half-maximal activity (AC50) of 149 μM; no other measures of ERα activity were evaluated. The only other detected activity was in a single cytotoxicity end-point (TOX21_P53_BLA_p2_viability), but all other 18 assays mapped to the same key characteristic were negative, including 4 others in similar assay conditions. [The Working Group considered this to be a false-positive response because activity was only detected at the lowest dose (0.001 μM), the calculated AC50 was orders of magnitude lower than this dose (i.e. 0.000 067 2 μM), and no bioactivity was reported in any other assay mapped to key characteristic 10.] The tested samples passed chemical quality control (QC) (purity, > 90%), although one sample had a caution indicating a lower-than-expected concentration (NIH, 2017).

Styrene-7,8-oxide (Chem. Abstr. Serv. Reg. No. 96-09-3): Styrene-7,8-oxide was inactive in all but 4 of the 236 ToxCast and Tox21 assay end-points mapped to the key characteristics of carcinogens. Styrene-7,8-oxide was active in an assay measuring aryl hydrocarbon receptor (AhR) activation in human cells (TOX21_AhR_LUC_Agonist) with an AC50 of 49.9 μM, and also induced transcription of PXR, a xenobiotic receptor that recognizes a diverse range of chemicals (ATG_PXRE_CIS_UP), with an AC50 of 33.2 μM, both mapped to key characteristic 8. However, styrene-7,8-oxide lacked bioactivity in a separate cell-based assay for AhR activity (ATG_Ahr_CIS_up) and in a biochemical receptor–ligand binding assay for PXR (NVS_NR_hPXR). Styrene-7,8-oxide also demonstrated activity in two other biochemical receptor–ligand binding assays, one for human progesterone (NVS_NR_hPR) and one for androgen receptor (AR) binding (NVS_NR_hAR), with AC50s of 3.44 μM and 8.36 μM, respectively; both also mapped to key characteristic 8. No activity was detected in cell-free assays with orthologous receptor isoforms (bovine progesterone receptor (PR), chimpanzee AR, or rat AR), or in numerous assays for AR

activity in human cells also mapped to key characteristic 8. Sample analysis was still under way. The QC grade was not determined; one sample had a caution indicating that the concentration may have been lower than expected, and another had a purity of 75–90% (NIH, 2017).

Styrene glycol (Chem. Abstr. Serv. Reg. No. 93-56-1): No bioactivity was found in the 129 tested Tox21 assays and subset of ToxCast assay end-points mapped to the key characteristics of carcinogens. The chemical QC information was not available for the Tox21 chemical library sample, as analysis was still under way (NIH, 2017).

2-Phenylethanol (Chem. Abstr. Serv. Reg. No. 60-12-8): 2-Phenylethanol was only active in 1 of the 129 tested Tox21 assays and subset of ToxCast assay end-points mapped to the key characteristics of carcinogens. A dose-responsive increase in the assay measuring hERβ-fragment protein-binding (OT_ER_ERbERb_1440) was observed, with an AC50 of 13.2 μM mapped to key characteristic 8. Although 2-phenylethanol lacked bioactivity in at least 10 other assays for ER binding or activity across various technology platforms, including both cell-based and biochemical receptor–ligand binding assays, the majority of these were selective for ERα versus ERβ. Although one sample had a purity of more than 90%, the chemical QC information was not available for the remaining three Tox21 chemical library samples as analysis was still under way (NIH, 2017).

Quinoline (Chem. Abstr. Serv. Reg. No. 91-22-5): Quinoline was inactive in all but 3 of the 234 ToxCast and Tox21 assay end-points mapped to the key characteristics of carcinogens. Quinoline was active in an assay measuring AhR activation (ATG_Ahr_CIS_up) with an AC50 of 42.8 μM mapped to key characteristic 8, but was not positive in a different cell-based assay for AhR activity from another technology platform (TOX21_AhR_LUC_Agonist). Activity was also detected in a cell-based assay end-point measuring changes in transcription of heat shock factor 1 (Tox21_HSE_BLA_agonist_ratio) with an AC50 of 75.6 μM and mapped to key characteristic 5, as well as an end-point following cellular adenosine triphosphate content as a measure of cytotoxicity (Tox21_VDR_BLA_Agonist_viability) with an AC50 of 67.8 μM and mapped to key characteristic 10. However, quinoline was not active in any other assay mapped to either key characteristic 5 or 10, despite significant assay coverage. [The Working Group considered it likely that these two responses were false positives because: (i) activity was only detected at the highest dose administered (~100 μM), which was qualitatively similar to that observed in the background assay performed (TOX21_HSE_BLA_agonist_ch2) for this assay platform; (ii) activity was quantitatively similar to that observed in the background assay, which had an AC50 of 72.9 μM; and (iii) there was an absence of activity in any other assay end-points mapped to key characteristics 5 or 10.] The chemical QC grade was not determined, but both samples had purities of more than 90% (NIH, 2017).

5. Summary of Data Reported

5.1 Exposure data

5.1.1 Styrene

Styrene is a colourless volatile liquid with an aromatic odour. It polymerizes easily in the presence of oxygen and oxidizes by air and light; in commercial styrene products a stabilizer, e.g. 4-*tert*-butylcatechol, may therefore be added. Styrene is a high production volume chemical, the production of which has increased in recent years (mainly in East Asia).

Styrene is primarily used as a monomer in the production of polystyrene polymers, including expandable polystyrene for packaging and building insulation, and copolymers, such

as styrene–butadiene rubber for the production of fibreglass-reinforced plastic products such as boats, industrial containers, and wind turbine blades.

The primary route of exposure in humans is inhalation. In the general population, exposure to styrene at a low concentration is widespread primarily because of its occurrence in tobacco smoke. Other sources include indoor and outdoor air pollution, and migration from styrene-based food packaging.

Occupational exposure to styrene occurs in the manufacture of fibreglass-reinforced plastic products, and in the production of styrene, polystyrene, and styrene-based plastics and rubbers. Occupational exposures are typically much higher than those measured in the general population.

Reliable and sensitive methods are available to measure styrene in environmental media and styrene biomarkers in exposed humans. The concentrations of styrene measured in air and the concentrations of styrene and its biomarkers in urine and blood are strongly correlated.

5.1.2 Styrene-7,8-oxide

Styrene-7,8-oxide is a clear liquid with a sweet odour. It is reactive and polymerizes easily. Styrene-7,8-oxide is industrially produced in many countries as a mixture of two optical isomers (enantiomers) by chemical oxidation of styrene. Styrene-7,8-oxide is used in epoxy resins and for the production of phenethyl alcohol and styrene glycol, which are intermediates in the production of many chemicals. It is formed in workplace air by the oxidation of styrene.

There are few data on human exposure to styrene-7,8-oxide. Occupational exposure has been documented in the reinforced plastics industry, where styrene-7,8-oxide co-occurs with styrene at concentrations that are typically 3 orders of magnitude lower than those of styrene.

Styrene-7,8-oxide and its albumin and haemoglobin adducts have been detected in the blood of occupationally exposed workers and of the general population.

5.2 Cancer in humans

There was a considerable body of research relating to cancers in humans available to the Working Group, mainly from cohort studies in the reinforced plastics industry (the industry with the highest exposures), the synthetic rubber industry, and the styrene monomer and polymer production industry, as well as from a series of population-based case–control studies. The stronger and more consistent evidence for cancer was found for leukaemias and, to a lesser extent, lymphomas in the reinforced plastics industry cohort studies. Only a small number of studies had sufficient power to analyse lymphoma and leukaemia subtypes; in these few studies, the stronger evidence was for AML and T-cell lymphoma, with less consistent results found for other leukaemia and lymphoma subtypes. However, effect estimates were often small with low precision (i.e., wide confidence intervals), and many different analyses were undertaken using several different exposure metrics, meaning that chance findings could have occurred. Although there was a strong signal in one large study for sinonasal adenocarcinoma, a rare cancer, this was based on only a few cases, and chance and confounding could not be discounted. Based on the internal analyses in the cohort studies and the findings from the case–control studies, there was no convincing evidence for an association with cancer of the lung. There was no convincing or consistent evidence reported for any other solid tumours in humans. Overall, the epidemiological studies provide some credible evidence that exposure to styrene causes lymphohaematopoietic malignancies in humans, but confounding, bias, or chance cannot be ruled out.

5.3 Cancer in experimental animals

5.3.1 *Styrene*

There were nine studies of carcinogenicity of styrene in mice: one study by gavage in males and females, five studies by inhalation (one in males and females, and four in males only), two studies of transplacental exposure followed by oral exposure by gavage in male and female pups, and one study by intraperitoneal injection in females.

In the study by gavage in B6C3F$_1$ mice, styrene significantly increased the incidence (with a significant positive trend) of bronchioloalveolar adenoma or carcinoma (combined) of the lung in males, and there was a significant positive trend in the incidence of hepatocellular adenoma in females. In one study of transplacental exposure followed by gavage in O20 mice, styrene significantly increased the incidence of lung carcinoma in female pups, and of lung adenoma or carcinoma (combined) in male and female pups. The other study by transplacental exposure followed by gavage in C57BL mice yielded negative results. Exposure to styrene significantly increased the incidence (with a significant positive trend) of bronchioloalveolar adenoma, and of bronchioloalveolar adenoma or carcinoma (combined) in male and female CD-1 mice in one study by inhalation; another result of this study was that exposure to styrene significantly increased the incidence (with a significant positive trend) of bronchioloalveolar carcinoma in females. Exposure to styrene also significantly increased the incidence of bronchioloalveolar carcinoma in male CD-1 mice in another study by inhalation. Three studies by inhalation, including two in genetically modified C57BL/6 mice, and the study by intraperitoneal injection all yielded negative results. Overall, exposure to styrene increased the incidence of lung tumours in the B6C3F$_1$, O20, and CD-1 strains of mice.

There were nine studies of the carcinogenicity of styrene in male and/or female rats.

In one lifetime inhalation study in male and female Sprague-Dawley rats exposed to styrene for 1 year, there was a significant increase (with a significant positive trend) in the incidences of malignant tumours of the mammary gland and of benign or malignant tumours (combined) of the mammary gland in females. In a 2-year inhalation study in male and female Sprague-Dawley rats, there was a significant dose-dependent decrease in the incidence of mammary gland adenocarcinoma in females. There was no significant increase in the incidence of any tumour type in three studies by gavage in males or females, in one study by transplacental exposure followed by gavage in male and female pups, in one study by drinking-water, in one study by intraperitoneal injection, and in one study by subcutaneous injection in males and females.

5.3.2 *Styrene-7,8-oxide*

There were three studies of the carcinogenicity of styrene-7,8-oxide in mice: one study by gavage in males and females, and two studies by skin application. In the study by gavage, styrene-7,8-oxide significantly increased the incidences (with a significant positive trend) of squamous cell papilloma, squamous cell carcinoma, and squamous cell papilloma or squamous cell carcinoma (combined) of the forestomach in males and females; exposure to styrene-7,8-oxide also significantly increased the incidence (with a significant positive trend) of hepatocellular adenoma or carcinoma (combined) in males. Both studies by skin application were inadequate for the evaluation.

There were three studies of the carcinogenicity of styrene-7,8-oxide in rats: two studies by gavage in males and females and one study by transplacental exposure followed by gavage in male and female pups. Exposure to styrene-7,8-oxide significantly increased the incidences

(with a significant positive trend) of squamous cell papilloma and squamous cell carcinoma of the forestomach in males and females in both studies by gavage, and of squamous cell papilloma or squamous cell carcinoma (combined) of the forestomach in males and females in one study by gavage; exposure to styrene-7,8-oxide also significantly increased the incidence (with a significant positive trend) of benign or malignant (combined) tumours of the mammary gland in males in one study by gavage. In the study by transplacental exposure followed by gavage in male and female pups, styrene-7,8-oxide caused a significantly increased incidence of forestomach papilloma in males and of forestomach carcinoma in males and females.

5.4 Mechanistic and other relevant data

In humans, styrene is absorbed after inhalation (the major route), skin contact, or ingestion, after which styrene is rapidly absorbed into the blood and has been shown to distribute to adipose tissue. In experimental animals, styrene is widely distributed to tissues. In both humans and experimental systems, styrene is metabolized mainly by CYP2E1, CYP2F, CYP2A13, and CYP2B to enantiomers of styrene-7,8-oxide, which are further metabolized by epoxide hydrolase to styrene glycol. Styrene, styrene-7,8-oxide, and styrene glycol have been measured in the blood of exposed humans. Approximately 60% of the excretion products formed from inhaled styrene come from styrene-7,8-oxide, the majority eliminated via urine as mandelic acid and phenylglyoxylic acid. The rates of metabolism of styrene to styrene-7,8-oxide were higher in microsomes from mouse lung compared with rat lung, and much higher compared with human lung. There are genetic polymorphisms in human cytochrome P450s, glutathione *S*-transferases, aldehyde dehydrogenase, and epoxide hydrolase that modulate excretion levels of metabolites.

Regarding the key characteristics of carcinogens, there is *strong* evidence that styrene is metabolically activated in animals and in exposed humans to an electrophile, styrene-7,8-oxide. Styrene-7,8-oxide is electrophilic and reacts directly with DNA to form adducts mainly at *N*7-guanine, followed by the N^2 and O^6 positions of guanine, as well as sites in adenine, cytosine, and thymine. In several rodent studies, styrene exposure by inhalation or intraperitoneal injection resulted in styrene-7,8-oxide–DNA adducts found in several tissues (e.g. liver and lung) and in mouse urine. In various human cells in vitro, DNA adduct formation was demonstrated after exposure to styrene or styrene-7,8-oxide. Several studies detected DNA adducts derived from styrene-7,8-oxide in the peripheral blood cells of workers exposed to styrene, at levels significantly higher than in unexposed controls.

Styrene-7,8-oxide reacts directly with globin and albumin to form various amino acid adducts. Adducts with valine and cysteine in globin, and with cysteine in albumin, were detected in some studies of workers exposed to styrene or to styrene and styrene-7,8-oxide.

There is *strong* evidence that both styrene and styrene-7,8-oxide are genotoxic, and this mechanism can also operate in humans. Styrene-7,8-oxide–DNA adducts are found in the blood and urine of workers exposed to styrene, although results of studies on other aspects of genotoxicity are mixed. In workers exposed to styrene, the majority (but not all) of the several available studies showed increased levels of DNA damage as measured by the comet assay. However, results were negative in studies using the comet assay to assess oxidative damage to DNA, studies measuring 8-hydroxy-2′-deoxyguanosine in DNA were inconsistent, and, in the few studies on gene mutation, no clear relationship was found with occupational exposure to styrene. Of the more than 30 studies available on chromosomal

end-points in blood cells in exposed humans, six studies of adequate size and design reported positive effects with good concordance among different indicators (in some cases, < 100 ppm). Several other studies with design limitations (e.g. small size) also reported positive results. A number of studies of adequate size and design did not report changes in chromosomal end-points. The remaining studies were less informative as a result of their small sample size or confounding co-exposures.

In human cells in vitro, styrene and styrene-7,8-oxide were consistently genotoxic. Cytogenetic effects, analysed by sister-chromatid exchange, chromosomal aberration, and micronucleus formation, mainly in whole-blood lymphocyte cultures, were consistently positive. Styrene-7,8-oxide induced *HPRT* gene mutations in human lymphocytes in two studies. Overall, results were negative or equivocal for cytogenetic effects in rodents exposed to styrene or styrene-7,8-oxide, although positive results for DNA damage (e.g. comet assay) were obtained in multiple tissues in several studies. In various non-human experimental systems (non-human mammalian cells in vitro, *Drosophila melanogaster*, yeast, bacteria, and plants), styrene or styrene-7,8-oxide was consistently positive across a variety of end-points (DNA damage, gene mutation, chromosomal aberration, micronucleus formation, and sister-chromatid exchange).

Inconsistent results from several studies investigating the possible influence of occupational exposure to styrene on DNA repair, or on the expression levels of DNA repair genes, mean that the evidence is *weak* that styrene alters DNA repair. DNA repair was measured by the comet assay in lymphocytes from exposed workers and from control individuals challenged ex vivo.

There is *strong* evidence that both styrene and styrene-7,8-oxide alter cell proliferation. There were a few studies in humans, in each of which styrene reduced cell proliferation in cultured lymphocytes in vivo and in vitro. Styrene-7,8-oxide induced cell proliferation in rat forestomach in two studies. In several studies, styrene induced cell proliferation in lung and liver in multiple strains of mice. In mouse lung, cell proliferation induced by styrene or styrene-7,8-oxide was dependent on the presence of CYP2F2. A mechanism has been proposed for the induction of mouse lung tumours that involves the metabolism of styrene to 4-vinylphenol by CYP2F2, cytotoxicity in club (Clara) cells, and regenerative epithelial proliferation in the terminal bronchioles. Cytotoxicity, lung cell proliferation, and bronchial hyperplasia were induced in CD-1 and C57BL/6 mouse strains; however, only the CD-1 mice developed lung tumours. Furthermore, lung cell proliferation did not persist beyond a short-term period, even with continuous exposure. Cytotoxicity, lung cell proliferation, bronchial hyperplasia, and lung tumour incidence were not increased in C57BL/6 *Cyp2f2*$^{(-/-)}$ mice, or in a humanized C57BL/6 strain, exposed to styrene. No in vivo metabolism data were available in the C57BL/6 mouse strains. The mechanistic events for mouse lung tumour induction by styrene in CD-1, B6C3F$_1$, and O20 mice are therefore not yet established.

There is *strong* evidence that styrene modulates receptor-mediated effects, and that these effects occur in humans, based on studies of increased serum prolactin. Exposure to styrene increased serum prolactin levels in four studies of workers in the reinforced plastics industry, including one study that made repeated measurements over the course of 2–3 years. For styrene-7,8-oxide, no data were available.

There is *moderate* evidence that styrene induces oxidative stress. No data other than on oxidative damage to DNA were available in exposed humans. In human cells in vitro, non-cytotoxic levels of styrene induced various measures of oxidative stress, including oxidation of lipids and proteins. Some responses were abrogated by *N*-acetylcysteine. In the lungs and livers of mice and rats, styrene increased lipid

peroxidation at concentrations that sufficiently depleted tissue glutathione levels, but results with *N*-acetylcysteine or buthionine sulfoximine pre-treatment were not supportive. For styrene-7,8-oxide, the available studies covered disparate end-points; the evidence that styrene-7,8-oxide induces oxidative stress is therefore *weak*.

There is *moderate* evidence that both styrene and styrene-7,8-oxide induce immunosuppression. Single studies of workers exposed to styrene reported the impairment of various measures of innate immune function ex vivo, in addition to the inhibition of lymphocyte proliferation. Several studies of workers exposed to styrene reported alterations in peripheral blood leukocyte populations. In several studies in rodents, each of which evaluated different end-points, subchronic exposure to styrene inhibited resolution of infection, and affected bone marrow progenitor cell populations, peripheral leukocyte populations, and/or splenic cellularity. For styrene-7,8-oxide, no in vivo data were available. In studies in human whole-blood cells conducted in vitro, and in mouse or rat lymphocytes, proliferation was inhibited. Murine natural killer cell lytic activity was decreased in a dose-responsive manner, and interferon response to viral infection was inhibited in murine embryonic fibroblasts.

There is *weak* evidence that styrene induces chronic inflammation. In multiple studies of workers exposed to styrene, alterations in immune cell populations consistent with pro-inflammatory responses were observed. In human lung carcinoma cells in vitro, non-cytotoxic concentrations of styrene induced a number of inflammatory responses. Inflammation was not consistently increased after long-term exposure in numerous studies in mice and rats. In several studies in mice, each of which evaluated different end-points, styrene stimulated different allergic or adaptive immune responses after short-term exposure. Data are sparse for styrene-7,8-oxide; the evidence that styrene-7,8-oxide induces chronic inflammation is *weak*.

Respiratory disease, haematological effects, altered liver function, and neurotoxicity have been reported in exposed workers. In rats and mice, styrene given by various exposure routes induced respiratory tract toxicity and hepatotoxicity. Although fewer data are available for styrene-7,8-oxide, the effects reported are similar; in addition, forestomach irritation was reported in rats after chronic oral exposure.

Results were largely null or negative in the Toxicity Forecaster and Toxicity Testing in the 21st Century high-throughput testing programmes of the United States government, and high-content gene expression studies were uninformative.

6. Evaluation

6.1 Cancer in humans

There is *limited evidence* in humans for the carcinogenicity of styrene. Positive associations have been observed between exposure to styrene and lymphohaematopoietic malignancies.

There is *inadequate evidence* in humans for the carcinogenicity of styrene-7,8-oxide.

6.2 Cancer in experimental animals

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

There is *sufficient evidence* in experimental animals for the carcinogenicity of styrene-7,8-oxide.

6.3 Overall evaluation

Styrene is *probably carcinogenic to humans (Group 2A)*.

Styrene-7,8-oxide is *probably carcinogenic to humans (Group 2A).*

6.4 Rationale

In making this overall evaluation, the Working Group noted that the mechanistic and other relevant data support the classification of styrene in Group 2A.

In making its overall evaluation, the Working Group took account of the mechanistic and other relevant data in classifying styrene-7,8-oxide (the major metabolite of styrene) in Group 2A. Styrene-7,8-oxide is an electrophile. There is strong evidence in human systems that it forms DNA adducts and is genotoxic. This mechanism can also operate in humans.

References

ACGIH (2017). TLVs and BEIs. Threshold limit values for chemical substances and physical agents and biological exposure indices. Cincinnati (OH), USA: American Conference of Governmental Industrial Hygienists.

Adgate JL, Eberly LE, Stroebel C, Pellizzari ED, Sexton K (2004). Personal, indoor, and outdoor VOC exposures in a probability sample of children. *J Expo Anal Environ Epidemiol*, 14(Suppl 1):S4–13. doi:10.1038/sj.jea.7500353 PMID:15118740

Aghayarzadeh M, Alizadeh R, Shafiei S (2014). Simulation and optimization of styrene monomer production using neural network. *Iran J Chem Eng*, 11(1):30–41.

Amacher DE, Turner GN (1982). Mutagenic evaluation of carcinogens and non-carcinogens in the L5178Y/TK assay utilizing postmitochondrial fractions (S9) from normal rat liver. *Mutat Res*, 97(1):49–65. doi:10.1016/0165-1161(82)90019-X PMID:7057798

Andersen ME, Cruzan G, Black MB, Pendse SN, Dodd D, Bus JS, et al. (2017). Assessing molecular initiating events (MIEs), key events (KEs) and modulating factors (MFs) for styrene responses in mouse lungs using whole genome gene expression profiling following 1-day and multi-week exposures. *Toxicol Appl Pharmacol*, 335:28–40. doi:10.1016/j.taap.2017.09.015 PMID:28951217

Andersson HC, Tranberg EA, Uggla AH, Zetterberg G (1980). Chromosomal aberrations and sister-chromatid exchanges in lymphocytes of men occupationally exposed to styrene in a plastic-boat factory. *Mutat Res*, 73(2):387–401. doi:10.1016/0027-5107(80)90203-1 PMID:7464847

Apostoli P, Alessandro G, Placidi D, Alessio L (1998). Metabolic interferences in subjects occupationally exposed to binary styrene-acetone mixtures. *Int Arch Occup Environ Health*, 71(7):445–52. doi:10.1007/s004200050304 PMID:9826076

Arfini G, Mutti A, Vescovi P, Ferroni C, Ferrari M, Giaroli C, et al. (1987). Impaired dopaminergic modulation of pituitary secretion in workers occupationally exposed to styrene: further evidence from PRL response to TRH stimulation. *J Occup Med*, 29(10):826–30. PMID:3119799

Arnedo-Pena A, Bellido-Blasco J, Villamarin-Vazquez JL, Aranda-Mares JL, Font-Cardona N, Gobba F, et al. (2003). Acute health effects after accidental exposure to styrene from drinking water in Spain. *Environ Health*, 2(1):6. doi:10.1186/1476-069X-2-6 PMID:12777181

Artuso M, Angotzi G, Bonassi S, Bonatti S, De Ferrari M, Gargano D, et al. (1995). Cytogenetic biomonitoring of styrene-exposed plastic boat builders. *Arch Environ Contam Toxicol*, 29(2):270–4. doi:10.1007/BF00212980 PMID:7661632

Association of Oregon Recyclers (2017). Agilyx to develop styrene monomer production plant in Oregon. Available from: http://oregonrecyclers.org/blog/agilyx-develop-styrene-monomer-production-plant-oregon, accessed 26 February 2018.

ATSDR (2010). Toxicological profile for styrene. Atlanta (GA), USA: Agency for Toxic Substances and Disease Registry, Division of Toxicology and Human Health Sciences. Available from: http://www.atsdr.cdc.gov/toxprofiles/tp53.pdf, accessed 1 March 2018.

Austin CC, Wang D, Ecobichon DJ, Dussault G (2001). Characterization of volatile organic compounds in smoke at municipal structural fires. *J Toxicol Environ Health A*, 63(6):437–58. doi:10.1080/152873901300343470 PMID:11482799

Bajpai U, Mukherjee A (2017). A project report on design a plant to manufacture styrene oxide. Available from: https://www.slideshare.net/ARITRAMUKHERJEE/styrene-oxide-a-brief-reiew, accessed 26 February 2018.

Bakke OM, Scheline RR (1970). Hydroxylation of aromatic hydrocarbons in the rat. *Toxicol Appl Pharmacol*, 16(3):691–700. doi:10.1016/0041-008X(70)90074-8 PMID:5422210

Ban M, Hettich D, Bonnet P (2003). Effect of inhaled industrial chemicals on systemic and local immune response. *Toxicology*, 184(1):41–50. doi:10.1016/S0300-483X(02)00553-X PMID:12505375

Ban M, Langonné I, Huguet N, Pépin E, Morel G (2006). Inhaled chemicals may enhance allergic airway inflammation in ovalbumin-sensitised mice. *Toxicology*, 226(2–3):161–71. doi:10.1016/j.tox.2006.06.012 PMID:16879906

Barlow T, Ding J, Vouros P, Dipple A (1997). Investigation of hydrolytic deamination of 1-(2-hydroxy-1-phenylethyl)adenosine. *Chem Res Toxicol*, 10(11):1247–9. doi:10.1021/tx970091m PMID:9403177

Barlow T, Dipple A (1998). Aralkylation of guanosine with para-substituted styrene oxides. *Chem Res Toxicol*, 11(1):44–53. doi:10.1021/tx970126i PMID:9477225

Barlow T, Dipple A (1999). Formation of deaminated products in styrene oxide reactions with deoxycytidine. *Chem Res Toxicol*, 12(10):883–6. doi:10.1021/tx990070n PMID:10525262

Barnes MC, Streips UN, Sonnenfeld G (1981). Effect of carcinogens and analogs on interferon induction. *Oncology*, 38(2):98–101. doi:10.1159/000225530 PMID:6162141

Basile A, Ferranti P, Mamone G, Manco I, Pocsfalvi G, Malorni A, et al. (2002). Structural analysis of styrene oxide/haemoglobin adducts by mass spectrometry: identification of suitable biomarkers for human exposure evaluation. *Rapid Commun Mass Spectrom*, 16(9):871–8. doi:10.1002/rcm.655 PMID:11948819

Bastlová T, Podlutsky A (1996). Molecular analysis of styrene oxide-induced *hprt* mutation in human T-lymphocytes. *Mutagenesis*, 11(6):581–91. doi:10.1093/mutage/11.6.581 PMID:8962428

Bastlová T, Vodička P, Peterková K, Hemminki K, Lambert B (1995). Styrene oxide-induced HPRT mutations, DNA adducts and DNA strand breaks in cultured human lymphocytes. *Carcinogenesis*, 16(10):2357–62. doi:10.1093/carcin/16.10.2357 PMID:7586135

Bausinger J, Speit G (2014). Induction and repair of DNA damage measured by the comet assay in human T lymphocytes separated by immunomagnetic cell sorting. *Mutat Res*, 769:42–8. doi:10.1016/j.mrfmmm.2014.07.005 PMID:25771724

Behr A (2017). Styrene production from ethylbenzene. Dortmund, Germany: Faculty of Biochemical and Chemical Engineering. Dortmund University. Available from: http://www.tc.bci.tu-dortmund.de/Downloads/Praktika/tc30_styrene_english.pdf, accessed 26 February 2018.

Beije B, Jenssen D (1982). Investigation of styrene in the liver perfusion/cell culture system. No indication of styrene-7,8-oxide as the principal mutagenic metabolite produced by the intact rat liver. *Chem Biol Interact*, 39(1):57–76. doi:10.1016/0009-2797(82)90006-0 PMID:7060221

Belanger PL, Elesh E (1980). Health hazard evaluation determination: Bell Helmets, Inc., Norwalk, CA. Cincinnati (OH), USA: National Institute for Occupational Safety and Health.

Beliles RP, Butala JH, Stack CR, Makris S (1985). Chronic toxicity and three-generation reproduction study of styrene monomer in the drinking water of rats. *Fundam Appl Toxicol*, 5(5):855–68. doi:10.1016/0272-0590(85)90168-X PMID:4065461

Bellander T, Kogevinas M, Pannett B, Bjerk JE, Breum NO, Ferro S, et al. (1994). Part II: Historical exposure to styrene in the manufacture of glass-reinforced polyester products in six European countries. In: Kogevinas M, Ferro G, Saracci R, Andersen A, Bellander T, Biocca M, et al., editors. IARC Historical Multicentric Cohort Study of Workers Exposed to Styrene. Report of the Epidemiological Study and the Industrial Hygiene Investigation (IARC Internal Technical Report 94/002). Lyon, France: International Agency for Research on Cancer; pp. 63–127.

Bergamaschi E, Mutti A, Cavazzini S, Vettori MV, Renzulli FS, Franchini I (1996). Peripheral markers of neurochemical effects among styrene-exposed workers. *Neurotoxicology*, 17(3–4):753–9. PMID:9086498

Bergamaschi E, Smargiassi A, Mutti A, Cavazzini S, Vettori MV, Alinovi R, et al. (1997). Peripheral markers of catecholaminergic dysfunction and symptoms of neurotoxicity among styrene-exposed workers. *Int Arch Occup Environ Health*, 69(3):209–14. doi:10.1007/s004200050138 PMID:9049672

Bergamaschi E, Smargiassi A, Mutti A, Franchini I, Lucchini R (1995). Immunological changes among workers occupationally exposed to styrene. *Int Arch Occup Environ Health*, 67(3):165–71. PMID:7591174

Bernardini S, Hirvonen A, Järventaus H, Norppa H (2002). Influence of GSTM1 and GSTT1 genotypes on sister chromatid exchange induction by styrene in cultured human lymphocytes. *Carcinogenesis*, 23(5):893–7. doi:10.1093/carcin/23.5.893 PMID:12016165

Berode M, Droz PO, Guillemin M (1985). Human exposure to styrene. VI. Percutaneous absorption in human volunteers. *Int Arch Occup Environ Health*, 55(4):331–6. doi:10.1007/BF00377691 PMID:4008057

Bertke SJ, Yiin JH, Daniels RD (2018). Cancer mortality update with an exposure response analysis among styrene-exposed workers in the reinforced plastics boatbuilding industry. *Am J Ind Med*, 61(7):566–71. doi:10.1002/ajim.22853 PMID:29638005

Bidoli F, Airoldi L, Pantarotto C (1980). Quantitative determination of styrene-7,8-oxide in blood by combined gas chromatography-multiple ion detection mass fragmentography. *J Chromatogr A*, 196(2):314–8. doi:10.1016/S0021-9673(00)80451-3 PMID:7410515

Bigbee WL, Grant SG, Langlois RG, Jensen RH, Anttila A, Pfäffli P, et al. (1996). Glycophorin A somatic cell mutation frequencies in Finnish reinforced plastics workers exposed to styrene. *Cancer Epidemiol Biomarkers Prev*, 5(10):801–10. PMID:8896891

Billionnet C, Gay E, Kirchner S, Leynaert B, Annesi-Maesano I (2011). Quantitative assessments of indoor air pollution and respiratory health in a population-based sample of French dwellings. *Environ Res*, 111(3):425–34. doi:10.1016/j.envres.2011.02.008 PMID:21397225

Bjørge C, Brunborg G, Wiger R, Holme JA, Scholz T, Dybing E, et al. (1996). A comparative study of chemically induced DNA damage in isolated human and rat testicular cells. *Reprod Toxicol*, 10(6):509–19. doi:10.1016/S0890-6238(96)00138-4 PMID:8946565

Blount BC, McElprang DO, Chambers DM, Waterhouse MG, Squibb KS, Lakind JS (2010). Methodology for collecting, storing, and analyzing human milk for volatile organic compounds. *J Environ Monit*, 12(6):1265–73. doi:10.1039/b927022a PMID:20358052

Boccellino M, Cuccovillo F, Napolitano M, Sannolo N, Balestrieri C, Acampora A, et al. (2003). Styrene-7,8-oxide activates a complex apoptotic response in neuronal PC12 cell line. *Carcinogenesis*, 24(3):535–40. doi:10.1093/carcin/24.3.535 PMID:12663515

Boffetta P, Adami HO, Cole P, Trichopoulos D, Mandel JS (2009). Epidemiologic studies of styrene and cancer: a review of the literature. *J Occup Environ Med*, 51(11):1275–87. doi:10.1097/JOM.0b013e3181ad49b2 PMID:19858749

Boffetta P, Sali D, Kolstad H, Coggon D, Olsen J, Andersen A, et al. (1998). Mortality of short-term workers in two international cohorts. *J Occup Environ Med*, 40(12):1120–6. doi:10.1097/00043764-199812000-00012 PMID:9871889

Bonanni RC, Gatto MP, Paci E, Gordiani A, Gherardi M, Tranfo G (2015). Biomonitoring for exposure assessment to styrene in the fibreglass reinforced plastic industry: determinants and interferents. *Ann Occup Hyg*, 59(8):1000–11. doi:10.1093/annhyg/mev047 PMID:26180262

Bond GG, Bodner KM, Olsen GW, Cook RR (1992). Mortality among workers engaged in the development or manufacture of styrene-based products – an update. *Scand J Work Environ Health*, 18(3):145–54. doi:10.5271/sjweh.1594 PMID:1615288

Bond JA, Bolt HM (1989). Review of the toxicology of styrene. *Crit Rev Toxicol*, 19(3):227–49. doi:10.3109/10408448909037472 PMID:2653733

Bonini MG, Rota C, Tomasi A, Mason RP (2006). The oxidation of 2′,7′-dichlorofluorescin to reactive oxygen species: a self-fulfilling prophesy? *Free Radic Biol Med*, 40(6):968–75. doi:10.1016/j.freeradbiomed.2005.10.042 PMID:16540392

Boogaard PJ, de Kloe KP, Sumner SC, van Elburg PA, Wong BA (2000a). Disposition of [Ring-U-^{14}C]styrene in rats and mice exposed by recirculating nose-only inhalation. *Toxicol Sci*, 58(1):161–72. doi:10.1093/toxsci/58.1.161 PMID:11053553

Boogaard PJ, de Kloe KP, Wong BA, Sumner SC, Watson WP, van Sittert NJ (2000b). Quantification of DNA adducts formed in liver, lungs, and isolated lung cells of rats and mice exposed to ^{14}C-styrene by nose-only inhalation. *Toxicol Sci*, 57(2):203–16. doi:10.1093/toxsci/57.2.203 PMID:11006351

Bowman JD, Held JL, Factor DR (1990). A field evaluation of mandelic acid in urine as a compliance monitor for styrene exposure. *Appl Occup Environ Hyg*, 5:526–35.

Boyd DP, Bodner KM, Bond GC, de la Cruz PL, Miller RR, Nolan RJ (1990). Styrene: perspectives on the carcinogen question. *SIRC Rev*, 1(1):9–23.

Brams A, Buchet JP, Crutzen-Fayt MC, De Meester C, Lauwerys R, Léonard A (1987). A comparative study, with 40 chemicals, of the efficiency of the *Salmonella* assay and the SOS chromotest (kit procedure). *Toxicol Lett*, 38(1–2):123–33. doi:10.1016/0378-4274(87)90120-2 PMID:3307023

Brenner DD, Jeffrey AM, Latriano L, Wazneh L, Warburton D, Toor M, et al. (1991). Biomarkers in styrene-exposed boatbuilders. *Mutat Res*, 261(3):225–36. doi:10.1016/0165-1218(91)90071-S PMID:1719415

Brockmann WG, Kostoryz EL, Eick JD (2006). Correlation of apoptotic potential of simple oxiranes with cytotoxicity. *Toxicol In Vitro*, 20(5):729–35. doi:10.1016/j.tiv.2005.10.009 PMID:16311010

Brondeau MT, Bonnet P, Guenier JP, Simon P, de Ceaurriz J (1990). Adrenal-dependent leucopenia after short-term exposure to various airborne irritants in rats. *J Appl Toxicol*, 10(2):83–6. doi:10.1002/jat.2550100204 PMID:2362083

Brooks SM, Anderson L, Emmett E, Carson A, Tsay JY, Elia V, et al. (1980). The effects of protective equipment on styrene exposure in workers in the reinforced plastics industry. *Arch Environ Health*, 35(5):287–94. doi:10.1080/00039896.1980.10667507 PMID:7447499

Brugnone F, Perbellini L, Wang GZ, Maranelli G, Raineri E, De Rosa E, et al. (1993). Blood styrene concentrations in a "normal" population and in exposed workers 16 hours after the end of the workshift. *Int Arch Occup Environ Health*, 65(2):125–30. doi:10.1007/BF00405731 PMID:8253510

Brunnemann KD, Rivenson A, Cheng SC, Saa V, Hoffmann D (1992). A study of tobacco carcinogenesis. XLVII. Bioassays of vinylpyridines for genotoxicity and for tumorigenicity in A/J mice. *Cancer Lett*, 65(2):107–13. doi:10.1016/0304-3835(92)90153-M PMID:1511414

Burroughs GE (1979). Health hazard evaluation determination: Piper Aircraft Corporation, Vero Beach, FL. Cincinnati (OH), USA: National Institute for Occupational Safety and Health.

Buschini A, De Palma G, Poli P, Martino A, Rossi C, Mozzoni P, et al. (2003). Genetic polymorphism of drug-metabolizing enzymes and styrene-induced DNA damage. *Environ Mol Mutagen*, 41(4):243–52. doi:10.1002/em.10150 PMID:12717779

Busk L (1979). Mutagenic effects of styrene and styrene oxide. *Mutat Res*, 67(3):291–8. PMID:384233

Byfält Nordqvist M, Löf A, Osterman-Golkar S, Walles SAS (1985). Covalent binding of styrene and styrene-7,8-oxide to plasma proteins, hemoglobin and DNA

in the mouse. *Chem Biol Interact*, 55(1–2):63–73. doi:10.1016/S0009-2797(85)80120-4 PMID:4064194

Camurri L, Codeluppi S, Pedroni C, Scarduelli L (1983). Chromosomal aberrations and sister-chromatid exchanges in workers exposed to styrene. *Mutat Res*, 119(3):361–9. doi:10.1016/0165-7992(83)90186-0 PMID:6828071

Cantor KP, Stewart PA, Brinton LA, Dosemeci M (1995). Occupational exposures and female breast cancer mortality in the United States. *J Occup Environ Med*, 37(3):336–48. doi:10.1097/00043764-199503000-00011 PMID:7796202

Cantoreggi S, Dietrich DR, Lutz WK (1993). Induction of cell proliferation in the forestomach of F344 rats following subchronic administration of styrene 7,8-oxide and butylated hydroxyanisole. *Cancer Res*, 53(15):3505–8. PMID:8339254

Cantoreggi S, Lutz WK (1992). Investigation of the covalent binding of styrene-7,8-oxide to DNA in rat and mouse. *Carcinogenesis*, 13(2):193–7. doi:10.1093/carcin/13.2.193 PMID:1740009

Cantoreggi S, Lutz WK (1993). Covalent binding of styrene to DNA in rat and mouse. *Carcinogenesis*, 14(3):355–60. doi:10.1093/carcin/14.3.355 PMID:8453710

Caperos JR, Humbert B, Droz PO (1979). Styrene exposure. II. Percentage studies of absorption, excretion, and metabolism by human subjects. *Int Arch Occup Environ Health*, 42(3–4):223–30. doi:10.1007/BF00377776 PMID:422263 [in French]

Carbonari D, Mansi A, Proietto AR, Paci E, Bonanni RC, Gherardi M, et al. (2015). Influence of genetic polymorphisms of styrene-metabolizing enzymes on the levels of urinary biomarkers of styrene exposure. *Toxicol Lett*, 233(2):156–62. doi:10.1016/j.toxlet.2015.01.002 PMID:25562543

Carlson G (2003). In vitro metabolism of styrene to styrene oxide in liver and lung of Cyp2E1 knockout mice. *J Toxicol Environ Health A*, 66(9):861–9. doi:10.1080/15287390306386 PMID:12746132

Carlson GP (1997a). Effects of inducers and inhibitors on the microsomal metabolism of styrene to styrene oxide in mice. *J Toxicol Environ Health*, 51(5):477–88. doi:10.1080/00984109708984038 PMID:9233381

Carlson GP (1997b). Comparison of mouse strains for susceptibility to styrene-induced hepatotoxicity and pneumotoxicity. *J Toxicol Environ Health*, 51(2):177–87. doi:10.1080/00984109708984020 PMID:9176557

Carlson GP (2004). Comparison of the susceptibility of wild-type and CYP2E1 knockout mice to the hepatotoxic and pneumotoxic effects of styrene and styrene oxide. *Toxicol Lett*, 150(3):335–9. doi:10.1016/j.toxlet.2004.02.004 PMID:15110085

Carlson GP (2008). Critical appraisal of the expression of cytochrome P450 enzymes in human lung and evaluation of the possibility that such expression provides evidence of potential styrene tumorigenicity in humans. *Toxicology*, 254(1–2):1–10. doi:10.1016/j.tox.2008.09.017 PMID:18951947

Carlson GP (2010a). Metabolism and toxicity of styrene in microsomal epoxide hydrolase-deficient mice. *J Toxicol Environ Health A*, 73(24):1689–99. doi:10.1080/15287394.2010.516240 PMID:21058172

Carlson GP (2010b). Depletion by styrene of glutathione in plasma and bronchioalveolar lavage fluid of non-Swiss albino (NSA) mice. *J Toxicol Environ Health A*, 73(11):766–72. doi:10.1080/15287391003689143 PMID:20391119

Carlson GP (2011). Hepatotoxicity and pneumotoxicity of styrene and its metabolites in glutathione *S*-transferase-deficient mice. *Drug Chem Toxicol*, 34(4):440–4. doi:10.3109/01480545.2011.562899 PMID:21749217

Carlson GP (2012). Modification of the metabolism and toxicity of styrene and styrene oxide in hepatic cytochrome P450 reductase deficient mice and CYP2F2 deficient mice. *Toxicology*, 294(2–3):104–8. doi:10.1016/j.tox.2012.02.006 PMID:22386858

Carlson GP, Turner M, Mantick NA (2006). Effects of styrene and styrene oxide on glutathione-related antioxidant enzymes. *Toxicology*, 227(3):217–26. doi:10.1016/j.tox.2006.08.006 PMID:16971035

CDC (2010). Tobacco Smoke Causes Disease: The Biology and Behavioral Basis for Smoking-Attributable Disease: A Report of the Surgeon General. Atlanta (GA), USA: US Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health.

CDC (2018). Fourth national report on human exposure to environmental chemicals. National Health and Nutrition Examination Survey. Atlanta (GA), USA: Centers for Disease Control and Prevention. Available from: https://www.cdc.gov/exposurereport/index.html, accessed 15 January 2018.

CEFIC (2007). Styrene monomer: environmental, health, safety, transport and storage guidelines. Available from: http://www.cefic.org/Documents/RESOURCES/Guidelines/Transport-and-Logistics/Best%20Practice%20Guidelines%20-%20Product%20Specific%20Guidelines/Styrene-Monomer-Environmental-health-Safety-and-Distribution-Guidelines.pdf, accessed 26 February 2018.

Cemeli E, Mirkova E, Chiuchiarelli G, Alexandrova E, Anderson D (2009). Investigation on the mechanisms of genotoxicity of butadiene, styrene and their combination in human lymphocytes using the Comet assay. *Mutat Res*, 664(1–2):69–76. doi:10.1016/j.mrfmmm.2009.02.010 PMID:19428383

Cerný S, Mráz J, Flek J, Tichý M (1990). Effect of ethanol on the urinary excretion of mandelic and phenylglyoxylic acids after human exposure to styrene. *Int Arch Occup Environ Health*, 62(3):243–7. doi:10.1007/BF00379441 PMID:2347648

Chakrabarti S, Duhr MA, Senécal-Quevillon M, Richer CL (1993). Dose-dependent genotoxic effects of styrene on human blood lymphocytes and the relationship to its oxidative and metabolic effects. *Environ Mol Mutagen*, 22(2):85–92. doi:10.1002/em.2850220206 PMID:8359157

Chakrabarti S, Zhang XX, Richer CL (1997). Influence of duration of exposure to styrene oxide on sister chromatid exchanges and cell-cycle kinetics in cultured human blood lymphocytes in vitro. *Mutat Res*, 395(1):37–45. doi:10.1016/S1383-5718(97)00140-X PMID:9465912

Chambers DM, Ocariz JM, McGuirk MF, Blount BC (2011). Impact of cigarette smoking on volatile organic compound (VOC) blood levels in the U.S. population: NHANES 2003–2004. *Environ Int*, 37(8):1321–8. doi:10.1016/j.envint.2011.05.016 PMID:21703688

Checkoway H, Williams TM (1982). A hematology survey of workers at a styrene-butadiene synthetic rubber manufacturing plant. *Am Ind Hyg Assoc J*, 43(3):164–9. doi:10.1080/15298668291409550 PMID:7064811

Cheh AM, Carlson RE (1981). Determination of potentially mutagenic and carcinogenic electrophiles in environmental samples. *Anal Chem*, 53(7):1001–6. doi:10.1021/ac00230a018

Chen Y, Pan W, Wu F (2013). Emission fluxes of volatile organic compounds from three heavily polluted rivers in Guangzhou, South China. *Water Air Soil Pollut*, 224(4):1509 doi:10.1007/s11270-013-1509-1

Cherry N, Waldron HA, Wells GG, Wilkinson RT, Wilson HK, Jones S (1980). An investigation of the acute behavioural effects of styrene on factory workers. *Br J Ind Med*, 37(3):234–40. PMID:6107122

Chevron Phillips Chemical Co. (2010). Safe handling and storage of styrene monomer. Available from: http://www.cpchem.com/bl/aromatics/enus/Documents/Safe Handling and Storage of Styrene Monomer.pdf, accessed 26 February 2018.

Chevron Phillips Chemical Co. (2017). Styrene monomer. Technical bulletin. Available from: http://www.cpchem.com/bl/aromatics/en-us/Pages/StyreneMonomer.aspx, accessed 26 February 2018.

Chiu WA, Guyton KZ, Martin MT, Reif DM, Rusyn I (2018). Use of high-throughput in vitro toxicity screening data in cancer hazard evaluations by IARC Monograph Working Groups. *ALTEX*, 35(1):51–64. doi:10.14573/altex.1703231 PMID:28738424

Chon MC (2003). Transformation of the old process: ethylbenzene to styrene with CO_2 dilution. Chemrawn XVI Conference Consultation Forum. Innovation stage: the way from pure to applied chemistry. Ottawa, Canada. 9 and 12 August 2003. Available from: http://list.iupac.org/symposia/conferences/chemrawn/crXVI/crXVI-N11-MinCheChon.pdf, accessed 26 February 2018.

Christakopoulos A, Bergmark E, Zorcec V, Norppa H, Mäki-Paakkanen J, Osterman-Golkar S (1993). Monitoring occupational exposure to styrene from hemoglobin adducts and metabolites in blood. *Scand J Work Environ Health*, 19(4):255–63. doi:10.5271/sjweh.1476 PMID:8235514

Christensen MS, Hansen J, Ramlau-Hansen CH, Toft G, Kolstad H (2017). Cancer incidence in workers exposed to styrene in the Danish-reinforced plastics industry, 1968-2012. *Epidemiology*, 28(2):300–10. doi:10.1097/EDE.0000000000000608 PMID:27984421

Christensen MS, Vestergaard JM, d'Amore F, Gørløv JS, Toft G, Ramlau-Hansen CH, et al. (2018). Styrene exposure and risk of lymphohematopoietic malignancies in 73,036 reinforced plastics workers. *Epidemiology*, 29(3):342–51. doi:10.1097/EDE.0000000000000819 PMID:29533250

Clay P (2004). Styrene monomer does not induce unscheduled DNA synthesis in the mouse liver following inhalation exposure. *Mutagenesis*, 19(6):489–92. doi:10.1093/mutage/geh062 PMID:15548762

Coccini T, Di Nucci A, Tonini M, Maestri L, Costa LG, Liuzzi M, et al. (1996). Effects of ethanol administration on cerebral non-protein sulfhydryl content in rats exposed to styrene vapour. *Toxicology*, 106(1–3):115–22. doi:10.1016/0300-483X(95)03170-K PMID:8571382

Coccini T, Fenoglio C, Nano R, De Piceis Polver P, Moscato G, Manzo L (1997). Styrene-induced alterations in the respiratory tract of rats treated by inhalation or intraperitoneally. *J Toxicol Environ Health*, 52(1):63–77. doi:10.1080/00984109708984053 PMID:9269323

Cocco P, t'Mannetje A, Fadda D, Melis M, Becker N, de Sanjosé S, et al. (2010). Occupational exposure to solvents and risk of lymphoma subtypes: results from the Epilymph case-control study. *Occup Environ Med*, 67(5):341–7. doi:10.1136/oem.2009.046839 PMID:20447988

Coggon D, Ntani G, Harris EC, Palmer KT (2015). Risk of cancer in workers exposed to styrene at eight British companies making glass-reinforced plastics. *Occup Environ Med*, 72(3):165–70. doi:10.1136/oemed-2014-102382 PMID:25358742

Coggon D, Osmond C, Pannett B, Simmonds S, Winter PD, Acheson ED (1987). Mortality of workers exposed to styrene in the manufacture of glass-reinforced plastics. *Scand J Work Environ Health*, 13(2):94–9. doi:10.5271/sjweh.2067 PMID:3602970

Cohen JT, Carlson G, Charnley G, Coggon D, Delzell E, Graham JD, et al. (2002). A comprehensive evaluation of the potential health risks associated with occupational and environmental exposure to styrene. *J Toxicol Environ Health B Crit Rev*, 5(1–2):1–265. doi:10.1080/10937400252972162 PMID:12012775

Collins JJ, Bodner KM, Bus JS (2013). Cancer mortality of workers exposed to styrene in the U.S. reinforced plastics and composite industry. *Epidemiology*,

24(2):195–203. doi:10.1097/EDE.0b013e318281a30f PMID:23344212

Collins JJ, Delzell E (2018). A systematic review of epidemiologic studies of styrene and cancer. *Crit Rev Toxicol*, 48(6):443–70. doi:10.1080/10408444.2018.1445700 PMID:29589466

Compton-Quintana PJ, Jensen RH, Bigbee WL, Grant SG, Langlois RG, Smith MT, et al. (1993). Use of the glycophorin A human mutation assay to study workers exposed to styrene. *Environ Health Perspect*, 99:297–301. doi:10.1289/ehp.9399297 PMID:8319648

Conner MK, Alarie Y, Dombroske RL (1980). Sister chromatid exchange in murine alveolar macrophages, bone marrow, and regenerating liver cells induced by styrene inhalation. *Toxicol Appl Pharmacol*, 55(1):37–42. doi:10.1016/0041-008X(80)90217-3 PMID:7423505

Conti B, Maltoni C, Perino G, Ciliberti A (1988). Long-term carcinogenicity bioassays on styrene administered by inhalation, ingestion and injection and styrene oxide administered by ingestion in Sprague-Dawley rats, and para-methylstyrene administered by ingestion in Sprague-Dawley rats and Swiss mice. *Ann N Y Acad Sci*, 534(1):203–34. doi:10.1111/j.1749-6632.1988.tb30112.x PMID:3389656

Cosnier F, Nunge H, Cossec B, Gaté L (2012). Simultaneous determination of aromatic acid metabolites of styrene and styrene-oxide in rat urine by gas chromatography-flame ionization detection. *J Anal Toxicol*, 36(5):312–8. doi:10.1093/jat/bks015 PMID:22492963

Costa C, Costa S, Silva S, Coelho P, Botelho M, Gaspar J, et al. (2012). DNA damage and susceptibility assessment in industrial workers exposed to styrene. *J Toxicol Environ Health A*, 75(13-15):735–46. doi:10.1080/15287394.2012.688488 PMID:22788361

Costa C, Pasquale RD, Silvari V, Barbaro M, Catania S (2006). In vitro evaluation of oxidative damage from organic solvent vapours on human skin. *Toxicol In Vitro*, 20(3):324–31. doi:10.1016/j.tiv.2005.08.007 PMID:16169704

Costello RJ (1983). Health hazard evaluation report: Chem-Dyne Hazardous Waste Site, Hamilton, OH. Cincinnati (OH), USA: National Institute for Occupational Safety and Health.

Crandall MS, Hartle RW (1985). An analysis of exposure to styrene in the reinforced plastic boat-making industry. *Am J Ind Med*, 8(3):183–92. doi:10.1002/ajim.4700080304 PMID:4050801

Cruzan G, Bus J, Hotchkiss J, Harkema J, Banton M, Sarang S (2012). CYP2F2-generated metabolites, not styrene oxide, are a key event mediating the mode of action of styrene-induced mouse lung tumors. *Regul Toxicol Pharmacol*, 62(1):214–20. doi:10.1016/j.yrtph.2011.10.007 PMID:22041433

Cruzan G, Bus J, Hotchkiss J, Sura R, Moore C, Yost G, et al. (2013). Studies of styrene, styrene oxide and 4-hydroxystyrene toxicity in CYP2F2 knockout and CYP2F1 humanized mice support lack of human relevance for mouse lung tumors. *Regul Toxicol Pharmacol*, 66(1):24–9. doi:10.1016/j.yrtph.2013.02.008 PMID:23454103

Cruzan G, Bus JS, Banton MI, Sarang SS, Waites R, Layko DB, et al. (2017). Complete attenuation of mouse lung cell proliferation and tumorigenicity in CYP2F2 knockout and CYP2F1 humanized mice exposed to inhaled styrene for up to 2 years supports a lack of human relevance. *Toxicol Sci*, 159(2):413–21. doi:10.1093/toxsci/kfx141 PMID:28962520

Cruzan G, Cushman JR, Andrews LS, Granville GC, Johnson KA, Bevan C, et al. (2001). Chronic toxicity/oncogenicity study of styrene in CD-1 mice by inhalation exposure for 104 weeks. *J Appl Toxicol*, 21(3):185–98. doi:10.1002/jat.737 PMID:11404830

Cruzan G, Cushman JR, Andrews LS, Granville GC, Johnson KA, Hardy CJ, et al. (1998). Chronic toxicity/oncogenicity study of styrene in CD rats by inhalation exposure for 104 weeks. *Toxicol Sci*, 46(2):266–81. doi:10.1093/toxsci/46.2.266 PMID:10048130

Cruzan G, Cushman JR, Andrews LS, Granville GC, Miller RR, Hardy CJ, et al. (1997). Subchronic inhalation studies of styrene in CD rats and CD-1 mice. *Fundam Appl Toxicol*, 35(2):152–65. doi:10.1006/faat.1996.2273 PMID:9038236

Csanády GA, Kessler W, Hoffmann HD, Filser JG (2003). A toxicokinetic model for styrene and its metabolite styrene-7,8-oxide in mouse, rat and human with special emphasis on the lung. *Toxicol Lett*, 138(1–2):75–102. doi:10.1016/S0378-4274(02)00409-5 PMID:12559694

Csanády GA, Mendrala AL, Nolan RJ, Filser JG (1994). A physiologic pharmacokinetic model for styrene and styrene-7,8-oxide in mouse, rat and man. *Arch Toxicol*, 68(3):143–57. doi:10.1007/BF03035414 PMID:8024461

Dalbey WE, Rodriguez SC, Cope C, Cruzan G (1996). Cell proliferation in rat forestomach following oral administration of styrene oxide. *Fundam Appl Toxicol*, 30(1):67–74. doi:10.1006/faat.1996.0044 PMID:8812228

Daré E, Tofighi R, Nutt L, Vettori MV, Emgård M, Mutti A, et al. (2004). Styrene 7,8-oxide induces mitochondrial damage and oxidative stress in neurons. *Toxicology*, 201(1–3):125–32. doi:10.1016/j.tox.2004.04.010 PMID:15297027

Darrall KG, Figgins JA, Brown RD, Phillips GF (1998). Determination of benzene and associated volatile compounds in mainstream cigarette smoke. *Analyst (Lond)*, 123(5):1095–101. doi:10.1039/a708664d PMID:9709493

de Meester C, Duverger-van Bogaert M, Lambotte-Vandepaer M, Mercier M, Poncelet F (1981). Mutagenicity of styrene in the *Salmonella typhimurium* test system. *Mutat Res*, 90(4):443–50. doi:10.1016/0165-1218(81)90066-5 PMID:7038463

de Meester C, Poncelet F, Roberfroid M, Rondelet J, Mercier M (1977). Mutagenicity of styrene and styrene oxide. *Mutat Res*, 56(2):147–52. doi:10.1016/0027-5107(77)90202-0

De Palma G, Manini P, Mozzoni P, Andreoli R, Bergamaschi E, Cavazzini S, et al. (2001). Polymorphism of xenobiotic-metabolizing enzymes and excretion of styrene-specific mercapturic acids. *Chem Res Toxicol*, 14(10):1393–400. doi:10.1021/tx015505x PMID:11599931

de Raat WK (1978). Induction of sister chromatid exchanges by styrene and its presumed metabolite styrene oxide in the presence of rat liver homogenate. *Chem Biol Interact*, 20(2):163–70. doi:10.1016/0009-2797(78)90050-9 PMID:647839

De Rosa E, Cellini M, Sessa G, Saletti C, Rausa G, Marcuzzo G, et al. (1993). Biological monitoring of workers exposed to styrene and acetone. *Int Arch Occup Environ Health*, 65(1 Suppl):S107–10. doi:10.1007/BF00381318 PMID:8406902

Del Carratore R, Bronzetti G, Bauer C, Corsi C, Nieri R, Paolini M, et al. (1983). Cytochrome P-450 factors determining synthesis in strain D7 *Saccharomyces cerevisiae*. An alternative system to microsomal assay. *Mutat Res*, 121(2):117–23. doi:10.1016/0165-7992(83)90109-4 PMID:6348531

Delbressine LP, Ketelaars HC, Seutter-Berlage F, Smeets FL (1980). Phenaceturic acid, a new urinary metabolite of styrene in the rat. *Xenobiotica*, 10(5):337–42. doi:10.3109/00498258009033765 PMID:7415215

Delzell E, Macaluso M, Sathiakumar N, Matthews R (2001). Leukemia and exposure to 1,3-butadiene, styrene and dimethyldithiocarbamate among workers in the synthetic rubber industry. *Chem Biol Interact*, 135–136:515–34. doi:10.1016/S0009-2797(01)00223-X PMID:11397410

Delzell E, Sathiakumar N, Hovinga M, Macaluso M, Julian J, Larson R, et al. (1996). A follow-up study of synthetic rubber workers. *Toxicology*, 113(1–3):182–9. doi:10.1016/0300-483X(96)03443-9 PMID:8901897

DFG (2017a). Deutsche Forschungsgemeinschaft. List of MAK and BAT Values 2017: Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area. Report 53. Weinheim, Germany: Wiley-VCH Verlag. doi:10.1002/9783527812127

DFG (2017b). Deutsche Forschungsgemeinschaft. Styrene [MAK Value Documentation, 2003]. The MAK-Collection for Occupational Health and Safety. Weinheim, Germany: Wiley-VCH Verlag; pp. 286–290.

Diodovich C, Bianchi MG, Bowe G, Acquati F, Taramelli R, Parent-Massin D, et al. (2004). Response of human cord blood cells to styrene exposure: evaluation of its effects on apoptosis and gene expression by genomic technology. *Toxicology*, 200(2–3):145–57. doi:10.1016/j.tox.2004.03.021 PMID:15212811

Diodovich C, Urani C, Maurici D, Malerba I, Melchioretto P, Orlandi M, et al. (2006). Modulation of different stress pathways after styrene and styrene-7,8-oxide exposure in HepG2 cell line and normal human hepatocytes. *J Appl Toxicol*, 26(4):317–25. doi:10.1002/jat.1142 PMID:16705669

Dogra RK, Chandra K, Chandra S, Gupta S, Khanna S, Srivastava SN, et al. (1992). Host resistance assays as predictive models in styrene immunomodulation. *Int J Immunopharmacol*, 14(6):1003–9. doi:10.1016/0192-0561(92)90144-A PMID:1330943

Dogra RK, Khanna S, Srivastava SN, Shukla LJ, Shanker R (1989). Styrene-induced immunomodulation in mice. *Int J Immunopharmacol*, 11(5):577–86. doi:10.1016/0192-0561(89)90188-4 PMID:2807632

Doherty BT, Kwok RK, Curry MD, Ekenga C, Chambers D, Sandler DP, et al. (2017). Associations between blood BTEXS concentrations and hematologic parameters among adult residents of the U.S. Gulf States. *Environ Res*, 156:579–87. doi:10.1016/j.envres.2017.03.048 PMID:28448810

Dolmierski R, Szczepanik M, Danielewicz-Garbalińska G, Kunikowska D, Mickiewicz W, Chomicz M, et al. (1983). Mutagenic action of styrene and its metabolites. 1. Chromosome aberration in persons exposed to the action of styrene. Introductory investigations. *Bull Inst Marit Trop Med Gdynia*, 34(1–2):89–93. PMID:6679470

Donner M, Sorsa M, Vainio H (1979). Recessive lethals induced by styrene and styrene oxide in *Drosophila melanogaster*. *Mutat Res*, 67(4):373–6. doi:10.1016/0165-1218(79)90035-1 PMID:113677

Dumas S, Parent M-É, Siemiatycki J, Brisson J (2000). Rectal cancer and occupational risk factors: a hypothesis-generating, exposure-based case-control study. *Int J Cancer*, 87(6):874–9. doi:10.1002/1097-0215(20000915)87:6<874::AID-IJC18>3.0.CO;2-L PMID:10956400

Dypbukt JM, Costa LG, Manzo L, Orrenius S, Nicotera P (1992). Cytotoxic and genotoxic effects of styrene-7,8-oxide in neuroadrenergic Pc 12 cells. *Carcinogenesis*, 13(3):417–24.doi:10.1093/carcin/13.3.417PMID:1547532

ECHA (2017). Styrene. Helsinki, Finland: European Chemicals Agency. Available from: https://echa.europa.eu/information-on-chemicals, accessed 26 February 2018.

Edwards RD, Jurvelin J, Saarela K, Jantunen M (2001). VOC concentrations measured in samples and residential indoor, outdoor and microenvironments in EXPOLIS-Helsinki, Finland. *Atmos Environ*, 35(27):4531–43. doi:10.1016/S1352-2310(01)00230-8

Ehret-Henry J, Ducruet V, Luciani A, Feigenbaum A (1994). Styrene and ethylbenzene migration from polystyrene into dairy products by dynamic purge-and-trap gas chromatography. *J Food Sci*, 59(5):990–2. doi:10.1111/j.1365-2621.1994.tb08174.x

Einistö P, Hooberman BH, Sinsheimer JE (1993). Base-pair mutations caused by six aliphatic epoxides in *Salmonella typhimurium* TA100, TA104, TA4001, and TA4006. *Environ Mol Mutagen*, 21(3):253–7. doi:10.1002/em.2850210308 PMID:8462529

El-Ziney MG, BaAbdoulah H, Tawfik MS, et al. (2016). Monostyrene intake in albino rats: accumulation in organs and effects on growth performance and oxidative stress. *Int J Nutr Food Sci*., 5(1):72–9. doi:10.11648/j.ijnfs.20160501.21

El-Ziney MG, Tawfik MS (2016). Migration levels of monostyrene from polystyrene containers to dairy products *Food process Technol*, 3(2):00066.

Elovaara E, Vainio H, Aitio A (1990). Pulmonary toxicity of inhaled styrene in acetone-, phenobarbital- and 3-methylcholanthrene-treated rats. *Arch Toxicol*, 64(5):365–9. doi:10.1007/BF01973457 PMID:2403287

Engelhardt G, Gamer A, Vodička P, Bárta I, Hoffmann HD, Veenstra G (2003). A re-assessment of styrene-induced clastogenicity in mice in a subacute inhalation study. *Arch Toxicol*, 77(1):56–61. doi:10.1007/s00204-002-0406-y PMID:12491042

Engström J, Astrand I, Wigaeus E (1978a). Exposure to styrene in a polymerization plant. Uptake in the organism and concentration in subcutaneous adipose tissue. *Scand J Work Environ Health*, 4(4):324–9. doi:10.5271/sjweh.2693 PMID:734393

Engström J, Bjurström R, Astrand I, Ovrum P (1978b). Uptake, distribution and elimination of styrene in man. Concentration in subcutaneous adipose tissue. *Scand J Work Environ Health*, 4(4):315–23. doi:10.5271/sjweh.2694 PMID:734392

EPA (1993). Locating and estimating air emissions from sources of styrene. EPA-454/R-93-011. Research Triangle Park (NC), USA: Emission Inventory Branch, United States Environmental Protection Agency. Available from: https://www3.epa.gov/ttn/chief/le/styrene.pdf, accessed 26 February 2018.

EPA (1996). Method 8260B. Volatile organic compounds by gas chromatography/mass spectrometry (GC/MS), Revision 2. Washington (DC), USA: United States Environmental Protection Agency. Available from: http://www.epa.gov/epaoswer/hazwaste/test/8260b.pdf, accessed 26 February 2018.

EPA (2001). National primary drinking water regulations. US Code Fed. Regul., Title 40, Subpart D, Parts 141.32, 141.61. Washington (DC), USA: United States Environmental Protection Agency; pp. 401, 430–43.

EPA (2015). 2015 Toxics Release Inventory. Washington (DC), USA: United States Environmental Protection Agency. Available from: https://www.epa.gov/toxics-release-inventory-tri-program/tri-national-analysis-archive, accessed 25 October 2017.

EPA (2017a). iCSS ToxCast Dashboard. Prod_dashboard_v2. Washington (DC), USA: United States Environmental Protection Agency. Available from: http://actor.epa.gov/dashboard2/, accessed 15 October 2017.

EPA (2017b). Toxicity Forecaster (ToxCast) data. Washington (DC), USA: United States Environmental Protection Agency. Available from: http://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data, accessed 15 October 2017.

EPA (2017c). Chemistry Dashboard. Washington (DC), USA: United States Environmental Protection Agency. Available from: https://comptox.epa.gov/dashboard/, accessed 27 December 2017.

EU (2011). Commission Regulation (EU) No. 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food. Available from: https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX%3A32011R0010.

Fabiani R, Rosignoli P, De Bartolomeo A, Fuccelli R, Morozzi G (2012). Genotoxicity of alkene epoxides in human peripheral blood mononuclear cells and HL60 leukaemia cells evaluated with the comet assay. *Mutat Res*, 747(1):1–6. doi:10.1016/j.mrgentox.2012.01.004 PMID:22285587

Fabry L, Léonard A, Roberfroid M (1978). Mutagenicity tests with styrene oxide in mammals. *Mutat Res*, 51(3):377–81. doi:10.1016/0027-5107(78)90125-2 PMID:714070

FDA (2017). Food and drugs. US Code Fed. Regul., Title 21, Parts 172.515, 172.615, 173.20, 173.25, 173.70, 175.105, 175.125, 175.300, 175.320, 176.170, 176.180, 176.300, 177.1010, 177.1020, 177.1030, 177.1040, 177.1050, 177.1200, 177.1210, 177.1430, 177.1630, 177.1635, 177.1640, 177.1810, 177.1820, 177.1830, 177.2260, 177.2420, 177.2600, 177.2710, 177.2800, 178.1005, 178.2010, 178.3480, 178.3610, 178.3790, 181.30, 181.32. Silver Spring (MD), USA: United States Food and Drug Administration; pp. 26–432, 443–447.

Fernández-Villarrenaga Martín V, López Mahía P, Muniategui Lorenzo S, Prada Rodríguez D (2000). Development of a thermal desorption-gas chromatography-mass spectrometry method for determination of styrene in air. Application to workplace air. *Analusis*, 28(8):737–42. doi:10.1051/analusis:2000144

Filer DL, Kothiya P, Setzer RW, Judson RS, Martin MT (2017). tcpl: the ToxCast pipeline for high-throughput screening data. *Bioinformatics*, 33(4):618–20. PMID:27797781

Filser JG, Gelbke HP (2016). An evaluation of concentrations of styrene-7,8-oxide in rats and humans resulting from exposure to styrene or styrene-7,8-oxide and potential genotoxicity. *Toxicol Lett*, 247:11–28. doi:10.1016/j.toxlet.2016.02.001 PMID:26851640

Filser JG, Kessler W, Csanády GA (2002). Estimation of a possible tumorigenic risk of styrene from daily intake via food and ambient air. *Toxicol Lett*, 126(1):1–18. doi:10.1016/S0378-4274(01)00420-9 PMID:11738266

Finnish Ministry of Social Affairs and Health (2016). Values known to be harmful. [Sosiaali- ja Terveysministeriö. HTP-arvot 2016. Haitallisiksi tunnetut pitoisuudet]. Helsinki, Finland: Ministry of Social Affairs and Health, Department of Occupational Health. Available from: https://julkaisut.valtioneuvosto.fi/bitstream/handle/10024/79109/08_2016_HTP-arvot_suomi_22122016_netti_kansilla.pdf?sequence=1.

Fischäder G, Röder-Stolinski C, Wichmann G, Nieber K, Lehmann I (2008). Release of MCP-1 and IL-8 from lung epithelial cells exposed to volatile organic compounds. *Toxicol In Vitro*, 22(2):359–66. doi:10.1016/j.tiv.2007.09.015 PMID:17993253

Fjeldstad PE, Thorud S, Wannag A (1979). Styrene oxide in the manufacture of reinforced polyester plastics. *Scand J Work Environ Health*, 5(2):162–3. doi:10.5271/sjweh.2655 PMID:472688

Fleig I, Thiess AM (1978). Mutagenicity study of workers employed in the styrene and polystyrene processing and manufacturing industry. *Scand J Work Environ Health*, 4(Suppl 2):254–8. doi:10.5271/sjweh.2740 PMID:734412

Fleming-Jones ME, Smith RE (2003). Volatile organic compounds in foods: a five year study. *J Agric Food Chem*, 51(27):8120–7. doi:10.1021/jf0303159 PMID:14690406

Flodin U, Fredriksson M, Persson B, Hardell L, Axelson O (1986). Background radiation, electrical work, and some other exposures associated with acute myeloid leukemia in a case-referent study. *Arch Environ Health*, 41(2):77–84. doi:10.1080/00039896.1986.9937413 PMID:3459400

Florin I, Rutberg L, Curvall M, Enzell CR (1980). Screening of tobacco smoke constituents for mutagenicity using the Ames' test. *Toxicology*, 15(3):219–32. doi:10.1016/0300-483X(80)90055-4 PMID:7008261

Fontaine FR, DeGraaf YC, Ghaoui R, Sallustio BC, Edwards J, Burcham PC (2004). Optimisation of the comet genotoxicity assay in freshly isolated murine hepatocytes: detection of strong in vitro DNA damaging properties for styrene. *Toxicol In Vitro*, 18(3):343–50. doi:10.1016/j.tiv.2003.10.003 PMID:15046782

Forni A, Goggi E, Cortisi E, Cecchetti R, Cortona G, Sesana G, et al. (1988). Cytogenetic findings in styrene workers in relation to exposure. In: Seemayer NH, Hadnagy W editors. Environmental hygiene. Berlin, Germany: Springer-Verlag; pp. 159–62. doi:10.1007/978-3-642-73766-4_34

Fracasso ME, Doria D, Carrieri M, Bartolucci GB, Quintavalle S, De Rosa E (2009). DNA single- and double-strand breaks by alkaline- and immuno-comet assay in lymphocytes of workers exposed to styrene. *Toxicol Lett*, 185(1):9–15. doi:10.1016/j.toxlet.2008.11.010 PMID:19095051

Frentzel-Beyme R, Thiess AM, Wieland R (1978). Survey of mortality among employees engaged in the manufacture of styrene and polystyrene at the BASF Ludwigshafen works. *Scand J Work Environ Health*, 4(Suppl 2):231–9. doi:10.5271/sjweh.2742 PMID:734410

Fukami T, Katoh M, Yamazaki H, Yokoi T, Nakajima M (2008). Human cytochrome P450 2A13 efficiently metabolizes chemicals in air pollutants: naphthalene, styrene, and toluene. *Chem Res Toxicol*, 21(3):720–5. doi:10.1021/tx700325f PMID:18266326

Fustinoni S, Campo L, Manini P, Buratti M, Waidyanatha S, De Palma G, et al. (2008). An integrated approach to biomonitoring exposure to styrene and styrene-(7,8)-oxide using a repeated measurements sampling design. *Biomarkers*, 13(6):560–78. doi:10.1080/13547500802062994 PMID:18608187

Fustinoni S, Colosio C, Colombi A, Lastrucci L, Yeowell-O'Connell K, Rappaport SM (1998). Albumin and hemoglobin adducts as biomarkers of exposure to styrene in fiberglass-reinforced-plastics workers. *Int Arch Occup Environ Health*, 71(1):35–41. doi:10.1007/s004200050247 PMID:9523247

Fustinoni S, Manini P, Campo L, De Palma G, Andreoli R, Mutti A, et al. (2010). Assessing variability and comparing short-term biomarkers of styrene exposure using a repeated measurements approach. *Toxicol Lett*, 192(1):40–4. doi:10.1016/j.toxlet.2009.02.004 PMID:20117324

Futamura M, Goto S, Kimura R, Kimoto I, Miyake M, Ito K, et al. (2009). Differential effects of topically applied formalin and aromatic compounds on neurogenic-mediated microvascular leakage in rat skin. *Toxicology*, 255(1–2):100–6. doi:10.1016/j.tox.2008.10.012 PMID:19014991

Gadberry MG, DeNicola DB, Carlson GP (1996). Pneumotoxicity and hepatotoxicity of styrene and styrene oxide. *J Toxicol Environ Health*, 48(3):273–94. doi:10.1080/009841096161339 PMID:8656450

Galassi C, Kogevinas M, Ferro G, Biocca M (1993). Biological monitoring of styrene in the reinforced plastics industry in Emilia Romagna, Italy. *Int Arch Occup Environ Health*, 65(2):89–95. doi:10.1007/BF00405725 PMID:8253516

Gamer AO, Leibold E, Deckardt K, Kittel B, Kaufmann W, Tennekes HA, et al. (2004). The effects of styrene on lung cells in female mice and rats. *Food Chem Toxicol*, 42(10):1655–67. doi:10.1016/j.fct.2004.06.002 PMID:15304312

Gao LB, Wang JZ, Zeng S (2009). Cloning, expression and the application of human, rat alcohol dehydrogenase and aldo-keto reductase. *Yao Xue Xue Bao*, 44(7):778–84. PMID:19806920 [in Chinese]

Gaté L, Micillino JC, Sébillaud S, Langlais C, Cosnier F, Nunge H, et al. (2012). Genotoxicity of styrene-7,8-oxide and styrene in Fischer 344 rats: a 4-week inhalation study. *Toxicol Lett*, 211(3):211–9. doi:10.1016/j.toxlet.2012.03.796 PMID:22507543

Genualdi S, Nyman P, Begley T (2014). Updated evaluation of the migration of styrene monomer and oligomers from polystyrene food contact materials to foods and food simulants. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess*, 31(4):723–33. doi:10.1080/19440049.2013.878040 PMID:24383702

Gérin M, Siemiatycki J, Désy M, Krewski D (1998). Associations between several sites of cancer and occupational exposure to benzene, toluene, xylene, and styrene: results of a case-control study in Montreal. *Am J Ind Med*, 34(2):144–56. doi:10.1002/(SICI)1097-0274(199808)34:2<144::AID-AJIM7>3.0.CO;2-X PMID:9651624

Geuskens RB, van der Klaauw MM, van der Tuin J, van Hemmen JJ (1992). Exposure to styrene and health complaints in the Dutch glass-reinforced plastics industry. *Ann Occup Hyg*, 36(1):47–57. PMID:1567113

Ghittori S, Maestri L, Imbriani M, Capodaglio E, Cavalleri A (1997). Urinary excretion of specific mercapturic acids in workers exposed to styrene. *Am J Ind Med*, 31(5):636–44. doi:10.1002/(SICI)1097-0274(199705)31:5<636::AID-AJIM20>3.0.CO;2-Z PMID:9099368

Gilbert J, Startin JR (1983). A survey of styrene monomer levels in foods and plastic packaging by coupled mass spectrometry–automatic headspace gas chromatography. *J Sci Food Agric*, 34(6):647–52. doi:10.1002/jsfa.2740340616 PMID:6887788

Głośnicka R, Dziadziuszko H (1986). Mutagenic action of styrene and its metabolites. II. Genotoxic activity of styrene, styrene oxide, styrene glycol and benzoic acid tested with the SOS chromotest. *Bull Inst Marit Trop Med Gdynia*, 37(3–4):295–302. PMID:3125874

Gobba F, Galassi C, Ghittori S, Imbriani M, Pugliese F, Cavalleri A (1993). Urinary styrene in the biological monitoring of styrene exposure. *Scand J Work Environ Health*, 19(3):175–82. doi:10.5271/sjweh.1484 PMID:8367695

Gobba F, Ghittori S, Imbriani M, Cavalleri A (2000). Evaluation of half-mask respirator protection in styrene-exposed workers. *Int Arch Occup Environ Health*, 73(1):56–60. doi:10.1007/PL00007938 PMID:10672492

Godderis L, Aka P, Mateuca R, Kirsch-Volders M, Lison D, Veulemans H (2006). Dose-dependent influence of genetic polymorphisms on DNA damage induced by styrene oxide, ethylene oxide and gamma-radiation. *Toxicology*, 219(1–3):220–9. doi:10.1016/j.tox.2005.11.021 PMID:16386346

Godderis L, De Boeck M, Haufroid V, Emmery M, Mateuca R, Gardinal S, et al. (2004). Influence of genetic polymorphisms on biomarkers of exposure and genotoxic effects in styrene-exposed workers. *Environ Mol Mutagen*, 44(4):293–303. doi:10.1002/em.20069 PMID:15468052

Godderis L, Thomas R, Hubbard AE, Tabish AM, Hoet P, Zhang L, et al. (2012). Effect of chemical mutagens and carcinogens on gene expression profiles in human TK6 cells. *PLoS One*, 7(6):e39205. doi:10.1371/journal.pone.0039205 PMID:22723965

González-Pérez M, Gómez-Bombarelli R, Pérez-Prior MT, Arenas-Valgañón J, García-Santos MP, Calle E, et al. (2014). Alkylating potential of styrene oxide: reactions and factors involved in the alkylation process. *Chem Res Toxicol*, 27(10):1853–9. doi:10.1021/tx5002892 PMID:25222830

Governa M, Valentino M, Visonà I (1994). Chemotactic activity of human polymorphonuclear leukocytes and industrial xenobiotics: a brief review. *Toxicology*, 91(2):165–77. doi:10.1016/0300-483X(94)90142-2 PMID:8059440

Graff JJ, Sathiakumar N, Macaluso M, Maldonado G, Matthews R, Delzell E (2005). Chemical exposures in the synthetic rubber industry and lymphohematopoietic cancer mortality. *J Occup Environ Med*, 47(9):916–32. doi:10.1097/01.jom.0000172866.16615.db PMID:16155477

Grayson MH, Gill SS (1986). Effect of in vitro exposure to styrene, styrene oxide, and other structurally related compounds on murine cell-mediated immunity. *Immunopharmacology*, 11(3):165–73. doi:10.1016/0162-3109(86)90018-4 PMID:3488303

Green T, Lee R, Toghill A, Meadowcroft S, Lund V, Foster J (2001a). The toxicity of styrene to the nasal epithelium of mice and rats: studies on the mode of action and relevance to humans. *Chem Biol Interact*, 137(2):185–202. doi:10.1016/S0009-2797(01)00236-8 PMID:11551533

Green T, Toghill A, Foster JR (2001b). The role of cytochromes P-450 in styrene induced pulmonary toxicity and carcinogenicity. *Toxicology*, 169(2):107–17. doi:10.1016/S0300-483X(01)00488-7 PMID:11718952

Guillemin MP, Bauer D (1979). Human exposure to styrene. III. Elimination kinetics of urinary mandelic and phenylglyoxylic acids after single experimental exposure. *Int Arch Occup Environ Health*, 44(4):249–63. doi:10.1007/BF00381658 PMID:536049

Guillemin MP, Bauer D, Martin B, Marazzi A (1982). Human exposure to styrene. IV. Industrial hygiene investigations and biological monitoring in the polyester industry. *Int Arch Occup Environ Health*, 51(2):139–50. doi:10.1007/BF00378158 PMID:7160913

Guyonnet D, Belloir C, Suschetet M, Siess MH, Le Bon AM (2001). Antimutagenic activity of organosulfur compounds from *Allium* is associated with phase II enzyme induction. *Mutat Res*, 495(1–2):135–45. doi:10.1016/S1383-5718(01)00205-4 PMID:11448651

Haberlein RA (1998). CFA emission models for the reinforced plastics industries. Available from: https://www3.epa.gov/ttnchie1/efdocs/cfa_rpt3.pdf, accessed 26 February 2018.

Hagmar L, Högstedt B, Welinder H, Karlsson A, Rassner F (1989). Cytogenetic and hematological effects in plastics workers exposed to styrene. *Scand J Work Environ Health*, 15(2):136–41. doi:10.5271/sjweh.1871 PMID:2772576

Hampton CV, Pierson WR, Harvey TM, Schuetzle D (1983). Hydrocarbon gases emitted from vehicles on the road. 2. Determination of emission rates from diesel and spark-ignition vehicles. *Environ Sci Technol*, 17(12):699–708. doi:10.1021/es00118a003 PMID:22283124

Han JH, Choi CS, Kim MY, Chun YJ (2007). Differential gene expression by styrene in rat reproductive tissue. *J Toxicol Environ Health A*, 70(15-16):1259–63. doi:10.1080/15287390701434414 PMID:17654243

Hanova M, Stetina R, Vodickova L, Vaclavikova R, Hlavac P, Smerhovsky Z, et al. (2010). Modulation of DNA repair capacity and mRNA expression levels of *XRCC1*, *hOGG1* and *XPC* genes in styrene-exposed workers. *Toxicol Appl Pharmacol*, 248(3):194–200. doi:10.1016/j.taap.2010.07.027 PMID:20692273

Hanova M, Vodickova L, Vaclavikova R, Smerhovsky Z, Stetina R, Hlavac P, et al. (2011). DNA damage, DNA repair rates and mRNA expression levels of cell cycle genes (*TP53*, *p21*^{CDKN1A}, *BCL2* and *BAX*) with respect to occupational exposure to styrene. *Carcinogenesis*, 32(1):74–9. doi:10.1093/carcin/bgq213 PMID:20966084

Hansteen IL, Jelmert O, Torgrimsen T, Førsund B (1984). Low human exposure to styrene in relation to chromosome breaks, gaps and sister chromatid exchanges. *Hereditas*, 100(1):87–91. doi:10.1111/j.1601-5223.1984.tb00109.x PMID:6725010

Hartman JH, Martin HC, Caro AA, Pearce AR, Miller GP (2015). Subcellular localization of rat CYP2E1 impacts metabolic efficiency toward common substrates. *Toxicology*, 338:47–58. doi:10.1016/j.tox.2015.10.001 PMID:26463279

Harvilchuck JA, Carlson GP (2006). Comparison of styrene and its metabolites styrene oxide and 4-vinylphenol on cytotoxicity and glutathione depletion in Clara cells of mice and rats. *Toxicology*, 227(1–2):165–72. doi:10.1016/j.tox.2006.08.001 PMID:16956708

Harvilchuck JA, Pu X, Klaunig JE, Carlson GP (2009). Indicators of oxidative stress and apoptosis in mouse whole lung and Clara cells following exposure to styrene and its metabolites. *Toxicology*, 264(3):171–8. doi:10.1016/j.tox.2009.08.001 PMID:19666080

Harvilchuck JA, Zurbrugg RJ, Carlson GP (2008). CC10 mRNA and protein expression in Clara cells of CD-1 mice following exposure to styrene or its metabolites styrene oxide or 4-vinylphenol. *Toxicol Lett*, 183(1–3):28–35. doi:10.1016/j.toxlet.2008.09.010 PMID:18926891

Haufroid V, Buchet JP, Gardinal S, Ghittori S, Imbriani M, Hirvonen A, et al. (2001). Importance of genetic polymorphisms of drug-metabolizing enzymes for the interpretation of biomarkers of exposure to styrene. *Biomarkers*, 6(3):236–49. doi:10.1080/13547500010014540 PMID:23886278

Haufroid V, Jakubowski M, Janasik B, Ligocka D, Buchet JP, Bergamaschi E, et al. (2002). Interest of genotyping and phenotyping of drug-metabolizing enzymes for the interpretation of biological monitoring of exposure to styrene. *Pharmacogenetics*, 12(9):691–702. doi:10.1097/00008571-200212000-00003 PMID:12464798

Health Canada (1993). Priority Substances List assessment report: Styrene. Ottawa (ON), Canada: Minister of Supply and Services Canada.

Health Canada (1999). Determination of pyridine, quinoline and styrene in mainstream tobacco smoke – official method. Ottawa (ON), Canada:Health Canada.

Heck JE, Park AS, Qiu J, Cockburn M, Ritz B (2013). An exploratory study of ambient air toxics exposure in pregnancy and the risk of neuroblastoma in offspring. *Environ Res*, 127:1–6. doi:10.1016/j.envres.2013.09.002 PMID:24139061

Heck JE, Park AS, Qiu J, Cockburn M, Ritz B (2014). Risk of leukemia in relation to exposure to ambient air toxics in pregnancy and early childhood. *Int J Hyg Environ Health*, 217(6):662–8. doi:10.1016/j.ijheh.2013.12.003 PMID:24472648

Heck JE, Park AS, Qiu J, Cockburn M, Ritz B (2015). Retinoblastoma and ambient exposure to air toxics in the perinatal period. *J Expo Sci Environ Epidemiol*, 25(2):182–6. doi:10.1038/jes.2013.84 PMID:24280682

Helal SF, Elshafy WS (2013). Health hazards among workers in plastic industry. *Toxicol Ind Health*, 29(9):812–9. doi:10.1177/0748233712442728 PMID:22609858

Hemminki K (1986). Binding of styrene oxide to amino acids, human serum proteins and hemoglobin. *Arch Toxicol Suppl*, 9:286–90. doi:10.1007/978-3-642-71248-7_46 PMID:3468906

Hemminki K, Hesso A (1984). Reaction products of styrene oxide with guanosine in aqueous media. *Carcinogenesis*, 5(5):601–7. doi:10.1093/carcin/5.5.601 PMID:6722980

Hemminki K, Vodička P (1995). Styrene: from characterisation of DNA adducts to application in styrene-exposed lamination workers. *Toxicol Lett*, 77(1–3):153–61. doi:10.1016/0378-4274(95)03285-1 PMID:7618130

Hennard C, Finneman J, Harris CM, Harris TM, Stone MP (2001). The nonmutagenic (*R*)- and (*S*)-beta-(N^6-adenyl)styrene oxide adducts are oriented in the major groove and show little perturbation to DNA structure. *Biochemistry*, 40(33):9780–91. doi:10.1021/bi010564v PMID:11502171

Higgins LG, Hayes JD (2011). The cap'n'collar transcription factor Nrf2 mediates both intrinsic resistance to environmental stressors and an adaptive response elicited by chemopreventive agents that determines

susceptibility to electrophilic xenobiotics. *Chem Biol Interact*, 192(1–2):37–45. doi:10.1016/j.cbi.2010.09.025 PMID:20932822

Hirasawa F, Kumagoe M, Wang JS, Arany S, Zhou XP, Kumagai A, et al. (2007). Gadolinium chloride suppresses styrene-induced cytochrome P450s expression in rat liver. *Biomed Res*, 28(6):323–30. doi:10.2220/biomedres.28.323 PMID:18202523

Hodgson AT (1999). Common indoor sources of volatile organic compounds: emission rates and techniques for reducing consumer exposures. Final report, Contract number 95-302. Sacramento (CA), USA: California Environmental Protection Agency, Air Resources Board, Research Division. Available from: https://www.arb.ca.gov/research/single-project.php?row_id=58743.

Hodgson AT, Daisey JM, Mahanama KRR, Ten Brinke J, Alevantis LE (1996). Use of volatile tracers to determine the contribution of environmental tobacco smoke to concentrations of volatile organic compounds in smoking environments. *Environ Int*, 3(3):295–307. doi:10.1016/0160-4120(96)00015-3

Hodgson JT, Jones RD (1985). Mortality of styrene production, polymerization and processing workers at a site in northwest England. *Scand J Work Environ Health*, 11(5):347–52. doi:10.5271/sjweh.2214 PMID:4070999

Hoet P, Lison D (2008). Ototoxicity of toluene and styrene: state of current knowledge. *Crit Rev Toxicol*, 38(2):127–70. doi:10.1080/10408440701845443 PMID:18259983

Hoff A, Jacobsson S, Pfäffli P, Zitting A, Frostling H (1982). Degradation products of plastics. Polyethylene and styrene-containing thermoplastics – analytical, occupational and toxicologic aspects. *Scand J Work Environ Health*, 8(Suppl 2):1–60. PMID:7134933

Hofmann C, Pütz C, Semder B, Faller TH, Csanády GA, Filser JG (2006). Styrene-7,8-oxide burden in ventilated, perfused lungs of mice and rats exposed to vaporous styrene. *Toxicol Sci*, 90(1):39–48. doi:10.1093/toxsci/kfj056 PMID:16322077

Högstedt B (1984). Micronuclei in lymphocytes with preserved cytoplasm. A method for assessment of cytogenetic damage in man. *Mutat Res*, 130(1):63–72. doi:10.1016/0165-1161(84)90007-4 PMID:6694656

Högstedt B, Akesson B, Axell K, Gullberg B, Mitelman F, Pero RW, et al. (1983). Increased frequency of lymphocyte micronuclei in workers producing reinforced polyester resin with low exposure to styrene. *Scand J Work Environ Health*, 9(3):241–6. doi:10.5271/sjweh.2414 PMID:6612264

Högstedt B, Hedner K, Mark-Vendel E, Mitelman F, Schütz A, Skerfving S (1979). Increased frequency of chromosome aberrations in workers exposed to styrene. *Scand J Work Environ Health*, 5(4):333–5. doi:10.5271/sjweh.2649 PMID:538423

Holmes MJ, Hart A, Northing P, Oldring PK, Castle L, Stott D, et al. (2005). Dietary exposure to chemical migrants from food contact materials: a probabilistic approach. *Food Addit Contam*, 22(10):907–19. doi:10.1080/02652030500307172 PMID:16236636

Holz O, Scherer G, Brodtmeier S, Koops F, Warncke K, Krause T, et al. (1995). Determination of low level exposure to volatile aromatic hydrocarbons and genotoxic effects in workers at a styrene plant. *Occup Environ Med*, 52(6):420–8. doi:10.1136/oem.52.6.420 PMID:7627321

Hopkins AL, Groom CR, Alex A (2004). Ligand efficiency: a useful metric for lead selection. *Drug Discov Today*, 9(10):430–1. doi:10.1016/S1359-6446(04)03069-7 PMID:15109945

Horvath E, Pongracz K, Rappaport S, Bodell WJ (1994). ^{32}P-post-labeling detection of DNA adducts in mononuclear cells of workers occupationally exposed to styrene. *Carcinogenesis*, 15(7):1309–15. doi:10.1093/carcin/15.7.1309 PMID:8033305

HSDB (2017b). Styrene-7,8-oxide. Hazardous Substances Data Bank. Available from: https://toxnet.nlm.nih.gov/cgi-bin/sis/search2/f?/temp/~ckmQKQ:3, accessed November 2017.

HSE (2011). EH40/2005 Workplace exposure limits. 2nd ed. Norwich, United Kingdom: Health and Safety Executive. Available from: http://www.hse.gov.uk/pUbns/priced/eh40.pdf.

Huang M (1992). Study of cytogenetic damages in peripheral blood of styrene exposed workers. *Zhonghua Yu Fang Yi Xue Za Zhi*, 26(5):272–4. PMID:1298602 [in Chinese]

Hynes DE, DeNicola DB, Carlson GP (1999). Metabolism of styrene by mouse and rat isolated lung cells. *Toxicol Sci*, 51(2):195–201. doi:10.1093/toxsci/51.2.195 PMID:10543021

IARC (1985). Allyl compounds, aldehydes, epoxides and peroxides. *IARC Monogr Eval Carcinog Risk Chem Hum*, 36:1–369. Available from: http://publications.iarc.fr/54.

IARC (1986). Tobacco smoking. *IARC Monogr Eval Carcinog Risk Chem Hum*, 38:1–421. Available from: http://publications.iarc.fr/56.

IARC (1994). Some industrial chemicals. *IARC Monogr Eval Carcinog Risks Hum*, 60:1–560. Available from: http://publications.iarc.fr/78 PMID:7869568

IARC (2002). Some traditional herbal medicines, some mycotoxins, naphthalene and styrene. *IARC Monogr Eval Carcinog Risks Hum*, 82:1–556. Available from: http://publications.iarc.fr/100 PMID:12687954

IARC (2004). Tobacco smoke and involuntary smoking. *IARC Monogr Eval Carcinog Risks Hum*, 83:1–1438. Available from: http://publications.iarc.fr/101 PMID:15285078

IARC (2012). Personal habits and indoor combustions. *IARC Monogr Eval Carcinog Risks Hum*, 100E:1–598. Available from: http://publications.iarc.fr/122 PMID:23193840

IARC (2012a). Arsenic, metals, fibres, and dusts. *IARC Monogr Eval Carcinog Risks Hum*, 100C:1–499. Available from: http://publications.iarc.fr/120 PMID:23189751

IARC (2012b). Chemical agents and related occupations. *IARC Monogr Eval Carcinog Risks Hum*, 100F:1–599. Available from: http://publications.iarc.fr/123 PMID:23189753

IARC (2018). Benzene. *IARC Monogr Eval Carcinog Risks Hum*, 120:1–300. Available from: http://publications.iarc.fr/576 PMID:31769947

ICIS (2014). Chemical profile: US styrene. Available from: https://www.icis.com/resources/news/2014/12/12/9846177/chemical-profile-us-styrene.

IFA (2017). GESTIS international limit values. Germany: Institute for Occupational Safety and Health of the German Social Accident Insurance. Available from: http://limitvalue.ifa.dguv.de/WebForm_gw2.aspx.

IHS Markit (2017). Chemical economics handbook. Available from: https://www.ihs.com/products/styrene-chemical-economics-handbook.html, accessed 26 February 2018.

Ikeda M, Koizumi A, Miyasaka M, Watanabe T (1982). Styrene exposure and biologic monitoring in FRP boat production plants. *Int Arch Occup Environ Health*, 49(3–4):325–39. doi:10.1007/BF00377941 PMID:7068244

ILO (2011). Chemical exposure limits. Geneva, Switzerland: International Labour Organization. Available from: http://www.ilo.org/safework/info/publications/WCMS_151534/lang--en/index.htm, accessed 26 March 2018.

Imbriani M, Ghittori S, Pezzagno G, Capodaglio E (1986). Toluene and styrene in urine as biological exposure indices. *Appl Ind Hyg J*, 1(4):172–6. doi:10.1080/08828032.1986.10390518

Ishidate M Jr, Yoshikawa K (1980). Chromosome aberration tests with Chinese hamster cells in vitro with and without metabolic activation – a comparative study on mutagens and carcinogens. *Arch Toxicol Suppl*, 4:41–4. doi:10.1007/978-3-642-67729-8_8 PMID:7002106

Jablonická A, Karelová J, Poláková H, Vargová M (1988). Analysis of chromosomes in peripheral blood lymphocytes of styrene-exposed workers. *Mutat Res*, 206(2):167–9. doi:10.1016/0165-1218(88)90156-5 PMID:3173388

Jágr M, Mráz J, Linhart I, Stránský V, Pospísil M (2007). Synthesis and characterization of styrene oxide adducts with cysteine, histidine, and lysine in human globin. *Chem Res Toxicol*, 20(10):1442–52. doi:10.1021/tx700057t PMID:17867648

Jahnová E, Tulinská J, Weissová S, Dusinská M, Fuortes L (2002). Effects of occupational exposure to styrene on expression of adhesion molecule on leukocytes. *Hum Exp Toxicol*, 21(5):235–40. doi:10.1191/0960327102ht251oa PMID:12141393

Jankovic J, Jones W, Burkhart J, Noonan G (1991). Environmental study of firefighters. *Ann Occup Hyg*, 35(6):581–602. PMID:1768008

Jansen JG, Vrieling H, van Teijlingen CM, Mohn GR, Tates AD, van Zeeland AA (1995). Marked differences in the role of O^6-alkylguanine in *hprt* mutagenesis in T-lymphocytes of rats exposed *in vivo* to ethylmethanesulfonate, *N*-(2-hydroxyethyl)-*N*-nitrosourea, or *N*-ethyl-*N*-nitrosourea. *Cancer Res*, 55(9):1875–82. PMID:7728755

Jantunen K, Mäki-Paakkanen J, Norppa H (1986). Induction of chromosome aberrations by styrene and vinylacetate in cultured human lymphocytes: dependence on erythrocytes. *Mutat Res*, 159(1–2):109–16. doi:10.1016/0027-5107(86)90119-3 PMID:3941660

Jensen AA, Breum NO, Bacher J, Lynge E (1990). Occupational exposures to styrene in Denmark 1955–88. *Am J Ind Med*, 17(5):593–606. doi:10.1002/ajim.4700170505 PMID:2337083

Johanson G, Ernstgård L, Gullstrand E, Löf A, Osterman-Golkar S, Williams CC, et al. (2000). Styrene oxide in blood, hemoglobin adducts, and urinary metabolites in human volunteers exposed to $^{13}C_8$-styrene vapors. *Toxicol Appl Pharmacol*, 168(1):36–49. doi:10.1006/taap.2000.9007 PMID:11000098

Jonsson F, Johanson G (2002). Physiologically based modeling of the inhalation kinetics of styrene in humans using a Bayesian population approach. *Toxicol Appl Pharmacol*, 179(1):35–49. doi:10.1006/taap.2001.9331 PMID:11884235

Judson R, Houck K, Martin M, Richard AM, Knudsen TB, Shah I, et al. (2016). Editor's highlight: analysis of the effects of cell stress and cytotoxicity on *in vitro* assay activity across a diverse chemical and assay space. *Toxicol Sci*, 152(2):323–39. doi:10.1093/toxsci/kfw092 PMID:27208079

Karami S, Boffetta P, Brennan P, Stewart PA, Zaridze D, Matveev V, et al. (2011). Renal cancer risk and occupational exposure to polycyclic aromatic hydrocarbons and plastics. *J Occup Environ Med*, 53(2):218–23. doi:10.1097/JOM.0b013e31820a40a3 PMID:21270648

Katoh T, Higashi K, Inoue N (1989). Sub-chronic effects of styrene and styrene oxide on lipid peroxidation and the metabolism of glutathione in rat liver and brain. *J Toxicol Sci*, 14(1):1–9. doi:10.2131/jts.14.1 PMID:2738962

Kaufmann W, Mellert W, van Ravenzwaay B, Landsiedel R, Poole A (2005). Effects of styrene and its metabolites on different lung compartments of the mouse – cell proliferation and histomorphology. *Regul Toxicol Pharmacol*, 42(1):24–36. doi:10.1016/j.yrtph.2005.01.002 PMID:15896440

Kaur S, Hollander D, Haas R, Burlingame AL (1989). Characterization of structural xenobiotic modifications in proteins by high sensitivity tandem mass spectrometry. Human hemoglobin treated *in vitro*

with styrene 7,8-oxide. *J Biol Chem*, 264(29):16981–4. PMID:2793839

Kavlock R, Chandler K, Houck K, Hunter S, Judson R, Kleinstreuer N, et al. (2012). Update on EPA's ToxCast program: providing high throughput decision support tools for chemical risk management. *Chem Res Toxicol*, 25(7):1287–302. doi:10.1021/tx3000939 PMID:22519603

Kawai T, Mizunuma K, Yasugi T, Horiguchi S, Moon CS, Zhang ZW, et al. (1995). Effects of methanol on styrene metabolism among workers occupationally exposed at low concentrations. *Arch Environ Contam Toxicol*, 28(4):543–6. doi:10.1007/BF00211640 PMID:7755408

Kelsey KT, Smith TJ, Hammond SK, Letz R, Little JB (1990). Sister-chromatid exchanges in lymphocytes from styrene-exposed boat builders. *Mutat Res*, 241(2):215–21. doi:10.1016/0165-1218(90)90123-J PMID:2345557

Khanna S, Rao GS, Dogra RK, Shukla LJ, Srivastava SN, Dhruv SP, et al. (1994). Styrene induced pancreatic changes in rodents. *Indian J Exp Biol*, 32(1):68–71. PMID:8045607

Kim H, Wang RS, Elovaara E, Raunio H, Pelkonen O, Aoyama T, et al. (1997). Cytochrome P450 isozymes responsible for the metabolism of toluene and styrene in human liver microsomes. *Xenobiotica*, 27(7):657–65. doi:10.1080/004982597240253 PMID:9253143

Kim HY, Finneman JI, Harris CM, Harris TM (2000). Studies of the mechanisms of adduction of 2′-deoxyadenosine with styrene oxide and polycyclic aromatic hydrocarbon dihydrodiol epoxides. *Chem Res Toxicol*, 13(7):625–37. doi:10.1021/tx000054m PMID:10898595

Kingsley I (1976). Health hazard evaluation determination: New York Telephone and Telegraph Company, NY. Cincinnati (OH), USA: National Institute for Occupational Safety and Health.

Kligerman AD, Allen JW, Erexson GL, Morgan DL (1993). Cytogenetic studies of rodents exposed to styrene by inhalation. *IARC Sci Publ*, 127:217–24. PMID:8070867

Knöppel H, Schauenburg H (1989). Screening of household products for the emission of volatile organic compounds. *Environ Int*, 15(1–6):413–8. doi:10.1016/0160-4120(89)90056-1

Kogevinas M, Ferro G, Andersen A, Bellander T, Biocca M, Coggon D, et al. (1994). Cancer mortality in a historical cohort study of workers exposed to styrene. *Scand J Work Environ Health*, 20(4):251–61. doi:10.5271/sjweh.1400 PMID:7801070

Kogevinas M, Ferro G, Saracci R, Andersen A, Biocca M, Coggon D, et al. (1993). Cancer mortality in an international cohort of workers exposed to styrene. *IARC Sci Publ*, 127:289–300. PMID:8070875

Köhlerová R, Stětina R (2003). The repair of DNA damage induced in human peripheral lymphocytes with styrene oxide. *Acta Medica (Hradec Kralove)*, 46(3):95–100. PMID:14677717

Kolstad HA, Juel K, Olsen J, Lynge E (1995). Exposure to styrene and chronic health effects: mortality and incidence of solid cancers in the Danish reinforced plastics industry. *Occup Environ Med*, 52(5):320–7. doi:10.1136/oem.52.5.320 PMID:7795754

Kolstad HA, Lynge E, Olsen J (1993). Cancer incidence in the Danish reinforced plastics industry. *IARC Sci Publ*, 127:301–8. PMID:8070877

Kolstad HA, Lynge E, Olsen J, Breum N (1994). Incidence of lymphohematopoietic malignancies among styrene-exposed workers of the reinforced plastics industry. *Scand J Work Environ Health*, 20(4):272–8. doi:10.5271/sjweh.1398 PMID:7801072

Kolstad HA, Pedersen B, Olsen J, Lynge E, Jensen G, Lisse I, et al. (1996). Clonal chromosome aberrations in myeloid leukemia after styrene exposure. *Scand J Work Environ Health*, 22(1):58–61. doi:10.5271/sjweh.110 PMID:8685676

Kolstad HA, Sønderskov J, Burstyn I (2005). Company-level, semi-quantitative assessment of occupational styrene exposure when individual data are not available. *Ann Occup Hyg*, 49(2):155–65. PMID:15734828

Korn M, Gfrörer W, Filser JG, Kessler W (1994). Styrene-7,8-oxide in blood of workers exposed to styrene. *Arch Toxicol*, 68(8):524–7. doi:10.1007/s002040050107 PMID:7802595

Korn M, Wodarz R, Drysch K, Schmahl FW (1987). Stereometabolism of styrene in man. Urinary excretion of chiral styrene metabolites. *Arch Toxicol*, 60(1–3):86–8. doi:10.1007/BF00296954 PMID:3619649

Korn M, Wodarz R, Drysch K, Schoknecht W, Schmahl FW (1985). Stereometabolism of styrene in man: gas chromatographic determination of phenylethyleneglycol enantiomers and phenylethanol isomers in the urine of occupationally-exposed persons. *Arch Toxicol*, 58(2):110–4. doi:10.1007/BF00348319 PMID:2418806

Koskinen M, Calebiro D, Hemminki K (2000b). Styrene oxide-induced 2 -deoxycytidine adducts: implications for the mutagenicity of styrene oxide. *Chem Biol Interact*, 126(3):201–13. doi:10.1016/S0009-2797(00)00165-4 PMID:10862818

Koskinen M, Hemminki K (1999). Separate deamination mechanisms for isomeric styrene oxide induced N1-adenine adducts. *Org Lett*, 1(8):1233–5. doi:10.1021/ol9909031 PMID:10825973

Koskinen M, Plná K (2000). Specific DNA adducts induced by some mono-substituted epoxides in vitro and in vivo. *Chem Biol Interact*, 129(3):209–29. doi:10.1016/S0009-2797(00)00206-4 PMID:11137062

Koskinen M, Vodička P, Hemminki K (2000a). Adenine N3 is a main alkylation site of styrene oxide in double-stranded DNA. *Chem Biol Interact*, 124(1):13–27. doi:10.1016/S0009-2797(99)00137-4 PMID:10658899

Koskinen M, Vodička P, Hemminki K (2001b). Identification of 1-adenine DNA adducts in workers occupationally exposed to styrene. *J Occup Environ Med*, 43(8):694–700. doi:10.1097/00043764-200108000-00007 PMID:11515252

Koskinen M, Vodicková L, Vodička P, Warner SC, Hemminki K (2001a). Kinetics of formation of specific styrene oxide adducts in double-stranded DNA. *Chem Biol Interact*, 138(2):111–24. doi:10.1016/S0009-2797(01)00254-X PMID:11672695

Kriebel D, Checkoway H, Pearce N (2007). Exposure and dose modelling in occupational epidemiology. *Occup Environ Med*, 64(7):492–8. doi:10.1136/oem.2006.030031 PMID:17582090

Kronoveter KJ, Boiano JM (1984a). Health hazard evaluation report: Charlie's Taxidermy and Gifts, Fleetwood, PA. Cincinnati (OH), USA: National Institute for Occupational Safety and Health.

Krost KJ, Pellizzari ED, Walburn SG, Hubbard SA (1982). Collection and analysis of hazardous organic emissions. *Anal Chem*, 54(4):810–7. doi:10.1021/ac00241a050

Kuricova M, Naccarati A, Kumar R, Koskinen M, Sanyal S, Dusinska M, et al. (2005). DNA repair and *cyclin D1* polymorphisms and styrene-induced genotoxicity and immunotoxicity. *Toxicol Appl Pharmacol*, 207(2 Suppl):302–9. doi:10.1016/j.taap.2004.12.023 PMID:15992842

Kwok RK, Engel LS, Miller AK, Blair A, Curry MD, Jackson WB 2nd, et al.; GuLF STUDY Research Team (2017). The GuLF STUDY: a prospective study of persons involved in the Deepwater Horizon oil spill response and clean-up. *Environ Health Perspect*, 125(4):570–8. doi:10.1289/EHP715 PMID:28362265

Laffon B, Pásaro E, Méndez J (2001a). Effects of styrene-7,8-oxide over *p53*, *p21*, *bcl-2* and *bax* expression in human lymphocyte cultures. *Mutagenesis*, 16(2):127–32. doi:10.1093/mutage/16.2.127 PMID:11230554

Laffon B, Pásaro E, Méndez J (2001b). Genotoxic effects of styrene-7,8-oxide in human white blood cells: comet assay in relation to the induction of sister-chromatid exchanges and micronuclei. *Mutat Res*, 491(1–2):163–72. doi:10.1016/S1383-5718(01)00135-8 PMID:11287309

Laffon B, Pásaro E, Méndez J (2002a). Evaluation of genotoxic effects in a group of workers exposed to low levels of styrene. *Toxicology*, 171(2–3):175–86. doi:10.1016/S0300-483X(01)00572-8 PMID:11836023

Laffon B, Pásaro E, Méndez J (2002b). DNA damage and repair in human leukocytes exposed to styrene-7,8-oxide measured by the comet assay. *Toxicol Lett*, 126(1):61–8. doi:10.1016/S0378-4274(01)00432-5 PMID:11738271

Laffon B, Pérez-Cadahía B, Pásaro E, Méndez J (2003a). Effect of epoxide hydrolase and glutathione *S*-tranferase genotypes on the induction of micronuclei and DNA damage by styrene-7,8-oxide in vitro. *Mutat Res*, 536(1–2):49–59. doi:10.1016/S1383-5718(03)00034-2 PMID:12694745

Laffon B, Pérez-Cadahía B, Pásaro E, Méndez J (2003b). Individual sensitivity to DNA damage induced by styrene in vitro: influence of cytochrome p450, epoxide hydrolase and glutathione *S*-transferase genotypes. *Toxicology*, 186(1–2):131–41. doi:10.1016/S0300-483X(02)00729-1 PMID:12604177

Lai S-T, Locke DC (1983). Stepwise pyrolysis-liquid chromatography and pyrolysis-gas chromatography of polystyrene. *J Chromatogr A*, 255:511–27. doi:10.1016/S0021-9673(01)88306-0

LaRegina J, Bozzelli JW, Harkov J, Gianti S (1986). Volatile organic compounds at hazardous waste sites and a sanitary landfill in New Jersey. An up-to-date review of the present situation. *Environ Prog*, 5(1):18–27. doi:10.1002/ep.670050109

Latham GJ, Forgacs E, Beard WA, Prasad R, Bebenek K, Kunkel TA, et al. (2000). Vertical-scanning mutagenesis of a critical tryptophan in the "minor groove binding track" of HIV-1 reverse transcriptase. Major groove DNA adducts identify specific protein interactions in the minor groove. *J Biol Chem*, 275(20):15025–33. doi:10.1074/jbc.M000279200 PMID:10747890

Latham GJ, Lloyd RS (1994). Deoxynucleotide polymerization by HIV-1 reverse transcriptase is terminated by site-specific styrene oxide adducts after translesion synthesis. *J Biol Chem*, 269(46):28527–30. PMID:7525577

Latham GJ, McNees AG, De Corte B, Harris CM, Harris TM, O'Donnell M, et al. (1996). Comparison of the efficiency of synthesis past single bulky DNA adducts *in vivo* and *in vitro* by the polymerase III holoenzyme. *Chem Res Toxicol*, 9(7):1167–75. doi:10.1021/tx9600558 PMID:8902273

Latham GJ, Zhou L, Harris CM, Harris TM, Lloyd RS (1993). The replication fate of *R*- and *S*-styrene oxide adducts on adenine N^6 is dependent on both the chirality of the lesion and the local sequence context. *J Biol Chem*, 268(31):23427–34. PMID:8226868

Lawton BW, Hoffmann J, Triebig G (2006). The ototoxicity of styrene: a review of occupational investigations. *Int Arch Occup Environ Health*, 79(2):93–102. doi:10.1007/s00420-005-0030-2 PMID:16151828

Lazutka JR, Lekevicius R, Dedonyte V, Maciuleviciute-Gervers L, Mierauskiene J, Rudaitiene S, et al. (1999). Chromosomal aberrations and sister-chromatid exchanges in Lithuanian populations: effects of occupational and environmental exposures. *Mutat Res*, 445(2):225–39. doi:10.1016/S1383-5718(99)00128-X PMID:10575432

Lee SH, Norppa H (1995). Effects of indomethacin and arachidonic acid on sister chromatid exchange induction by styrene and styrene-7,8-oxide. *Mutat Res*, 348(4):175–81. doi:10.1016/0165-7992(95)00051-8 PMID:7477057

Lenvik K, Osvoll PO, Woldbaek T (1999). Occupational exposure to styrene in Norway, 1972–1996. *Appl Occup Environ Hyg*, 14(3):165–70. doi:10.1080/104732299303124 PMID:10453630

Li L, Wei Y, Van Winkle L, Zhang QY, Zhou X, Hu J, et al. (2011). Generation and characterization of a *Cyp2f2*-null mouse and studies on the role of CYP2F2 in naphthalene-induced toxicity in the lung and nasal olfactory mucosa. *J Pharmacol Exp Ther*, 339(1):62–71. doi:10.1124/jpet.111.184671 PMID:21730012

Lickly TD, Breder CV, Rainey ML (1995). A model for estimating the daily dietary intake of a substance from food-contact articles: styrene from polystyrene food-contact polymers. *Regul Toxicol Pharmacol*, 21(3):406–17. doi:10.1006/rtph.1995.1055 PMID:7480894

Lijinsky W (1986). Rat and mouse forestomach tumors induced by chronic oral administration of styrene oxide. *J Natl Cancer Inst*, 77(2):471–6. PMID:3461208

Limasset JC, Simon P, Poirot P, Subra I, Grzebyk M (1999). Estimation of the percutaneous absorption of styrene in an industrial situation. *Int Arch Occup Environ Health*, 72(1):46–51. doi:10.1007/s004200050333 PMID:10029230

Linhart I, Gut I, Smejkal J, Novák J (2000). Biotransformation of styrene in mice. Stereochemical aspects. *Chem Res Toxicol*, 13(1):36–44. doi:10.1021/tx9900829 PMID:10649965

Linhart I, Mráz J, Dabrowská L, Mališ M, Krouželka J, Kořínek M (2012). Vinylphenylmercapturic acids in human urine as biomarkers of styrene ring oxidation. *Toxicol Lett*, 213(2):260–5. doi:10.1016/j.toxlet.2012.06.012 PMID:22750350

Linhart I, Mráz J, Scharff J, Krouzelka J, Dusková S, Nohová H, et al. (2010). New urinary metabolites formed from ring-oxidized metabolic intermediates of styrene. *Chem Res Toxicol*, 23(1):251–7. doi:10.1021/tx9004192 PMID:20020750

Linnainmaa K, Meretoja T, Sorsa M, Vainio H (1978a). Cytogenetic effects of styrene and styrene oxide on human lymphocytes and *Allium cepa*. *Scand J Work Environ Health*, 4(Suppl 2):156–62. doi:10.5271/sjweh.2751 PMID:734401

Linnainmaa K, Meretoja T, Sorsa M, Vainio H (1978b). Cytogenetic effects of styrene and styrene oxide. *Mutat Res*, 58(2–3):277–86. doi:10.1016/0165-1218(78)90020-4 PMID:745617

Lino Cardenas CL, Bourgine J, Cauffiez C, Allorge D, Lo-Guidice JM, Broly F, et al. (2010). Genetic polymorphisms of glycine N-acyltransferase (GLYAT) in a French Caucasian population. *Xenobiotica*, 40(12):853–61. doi:10.3109/00498254.2010.519407 PMID:20925583

Liu SF, Rappaport SM, Pongracz K, Bodell WJ (1988). Detection of styrene oxide-DNA adducts in lymphocytes of a worker exposed to styrene. *IARC Sci Publ*, (89):217–22. PMID:3198203

Loeb LA, Liu PK, Fry M (1986). DNA polymerase-alpha: enzymology, function, fidelity, and mutagenesis. *Prog Nucleic Acid Res Mol Biol*, 33:57–110. doi:10.1016/S0079-6603(08)60020-3 PMID:3797680

Löf A, Lundgren E, Nordqvist MB (1986). Kinetics of styrene in workers from a plastics industry after controlled exposure: a comparison with subjects not previously exposed. *Br J Ind Med*, 43(8):537–43. doi:10.1136/oem.43.8.537 PMID:3730303

Loomis D, Guha N, Kogevinas M, Fontana V, Gennaro V, Kolstad HA, et al. (2019). Cancer mortality in an international cohort of reinforced plastics workers exposed to styrene: a reanalysis. *Occup Environ Med*, 76(3):157–62. doi:10.1136/oemed-2018-105131 PMID:29669820

López P, Batlle R, Salafranca J, Nerín C (2008). Efficiency of whole and skimmed powdered milk for trapping volatile compounds released from plastic containers in high-temperature applications. *J Food Prot*, 71(9):1889–97. doi:10.4315/0362-028X-71.9.1889 PMID:18810874

Loprieno N, Abbondandolo A, Barale R, Baroncelli S, Bonatti S, Bronzetti G, et al. (1976). Mutagenicity of industrial compounds: styrene and its possible metabolite styrene oxide. *Mutat Res*, 40(4):317–24. doi:10.1016/0165-1218(76)90030-6 PMID:796697

Loprieno N, Presciuttini S, Sbrana I, Stretti G, Zaccaro L, Abbondandolo A, et al. (1978). Mutagenicity of industrial compounds. VII. Styrene and styrene oxide: II. Point mutations, chromosome aberrations and DNA repair induction analyses. *Scand J Work Environ Health*, 4(Suppl 2):169–78. doi:10.5271/sjweh.2760 PMID:366743

Lu X-H, Lei J, Zhou D, Fang S-Y, Dong Y-L, Xia Q-H (2010). Selective oxidation of styrene with air over Co_3O_4-MO_x and CoO_x-MO_x/SiO_2. *Indian J Chem*, 49A:1586–92. Available from: http://nopr.niscair.res.in/bitstream/123456789/10724/1/IJCA%2049A%2812%29%201586-1592.pdf.

Luderer U, Tornero-Velez R, Shay T, Rappaport S, Heyer N, Echeverria D (2004). Temporal association between serum prolactin concentration and exposure to styrene. *Occup Environ Med*, 61(4):325–33. doi:10.1136/oem.2002.005561 PMID:15031390

Lutz WK, Cantoreggi S, Velic I (1993). DNA binding and stimulation of cell division in the carcinogenicity of styrene 7,8-oxide. *IARC Sci Publ*, 127:245–52. PMID:8070870

Ma M, Umemura T, Mori Y, Gong Y, Saijo Y, Sata F, et al. (2005). Influence of genetic polymorphisms of styrene-metabolizing enzymes and smoking habits on levels of urinary metabolites after occupational exposure to styrene. *Toxicol Lett*, 160(1):84–91. doi:10.1016/j.toxlet.2005.06.011 PMID:16125881

Macaluso M, Larson R, Delzell E, Sathiakumar N, Hovinga M, Julian J, et al. (1996). Leukemia and cumulative exposure to butadiene, styrene and benzene among workers in the synthetic rubber industry. *Toxicology*,

113(1–3):190–202. doi:10.1016/0300-483X(96)03444-0 PMID:8901898

Macaluso M, Larson R, Lynch J, Lipton S, Delzell E (2004). Historical estimation of exposure to 1,3-butadiene, styrene, and dimethyldithiocarbamate among synthetic rubber workers. *J Occup Environ Hyg*, 1(6):371–90. doi:10.1080/15459620490452004 PMID:15238328

MAFF (1989). Survey of styrene levels in food contact materials and in foods. The Eleventh Report of the Steering Group on Food Surveillance. The Working Party on Styrene (Food Surveillance Paper No. 11). 2nd ed. London, United KingdomMinistry of Agriculture, Fisheries and Food.

Mahler JF, Price HC Jr, O'Connor RW, Wilson RF, Eldridge SR, Moorman MP, et al. (1999). Characterization of hepatocellular resistance and susceptibility to styrene toxicity in B6C3F1 mice. *Toxicol Sci*, 48(1):123–33. doi:10.1093/toxsci/48.1.123 PMID:10330692

Mäki-Paakkanen J (1987). Chromosome aberrations, micronuclei and sister-chromatid exchanges in blood lymphocytes after occupational exposure to low levels of styrene. *Mutat Res*, 189(4):399–406. doi:10.1016/0165-1218(87)90049-8 PMID:3683435

Mäki-Paakkanen J, Walles S, Osterman-Golkar S, Norppa H (1991). Single-strand breaks, chromosome aberrations, sister-chromatid exchanges, and micronuclei in blood lymphocytes of workers exposed to styrene during the production of reinforced plastics. *Environ Mol Mutagen*, 17(1):27–31. doi:10.1002/em.2850170105 PMID:1991456

Malkinson AM (1989). The genetic basis of susceptibility to lung tumors in mice. *Toxicology*, 54(3):241–71. doi:10.1016/0300-483X(89)90062-0 PMID:2650016

Maltoni C, Failla G, Kassapidis G (1979). First experimental demonstration of the carcinogenic effects of styrene oxide; long-term bioassays on Sprague-Dawley rats by oral administration. *Med Lav*, 70(5):358–62. PMID:554914

Mamaca E, Bechmann RK, Torgrimsen S, Aas E, Bjørnstad A, Baussant T, et al. (2005). The neutral red lysosomal retention assay and Comet assay on haemolymph cells from mussels (*Mytilus edulis*) and fish (*Symphodus melops*) exposed to styrene. *Aquat Toxicol*, 75(3):191–201. doi:10.1016/j.aquatox.2005.08.001 PMID:16221498

Manini P, Andreoli R, Poli D, De Palma G, Mutti A, Niessen WM (2002). Liquid chromatography/electrospray tandem mass spectrometry characterization of styrene metabolism in man and in rat. *Rapid Commun Mass Spectrom*, 16(24):2239–48. doi:10.1002/rcm.848 PMID:12478566

Manini P, Buzio L, Andreoli R, Goldoni M, Bergamaschi E, Jakubowski M, et al. (2003). Assessment of biotransformation of the arene moiety of styrene in volunteers and occupationally exposed workers. *Toxicol Appl Pharmacol*, 189(3):160–9. doi:10.1016/S0041-008X(03)00124-8 PMID:12791301

Manini P, De Palma G, Andreoli R, Goldoni M, Mutti A (2004). Determination of urinary styrene metabolites in the general Italian population by liquid chromatography-tandem mass spectrometry. *Int Arch Occup Environ Health*, 77(6):433–6. doi:10.1007/s00420-004-0526-1 PMID:15205964

Manini P, De Palma G, Andreoli R, Marczynski B, Hanova M, Mozzoni P, et al. (2009). Biomarkers of nucleic acid oxidation, polymorphism in, and expression of, *hOGG1* gene in styrene-exposed workers. *Toxicol Lett*, 190(1):41–7. doi:10.1016/j.toxlet.2009.06.862 PMID:19563869

Mansuy D, Leclaire J, Fontecave M, Momenteau M (1984). Oxidation of monosubstituted olefins by cytochromes P-450 and heme models: evidence for the formation of aldehydes in addition to epoxides and allylic alcohols. *Biochem Biophys Res Commun*, 119(1):319–25. doi:10.1016/0006-291X(84)91654-1 PMID:6704124

Marczynski B, Peel M, Baur X (1997b). Changes in high molecular weight DNA fragmentation following human blood exposure to styrene-7,8-oxide. *Toxicology*, 120(2):111–7. doi:10.1016/S0300-483X(97)03643-3 PMID:9184198

Marczynski B, Peel M, Baur X (2000). New aspects in genotoxic risk assessment of styrene exposure – a working hypothesis. *Med Hypotheses*, 54(4):619–23. doi:10.1054/mehy.1999.0907 PMID:10859650

Marczynski B, Rozynek P, Elliehausen HJ, Korn M, Baur X (1997a). Detection of 8-hydroxydeoxyguanosine, a marker of oxidative DNA damage, in white blood cells of workers occupationally exposed to styrene. *Arch Toxicol*, 71(8):496–500. doi:10.1007/s002040050418 PMID:9248627

Marhuenda D, Prieto MJ, Periago JF, Marti J, Perbellini L, Cardona A (1997). Biological monitoring of styrene exposure and possible interference of acetone co-exposure. *Int Arch Occup Environ Health*, 69(6):455–60. doi:10.1007/s004200050174 PMID:9215933

Matanoski G, Elliott E, Tao X, Francis M, Correa-Villasenor A, Santos-Burgoa C (1997). Lymphohematopoietic cancers and butadiene and styrene exposure in synthetic rubber manufacture. *Ann N Y Acad Sci*, 837(1):157–69. doi:10.1111/j.1749-6632.1997.tb56872.x PMID:9472338

Matanoski G, Francis M, Correa-Villasenor A, Elliott E, Santos-Burgoa C, Schwartz L (1993). Cancer epidemiology among styrene-butadiene rubber workers. In: Sorsa M, Peltonen K, Vainio H, Hemminki K, editors. Butadiene and styrene: assessment of health hazards (IARC Scientific Publications, No. 127). Lyon, France: IARC Press; pp. 363–74.

Matanoski GM, Santos-Burgoa C, Schwartz L (1990). Mortality of a cohort of workers in the styrene-butadiene polymer manufacturing industry (1943-1982).

Environ Health Perspect, 86:107–17. doi:10.1289/ehp.9086107 PMID:2401250

Matanoski GM, Schwartz L (1987). Mortality of workers in styrene-butadiene polymer production. *J Occup Med*, 29(8):675–80. PMID:3655951

Mateuca RA, Roelants M, Iarmarcovai G, Aka PV, Godderis L, Tremp A, et al. (2008). *hOGG1*[326], *XRCC1*[399] and *XRCC3*[241] polymorphisms influence micronucleus frequencies in human lymphocytes *in vivo*. *Mutagenesis*, 23(1):35–41. doi:10.1093/mutage/gem040 PMID:17971348

Matsuoka A, Hayashi M, Ishidate M Jr (1979). Chromosomal aberration tests on 29 chemicals combined with S9 mix in vitro. *Mutat Res*, 66(3):277–90. doi:10.1016/0165-1218(79)90089-2 PMID:375085

McDougal JN, Jepson GW, Clewell HJ 3rd, Gargas ML, Andersen ME (1990). Dermal absorption of organic chemical vapors in rats and humans. *Fundam Appl Toxicol*, 14(2):299–308. doi:10.1016/0272-0590(90)90209-3 PMID:2318354

Meinhardt TJ, Lemen RA, Crandall MS, Young RJ (1982). Environmental epidemiologic investigation of the styrene-butadiene rubber industry. Mortality patterns with discussion of the hematopoietic and lymphatic malignancies. *Scand J Work Environ Health*, 8(4):250–9. doi:10.5271/sjweh.2469 PMID:7170621

Merck (2017a). Merck online catalogue. No. 807679: styrene. Available from: http://www.merckmillipore.com/DK/en/search/styrene?search=&TrackingSearchType=SB+-+Search+Box&SearchContextPageletUUID=&SearchTerm=styrene, accessed 26 February 2018.

Merck (2017b). Merck online catalogue, No. 803202: epoxystyrene. Available from: http://www.merckmillipore.com/DK/en/product/Epoxystyrene,MDA_CHEM-803202#documentation, accessed 26 February 2018.

Meretoja T, Järventaus H, Sorsa M, Vainio H (1978). Chromosome aberrations in lymphocytes of workers exposed to styrene. *Scand J Work Environ Health*, 4(Suppl 2):259–64. doi:10.5271/sjweh.2739 PMID:734413

Meretoja T, Vainio H, Sorsa M, Härkönen H (1977). Occupational styrene exposure and chromosomal aberrations. *Mutat Res*, 56(2):193–7. doi:10.1016/0027-5107(77)90209-3

Merker GH, Metzner G, Raabe F (2006). T_{H1}-directed modulation of in vitro cytokine production in human peripheral blood mononuclear cells by styrene-7.8-oxide. *Toxicol Lett*, 160(2):105–11. doi:10.1016/j.toxlet.2005.06.014 PMID:16111840

Meszka-Jordan A, Mahlapuu R, Soomets U, Carlson GP (2009). Oxidative stress due to (*R*)-styrene oxide exposure and the role of antioxidants in non-Swiss albino (NSA) mice. *J Toxicol Environ Health A*, 72(10):642–50. doi:10.1080/15287390902769436 PMID:19308849

Mierauskiene J, Lekevicius R, Lazutka JR (1993). Anticlastogenic effects of Aevitum intake in a group of chemical industry workers. *Hereditas*, 118(3):201–4. doi:10.1111/j.1601-5223.1993.00201.x PMID:8376175

Migliore L, Naccarati A, Coppedè F, Bergamaschi E, De Palma G, Voho A, et al. (2006). Cytogenetic biomarkers, urinary metabolites and metabolic gene polymorphisms in workers exposed to styrene. *Pharmacogenet Genomics*, 16(2):87–99. doi:10.1097/01.fpc.0000182783.70006.44 PMID:16424821

Migliore L, Naccarati A, Zanello A, Scarpato R, Bramanti L, Mariani M (2002). Assessment of sperm DNA integrity in workers exposed to styrene. *Hum Reprod*, 17(11):2912–8. doi:10.1093/humrep/17.11.2912 PMID:12407048

Mikes P, Korínek M, Linhart I, Krouzelka J, Dabrowská L, Stránský V, et al. (2010). Urinary N3 adenine DNA adducts in humans occupationally exposed to styrene. *Toxicol Lett*, 197(3):183–7. doi:10.1016/j.toxlet.2010.05.015 PMID:20553827

Mikes P, Korínek M, Linhart I, Krouzelka J, Frantík E, Vodicková L, et al. (2009). Excretion of urinary N7 guanine and N3 adenine DNA adducts in mice after inhalation of styrene. *Toxicol Lett*, 184(1):33–7. doi:10.1016/j.toxlet.2008.10.010 PMID:19015015

Miligi L, Costantini AS, Benvenuti A, Kriebel D, Bolejack V, Tumino R, et al. (2006). Occupational exposure to solvents and the risk of lymphomas. *Epidemiology*, 17(5):552–61. doi:10.1097/01.ede.0000231279.30988.4d PMID:16878041

Miller SL, Branoff S, Nazaroff WW (1998). Exposure to toxic air contaminants in environmental tobacco smoke: an assessment for California based on personal monitoring data. *J Expo Anal Environ Epidemiol*, 8(3):287–311. PMID:9679213

Mizutani T, Irie Y, Nakanishi K (1994). Styrene-induced hepatotoxicity in mice depleted of glutathione. *Res Commun Mol Pathol Pharmacol*, 86(3):361–74. PMID:7712112

Mögel I, Baumann S, Böhme A, Kohajda T, von Bergen M, Simon J-C, et al. (2011). The aromatic volatile organic compounds toluene, benzene and styrene induce COX-2 and prostaglandins in human lung epithelial cells via oxidative stress and p38 MAPK activation. *Toxicology*, 289(1):28–37. doi:10.1016/j.tox.2011.07.006 PMID:21801798

Mörbt N, Mögel I, Kalkhof S, Feltens R, Röder-Stolinski C, Zheng J, et al. (2009). Proteome changes in human bronchoalveolar cells following styrene exposure indicate involvement of oxidative stress in the molecular-response mechanism. *Proteomics*, 9(21):4920–33. doi:10.1002/pmic.200800836 PMID:19862763

Morgan DL, Mahler JF, Moorman MP, Wilson RE, Price HC Jr, Richards JH, et al. (1995). Comparison of styrene hepatotoxicity in B6C3F1 and Swiss mice. *Fundam Appl Toxicol*, 27(2):217–22. doi:10.1006/faat.1995.1126 PMID:8529816

Morgan DL, Mahler JF, O'Connor RW, Price HC Jr, Adkins B Jr (1993). Styrene inhalation toxicity studies in mice. I. Hepatotoxicity in B6C3F1 mice. *Fundam Appl Toxicol*, 20(3):325–35. doi:10.1006/faat.1993.1042 PMID:8504906

Morgan DL, Mahler JF, Wilson RE, Moorman MP, Price HC Jr, Patrick KR (1997). Effects of various pretreatments on the hepatotoxicity of inhaled styrene in the B6C3F1 mouse. *Xenobiotica*, 27(4):401–11. doi:10.1080/004982597240550 PMID:9149379

Morris JB (2000). Uptake of styrene in the upper respiratory tract of the CD mouse and Sprague-Dawley rat. *Toxicol Sci*, 54(1):222–8. doi:10.1093/toxsci/54.1.222 PMID:10746949

Moschel RC, Hudgins WR, Dipple A (1986). Electrophilic attack at carbon-5 of guanine nucleosides: structure and properties of the resulting guanidinoimidazole products. *IARC Sci Publ*, (70):235–40. PMID:3793175

Mráz J, Hanzlíková I, Dušková Š, Dabrowská L, Chrástecká H, Vajtrová R, et al. (2016b). Biological fate of styrene oxide adducts with globin: elimination of cleavage products in the rat urine. *Toxicol Lett*, 261:26–31. doi:10.1016/j.toxlet.2016.08.022 PMID:27575569

Mráz J, Hanzlíková I, Moulisová A, Dušková Š, Hejl K, Bednářová A, et al. (2016a). Hydrolytic cleavage products of globin adducts in urine as possible biomarkers of cumulative dose. Proof of concept using styrene oxide as a model adduct-forming compound. *Chem Res Toxicol*, 29(4):676–86. doi:10.1021/acs.chemrestox.5b00518 PMID:26954110

Mutti A, Buzio C, Perazzoli F, Bergamaschi E, Bocchi MC, Selis L, et al. (1992). Lymphocyte subpopulations in workers exposed occupationally to styrene. *Med Lav*, 83(2):167–77. PMID:1630405 [in Italian]

Mutti A, Vescovi PP, Falzoi M, Arfini G, Valenti G, Franchini I (1984). Neuroendocrine effects of styrene on occupationally exposed workers. *Scand J Work Environ Health*, 10(4):225–8. doi:10.5271/sjweh.2340 PMID:6436966

Naccarati A, Zanello A, Landi S, Consigli R, Migliore L (2003). Sperm-FISH analysis and human monitoring: a study on workers occupationally exposed to styrene. *Mutat Res*, 537(2):131–40. doi:10.1016/S1383-5718(03)00081-0 PMID:12787818

Nagwekar JB, Kostenbauder HB (1970). Kinetics of elimination of optical isomers of mandelic acid and effect of probenecid on their elimination kinetics in humans. *J Pharm Sci*, 59(12):1775–80. doi:10.1002/jps.2600591214 PMID:5532902

Nakajima T, Elovaara E, Gonzalez FJ, Gelboin HV, Raunio H, Pelkonen O, et al. (1994a). Styrene metabolism by cDNA-expressed human hepatic and pulmonary cytochromes P450. *Chem Res Toxicol*, 7(6):891–6. doi:10.1021/tx00042a026 PMID:7696548

Nakajima T, Wang RS, Elovaara E, Gonzalez FJ, Gelboin HV, Vainio H, et al. (1994b). CYP2C11 and CYP2B1 are major cytochrome P450 forms involved in styrene oxidation in liver and lung microsomes from untreated rats, respectively. *Biochem Pharmacol*, 48(4):637–42. doi:10.1016/0006-2952(94)90039-6 PMID:8080435

Nakamura SI, Oda Y, Shimada T, Oki I, Sugimoto K (1987). SOS-inducing activity of chemical carcinogens and mutagens in *Salmonella typhimurium* TA1535/pSK1002: examination with 151 chemicals. *Mutat Res*, 192(4):239–46. doi:10.1016/0165-7992(87)90063-7 PMID:3317033

Nano R, Rossi A, Fenoglio C, de Piceis Polver P (2000). Evaluation of a possible styrene-induced damage to the haematopoietic tissues in the rat. *Anticancer Res*, 20:3A: 1615–9. PMID:10928080

National Research Council (2014). Review of the styrene assessment in the National Toxicology Program 12th Report on Carcinogens. Washington (DC), USA: The National Academies Press.

NTP (2008). Final report on carcinogens background document for styrene. *Rep Carcinog Backgr Doc*, (8-5978):i-462. PMID:20737009

Newhook R, Caldwell I (1993). Exposure to styrene in the general Canadian population. *IARC Sci Publ*, 127:27–33. PMID:8070873

Niaz K, Hassan FI, Mabqool F, Khan F, Momtaz S, Baeeri M, et al. (2017a). Effect of styrene exposure on plasma parameters, molecular mechanisms and gene expression in rat model islet cells. *Environ Toxicol Pharmacol*, 54:62–73. doi:10.1016/j.etap.2017.06.020 PMID:28688303

Niaz K, Mabqool F, Khan F, Ismail Hassan F, Baeeri M, Navaei-Nigjeh M, et al. (2017b). Molecular mechanisms of action of styrene toxicity in blood plasma and liver. *Environ Toxicol*, 32(10):2256–66. doi:10.1002/tox.22441 PMID:28678435

Nicholson WJ, Selikoff IJ, Seidman H (1978). Mortality experience of styrene-polystyrene polymerization workers. Initial findings. *Scand J Work Environ Health*, 4(Suppl 2):247–52. doi:10.5271/sjweh.2741 PMID:734411

NIH (2017). Tox21 Data Browser. Available from: https://tripod.nih.gov/tox21/samples, accessed 15 October 2017.

NIOSH (1983). Criteria for a recommended standard: occupational exposure to styrene. Cincinnati (OH), USA: United States Department of Health and Human Services, Public Health Service, Centers for Disease Control, National Institute for Occupational Safety and Health.

NIOSH (1994). *M*ethod No. 1501. NIOSH manual of analytical methods: hydrocarbons, aromatic, 4th ed. Cincinnati (OH), USA: National Institute for Occupational Safety and Health. Available from: https://www.cdc.gov/niosh/docs/2003-154/default.html.

Nishi Y, Hasegawa MM, Taketomi M, Ohkawa Y, Inui N (1984). Comparison of 6-thioguanine-resistant mutation and sister chromatid exchanges in Chinese hamster V79 cells with forty chemical and physical agents. *Cancer Res*, 44(8):3270–9. PMID:6744262

Nissen MS, Stokholm ZA, Christensen MS, Schlünssen V, Vestergaard JM, Iversen IB, et al. (2018). Sinonasal adenocarcinoma following styrene exposure in the reinforced plastics industry. *Occup Environ Med*, 75(6):412–4. doi:10.1136/oemed-2017-104974 PMID:29540567

Nordenson I, Beckman L (1984). Chromosomal aberrations in lymphocytes of workers exposed to low levels of styrene. *Hum Hered*, 34(3):178–82. doi:10.1159/000153458 PMID:6469264

Norppa H (1981). Styrene and vinyltoluene induce micronuclei in mouse bone marrow. *Toxicol Lett*, 8(4–5):247–51. doi:10.1016/0378-4274(81)90109-0 PMID:7268808

Norppa H, Elovaara E, Husgafvel-Pursiainen K, Sorsa M, Vainio H (1979). Effects of styrene oxide on chromosome aberrations, sister chromatid exchange and hepatic drug biotransformation in Chinese hamsters in vivo. *Chem Biol Interact*, 26(3):305–15. doi:10.1016/0009-2797(79)90033-4 PMID:509693

Norppa H, Sorsa M, Vainio H (1980). Chromosomal aberrations in bone marrow of Chinese hamsters exposed to styrene and ethanol. *Toxicol Lett*, 5(3–4):241–4. doi:10.1016/0378-4274(80)90066-1 PMID:7466852

Norppa H, Tursi F, Einistö P (1985). Erythrocytes as a metabolic activation system in mutagenicity tests. In: Janiaud P, Averbeck D, Moustacchi E, editors. Mutagenesis and genetic toxicology theoretical and practical results (INSERM Vol. 119). Paris, France: Editions INSERM; pp. 35–50.

Norppa H, Vainio H (1983a). Induction of sister-chromatid exchanges by styrene analogues in cultured human lymphocytes. *Mutat Res*, 116(3–4):379–87. doi:10.1016/0165-1218(83)90076-9 PMID:6835253

Norppa H, Vainio H (1983b). Genetic toxicity of styrene and some of its derivatives. *Scand J Work Environ Health*, 9(2 Spec No):108–14. doi:10.5271/sjweh.2436 PMID:6648407

Norppa H, Vainio H, Sorsa M (1983). Metabolic activation of styrene by erythrocytes detected as increased sister chromatid exchanges in cultured human lymphocytes. *Cancer Res*, 43(8):3579–82. PMID:6861129

NTP (1979a). Bioassay of styrene for possible carcinogenicity (CAS No. 100-42-5). *Natl Toxicol Program Tech Rep Ser*, 185:1–107. PMID:12778193

NTP (1979b). Bioassay of a solution of β-nitrostyrene and styrene for possible carcinogenicity. *Natl Cancer Inst Carcinog Tech Rep Ser*, 170:1–99. PMID:12799693

NTP (2016a). Styrene. Report on carcinogens. 14th ed. Research Triangle Park (NC), USA: United States Department of Health and Human Services, Public Health Service. Available from: https://ntp.niehs.nih.gov/ntp/roc/content/profiles/styrene.pdf, accessed 12 February 2018.

NTP (2016b). Styrene oxide. Report on carcinogens. 14th ed. Research Triangle Park (NC), USA: United States Department of Health and Human Services, Public Health Service. Available from: https://ntp.niehs.nih.gov/ntp/roc/content/profiles/styreneoxide.pdf, accessed 12 February 2018.

Nylander-French LA, Kupper LL, Rappaport SM (1999). An investigation of factors contributing to styrene and styrene-7,8-oxide exposures in the reinforced-plastics industry. *Ann Occup Hyg*, 43(2):99–105. doi:10.1016/S0003-4878(98)00080-5 PMID:10206038

Oberheitmann B, Frentzel-Beyme R, Hoffmann W (2001). An application of the challenge assay in boat builders exposed to low levels of styrene – a feasibility study of a possible biomarker for acquired susceptibility. *Int J Hyg Environ Health*, 204(1):23–9. doi:10.1078/1438-4639-00968 PMID:11725341

OEHHA (2010). Public health goal for styrene in drinking water. Sacramento (CA), USA: Office of Environmental Health Hazard Assessment, State of California Environmental Protection Agency. Available from: https://oehha.ca.gov/media/downloads/water/chemicals/phg/122810styrene.pdf, accessed 18 June 2018.

Oesch F, Herrero ME, Hengstler JG, Lohmann M, Arand M (2000). Metabolic detoxification: implications for thresholds. *Toxicol Pathol*, 28(3):382–7. doi:10.1177/019262330002800305 PMID:10862554

Oesch F, Thoenen H, Fahrlaender H (1974). Epoxide hydrase in human liver biopsy specimens: assay and properties. *Biochem Pharmacol*, 23(8):1307–17. doi:10.1016/0006-2952(74)90333-5 PMID:4207548

Okada Y, Nakagoshi A, Tsurukawa M, Matsumura C, Eiho J, Nakano T (2012). Environmental risk assessment and concentration trend of atmospheric volatile organic compounds in Hyogo Prefecture, Japan. *Environ Sci Pollut Res Int*, 19(1):201–13. doi:10.1007/s11356-011-0550-0 PMID:21717170

Okun AH, Beaumont JJ, Meinhardt TJ, Crandall MS (1985). Mortality patterns among styrene-exposed boatbuilders. *Am J Ind Med*, 8(3):193–205. doi:10.1002/ajim.4700080305 PMID:4050802

Ollikainen T, Hirvonen A, Norppa H (1998). Influence of GSTT1 genotype on sister chromatid exchange induction by styrene-7,8-oxide in cultured human lymphocytes. *Environ Mol Mutagen*, 31(4):311–5. doi:10.1002/(SICI)1098-2280(1998)31:4<311::AID-EM2>3.0.CO;2-L PMID:9654239

Ong CN, Shi CY, Chia SE, Chua SC, Ong HY, Lee BL, et al. (1994). Biological monitoring of exposure to low concentrations of styrene. *Am J Ind Med*, 25(5):719–30. doi:10.1002/ajim.4700250511 PMID:8030642

OSHA (2018). Occupational Safety and Health Standards, Toxic and Hazardous Substances, Annotated OSHA Z-2 Table. Washington (DC), USA: Occupational Safety and Health Administration; pp. 7–19. Available from: https://www.osha.gov/dsg/annotated-pels/tablez-2.html.
Osterman-Golkar S, Christakopoulos A, Zorcec V, Svensson K (1995). Dosimetry of styrene 7,8-oxide in styrene- and styrene oxide-exposed mice and rats by quantification of haemoglobin adducts. *Chem Biol Interact*, 95(1–2):79–87. doi:10.1016/0009-2797(94)03348-X PMID:7697755
Ott MG, Kolesar RC, Scharnweber HC, Schneider EJ, Venable JR (1980). A mortality survey of employees engaged in the development or manufacture of styrene-based products. *J Occup Med*, 22(7):445–60. PMID:7411258
Otteneder M, Eder E, Lutz WK (1999). ^{32}P-Postlabeling analysis of DNA adducts of styrene 7,8-oxide at the O^6-position of guanine. *Chem Res Toxicol*, 12(1):93–9. doi:10.1021/tx980028c PMID:9894023
Otteneder M, Lutz U, Lutz WK (2002). DNA adducts of styrene-7,8-oxide in target and non-target organs for tumor induction in rat and mouse after repeated inhalation exposure to styrene. *Mutat Res*, 500(1–2):111–6. doi:10.1016/S0027-5107(02)00008-8 PMID:11890940
Pachecka J, Gariboldi P, Cantoni L, Belvedere G, Mussini E, Salmona M (1979). Isolation and structure determination of enzymatically formed styrene oxide glutathione conjugates. *Chem Biol Interact*, 27(2–3):313–21. doi:10.1016/0009-2797(79)90134-0 PMID:498358
Panke S, Held M, Wubbolts MG, Witholt B, Schmid A (2002). Pilot-scale production of (*S*)-styrene oxide from styrene by recombinant *Escherichia coli* synthesizing styrene monooxygenase. *Biotechnol Bioeng*, 80(1):33–41. doi:10.1002/bit.10346 PMID:12209784
Pantarotto C, Fanelli R, Belletti I, Bidoli F (1980). Determination of styrene in biological specimens by gas chromatography-selected ion monitoring: distribution in mice. *Anal Biochem*, 105(2):340–7. doi:10.1016/0003-2697(80)90467-4 PMID:7457839
Paolini M, Sapigni E, Hrelia P, Grilli S, Cantelli-Forti G (1988). Isolation of S9 fractions from mouse and rat with increased enzyme activities after repeated administration of cytochrome P-450 and P-448 inducers. *Mutagenesis*, 3(3):239–43. doi:10.1093/mutage/3.3.239 PMID:3045486
Pauwels W, Veulemans H (1998). Comparison of ethylene, propylene and styrene 7,8-oxide in vitro adduct formation on N-terminal valine in human haemoglobin and on *N*-7-guanine in human DNA. *Mutat Res*, 418(1):21–33. doi:10.1016/S1383-5718(98)00106-5 PMID:9748485
Pauwels W, Vodicèka P, Severi M, Plná K, Veulemans H, Hemminki K (1996). Adduct formation on DNA and haemoglobin in mice intraperitoneally administered with styrene. *Carcinogenesis*, 17(12):2673–80. doi:10.1093/carcin/17.12.2673 PMID:9006105
Pekari K, Nylander-French L, Pfäffli P, Sorsa M, Aitio A (1993). Biological monitoring of exposure to styrene – assessment of different approaches. *J Occup Med Toxicol*, 2:115–26.
Pellizzari ED, Bunch JE, Berkley RE, McRae J (1976). Determination of trace hazardous organic vapor pollutants in ambient atmospheres by gas chromatography/mass spectrometry/computer. *Anal Chem*, 48(6):803–7. doi:10.1021/ac60370a003 PMID:57732
Penttilä M, Sorsa M, Vainio H (1980). Inability of styrene to induce nondisjunction in *Drosophila* or a positive micronucleus test in the Chinese hamster. *Toxicol Lett*, 6(2):119–23. doi:10.1016/0378-4274(80)90178-2 PMID:6774448
Perbellini L, Mozzo P, Turri PV, Zedde A, Brugnone F (1988). Biological exposure index of styrene suggested by a physiologico-mathematical model. *Int Arch Occup Environ Health*, 60(3):187–93. doi:10.1007/BF00378696 PMID:3384484
Petrova S, Mikhaĭlova A, Donchev N (1992). The effect of elevated amounts of ascorbic acid on the status of the vitamin and lung disorders in guinea pigs inhaling styrene. *Probl Khig*, 17:137–45. PMID:1364534 [in Bulgarian]
Pfäffli P, Hesso A, Vainio H, Hyvönen M (1981). 4-Vinylphenol excretion suggestive of arene oxide formation in workers occupationally exposed to styrene. *Toxicol Appl Pharmacol*, 60(1):85–90. doi:10.1016/0041-008X(81)90138-1 PMID:6792746
Pfäffli P, Säämänen A (1993). The occupational scene of styrene. In: Sorsa MKP, Vainio H, Hemminki K, editors. Butadiene and styrene: assessment of health hazards (IARC Scientific Publications, No. 127). Lyon, France: International Agency for Research on Cancer; pp. 15–26.
Pfäffli P, Vainio H, Hesso A (1979). Styrene and styrene oxide concentrations in the air during the lamination process in the reinforced plastics industry. *Scand J Work Environ Health*, 5(2):158–61. doi:10.5271/sjweh.2656 PMID:472687
Phillips DH, Farmer PB (1994). Evidence for DNA and protein binding by styrene and styrene oxide. *Crit Rev Toxicol*, 24(Suppl):S35–46. doi:10.3109/10408449409020139 PMID:7818770
Philo MR, Fordham PJ, Damant AP, Castle L (1997). Measurement of styrene oxide in polystyrenes, estimation of migration to foods, and reaction kinetics and products in food simulants. *Food Chem Toxicol*, 35(8):821–6. doi:10.1016/S0278-6915(97)00054-9 PMID:9350227
Pierce CH, Tozer TN (1992). Styrene in adipose tissue of nonoccupationally exposed persons. *Environ Res*, 58(2):230–5. doi:10.1016/S0013-9351(05)80218-X PMID:1511676

Plastemart.com (2017). China initiates anti-dumping investigations on styrene monomer imports from South Korea, Taiwan, US. Available from: http://www.plastemart.com/news-plastics-information/china-initiates-anti-dumping-investigations-on-styrene-monomer-imports-from-s-korea-taiwan-us/45050.

Plna K, Nilsson R, Koskinen M, Segerbäck D (1999). ^{32}P-postlabelling of propylene oxide 1- and N^{6}-substituted adenine and 3-substituted cytosine/uracil: formation and persistence *in vitro* and *in vivo*. *Carcinogenesis*, 20(10):2025–32. doi:10.1093/carcin/20.10.2025 PMID:10506120

Pohlová H, Rössner P, Srám RJ (1984). Cytogenetic analysis of human peripheral blood lymphocytes in culture exposed in vitro to styrene and styrene oxide. *J Hyg Epidemiol Microbiol Immunol*, 29(3):269–74. PMID:6443360

Pohlová H, Srám RJ (1985). Cytogenetic analysis of peripheral blood lymphocytes of workers occupationally exposed to styrene. *J Hyg Epidemiol Microbiol Immunol*, 29(2):155–61. PMID:4020119

Poirier M, Fournier M, Brousseau P, Morin A (2002). Effects of volatile aromatics, aldehydes, and phenols in tobacco smoke on viability and proliferation of mouse lymphocytes. *J Toxicol Environ Health A*, 65(19):1437–51. doi:10.1080/00984100290071342 PMID:12396875

Poncelet F, de Meester C, Duverger-van Bogaert M, Lambotte-Vandepaer M, Roberfroid M, Mercier M (1980). Influence of experimental factors on the mutagenicity of vinylic monomers. *Arch Toxicol Suppl*, 4:63–6. doi:10.1007/978-3-642-67729-8_14 PMID:7002109

Ponomarkov V, Cabral JRP, Wahrendorf J, Galendo D (1984). A carcinogenicity study of styrene-7,8-oxide in rats. *Cancer Lett*, 24(1):95–101. doi:10.1016/0304-3835(84)90085-5 PMID:6498803

Ponomarkov V, Tomatis L (1978). Effects of long-term oral administration of styrene to mice and rats. *Scand J Work Environ Health*, 4(Suppl 2):127–35. doi:10.5271/sjweh.2754 PMID:734398

Preston RJ, Abernethy DJ (1993). Studies of the induction of chromosomal aberration and sister chromatid exchange in rats exposed to styrene by inhalation. *IARC Sci Publ*, 127:225–33. PMID:8070868

Prieto MJ, Berenguer V, Marhuenda D, Cardona A (2000). Purge-and-trap gas chromatographic determination of styrene in urine and blood. Application to exposed workers. *J Chromatogr B Biomed Sci Appl*, 741(2):301–6. doi:10.1016/S0378-4347(00)00088-8 PMID:10872600

Prieto MJ, Marhuenda D, Cardona A (2002). Analysis of styrene and its metabolites in blood and urine of workers exposed to both styrene and acetone. *J Anal Toxicol*, 26(1):23–8. doi:10.1093/jat/26.1.23 PMID:11888014

Prieto-Castelló MJ, Cardona A, Marhuenda D, Roel JM, Corno A (2010). Use of the CYP2E1 genotype and phenotype for the biological monitoring of occupational exposure to styrene. *Toxicol Lett*, 192(1):34–9. doi:10.1016/j.toxlet.2009.01.011 PMID:20117323

PubChem (2017). Styrene oxide. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Styrene_oxide#section=Top.

Qian C, Dipple A (1995). Different mechanisms of aralkylation of adenosine at the 1- and N^{6}-positions. *Chem Res Toxicol*, 8(3):389–95. doi:10.1021/tx00045a010 PMID:7578925

Ramsey JC, Young JD, Karbowski RJ, Chenoweth MB, McCarty LP, Braun WH (1980). Pharmacokinetics of inhaled styrene in human volunteers. *Toxicol Appl Pharmacol*, 53(1):54–63. doi:10.1016/0041-008X(80)90381-6 PMID:7385239

Rappaport SM, Ting D, Jin Z, Yeowell-O'Connell K, Waidyanatha S, McDonald T (1993). Application of Raney nickel to measure adducts of styrene oxide with hemoglobin and albumin. *Chem Res Toxicol*, 6(2):238–44. doi:10.1021/tx00032a014 PMID:7682851

Rappaport SM, Yeowell-O'Connell K, Bodell W, Yager JW, Symanski E (1996). An investigation of multiple biomarkers among workers exposed to styrene and styrene-7,8-oxide. *Cancer Res*, 56(23):5410–6. PMID:8968094

Reed LD (1983). Health hazard evaluation report: Columbia College, Columbia, MO. Cincinnati (OH), USA: National Institute for Occupational Safety and Health.

Rehwagen M, Schlink U, Herbarth O (2003). Seasonal cycle of VOCs in apartments. *Indoor Air*, 13(3):283–91. doi:10.1034/j.1600-0668.2003.00206.x PMID:12950592

Rizzo R, Quintavalle S, Stignani M, Franzè F, Melchiorri L, De Rosa E, et al. (2009). Reduced production of anti-inflammatory soluble HLA-G molecules in styrene exposed workers. *Environ Toxicol Pharmacol*, 27(2):303–5. doi:10.1016/j.etap.2008.10.013 PMID:21783957

Röder-Stolinski C, Fischäder G, Oostingh GJ, Feltens R, Kohse F, von Bergen M, et al. (2008). Styrene induces an inflammatory response in human lung epithelial cells via oxidative stress and NF-kappaB activation. *Toxicol Appl Pharmacol*, 231(2):241–7. doi:10.1016/j.taap.2008.04.010 PMID:18554678

Rodriguez-Arnaiz R (1998). Biotransformation of several structurally related 2B compounds to reactive metabolites in the somatic w/w+ assay of *Drosophila melanogaster*. *Environ Mol Mutagen*, 31(4):390–401. doi:10.1002/(SICI)1098-2280(1998)31:4<390::AID-EM12>3.0.CO;2-7 PMID:9654249

Rota C, Chignell CF, Mason RP (1999). Evidence for free radical formation during the oxidation of 2′-7′-dichlorofluorescin to the fluorescent dye 2′-7′-dichlorofluorescein by horseradish peroxidase: possible implications for oxidative stress measurements. *Free Radic Biol Med*, 27(7-8):873–81. doi:10.1016/S0891-5849(99)00137-9 PMID:10515592

Ruder AM, Bertke SJ (2017). Cancer incidence among boatbuilding workers exposed to styrene. *Am J Ind Med*, 60(7):651–7. doi:10.1002/ajim.22735 PMID:28616886

Ruder AM, Meyers AR, Bertke SJ (2016). Mortality among styrene-exposed workers in the reinforced plastic boatbuilding industry. *Occup Environ Med*, 73(2):97–102. doi:10.1136/oemed-2015-102990 PMID:26574575

Ruder AM, Ward EM, Dong M, Okun AH, Davis-King K (2004). Mortality patterns among workers exposed to styrene in the reinforced plastic boatbuilding industry: an update. *Am J Ind Med*, 45(2):165–76. doi:10.1002/ajim.10349 PMID:14748047

Ruhe RL, Jannerfeldt ER (1980). Health hazard evaluation report: Metamora Products Corporation, Elkland, PA. Cincinnati (OH), USA: National Institute for Occupational Safety and Health.

Ruth JH (1986). Odor thresholds and irritation levels of several chemical substances: a review. *Am Ind Hyg Assoc J*, 47(3):A142–51. doi:10.1080/15298668691389595 PMID:3706135

Rutkowski JV, Levin BC (1986). Acrylonitrile-butadiene-styrene copolymers (ABS): pyrolysis and combustion products and their toxicity – a review of the literature. *Fire Mater*, 10(3–4):93–105. doi:10.1002/fam.810100303

Säämänen A (1998). Methods to control styrene exposure in the reinforced plastic industry [thesis]. Espoo, Finland: Technical Research Centre of Finland.

Säämänen A, Anttila A, Pfäffli P (1991a). Styreeni [Styrene]. Exposures at Work 9. Helsinki, Finland: Työterveyslaitos, Työsuojelurahasto [National Institute of Occupational Health, Work Environment Fund]. [in Finnish]

Säämänen A, Pfäffli P, Niemelä R (1993). Altistumishuippujen vähentäminen polyesterilujitemuovityössä [Reduction of peak exposures in the reinforced plastics industry]. Helsinki, Finland: Työsuojelurahasto Työterveyslaitos. [in Finnish]

Säämänen AJ, Niemelä RI, Blomqvist TK, Nikander EM (1991b). Emission of styrene during the hand lay-up molding of reinforced polyester. *Appl Occup Environ Hyg*, 6(9):790–3. doi:10.1080/1047322X.1991.10389730

Samimi B, Falbo L (1982). Monitoring of workers exposure to low levels of airborne monomers in a polystyrene production plant. *Am Ind Hyg Assoc J*, 43(11):858–62. doi:10.1080/15298668291410693 PMID:7168443

Santini F, Mantovani A, Cristaudo A, Rago T, Marsili A, Buselli R, et al. (2008). Thyroid function and exposure to styrene. *Thyroid*, 18(10):1065–9. doi:10.1089/thy.2008.0003 PMID:18816178

Santos-Burgoa C, Matanoski GM, Zeger S, Schwartz L (1992). Lymphohematopoietic cancer in styrene-butadiene polymerization workers. *Am J Epidemiol*, 136(7):843–54. doi:10.1093/aje/136.7.843 PMID:1442750

Sarangapani R, Teeguarden JG, Cruzan G, Clewell HJ, Andersen ME (2002). Physiologically based pharmacokinetic modeling of styrene and styrene oxide respiratory-tract dosimetry in rodents and humans. *Inhal Toxicol*, 14(8):789–834. doi:10.1080/08958370290084647 PMID:12122565

Sasaki YF, Izumiyama F, Nishidate E, Matsusaka N, Tsuda S (1997). Detection of rodent liver carcinogen genotoxicity by the alkaline single-cell gel electrophoresis (Comet) assay in multiple mouse organs (liver, lung, spleen, kidney, and bone marrow). *Mutat Res*, 391(3):201–14. doi:10.1016/S1383-5718(97)00072-7 PMID:9268046

Sathiakumar N, Brill I, Delzell E (2009). 1,3-butadiene, styrene and lung cancer among synthetic rubber industry workers. *J Occup Environ Med*, 51(11):1326–32. doi:10.1097/JOM.0b013e3181c3c663 PMID:19858739

Sathiakumar N, Brill I, Leader M, Delzell E (2015). 1,3-Butadiene, styrene and lymphohematopoietic cancer among male synthetic rubber industry workers – preliminary exposure-response analyses. *Chem Biol Interact*, 241:40–9. doi:10.1016/j.cbi.2015.09.003 PMID:26343807

Sathiakumar N, Delzell E (2009). A follow-up study of mortality among women in the North American synthetic rubber industry. *J Occup Environ Med*, 51(11):1314–25. doi:10.1097/JOM.0b013e3181bd8972 PMID:19858743

Sathiakumar N, Delzell E, Hovinga M, Macaluso M, Julian JA, Larson R, et al. (1998). Mortality from cancer and other causes of death among synthetic rubber workers. *Occup Environ Med*, 55(4):230–5. doi:10.1136/oem.55.4.230 PMID:9624276

Sathiakumar N, Graff J, Macaluso M, Maldonado G, Matthews R, Delzell E (2005). An updated study of mortality among North American synthetic rubber industry workers. *Occup Environ Med*, 62(12):822–9. doi:10.1136/oem.2004.018176 PMID:16299089

Savela K, Hesso A, Hemminki K (1986). Characterization of reaction products between styrene oxide and deoxynucleosides and DNA. *Chem Biol Interact*, 60(3):235–46. doi:10.1016/0009-2797(86)90055-4 PMID:3791490

Savolainen H, Vainio H (1977). Organ distribution and nervous system binding of styrene and styrene oxide. *Toxicology*, 8(2):135–41. doi:10.1016/0300-483X(77)90002-6 PMID:929621

Sbrana I, Lascialfari D, Rossi AM, Loprieno N, Bianchi M, Tortoreto M, et al. (1983). Bone marrow cell chromosomal aberrations and styrene biotransformation in mice given styrene on a repeated oral schedule. *Chem Biol Interact*, 45(3):349–57. doi:10.1016/0009-2797(83)90081-9 PMID:6883576

Scélo G, Constantinescu V, Csiki I, Zaridze D, Szeszenia-Dabrowska N, Rudnai P, et al. (2004). Occupational exposure to vinyl chloride, acrylonitrile and styrene and lung cancer risk (Europe). *Cancer Causes Control*, 15(5):445–52. doi:10.1023/B:CACO.0000036444.11655.be PMID:15286464

Schaller KH, Triebig G (2012). Deutsche Forschungsgemeinschaft. Styrene [BAT Value Documentation, 1998a]. The MAK-Collection for Occupational Health and Safety. Weinhem, Germany: Wiley-VCH Verlag; pp. 164–178.

Schladt L, Thomas H, Hartmann R, Oesch F (1988). Human liver cytosolic epoxide hydrolases. *Eur J Biochem*, 176(3):715–23. doi:10.1111/j.1432-1033.1988.tb14335.x PMID:3169021

Schmidt-Bleek F, Haberland W, Klein AW, Caroli S (1982). Steps towards environmental hazard assessment of new chemicals (including a hazard ranking scheme, based upon Directive 79/831/EEC). *Chemosphere*, 11(4):383–415. doi:10.1016/0045-6535(82)90044-3

Scholdberg TA, Nechev LV, Merritt WK, Harris TM, Harris CM, Lloyd RS, et al. (2004). Structure of a site specific major groove (2*S*,3*S*)-N^6-(2,3,4-trihydroxybutyl)-2 -deoxyadenosyl DNA adduct of butadiene diol epoxide. *Chem Res Toxicol*, 17(6):717–30. doi:10.1021/tx034271+ PMID:15206893

Schumacher RL, Breysse PA, Carlyon WR, Hibbard RP, Kleinman GD (1981). Styrene exposure in the fiberglass fabrication industry in Washington State. *Am Ind Hyg Assoc J*, 42(2):143–9. doi:10.1080/15298668191419488 PMID:7234679

Seidel HJ, Herkommer J, Seitz D, Weber L, Barthel E (1990). Haemopoietic stem cells in mice chronically exposed to styrene vapour. *Arch Toxicol*, 64(6):466–9. doi:10.1007/BF01977628 PMID:2275603

Seidler A, Möhner M, Berger J, Mester B, Deeg E, Elsner G, et al. (2007). Solvent exposure and malignant lymphoma: a population-based case-control study in Germany. *J Occup Med Toxicol*, 2(1):2. doi:10.1186/1745-6673-2-2 PMID:17407545

Seniori Costantini A, Benvenuti A, Vineis P, Kriebel D, Tumino R, Ramazzotti V, et al. (2008). Risk of leukemia and multiple myeloma associated with exposure to benzene and other organic solvents: evidence from the Italian Multicenter Case-control study. *Am J Ind Med*, 51(11):803–11. doi:10.1002/ajim.20592 PMID:18651579

Seniori Costantini A, Miligi L, Kriebel D, Ramazzotti V, Rodella S, Scarpi E, et al. (2001). A multicenter case-control study in Italy on hematolymphopoietic neoplasms and occupation. *Epidemiology*, 12(1):78–87. doi:10.1097/00001648-200101000-00014 PMID:11138825

Sepai O, Anderson D, Street B, Bird I, Farmer PB, Bailey E (1993). Monitoring of exposure to styrene oxide by GC-MS analysis of phenylhydroxyethyl esters in hemoglobin. *Arch Toxicol*, 67(1):28–33. doi:10.1007/BF02072031 PMID:8452476

Serdar B, Tornero-Velez R, Echeverria D, Nylander-French LA, Kupper LL, Rappaport SM (2006). Predictors of occupational exposure to styrene and styrene-7,8-oxide in the reinforced plastics industry. *Occup Environ Med*, 63(10):707–12. doi:10.1136/oem.2005.025205 PMID:16757507

Severi M, Pauwels W, Van Hummelen P, Roosels D, Kirsch-Volders M, Veulemans H (1994). Urinary mandelic acid and hemoglobin adducts in fiberglass-reinforced plastics workers exposed to styrene. *Scand J Work Environ Health*, 20(6):451–8. doi:10.5271/sjweh.1375 PMID:7701291

Sexton K, Adgate JL, Church TR, Ashley DL, Needham LL, Ramachandran G, et al. (2005). Children's exposure to volatile organic compounds as determined by longitudinal measurements in blood. *Environ Health Perspect*, 113(3):342–9. doi:10.1289/ehp.7412 PMID:15743726

Shamy MY, Osman HH, Kandeel KM, Abdel-Moneim NM, El Said KF (2002). DNA single strand breaks induced by low levels of occupational exposure to styrene: the gap between standards and reality. *J Environ Pathol Toxicol Oncol*, 21(1):57–61. doi:10.1615/JEnvironPatholToxicolOncol.v21.i1.40 PMID:11934013

Sharief Y, Brown AM, Backer LC, Campbell JA, Westbrook-Collins B, Stead AG, et al. (1986). Sister chromatid exchange and chromosome aberration analyses in mice after in vivo exposure to acrylonitrile, styrene, or butadiene monoxide. *Environ Mutagen*, 8(3):439–48. doi:10.1002/em.2860080312 PMID:3709426

Sharma RP, Smith FA, Gehring PJ (1981). Styrene epoxide intermediate as a possible stimulant of lymphocytic function. *J Immunopharmacol*, 3(1):67–78. doi:10.3109/08923978109026419 PMID:7252181

Shell (2017). Material Safety Data Sheet. Styrene monomer. Available from: https://ch-msds.shell.com/MSDS/000000000847_AU_EN.pdf.

Shen S, Li L, Ding X, Zheng J (2014). Metabolism of styrene to styrene oxide and vinylphenols in cytochrome P450 2F2- and P450 2E1-knockout mouse liver and lung microsomes. *Chem Res Toxicol*, 27(1):27–33. doi:10.1021/tx400305w PMID:24320693

Shen S, Zhang F, Gao L, Zeng S, Zheng J (2010). Detection of phenolic metabolites of styrene in mouse liver and lung microsomal incubations. *Drug Metab Dispos*, 38(11):1934–43. doi:10.1124/dmd.110.033522 PMID:20724499

Shield AJ, Sanderson BJ (2004). A recombinant model for assessing the role of GSTM1 in styrene-7,8-oxide toxicity and mutagenicity. *Toxicology*, 195(1):61–8. doi:10.1016/j.tox.2003.08.010 PMID:14698568

Siemiatycki J (1991). Risk factors for cancer in the workplace. Boca Raton (FL), USA: CRC Press.

Simeonov MF, Tamura PJ, Wilkinson AS, Harris CM, Harris TM, Stone MP (2000). Sequence- and stereospecific conformational rearrangement of styrene oxide adducts located at A x C mismatched base pairs. *Biochemistry*, 39(5):924–37. doi:10.1021/bi992080t PMID:10653636

Simula AP, Priestly BG (1992). Species differences in the genotoxicity of cyclophosphamide and styrene in three in vivo assays. *Mutat Res*, 271(1):49–58. doi:10.1016/0165-1161(92)90031-G PMID:1371829

Sina JF, Bean CL, Dysart GR, Taylor VI, Bradley MO (1983). Evaluation of the alkaline elution/rat hepatocyte assay as a predictor of carcinogenic/mutagenic potential. *Mutat Res*, 113(5):357–91. doi:10.1016/0165-1161(83)90228-5 PMID:6877265

Sinha AK, Jersey GC, Linscombe VA, Adams RL, Mueller AM, McClintock ML (1983). Cytogenetic evaluation of bone marrow cells from rats exposed to styrene vapor for one year. *Fundam Appl Toxicol*, 3(2):95–8. doi:10.1016/S0272-0590(83)80062-1 PMID:6347787

Sinsheimer JE, Chen R, Das SK, Hooberman BH, Osorio S, You Z (1993). The genotoxicity of enantiomeric aliphatic epoxides. *Mutat Res*, 298(3):197–206. doi:10.1016/0165-1218(93)90041-B PMID:7678154

Slyskova J, Dusinska M, Kuricova M, Soucek P, Vodickova L, Susova S, et al. (2007). Relationship between the capacity to repair 8-oxoguanine, biomarkers of genotoxicity and individual susceptibility in styrene-exposed workers. *Mutat Res*, 634(1–2):101–11. doi:10.1016/j.mrgentox.2007.06.012 PMID:17855160

Smejkalová J, Hassmanová V, Emminger S, Malír F (1989). Chromosome aberrations in peripheral blood lymphocytes in workers occupationally exposed to styrene. *Sb Ved Pr Lek Fak Karlovy Univerzity Hradci Kralove Suppl*, 32(4):471–80. PMID:2640361 [in Czech]

Smith MT, Guyton KZ, Gibbons CF, Fritz JM, Portier CJ, Rusyn I, et al. (2016). Key characteristics of carcinogens as a basis for organizing data on mechanisms of carcinogenesis. *Environ Health Perspect*, 124(6):713–21. doi:10.1289/ehp.1509912 PMID:26600562

Snyder CA, Valle CD (1991). Lymphocyte proliferation assays as potential biomarkers for toxicant exposures. *J Toxicol Environ Health*, 34(1):127–39. doi:10.1080/15287399109531553 PMID:1890689

Solveig Walles SA, Orsén I (1983). Single-strand breaks in DNA of various organs of mice induced by styrene and styrene oxide. *Cancer Lett*, 21(1):9–15. doi:10.1016/0304-3835(83)90076-9 PMID:6640517

Somorovská M, Jahnová E, Tulinská J, Zámecníková M, Sarmanová J, Terenová A, et al. (1999). Biomonitoring of occupational exposure to styrene in a plastics lamination plant. *Mutat Res*, 428(1–2):255–69. doi:10.1016/S1383-5742(99)00052-6 PMID:10517998

Sorsa M, Anttila A, Järventaus H, Kubiak R, Norppa H, Nylander L, et al. (1991). Styrene revisited – exposure assessment and risk estimation in reinforced plastics industry. *Prog Clin Biol Res*, 372:187–95. PMID:1956917

Speit G, Linsenmeyer R, Schütz P, Kuehner S (2012). Insensitivity of the in vitro cytokinesis-block micronucleus assay with human lymphocytes for the detection of DNA damage present at the start of the cell culture. *Mutagenesis*, 27(6):743–7. doi:10.1093/mutage/ges041 PMID:22869611

Srivastava S, Seth PK, Srivastava SP (1992). Altered activity of hepatic mixed function oxidase, cytochrome P-450 and glutathione-S-transferase by styrene in rat fetal liver. *Drug Chem Toxicol*, 15(3):233–44. doi:10.3109/01480549209014153 PMID:1425362

Srivastava SP, Das M, Seth PK (1983). Enhancement of lipid peroxidation in rat liver on acute exposure to styrene and acrylamide a consequence of glutathione depletion. *Chem Biol Interact*, 45(3):373–80. doi:10.1016/0009-2797(83)90083-2 PMID:6883577

Stampfer JF, Hermes RE (1981). Development of sampling and analytical method for styrene oxide. *Am Ind Hyg Assoc J*, 42(10):699–706. doi:10.1080/15298668191420567

Statista (2017). Production volume of styrene monomer in Japan from 2012 to 2016. Available from: https://www.statista.com/statistics/733912/japan-styrene-monomer-production-volume/, accessed 26 February 2018.

Stefaniak AB, Breysse PN, Murray MP, Rooney BC, Schaefer J (2000). An evaluation of employee exposure to volatile organic compounds in three photocopy centers. *Environ Res*, 83(2):162–73. doi:10.1006/enrs.2000.4061 PMID:10856189

Stefaniak AB, LeBouf RF, Yi J, Ham J, Nurkewicz T, Schwegler-Berry DE, et al. (2017). Characterization of chemical contaminants generated by a desktop fused deposition modeling 3-dimensional printer. *J Occup Environ Hyg*, 14(7):540–50. doi:10.1080/15459624.2017.1302589 PMID:28440728

Stengel B, Touranchet A, Boiteau HL, Harousseau H, Mandereau L, Hémon D (1990). Hematological findings among styrene-exposed workers in the reinforced plastics industry. *Int Arch Occup Environ Health*, 62(1):11–8. doi:10.1007/BF00397843 PMID:2295518

Stoltz DR, Whitey RJ (1977). Mutagenicity testing of styrene and styrene epoxide in *Salmonella typhimurium*. *Bull Environ Contam Toxicol*, 17(6):739–42. doi:10.1007/BF01685963 PMID:328090

Styrene Information & Research Center (2001). Styrene sources and exposure scenarios. Arlington (VA), USA.

Su FC, Mukherjee B, Batterman S (2011). Trends of VOC exposures among a nationally representative sample: analysis of the NHANES 1988 through 2004 data sets. *Atmos Environ (1994)*, 45(28):4858–67. doi:10.1016/j.atmosenv.2011.06.016 PMID:25705111

Su T, Bao Z, Zhang QY, Smith TJ, Hong JY, Ding X (2000). Human cytochrome P450 CYP2A13: predominant expression in the respiratory tract and its high efficiency metabolic activation of a tobacco-specific carcinogen, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone. *Cancer Res*, 60(18):5074–9. PMID:11016631

Sugiura K, Goto M (1981). Mutagenicities of styrene oxide derivatives on bacterial test systems: relationship between mutagenic potencies and chemical reactivity.

Chem Biol Interact, 35(1):71–91. doi:10.1016/0009-2797(81)90064-8 PMID:6451311
Sugiura K, Yamanaka S, Fukasawa S, Goto M (1978). The mutagenicity of substituted and unsubstituted styrene oxides in *E. coli*: relationship between mutagenic potencies and physicochemical properties. *Chemosphere*, 9(9):737–42. doi:10.1016/0045-6535(78)90112-1
Sullivan EA, Sullivan JL (1986). Styrene exposure in the reinforced plastics industry in Ontario. *Occup Health Ontario*, 7:38–55.
Sumner SC, Cattley RC, Asgharian B, Janszen DB, Fennell TR (1997). Evaluation of the metabolism and hepatotoxicity of styrene in F344 rats, B6C3F1 mice, and CD-1 mice following single and repeated inhalation exposures. *Chem Biol Interact*, 106(1):47–65. doi:10.1016/S0009-2797(97)00054-9 PMID:9305408
Sumner SJ, Fennell TR (1994). Review of the metabolic fate of styrene. *Crit Rev Toxicol*, 24(Suppl):S11–33. doi:10.3109/10408449409020138 PMID:7818768
Tang W, Hemm I, Eisenbrand G (2000). Estimation of human exposure to styrene and ethylbenzene. *Toxicology*, 144(1–3):39–50. doi:10.1016/S0300-483X(99)00188-2 PMID:10781869
Tates AD, Grummt T, van Dam FJ, de Zwart F, Kasper FJ, Rothe R, et al. (1994). Measurement of frequencies of HPRT mutants, chromosomal aberrations, micronuclei, sister-chromatid exchanges and cells with high frequencies of SCEs in styrene/dichloromethane-exposed workers. *Mutat Res*, 313(2–3):249–62. doi:10.1016/0165-1161(94)90054-X PMID:7523909
Tawfik MS, BaAbdullah H (2014). Migration levels of monostyrene in most vulnerable foods handled and stored in polystyrene containers and their impact on the daily intake. *Pak J Food Sci*, 24(1):57–63.
Taylor DG (1979). NIOSH Manual of analytical methods. 2nd ed. Volume 5. DHEW (NIOSH) Publ. No. 79-141. Washington (DC), USA: United States Government Printing Office; pp. 303-1–303-8.
Teixeira JP, Gaspar J, Coelho P, Costa C, Pinho-Silva S, Costa S, et al. (2010). Cytogenetic and DNA damage on workers exposed to styrene. *Mutagenesis*, 25(6):617–21. doi:10.1093/mutage/geq049 PMID:20729469
Teixeira JP, Gaspar J, Roma-Torres J, Silva S, Costa C, Roach J, et al. (2007). Styrene-oxide N-terminal valine haemoglobin adducts in reinforced plastic workers: possible influence of genetic polymorphism of drug-metabolising enzymes. *Toxicology*, 237(1–3):58–64. doi:10.1016/j.tox.2007.04.019 PMID:17566625
Teixeira JP, Gaspar J, Silva S, Torres J, Silva SN, Azevedo MC, et al. (2004). Occupational exposure to styrene: modulation of cytogenetic damage and levels of urinary metabolites of styrene by polymorphisms in genes *CYP2E1*, *EPHX1*, *GSTM1*, *GSTT1* and *GSTP1*. *Toxicology*, 195(2–3):231–42. doi:10.1016/j.tox.2003.10.010 PMID:14751678
The Styrene Forum (2017). Information on the styrene industry worldwide. Available from: http://www.styreneforum.org/industry_index.html, accessed 26 February 2018.
Thiess AM, Fleig I (1978). Chromosome investigations on workers exposed to styrene/polystyrene. *J Occup Med*, 20(11):747–9. doi:10.1097/00043764-197811000-00006 PMID:712442
Thiess AM, Friedheim M (1978). Morbidity among persons employed in styrene production, polymerization and processing plants. *Scand J Work Environ Health*, 4(Suppl 2):203–14. doi:10.5271/sjweh.2746 PMID:734406
Thiess AM, Schwegler H, Fleig I (1980). Chromosome investigations in lymphocytes of workers employed in areas in which styrene-containing unsaturated polyester resins are manufactured. *Am J Ind Med*, 1(2):205–10. doi:10.1002/ajim.4700010211 PMID:7342768
Tice RR, Austin CP, Kavlock RJ, Bucher JR (2013). Improving the human hazard characterization of chemicals: a Tox21 update. *Environ Health Perspect*, 121(7):756–65. doi:10.1289/ehp.1205784 PMID:23603828
Tomanin R, Ballarin C, Bartolucci GB, De Rosa E, Sessa G, Iannini G, et al. (1992). Chromosome aberrations and micronuclei in lymphocytes of workers exposed to low and medium levels of styrene. *Int Arch Occup Environ Health*, 64(3):209–15. doi:10.1007/BF00380911 PMID:1399034
Tomar RS, Lim S, Gill SS (1991). The effects of epoxidized xenobiotics on mitogen responsiveness and antibody-producing ability of murine spleen cells *in vitro*. *Int J Immunopharmacol*, 13(1):57–65. doi:10.1016/0192-0561(91)90026-4 PMID:2026476
Tornero-Velez R, Rappaport SM (2001). Physiological modeling of the relative contributions of styrene-7,8-oxide derived from direct inhalation and from styrene metabolism to the systemic dose in humans. *Toxicol Sci*, 64(2):151–61. doi:10.1093/toxsci/64.2.151 PMID:11719697
Tornero-Velez R, Waidyanatha S, Echeverria D, Rappaport SM (2000). Measurement of styrene-7,8-oxide and other oxidation products of styrene in air. *J Environ Monit*, 2(2):111–7. doi:10.1039/a908976d PMID:11253028
Tornero-Velez R, Waidyanatha S, Pérez HL, Osterman-Golkar S, Echeverria D, Rappaport SM (2001). Determination of styrene and styrene-7,8-oxide in human blood by gas chromatography-mass spectrometry. *J Chromatogr B Biomed Sci Appl*, 757(1):59–68. doi:10.1016/S0378-4347(01)00063-9 PMID:11419749
Tossavainen A (1978). Styrene use and occupational exposure in the plastics industry. *Scand J Work Environ Health*, 4(Suppl 2):7–13. doi:10.5271/sjweh.2732 PMID:734420

Tranfo G, Gherardi M, Paci E, Gatto M, Gordiani A, Caporossi L, et al. (2012). Occupational exposure to styrene in the fibreglass reinforced plastic industry: comparison between two different manufacturing processes. *Med Lav*, 103(5):402–12. PMID:23077800

Triebig G, Lehrl S, Weltle D, Schaller KH, Valentin H (1989). Clinical and neurobehavioural study of the acute and chronic neurotoxicity of styrene. *Br J Ind Med*, 46(11):799–804. PMID:2590645

Truchon G, Gérin M, Brodeur J (1990). Urinary excretion of mandelic, phenylglyoxylic, and specific mercapturic acids in rats exposed repeatedly by inhalation to various concentrations of styrene vapors. *Can J Physiol Pharmacol*, 68(5):556–61. doi:10.1139/y90-081 PMID:2340445

Truchon G, Ostiguy C, Drolet D, Mergler D, Campagna D, Bélanger S, et al. (1992). Neurotoxic effects among styrene-exposed workers. I. Environmental and biological monitoring of exposure. *Trav. Sante*, 8:S11–4. [in French]

Tsuda S, Matsusaka N, Madarame H, Miyamae Y, Ishida K, Satoh M, et al. (2000). The alkaline single cell electrophoresis assay with eight mouse organs: results with 22 mono-functional alkylating agents (including 9 dialkyl *N*-nitrosoamines) and 10 DNA crosslinkers. *Mutat Res*, 467(1):83–98. doi:10.1016/S1383-5718(00)00014-0 PMID:10771273

Tulinská J, Dusinská M, Jahnová E, Lisková A, Kuricova M, Vodička P, et al. (2000). Changes in cellular immunity among workers occupationally exposed to styrene in a plastics lamination plant. *Am J Ind Med*, 38(5):576–83. doi:10.1002/1097-0274(200011)38:5<576::AID-AJIM10>3.0.CO;2-F PMID:11025499

Turner M, Mantick NA, Carlson GP (2005). Comparison of the depletion of glutathione in mouse liver and lung following administration of styrene and its metabolites styrene oxide and 4-vinylphenol. *Toxicology*, 206(3):383–8. doi:10.1016/j.tox.2004.07.013 PMID:15588928

Uddin MS, Blount BC, Lewin MD, Potula V, Ragin AD, Dearwent SM (2014). Comparison of blood volatile organic compound levels in residents of Calcasieu and Lafayette Parishes, LA, with US reference ranges. *J Expo Sci Environ Epidemiol*, 24(6):602–7. doi:10.1038/jes.2013.94 PMID:24472757

US NLM (2018). Styrene-7,8-oxide (96–09–3), Hazardous Substances Data Bank. Toxicology Data Network (TOXNET). United States National Library of Medicine.

USDOE (2012). New process for producing styrene cuts costs, saves energy, and reduces greenhouse gas emissions. Available from: https://www1.eere.energy.gov/office_eere/pdfs/exelus_case_study.pdf, accessed 26 February 2018.

Uusküla M, Järventaus H, Hirvonen A, Sorsa M, Norppa H (1995). Influence of GSTM1 genotype on sister chromatid exchange induction by styrene-7,8-oxide and 1,2-epoxy-3-butene in cultured human lymphocytes. *Carcinogenesis*, 16(4):947–50. doi:10.1093/carcin/16.4.947 PMID:7728978

Vaghef H, Hellman B (1998). Detection of styrene and styrene oxide-induced DNA damage in various organs of mice using the comet assay. *Pharmacol Toxicol*, 83(2):69–74. doi:10.1111/j.1600-0773.1998.tb01446.x PMID:9783323

Vainio H, Järvisalo J, Taskinen E (1979). Adaptive changes caused by intermittent styrene inhalation on xenobiotic biotransformation. *Toxicol Appl Pharmacol*, 49(1):7–14. doi:10.1016/0041-008X(79)90270-9 PMID:112720

Vainio H, Pääkkönen R, Rönnholm K, Raunio V, Pelkonen O (1976). A study on the mutagenic activity of styrene and styrene oxide. *Scand J Work Environ Health*, 2(3):147–51. doi:10.5271/sjweh.2813 PMID:788148

Vanduuren BL, Nelson N, Orris L, Palmes ED, Schmitt FL (1963). Carcinogenicity of epoxides, lactones, and peroxy compounds. *J Natl Cancer Inst*, 31:41–55. PMID:14043038

Vettori MV, Caglieri A, Goldoni M, Castoldi AF, Darè E, Alinovi R, et al. (2005). Analysis of oxidative stress in SK-N-MC neurons exposed to styrene-7,8-oxide. *Toxicol In Vitro*, 19(1):11–20. doi:10.1016/j.tiv.2004.04.015 PMID:15582351

Vitrac O, Leblanc JC (2007). Consumer exposure to substances in plastic packaging. I. Assessment of the contribution of styrene from yogurt pots. *Food Addit Contam*, 24(2):194–215. doi:10.1080/02652030600888618 PMID:17364921

Vodička P, Bastlová T, Vodicková L, Peterková K, Lambert B, Hemminki K (1995). Biomarkers of styrene exposure in lamination workers: levels of O^6-guanine DNA adducts, DNA strand breaks and mutant frequencies in the hypoxanthine guanine phosphoribosyltransferase gene in T-lymphocytes. *Carcinogenesis*, 16(7):1473–81. doi:10.1093/carcin/16.7.1473 PMID:7614680

Vodička P, Hemminki K (1993). ^{32}P-postlabelling of DNA adducts in styrene oxide-modified DNA and in workers exposed to styrene. *IARC Sci Publ*, 127:109–18. PMID:8070856

Vodička P, Koskinen M, Arand M, Oesch F, Hemminki K (2002). Spectrum of styrene-induced DNA adducts: the relationship to other biomarkers and prospects in human biomonitoring. *Mutat Res*, 511(3):239–54. doi:10.1016/S1383-5742(02)00012-1 PMID:12088719

Vodička P, Koskinen M, Naccarati A, Oesch-Bartlomowicz B, Vodickova L, Hemminki K, et al. (2006a). Styrene metabolism, genotoxicity, and potential carcinogenicity. *Drug Metab Rev*, 38(4):805–53. doi:10.1080/03602530600952222 PMID:17145703

Vodička P, Koskinen M, Vodicková L, Stetina R, Smerák P, Bárta I, et al. (2001a). DNA adducts, strand breaks and micronuclei in mice exposed to styrene by inhalation. *Chem Biol Interact*, 137(3):213–27. doi:10.1016/S0009-2797(01)00253-8 PMID:11566290

Vodička P, Soucek P, Tates AD, Dusinska M, Sarmanova J, Zamecnikova M, et al. (2001b). Association between genetic polymorphisms and biomarkers in styrene-exposed workers. *Mutat Res*, 482(1–2):89–103. doi:10.1016/S0027-5107(01)00214-7 PMID:11535253

Vodička P, Stetina R, Kumar R, Plna K, Hemminki K (1996). 7-Alkylguanine adducts of styrene oxide determined by ^{32}P-postlabeling in DNA and human embryonal lung fibroblasts (HEL). *Carcinogenesis*, 17(4):801–8. doi:10.1093/carcin/17.4.801 PMID:8625494

Vodička P, Tuimala J, Stetina R, Kumar R, Manini P, Naccarati A, et al. (2004). Cytogenetic markers, DNA single-strand breaks, urinary metabolites, and DNA repair rates in styrene-exposed lamination workers. *Environ Health Perspect*, 112(8):867–71. doi:10.1289/ehp.6849 PMID:15175174

Vodička P, Tvrdik T, Osterman-Golkar S, Vodicková L, Peterková K, Soucek P, et al. (1999). An evaluation of styrene genotoxicity using several biomarkers in a 3-year follow-up study of hand-lamination workers. *Mutat Res*, 445(2):205–24. doi:10.1016/S1383-5718(99)00127-8 PMID:10575431

Vodička P, Vodickova L, Hemminki K (1993). ^{32}P-postlabeling of DNA adducts of styrene-exposed lamination workers. *Carcinogenesis*, 14(10):2059–61. doi:10.1093/carcin/14.10.2059 PMID:8222054

Vodička P, Vodicková L, Trejbalová K, Srám RJ, Hemminki K (1994). Persistence of *O*^{6}-guanine DNA adducts in styrene-exposed lamination workers determined by ^{32}P-postlabelling. *Carcinogenesis*, 15(9):1949–53. doi:10.1093/carcin/15.9.1949 PMID:7923589

Vodička PE, Linhart I, Novak J, Koskinen M, Vodickova L, Hemminki K (2006b). 7-Alkylguanine adduct levels in urine, lungs and liver of mice exposed to styrene by inhalation. *Toxicol Appl Pharmacol*, 210(1–2):1–8. doi:10.1016/j.taap.2005.04.020 PMID:15949832

Vogie K, Mantick N, Carlson G (2004). Metabolism and toxicity of the styrene metabolite 4-vinylphenol in CYP2E1 knockout mice. *J Toxicol Environ Health A*, 67(2):145–52. doi:10.1080/15287390490264785 PMID:14675903

von der Hude W, Seelbach A, Basler A (1990). Epoxides: comparison of the induction of SOS repair in *Escherichia coli* PQ37 and the bacterial mutagenicity in the Ames test. *Mutat Res*, 231(2):205–18. doi:10.1016/0027-5107(90)90027-2 PMID:2200956

von Ehrenstein OS, Heck JE, Park AS, Cockburn M, Escobedo L, Ritz B (2016). In utero and early-life exposure to ambient air toxics and childhood brain tumors: a population-based case-control study in California, USA. *Environ Health Perspect*, 124(7):1093–9. doi:10.1289/ehp.1408582 PMID:26505805

Wade DR, Airy SC, Sinsheimer JE (1978). Mutagenicity of aliphatic epoxides. *Mutat Res*, 58(2–3):217–23. doi:10.1016/0165-1218(78)90012-5 PMID:370573

Wallace L, Pellizzari E, Hartwell TD, Perritt R, Ziegenfus R (1987). Exposures to benzene and other volatile compounds from active and passive smoking. *Arch Environ Health*, 42(5):272–9. doi:10.1080/00039896.1987.9935820 PMID:3452294

Wallace LA (1986). Personal exposures, indoor and outdoor air concentrations, and exhaled breath concentrations of selected volatile organic compounds measured for 600 residents of New Jersey, North Dakota, North Carolina, and California. *Toxicol Environ Chem*, 12(3–4):215–36. doi:10.1080/02772248609357160

Wallace LA, Pellizzari ED, Hartwell TD, Davis V, Michael LC, Whitmore RW (1989). The influence of personal activities on exposure to volatile organic compounds. *Environ Res*, 50(1):37–55. doi:10.1016/S0013-9351(89)80047-7 PMID:2792060

Wallace LA, Pellizzari ED, Hartwell TD, Sparacino CM, Sheldon LS, Zelon H (1985). Personal exposures, indoor-outdoor relationships, and breath levels of toxic air pollutants measured for 355 persons in New Jersey. *Atmos Environ*, 19(10):1651–61. doi:10.1016/0004-6981(85)90217-3

Walles SA, Edling C, Anundi H, Johanson G (1993). Exposure dependent increase in DNA single strand breaks in leucocytes from workers exposed to low concentrations of styrene. *Br J Ind Med*, 50(6):570–4. doi:10.1136/oem.50.6.570 PMID:8329323

Wang MF, Han CL, Yin SJ (2009). Substrate specificity of human and yeast aldehyde dehydrogenases. *Chem Biol Interact*, 178(1–3):36–9. doi:10.1016/j.cbi.2008.10.002 PMID:18983993

Wang Y, Kupper LL, Löf A, Rappaport SM (1996). Comparison of average estimated metabolic rates for styrene in previously exposed and unexposed groups with pharmacokinetic modelling. *Occup Environ Med*, 53(9):601–5. doi:10.1136/oem.53.9.601 PMID:8882117

Watabe T, Hiratsuka A, Aizawa T, Sawahata T, Ozawa N, Isobe M, et al. (1982). Studies on metabolism and toxicity of styrene. IV. 1-Vinylbenzene 3, 4-oxide, a potent mutagen formed as a possible intermediate in the metabolism in vivo of styrene to 4-vinylphenol. *Mutat Res*, 93(1):45–55. doi:10.1016/0027-5107(82)90124-5 PMID:7038469

Watabe T, Isobe M, Sawahata T, Yoshikawa K, Yamada S, Takabatake E (1978). Metabolism and mutagenicity of styrene. *Scand J Work Environ Health*, 4(Suppl 2):142–55. doi:10.5271/sjweh.2769 PMID:32616

Watanabe T, Endo A, Kumai M, Ikeda M (1983). Chromosome aberrations and sister chromatid exchanges in styrene-exposed workers with reference

to their smoking habits. *Environ Mutagen*, 5(3):299–309. doi:10.1002/em.2860050308 PMID:6851971

Weil CS, Condra N, Haun C, Striegel JA (1963). Experimental carcinogenicity and acute toxicity of representative epoxides. *Am Ind Hyg Assoc J*, 24(4):305–25. doi:10.1080/00028896309343226 PMID:14042492

Weng Z, Suda M, Wan M, Zhang X, Guan D, Zhao P, et al. (2016). Significant association between decreased ALDH2 activity and increased sensitivity to genotoxic effects in workers occupationally exposed to styrene. *Oncotarget*, 7(25):38224–34. doi:10.18632/oncotarget.9502 PMID:27224914

Wenker MA, Kezić S, Monster AC, de Wolff FA (2000). Metabolism of styrene-7,8-oxide in human liver *in vitro*: interindividual variation and stereochemistry. *Toxicol Appl Pharmacol*, 169(1):52–8. doi:10.1006/taap.2000.9038 PMID:11076696

Wenker MA, Kezić S, Monster AC, De Wolff FA (2001a). Metabolism of styrene in the human liver *in vitro*: interindividual variation and enantioselectivity. *Xenobiotica*, 31(2):61–72. doi:10.1080/00498250010031638 PMID:11407535

Wenker MA, Kezić S, Monster AC, de Wolff FA (2001b). Stereochemical metabolism of styrene in volunteers. *Int Arch Occup Environ Health*, 74(5):359–65. doi:10.1007/PL00007953 PMID:11516070

WHO (1983). Styrene. Environmental Health Criteria 26. Geneva, Switzerland: International Programme on Chemical Safety, World Health Organization. Available from: http://www.inchem.org/documents/ehc/ehc/ehc26.htm, accessed 26 February 2018.

WHO (2004). Recommendations. Guidelines for drinking-water quality. Volume 1. 3rd ed. Geneva, Switzerland: World Health Organization.

Wieczorek H, Piotrowski JK (1985). Evaluation of low exposure to styrene. I. Absorption of styrene vapours by inhalation under experimental conditions. *Int Arch Occup Environ Health*, 57(1):57–69. doi:10.1007/BF00383546 PMID:4077282

Wigaeus E, Löf A, Bjurström R, Nordqvist MB (1983). Exposure to styrene. Uptake, distribution, metabolism and elimination in man. *Scand J Work Environ Health*, 9(6):479–88. doi:10.5271/sjweh.2385 PMID:6673105

Wigaeus E, Löf A, Nordqvist MB (1984). Uptake, distribution, metabolism, and elimination of styrene in man. A comparison between single exposure and co-exposure with acetone. *Br J Ind Med*, 41(4):539–46. doi:10.1136/oem.41.4.539 PMID:6498116

Willem H, Singer B (2010). Chemical emissions of residential materials and products: review of available information. Berkeley (CA), USA: Ernest Orlando Lawrence Berkeley National Laboratory, Environmental Energy Technologies Division. doi:10.2172/988998

Wilson HK, Robertson SM, Waldron HA, Gompertz D (1983). Effect of alcohol on the kinetics of mandelic acid excretion in volunteers exposed to styrene vapour. *Br J Ind Med*, 40(1):75–80. doi:10.1136/oem.40.1.75 PMID:6824604

Wolff MS, Lilis R, Lorimer WV, Selikoff IJ (1978). Biological indicators of exposure in styrene polymerization workers. Styrene in blood and adipose tissue and mandelic and phenylglyoxylic acids in urine. *Scand J Work Environ Health*, 4(Suppl 2):114–8. doi:10.5271/sjweh.2757 PMID:734395

Wong O (1990). A cohort mortality study and a case-control study of workers potentially exposed to styrene in the reinforced plastics and composites industry. *Br J Ind Med*, 47(11):753–62. PMID:2245187

Wong O, Trent LS, Whorton MD (1994). An updated cohort mortality study of workers exposed to styrene in the reinforced plastics and composites industry. *Occup Environ Med*, 51(6):386–96. doi:10.1136/oem.51.6.386 PMID:8044230

Wongvijitsuk S, Navasumrit P, Vattanasit U, Parnlob V, Ruchirawat M (2011). Low level occupational exposure to styrene: its effects on DNA damage and DNA repair. *Int J Hyg Environ Health*, 214(2):127–37. doi:10.1016/j.ijheh.2010.09.007 PMID:21030303

Yagen B, Hernandez O, Bend JR, Cox RH (1981). Synthesis and relative stereochemistry of the benzylic thioether diastereoisomers formed from glutathione and styrene oxide. *Bioorg Chem*, 10(3):299–310. doi:10.1016/0045-2068(81)90009-2

Yager JW, Paradisin WM, Rappaport SM (1993). Sister-chromatid exchanges in lymphocytes are increased in relation to longitudinally measured occupational exposure to low concentrations of styrene. *Mutat Res*, 319(3):155–65. doi:10.1016/0165-1218(93)90075-O PMID:7694137

Yang WP, Hu BH, Chen GD, Bielefeld EC, Henderson D (2009). Protective effect of N-acetyl-L-cysteine (L-NAC) against styrene-induced cochlear injuries. *Acta Otolaryngol*, 129(10):1036–43. doi:10.1080/00016480802566261 PMID:19051069

Yeowell-O'Connell K, Jin Z, Rappaport SM (1996). Determination of albumin and hemoglobin adducts in workers exposed to styrene and styrene oxide. *Cancer Epidemiol Biomarkers Prev*, 5(3):205–15. PMID:8833621

Zang H, Harris TM, Guengerich FP (2005). Kinetics of nucleotide incorporation opposite DNA bulky guanine N2 adducts by processive bacteriophage T7 DNA polymerase (exonuclease-) and HIV-1 reverse transcriptase. *J Biol Chem*, 280(2):1165–78. doi:10.1074/jbc.M405996200 PMID:15533946

Zeiger E, Anderson B, Haworth S, Lawlor T, Mortelmans K (1988). *Salmonella* mutagenicity tests: IV. Results from the testing of 300 chemicals. *Environ Mol Mutagen*, 11(Suppl 12):1–157. doi:10.1002/em.2850110602 PMID:3277844

Zeiger E, Anderson B, Haworth S, Lawlor T, Mortelmans K (1992). *Salmonella* mutagenicity tests: V. Results from the testing of 311 chemicals. *Environ Mol Mutagen*,

19(Suppl 21):2–141. doi:10.1002/em.2850190603 PMID:1541260

Zhang F, Lowe ER, Rick DL, Qiu X, Leibold E, Cruzan G, et al. (2011). In vitro metabolism, glutathione conjugation, and CYP isoform specificity of epoxidation of 4-vinylphenol. *Xenobiotica*, 41(1):6–23. doi:10.3109/00498254.2010.523735 PMID:20925585

Zhang F, Meng JL, Shao H, Zhang ZH, Feng B (2013). Influence of genetic polymorphisms of epoxide hydrolase 1 on metabolism of styrene in body. *Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi*, 31(7):496–9. PMID:24053912 [in Chinese]

Zhang W, Johnson F, Grollman AP, Shibutani S (1995). Miscoding by the exocyclic and related DNA adducts 3,N4-etheno-2′-deoxycytidine, 3,N4-ethano-2′-deoxycytidine, and 3-(2-hydroxyethyl)-2′-deoxyuridine. *Chem Res Toxicol*, 8(1):157–63. doi:10.1021/tx00043a021 PMID:7703360

Zhang XX, Chakrabarti S, Malick AM, Richer CL (1993). Effects of different styrene metabolites on cytotoxicity, sister-chromatid exchanges and cell-cycle kinetics in human whole blood lymphocytes in vitro. *Mutat Res*, 302(4):213–8. doi:10.1016/0165-7992(93)90107-7 PMID:7688860

Zhu J, Wong SL, Cakmak S (2013). Nationally representative levels of selected volatile organic compounds in Canadian residential indoor air: population-based survey. *Environ Sci Technol*, 47(23):13276–83. doi:10.1021/es403055e PMID:24164357

QUINOLINE

1. Exposure Data

1.1 Identification of the agent

1.1.1 Nomenclature

Chem. Abstr. Serv. (CAS) Reg. No.: 91-22-5

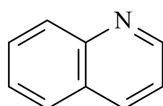
CAS name: 1-Azanaphthalene

IUPAC systematic name: Quinoline

Synonyms: 1-Benzazine, chinoline, quinolin, 2,3-benzopyridine, leucol

1.1.2 Structural and molecular formulae, and relative molecular mass

Structural formula:



Molecular formula: C_9H_7N

Relative molecular mass: 129.16 (Merck, 2017)

1.1.3 Chemical and physical properties of the pure substance

Description: Quinoline is a colourless, hygroscopic, weakly basic liquid with a characteristic unpleasant odour. It turns brown on exposure to light. It absorbs as much as 22% water (O'Neil, 2006).

Melting/freezing point: −15 °C (Merck, 2017)

Boiling point: 237–238 °C at 101 kPa (Merck, 2017)

Density: 1.09 g/cm³ at 25 °C (Merck, 2017)

Relative density: $d_{20/4}$, 1.0900 (water, 1) (Merck, 2017)

Solubility in organic solvents: Soluble in carbon tetrachloride and miscible with ethanol, ether, acetone, benzene, and carbon disulfide (Lide, 2003); dissolves sulfur, phosphorous, and arsenic trioxide (O'Neil, 2006)

Solubility in water: 6 g/L at 20 °C (Merck, 2017)

Dissociation constant: pK_a, 4.90 at 20 °C (Lide, 2003)

Vapour pressure: 11 Pa at 25 °C (Merck, 2017)

Relative vapour density: 4.5 (air, 1) (Weiss, 1986)

Odour threshold: 71 ppm = 375 mg/m³ (HSDB, 2017)

Reactivity: May attack some forms of plastic (Weiss, 1986); forms explosive mixtures with air on intense heating. Development of hazardous combustion gases (nitrogen oxides) or vapours possible in the event of fire (Merck, 2017). Protect from light and moisture (O'Neil, 2006).

Octanol/water partition coefficient (P): log K_{ow}, 2.03 (HSDB, 2017)

Conversion factor: 1 ppm = 5.28 mg/m³ at 25 °C and 101.3 kPa

Table 1.1 Examples of some quinolinium salts

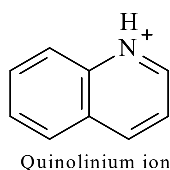
Salt	CAS No.	Formula	Relative molecular mass	Reference
Quinolinium hydrogen sulfate	530-66-5	$C_9H_9NO_4S$	227.23	O'Neil (2006)
Quinolinium chloride	530-64-3	C_9H_8ClN	165.62	O'Neil (2006)
Quinolinium bromide	ChemSpider ID: 378462	C_9H_8BrN	210.07	ChemSpider (2017)
Quinolinium dichromate	56549-24-7	$C_{18}H_{16}Cr_2N_2O_7$	476.32	PubChem (2017)
Quinolinium chlorochromate	108703-35-1	$C_9H_7ClCrNO_3$	264.61	PubChem (2017)

CAS, Chemical Abstracts Service.

1.1.4 Chemical and physical properties of some quinolinium salts

As a base, quinoline forms quinolinium salts upon contact with acids.

Basic structural formula of quinolinium salts:



Quinolinium ion

Some quinolinium salts are listed in Table 1.1.

Quinolinium chlorochromate is an efficient reagent for oxidative cleavage of oximes via the use of microwave irradiation, and pestle and mortar (Singh et al., 2003).

1.1.5 Technical products and impurities

Commercial quinoline has a purity of at least 90%. The chromatographic composition of this product is typically 92% quinoline and 5% isoquinoline by weight. Impurities include methylquinolines, 2,8-dimethylquinoline, and some homologues of isoquinoline (Finley, 1999).

1.2 Production and use

1.2.1 Production process

Many different methods currently exist for the synthesis of quinoline and its derivatives (Organic Chemistry Portal, 2017). Quinoline may be prepared by the classical Skraup synthesis from 1880 of heating aniline with glycerol in the presence of sulfuric acid and an oxidising agent such as nitrobenzene (O'Neil, 2006).

The Skraup synthesis is very energy intensive, and many modifications have been introduced (Batista et al., 2016). For example, quinoline can be continuously produced (42% yield) from aniline and glycerol in a reactor by microwaves under pressure (12 bar) and reduced temperature (200 °C) (Saggadi et al., 2015).

1.2.2 Production volume

About 35 years ago, the world production of quinoline was more than 2000 United States tons [2032 metric tonnes] annually. Annual production in the USA was at least 45.4 tons [> 40.8 tonnes] in 1978, and in 1982 the USA produced 2.27 tons [1.82 tonnes] and imported 39.6 tons [35.4 tonnes] (HSDB, 2017).

According to the United States Environmental Protection Agency (EPA) Chemical Data Access Tool, the aggregate production volume of quinoline in the USA was in the range of 100 000–500 000 pounds/year [~45–227 metric tonnes] for 2011. National production volume data for subsequent years are not publicly available. Data were provided for four companies, of which one produced 145 909 pounds [~66 metric tonnes] of quinoline per year. Quinoline is currently imported in confidential amounts into the USA (EPA, 2017).

This substance is manufactured and/or imported into the European Economic Area in

quantities of 100–1000 tonnes per year (ECHA, 2018). Data on exact quantities are not publicly available.

One or more companies in Canada reported the manufacture or import of quinoline in excess of 20 000 kg during the calendar year 2000 as part of chemical compounds comprising less than 1% quinoline; however, more recent data are not available (Government of Canada, 2011a).

Quinoline is included in the 2007 Organisation for Economic Co-operation and Development list of high production volume chemicals, which are those chemicals produced or imported at quantities greater than 1000 tonnes per year in at least one member country and/or region (OECD, 2009). In 2018, Chemical Sources International reported the following registered quinoline manufacturers: USA (19), Japan (2), United Kingdom (2), and 1 each in Canada, China, Hong Kong Special Administrative Region (China), France, Germany, and Switzerland (Chemical Sources International, 2018).

1.2.3 Uses

The main application of quinoline is the production of 8-quinolinol, which is obtained by alkaline fusion of quinoline-8-sulfonic acid.

Quinoline is used as a solvent in the production of dyes, paints, and other chemicals. A recently developed application is in the preparation of ionic liquid crystal solvents, such as *N*-alkylquinolinium bromide (Lava et al., 2012). It is also used as a reagent, a corrosion inhibitor, in metallurgical processes, and as an intermediate in the manufacture of pharmaceuticals and veterinary drugs (Gerhartz, 1993; O'Neil, 2006; Government of Canada, 2011b). Quinoline can be used to prepare and/or produce: nicotinic acid and its derivative niacin or vitamin B_3; antimalarial medicines (chloroquine, quinine, and mefloquine); 8-hydroxyquinoline sulfate (CAS No. 148-24-3), a metal chelating agent which is used in cosmetics; and dyes and pigments used in textiles, for example, Quinoline Yellow (CAS No. 8003-22-3). Quinoline Yellow is also used as a greenish-yellow food additive in certain countries. In the European Union (E-number E104) and Australia, Quinoline Yellow is permitted in beverages and is used in foods such as sauces, decorations, and coatings. Quinoline Yellow is not listed as a permitted food additive in Canada or the USA, but it is used in medicines and cosmetics and is known as D&C Yellow 10. The Codex Alimentarius does not list it (Abbey et al., 2013).

1.3 Measurement and analysis

1.3.1 Detection, separation, and quantification

Quinoline is an azaarene. Azaarenes are *N*-heterocyclic analogues of PAHs. Because azaarenes are more hydrophilic and have some basic (alkaline) properties as a result of nitrogen in the aromatic ring, the chromatography is considerably more difficult than analogous PAH separations (Steinheimer & Ondrus, 1986).

(a) Tars and fuels

Quinoline (and pyridine) has been pre-concentrated and determined in gasoline and diesel fuel by differential pulse voltammetry (Okumura & Ramos, 2007). The method had good agreement with an ultraviolet (UV) spectrometric technique based on the *F*-distribution and Student *t*-distribution. The limit of detection (LOD) for quinoline was 5 µg/L, and the spike recovery was 94%.

(b) Ambient air

Özel et al. (2011) developed a method to determine various nitrogen-containing compounds including quinoline in airborne particulate matter of diameter less than 2.5 µm ($PM_{2.5}$) from urban air. Two types of chemical analysis were performed on the collected samples, the

first using direct thermal desorption of analytes to comprehensive two-dimensional gas chromatography (GC×GC) and time-of-flight mass spectrometry (MS), and the second using water extraction of filters and solid-phase extraction (SPE) clean-up before GC×GC with nitrogen chemiluminescence detection. The LOD and limit of quantitation (LOQ) in standards for analysing quinoline by the first method were 4.36 µg/L and 18.9 µg/L, and by the second (more sensitive) method 2.24 µg/L and 9.71 µg/L, respectively. Quinoline was detected in the $PM_{2.5}$ air samples collected.

In the large Chinese city of Xian, azaarenes, including quinoline, bound to $PM_{2.5}$ were sampled on a filter. After being spiked with internal standards, the azaarene fraction of the sample was isolated by pressurized liquid extraction. The fraction was then extracted twice using dichloromethane. The analytes were measured by GC mass spectrometry (MS) in selected ion monitoring (SIM) mode. The average recovery of quinoline was 75 ± 5%. The relative standard deviation (RSD) for the replicate measurements (n = 3) of quinoline was 7–10%. The LOD of the analytical method was calculated as the mass of the target compound that produces a signal that is 3 times the baseline noise in the chromatogram (Bandowe et al., 2016).

(c) Water

A method to analyse several azaarenes, including quinoline, in various water sources was developed by Steinheimer & Ondrus (1986). The azaarene fraction was separated from its carbon analogues on n-octadecyl packing material by elution with acidified water and/or acetonitrile. The authors used bonded-phase extraction followed by high-performance liquid chromatography (HPLC) on flexible-walled, wide-bore columns with fluorescence and UV detection. The recovery of azaarenes at concentrations of parts per billion was close to the LOQ, and the detection of less than 1 ng quinoline (50 µg/L using a 20-µL injection) was possible. The method could be used to detect concentrations of parts per trillion in relatively pure water samples, and to assess azaarenes in complex, highly contaminated waters containing PAHs and other organics that might be expected to provide significant interference.

A high-sensitivity analytical method for assessing heteroaromatic compounds, including quinoline, in creosote-contaminated groundwater was developed with acceptable reproducibility (mean RSD, 19%), providing an LOQ of 50 ng/L (Johansen et al., 1996). The best technique (in terms of highest recovery and reproducibility) for sample preparation and analysis was determined to be the classic liquid-liquid extraction with dichloromethane from weakly basic solutions and GC-MS in SIM mode analysis of concentrated extracts. The recovery for spiked quinoline by extraction by dichloromethane was 98%; the recovery of quinoline analysed in groundwater was 71–74% and RSD varied over the range 2.6–20%.

Liquid chromatography tandem MS analysis of tar oil compounds in groundwater contaminated with tar oils in Germany revealed the occurrence of quinoline as well as its hydroxylated and hydrogenated metabolites (Reineke et al., 2007).

(d) Soil

Meyer et al. (1999) developed a simple and reproducible method which provided the simultaneous determination of PAHs and heteroaromatic compounds (N, S, O) and their degradation products in soils polluted with creosote. A sample of contaminated soil was acidified, extracted with dichloromethane and heptane, and transferred in concentrated extract on an SPE column. The fraction with quinoline was eluted with dichloromethane and/or methanol and transferred to an SPE cartridge. The basic fraction was then eluted with ammonia dissolved in methanol. The identification and quantification was performed

using either GC-MS or HPLC with diode array detection (DAD).

A method to determine azaarenes in soils using HPLC with UV-DAD or fluorescence detector (FD) was developed by Švábenský et al. (2007). Soil samples were extracted with acetonitrile and methanol (80:20, volume/volume), concentrated, filtered using a syringe filter, further concentrated under a stream of nitrogen, and analysed by HPLC. The LOD for quinoline was 2.14 ng per injection for UV-DAD and 12.7 ng per injection for FD. The LOD values obtained with FD were comparable with those published for GC flame ionization detector and GC-MS techniques.

(e) Textiles

Textiles may contain dyes based on quinoline. Luongo et al. (2016a) developed a method for the determination of aniline and quinoline compounds in textiles. Textile samples of cotton, polyamide, or polyester were extracted by dichloromethane, concentrated, and passed through graphitized carbon black SPE cartridges that selectively retain dyes and other interfering compounds present in the matrix, producing an extract suitable for GC-MS analysis. Recovered samples were assessed by spiking with a known amount of all the analytes before extraction. The recovery for quinoline was 79–83%, the LOD was 2.0 pg injected, and the LOQ was 5 ng/g.

1.3.2 Exposure assessment and biomarkers

No information was available to the Working Group on biomarkers of exposure to quinoline in humans.

1.4 Occurrence and exposure

1.4.1 Environmental occurrence

Quinoline occurs in small amounts (average, 0.3%) in coal tar and may be isolated by distillation (O'Neil, 2006; Seidel, 2006). Quinoline may enter the environment through atmospheric emissions and wastewaters of petroleum, shale oil, coal processing, and the application of coal tar creosote in wood preservation, and tobacco smoke. Quinoline is a major contaminant of soil and groundwater at sites where coal tar creosote has been used in wood preservation (Bennett et al., 1985; Pereira et al., 1987; Blum et al., 2011). The uses of quinoline in manufacturing, and as a corrosion inhibitor and as a solvent, (see Section 1.2.3) also provide avenues for its release to the environment through effluents and various waste streams (EPA, 1985, 2001). Environmental quinoline is often a component of complex mixtures, which include quinoline derivatives, volatile organic compounds, PAHs, and heteroaromatic compounds (N, S, O) (see Table 1.2).

Quinoline is soluble in water, mobile in groundwater, and subject to aerobic and anaerobic biodegradation processes; however, it has also been described as resistant to biodegradation (Thomsen et al., 1999; Deng et al., 2011; Bai et al., 2015; Xu et al., 2017). Some studies have investigated factors that control its persistence and mobility in the environment; for instance, soil characteristics and pH are known to affect quinoline mobility (Pereira et al., 1987; Fowler et al., 1994; Thomsen et al., 1999; Deng et al., 2011; Bai et al., 2015; Xu et al., 2017).

Quinoline is not known to bioaccumulate in mammals or fish (Novack & Brodie, 1950; Bean et al., 1985).

Table 1.2 Detection of quinoline and derivatives and other compounds in polluted groundwater

Pollutants	Origin of groundwater pollution	References
72 neutral, 41 basic, and 22 acidic compounds; quinoline, isoquinoline, methylquinolines, dimethylquinolines, (methyl)tetrahydroquinolines	Coal gasification site	Stuermer et al. (1982)
Quinoline, quinolinone, isoquinoline, isoquinolinone, 2- and 4-methylquinoline, (di)methylquinolinones, 2-methylisoquinolinone, benzoquinolines	Former wood-treatment plant site, Pensacola, Florida, USA	Pereira et al. (1987), Ondrus & Steinheimer (1990), Godsy et al. (1992)
111 polycyclic aromatic compounds, including N-/S-/O-heteroaromatic compounds, quinoline, 2-methylquinoline, (di)methylquinolines, isoquinoline, (iso)quinolinones, benzoquinolines, 1,2,3,4-tetrahydro(methyl)quinolines	Former coal tar distillation and wood-treatment plant at a Superfund site, St Louis Park, Minnesota, USA	Pereira et al. (1983), Rostad et al. (1985), Ondrus & Steinheimer (1990)
Polycyclic aromatic compounds and 17 N-heteroaromatic compounds; isoquinoline, quinolinones, methylquinolines, isoquinoline, quinolinones (quinoline not reported)	Coal and oil gasification site with non-aqueous phase liquids	Turney & Goerlitz (1990)
Isoquinolinone, methyl and dimethyl derivatives of quinolinone (quinoline not reported)	Former gas plant	Edler et al. (1997)
Several N-/S-/O-heteroaromatic compounds; quinoline, 2-methylquinoline, 2-hydroxyquinoline, 1-hydroxyisoquinoline, alkylquinolines	Three different creosote sites	Johansen et al. (1997)
16 polycyclic aromatic compounds and 23 N-heteroaromatic compounds; quinoline, isoquinoline, methylquinolines, dimethylquinolines, benzoquinolines	Subsurface non-aqueous-phase liquids at coke ovens site (steel production)	Baechler & MacFarlane (1992)

Compiled by the Working Group

(a) Water

Quinoline is often included in studies reporting the multitude of groundwater contaminants resulting from coal gasification or from the contamination of sites with creosote (Stuermer et al., 1982; Pereira et al., 1983; Rostad et al., 1985; Blum et al., 2011). For instance, 22 acidic, 72 neutral, and 41 basic compounds were isolated and identified in three groundwater samples collected near two underground coal gasification sites in north-east Wyoming, USA, 15 months after the end of gasification (Stuermer et al., 1982). Among the basic compounds, quinoline and other alkylated derivatives were identified; concentrations of 0.45, 7.1, and 14.0 μg/L were reported for quinoline and isoquinoline combined (Stuermer et al., 1982).

The need for rot-resistant wood products for railroad ties, pilings, poles, and other uses resulted in the establishment of about 400 creosote-treating facilities in the USA (EPA, 1981). Coal tar creosote, coal tar, and coal tar pitch have been found in at least 46 of the 1613 current or former sites identified in the EPA National Priorities List (ATSDR, 2002). In Germany, more than 1400 sites contaminated with coal tar have been identified (Blum et al., 2011). The United States Geological Survey extensively studied the fate of quinoline in two such creosote-contaminated sites in the USA: one in Pensacola, Florida (Bennett et al., 1985) and the other in St Louis Park, Minnesota (Rostad et al., 1985). In both cases the plants were operating for more than five decades, contaminating the groundwater and local aquifers.

In a wood-preserving facility occupying 18 acres within the city limits of Pensacola, Florida from 1902 to 1981, creosote and pentachlorophenol were solubilized with diesel and used to treat utility poles and lumber (Pereira et al., 1987). Wastes were discharged into two unlined surface impoundments in hydraulic connection with the sand and gravel aquifer (Pereira et al., 1987). A groundwater sample collected within the site, drawn from a depth of 6 m, indicated a concentration of 288 μg/L for quinoline and 5818 μg/L for the oxygenated derivative 2(1*H*)-quinolinone (Pereira et al., 1987). Ondrus & Steinheimer (1990) reported a quinoline concentration of 11.2 mg/L and a corresponding 2-hydroxyquinoline concentration of 42 mg/L in a single groundwater sample from the Pensacola site. The concentrations of isoquinoline and 1-hydroxyisoquinoline were 1.8 mg/L and 6.9 mg/L, respectively, suggesting microbial degradation.

The operation of a coal tar distillation and wood-preserving facility in St Louis Park, Minnesota from 1918 to 1972 resulted in extensive groundwater contamination and led to the closure of eight municipal wells in the vicinity; quinoline was qualitatively identified with 49 other compounds in the aqueous phase of a groundwater sample (Pereira et al., 1983). Azaarenes of high molecular weight were identified among 22 compounds in the oily tar phase of the groundwater sample (Pereira et al., 1983). Rostad et al. (1985) performed additional analysis on the St Louis Park groundwater, identifying 111 PAHs and determining octanol/water partition coefficients for a set of PAHs and N-/S-/O-heteroaromatic compounds including quinoline.

A groundwater sample from an active municipal well nearly 1 mile from the former site of the St Louis Park creosote plant yielded quinoline and 1-hydroxyisoquinoline concentrations of less than 15 ng/L; concentrations of isoquinoline and 2-hydroxyquinoline were measured at less than 70 ng/L and less than 10 ng/L, respectively (Ondrus & Steinheimer, 1990).

Adams & Giam (1984) identified 31 azaarenes in the wastewater collected from an onsite storage pond where creosote–pentachlorophenol was applied as a wood preservative in central Texas, USA. The quinoline concentration of 260 mg/L represented a sizeable fraction of the total azaarene concentration of 1300 mg/L (Adams & Giam, 1984).

Quinoline is associated with urban pollution and has been detected in urban rainwater. Concentrations of 1–4 μg/L were reported for quinoline, isoquinoline, and their substituted compounds combined for three rainwater samples collected in Los Angeles, USA during 1981–1982 (Kawamura & Kaplan, 1983).

Quinoline, methylquinolines, benzoquinoline, and methylbenzoquinolines were qualitatively identified in a sample taken from the River Waal at Brakel, Germany (Meijers & Van der Leer, 1976).

An EPA analysis of the FracFocus Chemical Disclosure Registry 1.0 indicated that quinoline was reported in 0.02% of chemical disclosures in 20 states in which hydraulic fracturing was conducted between 1 January 2011 and 28 February 2013 (Yost et al., 2017).

(b) Sediment and soil

Less than 5% of the sediment samples collected from 443 sites in 19 major United States river basins during 1992–1995 tested positive for quinoline (Lopes et al., 1997).

Analysis of the water-soluble fraction of creosote-contaminated sediment obtained from a Superfund site located on the Elizabeth River in Virginia, USA revealed the presence of naphthalene and other PAHs, but an absence of quinoline and isoquinoline (Padma et al., 1998). The authors attributed the absence of quinoline to its water solubility or microbial degradation.

Furlong & Carpenter (1982) confirmed the presence of quinoline in marine sediments of Puget Sound, north-west Washington, USA. Of the 39 sediment samples collected at six different

Puget Sound sites, quinoline was detected in 75% at a range of 160–6600 ng/g organic carbon. Quinoline was detected in all three samples from nearby Lake Washington at a concentration of 120–1300 ng/g organic carbon. Furlong & Carpenter (1982) attributed quinoline and other two- and three-ring azaarenes in the surface sediments of Puget Sound to air particulate matter arising from petroleum combustion (Furlong & Carpenter, 1982).

(c) Air

Chuang et al. (1991) measured the indoor air levels of PAHs in eight homes in Columbus, Ohio, USA during the winter of 1986/1987. Average 8-hour indoor concentrations of quinoline within the range 10–26 μg/m^3 were measured in homes occupied by non-smokers and 93–560 μg/m^3 in the homes of smokers (Chuang et al., 1991). The average outdoor concentration of these residences, in areas characterized as devoid of apparent contamination sources and low in traffic, was 3.3 μg/m^3 (range, 0.78–5.5 μg/m^3) (Chuang et al., 1991).

Quinoline was measured in two particulate matter samples collected in the urban air above New York City with high-volume samplers (Dong et al., 1977). Quinoline was found at concentrations of 69 and 22 ng per 1000 m^3, isoquinoline at 180 and 140 ng per 1000 m^3, and several alkyl derivatives of quinoline.

A low Henry Law constant is an indication of insignificant volatilization of quinoline from surface waters (EPA, 2001). Air samples collected from a pilot-scale shale oil wastewater treatment facility at the Logan Wash site, Colorado, USA in 1982 contained quinoline at 6 μg/m^3 in indoor air and 1 μg/m^3 in outdoor air (Hawthorne & Sievers, 1984). Concentrations below the LOD (0.05 μg/m^3) were measured in the rural air of an undeveloped region of the shale oil region and in the urban air of Boulder, Colorado, USA.

Quinoline emissions in the USA reported to the EPA decreased from 9.9 tonnes in 2000 (18 industry submissions) to 0.27 tonnes in 2015 (10 industry submissions) (EPA, 2015).

(d) Tobacco

Indoor concentrations of quinoline and isoquinoline were found to correlate closely with nicotine, and may serve as markers of indoor levels of environmental tobacco smoke (Chuang et al., 1991). The estimated correlation coefficients between quinoline and nicotine and between isoquinoline and nicotine were 0.96 ($P = 0.0001$) and 0.97 ($P = 0.0001$), respectively (Chuang et al., 1991).

1.4.2 Exposure of the general population

The general population may be exposed to quinoline by the inhalation of cigarette smoke or environmental tobacco smoke, or from particulate matter in urban air. Quinoline and isoquinoline are found in tobacco smoke, but not tobacco leaf (Stedman, 1968). Quinoline has been quantified in cigarette mainstream smoke at 0.17–1.30 μg per cigarette by Adams et al. (1983), at 0.19 μg per cigarette by White et al. (1990), and at 0.23–0.30 μg per cigarette by Chen & Moldoveanu (2003). Relative to non-filtered cigarettes, filters were found to reduce quinoline in smoke by 36–50% with a similar reduction in tar of 28–63% (Adams et al., 1983).

The potential for skin exposure exists from clothing containing dyes based on quinoline and for oral exposure through food colorants based on quinoline. Quinoline is used in the dyeing process of textiles (Lam et al., 2012), and the presence of quinoline and quinoline derivatives has been confirmed in clothing items (Luongo et al., 2014, 2016a,b; Antal et al., 2016). Luongo et al. (2014) detected quinoline and 10 quinoline derivatives in 31 textile samples purchased between 2011 and 2012 from different shops in Stockholm, Sweden. Quinoline was detected in all garments made of polyester at concentrations in the range 26–16 700 ng/g with a mean

concentration of 4700 ng/g, 600 times quinoline concentrations in cotton garments. In a subsequent study, the average washout of quinoline from clothing textiles was determined to be about 20% after the items had been washed 10 times (Luongo et al., 2016b). [This suggests a potential for skin exposure from clothing containing dyes based on quinoline. Furthermore, because dyes based on quinoline may have mutual food and textile usage (i.e. Quinoline Yellow), the potential for oral exposure through food colorants based on quinoline cannot be ruled out.]

Groundwater contamination may pose an additional risk of exposure to quinoline for populations accessing aquifers proximate to creosote wood preservation sites (Bennett et al., 1985; Pereira et al., 1987; Thomsen et al., 1999; Zhang et al., 2010).

1.4.3 Occupational exposure

The most probable route of occupational exposure to quinoline is by inhalation of particulates or vapours from the processing of petroleum, the processing and production of shale oil, or the use of coal-derived products (Gammage, 1983). There is also potential for exposure to quinoline in industries where quinoline is used as a solvent or chemical intermediate; however, no relevant occupational data were available to the Working Group. A Finnish study of workers involved in railway repair and construction found that the handling of wood impregnated with creosote resulted in the exposure of workers to quinoline at concentrations of less than 0.1 mg/m^3 (18 workers), and that the assembly of switch elements resulted in exposure to concentrations of less than 0.2 mg/m^3 (8 workers) (Heikkilä et al., 1987).

1.5 Regulations and guidelines

The American Industrial Hygiene Association set a 2011 Workplace Environmental Exposure Level for quinoline of 0.001 ppm (8-hour time-weighted average) with a “skin” notation, indicating that quinoline may be absorbed in toxicologically significant amounts through the skin (American Industrial Hygiene Association, 2013). The GESTIS database of International Limit Values for 30 countries, including various European Union Member States, specified a quinoline 8-hour time-weighted average of occupational limit for only one country (Latvia, 0.1 mg/m^3) (IFA 2017).

2. Cancer in Humans

No data on the carcinogenicity of quinoline in humans were available to the Working Group.

3. Cancer in Experimental Animals

See Table 3.1.

3.1 Mouse

3.1.1 Oral administration

(a) Feeding

Two groups of 40 male and 40 female ddY mice (age, 8 weeks) were given 0.2% quinoline [purity not reported] in commercial basal diet for 30 weeks (Shinohara et al., 1977). There were no untreated controls. One half of the number of males and females died of pneumonia within the first 6 weeks of the experiment. Only 10 males and 10 females survived after 30 weeks, and data were presented from these animals. The body weights of both male and female mice decreased during the experiment, but it was not reported

Table 3.1 Studies of carcinogenicity in experimental animals exposed to quinoline

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence and/or multiplicity of tumours	Significance	Comments
Full carcinogenicity Mouse, Crj: BDF_1 (M) 6 wk 55–65 wk Matsumoto et al. (2018)	Drinking-water Quinoline, > 99.6% Deionized water 0, 150, 300, 600 ppm ad libitum 50, 50, 50, 50 46 (at 65 wk), 15 (at 65 wk), 0 (at 65 wk), 0 (at 55 wk)	*Liver*		Principal strengths: studies in both males and females; data obtained from all treated animals; extensive histopathology; GLP study
		Hepatocellular carcinoma		
		0/50*, 4/50, 0/50, 1/50	*$P < 0.01$ (Peto trend test)	
		Histiocytic sarcoma		
		0/50*, 0/50, 3/50, 1/50	*$P < 0.01$ (Peto trend test)	
		Haemangiosarcoma		
		0/50*, 2/50, 1/50, 12/50**	*$P < 0.01$ (Peto trend test), **$P < 0.01$ (Fisher exact test)	
		Subcutis		
		Haemangioma		
		0/50, 0/50, 1/50, 0/50	NS	
		Haemangiosarcoma		
		0/50*, 2/50, 2/50, 3/50	*$P < 0.01$ (Peto trend test)	
		Retroperitoneum		
		Haemangioma		
		0/50*, 0/50, 0/50, 3/50	*$P < 0.01$ (Peto trend test)	
		Haemangiosarcoma		
		0/50*, 35/50**, 38/50**, 35/50**	*$P < 0.01$ (Peto trend test), **$P < 0.01$ (Fisher exact test)	
		Mesenterium		
		Haemangioma		
		0/50, 1/50, 1/50, 2/50	NS	
		Haemangiosarcoma		
		0/50*, 19/50**, 22/50**, 16/50**	*$P < 0.01$ (Peto trend test), **$P < 0.01$ (Fisher exact test)	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence and/or multiplicity of tumours	Significance	Comments
Matsumoto et al. (2018) (cont.)		*Mediastinum*: haemangiosarcoma		
		0/50, 2/50, 0/50, 1/50	NS	
		Peritoneum: haemangiosarcoma		
		0/50, 0/50, 0/50, 1/50	NS	
		All organs		
		Haemangioma		
		1/50*, 2/50, 3/50, 7/50**	*$P < 0.01$ (Peto trend test), **$P < 0.05$ (Fisher exact test)	
		Haemangiosarcoma		
		0/50*, 43/50**, 47/50**, 43/50**	*$P < 0.01$ (Peto trend test), **$P < 0.01$ (Fisher exact test)	
		Haemangioma or haemangiosarcoma (combined)		
		1/50*, 44/50**, 47/50**, 46/50**	*$P < 0.01$ (Peto trend test), **$P < 0.01$ (Fisher exact test)	
Full carcinogenicity Mouse, Crj: BDF_1 (F) 6 wk 44–50 wk Matsumoto et al. (2018)	Drinking-water Quinoline, > 99.6% Deionized water 0, 150, 300, 600 ppm ad libitum 50, 50, 50, 50 49 (at 50 wk), 20 (at 50 wk), 6 (at 50 wk), 0 (at 44 wk)	*Liver*		Principal strengths: studies in both males and females; data obtained from all treated animals; extensive histopathology; GLP study
		Hepatocellular adenoma		
		0/50, 0/50, 2/50, 1/50	NS	
		Histiocytic sarcoma		
		0/50*, 2/50, 6/50**, 4/50	*$P < 0.01$ (Peto trend test), **$P < 0.05$ (Fisher exact test)	
		Haemangioma		
		0/50*, 1/50, 2/50, 5/50**	*$P < 0.01$ (Peto trend test), **$P < 0.05$ (Fisher exact test)	
		Haemangiosarcoma		
		0/50, 0/50, 0/50, 2/50	NS	
		Subcutis		
		Haemangioma		
		0/50*, 0/50, 7/50**, 15/50**	*$P < 0.01$ (Peto trend test), **$P < 0.01$ (Fisher exact test)	
		Haemangiosarcoma		
		0/50*, 4/50, 15/50**, 33/50**	*$P < 0.01$ (Peto trend test), **$P < 0.01$ (Fisher exact test)	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence and/or multiplicity of tumours	Significance	Comments
Matsumoto et al. (2018) (cont.)		*Ovary*: haemangiosarcoma		
		0/50*, 1/50, 4/50, 1/50	*P < 0.01 (Peto trend test)	
		Retroperitoneum		
		Haemangioma		
		0/50*, 5/50**, 1/50, 1/50	*P < 0.05 (Peto trend test), **P < 0.05 (Fisher exact test)	
		Haemangiosarcoma		
		0/50*, 27/50**, 36/50**, 32/50**	*P < 0.01 (Peto trend test), **P < 0.01 (Fisher exact test)	
		Mesenterium		
		Haemangioma		
		0/50, 2/50, 2/50, 2/50	NS	
		Haemangiosarcoma		
		0/50*, 18/50**, 18/50**, 11/50**	*P < 0.01 (Peto trend test), **P < 0.01 (Fisher exact test)	
		Mediastinum		
		Haemangioma		
		0/50, 0/50, 0/50, 1/50	NS	
		Haemangiosarcoma		
		0/50*, 2/50, 3/50, 1/50	*P < 0.01 (Peto trend test)	
		Peritoneum		
		Haemangioma		
		0/50*, 2/50, 6/50**, 2/50	*P < 0.01 (Peto trend test), **P < 0.05 (Fisher exact test)	
		Haemangiosarcoma		
		0/50*, 3/50, 6/50**, 15/50***	*P < 0.01 (Peto trend test), **P < 0.05 (Fisher exact test), ***P < 0.01 (Fisher exact test)	
		All organs		
		Haemangioma		
		1/50*, 9/50**, 16/50**, 24/50**	*P < 0.01 (Peto trend test), **P < 0.01 (Fisher exact test)	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence and/or multiplicity of tumours	Significance	Comments
Matsumoto et al. (2018) (cont.)		Haemangiosarcoma		
		0/50*, 43/50**, 48/50**, 49/50**	*$P < 0.01$ (Peto trend test), **$P < 0.01$ (Fisher exact test)	
		Haemangioma or haemangiosarcoma (combined)		
		1/50*, 45/50**, 48/50**, 50/50**	*$P < 0.01$ (Peto trend test), **$P < 0.01$ (Fisher exact test)	
Full carcinogenicity Mouse, CD-1 (M) Newborn 52 wk LaVoie et al. (1987)	Intraperitoneal injection Quinoline, > 99% pure DMSO 0 (control), 1.75 μmol total dose 5, 10, and 20 μL of either DMSO (control) or a 0.05 mol/L solution of quinoline in DMSO on days 1, 8, and 15 of life, respectively 35, 41 17 (at 35 wk), 17 (at 35 wk)	*Liver*		Principal strengths: studies in both males and females Principal limitations: use of single dose; no body-weight data; no discussion of clinical signs; statistical test not specified Number of animals at start = M+F combined
		[Hepatocellular] adenoma		
		0/17, 4/17	NS	
		Total tumours: 0, 15		
		Hepatoma [hepatocellular carcinoma]		
		1/17, 8/17*	*$P < 0.01$	
		Total tumours: 1, 37		
		Hepatic tumours [hepatocellular tumours]		
		1/17, 12/17*	*$P < 0.005$	
		Haematopoietic and lymphoid tissues: lymphoma or leukaemia (combined)		
		1/17, 1/17	NS	
Full carcinogenicity Mouse, CD-1 (F) Newborn 52 wk LaVoie et al. (1987)	Intraperitoneal injection Quinoline, > 99% pure DMSO 0 (control), 1.75 μmol total dose 5, 10, and 20 μL of either DMSO (control) or a 0.05 mol/L solution of quinoline in DMSO on days 1, 8, and 15 of life, respectively 35, 41 18 (at 35 wk), 10 (at 35 wk)	*Liver*		Principal strengths: studies in both males and females Principal limitations: use of single dose; no body-weight data; no discussion of clinical signs; statistical test not specified Number of animals at start = M+F combined
		[Hepatocellular] adenoma		
		0/18, 1/10	NS	
		Total tumours: 0, 1		
		Hepatoma [hepatocellular carcinoma]		
		0/18, 0/10	NS	
		Haematopoietic and lymphoid tissues: lymphoma or leukaemia (combined)		
		0/18, 4/10*	*$P < 0.05$	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence and/or multiplicity of tumours	Significance	Comments
Full carcinogenicity Mouse, CD-1 (M) Newborn 52 wk LaVoie et al. (1988)	Intraperitoneal injection Quinoline, > 99% pure DMSO 0 (control), 1.75 µmol total dose 5, 10, and 20 µL of either DMSO (control) or a 0.05 mol/L solution of quinoline in DMSO on days 1, 8, and 15 of life, respectively 46, 56 21 (at 6 mo), 19 (at 6 mo)	*Liver* [Hepatocellular] adenoma 0/21, 13/19* Hepatoma [hepatocellular carcinoma] 0/21, 2/19	 *[$P < 0.0001$] NS; when hepatocellular adenomas and carcinomas are combined, the overall incidence of hepatocellular tumours (15/19) is significantly increased ($P < 0.05$, χ^2 test)	Principal strengths: studies in both males and females Principal limitations: use of single dose; no body-weight data; no discussion of clinical signs Repeat of the earlier study of LaVoie et al. (1987). Quinoline treatment produced a significant increase in the incidence of hepatocellular tumours in male mice only. In contrast to the 1987 study, there was a higher proportion of quinoline-induced hepatocellular adenomas than carcinomas in these mice. Number of animals at start = M+F combined
Full carcinogenicity Mouse, CD-1 (F) Newborn 52 wk LaVoie et al. (1988)	Intraperitoneal injection Quinoline, > 99% pure DMSO 0 (control), 1.75 µmol total dose 5, 10, and 20 µL of either DMSO (control) or a 0.05 mol/L solution of quinoline in DMSO on days 1, 8, and 15 of life, respectively 46, 56 21 (at 6 mo), 27 (at 6 mo)	*Liver* [Hepatocellular] adenoma 0/21, 0/27 Hepatoma [hepatocellular carcinoma] 0/21, 0/27	 NS NS	Principal strengths: studies in both males and females Principal limitations: use of single dose; no body-weight data; no discussion of clinical signs Repeat of the earlier study of LaVoie et al. (1987). No liver tumours were detected in quinoline-treated female mice; 3 mice with lung tumours and 5 mice with lymphomas or leukaemias in the 27 surviving treated female mice, but these results were not significant. Number of animals at start = M+F combined

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence and/or multiplicity of tumours	Significance	Comments
Full carcinogenicity Mouse, CD-1 (M) Newborn 52 wk Weyand et al. (1993)	Intraperitoneal injection Quinoline, > 98% pure DMSO 0 (control), 1.75 µmol total dose 5, 10, and 20 µL of either DMSO (control) or a 50 mmol/L solution of quinoline in DMSO on days 1, 8, and 15 of life, respectively 97, 85 38 (at 2 mo), 33 (at 2 mo)	*Liver* [Hepatocellular] adenoma 0/38, 15/33 (45%)* [Hepatocellular] carcinoma 0/38, 1/33 (3%)	 *[$P < 0.0001$, Fisher exact test] NS	Principal strengths: both sexes used Principal limitations: no body-weight data Number of animals at start = M+F combined
Full carcinogenicity Mouse, CD-1(F) Newborn 52 wk Weyand et al. (1993)	Intraperitoneal injection Quinoline, > 98% pure DMSO 0 (control), 1.75 µmol total dose 5, 10, and 20 µL of either DMSO (control) or a 50 mmol/L solution of quinoline in DMSO on days 1, 8, and 15 of life, respectively 97, 85 46 (at 2 mo), 37 (at 2 mo)	*Liver* [Hepatocellular] adenoma 0/46, 0/37 [Hepatocellular] carcinoma 0/46, 0/37	 NS NS	Principal strengths: both sexes used Principal limitations: no body-weight data Number of animals at start = M+F combined

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence and/or multiplicity of tumours	Significance	Comments
Initiation–promotion (tested as initiator) Mouse, SENCAR (F) 50–55 d 22 wk (initiation + TPA treatment) LaVoie et al. (1984)	Skin application Quinoline, > 99.8% pure Acetone 0 (control), 7.5 mg total dose 0 (control) or 0.75% in 0.1 mL acetone applied to skin in 10 doses (every other day) 40, 40 NR, NR	*Skin*: tumours (macroscopic examination) 3/39 (7.5%), 21/40 (53%)* Tumour multiplicity: 0.08, 0.73 Total tumours: 3, 29	 *$P < 0.01$, χ^2 test NR NR	Principal limitations: only one dose group; no histopathological examination Initiation–promotion study with quinoline being tested as an initiator (for 20 days) followed (after 10 days) by promotion with 2.0 µg TPA (2×/wk for 18 wk); for comparison, quinoline at 7.5 mg total dose produced 0.73 skin tumours per mouse and benzo[*a*]pyrene at 0.03 mg total dose produced 2.1 skin tumours per mouse
Full carcinogenicity Rat, Sprague-Dawley (M) NR 40 wk Hirao et al. (1976)	Oral administration Quinoline, > 99.8% pure Diet 0, 0.05, 0.1, 0.25% of diet 6, 20, 20, 20 6, 11 (at 16 wk), 16 (at 16 wk), 19 (at 16 wk)	*Liver* Haemangioendothelioma [haemangiosarcoma] 0/6, 6/11 (54%)*, 12/16 (75%)**, 18/19 (95%)*** Nodular hyperplasia 0/6, 6/11 (54%)*, 4/16 (25%), 0/19 (0%) Hepatocellular carcinoma 0/6, 3/11 (27%), 3/16 (19%), 0/19	 *[$P < 0.05$, Fisher exact test], **[$P < 0.005$], ***[$P < 0.0001$] *[$P < 0.05$, Fisher exact test] [NS]	Principal strengths: multiple dose study Principal limitations: no statistics reported in the article; dose selection criteria not given; data not taken from all animals; poor survival in high- and medium-dose animals
Carcinogenicity with other modifying factor Rat, Sprague-Dawley (M) 8 wk 30 wk Shinohara et al. (1977)	Oral administration Quinoline, NR Diet 0, 0.075% of diet NR 10 (at 26 wk), 20 (at 26 wk)	*Liver* Hepatocellular carcinoma 0/10, 0/20 Haemangioendothelioma [haemangiosarcoma] 0/10, 6/20 (30%)	 NS [NS]	Principal limitations: only one dose group; short duration of exposure; limited experimental details; only one sex used; number of animals at start unspecified

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence and/or multiplicity of tumours	Significance	Comments
Full carcinogenicity Rat, Wistar (M) 7 wk up to 20 wk Hasegawa et al. (1989)	Oral administration Quinoline, NR Diet 0 (20 wk, control), 0.25 (12 wk exposure), 0.25 (12 wk exposure + 4 wk), 0.25 (12 wk exposure + 8 wk), 0.25 (16 wk exposure), 0.25 (16 wk exposure + 4 wk), 0.25 (20 wk exposure) % of diet NR 12, 11, 12, 12, 14, 18, 16	Liver: haemangioendothelioma [haemangiosarcoma] 0/12, 1/11 [9%], 2/12 (17%), 5/12 (42%)*, 4/14 (29%)*, 4/18 (22%), 5/16 (31%)*	 *$P < 0.05$ (Fisher exact test)	Principal limitations: only one sex was used; only one time-matched control group; number of animals at start unspecified
Full carcinogenicity Rat, SHR (M) 5 wk 32 wk Futakuchi et al. (1996)	Oral administration Quinoline, NR Powdered diet 0, 0.2% of diet 10, 16 9, 15	*Liver* Haemangioendothelial sarcoma [haemangiosarcoma] 0/9, 1/15 (7%) Hyperplastic nodules 0/9, 3/15 (20%)	 NS NS	Principal strengths: chemical intake measured Principal limitations: only one dose group; only one sex used; short duration
Full carcinogenicity Rat, WKY (M) 5 wk 32 wk Futakuchi et al. (1996)	Oral administration Quinoline, NR Powdered diet 0, 0.2% of diet 10, 16 10, 8	*Liver* Haemangioendothelial sarcoma [haemangiosarcoma] 0/10, 14/15 (93%)* Hyperplastic nodules 0/10, 3/15 (20%)	 *$P < 0.001$, Fisher exact test NS	Principal strengths: chemical intake measured Principal limitations: only one dose group; only one sex used; short duration
Full carcinogenicity Rat, F344/DuCrj (M) 6 wk 76–96 wk Matsumoto et al. (2018)	Drinking-water Quinoline, > 99.6% Deionized water 0, 200, 400, 800 ppm ad libitum 50, 50, 50, 50 49 (at 96 wk), 19 (at 96 wk), 0 (at 95 wk), 0 (at 76 wk)	*Liver* Hepatocellular adenoma 1/50*, 10/50**, 10/50**, 9/50** Hepatocellular carcinoma 0/50*, 22/50**, 24/50**, 18/50**	 *$P < 0.05$ (Peto trend test), **$P < 0.01$ (Fisher exact test) *$P < 0.01$ (Peto trend test), **$P < 0.01$ (Fisher exact test)	Principal strengths: studies in both males and females; data obtained from all treated animals; extensive histopathology; GLP study

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence and/or multiplicity of tumours	Significance	Comments
Matsumoto et al. (2018) (cont.)		Hepatocellular adenoma or carcinoma (combined)		
		1/50*, 31/50**, 29/50**, 23/50**	*$P < 0.01$ (Peto trend test), **$P < 0.01$ (Fisher exact test)	
		Haemangiosarcoma		
		0/50*, 25/50**, 34/50**, 43/50**	*$P < 0.01$ (Peto trend test), **$P < 0.01$ (Fisher exact test)	
		Nasal cavity		
		Haemangioma		
		0/50, 0/50, 1/50, 0/50	NS	
		Sarcoma (NOS)		
		0/50*, 1/50, 5/50**, 1/50	*$P < 0.01$ (Peto trend test), **$P < 0.05$ (Fisher exact test)	
		Esthesioneuroepithelioma		
		0/50*, 0/50, 1/50, 6/50**	*$P < 0.01$ (Peto trend test), **$P < 0.05$ (Fisher exact test)	
		Lung		
		Haemangiosarcoma		
		0/50, 0/50, 2/50, 1/50	NS	
		Adenosquamous carcinoma		
		0/50, 0/50, 1/50, 0/50	NS	
		Mediastinum: sarcoma (NOS)		
		0/50*, 1/50, 2/50, 3/50	*$P < 0.01$ (Peto trend test)	
		Mesenterium: haemangiosarcoma		
		0/50*, 0/50, 2/50, 2/50	*$P < 0.05$ (Peto trend test)	
		Peritoneum: haemangiosarcoma		
		0/50*, 0/50, 0/50, 1/50	NS	
		Adipose tissue: haemangiosarcoma		
		0/50*, 2/50, 0/50, 3/50	*$P < 0.01$ (Peto trend test)	
		All organs: haemangiosarcoma		
		0/50*, 26/50**, 36/50**, 45/50**	*$P < 0.01$ (Peto trend test), **$P < 0.01$ (Fisher exact test)	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence and/or multiplicity of tumours	Significance	Comments
Full carcinogenicity Rat, F344/DuCrj (F) 6 wk 88–104 wk Matsumoto et al. (2018)	Drinking-water Quinoline, > 99.6% Deionized water 0, 150, 300, 600 ppm ad libitum 50, 50, 50, 50 41 (at 104 wk), 17 (at 104 wk), 2 (at 104 wk), 0 (at 88 wk)	*Liver* Hepatocellular adenoma		Principal strengths: studies in both males and females; data obtained from all treated animals; extensive histopathology; GLP study
		1/50*, 30/50**, 31/50**, 33/50**	*$P < 0.01$ (Peto trend test), **$P < 0.01$ (Fisher exact test)	
		Hepatocellular carcinoma		
		0/50*, 5/50**, 16/50***, 21/50***	*$P < 0.01$ (Peto trend test), **$P < 0.05$ (Fisher exact test), ***$P < 0.01$ (Fisher exact test)	
		Hepatocellular adenoma or carcinoma (combined)		
		1/50*, 32/50**, 38/50**, 42/50**	*$P < 0.01$ (Peto trend test), **$P < 0.01$ (Fisher exact test)	
		Haemangiosarcoma		
		0/50*, 15/50**, 27/50**, 41/50**	*$P < 0.01$ (Peto trend test), **$P < 0.01$ (Fisher exact test)	
		Nasal cavity: sarcoma (NOS)		
		0/50, 0/50, 1/50, 1/50	NS	
		Lung: haemangiosarcoma		
		0/50, 2/50, 0/50, 0/50	NS	
		Ovary: haemangioma		
		0/50, 1/50, 0/50, 0/50	NS	
		Retroperitoneum: haemangiosarcoma		
		0/50, 0/50, 0/50, 1/50	NS	
		Peritoneum: haemangiosarcoma		
		0/50, 0/50, 1/50, 0/50	NS	
		Adipose tissue: haemangiosarcoma		
		0/50, 0/50, 2/50, 0/50	NS	
		All organs: haemangiosarcoma		
		0/50*, 17/50**, 28/50**, 42/50**	*$P < 0.01$ (Peto trend test), **$P < 0.01$ (Fisher exact test)	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence and/or multiplicity of tumours	Significance	Comments
Full carcinogenicity Rat, Sprague-Dawley (M) Newborn 78 wk LaVoie et al. (1988)	Subcutaneous injection Quinoline, > 99% pure DMSO DMSO at 500 µL/kg bw 1×/wk for 8 wk (control), or quinoline at 200 µmol/kg bw at wk 1, at 100 µmol/kg bw at wk 2–7, and 200 µmol/kg bw at wk 8 50, 101 27, 25	*Liver* [Hepatocellular] adenoma 3/27, 1/25 Hepatoma [hepatocellular carcinoma] 2/27, 0/25	 NS NS	Principal strengths: studies in both males and females Principal limitations: high mortality after the initial dose; no body-weight data; only one variable dose group This study could have been compromised significantly by the high mortality rate following the initial injection of 200 µmol/kg bw of quinoline. Only 41 of the 101 pups survived, resulting in 59% mortality. In the surviving rats, carcinogenicity could have been reduced by the significant toxicity; no weight data were given to provide an assessment of toxicity in treated versus control animals during the study. Number of animals at start = M+F combined
Full carcinogenicity Rat, Sprague-Dawley (F) Newborn 78 wk LaVoie et al. (1988)	Subcutaneous injection Quinoline, > 99% pure DMSO DMSO at 500 µL/kg bw 1×/wk for 8 wk (control), or quinoline at 200 µmol/kg bw at wk 1, at 100 µmol/kg bw at wk 2–7, and 200 µmol/kg bw at wk 8 50, 101 22, 15	*Liver* [Hepatocellular] adenoma 1/22, 0/15 Hepatoma [hepatocellular carcinoma] 0/22, 0/15	 NS NS	Principal strengths: studies in both males and females Principal limitations: high mortality after the initial dose; no body-weight data; only one variable dose group This study could have been compromised significantly by the high mortality rate following the initial injection of 200 µmol/kg bw of quinoline. Only 41 of the 101 pups survived, resulting in 59% mortality. In the surviving rats, carcinogenicity could have been reduced by the significant toxicity; no weight data were given to provide an assessment of toxicity in treated versus control animals during the study. Number of animals at start = M+F combined

bw, body weight; d, day(s); DMSO, dimethyl sulfoxide; F, female; GLP, good laboratory practice; M, male; mo, month(s); NOS, not otherwise specified; NR, not reported; NS, not significant; ppm, parts per million; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; wk, week(s)

whether these weight losses were significant. The liver weight as a percentage of the body weight increased in mice of both sexes due to tumour development and/or swelling of the liver. Grossly, the livers of mice had multiple small and large nodules measuring up to 1.0 cm in diameter. Some tumorous nodules showed focal haemorrhagic change, but metastasis to other organs from liver tumours was not observed. The 10 surviving mice per sex were examined for changes to their livers; there were 8 male mice with haemangioendotheliomas [haemangiosarcomas] (80%) and 1 with hepatocellular carcinoma (10%), and there were 8 female mice with haemangioendotheliomas [haemangiosarcomas] (80%). [The Working Group noted that the principal limitations of the study included the use of a single dose, the short duration of exposure, the poor survival due to pneumonia, and the lack of controls. Although the occurrence of haemangiosarcomas in both male and female mice was 80%, the significance of this finding could not be determined due to the lack of controls. The Working Group concluded that this study was inadequate for the evaluation of the carcinogenicity of quinoline in experimental animals.]

(b) Drinking-water

In a good laboratory practice (GLP) study, four groups of 50 male and 50 female Crj:BDF$_1$ mice (SPF) (age, 6 weeks) were given quinoline (purity, > 99.6%) at 0, 150, 300, or 600 ppm in deionized water for varying periods of time (Matsumoto et al., 2018). Body weight and the consumption of food and water were measured once per week for the first 14 weeks of the administration period, and every 2 weeks thereafter.

The initial design was to expose mice to quinoline in their drinking-water for up to 104 weeks; however, dose-related decreases in survival occurred, necessitating the early termination of the experiment. All mice were observed daily for clinical signs and mortality, and animals found moribund were killed and their organs removed, weighed, and examined for macroscopic lesions at necropsy. All organs and tissues, including the entire respiratory tract, were examined for histopathology.

All male mice exposed to quinoline at 300 and 600 ppm were dead by the end of weeks 65 and 55, respectively, and there were only 15 surviving mice (30%) in the group exposed at 150 ppm; this group was terminated at week 65. The survival rate of the control males at week 65 was 92% (46/50). All female mice exposed to quinoline at 600 ppm were dead by the end of week 44, and there were only 6 surviving mice (12%) in the group exposed at 300 ppm; this group was terminated at week 50. The survival rates of the female controls and group exposed at 150 ppm at week 50 were 98% (49/50) and 40% (20/50), respectively. The decreased survival in treated males and females was attributed to deaths due to haemangiomas or haemangiosarcomas of the retroperitoneum, mesenterium, or subcutis. The earliest malignant tumour deaths were observed at weeks 36, 40, and 32 in the males and at weeks 33, 28, and 27 in the females exposed at 150, 300, and 600 ppm, respectively. The growth rates of all exposed males and the females exposed at 600 ppm were generally less than those of the controls throughout the study period.

The incidences of haemangiosarcoma and haemangioma were increased in exposed male mice, whereas no haemangiosarcomas and only one haemangioma (of the liver) were observed in 50 male controls. Quinoline significantly increased the incidence of haemangiosarcoma, in the liver of mice exposed at 600 ppm as well as in the retroperitoneum and in the mesenterium of mice exposed at all dose levels. In addition, in all organs combined, quinoline significantly increased the incidence of haemangioma in mice exposed at 600 ppm. Further, significant increases in the incidence of haemangiosarcoma and of haemangioma or haemangiosarcoma (combined) were seen in all organs combined at all dose levels. There was also a significant positive

trend in the incidences of hepatocellular carcinoma, liver histiocytic sarcoma, retroperitoneum haemangioma, and subcutis haemangiosarcoma.

In female mice, no haemangiosarcomas and only one haemangioma (of the ovary) were observed in 50 female controls. Quinoline significantly increased the incidence of histiocytic sarcoma in the liver (at 300 ppm), and of haemangioma in the liver of (at 600 ppm). The compound significantly increased the incidence of subcutis haemangioma and of subcutis haemangiosarcoma in mice exposed at 300 and 600 ppm. Quinoline also significantly increased the incidence of haemangioma in the retroperitoneum of mice exposed at 150 ppm, and of haemangiosarcoma in the retroperitoneum and of haemangiosarcoma in the mesenterium at all dose levels. In the peritoneum, quinoline significantly increased the incidence of haemangioma in mice exposed at 300 ppm, and of haemangiosarcoma in mice exposed at 300 and 600 ppm. For all organs combined, quinoline significantly increased the incidence of haemangioma, of haemangiosarcoma, and of haemangioma or haemangiosarcoma (combined) at all dose levels. There was also a significant positive trend in the incidences of ovary haemangiosarcoma and mediastinum haemangiosarcoma.

[The Working Group noted the early onset of rare tumours of various embryological origins at the lowest dose tested and the very poor survival due to tumour induction. The Working Group also noted that the principal strengths of this GLP study included: the use of both males and females, the use of multiple dose levels, the accurate determination of compound exposure, the reporting of body weight and survival data, the fact that results were obtained from all treated animals, and the extensive histopathological examination of all organs.]

3.1.2 Intraperitoneal injection

Groups of 41 male and 41 female CD-1 mouse pups were given intraperitoneal injections of quinoline (purity, > 99%) in dimethyl sulfoxide (DMSO) (LaVoie et al., 1987). Each pup was given 5, 10, and 20 µL of either DMSO (control) or of a 0.05 mol/L solution of quinoline on days 1, 8, and 15 of life, respectively. Each mouse received a total amount of 1.75 µmol of quinoline. Five of the mice given quinoline were killed at age 35 weeks, and there was no evidence of any lesions in these mice. The remaining mice exposed to quinoline and the DMSO controls were killed at age 52 weeks. A total of 27 (17 males and 10 females) of the 41 mice exposed to quinoline and all 35 (17 males and 18 females) of the mice given DMSO survived at 52 weeks. In the 17 male mice given quinoline, there were 4 mice with hepatic [hepatocellular] adenomas and 8 ($P < 0.01$) mice with hepatomas [considered by the Working Group to be hepatocellular carcinomas]. The incidence of hepatic tumours [hepatocellular tumours] (12/17, 71%) was significantly increased ($P < 0.005$) in male mice compared with controls. The tumour response in the 10 female mice given quinoline included 4 ($P < 0.05$) mice with lymphoma. There was 1 hepatoma [hepatocellular carcinoma] in the 17 male mice and no tumours in the 18 female mice treated with DMSO. [The Working Group noted the principal strength of the study was that both male and female mice were used. The principal limitations included the use of a single dose, the absence of body-weight data, the lack of a discussion of clinical signs, and the unspecified statistical test.]

In a second study in newborn CD-1 mice (LaVoie et al., 1988), 56 male and 56 female pups were given intraperitoneal injections of quinoline (purity, > 99%) in DMSO. Each pup was given 5, 10, and 20 µL of either DMSO (control) or of a 0.05 mol/L solution of quinoline on days 1, 8, and 15 of life, respectively. Each mouse was

given a total amount of 1.75 μmol of quinoline. The highest mortality was observed among the pups given quinoline, of which 18% had died by the third week of life. A total of 46 (19 males and 27 females) of the 56 mice given quinoline and 42 (21 males and 21 females) of the 46 mice given DMSO survived at 52 weeks, at which point they were killed. In the 19 male mice given quinoline, there were 13 mice with hepatic [hepatocellular] adenomas [$P < 0.0001$], 2 mice with hepatomas [hepatocellular carcinomas], and 1 with a lymphoma or leukaemia. The incidence of hepatic [hepatocellular] tumours in male mice (15/19, 79%) was significantly increased ($P < 0.05$) compared with controls. The tumour incidence in the 27 female mice given quinoline included 3 mice with lung tumours and 5 with lymphomas or leukaemias. In the 21 mice of each sex treated with DMSO, there was 1 lymphoma or leukaemia in females and 4 males with liver tumours (the types of liver tumours were not indicated, but they were not identified as liver adenomas or hepatomas). [The Working Group noted the principal strength of the study was that both male and female mice were used. However, the study was limited by the use of a single dose, the absence of body-weight data, and the lack of a discussion of clinical signs.]

In a third study (Weyand et al., 1993), 85 male and 85 female CD-1 mouse pups were given intraperitoneal injections of quinoline (purity, > 98%) in DMSO. Each pup received 5, 10, and 20 μL of a 50 mmol/L solution of quinoline in DMSO on days 1, 8, and 15, respectively. Each mouse was given a total of 1.75 μmol of quinoline. Negative control groups (97 males, 97 females) were given DMSO alone on days 1, 8, and 15 of life. A total of 70 (33 males and 37 females) of the 85 mice given quinoline and 84 (38 males and 46 females) of the 97 mice given DMSO survived at 52 weeks, at which point they were killed. After histopathological examination, the neoplastic response in the male mice given quinoline included 15 mice with liver adenomas [hepatocellular adenomas] (45%) [$P < 0.0001$] and 1 with a liver carcinoma [hepatocellular carcinoma] (3%). The neoplastic response in the female mice given quinoline included two mice with liver tumours (5%) (not diagnosed as hepatocellular adenomas or carcinomas), and one with a lung tumour (3%). No liver tumours were observed in female mice exposed to quinoline or in male and female controls. [The Working Group noted the principal strength of the study was that both males and females were used; however, the study was limited by the absence of body-weight data.]

3.1.3 Initiation–promotion

The tumour-initiating activity of quinoline (purity, ≥ 99.8%) was examined on the skin of 40 female HfD: SENCAR BR mice (age, 50–55 days) (LaVoie et al., 1984). Control mice (40 per group) were treated with either benzo[*a*]pyrene or acetone. Quinoline was applied at a 0.75% concentration in 0.1 mL of acetone in 10 separate doses every other day (total initiating dose, 7.5 mg). Negative control mice were treated with acetone only. Positive control mice were treated topically with benzo[*a*]pyrene at a total initiating dose of 0.03 mg. At 10 days after the last application of the initiator, promotion was started by applying 2.0 μg of 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in 0.1 mL acetone twice per week for 18 weeks. Skin tumours were counted each week. At experimental week 22, 53% ($P < 0.01$) of mice treated with quinoline plus TPA had gross skin tumours with an average of 0.73 tumours per mouse. In mice treated with acetone plus TPA, 7.5% had gross skin tumours with an average of 0.08 tumours per mouse. In mice treated with benzo[*a*]pyrene plus TPA, 63% ($P < 0.01$) had gross skin tumours with an average of 2.1 tumours per mouse. [The Working Group noted the principal limitations of the study were the use of a single dose and the lack of histopathological examination.]

3.2 Rat

3.2.1 Oral administration

(a) Feeding

Sixty male Sprague-Dawley rats [age not reported] weighing 160–185 g were given quinoline (purity, > 99.8%) in a semi-synthetic diet for 40 weeks (Hirao et al., 1976). The rats were divided into three groups of 20, and each group was treated with 0.05, 0.1, or 0.25% quinoline, respectively. Rats that died before week 16 of the study were excluded from the effective numbers of animals. Most rats treated with the medium or high dose of quinoline died before the end of the study due to toxicity of quinoline or to rupture of vascular tumours in the liver. The tumour response in the 11 surviving rats treated with 0.05% quinoline included 3 rats with hepatocellular carcinoma (27%) and 6 with liver haemangioendothelioma [haemangiosarcoma] (54%) [$P < 0.05$]. In the 16 surviving rats treated with 0.1% quinoline, 3 had hepatocellular carcinoma (19%) and 12 had liver haemangioendothelioma [haemangiosarcoma] (75%) [$P < 0.005$]. In the 19 surviving rats treated with 0.25% quinoline, there were no hepatocellular carcinomas and 18 rats had liver haemangioendothelioma [haemangiosarcoma] (95%) [$P < 0.0001$]. There were no tumours in the livers of the 6 control rats. [The Working Group noted that the study benefited from the multiple doses used. However, the study was limited by not providing the dose selection criteria, the poor survival in medium- and high-dose groups, and the lack of statistical analysis.]

Shinohara et al. (1977) evaluated the effects of dietary quinoline in Wistar rats and Sprague-Dawley rats. In a first experiment, 50 Wistar rats (age, 8 weeks; equal numbers of males and females) were given a basal diet containing 0.2% quinoline (Nakarai Pure Chemical Co., Japan) [purity unspecified] for up to 30 weeks. There were no rats on a control diet. A total of 15 of the 25 male rats and 22 of the 25 female rats survived after 26 weeks (effective number of animals). Gross examination showed that the livers of rats exposed to quinoline had numerous small and large nodules which measured up to 2.5 cm in diameter. Histologically, of the 15 surviving male rats given quinoline, there were 2 rats with hepatocellular carcinomas (13.3%) and 11 with haemangioendotheliomas [haemangiosarcomas] (73.3%) of the liver. Of the 22 surviving female rats, there were 2 with hepatocellular carcinoma (9.1%) and 7 with haemangioendothelioma [haemangiosarcoma] (31.8%) of the liver. Male rats had a higher incidence of haemangioendotheliomas [haemangiosarcomas] than female rats ($P < 0.02$).

In a second experiment, one group of male Sprague-Dawley rats (age, 8 weeks) received 0.075% quinoline in basal diet for 30 weeks; another group of male rats was given a control diet only [number of animals at start, unspecified]. The effective number of animals (those alive at 26 weeks) was 20 exposed and 10 control rats. Gross examination showed that the livers of rats exposed to quinoline had solitary or multiple spotted lesions in the liver measuring 1–2 mm in diameter; these lesions were not quantified. Histologically, 6 of the 20 rats (30.0%) exposed to quinoline [not significantly increased compared with controls] had haemangioendothelioma [haemangiosarcoma] of the liver; there were no treated rats with hepatocellular carcinoma. There were no liver tumours in the controls (0/10). [The Working Group noted that the principal strength of the study was that both males and females were used in the first experiment. The principal limitations included the lack of a control group in the first experiment, the use of only a single dose in both experiments, the short duration of exposure, the use of male rats only in the second experiment, and the limited reporting of experimental details in both experiments. The Working Group concluded that the first experiment was inadequate for the evaluation of the

carcinogenicity of quinoline in experimental animals.]

Five groups comprising a total of 170 male Wistar rats (age, 7 weeks) [number of animals at start unspecified] were given quinoline (Katayama Chemical Co., Japan) [purity not stated] in a powdered diet for 4, 8, 12, 16, or 20 weeks (Groups I–V, respectively). Subgroups (totalling 15 in number) of 5–18 rats from each of Groups I–V were killed at experimental weeks 4, 8, 12, 16, and 20 (Hasegawa et al., 1989). Group VI (Subgroup 16) comprised 12 rats that were not exposed to quinoline and killed at 20 weeks [tumour data for 7 of the 16 subgroups are reported in Table 3.1]. Several rats died before they were scheduled to be killed, as a result of either the toxic effects of the quinoline or the rupture of vascular tumours of the liver. Rats exposed to quinoline gained weight more slowly than controls, but normal body weights were restored within 4 weeks after cessation of treatment. In the 11 rats of Group III (exposed for 12 weeks) killed after 12 weeks, 1 (9%) had haemangioendothelioma [haemangiosarcoma] of the liver. In the 12 rats of Group III killed after 16 weeks, 2 (17%) had haemangioendotheliomas [haemangiosarcomas] of the liver. In the 12 rats of Group III killed after 20 weeks, 5 (42%, $P < 0.05$) had haemangioendotheliomas [haemangiosarcomas] of the liver. In the 14 rats of Group IV (exposed for 16 weeks) killed after 16 weeks, 4 (29%, $P < 0.05$) had haemangioendotheliomas [haemangiosarcomas] of the liver. In the 18 rats of Group IV killed after 20 weeks, 4 (22%) had haemangioendotheliomas [haemangiosarcomas] of the liver. In the 16 rats of Group V (exposed for 20 weeks), 5 (31%, $P < 0.05$) had haemangioendotheliomas [haemangiosarcomas] of the liver. None of the 12 control rats in Group VI on basal diet for 20 weeks developed any lesions in the liver. It was concluded that the critical period for the induction of tumours in animals treated with 0.25% dietary quinoline is 12 weeks. [The Working Group noted that the study was limited by the use of only male rats and only one time-matched control group.]

In a study of the effects of hypertension on vascular carcinogenesis induced by exposure to quinoline, two strains of male rats (age, 5 weeks), SHR (high hypertension) and its parent strain Wistar Kyoto (WKY) that differ in their tendency towards spontaneous hypertension, were given 0.2% quinoline (Wako Pure Chemical Industry, Ltd, Japan) [purity not stated] in their diet for 32 weeks (Futakuchi et al., 1996). There were 16 exposed and 10 control rats per strain at the beginning of the study. Body-weight gain was retarded by exposure to quinoline for both strains from the first week until the end of the experiment. After week 25, of the groups exposed to quinoline 8 WKY rats died of haemangiosarcoma and 1 SHR rat died of an unknown cause. Histological findings in the 15 SHR rats exposed to quinoline for at least 25 weeks included 1 (7%) with haemangiosarcoma of the liver and 3 (20%) with liver hyperplastic nodules. In contrast, in the 15 WKY rats exposed to quinolone for at least 25 weeks, 14 (93%, $P < 0.001$) had liver haemangiosarcomas and 3 (20%) had liver hyperplastic nodules. No liver lesions were observed in the 9 SHR and 10 WKY controls. [The Working Group noted that the measurement of quinoline intake was the principal strength of this study; however, the study was limited by the use of only one sex, the single dose, and its short duration.]

(b) Drinking-water

In a GLP study, four groups of 50 male F344/DuCrj rats (SPF) (age, 6 weeks) were given quinoline (purity, > 99.6%) either at 0 (control), 200, 400, or 800 ppm in drinking-water for various periods of time (Matsumoto et al., 2018). Similarly, four groups of 50 female rats of the same strain and age were given quinoline at 0, 150, 300, or 600 ppm in drinking-water for various periods of time. The initial design was to expose rats to quinoline for up to 104 weeks; however, dose-related decreases in survival necessitated the early

termination of the experiment. All rats were observed daily for clinical signs and mortality, and animals found moribund were killed and their organs removed, weighed, and examined for macroscopic lesions at necropsy. All organs and tissues, including the entire respiratory tract, were examined for histopathology.

All male rats given quinoline at 400 and 800 ppm were dead by the end of weeks 95 and 76, respectively. Further, there were only 19 surviving rats (38%) in the group exposed at 200 ppm at week 96; the study was therefore terminated at that point. The survival rate of the control males at week 96 was 98% (49/50). All females exposed to quinoline at 600 ppm were dead by the end of week 88. However, the other groups had a fairly high number of surviving animals at that point, so the study was continued to week 104. The survival rates of the control, low-dose (150 ppm), and medium-dose (300 ppm) females at the end of the 104 weeks were 82% (41/50), 34% (17/50), and 4% (2/50), respectively. The decreased survival in males and females was attributed to death due to tumours of the liver. The earliest deaths due to malignant tumours were observed at weeks 75, 37, and 22 in the male groups exposed at 200, 400, and 800 ppm, respectively, and at weeks 68, 33, and 40 in the female groups exposed at 150, 300, and 600 ppm, respectively. The growth rates of all exposed males and of the females exposed at 300 and 600 ppm were generally less than the controls throughout the study period.

In male rats, exposure to quinoline significantly increased the incidence of hepatocellular adenoma, of hepatocellular carcinoma, of hepatocellular adenoma or carcinoma (combined), of haemangiosarcoma in the liver, and of haemangiosarcomas in all organs at all dose levels. No haemangiosarcoma or hepatocellular carcinoma and only one hepatocellular adenoma were observed in 50 male controls. In addition to vascular and hepatic tumours, quinoline significantly increased the incidences of nasal cavity sarcoma (not otherwise specified) in rats exposed at 400 ppm, and of nasal esthesioneuroepithelioma in rats exposed at 800 ppm; no such tumours were observed in controls. There was also a significant positive trend in the incidences of mediastinum sarcoma (not otherwise specified), mesenterium haemangiosarcoma, and adipose tissue haemangiosarcoma.

In female rats, exposure to quinoline significantly increased the incidence of hepatocellular adenoma, of hepatocellular carcinoma, of hepatocellular adenoma or carcinoma (combined), of haemangiosarcoma in the liver, and of haemangiosarcoma in all organs combined at all dose levels. No haemangiosarcoma or hepatocellular carcinoma was observed, and only one hepatocellular adenoma was observed in 50 female controls.

[The Working Group noted the early onset of rare tumours of various embryological origins at the lowest dose tested and the very poor survival due to tumour induction. The Working Group also noted that the principal strengths of this GLP study included the use of both males and females, the multiple dose levels, the accurate determination of compound exposure, the reporting of body-weight and survival data, the results obtained from all treated animals, and the extensive histopathological examination of all organs.]

3.2.2 Subcutaneous injection

In a study of carcinogenicity in newborn Sprague-Dawley rats, 101 males and 101 females were given a subcutaneous injection of quinoline (purity, > 99%) in DMSO at a dose of 200 µmol/kg body weight (bw) within the first 24 hours of life (LaVoie et al., 1988). Control groups of 50 males and 50 females were given a subcutaneous injection of DMSO at 500 µL/kg bw. A mortality rate of 59% was observed among the rats exposed to quinoline following the first injection; only 41 out of the 101 survived. The subsequent injections given once per week during weeks 2–7

were therefore reduced to 100 μmol/kg bw. The final injection given at week 8 was at the high dose of 200 μmol/kg bw. All control rats were given DMSO at 500 μL/kg bw for weeks 2–8. All rats were killed at 78 weeks. One liver adenoma [hepatocellular adenoma] was observed in the 25 surviving males exposed to quinoline. There were no liver tumours in any of the 15 surviving females exposed to quinoline. Of the 50 rats given DMSO, 27 males and 22 females survived. One (4.5%) of the control females had a liver adenoma [hepatocellular adenoma], and five (18.5%) of the control males had liver tumours: three adenomas [hepatocellular adenomas] and two hepatomas [hepatocellular carcinomas]. [The Working Group noted the higher incidence of liver tumours in the DMSO control rats compared with treated rats. The Working Group also noted that the principal strengths of the study were the use of both males and females, and the fact that treatment was given over most of the lifespan. However, the study was limited by the use of only a single variable dose, the high mortality after the initial dose, the absence of body-weight data, and the lack of discussion of clinical signs.]

3.3 Syrian golden hamster

Fifty Syrian golden hamsters (age, 8 weeks) were given 0.2% quinoline [purity not stated] in the diet for 30 weeks (Shinohara et al., 1977). There were equal numbers of males and females in the study. There were no liver tumours in the 25 surviving males or the 19 surviving females (effective number of animals) after 26 weeks of exposure to quinoline. [The Working Group noted that the principal limitations of the study included: a lack of controls given the basal diet for 30 weeks, the use of only one dose group, the short duration of exposure, and the limited reporting of experimental details. The Working Group concluded that the study was inadequate for the evaluation of the carcinogenicity of quinoline in experimental animals.]

3.4 Guinea-pig

Forty-four Hartley guinea-pigs (age, 8 weeks) were given 0.2% quinoline [purity not stated] in their diet for 30 weeks (Shinohara et al., 1977). There were equal numbers of males and females in the study. There were no liver tumours in the 21 surviving males or the 17 surviving females (effective number of animals) after 26 weeks of exposure to quinoline. [The Working Group noted that the principal limitations of the study included: a lack of controls given the basal diet for 30 weeks, the use of only one dose group, the short duration of exposure, and the limited reporting of experimental details. The Working Group concluded that the study was inadequate for the evaluation of the carcinogenicity of quinoline in experimental animals.]

4. Mechanistic and Other Relevant Data

4.1 Toxicokinetic data

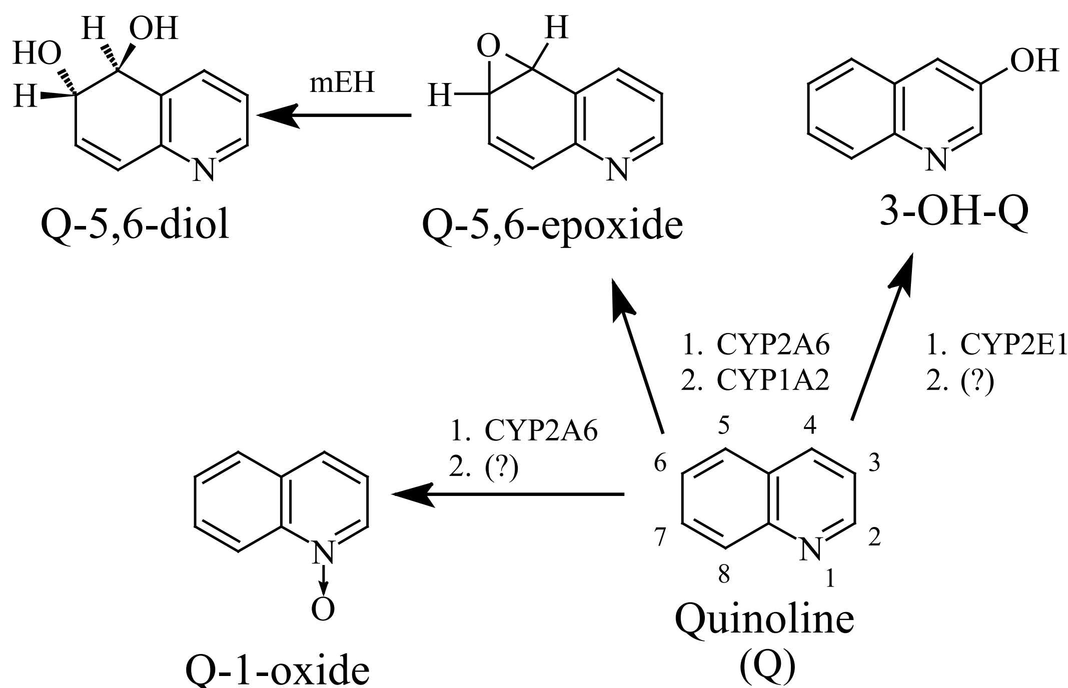
Relevant studies on the absorption, distribution, metabolism, and excretion of quinoline include in vitro studies in human and experimental systems, and in vivo studies in experimental animals. No data on humans exposed to quinoline, or on dermal absorption, were available to the Working Group. A specific focus in the published literature has been the biotransformation pathway underlying the mutagenicity of quinoline.

4.1.1 Humans

No data in exposed humans were available to the Working Group.

An in vitro metabolism study on quinoline was performed using individual cDNA-expressed cytochrome P450 (CYP) enzymes from human (and rat, see Section 4.1.2) hepatic microsomes

Fig. 4.1 Metabolic pathways of quinoline in human liver microsomes



CYP, cytochrome P450; mEH, microsomal epoxide hydrolase
Adapted from Reigh et al. (1996). Cytochrome P450 species involved in the metabolism of quinoline. *Carcinogenesis*, 1996, volume 17, issue 9, pages 1989–1996, by permission of Oxford University Press.

(Reigh et al., 1996). CYP2A6 was found to be the primary isozyme involved in the formation of quinoline-1-oxide, and CYP2E1 is the principal isozyme involved in the formation of 3-hydroxy-quinoline. CYP2A6 and CYP1A2 are responsible for the formation of 5,6-dihydroquinoline-5,6-epoxide (also reported as quinoline-5,6-epoxide or 5,6-dihydro-5,6-epoxyquinoline), a precursor of 5,6-dihydroxy-5,6-dihydroquinoline (also reported as quinoline-5,6-diol, 5,6-dihydro-quinoline-5,6-diol, 5,6-dihydroxyquinoline, 5,6-dihydro-5,6-dihydroxyquinoline) (see Fig. 4.1). Conversion of quinoline-5,6-epoxide to quinoline-5,6-diol was effectively mediated by cDNA-expressed human microsomal epoxide hydrolase. Kinetic analysis has shown that the formation of quinoline-5,6-diol is monophasic, and that of quinoline-1-oxide and 3-hydroxy-quinoline is biphasic.

4.1.2 *Experimental systems*

(a) Absorption, distribution, and excretion

In a study by Novack & Brodie (1950), dogs were given quinoline intravenously at 25 mg/kg body weight (bw). After dosing, quinoline plasma concentrations of 16.9, 5.1, 2.6, and 0.7 mg/L were measured at 0.25, 0.75, 2, and 4 hours, respectively, and less than 0.5% of quinoline was excreted with urine in a free form within 24 hours of dosing. These data indicate that quinoline

was distributed rapidly and metabolized almost completely.

Absorption and excretion were also demonstrated in rabbits given quinoline orally (Smith & Williams, 1955; see the following section).

(b) Metabolism

(i) In vivo studies

In the study in dogs mentioned in the previous section (Novack & Brodie, 1950), 3-hydroxyquinoline was identified as a major metabolite of quinoline, accounting for 29–32% of the given dose (25 mg/kg bw). Of this amount, 4% was excreted in a free form while the remainder was excreted as an acid-hydrolysable conjugate, perhaps glucuronide and/or sulfate. When 3-hydroxyquinoline was given intravenously to two dogs at a dose of 0.6 mg/kg bw, 34% and 35% was recovered in urine in a conjugated form, although the amount of excreted free 3-hydroxyquinoline was negligible.

Smith & Williams (1955) investigated the metabolism of quinoline in rabbits dosed orally at 250 mg/kg bw or at 0.5 g per animal. In 24-hour urine samples, glucuronide and sulfate fractions were separated and hydrolysed to obtain products identified as 3-hydroxyquinoline, 2,6-dihydroxy-2,6-dihydroquinoline, and 5,6-dihydroxy-5,6-dihydroquinoline. Formation of 2,6-dihydroxy-2,6-dihydroquinoline may be initiated by the oxidation at C-2 or C-6, since both possible intermediates, 2-quinolone (2-hydroxyquinoline) or 6-hydroxyquinoline, had been described previously (Scheunemann, 1923; Knox, 1946). 5,6-Dihydroxy-5,6-dihydroquinoline, accounting for 3–4% of the administered dose of quinoline, occurred in the urine as a monosulfate (6-hydroxy-5,6-dihydroquinolyl-5-sulfuric acid). In contrast, 3-hydroxyquinoline and 2,6-dihydroxy-2,6-dihydroquinoline were excreted as glucuronides. About 10% of quinoline was excreted as an unknown labile compound that yielded the parent compound on heating with acid. Compounds 3-, 5-, and 6-hydroxyquinoline were mainly metabolized by direct conjugation. Further, 3-hydroxyquinoline was converted in a small extent to 2,3-dihydroxy-2,3-dihydroquinoline, and 6-hydroxyquinoline to 2,6-dihydroxy-2,6-dihydroquinoline and 5,6-dihydroxy-5,6-dihydroquinoline, although no oxidative product of 5-hydroxyquinoline has been detected (Smith & Williams, 1955).

(ii) In vitro studies

In vitro N-oxidation of quinoline by the hepatic and pulmonary microsomal preparations was studied by Cowan et al. (1978). Quinoline-1-oxide was detected in hepatic microsomal preparations from four rodent species and rabbits. Pulmonary microsomes isolated from rabbits, but not from guinea-pigs, exhibited oxidative activity. Later studies with hepatic microsomal fractions from rats treated with specific enzymatic inducers or inhibitors indicated that N-oxidation is catalysed by (phenobarbital-inducible) CYP monooxygenases, whereas oxidation at the 5,6-position is catalysed by CYP1A1 (Tada et al., 1982). Metabolism of quinoline and isoquinoline in rat liver microsomes was compared by LaVoie et al. (1983). The major metabolite of quinoline was identified as 5,6-dihydroxy-5,6-dihydroquinoline, while 3-hydroxyquinoline and quinoline-1-oxide were among the minor metabolites.

As noted in Section 4.1.1, an in vitro study compared metabolism of quinoline in rat hepatic microsomes (Reigh et al., 1996) with that in human hepatic microsomes. The types of CYP isoenzymes involved in the corresponding metabolic pathways differed notably between the species. The formation of quinoline-1-oxide in rat hepatic microsomes was negligible but it was enhanced by pre-treatment with phenobarbital, acting as CYP3A2 inducer. The enzymes responsible for the formation of quinoline-5,6-epoxide were CYP1A2 and CYP1A1, and the formation of 3-hydroxyquinoline was mediated by CYP2E1.

Similarly, in vitro formation of quinoline metabolites as catalysed by five purified mammalian (species unspecified) CYP450 enzymes was described by Dowers et al. (2004). Quinoline-1-oxide was a major metabolite upon incubation with CYP3A4 and 2A6, a relevant but not major metabolite with CYP2B4, but was not detected with CYP1A2 and only traces were found in incubations with CYP2E1. 3-Hydroxyquinoline was a major metabolite upon incubation with CYP2E1, 1A2, and 2B4. With all isozymes tested, 5- and 8-hydroxyquinoline were produced. Small amounts of 6-hydroxyquinoline were produced with all isoenzymes except CYP1A2.

Quinoline metabolism was also studied in incubations with rat olfactory mucosa and NADPH in vitro. Quinoline-1-oxide and quinoline-5,6-epoxide appeared to be the main metabolites; other products were unspecified diols. The rate of quinoline-1-oxide formation in microsomes from olfactory mucosa was about 3-fold that in hepatic microsomes. Inhibition studies confirmed the dominant role of CYP isoenzymes in the biotransformation of quinoline (Thiebaud et al., 2013).

4.2 Mechanisms of carcinogenesis

Quinoline has been studied for genotoxic potential primarily using non-human mammalian in vivo and in vitro models, as well as bacterial mutagenicity assays. These studies are summarized in Table 4.1, Table 4.2, and Table 4.3.

4.2.1 Genetic and related effects

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

(i) Non-human mammals in vivo

No effect was seen on unscheduled DNA synthesis in hepatocytes isolated from rats following single oral gavage doses of quinoline at up to 500 mg/kg bw (Ashby et al. 1989).

Quinoline given to transgenic mice (MutaMouse) by intraperitoneal injection at 50 mg/kg bw per day for 4 days consistently elevated the mutation frequency of the *lacZ* and *cII* transgenes in liver tissue 14 days after treatment (Miyata et al., 1998; Suzuki et al., 1998; Suzuki et al., 2000), but did not change the mutation frequency of the *lacZ* gene in kidney, lung, spleen (Suzuki et al., 1998), bone marrow, or testis (Miyata et al., 1998). When the *cII* gene was sequenced from liver DNA, the majority of quinoline-induced mutations were G:C to C:G transversions (Suzuki et al., 2000).

Micronuclei were significantly increased in the livers of rats given quinoline by oral gavage at 15 mg/kg bw per day for 14 days or 30 mg/kg bw per day 28 days (Uno et al., 2015). However, micronuclei were not increased in the rat bone marrow (immature erythrocytes), colon, or stomach after a higher daily gavage dose (up to 120 mg/kg bw for 14 days or 28 days) (Uno et al., 2015), or in the rat bone marrow (immature erythrocytes) after a single dose by gavage at 200 mg/kg bw or after treatment every day for 28 days (Asakura et al., 1997). Chromosomal aberrations were significantly increased in hepatocytes isolated from rats following a single dose (100 mg/kg bw) or a dose (25 mg/kg bw) once per day for 28 days by gavage (Asakura et al., 1997). In the same study, sister-chromatid exchanges were significantly increased in rat hepatocytes after a single dose (50 mg/kg bw) or a dose (25 mg/kg bw) once per day for 28 days by gavage. Quinoline given intravenously at 500 µmol/kg bw significantly increased micronuclei in the livers of mice that underwent partial hepatectomy when sampled 5 or 10 days after exposure (Saeki et al., 2000),

Table 4.1 Genetic and related effects of quinoline in non-human mammals in vivo

End-point	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Unscheduled DNA synthesis	Rat, Alpk:AP (M)	Liver	–	500 mg/kg	Oral gavage ×1, sampled at 16 h		Ashby et al. (1989)
Mutation	MutaMouse, CD2 (M)	Liver	+	50 mg/kg	Intraperitoneal injection 1×/d for 4 d, sampled at 14 d		Miyata et al. (1998)
Mutation	MutaMouse, CD2 (M)	Bone marrow, testis	–	50 mg/kg	Intraperitoneal injection, 1×/d for 4 d, sampled at 14 d		Miyata et al. (1998)
Micronuclei	MutaMouse, CD2 (M)	Peripheral blood	–	50 mg/kg	Intraperitoneal injection, 1×/d for 2 d, sampled at 24 h		Miyata et al. (1998)
Mutation	MutaMouse, CD2 (F)	Liver and after partial hepatectomy	+	50 mg/kg	Intraperitoneal injection, 1×/d for 4 d, sampled at 14 d		Suzuki et al. (1998)
Mutation	MutaMouse, CD2 (M)	Liver	+	50 mg/kg	Intraperitoneal injection, 1×/d for 4 d, sampled at 14 d	The majority of quinoline-induced mutations were G:C to C:G transversions	Suzuki et al. (2000)
Mutation	MutaMouse, CD2 (F)	Kidney, lung, spleen	–	50 mg/kg	Intraperitoneal injection, 1×/d for 4 d, sampled at 14 d		Suzuki et al. (1998)
Micronuclei	Rat, Crl:CD(SD) (M)	Liver	+	15 mg/kg	Oral gavage ×1/d for 14 d (or at 30 mg/kg/d for 28 d)		Uno et al. (2015)
Micronuclei	Rat, Crl:CD(SD) (M)	Bone marrow, colon, stomach	–	60 mg/kg	Oral gavage ×1/d, 28 d (or 120 for 14 d)		Uno et al. (2015)
Micronuclei	Rat, F344/Du Crj (M)	Bone marrow	–	200 mg/kg bw	Oral gavage ×1, or ×1/d for 28 d, sampled at 24 hours		Asakura et al. (1997)
Chromosomal aberrations	Rat, F344/Du Crj (M)	Liver	+	25 mg/kg bw	Oral gavage ×1/d for 28 d, sampled at 24 h	Dose-dependent increases in chromosomal aberrations (0, 25, 50, 100, and 200 mg/kg bw)	Asakura et al. (1997)
Sister-chromatid exchange	Rat, F344/Du Crj (M)	Liver	+	50 mg/kg bw	Oral gavage ×1 or 25 mg/kg bw for 28 d, dose–response analysis; sampled at 24 h		Asakura et al. (1997)
Micronuclei	Mouse, ICR (M)	Liver	+	0.5 mmol/kg bw	Intraperitoneal injection, ×1, sampled at 5 or 10 d	Mice underwent partial hepatectomy	Saeki et al. (2000)
Micronuclei	Mouse, ICR (M)	Liver	–	0.5 mmol/kg bw	Intraperitoneal injection, ×3, sampled at 6 or 11 d	Mice did not undergo partial hepatectomy	Saeki et al. (2000)
Micronuclei	Rat, F344 (M)	Liver	±	0.5 mmol/kg	Intraperitoneal injection, ×3, sampled at 6 or 11 d	Rats did not undergo partial hepatectomy	Hakura et al. (2007)

Table 4.1 (continued)

End-point	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Micronuclei	Mouse, CD-1 (M)	Bone marrow	+	25 mg/kg	Intraperitoneal injection, ×1, sampled at 48 h		Hamoud et al. (1989)
Sister-chromatid exchange	Mouse, $B6C3F_1$ (M)	Bone marrow	–	100 mg/kg	Intraperitoneal injection, ×1, sampled at 23 and 42 h	MTD, 100 mg/kg; ≥ 200 mg/kg lethal	McFee (1989)
Chromosomal aberrations	Mouse, $B6C3F_1$ (M)	Bone marrow	–	100 mg/kg	Intraperitoneal injection ×1; sampled at 17 and 36 h	MTD, 100 mg/kg; ≥ 200 mg/kg lethal	McFee (1989)

bw, body weight; d, day(s); F, female; h, hour(s); HID, highest ineffective dose; LED, lowest effective dose (units as reported); M, male; MTD, maximum tolerated dose; SD, standard deviation

[a] +, positive; –, negative; ±, equivocal (variable response in several experiments within an adequate study)

Table 4.2 Genetic and related effects of quinoline in non-human mammalian cells in vitro

Endpoint	Species, strain	Tissue, cell line	Results[a]		Concentration (LEC or HIC)	Comments	Reference
			Without metabolic activation	With metabolic activation			
Chromosomal aberrations	Chinese hamster	Lung, Don cells	–	NT	1 mM		Abe & Sasaki (1977)
Chromosomal aberrations	Chinese hamster	Lung fibroblasts	±	+	0.3 mg/mL	3-h incubation followed by 24-h expression period; chromosomal aberrations analysis included gaps	Matsuoka et al. (1979)
Chromosomal aberrations	Chinese hamster	Ovary, CHO-W-B1	–	+	500 µg/mL	2-h incubation followed by 8–12-h expression period; results obtained at one of two laboratories	Galloway et al. (1985)
Chromosomal aberrations	Chinese hamster	Ovary, CHO-W-B1	–	–	550 µg/mL	2-h incubation followed by 8–12-h expression period; results obtained at one of two laboratories	Galloway et al. (1985)
Chromosomal aberrations	Chinese hamster	Lung fibroblasts	NT	+	0.03 mg/mL	6-h incubation followed by 18-h expression period	Suzuki et al. (2007)
Micronuclei	Chinese hamster	Lung fibroblasts	NT	+	0.05 mg/mL	6-h incubation followed by 72-h expression period	Suzuki et al. (2007)
Sister-chromatid exchange	Chinese hamster	Lung, Don cells	–	NT	1 mM	26-h exposure	Abe & Sasaki (1977)
Sister-chromatid exchange	Chinese hamster	Ovary, CHO-W-B1	–	+	4.4 µg/mL	2-h exposure followed by 24-h expression period; study compared concurrently produced results between two laboratories	Galloway et al. (1985)
DNA strand breaks	Rat, NR	Isolated hepatocytes	+	NT	1 mM	Primary hepatocytes exposed for 3 h	Sina et al. (1983)
Unscheduled DNA synthesis	Rat, Sprague-Dawley	Isolated hepatocytes	+	NT	1 mM	Exact duration of exposure was not reported (18–20 h)	LaVoie et al. (1991)

h, hour(s); HIC, highest ineffective concentration; LEC, lowest effective concentration; NR, not reported; NT, not tested

[a] +, positive; –, negative; ±, equivocal (variable response in several experiments within an adequate study)

Table 4.3 Genetic and related effects of quinoline in non-mammalian species

Test system (species, strain)	End-point	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Drosophila melanogaster, Canton-S males mated to Basc females	Sex-linked recessive lethal mutations	–	NT	600 ppm, 1 injection	Adult male flies were treated for the study	Zimmering et al. (1985)
Drosophila melanogaster, Canton-S males mated to Basc females	Sex-linked recessive lethal mutations	–	NT	130 ppm in feed	Flies were exposed throughout the larval stage of development	Valencia et al. (1989)
Salmonella typhimurium, TA100	Mutation	–	+	40 µg/plate		Talcott et al. (1976)
Salmonella typhimurium, TA98, TA1535, TA1537	Mutation	–	–	100 µg/plate		Talcott et al. (1976)
Salmonella typhimurium, TA100	Mutation	–	+	50 µg/plate		Epler et al. (1977)
Salmonella typhimurium, TA98	Mutation	–	±	100 µg/plate		Epler et al. (1977)
Salmonella typhimurium, TA1535, TA1537	Mutation	–	–	200 µg/plate		Epler et al. (1977)
Salmonella typhimurium, TA100, TA98	Mutation	–	+	1 µM/plate		Nagao et al. (1977)
Salmonella typhimurium, TA100	Mutation	–	+	100 µg/plate		Hollstein et al. (1978)
Salmonella typhimurium, TA98, TA1535, TA1537	Mutation	–	–	100 µg/plate		Hollstein et al. (1978)
Salmonella typhimurium, TA100, TA98	Mutation	NT	+	50 µg/plate		Haworth et al. (1983)
Salmonella typhimurium, TA100	Mutation	NT	+	30 µg/plate		LaVoie et al. (1991)
Salmonella typhimurium, TA100	Mutation	NT	+	25 µg/plate		Debnath et al. (1992)
Salmonella typhimurium, TA100	Mutation	NT	+	0.4 µmol/plate		Willems et al. (1992)
Salmonella typhimurium, TA198	Mutation	NT	+	NR	LED not reported	Willems et al. (1992)
Salmonella typhimurium, TA1535, TA1537	Mutation	NT	–	200 µg/plate		Willems et al. (1992)
Salmonella typhimurium, TA100	Mutation	NT	+	0.2 µmol/plate		Kato et al. (1999)
Salmonella typhimurium, TA100	Mutation	–	+	79 mg/L		Neuwoehner et al. (2009)
Salmonella typhimurium, TA98	Mutation	–	–	158.1 mg/L		Neuwoehner et al. (2009)

HIC, highest ineffective concentration; LEC, lowest effective concentration; LED, lowest effective dose; NR, not reported; NT, not tested; ppm, parts per million

[a] +, positive; –, negative; ±, equivocal (variable response in several experiments within an adequate study)

but not when non-hepatectomized mice were intravenously exposed to quinoline at 0.5 mmol/kg bw, once a day for three consecutive days, and sampled 6 days after exposure (Hakura et al., 2007). Results for non-hepatectomized rats that underwent the same experimental exposure were equivocal (Hakura et al., 2007).

Micronuclei were significantly increased in immature erythrocytes taken from the bone marrow of mice 48 hours after intraperitoneal injection with quinoline at 25 mg/kg bw; however, the increase was less than 2-fold that in controls (Hamoud et al., 1989). Micronuclei were not increased in peripheral blood of the MutaMouse 24 hours after treatment with quinoline by intraperitoneal injection at 50 mg/kg bw once per day for 2 days (Miyata et al., 1998). Chromosomal aberrations and sister-chromatid exchanges were not increased in the bone marrow of mice given quinoline by intraperitoneal injection at 100 mg/kg bw (McFee, 1989).

(ii) Non-human mammalian cells in vitro

Quinoline at 1 mM significantly increased unscheduled DNA synthesis in isolated rat hepatocytes (LaVoie et al., 1991). DNA single-strand breaks were detected in the alkaline elution assay when isolated rat hepatocytes were exposed to quinoline (1 mM) (Sina et al., 1983). In a study comparing results between two laboratories, both reported significantly increased sister-chromatid exchanges, but only one laboratory reported significantly increased chromosomal aberrations in the presence of exogenous metabolic activation in Chinese hamster ovary cells (Galloway et al., 1985). Sister-chromatid exchanges and chromosomal aberrations were not increased in Chinese hamster lung Don cells by quinoline (1 mM) in the absence of exogenous metabolic activation (Abe & Sasaki, 1977). Chromosomal aberrations (Matsuoka et al., 1979; Suzuki et al., 2007) and micronuclei (Suzuki et al., 2007) were significantly increased in Chinese hamster lung fibroblasts in the presence of exogenous metabolic activation.

(iii) Non-mammalian systems in vivo

Quinoline was negative in the *Drosophila melanogaster* sex-linked recessive lethal test in adult flies (Zimmering et al., 1985), or when larvae were exposed throughout development (Valencia et al., 1989).

(iv) Non-mammalian systems in vitro

In *Salmonella typhimurium*, quinoline was positive in TA100 in the assay for reverse mutation in the presence of metabolic activation in studies conducted by various research groups (Talcott et al., 1976; Epler et al., 1977; Nagao et al., 1977; Hollstein et al., 1978; Haworth et al., 1983; LaVoie et al., 1991; Debnath et al., 1992; Willems et al., 1992; Kato et al., 1999; Neuwoehner et al., 2009). Willems et al. (1992) demonstrated that the mutagenic activity of quinoline in TA100 increased with increasing concentrations of induced rat liver S9 mix (Willems et al., 1992). Results were variable for TA98 (Epler et al., 1977; Nagao et al., 1977; Hollstein et al., 1978; Haworth et al., 1983; Willems et al., 1992; Neuwoehner et al., 2009) and TA1537 (Talcott et al., 1976; Epler et al., 1977; Hollstein et al., 1978; Willems et al., 1992) in the presence of exogenous metabolic activation. Negative results were obtained for quinoline in TA1535 with exogenous metabolic activation (Talcott et al., 1976; Epler et al., 1977; Hollstein et al., 1978; Willems et al., 1992). Quinoline was negative in TA100, TA98, TA1535, and TA1537 in the absence of exogenous metabolic activation (Talcott et al., 1976; Epler et al., 1977; Nagao et al., 1977; Hollstein et al., 1978; Haworth et al., 1983; Neuwoehner et al., 2009).

4.2.2 Other mechanistic data

To identify the structural requirements for the mutagenicity of quinoline, the activities of quinoline and 23 quinoline derivatives were compared in the Ames assay in the presence

of exogenous metabolic activation (Hollstein et al., 1978). It was suggested that C-2 and C-3 of quinoline are critical sites for the production of the proposed mutagenic intermediate, quinoline-2,3-epoxide. Alternate routes of activation, possibly independent of C-2 and C-3, may also play a minor role in quinoline mutagenicity.

The structure of the reactive intermediate that forms quinoline–nucleic-acid adducts was investigated by Tada et al. (1980). Adducts were produced by in vitro incubation of quinoline with yeast RNA, RNA polynucleotides, or calf thymus DNA in the presence of NADPH and rat liver microsomes, and were split by acid or alkali hydrolysis. Most of the quinoline residues, whether reacted with RNA or DNA, were released as 3-hydroxyquinoline. This suggests that a 2,3- or 3,4-epoxy derivative of quinoline is the reactive intermediate for nucleic acid modification. However, similar mutagenic potency of 4-methylquinoline and its tumorigenic activity on mouse skin suggests that formation of an electrophilic oxide at C-3 and C-4 is unlikely to be involved in the ultimate activation of quinoline (LaVoie et al., 1983, 1984).

Additional support for C-3 of quinoline being critical for mutagenicity was provided by Takahashi et al. (1988). Substituting C-3 with fluorine abolished the mutagenicity of quinoline, whereas mutagenicity was maintained but reduced when C-6 and C-8 were substituted with fluorine. Conversely, substitution of C-5 enhanced the mutagenicity of quinoline. In the same study, quinoline substituted with chlorine at C-2 or C-3 was not mutagenic but 4-chloroquinoline was slightly mutagenic. The 2,3-epoxide of the 1,4-hydrate form of quinoline was proposed as the intermediate responsible for mutagenicity.

Saeki et al. (1993) confirmed that the non-mutagenic 3-fluoroquinoline yielded metabolites at its benzene ring similar in type and quantity to the metabolites of mutagenic quinoline (5,6-dihydroxy-5,6-dihydro derivatives). This strongly suggests that the mutagenic activity of quinoline is prevented by fluorination at C-3, which then cannot undergo oxidation to the proposed mutagen, quinoline-2,3-epoxide. Oxidation at the benzene ring is considered to be a detoxification pathway of quinoline biotransformation. A similar conclusion was made in another study with 12 various di-, tri-, and tetra-fluoroquinolines (Kato et al., 1999). None of the quinoline derivatives with fluorine substituting for C-3 were mutagenic. In contrast, the mutagenicity of quinoline was enhanced when fluorine was substituted at C-5 or C-7, possibly because of inhibition of the major detoxification pathway affecting the benzene ring of quinoline.

The observation that C-3 fluorination abolishes the mutagenicity of quinoline in the Ames assay was investigated further using additional in vitro assays for genotoxicity. In Chinese hamster lung fibroblasts, fluorine substitution at C-3 clearly reduced the potency of quinoline in the micronucleus and chromosomal aberration assays, whereas substitution at C-5, C-6, or C-8 had comparatively modest effects (Suzuki et al., 2007); this pattern of responses was similar to those observed using the Ames assay. Furthermore, unscheduled DNA synthesis was induced in isolated rat hepatocytes when quinoline was fluorinated at C-5, C-6, C-7, or C-8 or methylated at C-4 or C-8, but not when fluorinated at C-2, C-3, or C-4, or methylated at C-2 or C-6 (methylations at other carbons were not tested) (LaVoie et al., 1991).

The apparent requirement of C-3 for the genotoxic activity of quinoline was tested in vivo. Quinoline and 5-fluoroquinoline, but not 3-fluoroquinoline, given by intraperitoneal injection at 50 mg/kg bw, once per day for 4 days, significantly increased the *lacZ* transgene mutation frequency in the liver tissue of the MutaMouse by 4–5-fold (Miyata et al., 1998). However, 3-fluoroquinoline given by a single intraperitoneal injection at 500 µmol/kg bw significantly increased micronuclei in the livers of mice that underwent

partial hepatectomy when sampled 5 or 10 days after exposure, although the increase was not as high as that obtained with quinoline (Saeki et al., 2000).

One study used quantitative real-time polymerase chain reaction to evaluate gene expression in the liver tissue of male B6C3F$_1$ mice 4 or 48 hours after treatment with quinoline by intraperitoneal injection at 100 mg/kg bw; quinoline was one of eight chemicals considered to be genotoxic hepatocarcinogens and one of four chemicals considered to be non-genotoxic hepatocarcinogens evaluated in the study (Watanabe et al., 2012). The set of genes evaluated by Watanabe et al. (2012) was previously shown to be associated with exposure to a different set of well-characterized genotoxicants and non-genotoxicants using the same mouse model and tissue (Watanabe et al., 2009). A principal component analysis of the gene expression data classified quinoline within the category of "genotoxic hepatocarcinogen" (Watanabe et al., 2012).

4.3 Other adverse effects

In a cancer bioassay conducted using male and female Crj:BDF1 mice and F344/DuCrj rats (Matsumoto et al., 2018), quinoline given orally via drinking-water induced non-neoplastic lesions in the nasal cavities of mice and angiectasis in the liver, a lesion that was associated with liver haemangiocarcinogenicity induced by quinoline. In rats, quinoline induced acidophilic foci, basophilic foci, and clear cell foci in the liver, central necrosis and focal necrosis in the liver, and basal cell hyperplasia and atrophy of the olfactory epithelium.

5. Summary of Data Reported

5.1 Exposure data

Quinoline is a colourless liquid with an unpleasant odour. It is a heterocyclic aromatic compound belonging to the group of azaarenes, and is classed as a high production volume chemical. It is used as a solvent or intermediate in the production of vitamin B$_3$, pharmaceuticals and veterinary drugs, anticorrosive agents, and dyes used for textiles, cosmetics, foods, and drinks.

Quinoline is a major pollutant of soil and groundwater at sites contaminated by coal tar and creosote. The most probable route of worker exposure to quinoline is by inhalation of particulates or vapours. Occupational exposure to quinoline may occur during petroleum and shale oil processing, the production or use of products derived from coal tar, and in industries where quinoline is used as a solvent or chemical intermediate. Very few data on occupational exposure were available to the Working Group.

Tobacco smoke is an important source of quinoline exposure. Environmental monitoring data indicate that the general population may be exposed to quinoline in particulate matter in urban air. Groundwater contamination may pose an additional risk of quinoline exposure for populations accessing aquifers near sites of creosote wood preservation. The potential for skin exposure exists from clothing containing quinoline-based dyes.

5.2 Cancer in humans

No data were available to the Working Group.

5.3 Cancer in experimental animals

There were five studies of the carcinogenicity of quinoline in mice: one good laboratory practice (GLP) study by drinking-water in males and

females, three studies by intraperitoneal injection in males and females, and one initiation–promotion study by skin application in females.

In the study by drinking-water, quinoline significantly increased the incidences (with a significant positive trend) of haemangioma of the liver, subcutis, peritoneum, and retroperitoneum, and haemangioma in all organs combined in females, of haemangioma in all organs combined in males, of haemangiosarcoma of the liver in males, of haemangiosarcoma of the peritoneum and subcutis in females, of haemangiosarcoma of the retroperitoneum and mesenterium, and haemangiosarcoma in all organs combined in males and females, of haemangioma or haemangiosarcoma (combined) in all organs combined in males and females, and of histiocytic sarcoma of the liver in females. There was also a significant positive trend in the incidences of hepatocellular carcinoma, histiocytic sarcoma of the liver, haemangioma of the retroperitoneum, and haemangiosarcoma of the subcutis in males, and in the incidences of haemangiosarcoma of the ovary and mediastinum in females. For many of these rare tumour types of various embryological origins, tumours in both males and females occurred at an early onset, at the lowest dose tested, and caused the early death of the mice.

In the studies by intraperitoneal injection, quinoline significantly increased the incidence of lymphoma in females in one study, of hepatocellular adenoma in males in two studies, of hepatocellular carcinoma in males in one study, and of hepatocellular adenoma or carcinoma (combined) in males in two studies. Quinoline initiated skin tumours in the initiation–promotion study.

There were seven studies of the carcinogenicity of quinoline in rats: five studies of exposure by feed in males, one GLP study by drinking-water in males and females, and one study by subcutaneous injection in males and females.

Quinoline significantly increased the incidence of haemangiosarcoma of the liver in males in three studies of exposure to quinoline via feed. Two studies of exposure to quinoline via feed and the study by subcutaneous injection yielded negative results.

In the study with drinking-water, quinoline significantly increased the incidences (with a significant positive trend) of haemangiosarcoma of the liver and in all organs combined in males and females, of hepatocellular adenoma, hepatocellular carcinoma, and hepatocellular adenoma or carcinoma (combined) in males and females, and of sarcoma (not otherwise specified) of the nasal cavity and of nasal esthesioneuroepithelioma in males. There was also a significant positive trend in the incidences of sarcoma (not otherwise specified) of the mediastinum as well as haemangiosarcoma of the mesenterium and of the adipose tissue in males. For many of these rare tumour types of various embryological origins, tumours in both males and females occurred at an early onset, at the lowest dose tested, and caused the early death of the rats.

5.4 Mechanistic and other relevant data

No data on absorption, distribution, metabolism, or excretion in exposed humans were available. No data on dermal absorption were available. Absorption and excretion of quinoline was demonstrated in orally dosed rabbits. Quinoline was distributed rapidly and metabolized almost completely following intravenous exposure in dogs.

Regarding the key characteristics of carcinogens, there is *moderate* evidence that quinoline is metabolized to an electrophile based on the indirect observation that the genotoxic effects of quinoline (see paragraph below) appear to require metabolic activation. No studies were available in humans or in human cells. In two studies conducted in vivo, one in dogs and one in rabbits, and in studies conducted in vitro in

different species, rapid oxidation dependent on cytochrome P450 (CYP) produced 3-hydroxy-quinoline, quinoline-5,6-diol, and quinoline-1-oxide as major metabolites in mammals. These metabolites were also produced in vitro in a study in which human CYPs were expressed. Mutagenicity studies in vivo and in vitro using quinoline derivatives suggested an azaarene oxide on the pyridine ring as a mutagenic intermediate; however, DNA adducts formed by quinoline have not been characterized.

There is *strong* evidence that quinoline is genotoxic. No data are available in exposed humans or in human systems. Quinoline induced chromosomal damage, including micronuclei, chromosomal aberrations, and sister-chromatid exchanges, in the liver of rats, but chromosomal damage (micronuclei) was not induced in other rat tissues including bone marrow, colon, and stomach. Quinoline induced mutations in the liver of transgenic mice, but not in the bone marrow, kidney, lung, spleen, or testes. Following metabolic activation, quinoline induced chromosomal damage (micronuclei, chromosomal aberrations, and sister-chromatid exchanges) in mammalian cells in vitro, and mutagenicity in the Ames assay.

No additional information in humans or in experimental systems, including on the eight remaining key characteristics of carcinogens, was available.

6. Evaluation

6.1 Cancer in humans

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

6.2 Cancer in experimental animals

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

6.3 Overall evaluation

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

References

Abbey J, Fields B, O'Mullane M, Tomaska LD (2013). Food additives: colorants. In: Motarjemi Y, Moy GG, Todd ECD, editors. Encyclopedia of food safety. Volume 2: Hazards and diseases. Academic Press; pp. 459–465.

Abe S, Sasaki M (1977). Chromosome aberrations and sister chromatid exchanges in Chinese hamster cells exposed to various chemicals. *J Natl Cancer Inst*, 58(6):1635–41. doi:10.1093/jnci/58.6.1635 PMID:864744

Adams J, Giam CS (1984). Polynuclear azaarenes in wood preservative wastewater. *Environ Sci Technol*, 18(5):391–4. doi:10.1021/es00123a020 PMID:22280091

Adams JD, LaVoie EJ, Shigematsu A, Owens P, Hoffmann D (1983). Quinoline and methylquinolines in cigarette smoke: comparative data and the effect of filtration. *J Anal Toxicol*, 7(6):293–6. doi:10.1093/jat/7.6.293 PMID:6664084

American Industrial Hygiene Association (2013). Workplace Environmental Exposure Level (WEEL) values 2011. ERPG/WEEL Handbook. Fairfax (VA), USA: AIHA Publications.

Antal B, Kuki Á, Nagy L, Nagy T, Zsuga M, Kéki S (2016). Rapid detection of hazardous chemicals in textiles by direct analysis in real-time mass spectrometry (DART-MS). *Anal Bioanal Chem*, 408(19):5189–98. doi:10.1007/s00216-016-9603-z PMID:27236310

Asakura S, Sawada S, Sugihara T, Daimon H, Sagami F (1997). Quinoline-induced chromosome aberrations and sister chromatid exchanges in rat liver. *Environ Mol Mutagen*, 30(4):459–67. doi:10.1002/(SICI)1098-2280(1997)30:4<459::AID-EM11>3.0.CO;2-C PMID:9435887

Ashby J, Mohammed R, Lefevre PA, Bandara L (1989). Quinoline: unscheduled DNA synthesis and mitogenesis data from the rat liver in vivo. *Environ Mol Mutagen*, 14(4):221–8. doi:10.1002/em.2850140403 PMID:2511010

ATSDR (2002). Toxicological profile for wood creosote, coal tar creosote, coal tar, coal tar pitch, and coal tar pitch volatiles. Agency for Toxic Substances

and Disease Registry, Public Health Service, U.S. Department of Health and Human Services.

Baechler FE, MacFarlane DS (1992). Sydney Tar Ponds clean up: hydrogeologic assessment. In: Weyer KU, editor. Proceedings of the 1992 International Conference on Coke Oven Complexes and Subsurface Contaminating Immiscible Fluids. Rotterdam, The Netherlands: Balkema; pp. 543–550.

Bai Q, Yang L, Li R, Chen B, Zhang L, Zhang Y, et al. (2015). Accelerating quinoline biodegradation and oxidation with endogenous electron donors. *Environ Sci Technol*, 49(19):11536–42. doi:10.1021/acs.est.5b03293 PMID:26327306

Bandowe BAM, Meusel H, Huang R, Hoffmann T, Cao J, Ho K (2016). Azaarenes in fine particulate matter from the atmosphere of a Chinese megacity. *Environ Sci Pollut Res Int*, 23(16):16025–36. doi:10.1007/s11356-016-6740-z PMID:27146538

Batista VF, Pinto DCGA, Silva AMS (2016). Synthesis of quinolines: a green perspective. *ACS Sustain Chem& Eng*, 4(8):4064–78. doi:10.1021/acssuschemeng.6b01010

Bean RM, Dauble DD, Thomas BL, Hanf RW Jr, Chess EK (1985). Uptake and biotransformation of quinoline by rainbow trout. *Aquat Toxicol*, 7(4):221–39. doi:10.1016/0166-445X(85)90041-4

Bennett JL, Updegraff JM, Pereira WE, Rostad CE (1985). Isolation and identification of four species of quinoline degrading pseudomonads from a creosote-contaminated site at Pensacola, Florida. *Microbios Lett*, 29:147–154.

Blum P, Sagner A, Tiehm A, Martus P, Wendel T, Grathwohl P (2011). Importance of heterocylic aromatic compounds in monitored natural attenuation for coal tar contaminated aquifers: A review. *J Contam Hydrol*, 126(3–4):181–94. doi:10.1016/j.jconhyd.2011.08.004 PMID:22115084

Chemical Sources International (2018). Chem Sources Online: quinoline. Available from: http://www.chemsources.com/index.html.

ChemSpider (2017). ChemSpider: quinolinium bromide. Royal Society of Chemistry. Available from: http://www.chemspider.com/Chemical-Structure.378462.html?rid=be227b8e-7868-4718-8838-7e00fcec8f8d.

Chen PX, Moldoveanu SC (2003). Mainstream smoke chemical analyses for 2R4F Kentucky reference cigarette. *Contrib Tob Res*, 20(7):448–58. doi:10.2478/cttr-2013-0760

Chuang JC, Mack GA, Kuhlman MR, Wilson NK (1991). Polycyclic aromatic hydrocarbons and their derivatives in indoor and outdoor air in an eight-home study. *Atmos Environ, B Urban Atmos*, 25(3):369–80. doi:10.1016/0957-1272(91)90008-3

Cowan DA, Damani LA, Gorrod JW (1978). Metabolic *N*-oxidation of 3-substituted pyridines: identification of products by mass spectrometry. *Biomed Mass Spectrom*, 5(9):551–6. doi:10.1002/bms.1200050909 PMID:708858

Debnath AK, de Compadre RL, Hansch C (1992). Mutagenicity of quinolines in *Salmonella typhimurium* TA100. A QSAR study based on hydrophobicity and molecular orbital determinants. *Mutat Res*, 280(1):55–65. doi:10.1016/0165-1218(92)90018-U PMID:1377345

Deng X, Chai X, Wei C, Fu L (2011). Rapid determination of quinoline and 2-hydroxyquinoline in quinoline biodegradation process by tri-wavelength UV/Vis spectroscopy. *Anal Sci*, 27(5):493–493. doi:10.2116/analsci.27.493 PMID:21558655

Dong MW, Locke DC, Hoffmann D (1977). Characterization of aza-arenes in basic organic portion of suspended particulate matter. *Environ Sci Technol*, 11(6):612–8. doi:10.1021/es60129a007

Dowers TS, Rock DA, Rock DA, Perkins BNS, Jones JP (2004). An analysis of the regioselectivity of aromatic hydroxylation and *N*-oxygenation by cytochrome P450 enzymes. *Drug Metab Dispos*, 32(3):328–32. doi:10.1124/dmd.32.3.328 PMID:14977867

ECHA (2018). Quinoline. Helsinki, Finland: European Chemicals Agency. Available from: https://echa.europa.eu/information-on-chemicals, accessed 7 March 2018.

Edler B, Zwiener C, Frimmel FH (1997). Particle beam LC/MS screening of polar compounds of contaminated ground water samples from a former gas plant. *Fresenius J Anal Chem*, 359(3):288–92. doi:10.1007/s002160050575

EPA (1981). Development documents for effluent limitations guidelines and standards for the timber products point source category. EPA 440/1-81/0123. Washington (DC), USA: U.S. Environmental Protection Agency.

EPA (1985). Health and environmental effects profile for quinoline. EPA/600/X-85/355 (NTIS PB88183124). Washington (DC), USA: U.S. Environmental Protection Agency. Available from: https://cfpub.epa.gov/ncea/risk/recordisplay.cfm?deid=48746.

EPA (2001). Toxicological review of quinoline (CAS No. 91-22-5). Support of summary information on the Integrated Risk Information System (IRIS). EPA/635/R-01/005. Washington (DC), USA: U.S. Environmental Protection Agency. Available from: https://cfpub.epa.gov/ncea/iris/iris_documents/documents/toxreviews/1004tr.pdf.

EPA (2015). 2015 Toxics Release Inventory. Washington (DC), USA: U.S. Environmental Protection Agency. Available from: https://www.epa.gov/toxics-release-inventory-tri-program/tri-national-analysis-archive, accessed 25 October 2017.

EPA (2017). Chemical Data Access Tool (CDAT). Washington (DC), USA: U.S. Environmental Protection Agency. Available from: https://chemview.epa.gov, accessed 7 March 2018.

Epler JL, Winton W, Ho T, Larimer FW, Rao TK, Hardigree AA (1977). Comparative mutagenesis of quinolines. *Mutat Res*, 39(3–4):285–96. doi:10.1016/0165-1110(77)90009-4 PMID:408671

Finley KT (1999). Quinolines and isoquinolines. In: Kroschwitz JI, Howe-Grant M, editors. Kirk-Othmer encyclopedia of chemical technology. 4th ed. Volume 20, Part II. New York (NY), USA: John Wiley; pp. 1698–1700.

Fowler MG, Brooks PW, Northcott M, King MW, Barker JF, Snowdon LR (1994). Preliminary results from a field experiment investigating the fate of some creosote components in a natural aquifer. *Org Geochem*, 22(3–5):641–9. doi:10.1016/0146-6380(94)90130-9

Furlong ET, Carpenter R (1982). Azaarenes in Puget Sound sediments. *Geochim Cosmochim Acta*, 46(8):1385–96. doi:10.1016/0016-7037(82)90274-5

Futakuchi M, Hasegawa R, Yamamoto A, Cui L, Ogiso T, Ito N, et al. (1996). Low susceptibility of the spontaneously hypertensive rat (SHR) to quinoline-induction of hepatic hemangioendothelial sarcomas. *Cancer Lett*, 104(1):37–41. doi:10.1016/0304-3835(96)04220-6 PMID:8640743

Galloway SM, Bloom AD, Resnick M, Margolin BH, Nakamura F, Archer P, et al. (1985). Development of a standard protocol for in vitro cytogenetic testing with Chinese hamster ovary cells: comparison of results for 22 compounds in two laboratories. *Environ Mutagen*, 7(1):1–51. doi:10.1002/em.2860070102 PMID:3967632

Gammage RB (1983). Polycyclic aromatic hydrocarbons in work atmospheres. In: Bjørseth A, editor. Handbook of polycyclic aromatic hydrocarbons. New York (NY), USA: Marcel Dekker; pp. 653–707.

Gerhartz W, editor (1993). Ullmann's encyclopedia of industrial chemistry. Volume A22. 5th ed. Deerfield Beach (FL), USA: VCH Publishers; p. 466.

Godsy EM, Goerlitz DF, Grbic-Galic D (1992). Methanogenic biodegradation of creosote contaminants in natural and simulated ground-water ecosystems. *Ground Water*, 30(2):232–42. doi:10.1111/j.1745-6584.1992.tb01795.x

Government of Canada (2011a). Screening assessment. Quinoline. Environment and Climate Change Canada. Available from: http://www.ec.gc.ca/ese-ees/default.asp?lang=En&n=202BA073-1.

Government of Canada (2011b). Chemicals at a glance. Quinoline. Available from: https://www.canada.ca/en/health-canada/services/chemical-substances/fact-sheets/chemicals-glance/quinoline.html.

Hakura A, Kadoi M, Suzuki T, Saeki K (2007). Clastogenicity of quinoline derivatives in the liver micronucleus assay using rats and mice. *J Health Sci*, 53(4):470–4. doi:10.1248/jhs.53.470

Hamoud MA, Ong T, Petersen M, Nath J (1989). Effects of quinoline and 8-hydroxyquinoline on mouse bone marrow erythrocytes as measured by the micronucleus assay. *Teratog Carcinog Mutagen*, 9(2):111–8. doi:10.1002/tcm.1770090206 PMID:2568020

Hasegawa R, Furukawa F, Toyoda K, Sato H, Imaida K, Takahashi M (1989). Sequential analysis of quinoline-induced hepatic hemangioendothelioma development in rats. *Carcinogenesis*, 10(4):711–6. doi:10.1093/carcin/10.4.711 PMID:2702719

Haworth S, Lawlor T, Mortelmans K, Speck W, Zeiger E (1983). *Salmonella* mutagenicity test results for 250 chemicals. *Environ Mutagen*, 5(Suppl 1):1–142. doi:10.1002/em.2860050703 PMID:6365529

Hawthorne SB, Sievers RE (1984). Emission of organic air pollutants from shale oil wastewaters. *Environ Sci Technol*, 18(6):483–90. doi:10.1021/es00124a016 PMID:22247953

Heikkilä PR, Hämeilä M, Pyy L, Raunu P (1987). Exposure to creosote in the impregnation and handling of impregnated wood. *Scand J Work Environ Health*, 13(5):431–7. doi:10.5271/sjweh.2017 PMID:3433045

Hirao K, Shinohara Y, Tsuda H, Fukushima S, Takahashi M (1976). Carcinogenic activity of quinoline on rat liver. *Cancer Res*, 36(2 Pt 1):329. PMID:177193

Hollstein M, Talcott R, Wei E (1978). Quinoline: conversion to a mutagen by human and rodent liver. *J Natl Cancer Inst*, 60(2):405–10. doi:10.1093/jnci/60.2.405 PMID:340706

HSDB (2017). Hazardous Substances Data Bank. Available from: https://toxnet.nlm.nih.gov/cgi-bin/sis/search2/f?/temp/~tsBJ07:1, accessed October 2017.

IFA (2017). Quinoline. GESTIS International Limit Values. Institute for Occupational Safety and Health of the German Social Accident Insurance. Available from: https://limitvalue.ifa.dguv.de/WebForm_ueliste2.aspx/, accessed 15 February 2018.

Johansen SS, Hansen AB, Mosbaek H, Arvin E (1996). Method development for trace analysis of heteroaromatic compounds in contaminated groundwater. *J Chromatogr A*, 738(2):295–304. doi:10.1016/0021-9673(96)00114-8 PMID:8696509

Johansen SS, Hansen AB, Mosbaek H, Arvin E (1997). Identification of heteroaromatic and other organic compounds in ground water at creosote-contaminated sites in Denmark. *Ground Water Monit Remediat*, 17(2):106–15. doi:10.1111/j.1745-6592.1997.tb01283.x

Kato T, Saeki K, Kawazoe Y, Hakura A (1999). Effects of oligofluorine substitution on the mutagenicity of quinoline: a study with twelve fluoroquinoline derivatives. *Mutat Res*, 439(2):149–57. doi:10.1016/S1383-5718(98)00188-0 PMID:10023045

Kawamura K, Kaplan IR (1983). Organic compounds in the rainwater of Los Angeles. *Environ Sci Technol*, 17(8):497–501.doi:10.1021/es00114a011PMID:22283170

Knox WE (1946). The quinine-oxidizing enzyme and liver aldehyde oxidase. *J Biol Chem*, 163:699–711. PMID:20985642

LaVoie EJ, Adams EA, Shigematsu A, Hoffmann D (1983). On the metabolism of quinoline and isoquinoline: possible molecular basis for differences in biological activities. *Carcinogenesis*, 4(9):1169–73. doi:10.1093/carcin/4.9.1169 PMID:6883639

LaVoie EJ, Defauw J, Fealy M, Way BM, McQueen CA (1991). Genotoxicity of fluoroquinolines and methylquinolines. *Carcinogenesis*, 12(2):217–20. doi:10.1093/carcin/12.2.217 PMID:1995188

LaVoie EJ, Dolan S, Little P, Wang CX, Sugie S, Rivenson A (1988). Carcinogenicity of quinoline, 4- and 8-methylquinoline and benzoquinolines in newborn mice and rats. *Food Chem Toxicol*, 26(7):625–9. doi:10.1016/0278-6915(88)90233-5 PMID:3181837

LaVoie EJ, Shigematsu A, Adams EA, Rigotty J, Hoffmann D (1984). Tumor-initiating activity of quinoline and methylated quinolines on the skin of SENCAR mice. *Cancer Lett*, 22(3):269–73. doi:10.1016/0304-3835(84)90162-9 PMID:6324986

LaVoie EJ, Shigematsu A, Rivenson A (1987). The carcinogenicity of quinoline and benzoquinolines in newborn CD-1 mice. *Jpn J Cancer Res*, 78(2):139–43. PMID:3104257

Lam P, Kan C, Yuen MC, Cheung S, Gambari R, Lam K, et al. (2012). Studies on quinoline type dyes and their characterisation studies on acrylic fabric. *Color Technol*, 128(3):192–8. doi:10.1111/j.1478-4408.2012.00363.x

Lava K, Evrard Y, Van Hecke K, Van Meervelta L, Binnemans K (2012). Quinolinium and isoquinolinium ionic liquid crystals. *RSC Advances*, 2(21):8061–70. Available from: https://lirias.kuleuven.be/bitstream/123456789/367006/2/262QuinoliniumILCs.pdf doi:10.1039/c2ra21208k

Lide DR, editor (2003). CRC handbook of chemistry and physics. 84th ed. Boca Raton (FL), USA: CRC Press; pp. 2003–4.

Lopes TJ, Furlong ET, Pritt JW (1997). Occurrence and distribution of semivolatile organic compounds in stream bed sediments in United States, 1992-95. In: Little EE, Greenberg BM, Delonay AJ, editors. Environmental toxicology and risk assessment. Volume 7. ASTM STP1333. West Conshohocken (PA), USA; pp. 105–119.

Luongo G, Avagyan R, Hongyu R, Östman C (2016b). The washout effect during laundry on benzothiazole, benzotriazole, quinoline, and their derivatives in clothing textiles. *Environ Sci Pollut Res Int*, 23(3):2537–48. doi:10.1007/s11356-015-5405-7 PMID:26429136

Luongo G, Iadaresta F, Moccia E, Östman C, Crescenzi C (2016a). Determination of aniline and quinoline compounds in textiles. *J Chromatogr A*, 1471:11–8. doi:10.1016/j.chroma.2016.09.068 PMID:27745926

Luongo G, Thorsén G, Ostman C (2014). Quinolines in clothing textiles–a source of human exposure and wastewater pollution? *Anal Bioanal Chem*, 406(12):2747–56. doi:10.1007/s00216-014-7688-9 PMID:24604325

Matsumoto M, Kano H, Suzuki M, Noguchi T, Umeda Y, Fukushima S (2018). Carcinogenicity of quinoline by drinking-water administration in rats and mice. *J Toxicol Sci*, 43(2):113–27. doi:10.2131/jts.43.113 PMID:29479033

Matsuoka A, Hayashi M, Ishidate M Jr (1979). Chromosomal aberration tests on 29 chemicals combined with S9 mix in vitro. *Mutat Res*, 66(3):277–90. doi:10.1016/0165-1218(79)90089-2 PMID:375085

McFee AF (1989). Genotoxic potency of three quinoline compounds evaluated in vivo in mouse marrow cells. *Environ Mol Mutagen*, 13(4):325–31. doi:10.1002/em.2850130408 PMID:2500337

Meijers AP, Van der Leer RC (1976). The occurrence of organic micropollutants in the river Rhine and the river Maas in 1974. *Water Res*, 10(7):597–604. doi:10.1016/0043-1354(76)90140-8

Merck (2017). Merck Catalogue No. 802407. Quinoline. Available from: http://www.merckmillipore.com/DK/en/search/Quinoline?search=&TrackingSearchType=SB+-+Search+Box&SearchContextPageletUUID=&SearchTerm=Quinoline.

Meyer S, Cartellieri S, Steinhart H (1999). Simultaneous determination of PAHs, hetero-PAHs (N, S, O), and their degradation products in creosote-contaminated soils. Method development, validation, and application to hazardous waste sites. *Anal Chem*, 71(18):4023–9. doi:10.1021/ac990136j

Miyata Y, Saeki K, Kawazoe Y, Hayashi M, Sofuni T, Suzuki T (1998). Antimutagenic structural modification of quinoline assessed by an in vivo mutagenesis assay using *lacZ*-transgenic mice. *Mutat Res*, 414(1–3):165–9. doi:10.1016/S1383-5718(98)00029-1 PMID:9630605

Nagao M, Yahagi T, Seino Y, Sugimura T, Ito N (1977). Mutagenicities of quinoline and its derivatives. *Mutat Res*, 42(3):335–42. doi:10.1016/S0027-5107(77)80037-7 PMID:323699

Neuwoehner J, Reineke AK, Hollender J, Eisentraeger A (2009). Ecotoxicity of quinoline and hydroxylated derivatives and their occurrence in groundwater of a tar-contaminated field site. *Ecotoxicol Environ Saf*, 72(3):819–27. doi:10.1016/j.ecoenv.2008.04.012 PMID:18550163

Novack L, Brodie BB (1950). Quinoline and its transformation products found in urine. *J Biol Chem*, 187(2):787–92. PMID:14803463

O'Neil MJ, editor (2006). The Merck index - an encyclopedia of chemicals, drugs, and biologicals. 18th ed. Whitehouse Station (NJ), USA: Merck and Co.; p. 1445.

OECD (2009). 2007 OECD list of high production volume chemicals. Organisation for Economic Co-operation and Development. Available from: http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?

cote=ENV/JM/MONO%282009%2940&doclanguage=en.

Okumura L, Ramos SN (2007). Simultaneous determination of quinoline and pyridine compounds in gasoline and diesel by differential pulse voltammetry. *Electroanalysis*, 19(6):709–16. doi:10.1002/elan.200603804

Ondrus MG, Steinheimer TR (1990). High-performance liquid chromatographic determination of azaarenes and their metabolites in groundwater affected by creosote wood preservatives. *J Chromatogr Sci*, 28(6):324–30. doi:10.1093/chromsci/28.6.324 PMID:2246357

Organic Chemistry Portal (2017). Synthesis of quinolines. Available from: https://www.organic-chemistry.org/synthesis/heterocycles/benzo-fused/quinolines.shtm.

Özel MZ, Hamilton JF, Lewis AC (2011). New sensitive and quantitative analysis method for organic nitrogen compounds in urban aerosol samples. *Environ Sci Technol*, 45(4):1497–505. doi:10.1021/es102528g PMID:21210660

Padma TV, Hale RC, Roberts MH Jr (1998). Toxicity of water-soluble fractions derived from whole creosote and creosote-contaminated sediments. *Environ Toxicol Chem*, 17(8):1606–10. doi:10.1002/etc.5620170823

Pereira WE, Rostad CE, Garbarino JR, Hult MF (1983). Groundwater contamination by organic bases derived from coal-tar wastes. *Environ Toxicol Chem*, 2:283–94.

Pereira WE, Rostad CE, Updegraff DM, Bennett JL (1987). Fate and movement of azaarenes and their anaerobic biotransformation products in an aquifer contaminated by wood-treatment chemicals. *Environ Toxicol Chem*, 6(3):163–76. doi:10.1002/etc.5620060302

PubChem (2017). PubChem database. U.S. National Center for Biotechnology Information (NCBI). Available from: https://pubchem.ncbi.nlm.nih.gov.

Reigh G, McMahon H, Ishizaki M, Ohara T, Shimane K, Esumi Y, et al. (1996). Cytochrome P450 species involved in the metabolism of quinoline. *Carcinogenesis*, 17(9):1989–96. doi:10.1093/carcin/17.9.1989 PMID:8824525

Reineke AK, Göen T, Preiss A, Hollender J (2007). Quinoline and derivatives at a tar oil contaminated site: hydroxylated products as indicator for natural attenuation? *Environ Sci Technol*, 41(15):5314–22. doi:10.1021/es070405k PMID:17822096

Rostad CE, Pereira WE, Hult MF (1985). Partitioning studies of coal-tar constituents in a two-phase contaminated ground-water system. *Chemosphere*, 14(8):1023–36. doi:10.1016/0045-6535(85)90023-2

Saeki K, Kadoi M, Kawazoe Y, Igarashi M, Shimada H (2000). Clastogenicity of quinoline derivatives tested by micronucleus induction in vivo in the hepatocytes of partially hepatectomized mice. *Biol Pharm Bull*, 23(2):219–21. doi:10.1248/bpb.23.219 PMID:10706388

Saeki K, Takahashi K, Kawazoe Y (1993). Metabolism of mutagenicity-deprived 3-fluoroquinoline: comparison with mutagenic quinoline. *Biol Pharm Bull*, 16(3):232–4. doi:10.1248/bpb.16.232 PMID:8364465

Saggadi H, Polaert I, Luart D, Lenb C, Estel L (2015). Microwaves under pressure for the continuous production of quinoline from glycerol. *Catal Today*, 255:66–74. doi:10.1016/j.cattod.2014.10.050

Scheunemann B (1923). Über das Verhalten des Chinolins im Tierkörper. *Arch Exp Pathol Pharmakol*, 100(1–2):51–60. doi:10.1007/BF01863054

Seidel A, editor (2006). Kirk–Othmer encyclopedia of chemical technology. Volume 21. 5th ed. Hoboken (NJ), USA: John Wiley & Sons; pp. 182–214.

Shinohara Y, Ogiso T, Hananouchi M, Nakanishi K, Yoshimura T, Ito N (1977). Effect of various factors on the induction of liver tumors in animals by quinoline. *Gan*, 68(6):785–96. PMID:598648

Sina JF, Bean CL, Dysart GR, Taylor VI, Bradley MO (1983). Evaluation of the alkaline elution/rat hepatocyte assay as a predictor of carcinogenic/mutagenic potential. *Mutat Res*, 113(5):357–91. doi:10.1016/0165-1161(83)90228-5 PMID:6877265

Singh J, Bhandari M, Kaur J, Kad GL (2003). Quinolinium chlorochromate as an efficient reagent for oxidative cleavage of oximes via the use of microwave irradiation and pestle/mortar. *Indian J Chem*, 42B(02):405–7. Available from: http://nopr.niscair.res.in/bitstream/123456789/21524/1/IJCB%2042B%282%29%20405-407.pdf doi:10.1002/chin.200321072

Smith JN, Williams RT (1955). Studies in detoxication. 65. The metabolism of quinoline; new metabolites of quinoline, with observations on the metabolism of 3-, 5- and 6-hydroxyquinoline and 2:4-dihydroxyquinoline. *Biochem J*, 60(2):284–90. doi:10.1042/bj0600284 PMID:14389238

Stedman RL (1968). The chemical composition of tobacco and tobacco smoke. *Chem Rev*, 68(2):153–207. doi:10.1021/cr60252a002 PMID:4868017

Steinheimer TR, Ondrus MG (1986). Determination of selected azaarenes in water by bonded-phase extraction and liquid chromatography. *Anal Chem*, 58(8):1839–44. doi:10.1021/ac00121a052

Stuermer DH, Ng DJ, Morris CJ (1982). Organic contaminants in groundwater near an underground coal gasification site in northeastern Wyoming. *Environ Sci Technol*, 16(9):582–7. doi:10.1021/es00103a009 PMID:22284199

Suzuki T, Miyata Y, Saeki K, Kawazoe Y, Hayashi M, Sofuni T (1998). In vivo mutagenesis by the hepatocarcinogen quinoline in the *lacZ* transgenic mouse: evidence for its in vivo genotoxicity. *Mutat Res*, 412(2):161–6. doi:10.1016/S1383-5718(97)00185-X PMID:9539970

Suzuki T, Takeshita K, Saeki KI, Kadoi M, Hayashi M, Sofuni T (2007). Clastogenicity of quinoline and monofluorinated quinolines in Chinese hamster lung cells. *J Health Sci*, 53(3):325–8. doi:10.1248/jhs.53.325

Suzuki T, Wang X, Miyata Y, Saeki K, Kohara A, Kawazoe Y, et al. (2000). Hepatocarcinogen quinoline induces G:C to C:G transversions in the *cII* gene in the liver of lambda/*lacZ* transgenic mice (MutaMouse). *Mutat Res*, 456(1–2):73–81. doi:10.1016/S0027-5107(00)00128-7 PMID:11087898

Švábenský R, Kočí K, Šimek Z (2007). The study of properties of HPLC determination of polycyclic aromatic nitrogen heterocycles. *Int J Environ Anal Chem*, 87(5):337–49. doi:10.1080/03067310601087130

Tada M, Takahashi K, Kawazoe Y (1982). Metabolites of quinoline, a hepatocarcinogen, in a subcellular microsomal system. *Chem Pharm Bull (Tokyo)*, 30(10):3834–7. doi:10.1248/cpb.30.3834 PMID:7160026

Tada M, Takahashi K, Kawazoe Y, Ito N (1980). Binding of quinoline to nucleic acid in a subcellular microsomal system. *Chem Biol Interact*, 29(3):257–66. doi:10.1016/0009-2797(80)90145-3 PMID:6766813

Takahashi K, Kamiya M, Sengoku Y, Kohda K, Kawazoe Y (1988). Deprivation of the mutagenic property of quinoline: inhibition of mutagenic metabolism by fluorine substitution. *Chem Pharm Bull (Tokyo)*, 36(11):4630–3. doi:10.1248/cpb.36.4630 PMID:3246033

Talcott R, Hollstein M, Wei E (1976). Mutagenicity of 8-hydroxyquinoline and related compounds in the *Salmonella typhimurium* bioassay. *Biochem Pharmacol*, 25(11):1323–8. doi:10.1016/0006-2952(76)90097-6 PMID:820351

Thiebaud N, Veloso Da Silva S, Jakob I, Sicard G, Chevalier J, Ménétrier F, et al. (2013). Odorant metabolism catalyzed by olfactory mucosal enzymes influences peripheral olfactory responses in rats. *PLoS One*, 8(3):e59547. doi:10.1371/journal.pone.0059547 PMID:23555703

Thomsen AB, Henriksen K, Grøn C, Møldrup P (1999). Sorption, transport, and degradation of quinoline in unsaturated soil. *Environ Sci Technol*, 33(17):2891–8. doi:10.1021/es981065t

Turney GL, Goerlitz OF (1990). Organic contamination of ground water at gas works park, Seattle, Washington. *Ground Water Monit Rev*, 10(3):187–98. doi:10.1111/j.1745-6592.1990.tb00014.x

Uno F, Tanaka J, Ueda M, Nagai M, Fukumuro M, Natsume M, et al. (2015). Repeated-dose liver and gastrointestinal tract micronucleus assays for quinoline in rats. *Mutat Res Genet Toxicol Environ Mutagen*, 780-781:51–5. doi:10.1016/j.mrgentox.2015.01.003 PMID:25892622

Valencia R, Mason JM, Zimmering S (1989). Chemical mutagenesis testing in *Drosophila*. VI. Interlaboratory comparison of mutagenicity tests after treatment of larvae. *Environ Mol Mutagen*, 14(4):238–44. doi:10.1002/em.2850140405 PMID:2511011

Watanabe T, Suzuki T, Natsume M, Nakajima M, Narumi K, Hamada S, et al. (2012). Discrimination of genotoxic and non-genotoxic hepatocarcinogens by statistical analysis based on gene expression profiling in the mouse liver as determined by quantitative real-time PCR. *Mutat Res*, 747(2):164–75. doi:10.1016/j.mrgentox.2012.04.011 PMID:22634710

Watanabe T, Tanaka G, Hamada S, Namiki C, Suzuki T, Nakajima M, et al. (2009). Dose-dependent alterations in gene expression in mouse liver induced by diethylnitrosamine and ethylnitrosourea and determined by quantitative real-time PCR. *Mutat Res*, 673(1):9–20. doi:10.1016/j.mrgentox.2008.11.004 PMID:19100860

Weiss G (1986). Hazardous chemicals handbook. Park Ridge (NJ), USA: Noyes Data Corporation; p. 880.

Weyand EH, Defauw J, McQueen CA, Meschter CL, Meegalla SK, LaVoie EJ (1993). Bioassay of quinoline, 5-fluoroquinoline, carbazole, 9-methylcarbazole and 9-ethylcarbazole in newborn mice. *Food Chem Toxicol*, 31(10):707–15. doi:10.1016/0278-6915(93)90141-K PMID:8225128

White EL, Uhrig MS, Johnson TJ, Gordon BM, Hicks RD, Borgerding MF, et al. (1990). Quantitative determination of selected compounds in a Kentucky 1R4F reference cigarette smoke by multidimensional gas chromatography and selected ion monitoring-mass spectrometry. *J Chromatogr Sci*, 28(8):393–9. doi:10.1093/chromsci/28.8.393 PMID:2229269

Willems MI, Dubois G, Boyd DR, Davies RJ, Hamilton L, McCullough JJ, et al. (1992). Comparison of the mutagenicity of quinoline and all monohydroxyquinolines with a series of arene oxide, *trans*-dihydrodiol, diol epoxide, *N*-oxide and arene hydrate derivatives of quinoline in the Ames/*Salmonella* microsome test. *Mutat Res*, 278(4):227–36. doi:10.1016/S0165-1218(10)80002-3 PMID:1373859

Xu H, Sun W, Yan N, Li D, Wang X, Yu T, et al. (2017). Competition for electrons between pyridine and quinoline during their simultaneous biodegradation. *Environ Sci Pollut Res Int*, 24(32):25082–91. doi:10.1007/s11356-017-0082-3 PMID:28921046

Yost EE, Stanek J, Burgoon LD (2017). A decision analysis framework for estimating the potential hazards for drinking water resources of chemicals used in hydraulic fracturing fluids. *Sci Total Environ*, 574:1544–58. doi:10.1016/j.scitotenv.2016.08.167 PMID:27666475

Zhang C, Liu G, Zhang R, Luo H (2010). Electricity production from and biodegradation of quinoline in the microbial fuel cell. *J Environ Sci Health A Tox Hazard Subst Environ Eng*, 45(2):250–6. doi:10.1080/10934520903430061 PMID:20390865

Zimmering S, Mason JM, Valencia R, Woodruff RC (1985). Chemical mutagenesis testing in *Drosophila*. II. Results of 20 coded compounds tested for the National Toxicology Program. *Environ Mutagen*, 7(1):87–100. doi:10.1002/em.2860070105 PMID:3917911

LIST OF ABBREVIATIONS

8-OHdG	8-hydroxy-2′-deoxyguanosine
8-oxodGuo	8-oxo-7,8-dihydro-2′-deoxyguanosine
ABS	acrylonitrile–butadiene–styrene
AC50	concentration for half-maximal activity
AhR	aryl hydrocarbon receptor
ALL	acute lymphoblastic/lymphocytic leukaemia
AML	acute myeloid leukaemia
AR	androgen receptor
BALF	bronchioloalveolar lavage fluid
BrdU	bromodeoxyuridine
BSO	buthionine sulfoximine
bw	body weight
CAS	Chemical Abstracts Service
CI	confidence interval
CLL	chronic lymphocytic leukaemia
CML	chronic myeloid leukaemia
CYP	cytochrome P450
DAD	diode array detection
DEG	differentially expressed gene
DMDTC	dimethyldithiocarbamate
DMSO	dimethylsulfoxide
EPA	United States Environmental Protection Agency
EPA	expandable polystyrene
ER	estrogen receptor
FCM	food-contact material
FD	fluorescence detector
FDA	United States Food and Drug Administration
FID	flame ionization detector
Fpg	formamido pyrimidine glycosylase
FT3	free triiodothyronine
FT4	free thyroxine
GC	gas chromatography
GC×GC	two-dimensional gas chromatography

GLP	good laboratory practice
GM	geometric mean
GSH	glutathione
GSSG	oxidized glutathione
GST	glutathione *S*-transferase
HL	Hodgkin lymphoma
HLA-G	human leukocyte antigen G
HPLC	high-performance liquid chromatography
HPRT	hypoxanthine-guanine phosphoribosyl transferase
HR	hazard ratio
IARC	International Agency for Research on Cancer
JEM	job-exposure matrix
LALN	lung-associated lymph node
LC	liquid chromatography
L-NAC	L-*N*-acetylcysteine
LOD	limit of detection
LOQ	limit of quantitation
LPO	lipid peroxidation
LPS	lipopolysaccharide
LSE	low styrene emission
M1	*N*-acetyl-*S*-(1-phenyl-2-hydroxyethyl)-L-cysteine
M2	*N*-acetyl-*S*-(2-phenyl-2-hydroxyethyl)-L-cysteine
MA	mandelic acid
MDA	malondialdehyde
MEF	murine embryonic fibroblast
MM	multiple myeloma
MN	micronucleus
MNCPES	Minnesota Children's Pesticide Exposure Study
MS	mass spectrometry
MS/MS	tandem mass spectrometry
*N*3αA	3-(2-hydroxy-1-phenylethyl)adenine
*N*3βA	3-(2-hydroxy-2-phenylethyl)adenine
NAC	*N*-acetylcysteine
NHANES	United States National Health and Nutrition Examination Survey
NHL	non-Hodgkin lymphoma
NIOSH	United States National Institute for Occupational Safety and Health
NK	natural killer
NSA	non-Swiss albino
NVS	NovaScreen
OR	odds ratio
PAC	polycyclic aromatic compounds
PAH	polycyclic aromatic hydrocarbon
PFC	plaque-forming cell
PGA	phenylglyoxylic acid
PGE_2	prostaglandin E_2
$PGF_{2\alpha}$	prostaglandin $F_{2\alpha}$
PHEMA	phenylhydroxyethylmercapturic acid
$PM_{2.5}$	airborne particulate matter of diameter less than 2.5 µm
ppb	parts per billion
ppm	parts per million

PS	polystyrene
PT	purge-and-trap
QC	quality control
ROS	reactive oxygen species
RR	relative risk
RSD	relative standard deviation
SAN	styrene–acrylonitrile
SBL	styrene–butadiene latex
SBR	styrene–butadiene rubber
sHLA-G	soluble human leukocyte antigen G
SIM	selected ion monitoring
SIR	standardized incidence ratio
SMR	standardized mortality ratio
SOD	superoxide dismutase
SPE	solid-phase extraction
Tox21	Toxicity Testing in the 21st Century
ToxCast	Toxicity Forecaster
TPA	12-*O*-tetradecanoylphorbol-13-acetate
TRH	thyrotrophin-releasing hormone
TSH	thyroid-stimulating hormone
TWA	time-weighted average
UPR	unsaturated polyester resins
UV	ultraviolet
VOC	volatile organic carbon

ANNEX 1. SUPPLEMENTAL MATERIAL FOR TOXCAST/TOX21

This supplemental material (which is available online at: http://publications.iarc.fr/582) contains a spreadsheet (.xlsx) analysed by the Working Group for Volume 121 of the *IARC Monographs*. The spreadsheet lists the ToxCast/Tox21 assay end-points, the associated target and/or model system (e.g. cell type, species, detection technology, etc.), their mapping to 6 of the 10 “key characteristics” of known human carcinogens, and whether each chemical was “active” or “inactive” (EPA, 2015).

Reference

EPA (2015). ToxCast™ Data. Washington (DC), USA: United States Environmental Protection Agency. Available from: https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data. Data released December 2014.

This volume of the *IARC Monographs* provides evaluations of the carcinogenicity of quinoline, styrene, and styrene-7,8-oxide.

Quinoline and styrene are present in air pollution and in tobacco smoke. Quinoline also occurs in the processing of petroleum and shale oil, and is found in groundwater and soil at sites contaminated by coal tar and creosote. Quinoline and styrene are high production volume chemicals. Quinoline is used to produce various drugs and dyes. Styrene is primarily used in the production of polystyrene polymers. Styrene-7,8-oxide is primarily used to produce epoxy resins. Styrene-7,8-oxide is the primary metabolite of styrene in humans. Styrene and styrene-7,8-oxide are found in workplace air, particularly in the reinforced plastics industry and the rubber industry. Exposure to these agents may occur in the general population as well as in various occupational settings.

An *IARC Monographs* Working Group reviewed epidemiological evidence, animal bioassays, and mechanistic and other relevant data to reach conclusions as to the carcinogenic hazard to humans of environmental or occupational exposure to these agents.

© NIOSH/2006