

# **TOXICOLOGICAL PROFILE FOR 1,3-BUTADIENE**

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES  
Public Health Service  
Agency for Toxic Substances and Disease Registry

September 2012

## **DISCLAIMER**

Use of trade names is for identification only and does not imply endorsement by the Agency for Toxic Substances and Disease Registry, the Public Health Service, or the U.S. Department of Health and Human Services.

## UPDATE STATEMENT

A Toxicological Profile for 1,3-Butadiene, Draft for Public Comment was released in September 2009. This edition supersedes any previously released draft or final profile.

Toxicological profiles are revised and republished as necessary. For information regarding the update status of previously released profiles, contact ATSDR at:

Agency for Toxic Substances and Disease Registry  
Division of Toxicology and Human Health Sciences (proposed)  
Environmental Toxicology Branch (proposed)  
1600 Clifton Road NE  
Mailstop F-62  
Atlanta, Georgia 30333

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## FOREWORD

This toxicological profile is prepared in accordance with guidelines\* developed by the Agency for Toxic Substances and Disease Registry (ATSDR) and the Environmental Protection Agency (EPA). The original guidelines were published in the *Federal Register* on April 17, 1987. Each profile will be revised and republished as necessary.

The ATSDR toxicological profile succinctly characterizes the toxicologic and adverse health effects information for the toxic substances each profile describes. Each peer-reviewed profile identifies and reviews the key literature that describes a substance's toxicologic properties. Other pertinent literature is also presented but is described in less detail than the key studies. The profile is not intended to be an exhaustive document; however, more comprehensive sources of specialty information are referenced.

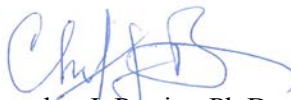
The profiles focus on health and toxicologic information; therefore, each toxicological profile begins with a public health statement that describes, in nontechnical language, a substance's relevant toxicological properties. Following the public health statement is information concerning levels of significant human exposure and, where known, significant health effects. A health effects summary describes the adequacy of information to determine a substance's health effects. ATSDR identifies data needs that are significant to protection of public health.

Each profile:

- (A) Examines, summarizes, and interprets available toxicologic information and epidemiologic evaluations on a toxic substance to ascertain the levels of significant human exposure for the substance and the associated acute, subacute, and chronic health effects;
- (B) Determines whether adequate information on the health effects of each substance is available or being developed to determine levels of exposure that present a significant risk to human health of acute, subacute, and chronic health effects; and
- (C) Where appropriate, identifies toxicologic testing needed to identify the types or levels of exposure that may present significant risk of adverse health effects in humans.

The principal audiences for the toxicological profiles are federal, state, and local health professionals; interested private sector organizations and groups; and members of the public.

This profile reflects ATSDR's assessment of all relevant toxicologic testing and information that has been peer-reviewed. Staff of the Centers for Disease Control and Prevention and other federal scientists also have reviewed the profile. In addition, this profile has been peer-reviewed by a nongovernmental panel and was made available for public review. Final responsibility for the contents and views expressed in this toxicological profile resides with ATSDR.



Christopher J. Portier, Ph.D.  
Assistant Administrator

Agency for Toxic Substances and Disease Registry

### \*Legislative Background

The toxicological profiles are developed under the Comprehensive Environmental Response, Compensation, and Liability Act of 1980, as amended (CERCLA or Superfund). CERCLA section 104(i)(1) directs the Administrator of ATSDR to "...effectuate and implement the health related authorities" of the statute. This includes the preparation of toxicological profiles for hazardous substances most commonly found at facilities on the CERCLA National Priorities List and that pose the most significant potential threat to human health, as determined by ATSDR and the EPA. Section 104(i)(3) of CERCLA, as amended, directs the Administrator of ATSDR to prepare a toxicological profile for each substance on the list. In addition, ATSDR has the authority to prepare toxicological profiles for substances not found at sites on the National Priorities List, in an effort to "...establish and maintain inventory of literature, research, and studies on the health effects of toxic substances" under CERCLA Section 104(i)(1)(B), to respond to requests for consultation under section 104(i)(4), and as otherwise necessary to support the site-specific response actions conducted by ATSDR.

## QUICK REFERENCE FOR HEALTH CARE PROVIDERS

Toxicological Profiles are a unique compilation of toxicological information on a given hazardous substance. Each profile reflects a comprehensive and extensive evaluation, summary, and interpretation of available toxicologic and epidemiologic information on a substance. Health care providers treating patients potentially exposed to hazardous substances will find the following information helpful for fast answers to often-asked questions.

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### *Primary Chapters/Sections of Interest*

**Chapter 1: Public Health Statement:** The Public Health Statement can be a useful tool for educating patients about possible exposure to a hazardous substance. It explains a substance's relevant toxicologic properties in a nontechnical, question-and-answer format, and it includes a review of the general health effects observed following exposure.

**Chapter 2: Relevance to Public Health:** The Relevance to Public Health Section evaluates, interprets, and assesses the significance of toxicity data to human health.

**Chapter 3: Health Effects:** Specific health effects of a given hazardous compound are reported by type of health effect (death, systemic, immunologic, reproductive), by route of exposure, and by length of exposure (acute, intermediate, and chronic). In addition, both human and animal studies are reported in this section.

**NOTE:** Not all health effects reported in this section are necessarily observed in the clinical setting. Please refer to the Public Health Statement to identify general health effects observed following exposure.

**Pediatrics:** Four new sections have been added to each Toxicological Profile to address child health issues:

|                    |                                                                      |
|--------------------|----------------------------------------------------------------------|
| <b>Section 1.6</b> | <b>How Can (Chemical X) Affect Children?</b>                         |
| <b>Section 1.7</b> | <b>How Can Families Reduce the Risk of Exposure to (Chemical X)?</b> |
| <b>Section 3.7</b> | <b>Children's Susceptibility</b>                                     |
| <b>Section 6.6</b> | <b>Exposures of Children</b>                                         |

### **Other Sections of Interest:**

|                     |                                           |
|---------------------|-------------------------------------------|
| <b>Section 3.8</b>  | <b>Biomarkers of Exposure and Effect</b>  |
| <b>Section 3.11</b> | <b>Methods for Reducing Toxic Effects</b> |

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### **ATSDR Information Center**

**Phone:** 1-800-CDC-INFO (800-232-4636) or 1-888-232-6348 (TTY) **Fax:** (770) 488-4178  
**E-mail:** [cdcinfo@cdc.gov](mailto:cdcinfo@cdc.gov) **Internet:** <http://www.atsdr.cdc.gov>

The following additional material can be ordered through the ATSDR Information Center:

*Case Studies in Environmental Medicine: Taking an Exposure History*—The importance of taking an exposure history and how to conduct one are described, and an example of a thorough exposure history is provided. Other case studies of interest include *Reproductive and Developmental Hazards*; *Skin Lesions and Environmental Exposures*; *Cholinesterase-Inhibiting Pesticide Toxicity*; and numerous chemical-specific case studies.

*Managing Hazardous Materials Incidents* is a three-volume set of recommendations for on-scene (prehospital) and hospital medical management of patients exposed during a hazardous materials incident. Volumes I and II are planning guides to assist first responders and hospital emergency department personnel in planning for incidents that involve hazardous materials. Volume III—*Medical Management Guidelines for Acute Chemical Exposures*—is a guide for health care professionals treating patients exposed to hazardous materials.

*Fact Sheets (ToxFAQs)* provide answers to frequently asked questions about toxic substances.

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### ***Other Agencies and Organizations***

*The National Center for Environmental Health (NCEH)* focuses on preventing or controlling disease, injury, and disability related to the interactions between people and their environment outside the workplace. Contact: NCEH, Mailstop F-29, 4770 Buford Highway, NE, Atlanta, GA 30341-3724 • Phone: 770-488-7000 • FAX: 770-488-7015.

*The National Institute for Occupational Safety and Health (NIOSH)* conducts research on occupational diseases and injuries, responds to requests for assistance by investigating problems of health and safety in the workplace, recommends standards to the Occupational Safety and Health Administration (OSHA) and the Mine Safety and Health Administration (MSHA), and trains professionals in occupational safety and health. Contact: NIOSH, 200 Independence Avenue, SW, Washington, DC 20201 • Phone: 800-356-4674 or NIOSH Technical Information Branch, Robert A. Taft Laboratory, Mailstop C-19, 4676 Columbia Parkway, Cincinnati, OH 45226-1998 • Phone: 800-35-NIOSH.

*The National Institute of Environmental Health Sciences (NIEHS)* is the principal federal agency for biomedical research on the effects of chemical, physical, and biologic environmental agents on human health and well-being. Contact: NIEHS, PO Box 12233, 104 T.W. Alexander Drive, Research Triangle Park, NC 27709 • Phone: 919-541-3212.

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### ***Referrals***

*The Association of Occupational and Environmental Clinics (AOEC)* has developed a network of clinics in the United States to provide expertise in occupational and environmental issues. Contact: AOEC, 1010 Vermont Avenue, NW, #513, Washington, DC 20005 • Phone: 202-347-4976 • FAX: 202-347-4950 • e-mail: [AOEC@AOEC.ORG](mailto:AOEC@AOEC.ORG) • Web Page: <http://www.aoec.org/>.

*The American College of Occupational and Environmental Medicine (ACOEM)* is an association of physicians and other health care providers specializing in the field of occupational and environmental medicine. Contact: ACOEM, 25 Northwest Point Boulevard, Suite 700, Elk Grove Village, IL 60007-1030 • Phone: 847-818-1800 • FAX: 847-818-9266.

## CONTRIBUTORS

### CHEMICAL MANAGER(S)/AUTHOR(S):

Annette Ashizawa, Ph.D.  
Nickolette Roney, M.P.H.  
Pamela G. Tucker, M.D.  
Carolyn Harper, Ph.D.  
Diana Cronin  
ATSDR, Division of Toxicology and Human Health Sciences (proposed), Atlanta, GA

Lisa Ingerman, Ph.D., DABT  
Julie Klotzbach, Ph.D.  
Gary L. Diamond, Ph.D.  
Mike Lumpkin, Ph.D.  
Daniel J. Plewak, B.S.  
SRC, Inc., North Syracuse, NY

### THE PROFILE HAS UNDERGONE THE FOLLOWING ATSDR INTERNAL REVIEWS:

1. Health Effects Review. The Health Effects Review Committee examines the health effects chapter of each profile for consistency and accuracy in interpreting health effects and classifying end points.
2. Minimal Risk Level Review. The Minimal Risk Level Workgroup considers issues relevant to substance-specific Minimal Risk Levels (MRLs), reviews the health effects database of each profile, and makes recommendations for derivation of MRLs.
3. Data Needs Review. The Environmental Toxicology Branch (proposed) reviews data needs sections to assure consistency across profiles and adherence to instructions in the Guidance.
4. Green Border Review. Green Border review assures the consistency with ATSDR policy.

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## PEER REVIEW

A peer review panel was assembled for 1,3-butadiene. The panel consisted of the following members:

1. Sherif Abdel-Rahman, Ph.D., Department of Preventative Medicine and Community Health, University of Texas Medical Branch, Galveston, Texas
2. Genevieve Matanoski, M.D., Dr.PH, Bloomberg School of Public Health, The Johns Hopkins University, Baltimore, Maryland
3. Amir Sapkota, Ph.D., Maryland Institute for Applied Environmental Health, University of Maryland, School of Public Health, College Park, Maryland

These experts collectively have knowledge of 1,3-butadiene's physical and chemical properties, toxicokinetics, key health end points, mechanisms of action, human and animal exposure, and quantification of risk to humans. All reviewers were selected in conformity with the conditions for peer review specified in Section 104(I)(13) of the Comprehensive Environmental Response, Compensation, and Liability Act, as amended.

Scientists from the Agency for Toxic Substances and Disease Registry (ATSDR) have reviewed the peer reviewers' comments and determined which comments will be included in the profile. A listing of the peer reviewers' comments not incorporated in the profile, with a brief explanation of the rationale for their exclusion, exists as part of the administrative record for this compound.

The citation of the peer review panel should not be understood to imply its approval of the profile's final content. The responsibility for the content of this profile lies with the ATSDR.

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## CONTENTS

|                                                                                                 |        |
|-------------------------------------------------------------------------------------------------|--------|
| DISCLAIMER .....                                                                                | ii     |
| UPDATE STATEMENT .....                                                                          | iii    |
| FOREWORD .....                                                                                  | v      |
| QUICK REFERENCE FOR HEALTH CARE PROVIDERS .....                                                 | vii    |
| CONTRIBUTORS .....                                                                              | ix     |
| PEER REVIEW .....                                                                               | xi     |
| CONTENTS .....                                                                                  | xiii   |
| LIST OF FIGURES .....                                                                           | xvii   |
| LIST OF TABLES .....                                                                            | xix    |
| <br>1. PUBLIC HEALTH STATEMENT .....                                                            | <br>1  |
| 1.1 WHAT IS 1,3-BUTADIENE? .....                                                                | 1      |
| 1.2 WHAT HAPPENS TO 1,3-BUTADIENE WHEN IT ENTERS THE ENVIRONMENT? .....                         | 2      |
| 1.3 HOW MIGHT I BE EXPOSED TO 1,3-BUTADIENE? .....                                              | 2      |
| 1.4 HOW CAN 1,3-BUTADIENE ENTER AND LEAVE MY BODY? .....                                        | 3      |
| 1.5 HOW CAN 1,3-BUTADIENE AFFECT MY HEALTH? .....                                               | 3      |
| 1.6 HOW CAN 1,3-BUTADIENE AFFECT CHILDREN? .....                                                | 4      |
| 1.7 HOW CAN FAMILIES REDUCE THE RISK OF EXPOSURE TO 1,3-BUTADIENE? .....                        | 4      |
| 1.8 IS THERE A MEDICAL TEST TO DETERMINE WHETHER I HAVE BEEN EXPOSED<br>TO 1,3-BUTADIENE? ..... | 5      |
| 1.9 WHAT RECOMMENDATIONS HAS THE FEDERAL GOVERNMENT MADE TO<br>PROTECT HUMAN HEALTH? .....      | 5      |
| 1.10 WHERE CAN I GET MORE INFORMATION? .....                                                    | 6      |
| <br>2. RELEVANCE TO PUBLIC HEALTH .....                                                         | <br>7  |
| 2.1 BACKGROUND AND ENVIRONMENTAL EXPOSURES TO 1,3-BUTADIENE IN<br>THE UNITED STATES .....       | 7      |
| 2.2 SUMMARY OF HEALTH EFFECTS .....                                                             | 8      |
| 2.3 MINIMAL RISK LEVELS (MRLs) .....                                                            | 10     |
| <br>3. HEALTH EFFECTS .....                                                                     | <br>19 |
| 3.1 INTRODUCTION .....                                                                          | 19     |
| 3.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE .....                                     | 19     |
| 3.2.1 Inhalation Exposure .....                                                                 | 20     |
| 3.2.1.1 Death .....                                                                             | 20     |
| 3.2.1.2 Systemic Effects .....                                                                  | 21     |
| 3.2.1.3 Immunological and Lymphoreticular Effects .....                                         | 39     |
| 3.2.1.4 Neurological Effects .....                                                              | 40     |
| 3.2.1.5 Reproductive Effects .....                                                              | 40     |
| 3.2.1.6 Developmental Effects .....                                                             | 42     |
| 3.2.1.7 Cancer .....                                                                            | 42     |
| 3.2.2 Oral Exposure .....                                                                       | 46     |
| 3.2.3 Dermal Exposure .....                                                                     | 47     |
| 3.3 GENOTOXICITY .....                                                                          | 47     |
| 3.4 TOXICOKINETICS .....                                                                        | 55     |
| 3.4.1 Absorption .....                                                                          | 55     |
| 3.4.1.1 Inhalation Exposure .....                                                               | 55     |
| 3.4.1.2 Oral Exposure .....                                                                     | 56     |
| 3.4.1.3 Dermal Exposure .....                                                                   | 56     |

|         |                                                                                |     |
|---------|--------------------------------------------------------------------------------|-----|
| 3.4.2   | Distribution .....                                                             | 56  |
| 3.4.2.1 | Inhalation Exposure .....                                                      | 56  |
| 3.4.2.2 | Oral Exposure .....                                                            | 57  |
| 3.4.2.3 | Dermal Exposure.....                                                           | 57  |
| 3.4.3   | Metabolism .....                                                               | 57  |
| 3.4.4   | Elimination and Excretion .....                                                | 62  |
| 3.4.4.1 | Inhalation Exposure .....                                                      | 62  |
| 3.4.4.2 | Oral Exposure .....                                                            | 63  |
| 3.4.4.3 | Dermal Exposure.....                                                           | 63  |
| 3.4.5   | Physiologically Based Pharmacokinetic (PBPK)/Pharmacodynamic (PD) Models ..... | 64  |
| 3.5     | MECHANISMS OF ACTION.....                                                      | 85  |
| 3.5.1   | Pharmacokinetic Mechanisms.....                                                | 85  |
| 3.5.2   | Mechanisms of Toxicity.....                                                    | 87  |
| 3.5.3   | Animal-to-Human Extrapolations.....                                            | 89  |
| 3.6     | TOXICITIES MEDIATED THROUGH THE NEUROENDOCRINE AXIS .....                      | 90  |
| 3.7     | CHILDREN'S SUSCEPTIBILITY .....                                                | 91  |
| 3.8     | BIOMARKERS OF EXPOSURE AND EFFECT.....                                         | 92  |
| 3.8.1   | Biomarkers Used to Identify or Quantify Exposure to 1,3-Butadiene.....         | 93  |
| 3.8.2   | Biomarkers Used to Characterize Effects Caused by 1,3-Butadiene .....          | 95  |
| 3.9     | INTERACTIONS WITH OTHER CHEMICALS.....                                         | 96  |
| 3.10    | POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE .....                               | 97  |
| 3.11    | METHODS FOR REDUCING TOXIC EFFECTS .....                                       | 98  |
| 3.11.1  | Reducing Peak Absorption Following Exposure .....                              | 98  |
| 3.11.2  | Reducing Body Burden.....                                                      | 99  |
| 3.11.3  | Interfering with the Mechanism of Action for Toxic Effects.....                | 99  |
| 3.12    | ADEQUACY OF THE DATABASE.....                                                  | 99  |
| 3.12.1  | Existing Information on Health Effects of 1,3-Butadiene.....                   | 100 |
| 3.12.2  | Identification of Data Needs .....                                             | 100 |
| 3.12.3  | Ongoing Studies.....                                                           | 108 |
| 4.      | CHEMICAL AND PHYSICAL INFORMATION.....                                         | 111 |
| 4.1     | CHEMICAL IDENTITY .....                                                        | 111 |
| 4.2     | PHYSICAL AND CHEMICAL PROPERTIES .....                                         | 111 |
| 5.      | PRODUCTION, IMPORT/EXPORT, USE, AND DISPOSAL .....                             | 114 |
| 5.1     | PRODUCTION.....                                                                | 115 |
| 5.2     | IMPORT/EXPORT.....                                                             | 119 |
| 5.3     | USE.....                                                                       | 119 |
| 5.4     | DISPOSAL .....                                                                 | 120 |
| 6.      | POTENTIAL FOR HUMAN EXPOSURE .....                                             | 121 |
| 6.1     | OVERVIEW .....                                                                 | 121 |
| 6.2     | RELEASES TO THE ENVIRONMENT .....                                              | 123 |
| 6.2.1   | Air.....                                                                       | 124 |
| 6.2.2   | Water.....                                                                     | 129 |
| 6.2.3   | Soil.....                                                                      | 129 |
| 6.3     | ENVIRONMENTAL FATE.....                                                        | 130 |
| 6.3.1   | Transport and Partitioning .....                                               | 130 |
| 6.3.2   | Transformation and Degradation .....                                           | 131 |
| 6.3.2.1 | Air.....                                                                       | 131 |
| 6.3.2.2 | Water.....                                                                     | 132 |

|            |                                                        |     |
|------------|--------------------------------------------------------|-----|
| 6.3.2.3    | Sediment and Soil .....                                | 133 |
| 6.3.2.4    | Other Media .....                                      | 133 |
| 6.4        | LEVELS MONITORED OR ESTIMATED IN THE ENVIRONMENT ..... | 133 |
| 6.4.1      | Air .....                                              | 133 |
| 6.4.2      | Water.....                                             | 134 |
| 6.4.3      | Sediment and Soil .....                                | 134 |
| 6.4.4      | Other Environmental Media.....                         | 137 |
| 6.5        | GENERAL POPULATION AND OCCUPATIONAL EXPOSURE.....      | 137 |
| 6.6        | EXPOSURES OF CHILDREN .....                            | 141 |
| 6.7        | POPULATIONS WITH POTENTIALLY HIGH EXPOSURES .....      | 142 |
| 6.8        | ADEQUACY OF THE DATABASE .....                         | 142 |
| 6.8.1      | Identification of Data Needs .....                     | 143 |
| 6.8.2      | Ongoing Studies.....                                   | 145 |
| 7.         | ANALYTICAL METHODS.....                                | 146 |
| 7.1        | BIOLOGICAL MATERIALS .....                             | 147 |
| 7.2        | ENVIRONMENTAL SAMPLES .....                            | 148 |
| 7.3        | ADEQUACY OF THE DATABASE .....                         | 150 |
| 7.3.1      | Identification of Data Needs .....                     | 151 |
| 7.3.2      | Ongoing Studies.....                                   | 152 |
| 8.         | REGULATIONS, ADVISORIES, AND GUIDELINES.....           | 153 |
| 9.         | REFERENCES .....                                       | 157 |
| 10.        | GLOSSARY .....                                         | 185 |
| APPENDICES |                                                        |     |
| A.         | ATSDR MINIMAL RISK LEVELS AND WORKSHEETS .....         | A-1 |
| B.         | USER’S GUIDE.....                                      | B-1 |
| C.         | ACRONYMS, ABBREVIATIONS, AND SYMBOLS.....              | C-1 |
| D.         | INDEX .....                                            | D-1 |

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## LIST OF FIGURES

|                                                                                                                                       |     |
|---------------------------------------------------------------------------------------------------------------------------------------|-----|
| 3-1. Levels of Significant Exposure to 1,3-Butadiene – Inhalation .....                                                               | 32  |
| 3-2. Metabolism of 1,3-Butadiene .....                                                                                                | 58  |
| 3-3. Conceptual Representation of a Physiologically Based Pharmacokinetic (PBPK) Model for a<br>Hypothetical Chemical Substance ..... | 66  |
| 3-4. Existing Information on Health Effects of 1,3-Butadiene .....                                                                    | 101 |
| 6-1. Frequency of NPL Sites with 1,3-Butadiene Contamination .....                                                                    | 122 |

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## LIST OF TABLES

|                                                                                                                                 |     |
|---------------------------------------------------------------------------------------------------------------------------------|-----|
| 2-1. Summary of Available Chronic Risk Assessment Values for 1,3-Butadiene.....                                                 | 16  |
| 3-1. Levels of Significant Exposure to 1,3-Butadiene – Inhalation.....                                                          | 22  |
| 3-2. Genotoxicity of 1,3-Butadiene <i>In Vitro</i> .....                                                                        | 48  |
| 3-3. Genotoxicity of 1,3 Butadiene <i>In Vivo</i> .....                                                                         | 49  |
| 3-4. Physiological and Chemical Parameters Used in the Johanson and Filser (1993) PBPK Model<br>for 1,3-Butadiene.....          | 68  |
| 3-5. Physiological Parameter Values Used in the Kohn and Melnick (2001) PBPK Model for<br>1,3-Butadiene .....                   | 70  |
| 3-6. Chemical Partition Coefficients Parameter Values Used in the Kohn and Melnick (2001) PBPK<br>Model for 1,3-Butadiene ..... | 71  |
| 3-7. Chemical Metabolism Parameter Values Used in the Kohn and Melnick (2001) PBPK Model for<br>1,3-Butadiene .....             | 72  |
| 3-8. Physiological and Chemical Parameters Used in the Brochot et al. 2007 PBPK Model for<br>1,3-Butadiene Humans .....         | 75  |
| 3-9. Physiological Parameters Used in Sweeney et al. (1997) 1,3-Butadiene PBPK Model.....                                       | 78  |
| 3-10. Partition Coefficients Used in Sweeney et al. (1997) PBPK Model for 1,3-Butadiene .....                                   | 79  |
| 3-11. Nonenzymatic Reaction Rate Constants Used in Sweeney et al. (1997) PBPK Model for<br>1,3-Butadiene .....                  | 80  |
| 3-12. Metabolism Rate Constants Used in the Sweeney et al. (1997) PBPK Model for<br>1,3-Butadiene .....                         | 81  |
| 3-13. Physiological Parameters Used in the Sweeney et al. (2010) PBPK Model for 1,3-Butadiene<br>in Humans .....                | 82  |
| 3-14. Chemical Partition Coefficients Used in the Sweeney et al. (2010) PBPK Model for<br>1,3-Butadiene in Humans .....         | 83  |
| 3-15. Chemical Metabolism Parameters Used in the Sweeney et al. (2010) PBPK Model for<br>1,3-Butadiene in Humans.....           | 84  |
| 4-1. Chemical Identity of 1,3-Butadiene.....                                                                                    | 112 |
| 4-2. Physical and Chemical Properties of 1,3-Butadiene.....                                                                     | 113 |
| 5-1. Companies that Produce 1,3-Butadiene in the United States and Annual Capacities During<br>2008.....                        | 116 |

|                                                                                                                                      |     |
|--------------------------------------------------------------------------------------------------------------------------------------|-----|
| 5-2. Facilities that Produce, Process, or Use 1,3-Butadiene .....                                                                    | 117 |
| 6-1. Releases to the Environment from Facilities that Produce, Process, or Use 1,3-Butadiene.....                                    | 125 |
| 6-2. 1,3-Butadiene Emission Data for 2005.....                                                                                       | 127 |
| 6-3. 1,3-Butadiene Concentrations in Outdoor Air.....                                                                                | 135 |
| 6-4. 1,3-Butadiene Concentrations in Indoor Air .....                                                                                | 136 |
| 6-5. Air Concentrations of 1,3-Butadiene Corresponding to Typical Operations Within a Styrene-<br>Butadiene Rubber (SBR) Plant ..... | 140 |
| 7-1. Analytical Methods For Determining 1,3-Butadiene in Environmental Samples .....                                                 | 149 |
| 8-1. Regulations, Advisories, and Guidelines Applicable to 1,3-Butadiene.....                                                        | 154 |



## 1. PUBLIC HEALTH STATEMENT

This public health statement tells you about 1,3-butadiene and the effects of exposure to it.

The Environmental Protection Agency (EPA) identifies the most serious hazardous waste sites in the nation. These sites are then placed on the National Priorities List (NPL) and are targeted for long-term federal clean-up activities. 1,3-Butadiene has been found in at least 13 of the 1,699 current or former NPL sites. Because not all NPL sites were tested for 1,3-butadiene, the number of sites where this chemical is found may increase in the future as more sites are evaluated. This information is important because these sites may be sources of exposure and exposure to this substance may be harmful.

When a substance is released either from a large area, such as an industrial plant, or from a container, such as a drum or bottle, it enters the environment. Such a release does not always lead to exposure. You can be exposed to a substance only when you come in contact with it. You may be exposed by breathing, eating, or drinking the substance, or by skin contact.

If you are exposed to 1,3-butadiene, many factors will determine whether you will be harmed. These factors include the dose (how much), the duration (how long), and how you come in contact with it. You must also consider any other chemicals you are exposed to and your age, sex, diet, family traits, lifestyle, and state of health.

### 1.1 WHAT IS 1,3-BUTADIENE?

|                    |                                                                                                                                                                                               |
|--------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Description</b> | 1,3-Butadiene is a colorless gas with a mild gasoline-like odor.                                                                                                                              |
| <b>Uses</b>        | About 60% of 1,3-butadiene is used to make man-made rubber, which is then used mostly for car and truck tires. 1,3-Butadiene is also used to make certain types of plastics such as acrylics. |

See Chapters 4 and 5 for more information on the sources, properties, and uses of 1,3-butadiene.

## 1. PUBLIC HEALTH STATEMENT

**1.2 WHAT HAPPENS TO 1,3-BUTADIENE WHEN IT ENTERS THE ENVIRONMENT?**

|                                                                                                  |                                                                                                                                                                                                                                                                                                                                                                                                                   |
|--------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Sources</b>                                                                                   | <p>Large amounts of 1,3-butadiene are released into the air by industrial sources. Industrial releases to water and soil are relatively low.</p> <p>Automobile exhaust is a constant source of 1,3-butadiene release into the air. Other sources of 1,3-butadiene include cigarette smoke and the smoke of wood fires.</p> <p>Forest fires are considered to be a natural source of 1,3-butadiene in the air.</p> |
| <b>Break-down</b> <ul style="list-style-type: none"><li>• Air</li><li>• Water and soil</li></ul> | <p>Half of the 1,3-butadiene in the air will likely be broken down in about 6 hours.</p> <p>1,3-Butadiene that is spilled onto water or soil will likely evaporate quickly into the air based on its physical and chemical properties.</p>                                                                                                                                                                        |

See Chapters 5 and 6 for more information on 1,3-butadiene in the environment.

**1.3 HOW MIGHT I BE EXPOSED TO 1,3-BUTADIENE?**

|                      |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
|----------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Air</b>           | <p>The primary way you can be exposed to 1,3-butadiene is by breathing air containing it. Releases of 1,3-butadiene into the air occur from:</p> <ul style="list-style-type: none"><li>• vehicle exhaust</li><li>• tobacco smoke</li><li>• wood burning</li><li>• burning of rubber and plastic</li><li>• forest fires</li><li>• accidental or intentional release at manufacturing plants</li></ul> <p>The average amount of 1,3-butadiene in the air is between 0.04 and 0.9 parts of 1,3-butadiene per billion parts of air (ppb) in cities and suburban areas.</p> |
| <b>Workplace air</b> | <p>Workers in the production of rubber, plastics, and resins are likely exposed to higher levels of 1,3-butadiene.</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                 |

## 1. PUBLIC HEALTH STATEMENT

|                                |                                                                                                                                                                                                                                                                                                                       |
|--------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Food and drinking water</b> | <p>1,3-Butadiene has been measured at very low levels in plastic or rubber of food containers, but it has not been found often in food samples.</p> <p>Exposure to 1,3-butadiene through ingestion of food and drinking water is expected to be very low compared to exposure through breathing contaminated air.</p> |
| <b>Gasoline</b>                | <p>People may be exposed to small amounts of 1,3-butadiene if gasoline gets on their skin or by breathing air that contains gasoline fumes.</p>                                                                                                                                                                       |

## 1.4 HOW CAN 1,3-BUTADIENE ENTER AND LEAVE MY BODY?

|                        |                                                                                                                                                                                                                                                                                 |
|------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Enter your body</b> | <p>1,3-Butadiene in air can be absorbed from the lungs and enter the blood stream.</p>                                                                                                                                                                                          |
| <b>Leave your body</b> | <p>1,3-Butadiene is broken down to other chemicals in the liver.</p> <p>About half of inhaled 1,3-butadiene is broken down and exhaled, while most of the remaining chemical is broken down and excreted in the urine. 1,3-Butadiene typically leaves the body by 10 hours.</p> |

For more information on how 1,3-butadiene enters and leaves the body, see Chapter 3.

## 1.5 HOW CAN 1,3-BUTADIENE AFFECT MY HEALTH?

This section looks at studies concerning potential health effects in animal and human studies.

|                  |                                                                                                                                                                              |
|------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Noncancer</b> | <p>In laboratory animals, 1,3-butadiene causes inflammation of nasal tissues, changes to lung, heart, and reproductive tissues, neurological effects, and blood changes.</p> |
|------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

## 1. PUBLIC HEALTH STATEMENT

|               |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
|---------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Cancer</b> | <p>Studies of workers exposed to 1,3-butadiene suggest that workers may have an increased risk for cancers of the blood and lymphatic system.</p> <p>Laboratory animals have developed cancer in multiple body tissues after exposure to 1,3-butadiene for 13 weeks or longer. Animals appear to be most sensitive to blood and lymphatic system cancers.</p> <p>The International Agency for Research on Cancer (IARC), National Toxicology Program (NTP), and EPA all classify 1,3-butadiene as a human carcinogen.</p> |
|---------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

**1.6 HOW CAN 1,3-BUTADIENE AFFECT CHILDREN?**

This section discusses potential health effects in humans from exposures during the period from conception to maturity at 18 years of age.

|                            |                                                                                                                                                                                             |
|----------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Effects in children</b> | It is likely that children would show the same health effects as adults. We do not know whether children are more sensitive to the effects of 1,3-butadiene.                                |
| <b>Birth defects</b>       | We do not know whether 1,3-butadiene causes birth defects in people. Some studies have found decreases in fetal weight and skeletal defects in laboratory animals exposed to 1,3-butadiene. |

**1.7 HOW CAN FAMILIES REDUCE THE RISK OF EXPOSURE TO 1,3-BUTADIENE?**

|                        |                                                                                                    |
|------------------------|----------------------------------------------------------------------------------------------------|
| <b>Wood burning</b>    | Take precautions to minimize the amount of smoke released into the home during wood burning.       |
| <b>Vehicle engines</b> | Make sure vehicle engines are turned off when in an enclosed space such as a garage.               |
| <b>Vehicle traffic</b> | Minimize time spent near areas of heavy vehicle traffic and avoid living very close to busy roads. |
| <b>Tobacco smoke</b>   | Families can reduce exposure to 1,3-butadiene by avoiding tobacco smoke, particularly indoors.     |

## 1. PUBLIC HEALTH STATEMENT

**1.8 IS THERE A MEDICAL TEST TO DETERMINE WHETHER I HAVE BEEN EXPOSED TO 1,3-BUTADIENE?**

|                                   |                                                                                                                                                                                                                                                              |
|-----------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Development of blood tests</b> | We currently have no reliable medical test to determine if someone has been exposed to 1,3-butadiene. However, scientists are working on tests to show if 1,3-butadiene attaches to compounds in the blood, such as proteins or deoxyribonucleic acid (DNA). |
|-----------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

**1.9 WHAT RECOMMENDATIONS HAS THE FEDERAL GOVERNMENT MADE TO PROTECT HUMAN HEALTH?**

The federal government develops regulations and recommendations to protect public health. Regulations can be enforced by law. The EPA, the Occupational Safety and Health Administration (OSHA), and the Food and Drug Administration (FDA) are some federal agencies that develop regulations for toxic substances. Recommendations provide valuable guidelines to protect public health, but cannot be enforced by law. The Agency for Toxic Substances and Disease Registry (ATSDR) and the National Institute for Occupational Safety and Health (NIOSH) are two federal organizations that develop recommendations for toxic substances.

Regulations and recommendations can be expressed as “not-to-exceed” levels. These are levels of a toxic substance in air, water, soil, or food that do not exceed a critical value. This critical value is usually based on levels that affect animals; they are then adjusted to levels that will help protect humans. Sometimes these not-to-exceed levels differ among federal organizations because they used different exposure times (an 8-hour workday or a 24-hour day), different animal studies, or other factors.

Recommendations and regulations are also updated periodically as more information becomes available. For the most current information, check with the federal agency or organization that provides it.

Some regulations and recommendations for 1,3-butadiene include the following:

|                                            |                                                                                            |
|--------------------------------------------|--------------------------------------------------------------------------------------------|
| <b>Levels in breathing air set by EPA</b>  | EPA has set a reference concentration in breathing air of 0.9 ppb for 1,3-butadiene.       |
| <b>Levels in drinking water set by EPA</b> | EPA has not set levels in drinking water for 1,3-butadiene.                                |
| <b>Levels in workplace air set by OSHA</b> | OSHA set a legal limit of 1 ppm for 1,3-butadiene in air averaged over an 8-hour work day. |

## 1. PUBLIC HEALTH STATEMENT

**1.10 WHERE CAN I GET MORE INFORMATION?**

If you have any more questions or concerns, please contact your community or state health or environmental quality department, or contact ATSDR at the address and phone number below.

ATSDR can also tell you the location of occupational and environmental health clinics. These clinics specialize in recognizing, evaluating, and treating illnesses that result from exposure to hazardous substances.

Toxicological profiles are also available on-line at [www.atsdr.cdc.gov](http://www.atsdr.cdc.gov) and on CD-ROM. You may request a copy of the ATSDR ToxProfiles™ CD-ROM by calling the toll-free information and technical assistance number at 1-800-CDCINFO (1-800-232-4636), by e-mail at [cdcinfo@cdc.gov](mailto:cdcinfo@cdc.gov), or by writing to:

Agency for Toxic Substances and Disease Registry  
Division of Toxicology and Human Health Sciences (proposed)  
1600 Clifton Road NE  
Mailstop F-62  
Atlanta, GA 30333  
Fax: 1-770-488-4178

Organizations for-profit may request copies of final Toxicological Profiles from the following:

National Technical Information Service (NTIS)  
5285 Port Royal Road  
Springfield, VA 22161  
Phone: 1-800-553-6847 or 1-703-605-6000  
Web site: <http://www.ntis.gov/>

## 2. RELEVANCE TO PUBLIC HEALTH

### 2.1 BACKGROUND AND ENVIRONMENTAL EXPOSURES TO 1,3-BUTADIENE IN THE UNITED STATES

1,3-Butadiene is a highly volatile gas that is used in the production of synthetic rubber; the major end use of the synthetic rubber is automobile tires. 1,3-Butadiene is also used for the production of high impact polystyrene and acrylonitrile-butadiene-styrene (ABS) resin plastics. The predominant source of 1,3-butadiene in the atmosphere is industrial releases, which can occur during manufacturing, use, transport, and storage of the chemical. Automobile exhaust is a constant source of low levels of 1,3-butadiene release to the atmosphere. Minor sources of 1,3-butadiene in the atmosphere include cigarette smoke, wood burning (including forest fires), and the burning of rubber and plastics. In the atmosphere, 1,3-butadiene is expected to undergo photo-initiated destruction with a half-life of approximately 6 hours. Relatively low levels of 1,3-butadiene are released to water and soil. 1,3-Butadiene in water or soil is expected to rapidly evaporate to the atmosphere.

Inhalation is the predominant route of exposure for the general population. Mean concentrations of 1,3-butadiene in the air in cities and suburban areas ranges from 0.1 to 2  $\mu\text{g}/\text{m}^3$  (0.04–1 ppb); the average background concentration of 0.13  $\mu\text{g}/\text{m}^3$  (0.59 ppb) has been estimated. Higher atmospheric concentrations have been measured in areas near oil refineries, chemical manufacturing plants, and plastic and rubber factories where 1,3-butadiene is manufactured or used; concentrations as high as 40  $\mu\text{g}/\text{m}^3$  (18 ppb) have been measured near industrial sites. Within the general population, smokers (and individuals exposed to secondhand smoke) and individuals inhaling smoke from wood fires are likely to be exposed to higher levels of 1,3-butadiene. Workers involved in the production of rubber, plastics, and resins are most likely to receive the largest exposures. No data are available to quantify general population exposure to 1,3-butadiene by other routes of exposure, but it is expected to be very low compared to breathing contaminated air. Low levels of 1,3-butadiene have been detected in U.S. drinking water supplies; however, specific quantitative data were not located. 1,3-Butadiene has also been measured at very low levels in the plastic or rubber of food containers and has been found in a few food samples.

Several biomarkers of exposure have been identified for 1,3-butadiene; these include 1,3-butadiene urinary metabolites, M1 and M2, and three hemoglobin adducts, *N*-(2-hydroxy-3-butenyl)valine (MHB-Val), *N*-(2,3,4-trihydroxybutyl)valine (THB-Val), and *N,N*-(2,3-dihydroxy-1,4-butadiyl)valine (*pyr*-Val), which are surrogate biomarkers for the 1,3-butadiene metabolites 1,2 epoxy-3-butene (EB),

## 2. RELEVANCE TO PUBLIC HEALTH

1,2-dihydroxy-3,4-epoxybutane (EBD), and 1,2:3,4-diepoxbutane (DEB), respectively. In workers, the levels of urinary metabolites and hemoglobin adducts have been shown to correlate with 1,3-butadiene exposure levels. However, background levels for the general population have not been established for these biomarkers of exposure.

**2.2 SUMMARY OF HEALTH EFFECTS**

The available data for 1,3-butadiene exposure and toxicity in humans and animals are limited to inhalation exposures; the effects from significant oral or dermal exposures are not known. Information on the toxicity of 1,3-butadiene in humans comes from case reports and epidemiology studies that primarily focused on the potential carcinogenicity of 1,3-butadiene. Slight eye irritation and difficulty in focusing on instrument scales were reported by two men exposed to 2,000 or 4,000 ppm 1,3-butadiene for 6–7 hours; however, this was not reported when the two men were exposed to 8,000 ppm for 8 hours. Psychomotor tests conducted in these subjects did not find alterations at 2,000–8,000 ppm. Numerous epidemiological studies of multiple occupational cohorts, including one encompassing 15,000 workers, have associated a higher incidence of hemato-lymphopoietic cancer mortality among exposed workers. Although most of these workers were co-exposed to other organic compounds, including styrene, benzene, and dithiocarbamates, multivariate analysis suggested that the estimates of 1,3-butadiene exposure provided the best correlation with the rates of lympho-hematopoietic cancers.

Numerous target organs for 1,3-butadiene toxicity have been identified in well-conducted laboratory studies ranging from single episode to lifetime exposures. Observed effects include death, neurological dysfunction, reproductive and developmental effects, hematological and lymphoreticular effects, and cancer. Evaluation of the relevance of adverse health effects observed in laboratory animals to human health is encumbered by large species differences in the metabolism of 1,3-butadiene. The metabolism of 1,3-butadiene in humans and laboratory animals involves the same enzymatic pathways; however, there are notable quantitative differences in the production and detoxification of several reactive metabolites, particularly, EB, DEB, and EBD; see Sections 3.4.2 and 3.5.3 for more information on species differences. Mice, the most sensitive species, are more efficient at converting 1,3-butadiene to EB and converting EB to DEB. Using *pyr*-Val hemoglobin adduct levels as a biomarker for blood DEB levels, an exposure to approximately 1 ppm 1,3-butadiene resulted in mouse DEB levels that were 50 times higher than rats and 1,000 times higher than humans. Although the mode of action has not been elucidated for all toxic end points, there are strong data to support the reactive metabolites as the causative agents for the ovarian atrophy, cancer, and genotoxic effects observed in laboratory animals. Without information on



## 2. RELEVANCE TO PUBLIC HEALTH

the mode of action, particularly the causative agent, the reader should use caution in evaluating the relevance of the animal data presented in this section to human health.

Lesions of the respiratory tract (olfactory tissues and lungs), liver, kidney, stomach, and eyes have been seen in mice exposed to  $\geq 200$  ppm for intermediate durations, but these lesions are typically epithelial or endothelial hyperplasias and are precancerous in nature. Non-neoplastic lesions of the liver (necrosis) in rats and kidney (renal nephrosis) in mice occurred following intermediate-duration exposure to 625 or 8,000 ppm, respectively.

Although no biologically relevant alterations in hematological parameters have been observed in 1,3-butadiene workers, changes in the blood and lymphoid tissues are common observations in rodents exposed for intermediate and chronic durations. Decreases in red blood cell counts and hemoglobin concentration occurred at 65 ppm in mice, progressing to macrocytic megaloblastic anemia from exposures of 200 ppm. These effects are likely associated with observed changes in normal bone marrow function, as indicated by reduced circulation of erythrocytes and leukocytes, and increased proliferative activity with no associated change in bone marrow cellularity. Lymphoreticular toxicity in mice was indicated by significant changes in thymus weight and lesions in lymphoid organs following intermediate-duration exposures to 625–1,250 ppm in mice. A reversible suppression of cytotoxic T-lymphocyte generation to mastocytoma cells and a depression of spleen cellularity were observed at these exposures. The changes in spleen and thymus weights, lymphocytic differentiation, and appearance of lymphoid lesions comport with the onset of lymphoma in mice after chronic exposure to 1,3-butadiene.

Reproductive and developmental effects are the most sensitive non-cancer effects observed in rodents. Wavy ribs and skeletal abnormalities occurred in offspring of rats exposed to 1,000–8,000 ppm during gestation days (GDs) 6–15. In mice, exposure of pregnant dams to 40 ppm on GDs 6–15 resulted in a 5% decrease in fetal body weight among male mice. Exposure of mice to  $\geq 200$  ppm resulted in  $\geq 19\%$  reductions in fetal weight. A possible dominant lethal effect was observed in mice in which increased fetal deaths occurred from exposure to 200 ppm. The lowest lowest-observed-adverse-effect level (LOAEL) identified for intermediate-duration exposures was 12.5 ppm in male mice mated with unexposed females, resulting in increased late fetal death, exencephaly, and skull abnormalities of fetuses. Serious lesions of reproductive tissues in male and female mice have arisen from intermediate- and chronic-duration exposures. Ovarian atrophy, including complete loss of oocytes, follicles, and corpora lutea, occurred in mice exposed to 200 ppm for 9 months and as low as 6.25 ppm for 2 years. Male mice

## 2. RELEVANCE TO PUBLIC HEALTH

were somewhat less sensitive, with testicular atrophy observed after 15-month exposures to 625 ppm 1,3-butadiene.

The consistent carcinogenic responses in rodent bioassays support the associations derived in epidemiological studies between hemato-lymphopoietic cancer and 1,3-butadiene exposure. In rats, 2-year exposure to 1,000 or 8,000 ppm resulted in increased incidences of tumors of the testes, pancreas, uterus, mammary gland, Zymbal gland, and thyroid. In mice, exposure to 200 ppm for 40 weeks resulted in increased tumor incidences of lymphopoietic system, heart, lung, stomach, liver, and eye. These same tumors developed in mice in as little as 13 weeks after exposure to 625 ppm. Chronic exposure of mice to concentrations of 20 ppm (males) and 6.25 ppm (females) of 1,3-butadiene resulted in increased tumor development in the lymphopoietic system, heart, lung, stomach, liver, eye, mammary glands, and ovaries.

### 2.3 MINIMAL RISK LEVELS (MRLs)

#### *Inhalation MRLs*

The toxicity of 1,3-butadiene following inhalation exposure has been examined in epidemiology studies, intermediate- and chronic-duration studies in rats and mice, reproductive toxicity studies in mice, and developmental toxicity studies in rats and mice. The epidemiological studies have primarily focused on carcinogenicity and have found increases in lympho-hematopoietic cancers. Observed effects found in animal studies include neurological dysfunction, reproductive and developmental effects, hematological and lymphoreticular effects, and cancer. Acute exposures have resulted in fetal effects (decreased growth and skeletal defects) (DOE/NTP 1987b; Irvine 1981) and reproductive effects (increased intrauterine death following male-only exposure) (DOE 1988b). Intermediate-duration exposures in mice resulted in precancerous lesions of the respiratory tract (olfactory tissues and lungs), liver, kidney, stomach, and eyes (NTP 1984, 1993). Non-neoplastic lesions of the liver (necrosis) in rats and kidney (renal nephrosis) in mice occurred following intermediate-duration inhalation exposure. In mice, intermediate-duration inhalation exposure also resulted in decreases in red blood cell counts and hemoglobin concentration, progressing to macrocytic megaloblastic anemia (NTP 1993), decreases in spleen and thymus weight (NTP 1993), and depressed splenic cellularity (Thurmond et al. 1986). Chronic-duration inhalation exposure studies identified a number of targets of toxicity in mice including, bone marrow, lungs, heart, forestomach, Harderian gland, testes, ovaries, and uterus (NTP 1984, 1993); neoplastic lesions were also observed in a number of tissues. In rats, chronic exposure resulted in histological alterations in the lungs and increased severity of nephropathy (Owen et al. 1987).

## 2. RELEVANCE TO PUBLIC HEALTH

Comparison of rat and mouse data identifies large differences in sensitivity to 1,3-butadiene, which are due to metabolic differences between species. As discussed in Sections 3.4.2 and 3.5.3, quantitative differences between humans, rats, and mice in the rate of formation of reactive metabolites, particularly EB and DEB have been found. These differences result in higher tissue levels of reactive metabolites in rodents than in humans (Bond et al. 1993; Csanády et al. 1992; Dahl et al. 1991; Filser et al. 2001, 2007, 2010; Henderson et al. 1996, 2001; Himmelstein et al. 1997; Kirman et al. 2010a; Krause and Elfarrar 1997; Schmidt and Loeser 1985; Thornton-Manning et al. 1995b). Following inhalation exposure to 1,3-butadiene, blood EB levels were 2–8 times higher in mice as compared to rats (Filser et al. 2007) and the maximum butadiene-diol levels were 4 times higher in mice than rats (Filser et al. 2007). The DEB levels were >100-fold higher in mice as compared to rats (Filser et al. 2007). At a similar exposure level (1 ppm), mice produce approximately 1,000 times as much DEB as humans, as measured using *pyr*-Val hemoglobin adduct as a biomarker and 50 times as much DEB as rats (Swenberg et al. 2011).

The Agency usually considers humans more sensitive than animals and makes an adjustment to the point of departure to account for species differences when deriving an MRL from an animal study. If possible, chemical-specific data, such as physiologically based pharmacokinetic (PBPK) modeling, is used to account for toxicokinetic differences between species. Although PBPK models for 1,3-butadiene have been developed in rodents (Johanson and Filser 1993; Kohn and Melnick 1993, 1996, 2000) and a preliminary model has been developed in humans (Brochot et al. 2007), the models are limited in their ability to predict internal doses for key metabolites (Kirman and Grant 2012). An alternative to using PBPK models would be to use a biomarker of exposure to reactive metabolites. Several biomarkers of exposure have been identified for reactive 1,3-butadiene metabolites including MHB-Val hemoglobin adducts, THB-Val hemoglobin adducts, and *pyr*-Val hemoglobin adducts, which have been shown to be good surrogate biomarkers for EB, EBD, and DEB, respectively (Georgieva et al. 2010; Slikker et al. 2004). However, there are limited mechanistic data that would allow identification of the 1,3-butadiene metabolite(s) (or parent compound) that is responsible for the non-neoplastic effects, with the exception of ovarian atrophy observed in mice, which is likely due to DEB.

In the absence of chemical-specific data, the Agency generally applies an uncertainty factor of 10 to account for interspecies differences in toxicokinetic and toxicodynamic properties. However, the toxicokinetic data for 1,3-butadiene indicate that mice are many-fold more sensitive than humans. Thus, the Agency can only use an uncertainty factor of 1 (or not apply an uncertainty factor [UF]), which in the case of 1,3-butadiene, may cause the MRL to overestimate the risk to humans. Therefore, in this instance,

## 2. RELEVANCE TO PUBLIC HEALTH

the Agency has elected to not derive inhalation MRLs for 1,3-butadiene. Brief discussions of the available literature for each duration period are presented below.

**Acute-Duration Inhalation MRL.** Death and neurological effects have been observed in rats, mice, and rabbits exposed to 8,000–250,000 ppm from <1 to 4 hours (Carpenter et al. 1944; Shugaev 1969). Studies examining nonlethal effects were limited to three developmental toxicity studies and a reproductive toxicity study. Significant increases in the occurrence of major skeletal defects, predominantly wavy ribs, were observed in the offspring of Sprague-Dawley rats exposed to 1,000 or 8,000 ppm 1,3-butadiene 6 hours/day on GDs 6–15 (Irvine 1981). Other non-concentration-related effects included an increase in minor skeletal defects at 200 ppm, but not at higher concentrations, and increases in the occurrence of minor external/visceral defects at 1,000 ppm, but not at 8,000 ppm. The study also found decreases in fetal growth (body weight and crown-rump length) at 8,000 ppm and decreases in maternal weight gain at  $\geq 200$  ppm; at 8,000 ppm, maternal body weight gain was 45% lower than controls. The investigators noted that the increase in the occurrence of wavy ribs was likely secondary to the decrease in maternal weight gain. In a second rat developmental toxicity study, no developmental effects (including alterations in occurrence of skeletal defects, fetal body weight, or maternal body weight) were observed in Sprague-Dawley rats exposed to 40–1,000 ppm 6 hours/day on GDs 6–15 (DOE/NTP 1987a). In a mouse developmental toxicity study, decreases in fetal body weight were observed in the offspring of CD-1 mice exposed to  $\geq 40$  ppm 6 hours/day on GDs 6–15 (DOE/NTP 1987b). The male fetal body weights were 5, 18, and 23% lower than controls and no significant alterations in female body weight were observed; interpretation of these results is limited by the lack of statistical adjustment for litter size. In the reproductive toxicity study, the mating of male CD-1 mice exposed to  $\geq 200$  ppm 6 hours/day for 5 days with unexposed females resulted in significant increases in dams with two or more intrauterine deaths (DOE/NTP 1988b). This effect was only observed when the mating occurred 1 week post-exposure, suggesting that the mature spermatozoa and/or spermatids were the targets.

The limited available data on the toxicity of 1,3-butadiene following acute-duration inhalation exposure suggest that mice are more sensitive than rats for developmental effects. Exposures to 1,3-butadiene has resulted in decreases in fetal body weights in mice at  $\geq 40$  ppm (DOE/NTP 1987b) and rats at  $\geq 200$  ppm (Irvine 1981), dominant lethal effects in mice at  $\geq 200$  ppm (DOE/NTP 1988b), and increases in skeletal malformations in rat fetuses at  $\geq 1,000$  ppm (Irvine 1981). The no-observed-adverse-effect levels (NOAELs) were 40 ppm for dominant lethal effects in mice and 200 ppm for skeletal defects in rats. As noted previously, the Agency has elected to not derive an acute-duration inhalation MRL for 1,3-butadiene due to the large species differences in the metabolism of 1,3-butadiene and the lack of

## 2. RELEVANCE TO PUBLIC HEALTH

chemical-specific data to adjust for these differences, which may result in the MRL overestimating the risk to humans.

***Intermediate-Duration Inhalation MRL.*** Intermediate-duration exposures resulted in death in mice exposed to 5,000 ppm, 6 hours/day for 5 weeks (NTP 1984) and 200 ppm, 6 hours/day for 40 weeks (NTP 1993). No systemic effects were seen in rats or mice exposed to 8,000 ppm, 6 hours/day for 13–14 weeks, with the exception of a 13% body weight reduction in mice exposed to 2,500 ppm (NTP 1984). Exposure of mice to 625 ppm, 6 hours/day for 40 weeks resulted in pre-cancerous hyperplasia of the respiratory and gastrointestinal systems (epithelial hyperplasia), as well as a 19% reduction in thymus weight. Multi-site cancer was observed in mice after 13–52 weeks of exposure to 200 ppm for 6 hours/day (NTP 1993). Hematological effects included decreased erythrocyte counts, hemoglobin concentration, and red blood cell volume in mice at 62.5 ppm and macrocytic megaloblastic anemia at 200 ppm, administered 6 hours/day for 40 weeks (NTP 1993). Reproductive effects in mice were the most sensitive effects observed, with ovarian atrophy occurring at exposures of 200 ppm, 6 hours/day for 40 weeks (NTP 1993). The most sensitive developmental effects observed were exencephalies, skull abnormalities, and late fetal death in the offspring of unexposed female mice mated with male mice exposed to 12.5 ppm for 10 weeks (Anderson et al. 1996).

The Agency has elected to not derive an intermediate-duration inhalation MRL for 1,3-butadiene due to the large species differences in the metabolism of 1,3-butadiene and the lack of chemical-specific data to adjust for these differences, which may result in the MRL overestimating the risk to humans.

***Chronic-Duration Inhalation MRL.*** Chronic-duration exposures resulted in increased mortality in rats and mice exposed to 8,000 or 20 ppm, 6 hours/day for 2 years. Rats exposed to 8,000 ppm, 6 hours/day for 2 years exhibited increased lung weight and metaplasia and kidney nephrosis (Owen and Glaister 1990; Owen et al. 1987). In mice, exposure to 1,250 ppm for 65 weeks resulted in nasal olfactory epithelial atrophy in mice (NTP 1984). Hepatic necrosis, forestomach epithelial hyperplasia, megaloblastic anemia, and endothelial hyperplasia of the heart were observed in mice exposed to 625 ppm (6 hours/day, 5 days/week) for 61–65 weeks (NTP 1984, 1993); testicular atrophy and preputial gland hyperplasia were observed in mice exposed to 625 ppm for 2 years (NTP 1993). Ovarian atrophy was observed in mice exposed to 62.5 ppm for 65 weeks or 6.25 ppm for 2 years (NTP 1993); complete destruction of oocytes, follicles, and corpora lutea was also observed. Alveolar epithelial hyperplasia was observed in mice following a 2-year exposure to 6.25 ppm (NTP 1993). In addition to the noncancerous effects, mammary gland tumors developed in rats exposed to 1,000 ppm, 6 hours/day for 2 years (Owen

## 2. RELEVANCE TO PUBLIC HEALTH

and Glaister 1990; Owen et al. 1987), while multi-site cancer was observed in mice at 625 ppm, 6 hours/day for 61 weeks (NTP, 1984) and lung cancer occurred in mice following exposure to 6.25 ppm, 6 hours/day for 2 years (NTP 1993).

Considerable species differences were observed in the chronic-duration studies in terms of observed effects and sensitivity. The lowest LOAEL in rats is 8,000 ppm for lung and kidney effects and the lowest LOAEL in mice is 6.25 ppm for ovarian and lung effects. Renal effects have not been observed in mice exposed to up to 625 ppm for 2 years (NTP 1993) and ovarian effects were not observed in rats exposed to concentrations as high as 8,000 ppm for 2 years (Owen et al. 1987). The differences in sensitivity and possibly critical targets are most likely related to species differences in 1,3-butadiene metabolism. As noted previously, mice produce substantially more DEB than rats; one study (Thornton-Manning et al. 1995b) found that peak tissue levels of DEB were 40–160-fold greater in mice than rats. A comparison of blood DEB levels estimated from *pyr*-Val hemoglobin adduct levels found that at similar exposure levels (approximately 1 ppm), mouse DEB levels were 50 times higher than in rats and 1,000 times higher than in humans (Swenberg et al. 2011). In the absence of human data for noncarcinogenic effects following chronic exposure, the species differences in metabolism necessitate estimating human equivalent concentrations for each end point and comparing these values in order to identify the most likely critical target in humans. The available data provide strong evidence that the 1,3-butadiene metabolite, DEB, is the causative agent of the ovarian atrophy observed in mice (Doerr et al. 1996). Mechanistic data that could be used to identify relevant internal dose metrics for other sensitive end points in rats and mice were not identified, which precludes a comparison of human equivalent concentrations for each sensitive target. Thus, the Agency has elected to not derive a chronic-duration inhalation MRL for 1,3-butadiene; the lack of chemical-specific data to adjust for the large species differences in metabolism may result in the MRL overestimating the risk to humans.

Although ATSDR considers that the lack of data that can be used to evaluate the most sensitive target of chronic toxicity in humans precludes derivation of a chronic-duration inhalation MRL, the U.S. EPA (IRIS 2012), the Texas Commission on Environmental Quality (TCEQ) (Grant et al. 2010), and Kirman and Grant (2012) have derived chronic risk assessment values based on ovarian atrophy in mice. These three approaches share several commonalities, but also have several differences. All three approaches use a time-to-response benchmark dose (BMD) model; EPA and TCEQ used incidence data from the National Toxicology Program (NTP 1993) chronic mouse study and Kirman and Grant (2012) used incidence data from intermediate- and chronic-duration rat and mouse studies. The EPA approach did not make any adjustments for chemical-specific differences in metabolism. TCEQ derived chemical-specific

## 2. RELEVANCE TO PUBLIC HEALTH

uncertainty factors to account for species differences in DEB formation, whereas Kirman and Grant (2012) ran the BMD modeling using an internal dose metric for DEB. A summary of these risk assessment values are presented in [Table 2-1](#) and a more detailed discussion of the three approaches follows.

**EPA (IRIS 2012).** In 2002, EPA derived a reference concentration (RfC) of 0.0009 ppm based on a BMCL<sub>10</sub> of 0.88 ppm using the concentration-response data for ovarian atrophy in mice exposed to 1,3-butadiene for 2 years (NTP 1993) and an uncertainty factor of 1,000 (3 for interspecies extrapolation with dosimetric adjustments, 10 for intraspecies variability, 3 for incomplete database, and 10 for extrapolation to a level below the 10% effect level). The BMD modeling used the Weibull time-to-response model and incorporated the incidence data from the interim and final sacrifices; the data were modeled to include extra risk only until age 50 years. Human equivalent concentrations were calculated by adjusting the BMCL<sub>10</sub> for intermittent exposure (6 hours/day, 5 days/week) and multiplying the adjusted BMCL<sub>10</sub> by an RGDR (ratio of blood:gas partition coefficients) of 1.

**Texas Commission on Environmental Quality (Grant et al. 2010).** The TCEQ (Grant et al. 2010) derived a chronic reference value of 0.0154 ppm based on a BMCL<sub>05</sub> of 0.462 ppm for ovarian atrophy in mice (NTP 1993) and a total uncertainty factor of 30. Similar to EPA, the Weibull time-to-response model was used for BMD analysis of the ovarian atrophy incidence data for mice exposed to 1,3-butadiene for 2 years (9- and 15-month interim sacrifice data were also included in the model). The component uncertainty factors were 1 for animal to human extrapolation, 10 for intraspecies variability, and 3 for database deficiencies (lack of a multigenerational reproductive study). Both the intraspecies and the interspecies uncertainty factors were divided into toxicokinetic and toxicodynamic components. For the intraspecies uncertainty factor, a default value of 3 was used to account for toxicodynamic factors because data are lacking on the key sequence of events and how DEB interacts in different subpopulations to produce ovarian atrophy; a toxicokinetic factor of 3 was used because metabolic genetic polymorphisms may account for differences in susceptibility of 2–3.5-fold in humans. For the interspecies uncertainty factor, 3 was used for toxicodynamic differences because data are not available on possible differences on how DEB would react in different species to produce ovarian atrophy; a toxicokinetic factor of 0.3 was selected to account for species differences in 1,3-butadiene metabolism. The basis of this 0.3 factor was: (1) a comparison of the levels of DEB-specific hemoglobin adduct (pyr-Val adduct) formation in mice and humans; (2) a comparison of total 1,3-butadiene metabolite levels in the blood; and (3) comparisons of DEB blood concentrations, DEB tissue levels, and blood area-under-

## 2. RELEVANCE TO PUBLIC HEALTH

**Table 2-1. Summary of Available Chronic Risk Assessment Values for 1,3-Butadiene**

| Source                   | Source of data for benchmark analysis             | POD                            | UF (UF <sub>L</sub> , UF <sub>A</sub> , UF <sub>H</sub> , UF <sub>DB</sub> ) | Issues and considerations                                                                                                                                                                                                                                                                                                                                                                                                        |
|--------------------------|---------------------------------------------------|--------------------------------|------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| EPA (IRIS 2012)          | 2-Year mouse study (including interim sacrifices) | BMCL <sub>10</sub> : 0.88 ppm  | 1,000 (10, 3, 10, 3)                                                         | The RfC does not have a chemical-specific adjustment for the increased production of DEB (reactive metabolite) in mice, as compared to humans.<br><br>Does not take into consideration that ovarian atrophy may not be the most sensitive target in humans and that effects due to exposure to other reactive metabolites may occur at lower doses.                                                                              |
| TCEQ (Grant et al. 2010) | 2-Year mouse study (including interim sacrifices) | BMCL <sub>05</sub> : 0.462 ppm | 30 (NA, 1, 10, 3)                                                            | Accounts for species differences by quantifying toxicokinetic differences between mice and humans and selecting the low end of the range as an uncertainty factor to account for interspecies toxicokinetic differences.<br><br>Does not take into consideration that ovarian atrophy may not be the most sensitive end point in humans and that effects due to exposure to other reactive metabolites may occur at lower doses. |
| Kirman and Grant 2012    | Intermediate and chronic rat and mouse studies    | BMCL <sub>01</sub> : 1.5 ppm   | 10 (NA, 3, 1, 3)                                                             | Accounts for species differences by including rat incidence data in BMD model and using <i>pyr</i> -Val hemoglobin adduct levels as a biomarker of DEB levels.<br><br>Does not take into consideration that ovarian atrophy may not be the most sensitive end point in humans and that effects due to exposure to other reactive metabolites may occur at lower doses.                                                           |

BMCL = 95% lower confidence limit of the benchmark concentration; BMD = benchmark dose; DEB = 1,2:3,4-diepoxybutane; LOAEL = lowest-observed-adverse-effect level; NA = not applicable; NOAEL = no-observed-adverse-effect level; POD = point of departure; RfC = reference concentration; UF = uncertainty factor: UF<sub>L</sub> = extrapolation from NOAEL to LOAEL; UF<sub>A</sub> = extrapolation from animals to humans; UF<sub>H</sub> = human variability; UF<sub>DB</sub> = database limitations



## 2. RELEVANCE TO PUBLIC HEALTH

the-curve levels in rats and mice; these comparisons resulted in a range of toxicokinetic uncertainty factors of 0.01–0.2 and the value of 0.3 was selected.

**Kirman and Grant (2012).** Kirman and Grant (2012) based their RfC of 0.2 ppm on a BMCL<sub>01</sub> of 1.5 ppm for ovarian atrophy and an uncertainty factor of 10 (3 for extrapolation from animals to humans and 3 for database deficiencies [lack of a multigenerational study and lack of dose-response data for follicle depletion]). A multi-stage Weibull time-to-response BMD model was applied to the combined dose-response data for ovarian atrophy in mice exposed for 2 years (including 40- and 65-week interim sacrifices (NTP 1993), mice exposed for 61 weeks (NTP 1984), mice exposed for 13 weeks (Bevan et al. 1996), rats exposed for 2 years (Owen et al. 1987), and rats exposed for 13 weeks (Bevan et al. 1996). To account for species differences in the metabolism of 1,3-butadiene, the BMD model was run using blood DEB levels as the internal dose metric. Blood DEB levels were estimated using a multistep process that used *pyr*-Val adduct burden as a biomarker for DEB levels. *Pyr*-Val adduct burdens were estimated using data on *pyr*-Val adduct efficiency (amount of adducts formed per ppm of 1,3-butadiene in air) in rats and mice as a function of 1,3-butadiene exposure concentration following a 4-week exposure (6 hours/day, 5 days/week). The estimated *pyr*-Val adduct burden were then used to calculate blood DEB concentrations using species-specific rate constants for the reaction of DEB with the terminal valine of hemoglobin and erythrocyte lifespan. For the time-to-response model, the exposure duration of interest was set equal to the window of susceptibility for ovotoxicity. Since the window of susceptibility is dependent on the number of follicles present at birth, the model was run for three scenarios: an average number of follicles at birth, the lower bound of central tendency for number of follicles, and the upper bound of the central tendency for the number of follicles; the range of susceptibility for depletion of follicle reserves for 95% of the population ranges from 8.5 higher and 8.5 lower than the average individual. The BMD model also included a 3-fold shift to account for toxicokinetic variation among humans. Since the model accounts for toxicokinetic and toxicodynamic differences in humans, no additional uncertainty factors were added to account for human variability.

**Oral MRLs**

There are no data available for effects in humans or animals exposed orally to 1,3-butadiene. For this reason, no acute-, intermediate-, or chronic-duration oral MRLs could be derived.

## 2. RELEVANCE TO PUBLIC HEALTH

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### 3. HEALTH EFFECTS

#### 3.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective on the toxicology of 1,3-butadiene. It contains descriptions and evaluations of toxicological studies and epidemiological investigations and provides conclusions, where possible, on the relevance of toxicity and toxicokinetic data to public health.

A glossary and list of acronyms, abbreviations, and symbols can be found at the end of this profile.

#### 3.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

To help public health professionals and others address the needs of persons living or working near hazardous waste sites, the information in this section is organized first by route of exposure (inhalation, oral, and dermal) and then by health effect (death, systemic, immunological, neurological, reproductive, developmental, genotoxic, and carcinogenic effects). These data are discussed in terms of three exposure periods: acute (14 days or less), intermediate (15–364 days), and chronic (365 days or more).

Levels of significant exposure for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELs have been classified into "less serious" or "serious" effects. "Serious" effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). "Less serious" effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an end point should be classified as a NOAEL, "less serious" LOAEL, or "serious" LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant dysfunction. However, the Agency has established guidelines and policies that are used to classify these end points. ATSDR believes that there is sufficient merit in this approach to warrant an attempt at distinguishing between "less serious" and "serious" effects. The distinction between "less serious" effects and "serious" effects is considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear. LOAELs or NOAELs should also help in determining whether or not

### 3. HEALTH EFFECTS

the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

The significance of the exposure levels shown in the Levels of Significant Exposure (LSE) tables and figures may differ depending on the user's perspective. Public health officials and others concerned with appropriate actions to take at hazardous waste sites may want information on levels of exposure associated with more subtle effects in humans or animals (LOAELs) or exposure levels below which no adverse effects (NOAELs) have been observed. Estimates of levels posing minimal risk to humans (Minimal Risk Levels or MRLs) may be of interest to health professionals and citizens alike.

Levels of exposure associated with carcinogenic effects (Cancer Effect Levels, CELs) of 1,3-butadiene are indicated in [Table 3-1](#) and [Figure 3-1](#). Because cancer effects could occur at lower exposure levels, [Figure 3-1](#) also shows a range for the upper bound of estimated excess risks, ranging from a risk of 1 in 10,000 to 1 in 1,000,000 ( $10^{-4}$  to  $10^{-6}$ ), as developed by EPA.

A User's Guide has been provided at the end of this profile (see Appendix B). This guide should aid in the interpretation of the tables and figures for Levels of Significant Exposure and the MRLs.

#### 3.2.1 Inhalation Exposure

##### 3.2.1.1 Death

Information on the lethality of 1,3-butadiene in humans is limited. A number of occupational exposure studies have examined mortality ratios in 1,3-butadiene workers, the results of these studies are discussed in subsequent sections on the primary effects.

No deaths were seen in B6C3F1 mice exposed to  $\leq 8,000$  ppm 1,3-butadiene 6 hours/day, 5 days/week, for 2 weeks (NTP 1984). The majority of rabbits died when exposed to 250,000 ppm 1,3-butadiene for an average of 23 minutes (Carpenter et al. 1944). The  $LC_{50}$  values calculated for mice and rats exposed for 2 and 4 hours, respectively, was 122,000 and 129,000 ppm, respectively (Shugaev 1969).

Intermediate-duration exposures produced no deaths in rats exposed for 6 hours/day, 5/days/week, for 13 weeks to 8,000 ppm (Crouch et al. 1979), or in rats, guinea pigs, rabbits, and dogs during 8 months of exposure to 6,700 ppm (Carpenter et al. 1944). Increased mortality was seen in mice exposed to 5,000 ppm, but not 2,500 ppm, for 6 hours/day, 5 days/week, for 14 weeks (NTP 1984). The lowest

## 3. HEALTH EFFECTS

intermediate-duration exposure resulting in death was observed in mice receiving 200 ppm for 6-hours/day, 5 days/week, for 40 weeks (NTP 1993), ostensibly from the early development of neoplasms.

During chronic exposure to 625 and 1,250 ppm of 1,3-butadiene for 61 weeks, significantly increased mortality, primarily due to cancer, was found in B6C3F1 mice (NTP 1984). Similar results were obtained in another study using a much lower concentration (20 ppm) (NTP 1993). Exposure of rats to 8,000 ppm 1,3-butadiene resulted in statistically significant increased mortality from cancer when compared with controls (Owen et al. 1987). The  $LC_{50}$  values and all reliable LOAEL values for death in each species and duration category are recorded in [Table 3-1](#) and plotted in [Figure 3-1](#).

### 3.2.1.2 Systemic Effects

**Respiratory Effects.** Workers exposed to 1,3-butadiene gas during the manufacture of rubber complained of irritation of the eyes, nasal passages, throat, and lungs (Wilson 1944). In some, coughing, fatigue, and drowsiness developed. All symptoms disappeared on removal from the gas. The associated exposure levels were not reported.

No effects in respiratory tissues were observed in rats, guinea pigs, rabbits, or dogs inhaling up to 6,700 ppm 1,3-butadiene for 7.5 hours/day, 6 days/week, for 8 months (Carpenter et al. 1944) or in rats or mice exposed to 8,000 ppm 1,3-butadiene for 6 hours/day, 5 days/week, for 13–14 weeks (Crouch et al. 1979; NTP 1984). No effects were observed in lungs of mice exposed to concentrations as high as 625 ppm for 6 hours/day, 5 days/week, for 9 months (NTP 1993).

An increase in chronic inflammation of the nasal cavity, fibrosis, cartilaginous metaplasia, osseous metaplasia, atrophy of the sensory epithelium, and hyperplasia of the respiratory epithelium were observed in mice exposed to 1,250 ppm for 2 years (NTP 1984). Lungs of rats exposed chronically to 8,000 ppm 1,3-butadiene exhibited metaplasia (Owen and Glaister 1990; Owen et al. 1987). Atrophy of the nasal olfactory epithelium was observed in mice exposed to concentrations as high as 1,250 ppm 1,3-butadiene for 6 hours/day, 5 days/week, for 61 weeks (NTP 1993), while alveolar epithelial hyperplasia (a possible precancerous lesion) occurred in mice exposed to 6.25 ppm 6 hours/day, 5 days/week for 2 years (NTP 1993).

Table 3-1 Levels of Significant Exposure to 1,3-butadiene - Inhalation

| Key to<br>Figure <sup>a</sup> | Species<br>(Strain) | Exposure/<br>Duration/<br>Frequency<br>(Route) | System | NOAEL<br>(ppm) | LOAEL                                                         |                                                        | Reference<br>Chemical Form | Comments |
|-------------------------------|---------------------|------------------------------------------------|--------|----------------|---------------------------------------------------------------|--------------------------------------------------------|----------------------------|----------|
|                               |                     |                                                |        |                | Less Serious<br>(ppm)                                         | Serious<br>(ppm)                                       |                            |          |
| ACUTE EXPOSURE                |                     |                                                |        |                |                                                               |                                                        |                            |          |
| Death                         |                     |                                                |        |                |                                                               |                                                        |                            |          |
| 1                             | Rat                 | 1 d<br>4 h/d                                   |        |                |                                                               | 129000 (LC50)                                          | Shugaev 1969               |          |
| 2                             | Mouse               | 1 d<br>2h/d                                    |        |                |                                                               | 122000 (LC50)                                          | Shugaev 1969               |          |
| 3                             | Rabbit              | 1 d<br>23 min/d                                |        |                |                                                               | 250000                                                 | Carpenter et al. 1944      |          |
| Systemic                      |                     |                                                |        |                |                                                               |                                                        |                            |          |
| 4                             | Rat                 | 10 d<br>6 hr/d<br>Gd 6-15                      | Bd Wt  |                | 200 F (decreased maternal<br>body weight gain)                | 8000 F (45% decreased<br>maternal body weight<br>gain) | Irvine 1981                |          |
| Neurological                  |                     |                                                |        |                |                                                               |                                                        |                            |          |
| 5                             | Human               | 1 d<br>6-8 hr/d                                |        | 8000           |                                                               |                                                        | Carpenter et al. 1944      |          |
| 6                             | Rabbit              | 1 d<br>23 min/d                                |        |                |                                                               | 250000 (anesthesia)                                    | Carpenter et al. 1944      |          |
| Reproductive                  |                     |                                                |        |                |                                                               |                                                        |                            |          |
| 7                             | Mouse<br>(B6C3F1)   | 5 d<br>6 h/d                                   |        |                | 1000 M (73% increase in number<br>of abnormal sperm<br>heads) |                                                        | DOE/NTP 1988a              |          |
| 8                             | Mouse<br>CD-1       | 6 hr/day<br>5 days                             |        |                | 200 M (increased intrauterine<br>death)                       |                                                        | DOE/NTP 1988b              |          |

Table 3-1 Levels of Significant Exposure to 1,3-butadiene - Inhalation

(continued)

| Key to<br>Figure <sup>a</sup> | Species<br>(Strain)     | Exposure/<br>Duration/<br>Frequency<br>(Route) | System | NOAEL<br>(ppm) | LOAEL                               |                                                     | Reference<br>Chemical Form | Comments |
|-------------------------------|-------------------------|------------------------------------------------|--------|----------------|-------------------------------------|-----------------------------------------------------|----------------------------|----------|
|                               |                         |                                                |        |                | Less Serious<br>(ppm)               | Serious<br>(ppm)                                    |                            |          |
| Developmental                 |                         |                                                |        |                |                                     |                                                     |                            |          |
| 9                             | Rat<br>(Sprague-Dawley) | 10 d<br>6 hr/d<br>Gd 6-15                      |        | 1000           |                                     |                                                     | DOE/NTP 1987a              |          |
| 10                            | Rat                     | 10 d<br>6 hr/d<br>Gd 6-15                      |        | 200            | 8000 (decreased fetal growth)       |                                                     | Irvine 1981                |          |
|                               |                         |                                                |        |                | 1000 (major skeletal malformations) |                                                     |                            |          |
| 11                            | Mouse<br>(CD-1)         | 10 d<br>6 hr/d<br>GD 6-15                      |        |                | 40 M (decreased fetal BW in males)  |                                                     | DOE/NTP 1987b              |          |
| INTERMEDIATE EXPOSURE         |                         |                                                |        |                |                                     |                                                     |                            |          |
| Death                         |                         |                                                |        |                |                                     |                                                     |                            |          |
| 12                            | Mouse                   | 14 wk<br>5 d/wk<br>6 hr/d                      |        |                |                                     | 5000 (increased mortality)                          | NTP 1984                   |          |
| 13                            | Mouse<br>(B6C3F1)       | 13-52 wk<br>6 hr/d<br>5 d/wk                   |        |                |                                     | 200 (increased mortality from 40 weeks of exposure) | NTP 1993                   |          |

Table 3-1 Levels of Significant Exposure to 1,3-butadiene - Inhalation

(continued)

| Key to<br>Figure <sup>a</sup> | Species<br>(Strain) | Exposure/<br>Duration/<br>Frequency<br>(Route) | System    | NOAEL<br>(ppm) | LOAEL                 |                                                                    | Reference<br>Chemical Form            | Comments |
|-------------------------------|---------------------|------------------------------------------------|-----------|----------------|-----------------------|--------------------------------------------------------------------|---------------------------------------|----------|
|                               |                     |                                                |           |                | Less Serious<br>(ppm) | Serious<br>(ppm)                                                   |                                       |          |
| Systemic                      |                     |                                                |           |                |                       |                                                                    |                                       |          |
| 14                            | Rat                 | 13 wk<br>5 d/wk<br>6 hr/d                      | Resp      | 8000           |                       |                                                                    | <a href="#">Crouch et al. 1979</a>    |          |
|                               |                     |                                                | Cardio    | 8000           |                       |                                                                    |                                       |          |
|                               |                     |                                                | Hemato    | 8000           |                       |                                                                    |                                       |          |
|                               |                     |                                                | Musc/skel | 8000           |                       |                                                                    |                                       |          |
|                               |                     |                                                | Hepatic   | 8000           |                       |                                                                    |                                       |          |
|                               |                     |                                                | Renal     | 8000           |                       |                                                                    |                                       |          |
|                               |                     |                                                | Dermal    | 8000           |                       |                                                                    |                                       |          |
|                               |                     |                                                | Ocular    | 8000           |                       |                                                                    |                                       |          |
| 15                            | Mouse               | 3-24 wk<br>6 d/wk<br>6 hr/d                    | Hemato    |                |                       | 1250 M (macrocytic<br>megaloblastic anemia<br>starting at 6 weeks) | <a href="#">Irons et al. 1986a, b</a> |          |



Table 3-1 Levels of Significant Exposure to 1,3-butadiene - Inhalation

(continued)

| Key to<br>Figure <sup>a</sup> | Species<br>(Strain) | Exposure/<br>Duration/<br>Frequency<br>(Route) | System    | NOAEL<br>(ppm) | LOAEL                                                  |                  | Reference<br>Chemical Form | Comments |
|-------------------------------|---------------------|------------------------------------------------|-----------|----------------|--------------------------------------------------------|------------------|----------------------------|----------|
|                               |                     |                                                |           |                | Less Serious<br>(ppm)                                  | Serious<br>(ppm) |                            |          |
| 16                            | Mouse               | 14 wk<br>5 d/wk<br>6 hr/d                      | Resp      | 8000           |                                                        |                  | NTP 1984                   |          |
|                               |                     |                                                | Cardio    | 8000           |                                                        |                  |                            |          |
|                               |                     |                                                | Gastro    | 8000           |                                                        |                  |                            |          |
|                               |                     |                                                | Musc/skel | 8000           |                                                        |                  |                            |          |
|                               |                     |                                                | Hepatic   | 8000           |                                                        |                  |                            |          |
|                               |                     |                                                | Renal     | 8000           |                                                        |                  |                            |          |
|                               |                     |                                                | Dermal    | 8000           |                                                        |                  |                            |          |
|                               |                     |                                                | Bd Wt     | 1250           | 2500 M (13% decreased body weight)                     |                  |                            |          |
| 17                            | Mouse<br>(B6C3F1)   | 13-52 wk<br>6 hr/d<br>5 d/wk                   | Resp      |                | 200 M (alveolar epithelial hyperplasia after 40 weeks) |                  | NTP 1993                   |          |
|                               |                     |                                                | Cardio    |                | 200 M (endothelial hyperplasia after 40 weeks)         |                  |                            |          |
|                               |                     |                                                | Ocular    | 200 M          | 625 M (Harderian gland hyperplasia after 26 weeks)     |                  |                            |          |

Table 3-1 Levels of Significant Exposure to 1,3-butadiene - Inhalation

(continued)

| Key to<br>Figure <sup>a</sup> | Species<br>(Strain) | Exposure/<br>Duration/<br>Frequency<br>(Route) | System    | NOAEL<br>(ppm) | LOAEL                                                                                |                                         | Reference<br>Chemical Form | Comments |
|-------------------------------|---------------------|------------------------------------------------|-----------|----------------|--------------------------------------------------------------------------------------|-----------------------------------------|----------------------------|----------|
|                               |                     |                                                |           |                | Less Serious<br>(ppm)                                                                | Serious<br>(ppm)                        |                            |          |
| 18                            | Mouse<br>(B6C3F1)   | 40 wk<br>6 hr/d<br>5 d/wk                      | Resp      | 200 M          | 625 M (alveolar epithelial hyperlasia)                                               |                                         | NTP 1993                   |          |
|                               |                     |                                                | Cardio    | 625            |                                                                                      |                                         |                            |          |
|                               |                     |                                                | Gastro    | 200            | 625 (forestomach epithelial hyperplasia)                                             |                                         |                            |          |
|                               |                     |                                                | Hemato    |                | 62.5 M (decreased erythrocyte counts, hemoglobin concentration, and red cell volume) | 200 F (macrocytic megaloblastic anemia) |                            |          |
|                               |                     |                                                | Musc/skel | 625            |                                                                                      |                                         |                            |          |
|                               |                     |                                                | Hepatic   | 625 F          |                                                                                      |                                         |                            |          |
|                               |                     |                                                | Bd Wt     | 625            |                                                                                      |                                         |                            |          |
| <b>Immuno/ Lymphoret</b>      |                     |                                                |           |                |                                                                                      |                                         |                            |          |
| 19                            | Mouse<br>(B6C3F1)   | 40 wk<br>6 hr/d<br>5 d/wk                      |           |                | 625 F (19% reduction in relative thymus weight)                                      |                                         | NTP 1993                   |          |
| 20                            | Mouse               | 6-24 wk<br>5 d/wk<br>6 hr/d                    |           |                | 1250 (lymphoid organ histopathology)                                                 |                                         | Thurmond et al. 1986       |          |
| <b>Neurological</b>           |                     |                                                |           |                |                                                                                      |                                         |                            |          |
| 21                            | Rat                 | 13 wk<br>5 d/wk<br>6 hr/d                      |           | 8000           |                                                                                      |                                         | Crouch et al. 1979         |          |

Table 3-1 Levels of Significant Exposure to 1,3-butadiene - Inhalation

(continued)

| Key to<br>Figure <sup>a</sup> | Species<br>(Strain) | Exposure/<br>Duration/<br>Frequency<br>(Route) | System | NOAEL<br>(ppm) | LOAEL                                  |                                                                                                                                                                                                                                                                  | Reference<br>Chemical Form | Comments |
|-------------------------------|---------------------|------------------------------------------------|--------|----------------|----------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------|----------|
|                               |                     |                                                |        |                | Less Serious<br>(ppm)                  | Serious<br>(ppm)                                                                                                                                                                                                                                                 |                            |          |
| 22                            | Mouse               | 14 wk<br>5 d/wk<br>6 hr/d                      |        | 8000           |                                        |                                                                                                                                                                                                                                                                  | NTP 1984                   |          |
| <b>Reproductive</b>           |                     |                                                |        |                |                                        |                                                                                                                                                                                                                                                                  |                            |          |
| 23                            | Mouse<br>(CD)       | 10 wk<br>6 h/d<br>5 d/wk                       |        |                |                                        | 12.5 M (increase in late fetal deaths, exencephalies, and skull abnormalities)                                                                                                                                                                                   | Anderson et al. 1996       |          |
| 24                            | Mouse<br>(CD)       | 4 wk<br>6 h/d<br>5 d/wk                        |        | 12.5 M         | 65 M (increases in early fetal deaths) |                                                                                                                                                                                                                                                                  | Anderson et al. 1998       |          |
| 25                            | Mouse<br>(B6C3F1)   | 40 wk<br>6 hr/d<br>5 d/wk                      |        | 62.5 F         |                                        | 200 F (ovarian atrophy)                                                                                                                                                                                                                                          | NTP 1993                   |          |
| <b>Cancer</b>                 |                     |                                                |        |                |                                        |                                                                                                                                                                                                                                                                  |                            |          |
| 26                            | Mouse<br>(B6C3F1)   | 13-52 wk<br>6 hr/d<br>5 d/wk                   |        |                |                                        | 200 M (CEL:lymphocytic lymphoma, histiocytic sarcoma, cardiac hemangiosarcoma, alveolar/bronchiolar adenoma/carcinoma, forestomach squamous cell papilloma/carcinoma, hepatocellular adenoma, Harderian gland adenoma/adenocarcinoma, preputial gland carcinoma) | NTP 1993                   |          |

Table 3-1 Levels of Significant Exposure to 1,3-butadiene - Inhalation

(continued)

| Key to<br>Figure <sup>a</sup> | Species<br>(Strain) | Exposure/<br>Duration/<br>Frequency<br>(Route) | System  | NOAEL<br>(ppm) | LOAEL                                        |                            | Reference<br>Chemical Form                  | Comments |
|-------------------------------|---------------------|------------------------------------------------|---------|----------------|----------------------------------------------|----------------------------|---------------------------------------------|----------|
|                               |                     |                                                |         |                | Less Serious<br>(ppm)                        | Serious<br>(ppm)           |                                             |          |
| CHRONIC EXPOSURE              |                     |                                                |         |                |                                              |                            |                                             |          |
| Death                         |                     |                                                |         |                |                                              |                            |                                             |          |
| 27                            | Rat                 | 105-111 wk<br>5 d/wk<br>6 hr/d                 |         | 1000           |                                              | 8000 (increased mortality) | Owen et al. 1987, Owen and<br>Glaister 1990 |          |
| 28                            | Mouse               | 61 wk<br>5 d/wk<br>6 hr/d                      |         |                |                                              | 625 (increased mortality)  | NTP 1984                                    |          |
| 29                            | Mouse<br>(B6C3F1)   | 2 yr<br>6 hr/d<br>5 d/wk                       |         |                |                                              | 20 (increased mortality)   | NTP 1993                                    |          |
| Systemic                      |                     |                                                |         |                |                                              |                            |                                             |          |
| 30                            | Rat                 | 105-111 wk<br>5 d/wk<br>6 hr/d                 | Resp    | 1000           | 8000 (increased organ weight,<br>metaplasia) |                            | Owen et al. 1987, Owen and<br>Glaister 1990 |          |
|                               |                     |                                                | Cardio  | 8000           |                                              |                            |                                             |          |
|                               |                     |                                                | Gastro  | 8000           |                                              |                            |                                             |          |
|                               |                     |                                                | Hemato  | 8000           |                                              |                            |                                             |          |
|                               |                     |                                                | Hepatic | 8000           |                                              |                            |                                             |          |
|                               |                     |                                                | Renal   | 1000           |                                              | 8000 (nephrosis)           |                                             |          |
|                               |                     |                                                | Dermal  | 8000           |                                              |                            |                                             |          |
|                               |                     |                                                | Ocular  | 8000           |                                              |                            |                                             |          |

Table 3-1 Levels of Significant Exposure to 1,3-butadiene - Inhalation

(continued)

| Key to<br>Figure <sup>a</sup> | Species<br>(Strain) | Exposure/<br>Duration/<br>Frequency<br>(Route) | System    | NOAEL<br>(ppm) | LOAEL                 |                                         | Reference<br>Chemical Form | Comments                                   |
|-------------------------------|---------------------|------------------------------------------------|-----------|----------------|-----------------------|-----------------------------------------|----------------------------|--------------------------------------------|
|                               |                     |                                                |           |                | Less Serious<br>(ppm) | Serious<br>(ppm)                        |                            |                                            |
| 31                            | Mouse               | 61 wk<br>5 d/wk<br>6 hr/d                      | Resp      | 625            |                       | 1250                                    | NTP 1984                   | (atrophy of nasal<br>olfactory epithelium) |
|                               |                     |                                                | Cardio    |                | 625                   | (endothelial hyperplasia)               |                            |                                            |
|                               |                     |                                                | Gastro    |                | 625                   | (epithelial hyperplasia)                |                            |                                            |
|                               |                     |                                                | Musc/skel | 1250           |                       |                                         |                            |                                            |
|                               |                     |                                                | Hepatic   |                | 625                   | (hepatic necrosis)                      |                            |                                            |
|                               |                     |                                                | Renal     | 1250           |                       |                                         |                            |                                            |
|                               |                     |                                                | Dermal    | 1250           |                       |                                         |                            |                                            |
| 32                            | Mouse<br>(B6C3F1)   | 2 yr<br>6 hr/d<br>5 d/wk                       | Resp      |                | 6.25 M                | (alveolar epithelial<br>hyperplasia)    | NTP 1993                   |                                            |
|                               |                     |                                                | Cardio    | 200            | 625                   | (endothelial hyperplasia)               |                            |                                            |
|                               |                     |                                                | Gastro    |                | 625 F                 | (forestomach epithelial<br>hyperplasia) |                            |                                            |
|                               |                     |                                                |           |                | 200 F                 | (forestomach epithelial<br>hyperplasia) |                            |                                            |
|                               |                     |                                                | Musc/skel | 625            |                       |                                         |                            |                                            |
|                               |                     |                                                | Hepatic   |                | 62.5                  | (liver necrosis)                        |                            |                                            |
|                               |                     |                                                | Bd Wt     | 625            |                       |                                         |                            |                                            |

Table 3-1 Levels of Significant Exposure to 1,3-butadiene - Inhalation

(continued)

| Key to<br>Figure <sup>a</sup> | Species<br>(Strain) | Exposure/<br>Duration/<br>Frequency<br>(Route) | System | NOAEL<br>(ppm) | LOAEL                 |                                          | Reference<br>Chemical Form                  | Comments                        |
|-------------------------------|---------------------|------------------------------------------------|--------|----------------|-----------------------|------------------------------------------|---------------------------------------------|---------------------------------|
|                               |                     |                                                |        |                | Less Serious<br>(ppm) | Serious<br>(ppm)                         |                                             |                                 |
| 33                            | Mouse<br>(B6C3F1)   | 65 wk<br>6 hr/d<br>5 d/wk                      | Hemato | 200            |                       | 625 (macrocytic<br>megaloblastic anemia) | NTP 1993                                    |                                 |
|                               |                     |                                                | Bd Wt  | 625            |                       |                                          |                                             |                                 |
| <b>Neurological</b>           |                     |                                                |        |                |                       |                                          |                                             |                                 |
| 34                            | Rat                 | 105-111 wk<br>5 d/wk<br>6 hr/d                 |        | 8000           |                       |                                          | Owen et al. 1987, Owen and<br>Glaister 1990 |                                 |
| 35                            | Mouse               | 61 wk<br>5 d/wk<br>6 hr/d                      |        | 1250           |                       |                                          | NTP 1984                                    |                                 |
| <b>Reproductive</b>           |                     |                                                |        |                |                       |                                          |                                             |                                 |
| 36                            | Rat                 | 105-111 wk<br>5 d/wk<br>6 hr/d                 |        | 8000           |                       |                                          | Owen et al. 1987, Owen and<br>Glaister 1990 | Only examined<br>histopathology |
| 37                            | Mouse               | 61 wk<br>5 d/wk<br>6 hr/d                      |        |                |                       | 625 (gonadal atrophy)                    | NTP 1984                                    |                                 |
| 38                            | Mouse<br>(B6C3F1)   | 2 yr<br>6 hr/d<br>5 d/wk                       |        |                |                       | 6.25 F (ovarian atrophy)                 | NTP 1993                                    |                                 |
| 39                            | Mouse<br>(B6C3F1)   | 65 wk<br>6 hr/d<br>5 d/wk                      |        | 20 F           |                       | 62.5 F (ovarian atrophy)                 | NTP 1993                                    |                                 |

Table 3-1 Levels of Significant Exposure to 1,3-butadiene - Inhalation

(continued)

| Key to<br>Figure <sup>a</sup> | Species<br>(Strain) | Exposure/<br>Duration/<br>Frequency<br>(Route) | System | NOAEL<br>(ppm) | LOAEL                 |                  | Reference<br>Chemical Form                                                                                                                                                                    | Comments                                 |
|-------------------------------|---------------------|------------------------------------------------|--------|----------------|-----------------------|------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------|
|                               |                     |                                                |        |                | Less Serious<br>(ppm) | Serious<br>(ppm) |                                                                                                                                                                                               |                                          |
| Cancer                        |                     |                                                |        |                |                       |                  |                                                                                                                                                                                               |                                          |
| 40                            | Rat                 | 105-111 wk<br>5 d/wk<br>6 hr/d                 |        |                |                       | 1000             | (CEL: mammary gland adenoma and sarcoma)                                                                                                                                                      | Owen et al. 1987, Owen and Glaister 1990 |
| 41                            | Mouse               | 61 wk<br>5 d/wk<br>6 hr/d                      |        |                |                       | 625              | (CEL:alveolar/bronchiolar adenoma or carcinoma, malignant lymphoma, hemangiosarcoma, forestomach squamous cell papilloma or carcinoma, mammary gland carcinoma, ovarian granulosa cell tumor) | NTP 1984                                 |
| 42                            | Mouse<br>(B6C3F1)   | 2 yr<br>6 hr/d<br>5 d/wk                       |        |                |                       | 6.25 F           | (CEL: alveolar/bronchiolar adenoma/carcinoma)                                                                                                                                                 | NTP 1993                                 |

<sup>a</sup> The number corresponds to entries in Figure 3-1.

Bd Wt = body weight; Cardio = cardiovascular; CEL = cancer effect level; d = day(s); F = Female; Gastro = gastrointestinal; Gd = gestational day; Hemato = hematological; hr = hour(s); Immuno/Lymphoret = immunological; LC50 = lethal concentration, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; min = minute(s); Musc/skel = musculoskeletal; NOAEL = no-observed-adverse-effect level; Resp = respiratory; wk = week(s); yr = year(s)

Figure 3-1 Levels of Significant Exposure to 1,3-Butadiene - Inhalation  
Acute ( $\leq 14$  days)

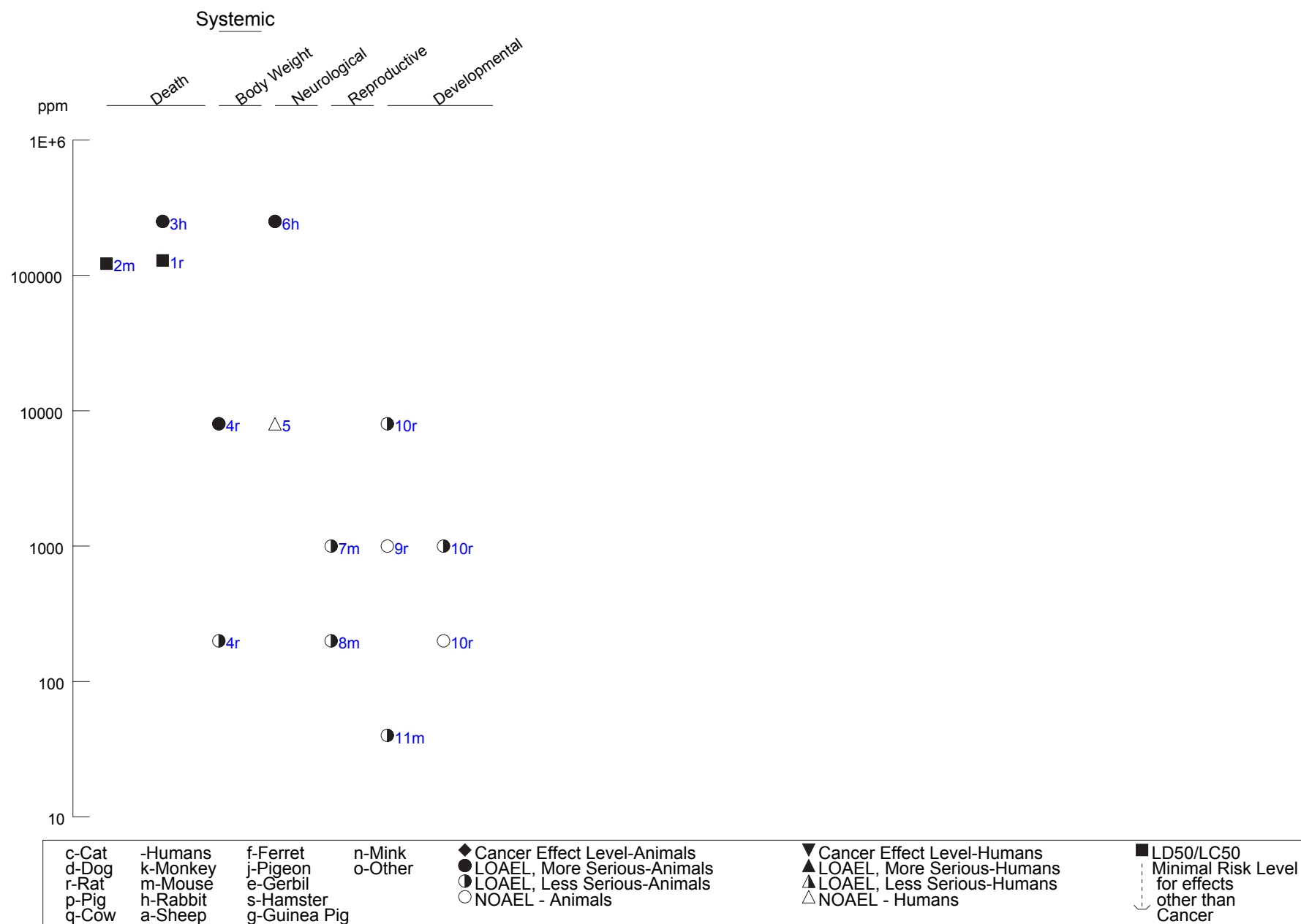




Figure 3-1 Levels of Significant Exposure to 1,3-Butadiene - Inhalation (Continued)

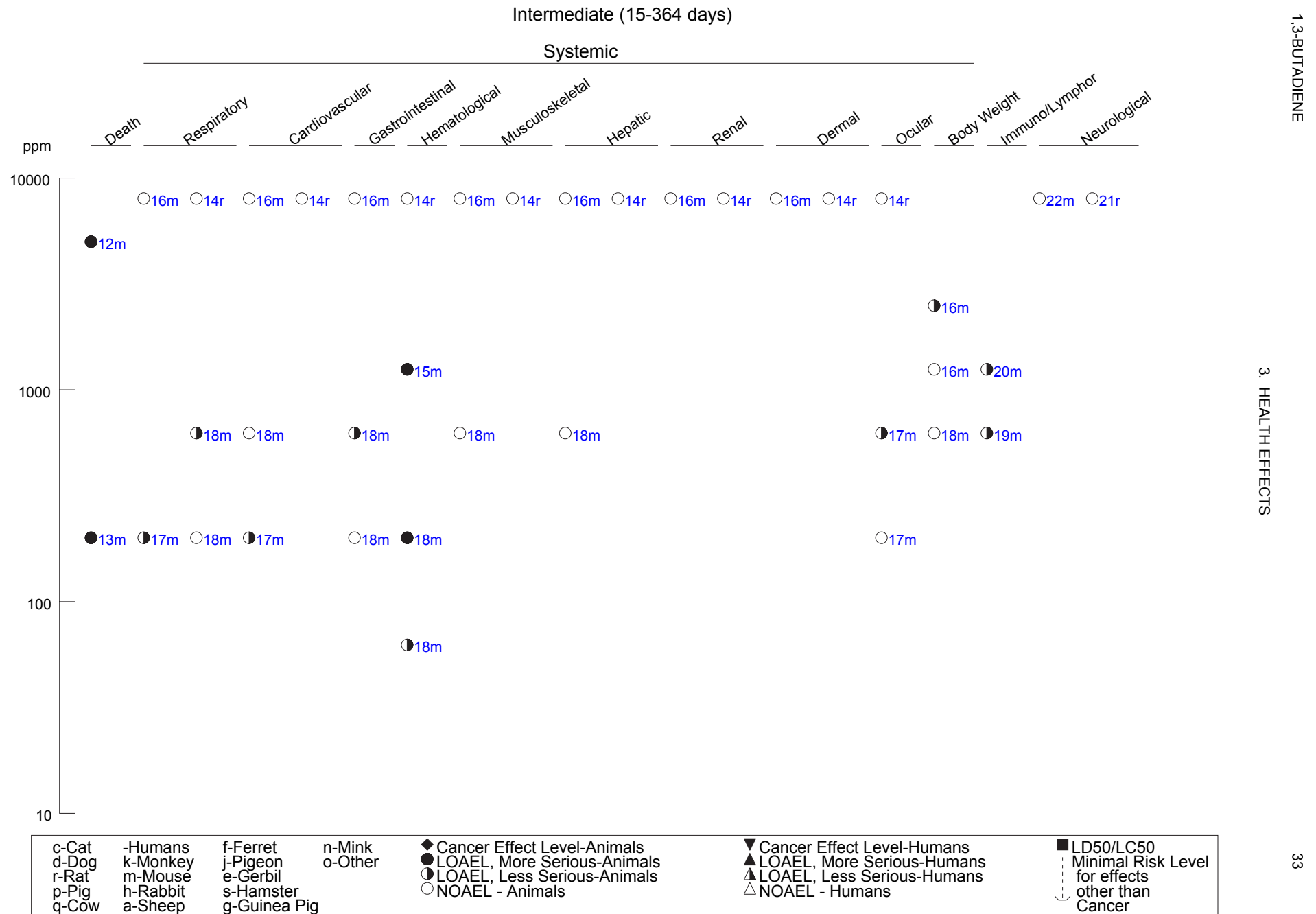
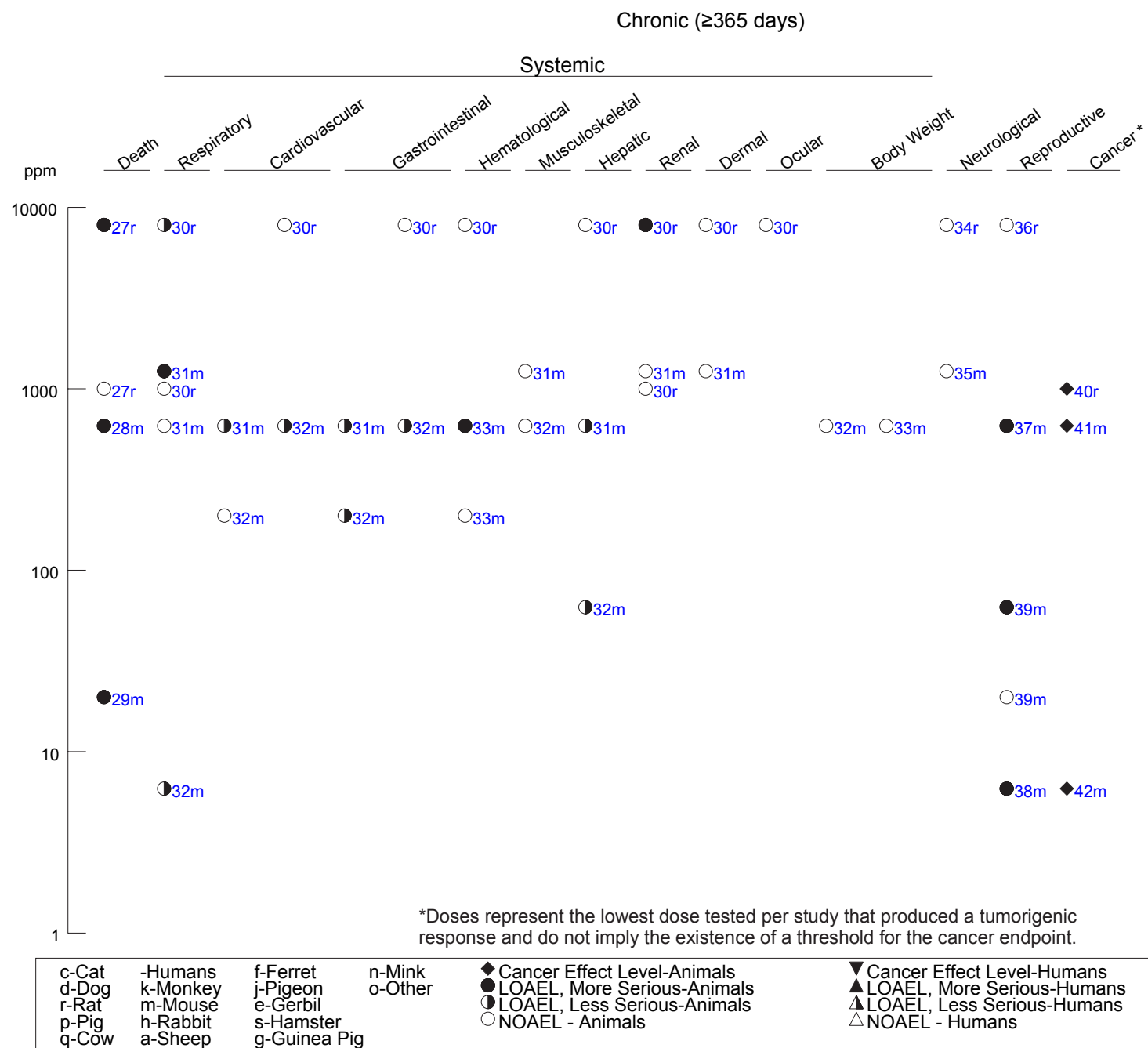


Figure 3-1 Levels of Significant Exposure to 1,3-Butadiene - Inhalation (*Continued*)

Intermediate (15-364 days)



Figure 3-1 Levels of Significant Exposure to 1,3-Butadiene - Inhalation (Continued)



## 3. HEALTH EFFECTS

**Cardiovascular Effects.** In a retrospective epidemiological study of middle-aged workers in the rubber industry, excessive mortality was noted for certain types of cardiovascular diseases, mainly chronic rheumatic and arteriosclerotic heart diseases (McMichael et al. 1974). Furthermore, increased mortality for arteriosclerotic heart disease was reported among black males in the rubber industry (Matanoski and Schwartz 1987). This result was also reported in an update of the original study (Matanoski et al. 1988, 1990). However, increased mortality from cardiovascular disease was not observed in three other cohorts of SBR and 1,3-butadiene monomer workers (Cowles et al. 1994; Divine and Hartman 1996, 2001; Ward et al. 1995). Thus, it is unclear if cardiovascular disease is likely to be caused by 1,3-butadiene exposure.

No cardiovascular lesions were found in mice or rats exposed to 8,000 ppm 1,3-butadiene 6 hours/day, 5 days/week, for 13–14 weeks (Crouch et al. 1979; NTP 1984). Endothelial hyperplasia in the heart (an early preneoplastic lesion) was observed in mice after exposure to 200 ppm for 6 hours/day, 5 days/week for 40 weeks (NTP 1993) or 625 ppm 6 hours/day, 5 days/week for 61 weeks or 2 years (NTP 1984, 1993). No exposure-related histopathological cardiac lesions were found in rats exposed chronically to up to 8,000 ppm for 2 years (Owen et al. 1987).

**Gastrointestinal Effects.** No studies were located regarding noncancer gastrointestinal effects in humans after inhalation exposure to 1,3-butadiene.

No significant incidences of gastrointestinal tract lesions were observed in mice following exposure to 8,000 ppm 1,3-butadiene for 6 hours/day, 5 days/week, for 14 weeks (NTP 1984) or 200 ppm for 6 hours/day, 5 days/week for 40 weeks (NTP 1993). In a chronic-duration study, high incidences of forestomach epithelial hyperplasia (a possible preneoplastic lesion) were observed in mice exposed to 625 ppm for 6 hours/day, 5 days/week for 61 weeks (Melnick et al. 1990a; NTP 1984) and for 2 years (NTP 1993). No exposure-related nonneoplastic gastrointestinal lesions were found in rats exposed chronically to up to 8,000 ppm for 6 hours/day, 5 days/week for 2 years (Owen et al. 1987).

**Hematological Effects.** A hematological survey of workers at a styrene-butadiene rubber plant revealed little indication of bone marrow toxicity among the workers (Checkoway and Williams 1982). Styrene and 1,3-butadiene were the most significant chemicals in the atmosphere; benzene and toluene were present in much lower concentrations. A group of eight tank farm workers (workers who load and unload chemicals from storage tanks; mean level exposure of 20 ppm) demonstrated slightly lower levels

## 3. HEALTH EFFECTS

of red blood cells, hemoglobin, platelets, and neutrophils compared with other workers, but these findings were within the normal range. Tsai et al. (2005) examined a number of hematological end points in a petrochemical production facility in which the current time-weighted average (TWA) 1,3-butadiene exposure level was 0.25 ppm; prior to 1997, the TWA concentration was 4.55 ppm. As compared to unexposed controls, no significant alterations in total or differential leukocyte levels, erythrocyte levels, hemoglobin levels, mean corpuscular volume, or platelet levels were found. An older study of workers from one of the facilities examined by Tsai et al. (2005) also found no significant alterations in total and differential leukocyte levels, erythrocyte levels, hemoglobin levels, platelet levels, or mean corpuscular volume (Cowles et al. 1994); the mean 1,3-butadiene concentration was 3.5 ppm.

No signs of blood dyscrasias were found among 164 animals (rats, rabbits, guinea pigs, dogs) exposed to concentrations up to 6,700 ppm of 1,3-butadiene for 8 months (Carpenter et al. 1944). The results were supported by a 3-month study in which no effects on hematological indices were found in rats after exposure to 8,000 ppm of 1,3-butadiene (Crouch et al. 1979).

A treatment-related macrocytic megaloblastic anemia was observed in B6C3F1 and NIH mice exposed to 1,250 ppm 1,3-butadiene for 6 hours/day, 6 days/week for 6–24 weeks, but not after a 3-week exposure to the same concentration (Irons et al. 1986a, 1986b). The bone marrow damage was expressed as reduced numbers of red blood cells, decreased hemoglobin concentration and hematocrit, and increased mean corpuscular volume of circulating erythrocytes. The changes were observed in both strains, independently of the occurrence of murine leukemia viruses in the animals. Male mice exposed to  $\geq 62.5$  ppm for 6 hours/day, 5 days/week for 40 weeks exhibited decreased red blood cell counts, hemoglobin concentration, and hematocrit (NTP 1993). Leukopenia and lymphopenia occurred at  $\geq 200$  ppm. Females exhibited macrocytic megaloblastic anemia and bone marrow atrophy after exposure to 200 and 625 ppm, respectively, 6 hours/day, 5 days/week for 40 weeks (NTP 1993).

Macrocytic megaloblastic anemia and bone marrow hyperplasia was also observed in mice exposed chronically to 625 ppm 1,3-butadiene 6 hours/day, 5 days/week for 65 weeks (NTP 1993). Surviving females, but not males, in this study exhibited increased bone marrow cellularity.

In contrast to the findings in mice, no effects on hematology or blood chemistry of Sprague-Dawley rats were observed after exposure to 1,000 and 8,000 ppm of 1,3-butadiene for 105–111 weeks (Owen et al. 1987).

## 3. HEALTH EFFECTS

**Musculoskeletal Effects.** No studies were located regarding musculoskeletal effects of 1,3-butadiene in humans after inhalation exposure.

No musculoskeletal effects were observed in mice and rats exposed to 8,000 ppm 1,3-butadiene 6 hours/day, 5 days/week for 13–14 weeks (Crouch et al. 1979; NTP 1984) or in mice exposed to 625 ppm 6 hours/day, 5 days/week from 40 or 65 weeks or 2 years (NTP 1993).

**Hepatic Effects.** No studies were located regarding hepatic effects of 1,3-butadiene in humans after inhalation exposure.

No histopathological changes in livers of rats (Crouch et al. 1979) or mice (NTP 1984) were found after intermediate-duration exposure to 1,3-butadiene. The relative liver weights of both sexes of Sprague-Dawley rats were elevated after the chronic exposure to 1,3-butadiene (1,000 and 8,000 ppm); however, this finding was not associated with any pathological changes (Owen et al. 1987). Mice exposed to  $\geq 625$  ppm 6 hours/day, 5 days/week for 61 weeks had a significant increase in liver necrosis (NTP 1984).

**Renal Effects.** No studies were located regarding renal effects in humans after inhalation exposure to 1,3-butadiene.

The results of urinalysis in 164 animals, including rats, guinea pigs, rabbits, and dogs were all normal after an 8-month exposure to concentrations up to 6,700 ppm of 1,3-butadiene (Carpenter et al. 1944), but the methods were poorly described. These results were supported, however, in rats after 13 weeks of exposure to concentrations up to 8,000 ppm of 1,3-butadiene (Crouch et al. 1979). Nephrosis was found among male rats after 111 weeks of exposure to 8,000 ppm, but not 1,000 ppm, of 1,3-butadiene (Owen et al. 1987). No non-neoplastic renal lesions were observed in mice exposed to 8,000 ppm 6 hours/day, 5 days/week for 14 weeks (NTP 1984) or 625 ppm 6 hrs/day, 5 days/week, for 40 weeks to 2 years.

**Dermal Effects.** No studies were located regarding dermal effects in humans after inhalation exposure to 1,3-butadiene.

No histopathological dermal changes were found in rats or mice after 13–14 weeks exposure to 8,000 ppm 1,3-butadiene (Crouch et al. 1979; NTP 1984), in rats after 111 weeks exposure to 8,000 ppm (Owen et al. 1987), or in mice after 40 weeks to 2 years exposure to 625 ppm (NTP 1993).

## 3. HEALTH EFFECTS

**Ocular Effects.** Two men reported slight irritation of the eyes and difficulty in focusing on instrument scales during 6–7 hours exposure to 2,000 and 4,000 ppm 1,3-butadiene (Carpenter et al. 1944).

Ophthalmologic examination of the eyes of dogs and rabbits disclosed no signs of injury during the course of exposure to up to 6,700 ppm 1,3-butadiene for 8 months (Carpenter et al. 1944). After the termination of the experiment, histological examination revealed that the sclera, cornea, and ciliary body were normal. Sections of the optic nerve with adjacent retina showed no myelin sheath degeneration. Although the ophthalmological examination was described in detail, the study was limited by the small number of animals used.

No histopathological ocular changes were found in rats or mice after 13–14 weeks exposure to 8,000 ppm (Crouch et al. 1979; NTP 1984) or in rats after 111 weeks exposure to 8,000 ppm 1,3-butadiene (Owen et al. 1987). Increased Harderian gland hyperplasia (a precancerous lesion) was observed in male mice exposed to  $\geq 62.5$  ppm 6 hours/day, 5 days/week for 65 weeks and 2 years (NTP 1993); this effect is not relevant to humans because they do not have Harderian glands.

**Body Weight Effects.** Body weights were reduced by 13% in male mice exposed to 2,500 ppm 1,3-butadiene 6 hours/day, 5 days/week for 14 weeks, but were not significantly different from controls when exposed to 1,250 ppm 6 hours/day, 5 days/week for 61 weeks or when exposed to 625 ppm 6 hours/day, 5 days/week for 2 years (NTP 1993)

### 3.2.1.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological effects of 1,3-butadiene in humans after inhalation exposure.

After 3–21 weeks of exposure to 1,250 ppm 1,3-butadiene, an increased expression of murine leukemia virus (MuLV) was observed in hematopoietic tissues of B6C3F1 mice, but not in NIH mice (Irons et al. 1987a). Furthermore, altered regulation of the stem cell development in B6C3F1 mice was reported after a similar exposure (Leiderman et al. 1986).

Intermediate-duration exposure of female mice to 62.5 ppm 1,3-butadiene 6 hours/day, 5 days/week for 40 weeks resulted in a 17% reduction in relative spleen weight, while exposure to 625 ppm for the same

### 3. HEALTH EFFECTS

duration resulted in a 19% reduction in thymus weight (NTP 1993). By 65 weeks, relative spleen weights in 625 ppm females had increased to 57% higher than controls.

Immunological changes were detected after evaluation of specific humoral and cell-mediated immunity in B6C3F1 mice exposed to 1,250 ppm 1,3-butadiene for 6, 12, or 24 weeks (Thurmond et al. 1986). Suppression of cytotoxic T-lymphocyte generation to mastocytoma cells was observed after 6 weeks, but recovered after 12 weeks of exposure. The histological examination of lymphoid organs showed depressed spleen cellularity after 24 weeks of exposure; this value is recorded as a LOAEL for immunological effects in [Table 3-1](#) and plotted in [Figure 3-1](#), although it is not known how these changes affect immunocompetency.

#### 3.2.1.4 Neurological Effects

Psychomotor responses of two men inhaling 2,000, 4,000, or 8,000 ppm 1,3-butadiene for 6–8 hours/day on different days were evaluated by Carpenter et al. (1944). At the two higher concentrations, the subjects performed a steadiness test; at the highest concentration, a tapping rate test was also performed. Results after 1,3-butadiene exposure were identical to those obtained before exposure.

Rabbits exposed to 250,000 ppm of 1,3-butadiene went through all stages of anesthesia to death in the average time of 23 minutes (Carpenter et al. 1944). Less than 2 minutes of exposure was required for loss of motor and labyrinth reflexes.

No effects on erythrocyte or brain cholinesterase or on neuromuscular function tests were found in rats exposed to up to 8,000 ppm for 13 weeks (Crouch et al. 1979). In intermediate and chronic exposure studies in mice and rats, no treatment-related histopathological lesions were found in organs and tissues of the nervous system (brain, spinal cord, sciatic nerves) (Crouch et al. 1979; NTP 1984; Owen and Glaister 1990; Owen et al. 1987). Tests results for neurological function (i.e., loss of balance on a rotating cone) were possibly confounded by the mammary tumors interfering with the mobility of rats (NTP, 1984). The highest NOAEL values and all reliable LOAEL values for neurological effects in each species and duration category are recorded in [Table 3-1](#) and plotted in [Figure 3-1](#).

#### 3.2.1.5 Reproductive Effects

No studies were located regarding reproductive effects in humans after inhalation exposure to 1,3-butadiene. A concentration-related increase in the incidence of sperm-head abnormalities occurred in



## 3. HEALTH EFFECTS

B6C3F1 mice after exposure to 1,000 (73% increase) and 5,000 ppm (129% increase) of 1,3-butadiene 6 hours/day for 5 days (DOE/NTP 1988a). No impairment of fertility was noted when groups of male and female rats, rabbits, or guinea pigs were exposed to  $\leq 6,700$  ppm 1,3-butadiene (Carpenter et al. 1944).

No treatment-related histopathological effects were seen in reproductive organs of rats or mice exposed to 8,000 ppm 1,3-butadiene 6 hours/day, 5 days/week for 13–14 weeks (Crouch et al. 1979; NTP 1984). Reduction in the number of round and elongated sperm heads was seen in mice exposed to 130 ppm 6 hours/day, 5 days/week for 4 weeks, but this was not associated with changes in fertility (Anderson et al. 1998). Ovarian and uterine atrophy occurred in female mice exposed to 200 ppm 6 hours/day, 5 days/week for 40 weeks (NTP 1993), while testicular atrophy was seen in male mice exposed to 625 ppm for the same duration. Exposure of female mice to 62.5 or  $\geq 6.25$  ppm 1,3-butadiene 6 hours/day, 5 days/week for 65 weeks or 2 years, respectively, resulted in an increased incidence of ovarian atrophy (NTP 1993). Affected females had no evidence of oocytes, follicles, or corpora lutea. An increase in testicular atrophy and preputial gland hyperplasia was observed in males only after exposure to 625 ppm 6 hours/day, 5 days/week for 2 years (NTP 1993). In contrast, no histological alterations were observed in the gonads of rats exposed to up to 8,000 ppm 6 hours/day, 5 days/week for 2 years (Owen et al. 1987).

In untreated female mice mated with males exposed to  $\geq 200$  ppm 6 hours/day for 5 days, a significant increase in the number of females with two or more intrauterine deaths were observed (DOE/NTP 1988b). This effect was only observed in animals mated during the first week post-exposure. Additional effects included increases in early implantation loss at 1,000 ppm during the first post-week of exposure and at 200 and 1,000 ppm during the second week post-exposure; implantation losses were not significantly increased in the mice exposed to 5,000 ppm. Early fetal death was also observed in untreated female mice mated to males exposed to 65 ppm for 6 hours/day, 5 days/week for 4 weeks (DOE/NTP 1988a). Fetal toxicity was observed following the mating of untreated female mice with males exposed to 12.5 ppm 1,3-butadiene 6 hours/day, 5 days/week for 10 weeks (Anderson et al. 1996). Observed effects included an increase in late fetal death, exencephaly, and skull abnormalities.

## 3. HEALTH EFFECTS

**3.2.1.6 Developmental Effects**

No studies were located regarding developmental effects in humans after inhalation exposure to 1,3-butadiene.

When exposed to concentrations up to 8,000 ppm 1,3-butadiene for 6 hours/day, 5 days/week during GDs 6–15, Sprague-Dawley rats showed signs of dose-related maternal and fetal toxicity (Irvine 1981). Depressed body weight gain among dams was observed at  $\geq 200$  ppm, and fetal growth (body weight and crown-rump length) was significantly decreased in the 8,000 ppm group. A significant increase in the number of litters with fetuses showing minor skeletal defects was observed at 200 ppm, but not at 1,000 or 8,000 ppm; however, an increase in the number of fetuses with irregular ossification was observed at 8,000 ppm. Significant increases in the number of litters with fetuses showing major skeletal defects were observed at 1,000 and 8,000 ppm. The majority of the major skeletal defects were wavy ribs; abnormalities of the skull, spine, sternum, long bones, and ribs were also observed at 8,000 ppm. A significant increase in the number of litters with fetuses showing minor external/visceral defects was observed at 1,000 ppm, but not at 8,000 ppm. In a study in which female Sprague-Dawley rats were exposed to 40–1,000 ppm during GDs 6–15 (DOE/NTP 1987a), some skeletal abnormalities and ossification reductions were found in the fetuses, but were not statistically significant and were not considered to be treatment-related. In mice, a 5–23% decrease in fetal body weight gain, primarily among male mice, was observed after exposure of dams during GDs 6–15 to 40–1,000 ppm 1,3-butadiene. The magnitude of the decreased fetal body weight in males was 5, 18, and 23% in the 40, 200, and 1,000 ppm groups, respectively. The investigators reported that the decreased fetal body weight was statistically significant in males at all dose levels; however, the statistical method used (ANOVA) did not account for differences in litter size. Increased incidences of extra ribs and reduced ossification of sternebrae were found in fetuses from groups exposed to 200 ppm and 1,000 ppm, respectively (DOE/NTP 1987b).

The highest NOAEL value and all reliable LOAEL values for developmental effects in rats for the acute duration category are recorded in [Table 3-1](#) and plotted in [Figure 3-1](#).

**3.2.1.7 Cancer**

Retrospective epidemiological studies of mortality among workers in the rubber industry were conducted in SBR (polymer) production workers and 1,3-butadiene monomer workers. For SBR workers, the primary cohort is comprised of largely overlapping cohorts from multiple SBR facilities in the United

## 3. HEALTH EFFECTS

States and Canada examined by investigators at John Hopkins (Matanoski and Schwartz 1987; Matanoski et al. 1989b, 1990) and the University of Alabama at Birmingham (Cheng et al. 2007; Delzell et al. 1996; Graff et al. 2005; Macaluso et al. 1996; Sathiakumar et al. 2005, 2007). Mortality among three independent cohorts of male 1,3-butadiene monomer production workers has been studied and updated on several occasions, including cohorts from Union Carbide (Ward et al. 1995), Texaco (Divine 1990; Divine et al. 1993), and Shell (Cowles et al. 1994).

Matanoski et al. 1990 (in an update of the Matanoski and Schwartz 1987 cohort) found increased standardized mortality ratios (SMRs) of 532 (1 case), 656 (95% confidence interval [CI]=135–1,906), and 482 (95% CI=59–1,762) for lymphosarcoma, leukemia, and other lymphatic neoplasms, respectively, in black SBR production workers; white workers exhibited an SMR of 230 (95% CI=92–473) for other lymphatic neoplasms. In white maintenance workers, SMRs of 144 (95% CI=53–314) and 166 (95% CI=93–275) were observed for esophageal and stomach cancer, respectively. An odds ratio of 9.4 for leukemia was observed in SBR workers in a nested case-control study of the Matanoski and Schwartz (1987) cohort of SBR workers (Matanoski et al. 1989b). No such association was found for exposure to styrene. Several investigators at the University of Alabama at Birmingham examined cancer mortality in a cohort of over 15,000 North American SBR workers (Cheng et al. 2007; Delzell et al. 1996; Graff et al. 2005; HEI 2006; Macaluso et al. 1996; Sathiakumar et al. 2005, 2007). Delzell et al. (1996) found increased SMRs for leukemia of 265 (95% CI=141–453) for maintenance and 431 (95% CI=207–793) for laboratory workers. Macaluso et al. (1996) derived a relative rate value of 4.5 for leukemia development (no confidence interval reported) associated with a cumulative exposure of 80 ppm-years. In an update of these studies (Sathiakumar et al. 2005), an increase in deaths from all types of leukemia (SMR of 258; 95% CI=156–403) was found among hourly employees with 20–29 years since hire and >10 years of employment. Increases in leukemia deaths were also found among workers in the polymerization (SMR of 204; 95% CI=121–322), maintenance labor (SMR of 326; 95% CI=178–456), and laboratory (SMR of 326; 95% CI=178–546) operations. However, no increases in a specific type of leukemia were found. Similarly, Graff et al. (2005) reported increased relative risks for leukemia of 2.9 (95% CI=1.4–6.4) and 3.7 (95% CI=1.7–8.0) among workers with cumulative 1,3-butadiene exposures of 184.7–<435.0 and 425.0 ppm-years. When the relative risks were adjusted for exposure to styrene and dimethyldithiocarbamate, age, and years since hire, only the highest cumulative exposure group had a confidence interval that included 1 (relative risk of 3.0, 95% CI=1.0–9.2). When workers were divided into two categories based on exposure to >100 and ≤100 ppm, associations between 1,3-butadiene cumulative exposure and leukemia were found for both groups, although the association was weaker at the lower concentration (relative risk of 2.0; 95% CI=0.6–6.0 for cumulative exposure to >124.7 ppm-years) than at

## 3. HEALTH EFFECTS

the higher concentration (relative risk of 3.7; 95% CI=1.3–11.1 for cumulative exposure of >247.6 ppm-years) and the trend was only statistically significant in the >100 ppm workers (HEI 2006). A more recent paper by Graff and associates (Graff et al. 2009) used uncertainty analysis to evaluate the impact of potential exposure estimate inaccuracies on the leukemia relative risks. The investigators concluded that analysis of the complete probability distribution of 1,3-butadiene exposure estimate supported the association between 1,3-butadiene cumulative exposure and increased leukemia risk. Using personal monitoring device data collected from 1977 to 1991, Sathiakumar et al. (2007) compared measured 1,3-butadiene levels with estimated levels. The mean measured 1,3-butadiene level (across all years and job categories) was 5.2 ppm and the mean estimated concentration was 4.7 ppm; the estimated 1,3-butadiene levels tended to underestimate concentrations that were greater than 7 ppm. Cheng et al. (2007) found a significant trend for the association of leukemia in SBR workers and increasing cumulative exposure or number of “peak” exposures (>100 ppm). They also reported a minimal association (relative rate of 1.03) of leukemia in SBR workers receiving an estimated 5 ppm 1,3-butadiene exposure for 20 years (100 ppm-years). Examination of possible associations between 1,3-butadiene exposure and increased deaths from other cancer types, including non-Hodgkin’s lymphoma, Hodgkin’s lymphoma, and multiple myeloma were not found in this cohort (HEI 2006). Increases in the risk of colorectal and prostate cancer were observed in some subgroups of SBR workers; however, no consistent exposure-response trends were found (HEI 2006).

These results in SBR workers with 1,3-butadiene exposure are supported by studies in 1,3-butadiene monomer production workers. Downs et al. (1987) calculated an SMR of 235 (no CI reported) for lymphosarcoma and reticular cell sarcoma. No increase in mortality from cancer of gastrointestinal, respiratory, urinary, and skeletal systems was associated with monomer exposure. Divine (1990) and Divine et al. (1993) reported similar results in a follow-up study of this cohort (SMR of 452 [95% CI=165–984]) for lymphosarcoma. Divine and Hartman (2001) followed this cohort for an additional 5 years and found a statistically significant increase in SMR (141; 95% CI=105–186) for all lymphohematopoietic cancer. Subcohort analyses showed that increases in lymphohematopoietic cancers appear to be restricted to monomer workers employed before 1950 and for the shortest duration (Divine and Hartman 1996, 2001). Elevations were also seen for leukemia (SMR of 129; 95% CI=77–204) and non-Hodgkin’s lymphoma (SMR of 148; 95% CI=89–231), but the elevation was not statistically significant. Additionally, higher SMRs for leukemia and non-Hodgkin’s lymphoma were found in workers with shorter employment durations. No significant associations were found between cumulative 1,3-butadiene exposure (defined as a combination of job exposure class, calendar time, and length of time in job) and the relative risk for lymphohematopoietic cancers, leukemia, or non-Hodgkin’s lymphoma

## 3. HEALTH EFFECTS

(Divine and Hartman 2001). Analysis of another cohort of 364 monomer workers, 277 of which worked in a U.S. Rubber Reserve plant during World War II, found an SMR of 577 (95% CI=157–1,480) for lymphosarcoma and reticulosarcoma (Ward et al. 1995). No significant association was found between 1,3-butadiene monomer exposure and all, lung, or lympho-hematopoietic cancers in a cohort of 614 monomer workers employed between 1948 and 1989 who were exposed to a relatively low mean concentration of 3.5 ppm, with many measurements below 1 ppm (Cowles et al. 1994).

The major limitations of the epidemiological studies described so far include the lack of precise historic exposure data to 1,3-butadiene, lack of adjustment for smoking, and possible exposure to other chemicals. Irons and Pyatt (1998) suggest that dithiocarbamates, such as dimethyldithiocarbamate (DMDTC), used in the SBR vulcanization process from 1950 to 1965, may have played a significant role in leukemia development that was concentrated in workers employed during this period. However, Santos-Burgoa et al. (1992) and Delzell et al. (2001) and HEI (2006) used multivariate analysis to suggest that the estimates of 1,3-butadiene exposure provided the best correlation with the rates of leukemia, even in the presence of styrene and DMDTC.

Several investigators have evaluated associations between childhood leukemias and ambient 1,3-butadiene emissions. Knox et al. (2005, 2006) associated the occurrence of childhood cancer with proximity of birthplace to industrial 1,3-butadiene and benzene emissions, and roads, railways, waterways, and bus, ferry, or train stations in Great Britain; however, no actual exposure data or estimates were available. Reynolds et al. (2003) calculated leukemia rate ratios (RR), adjusted for age, ethnicity, and sex, of 1.21 (95% CI=1.03–1.42) and 1.32 (95% CI=1.11–1.57) for children in California census tracts ranked highest for combined exposure to 25 hazardous air pollutants (HAPs, including 1,3-butadiene) and highest for point-source HAP exposures, respectively. Likewise, Whitworth et al. (2008) reported a significant association of childhood leukemia incidence with residence in census tracts close to the ship channel (which is in close proximity to petrochemical and chemical manufacturing facilities) in Houston, Texas. These studies do not indicate strong causality between 1,3-butadiene exposure and childhood leukemia, as many chemicals may have contributed to exposure and the actual exposure to 1,3-butadiene, if any, is unknown.

The rodent bioassay database corroborates the association of lympho-hematopoietic neoplasms and occupational exposure to 1,3-butadiene reported in the epidemiology literature. Mice were clearly more sensitive to 1,3-butadiene-mediated tumor development than rats. In rats, increased tumors were not observed following intermediate-duration exposures of up to 8,000 ppm 1,3-butadiene 6 hours/day,

## 3. HEALTH EFFECTS

5 days/week for 13 weeks (Crouch et al. 1979). Two-year exposure of rats to 1,000 or 8,000 ppm 6 hours/day, 5 days/week resulted in increased incidences of Leydig cell adenoma, pancreatic exocrine adenoma, uterine sarcoma, mammary gland adenoma and carcinoma, Zymbal gland carcinoma, and thyroid follicular cell tumors (Owen and Glaister 1990; Owen et al. 1987). In mice, exposure to 200 ppm for 40 weeks resulted in increased incidences of lymphocytic lymphoma, histiocytic sarcoma, cardiac hemangiosarcoma, alveolar/bronchiolar adenoma/carcinoma, forestomach squamous cell papilloma/carcinoma, hepatocellular adenoma, hardarian gland adenoma/adenocarcinoma, and preputial gland carcinoma (NTP 1993). These same tumors developed in mice in as little as 13 weeks after exposure to 625 ppm 6 hours/day, 5 days/week (NTP 1993). Chronic exposure of mice to lower concentrations of 1,3-butadiene also resulted in multi-target organ neoplasm development. Two-year 6-hour/day, 5-day/week exposures of 20 ppm for male mice and 6.25 ppm (the lowest exposure level tested) for female mice resulted in increased incidences of lymphocytic lymphoma, histiocytic sarcoma, cardiac hemangiosarcoma, alveolar/bronchiolar adenoma/carcinoma, forestomach squamous cell papilloma/carcinoma, hepatocellular adenoma, hardarian gland adenoma/adenocarcinoma, mammary gland carcinoma, adenocanthoma, malignant mixed tumor, and malignant ovarian granulosa cell tumor.

The cancer effect levels (CELs) are recorded in [Table 3-1](#) and plotted in [Figure 3-1](#).

Using the Poisson regression analysis by Health Canada (2000) of the leukemia risk data from a cohort of 15,000 SBR production workers (Delzell et al. 1996), EPA derived a unit risk for inhalation exposure of  $0.08 \text{ ppm}^{-1}$  (IRIS 2012). This unit risk corresponds to upper bound individual lifetime cancer risks at  $10^{-4}$ – $10^{-6}$  for exposure levels of  $1 \times 10^{-3}$ – $1 \times 10^{-6}$  ppm, which are plotted in [Figure 3-1](#). It should be noted that EPA derived the cancer risk levels in 2001, and thus, these values may not reflect the results of more recent studies of SBR workers.

The International Agency for Research on Cancer (IARC) has classified 1,3-butadiene as a Group 1 carcinogen (carcinogenic to humans) (IARC 2009). EPA has classified 1,3-butadiene as carcinogenic to humans (EPA 2002; IRIS 2012). The Department of Health and Human Services (NTP 2005) also identified 1,3-butadiene as a “known human carcinogen”.

### 3.2.2 Oral Exposure

No studies were located regarding health effects in humans or animals after oral exposure to 1,3-butadiene.

## 3. HEALTH EFFECTS

**3.2.3 Dermal Exposure**

Dermal contact with liquid 1,3-butadiene causes a sensation of cold followed by a sensation of burning, which is the result of rapid expansion of pressurized 1,3-butadiene from liquid to gas states (NIOSH, 2005). Although this may cause frostbite, it is specific to an unusual exposure scenario and is not a toxic endpoint. However, the possible toxic effects from dermal absorption of such a concentrated amount of 1,3-butadiene are unknown. High gas concentrations may cause mild skin irritation as well (NIOSH, 2005). No other studies were located regarding health effects in humans or animals following dermal exposure to 1,3-butadiene.

**3.3 GENOTOXICITY**

1,3-Butadiene has been tested for genotoxicity in a number of *in vitro* and *in vivo* studies (Tables 3-2 and 3-3). Positive results have been found in the reverse mutation assay in *Salmonella typhimurium* TA1530 and TA1535 in the absence or in the presence of metabolic activation system (de Meester et al. 1978; Madhusree et al. 2002). However, the interpretation of these results was confounded by the fact that the Petri dishes not containing S-9 mix were contaminated by volatile active metabolites. It was concluded that S-9 mix was necessary to activate 1,3-butadiene into mutagen(s) (De Meester 1988). TA1530 was the most sensitive strain, but 1,3-butadiene mutagenicity was detectable only with metabolic activation in the subsequent study (de Meester et al. 1980). No significant mutagenic effect on *S. typhimurium* strain TA100 with metabolic activation was observed (Victorin and Stahlberg 1988). A weak genotoxic activity was detected in strain TA1535 with rat S-9 (Arce et al. 1989). A weak increase in sister chromatid exchanges was observed in Chinese hamster ovary cells, but only with metabolic activation (Sasiadek et al. 1991). Increased mutations occurred in the hypoxanthine-guanine phosphoribosyl transferase (*hprt*) and *tk* gene loci of human TK6 lymphoblastoid cells (Cochrane and Skopek 1993). On the basis of these data, 1,3-butadiene appears to require metabolic activation to produce genotoxicity.

The genotoxicity of 1,3-butadiene has been examined in several occupational exposure cohorts. Ward et al. (1994) reported a significant increase in hypoxanthine-guanine phosphoribosyltransferase (*hprt*) mutant frequency (measured in peripheral lymphocytes) among eight workers at a Texas 1,3-butadiene production plant working in the area of the plant where the highest 1,3-butadiene exposure occurred, as compared to levels in five workers in an area with low 1,3-butadiene exposures or in six controls who did not work at the 1,3-butadiene production plant. The mean area and personal 1,3-butadiene levels were 3.5 ppm (although most individual samples were <1 ppm) in the high-exposure area and 0.03 ppm in the

## 3. HEALTH EFFECTS

**Table 3-2. Genotoxicity of 1,3-Butadiene *In Vitro***

| Species (test system)          | End point                                | Results         |                    | Reference                               |
|--------------------------------|------------------------------------------|-----------------|--------------------|-----------------------------------------|
|                                |                                          | With activation | Without activation |                                         |
| Prokaryotic organisms:         |                                          |                 |                    |                                         |
| <i>Salmonella typhimurium</i>  |                                          |                 |                    |                                         |
| TA1530                         | Gene mutation                            | +               | –                  | de Meester et al. 1980                  |
| TA100                          | Gene mutation                            | –               | –                  | Victorin and Stahlberg 1988             |
| TA1535                         | Gene mutation                            | +               | –                  | Arce et al. 1989; Madhusree et al. 2002 |
| Eukaryotic organisms:          |                                          |                 |                    |                                         |
| Chinese hamster ovary          | SCE                                      | +               | –                  | Sasiadek et al. 1991                    |
| Human TK6 lymphoblastoid cells | <i>hprt</i> and <i>tk</i> loci mutations | NA              | NA                 | Cochrane and Skopek 1993                |

– = negative result; + = positive result; NA = not applicable; SCE = sister chromatid exchange



## 3. HEALTH EFFECTS

**Table 3-3. Genotoxicity of 1,3 Butadiene *In Vivo***

| Species (test system)                                                            | End point                                                               | Results | Reference                                                    |
|----------------------------------------------------------------------------------|-------------------------------------------------------------------------|---------|--------------------------------------------------------------|
| B6C3F1 mice (inhalation)                                                         | Bone marrow: Dose-dependent increase in SCEs                            | +       | Cunningham et al. 1986                                       |
| Sprague-Dawley rats (inhalation)                                                 |                                                                         | -       |                                                              |
| B6C3F1 mice (inhalation)                                                         | Bone marrow: increase in CAs, SCEs, and AGT, and depression of MI       | +       | Tice et al. 1987                                             |
| Swiss mice (inhalation)                                                          | Peripheral blood erythrocytes: induction of micronuclei                 | +       | Irons et al. 1986b                                           |
| B6C3F1 mice (inhalation)                                                         | Bone marrow: alteration of hematopoietic stem cell development          | +       | Leiderman et al. 1986                                        |
| B6C3F1 mice (inhalation)                                                         | Peripheral blood erythrocytes: induction of micronuclei                 | +       | Jauhar et al. 1988                                           |
| B6C3F1 mice (inhalation)                                                         | Induction of MN; induction of SCEs; CAs                                 | +       | Tice et al. 1988                                             |
| B6C3F1 mice (inhalation)                                                         | Sperm abnormalities; dominant lethality                                 | +       | DOE/NTP 1988a                                                |
| C57B1/6 mice (intraperitoneal injection)                                         | Bone marrow increase in CAs and SCEs                                    | +       | Sharief et al. 1986                                          |
| B6C3F1 mice (inhalation)                                                         | <i>lacZ</i> <sup>-</sup> mutant frequency in lung                       | +       | Recio et al. 1992                                            |
| B6C3F1 mice (inhalation)                                                         | <i>lacZ</i> <sup>-</sup> mutant frequency in liver and bone marrow      | -       | Recio et al. 1992                                            |
| B6C3F1 mice and F344 rats (inhalation)                                           | <i>hprt</i> loci mutations in splenic T lymphocytes                     | +       | Cochrane and Skopek 1993; Meng et al. 1999, 2000, 2004, 2007 |
| B6C3F1 mice (inhalation)                                                         | Peripheral blood erythrocytes and bone marrow: induction of micronuclei | +       | Autio et al. 1994                                            |
| Wistar rats                                                                      | Peripheral blood erythrocytes and bone marrow: induction of micronuclei | -       | Autio et al. 1994                                            |
| C3H mice (inhalation)                                                            | Heritable spermatid chromosomal translocations; dominant lethality      | +       | Adler et al. 1998                                            |
| C3H mice (inhalation)                                                            | Spermatocytes: induction of micronuclei                                 | +       | Xiao and Tate 1995                                           |
| CD-1 mice (inhalation)                                                           | Dominant lethality                                                      | -       | Brinkworth et al. 1998                                       |
| CAST/EiJ, NOD/LTj, A/J, WSB/EiJ, PWK/PhJ, 129S/SvImJ, C57BL/6J mice (inhalation) | DNA adduct formation                                                    | +       | Koturbash et al. 2011a                                       |
| C57BL/6J mice (inhalation)                                                       | DNA adduct formation                                                    | +       | Koturbash et al. 2011b                                       |
| Humans (inhalation)                                                              | CA; SCE                                                                 | +       | Sram et al. 1998                                             |
| Humans (inhalation)                                                              | CA; SCE                                                                 | -       | Lovreglio et al. 2006                                        |
| Humans (inhalation)                                                              | <i>hprt</i> loci in peripheral lymphocytes                              | -       | Hayes et al. 1996, 2000                                      |
| Humans (inhalation)                                                              | <i>hprt</i> loci in peripheral lymphocytes                              | -       | Tates et al. 1996                                            |
| Humans (inhalation)                                                              | <i>hprt</i> loci in peripheral lymphocytes                              | -       | Albertini et al. 2001, 2007; HEI 2003                        |

## 3. HEALTH EFFECTS

**Table 3-3. Genotoxicity of 1,3 Butadiene *In Vivo***

| Species (test system)    | End point                                  | Results | Reference                                                                    |
|--------------------------|--------------------------------------------|---------|------------------------------------------------------------------------------|
| Humans (inhalation)      | <i>hprt</i> loci in peripheral lymphocytes | -       | Liu et al. 2008                                                              |
|                          | <i>hprt</i> exon deletion                  | +       | Liu et al. 2008                                                              |
| Humans (inhalation)      | <i>hprt</i> loci in peripheral lymphocytes | +       | Ward et al. 1994                                                             |
| Humans (inhalation)      | <i>hprt</i> loci in peripheral lymphocytes | +       | Abdel-Rahman et al. 2001, 2003, 2005; Ma et al. 2000; Ward et al. 1996, 2001 |
| Humans (inhalation)      | <i>hprt</i> loci in peripheral lymphocytes | +       | Wickliffe et al. 2009                                                        |
| C3H mice (inhalation)    | Induction of spermatid micronuclei         | +       | Tommasi et al. 1998                                                          |
| B6C3F1 mice (inhalation) | H- and K-ras mutation frequency            | +       | Sills et al. 2001                                                            |
| NMRI mice (inhalation)   | Bone marrow: induction of micronuclei      | +       | Vodicka et al. 2006                                                          |

- = negative result; + = positive result; AGT = average generation time; CA = chromosomal aberration; MI = mitotic index; MN = micronucleated cell; SCEs = sister chromatid exchange

## 3. HEALTH EFFECTS

low-exposure area. A significant correlation between *hprt* variant frequency and urinary levels of the 1,3-butadiene-specific metabolite, M1, was also found. No significant correlations between *hprt* variant frequency and age or employment length were found. A subsequent study of these workers reported 2.5-fold higher *hprt* mutation frequencies in workers exposed to airborne concentrations of 0.3 ppm 1,3-butadiene as compared to workers exposed to mean levels of 0.12 ppm (Ward et al. 1996), indicating good correlation between exposure and mutation frequency. Unlike the earlier study, no correlation between *hprt* variant frequency and urinary metabolite levels were found. A follow-up study of the southeast Texas SBR workers found a 3-fold increase in *hprt* mutation frequency in workers exposed to 1.7 ppm, compared to workers exposed to 0.07 ppm (Ward et al. 2001). A re-analysis of the blood samples from the Ward et al. (1996) study using a cloning assay, rather than the autoradiographic assay used in the Ward analyses, confirmed the significant difference in *hprt* variant frequency between high-exposure workers and the outside controls and found a significant difference in *hprt* mutation frequency between the groups (Ma et al. 2000). However, the investigators did not examine the possible association between 1,3-butadiene exposure level and mutation frequency. Neither Ma et al. (2000) nor Ward et al. (1994, 1996) provided demographic information on the outside control group, which consisted of workers in the Department of Preventive Medicine and Community Health at the University of Texas Medical Branch; thus, the appropriateness of this comparison group to the SBR workers cannot be evaluated. A more recent study of these SBR workers did not find a significant association between 1,3-butadiene exposure level and *hprt* variant frequency, after removal of an outlier (Wickliffe et al. 2009). The current 1,3-butadiene exposure levels of the 30 subjects examined were low with only six subjects having levels of >0.1 ppm. A significant association between employment length and *hprt* mutant frequency was found. Another study conducted by this group (Ammenheuser et al. 2001) at a different SBR facility found a 3-fold increase in *hprt* mutation frequency in 22 workers in the high-exposure group (mean 1,3-butadiene exposure level was 1.48 ppm) compared to low-exposure workers (mean exposure level of 0.15 ppm). Significant correlations between *hprt* variant frequency and 1,3-butadiene levels and urinary M1 levels were found. Abdel-Rahman et al. (2001, 2003, 2005) examined the association between polymorphisms, 1,3-butadiene exposure, and *hprt* variant frequency among workers at the two Texas SBR facilities. Individuals with a polymorphism for microsomal epoxide hydrolase (EH), an enzyme important for the hydrolysis of epoxide metabolites of 1,3-butadiene (see Section 3.4.3), exhibited a 3-fold higher *hprt* mutation frequency than workers without the polymorphism (Abdel-Rahman et al. 2001). Further, polymorphisms in the glutathione-S-transferase (another important enzyme in epoxide metabolite metabolism) genotypes GSTM1 or GSTT1 did not impact *hprt* mutation rates, but a combination of EH and GST polymorphism did result in an increase in *hprt* mutation frequency (Abdel-Rahman et al. 2001, 2003, 2005). Several studies that examined the possible association between

## 3. HEALTH EFFECTS

1,3-butadiene exposure and the frequency of chromosomal aberrations and/or sister chromatid exchanges among Texas 1,3-butadiene workers have not found significant associations (Au et al. 1995; Hallberg et al. 1997; Kelsey et al. 1995).

Unlike the results of Texas cohorts, studies of Chinese rubber production workers have not found alterations in *hprt* mutation frequency. No significant difference in *hprt* gene mutation frequency was observed in male and female Chinese polybutadiene rubber production workers exposed to an average of 1.0–3.5 ppm (median of 2.0 ppm) and unexposed controls (Hayes et al. 1996, 2000, 2001). Additionally, no exposure-related significant associations between *hprt* mutation frequency and 1,3-butadiene exposure (as assessed by exposure levels, urinary metabolite levels, or hemoglobin adducts) were found. Similarly, no significant alterations in glycophorin A variant frequencies were observed (Hayes et al. 2000). In another Chinese study, *hprt* mutation frequencies in petrochemical workers exposed to mean levels of 10 ppm were higher, but were not significantly different than unexposed controls, while the percentage of workers exhibiting *hprt* exon deletions (27%) was significantly higher than levels found in controls (13%) (Liu et al. 2008).

Several investigators have examined *hprt* mutation frequency in cohorts of 1,3-butadiene workers in the Czech Republic. Tates et al. (1996) did not find significant alterations in *hprt* mutation frequency among male workers from a 1,3-butadiene production plant exposed to a mean concentration of 1.76 ppm, as compared to unexposed workers at the plant. However, a significant increase in the percentage of lymphocytes with chromosomal aberrations was observed in the exposed workers; increases in DNA damage (as assessed using the comet assay) and micronuclei frequency were observed in exposed smokers, as compared to unexposed smokers (Šrám et al. 1998; Tates et al. 1996). A significant increase in the frequency of sister chromatid exchanges was also observed (Šrám et al. 1998). When the exposed workers and unexposed workers were subdivided based on glutathione-S-transferase polymorphism for M1 gene (GSTM1) and glutathione-S-transferase polymorphism for T1 gene (GSTT1) genotypes, a significant increase in the frequency of chromosomal aberrations were observed in exposed workers with the GSTM1-positive genotype, as compared to exposed workers with GSTM1-null genotype; no effects was observed for the GSTT1 genotype. Multifactorial analysis (accounting for 1,3-butadiene exposure, smoking, GSTM1, GSTT1, and age) showed a significant association between the frequency of chromosomal aberrations and the number of cells with a high frequency of sister chromatid exchanges (Šrám et al. 1998). However, when 1,3-butadiene exposure was evaluated using N-1-(2,3,4-trihydroxy-butyl)adenine adduct levels, there were no significant associations between chromosomal aberration frequency, micronuclei formation, or sister chromatid exchange (Zhao et al. 2001). In another study of

## 3. HEALTH EFFECTS

Czech 1,3-butadiene workers, no alterations in chromosome aberrations, sister chromatid exchanges, or micronuclei formation were observed in 1,3-butadiene production workers or workers in 1,3-butadiene polymer production (Sorsa et al. 1994). However, when workers were subdivided based on GSTM1 and GSTT1 genotypes, a significantly higher frequency of chromosomal aberrations were observed in 1,3-butadiene workers lacking the GSTT1 gene (Sorsa et al. 1996). In a larger-scale study of 24 workers at a 1,3-butadiene production facility (mean exposure level of 0.64 mg/m<sup>3</sup> [0.29 ppm]) and 34 workers at a polymerization facility (mean exposure level of 1.76 mg/m<sup>3</sup> [0.79 ppm]), no significant association between 1,3-butadiene exposure (as assessed using air concentrations, urinary metabolites, or hemoglobin adducts) and *hprt* mutation frequency (assessed using cloning assay) were found; no associations were found when workers divided by a number of genotypes including GSTM1 or GSTT1 (Albertini et al. 2001; HEI 2003). No significant alterations in the frequency of chromosomal aberrations or sister chromatid exchanges were found. In a follow-up study at the polymerization facility, male and female workers were examined. The mean 1,3-butadiene exposure levels were 0.397 mg/m<sup>3</sup> (0.18 ppm) and 0.808 mg/m<sup>3</sup> (0.36 ppm) in the females and males, respectively (Albertini et al. 2007). No significant associations between 1,3-butadiene exposure and *hprt* mutation frequency were found; similarly, there were no significant associations between exposure and sister chromatid exchanges or chromosomal aberrations.

In a cohort of workers at an Italian petrochemical plant exposed to very low levels of 1,3-butadiene (mean concentration of 0.0115 mg/m<sup>3</sup> [0.005 ppm]), no significant relationship between 1,3-butadiene exposure and alterations in the frequency of chromosomal aberrations or sister chromatid exchanges was observed (Fustinoni et al. 2004; Lovreglio et al. 2006).

In summary, studies of workers at Texas 1,3-butadiene production facilities or SBR facilities have found significantly higher frequencies of *hprt* variants in the lymphocytes in men working in areas of the facility with high 1,3-butadiene exposure levels (Ammenheuser et al. 2001; Ma et al. 2000; Ward et al. 1996, 2001). However, no significant associations were found in Czech cohorts (Albertini et al. 2001, 2007; HEI 2003; Tate et al. 1996) or Chinese cohorts (Hayes et al. 1996, 2000, 2001). The reasons for dissimilar outcomes in lymphocyte *hprt* gene mutation frequency between the different cohorts are not clear, but may be the result of varying exposure levels, experimental techniques in exposures assessment (active vs. passive sampling) and mutation analysis (autoradiography vs. cloning *hprt* assays). The mean exposure levels in the Texas cohort studies tended to be higher than other cohorts; the mean levels in the Chinese cohort are elevated due to intermittent high exposures rather than a high TWA level. This is supported by a study conducted by Wickliffe et al. (2009) that did not find significant increases in *hprt*

## 3. HEALTH EFFECTS

frequency in a Texas cohort with low 1,3-butadiene exposure levels (only six subjects were exposed to levels >0.1 ppm).

A number of rodent inhalation studies report genotoxic effects. Mice and rats exhibited increased *hprt* locus mutations in splenic T cells (Cochrane and Skopek 1993; Meng et al. 1999, 2000, 2004, 2007). Inhaled 1,3-butadiene also induce an increase in micronucleus induction in erythrocytes (Irons et al. 1986b; Jauhar et al. 1988; Tice et al. 1987; Vodicka et al. 2006), spermatocytes (Tommasi et al. 1998; Xiao and Bates 1995), and bone marrow cells (Autio et al. 1994), increased frequency of sister chromatid exchanges (Tice et al. 1987) and chromosomal aberration frequencies (Cunningham et al. 1986; Tice et al. 1987) in mice. Transgenic B6C3F1 mice exhibited an increased *lacZ* mutant frequency in the lungs (Recio et al. 1992). Increased percentages of H- and K-*ras* proto-oncogene mutations were found in forestomach neoplasms from mice inhaling 1,3-butadiene for 2 years (83% in exposed mice compared to 24% in spontaneous neoplasms from controls) (Sills et al. 2001). Increases in N-7-(2,3,4-trihydroxybut-1-yl) guanine adduct formation in liver DNA were found in various mouse strains exposed to 1,3-butadiene (Koturbash et al. 2011a, 2011b); the increase DNA adduct formation was concentration-related (Koturbash et al. 2011b). No genotoxic effects (micronucleus induction, chromosomal aberrations, or sister chromatid exchanges) were found in bone marrow of rats or liver of mice exposed by inhalation to 1,3-butadiene (Autio et al. 1994; Cunningham et al. 1986; Recio et al. 1992).

In a dominant lethal study in which male CD-1 mice inhaled 1,3-butadiene for 5 days and were mated to nonexposed females, an increased number of dead implantations per pregnancy occurred at 200 and 1,000 ppm, but not at 5,000 ppm during the first 2 weeks postexposure (DOE/NTP 1988b). These results were considered to be inconclusive because of the lack of a strict dose-response relationship. Increased numbers of dead fetuses were also observed in offspring of CH3 males inhaling 130 ppm (Adler et al. 1998), but not in CD-1 mice exposed to 125 ppm (Brinkworth et al. 1998). Heritable spermatid chromosomal translocations occurred in F<sub>1</sub> offspring of male CH3 mice inhaling 1,3-butadiene (Adler et al. 1995a, 1998).

Although cytogenetic monitoring of 1,3-butadiene rubber workers for chromosomal aberrations revealed no or slight differences between exposed and control groups (Lovreglio et al. 2006; Sram et al. 1998; Zhou et al. 1986), 1,3-butadiene is clearly genotoxic in mice. As discussed in Section 3.4.3, species differences exist in the metabolism of 1,3-butadiene, and data suggest that humans may metabolize this compound at different metabolic rates than do rodents. If the genotoxic and clastogenic response of 1,3-butadiene requires activation to an active metabolite that is formed more slowly or deactivated more

## 3. HEALTH EFFECTS

rapidly in humans than in rats and mice, the genotoxicity observed in animals may only be observed after much higher exposures in humans. The data in humans are too limited, however, to rule out the possibility of a genotoxic potential in humans exposed to 1,3-butadiene.

**3.4 TOXICOKINETICS****3.4.1 Absorption****3.4.1.1 Inhalation Exposure**

In human volunteers inhaling 2 ppm 1,3-butadiene for 20 minutes, the absorbed fraction varied from 18 to 74% (Lin et al. 2001). Neither sex nor age ( $30 \pm 8$  years for males,  $29 \pm 9$  years for females) were factors in this variation. Fractional absorption in Asian volunteers was about 20% greater than in Caucasians, African-Americans, or Hispanics. Blood triglyceride levels may influence absorption, as blood:air partition coefficients increased 20–40% in humans having borderline higher triglyceride levels after ingestion of fat in the diet (Lin et al. 2002).

In male Sprague-Dawley rats and male B6C3F1 mice exposed to 20 ppm  $^{14}\text{C}$ -radiolabeled 1,3-butadiene for 6 hours in a dynamic system, the total absorbed radioactivity, as estimated by the sum of  $^{14}\text{C}$  in urine, feces, carcass, and expired air, was 2.2% in rats and 1.6% in mice (Swain et al. 2003). In close-chamber studies, the uptake of inhaled 1,3-butadiene in mice and rats was linear to 2,000 and 1,000 ppm, respectively, above which metabolism is saturated (Kohn and Melnick 2001). Absorption of 1,3-butadiene was demonstrated by measurements of the metabolite, 1,2-epoxy-3-butene (EB), in the test chamber (due to exhaled air) and measurement of 1,3-butadiene metabolites in blood of male Sprague-Dawley rats exposed 1–10,000 ppm and male B6C3F1 mice exposed to 1–6,000 ppm for 6–8 hours in closed chambers (Filser et al. 2007). In rats, EB concentrations in the test chamber reached a plateau at all exposure concentrations. In mice, chamber concentrations of EB were higher than for rats; EB levels reached a plateau at exposure concentrations up to 1,000 ppm, but no plateau was observed at exposure concentrations of 2,000–6,000 ppm. The study authors suggest that this concentration-dependence is due to “breakdown” of hepatic glutathione-S-transferase mediated 1,3-butadiene conjugation. Filsner et al. (2007) did not report an absorption fraction for either species. Absorption of 1,3-butadiene was also demonstrated by measurement of metabolites (butadiene monoepoxide and butadiene diepoxide) in blood and tissues of male Sprague-Dawley rats and male B6C3F1 mice exposed (nose only) to 62.5 ppm 1,3-butadiene for 2 or 4 hours (Thornton-Manning et al. 1995a). Similar results were observed in male and female rats exposed (nose only) to 62.5 ppm 1,3-butadiene for 6 hours (Thornton-Manning et al.

## 3. HEALTH EFFECTS

1995b). The distribution coefficient for 1,3-butadiene between rabbit blood and air was 0.603 *in vitro* and 0.654 *in vivo*, suggesting simple passive diffusion of the gas from the alveoli to the blood (Carpenter et al. 1944). After 9 minutes of exposure of rabbits to 250,000 ppm, the concentration of 1,3-butadiene was 0.26 mg/mL in the femoral artery and 0.18 mg/mL in the femoral vein. Pulmonary absorption, therefore, appears to be rapid. Distribution studies in rats and mice following inhalation exposure to 1,3-butadiene indicate that it is absorbed from the lungs in these species as well (see Section 3.4.2.1). When *Macaca fascicularis* monkeys were exposed to radioactively labeled 1,3-butadiene, the uptake was calculated as 16.40  $\mu\text{mol}/\text{hour}/10\text{ ppm}$  of inhaled and 3.20  $\mu\text{mol}/\text{hour}/10\text{ ppm}$  of retained 1,3-butadiene (Dahl et al. 1990).

**3.4.1.2 Oral Exposure**

No studies were located regarding absorption in humans or animals after oral exposure to 1,3-butadiene.

**3.4.1.3 Dermal Exposure**

No studies were located regarding absorption in humans or animals after dermal exposure to 1,3-butadiene.

**3.4.2 Distribution**

*In vitro* measurements of tissue:blood equilibrium partition coefficients suggest that 1,3-butadiene distributes to a variety of tissues. Partition coefficients in humans were highest in fat (18.4) and were similar in well- and poorly-perfused tissues (0.69 and 0.72, respectively) (Brochot et al. 2007). In rats, partition coefficients were highest for fat (21.9), similar for liver, kidney, muscle, and spleen (0.87–0.94), and lowest in brain (0.43) (Johanson and Filser 1993).

**3.4.2.1 Inhalation Exposure**

In volunteers inhaling 2 ppm 1,3-butadiene for 20 minutes, blood levels approached equilibrium by 5 minutes (Smith et al. 2001). In mice and rats inhaling up to 625 ppm 1,3-butadiene, equilibrium in blood concentrations was reached by 2 hours, with blood levels in mice being three- to 4-fold higher than in rats at all times (Himmelstein et al. 1994). The distribution of 1,3-butadiene in several tissues in rats was measured following a 1-hour inhalation exposure to 129,000 ppm (Shugaev 1969). Perinephric fat



### 3. HEALTH EFFECTS

contained 152 mg 1,3-butadiene/100 cc tissue, compared to levels of 36–51 mg 1,3-butadiene/100 mL in the brain, liver, septum, and kidney.

Species differences in the distribution of inhaled 1,3-butadiene were studied in Sprague-Dawley rats and B6C3F1 mice (Bond et al. 1986, 1987). When normalized for amount of inhaled <sup>14</sup>C-1,3-butadiene, molar tissue concentrations of radioactive material at 1 hour postexposure were 17-fold (thyroid) to 80-fold higher (lung) in mice than in rats. In blood, the normalized radioactivity concentration was 57-fold higher in mice than rats, while 110- to 120-fold more radioactivity was found in mouse intestine than in rat intestine.

#### 3.4.2.2 Oral Exposure

No studies were located regarding distribution in humans or animals after oral exposure to 1,3-butadiene.

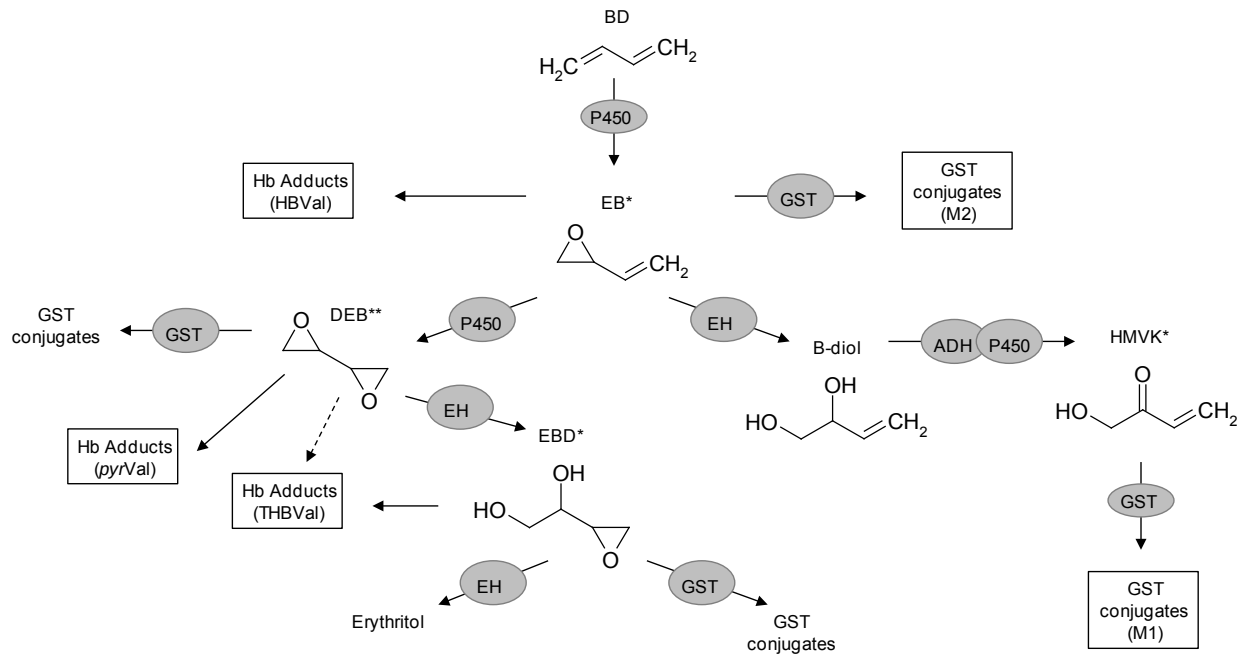
#### 3.4.2.3 Dermal Exposure

No studies were located regarding distribution in humans or animals after dermal exposure to 1,3-butadiene.

#### 3.4.3 Metabolism

1,3-Butadiene is metabolized by oxidation, hydrolysis, and conjugation reactions, with oxidation and hydrolysis reactions leading to the formation of several reactive epoxide intermediates (Figure 3-2). Of the reactive intermediates formed, EB (formed by oxidation of 1,3-butadiene), 1,2:3,4-diepoxybutane (DEB; formed by oxidation of EB), and 1,2-dihydroxy-3,4-epoxybutane (EBD; formed by hydrolysis reactions of DEB) are reactive electrophilic compounds. Metabolism of 1,3-butadiene appears to follow the same enzymatic pathways in all species, including humans, with production of the same reactive intermediates. However, important species differences exist in the rates of formation and detoxification of reactive metabolites (Kirman et al. 2010a). As a result, rodents, particularly mice, have much higher tissue levels of reactive metabolites than nonhuman primates and humans. Evidence for species differences in metabolism of 1,3-butadiene is available from *in vitro* studies, studies using isolated perfused livers, and *in vivo* studies measuring tissue and urine metabolite levels and blood hemoglobin adduct levels. Metabolism of 1,3-butadiene exhibits nonlinear kinetics (Kirman et al. 2010a), with both dose- and exposure duration-dependent effects. Several processes have been proposed as sources of

## 3. HEALTH EFFECTS

**Figure 3-2. Metabolism of 1,3-Butadiene**

□ = boxes indicate biomarkers of exposure that have been measured in exposed workers (Albertini et al. 2003);

\* = monofunctional alkylating agent; \*\* = bifunctional alkylating agent; ADH = alcohol dehydrogenase;

B-diol = butanediol; BD = 1,3-butadiene; DEB = diepoxybutane; EB = epoxybutene; EBD = epoxybutane diol;

EH = epoxide hydrolase; GST = glutathione S-transferase; HBVal = *N*-(2-hydroxy-3-butenyl)-valine;

HMVK = hydroxymethylvinyl ketone; M1 = 1,2-dihydroxy-4-(*N*-acetylcysteinyl)-butane (urinary metabolite);

M2 = 1-(*N*-acetylcysteinyl)-2-hydroxy-3-butene (urinary metabolite); P450 = cytochrome P450;

pyrVal = *N,N*-(2,3-dihydroxy-1,4-butadiyl)-valine; THBVal = *N*-(2,3,4-trihydroxybutyl)-valine

Source: Kirman et al. 2010

## 3. HEALTH EFFECTS

nonlinear kinetics; these include inhibition, induction, and saturation of various metabolizing enzymes and depletion of glutathione.

The metabolism of 1,3-butadiene has been observed in the liver, lung, and kidneys. The liver is the predominant site of 1,3-butadiene metabolism (Elfarra et al. 2001; Schmidt and Loeser 1985, 1986). 1,3-Butadiene is initially oxidized (Figure 3-2) by cytochrome P450 (CYP) to 2-butenal or EB (Bolt et al. 1983; Csanady et al. 1992; Duescher and Elfarra 1994; Himmelstein et al. 1994, 1995; Kirman et al. 2010; Malvoisin and Roberfroid 1982; Malvoisin et al. 1979; Thornton-Manning et al. 1995b, 1997). Metabolism of EB is mediated by three competing oxidative, hydrolytic, or conjugation pathways. The flux of 1,3-butadiene through the various pathways is concentration- and species-dependent. Successive oxidation steps of EB result in DEB and 3,4-epoxy-1,2-diol (EBdiol). EB can also be conjugated to glutathione by glutathione-S-transferase (GST) to form 1-glutathionyl-3-buten-2-ol, or can be hydrolyzed via epoxide hydrolase (EH) to 3-butene-1,2-diol (BDdiol). BDdiol is metabolized via CYP or aldehyde dehydrogenase (ADH) to the ketone 1-hydroxy-3-buten-2-one (hydroxymethylvinyl ketone, or HMVK) or EBdiol. GST can conjugate glutathione to HMVK and EBdiol to form 4-glutathionyl-1-hydroxy-2-butanone and 4-glutathionylbutane-1,2,3-triol. Several isoforms of CYP have been implicated in the oxidative metabolism of 1,3-butadiene and resulting epoxides in various tissues. In human liver microsomes, CYP2E1 dominates metabolism at low concentrations (i.e., 0.16 mM), while CYP2A6 dominates at higher concentrations (i.e., 4.4 mM) (Elfarra et al. 1996). In mice, CYP2E1 and 2A5 are active in 1,3-butadiene oxidation in lung and liver microsomes, but CYP4B1 dominates metabolism in the kidneys (Elfarra et al. 2001).

Examination of blood levels of 1,3-butadiene metabolites in male Sprague-Dawley rats exposed to 1–10,000 ppm and male B6C3F1 mice exposed to 1–6,000 ppm for 6–8 hours in closed chambers indicates species differences in the predominance of metabolic pathways (Filsner et al. 2007). In rats and mice, EB, EBD, and 3-butene-1,2-diol concentrations in blood increased with increasing exposure concentrations. Ratios of mouse:rat EB blood levels ranged from 2.0 to 8.6 over 1,3-butadiene exposure concentrations of 1–1,250 ppm. DEB was detected in blood of mice, but not in rats. Similar results were observed in male Sprague-Dawley rats and male B6C3F1 mice exposed (nose only) to 62.5, 625, or 1,250 ppm 1,3-butadiene for 6 hours; DEB was detected in mouse, but not rat, blood, and higher levels of butadiene monoepoxide were present in mouse blood, compared to rat blood (Himmelstein et al. 1994). Results of these studies are consistent with enhanced formation and/or lower metabolism of DEB in mice compared to rats. Differences also were noted between rodents and monkeys in 1,3-butadiene metabolism (Dahl et al. 1990; Sun et al. 1989a). At 10 ppm, blood levels of EB, DEB, and EBdiol were

## 3. HEALTH EFFECTS

lower in monkeys inhaling 10 ppm than in rodents. The difference was not so great at 8,000 ppm (Sun et al. 1989a). Similar exposures of 10 ppm 1,3-butadiene resulted in blood concentrations of total 1,3-butadiene metabolites in monkeys that were about 5–50 times lower than in mice and about 4–14 times lower than in rats (Dahl et al. 1991). The results indicated possible lower susceptibility to toxic effects of low levels of 1,3-butadiene in primates.

Species differences in metabolism are also supported by results of studies examining tissue levels of metabolites (Filser et al. 2007; Himmelstein et al. 1995; Thorton-Manning et al. 1995a, 1995b).

Comparison of butadiene epoxide levels in livers and lungs of male Sprague-Dawley rats and male B6C3F1 exposed (nose only) to 62.5, 625, 1,250, or 8,000 (rats only) ppm 1,3-butadiene shows higher levels of butadiene monoepoxide in mice compared to rats, and the presence of butadiene diepoxide in mice, but not rats (Himmelstein et al. 1995). In male Sprague-Dawley rats and male B6C3F1 mice exposed to 62.5 ppm 1,3-butadiene for 4 hours, tissue levels of butadiene monoepoxide were higher in tissues (blood, heart, lung, liver, fat, spleen, and bone marrow) of mice compared to rats, with mouse:rat ratios ranging from 3.0 (heart) to 11.5 (bone marrow); butadiene diepoxide was not detected in lung or liver of rats (Thorton-Manning et al. 1995a). In mice, butadiene diepoxide levels were similar to monoepoxide levels in blood, heart, thymus and bone marrow; diepoxide levels in mouse lung, liver, and spleen were higher than monoepoxide levels, but were lower in fat. Butadiene diepoxide levels in rats were very low compared to levels in mice, and diepoxide was not detected in liver or bone marrow. Comparison of butadiene epoxide levels in tissues of male and female Sprague-Dawley rats exposed to 1,3-butadiene for 6 hours (nose only) suggests gender differences in metabolism (Thorton-Manning et al. 1995b). Butadiene monoepoxide levels in lung were approximately 5-fold higher in males compared to females; whereas similar monoepoxide levels for males and females were observed for blood, femur, and fat.

Results of studies using isolated perfused livers provide additional evidence of species differences in metabolism of 1,3-butadiene. Following single pass, isolated perfusion of livers from male Sprague-Dawley rats and male B6C3F1 mice with 1,3-butadiene (at concentrations that approached saturation of 1,3-butadiene metabolism), differences were observed in 1,3-butadiene metabolites in liver effluent (Filser et al. 2001). In mice, three epoxides (EB, DEB, and EBD) and 3-butane-1,2-diol (B-diol) were detected, whereas only EB and B-diol were detected in effluent from rat livers. Furthermore, the concentration of EB in effluent from rat livers was approximately 8.5-fold less than that in effluent from mouse livers. Additional species differences were observed in a study evaluating single-pass perfusion of the 1,3-butadiene metabolites, EB, DEB and B-diol (Filser et al. 2010). For perfusion with EB, EBD,

## 3. HEALTH EFFECTS

DEB, and B-diol were formed in rats and mice, with an approximately 4-fold higher percentage of DEB in mice compared to rats. The major metabolite of DEB in rats and mice was EBD. For perfusion with B-diol, EBD was detected in effluent of rats, but not in mice. Results of studies using hepatic microsomes from mice and rats indicate differences in stereochemistry of 1,3-butadiene metabolites (Nieusma et al. 1997). Mouse microsomes form more (S)-EB than (R)-EB; rat microsomes initially formed more (S)-EB than (R)-EB, although the S:R fell below 1.0 as incubation time increased to 30 minutes. For DEB formation, mice microsomes formed more DEB when starting with (S)-EB compared to when starting with (R)-EB; the opposite was observed with rat microsomes.

*In vitro* studies indicate that mouse lung and liver have a higher capacity than other species, including humans, to oxidize 1,3-butadiene to EB and DEB, but have much less ability to detoxify the epoxides via the EH pathway (Jackson et al. 2000b). Female mouse tissue homogenates resulted in higher EB generation than in males or in rat, human, or monkey tissues, while human and monkey tissues hydrolyzed the epoxides to diols approximately 20-fold more extensively than rodents (Schmidt and Loeser 1985, 1986). In studies of liver microsomes, mice had intrinsic clearance ( $V_{\max}/K_m$ ) values of 57.5 and 0.77  $\text{minute}^{-1}$  for oxidation of 1,3-butadiene to EB and EB to DEB, respectively. These values are 3–4-fold higher than the respective rat values of 1,637 and 0.21  $\text{minute}^{-1}$  (Elfarra et al. 2001). Conversely, intrinsic clearance via EH-mediated hydrolysis of EB was 34.4  $\text{minute}^{-1}$  in rats, compared to 12.4  $\text{minute}^{-1}$  in mice. Clearance by EB conjugation with GSH was similar (21.0 and 22.0  $\text{minute}^{-1}$ ) in both species. These *in vitro* findings comport with metabolic differences observed between rats and mice after inhalation exposure to EB (Kreiling et al. 1987; Laib et al. 1990). A limited rate of EB removal and its subsequent accumulation was observed in mice at 500 ppm exposure, but not in rats at exposures up to 5,000 ppm. This may partially account for the differing levels of toxicity and carcinogenicity between rats and mice in long-term studies. For additional information, see Section 3.5 (Mechanisms of Action).

Based on evaluation of hemoglobin adduct biomarkers (adducts formed by interaction of 1,3-butadiene metabolites with hemoglobin), mice appear to have higher DEB levels than rats and much higher levels than humans (Swenberg et al. 2007). Analysis of hemoglobin adducts in mice and rats exposed to 1,3-butadiene by inhalation show much higher levels of the hemoglobin adducts, pyr-Val and THB-Val (formed by interaction of DEB with hemoglobin), in mice than in rats (Boysen et al. 2004). Results are consistent with results of studies showing higher levels of DEB in tissues of mice compared to rats. In polymerization workers exposed to 0.81 ppm 1,3-butadiene, THB-Val was the predominant hemoglobin adduct, comprising 99.6% of the total; the mean percent of HB-Val was 0.33% and the mean percent of pyr-Val was 0.05% (Boysen et al. 2012). In monomer workers exposed to lower concentrations of

## 3. HEALTH EFFECTS

1,3-butadiene (0.29 ppm), lower levels of HB-Val (0.26%) and higher levels of *pyr*-Val (0.11%) were found; the decrease in *pyr*-Val levels was significantly related to the increasing 1,3-butadiene concentrations. The lower percentage of *pyr*-Val formed in the workers exposed to higher 1,3-butadiene levels may be suggestive of saturation of the formation of *pyr*-Val in humans (Boysen et al. 2012).

As noted in the introduction to Section 3.4.3, metabolism of 1,3-butadiene exhibits nonlinear kinetics (Kirman et al. 2010a), with both dose- and exposure duration-dependent effects. Glutathione depletion and saturable kinetics have been proposed as possible sources of nonlinear kinetics. Studies evaluating effects of hepatic glutathione levels show that glutathione depletion exhibited concentration-dependence and was greater in mice than in rats (Deutschman and Laib, 1989; Kreiling et al. 1988). Glutathione depletion was also observed in livers and lungs of rats and mice exposed to 1,3-butadiene, with more extensive depletion in mice than in rats (Himmelstein et al. 1995). Regarding saturable kinetics, blood levels of EB reached a plateau in rats, but not mice (Filser et al. 2007). The study authors suggested that the results are consistent with competitive inhibition of CYP450 isozymes.

### 3.4.4 Elimination and Excretion

#### 3.4.4.1 Inhalation Exposure

The monoepoxide metabolite, EB, can be conjugated to glutathione by GST to form monohydroxybutenylmercaptic acid (MHBMA, or M2), a mixture of N-acetyl-S-([1-hydroxymethyl]-2-propenyl)cysteine and N-acetyl-S-([2-hydroxymethyl]-3-propenyl)cysteine. EBdiol, formed by hydrolysis of EB, may also be conjugated by GST to glutathione to form N-acetyl-S-(3,4-dihydroxybutyl)cysteine (DHBMA, or M1). Both mercaptic acids are excreted in the urine (Boogaard et al. 2001a; McDonald et al. 2004). These excretion products have been used as biomarkers of 1,3-butadiene exposures in both environmental and occupational settings (Albertini et al. 2001, 2007; Ammenheuser et al. 2001; Boogaard et al. 2001a) (see Section 3.8). The relative abundance of MHBMA and DHBMA in urine indicates the flux of EB through the competing GST and EH metabolic pathways. In humans, >97% of urinary mercaptic acid measured following 1,3-butadiene inhalation is DHBMA, indicating that most EB proceeds to hydrolysis via EH rather than to formation of the diepoxide (Henderson et al. 1996). Albertini et al. (2007) showed that women excrete lower levels of both mercaptic acids than men per unit of 1,3-butadiene exposure; however, they maintain the ratio of M1 and M2, suggesting sex differences in metabolic activity, but not pathway flux.

## 3. HEALTH EFFECTS

In rats exposed to 1,3-butadiene, 1,2-epoxybutene-3 and acetone were exhaled as suspected metabolites of the administered compound (Bolt et al. 1983). The pharmacokinetic profile of inhaled 1,3-butadiene was studied in mice (Kreiling et al. 1986b) and in rats (Bolt et al. 1984; Filser and Bolt 1984). Following exposure of mice and rats to  $^{14}\text{C}$ -1,3-butadiene, the elimination of radioactivity was rapid, and 77–99% of the initial tissue amount was eliminated with half-lives of between 2 and 10 hours (Bond et al. 1987). At concentrations of approximately  $\leq 1,000$  ppm, the elimination of 1,3-butadiene followed first-order kinetics in both species. The first-order metabolic clearance of inhaled 1,3-butadiene per kg body weight was 4,500 mL/hour for rats (Laib et al. 1988) and 7,300 mL/hour for mice (Kreiling et al. 1986b). The maximal metabolic elimination rate was calculated as 220  $\mu\text{mol}/\text{hour}/\text{kg}$  for rats (Laib et al. 1988) and 400  $\mu\text{mol}/\text{hour}/\text{kg}$  for mice (Kreiling et al. 1986b). With increasing concentrations of  $^{14}\text{C}$ -1,3-butadiene, exhalation of radiolabeled carbon was a major pathway for elimination of  $^{14}\text{C}$  in mice and rats (Bond et al. 1986). Similar results were observed in mice and rats following inhalation of 62.5 ppm 1,3-butadiene for 6 hours (Himmelstein et al. 1996). Blood 1,3-butadiene concentrations in mice fell from about 3  $\mu\text{M}$  at the end of exposure to 0.03  $\mu\text{M}$  15 minutes later. Rat elimination of 1,3-butadiene from blood was slower, falling from a post-exposure maximum of about 1.5–0.1  $\mu\text{M}$  30 minutes later.

About 2% of the total inhaled amount of 1,3-butadiene was excreted as metabolites in *Cynomolgus* monkeys (Sun et al. 1989a). Carbon dioxide was the major exhalation product at 10 ppm, while epoxy-metabolites (specific compounds not determined) were predominant in exhaled breath at 300 and 8,000 ppm. Urinary excretion of total metabolites was not influenced by exposure levels. In *Macaca fascicularis* monkeys, about 39% of metabolite radioactivity (specific compounds not determined) were eliminated in the urine, 0.8% in feces, and 56% were exhaled as carbon dioxide during the first 70 hours postexposure (Dahl et al. 1990).

#### 3.4.4.2 Oral Exposure

No studies were located regarding excretion in humans or animals after oral exposure to 1,3-butadiene.

#### 3.4.4.3 Dermal Exposure

No studies were located regarding excretion in humans or animals after dermal exposure to 1,3-butadiene.

## 3. HEALTH EFFECTS

**3.4.5 Physiologically Based Pharmacokinetic (PBPK)/Pharmacodynamic (PD) Models**

Physiologically based pharmacokinetic (PBPK) models use mathematical descriptions of the uptake and disposition of chemical substances to quantitatively describe the relationships among critical biological processes (Krishnan et al. 1994). PBPK models are also called biologically based tissue dosimetry models. PBPK models are increasingly used in risk assessments, primarily to predict the concentration of potentially toxic moieties of a chemical that will be delivered to any given target tissue following various combinations of route, dose level, and test species (Clewett and Andersen 1985). Physiologically based pharmacodynamic (PBPD) models use mathematical descriptions of the dose-response function to quantitatively describe the relationship between target tissue dose and toxic end points.

PBPK/PD models refine our understanding of complex quantitative dose behaviors by helping to delineate and characterize the relationships between: (1) the external/exposure concentration and target tissue dose of the toxic moiety, and (2) the target tissue dose and observed responses (Andersen and Krishnan 1994; Andersen et al. 1987). These models are biologically and mechanistically based and can be used to extrapolate the pharmacokinetic behavior of chemical substances from high to low dose, from route to route, between species, and between subpopulations within a species. The biological basis of PBPK models results in more meaningful extrapolations than those generated with the more conventional use of uncertainty factors.

The PBPK model for a chemical substance is developed in four interconnected steps: (1) model representation, (2) model parameterization, (3) model simulation, and (4) model validation (Krishnan and Andersen 1994). In the early 1990s, validated PBPK models were developed for a number of toxicologically important chemical substances, both volatile and nonvolatile (Krishnan and Andersen 1994; Leung 1993). PBPK models for a particular substance require estimates of the chemical substance-specific physicochemical parameters, and species-specific physiological and biological parameters. The numerical estimates of these model parameters are incorporated within a set of differential and algebraic equations that describe the pharmacokinetic processes. Solving these differential and algebraic equations provides the predictions of tissue dose. Computers then provide process simulations based on these solutions.

The structure and mathematical expressions used in PBPK models significantly simplify the true complexities of biological systems. If the uptake and disposition of the chemical substance(s) are adequately described, however, this simplification is desirable because data are often unavailable for



## 3. HEALTH EFFECTS

many biological processes. A simplified scheme reduces the magnitude of cumulative uncertainty. The adequacy of the model is, therefore, of great importance, and model validation is essential to the use of PBPK models in risk assessment.

PBPK models improve the pharmacokinetic extrapolations used in risk assessments that identify the maximal (i.e., the safe) levels for human exposure to chemical substances (Andersen and Krishnan 1994). PBPK models provide a scientifically sound means to predict the target tissue dose of chemicals in humans who are exposed to environmental levels (for example, levels that might occur at hazardous waste sites) based on the results of studies where doses were higher or were administered in different species.

Figure 3-3 shows a conceptualized representation of a PBPK model.

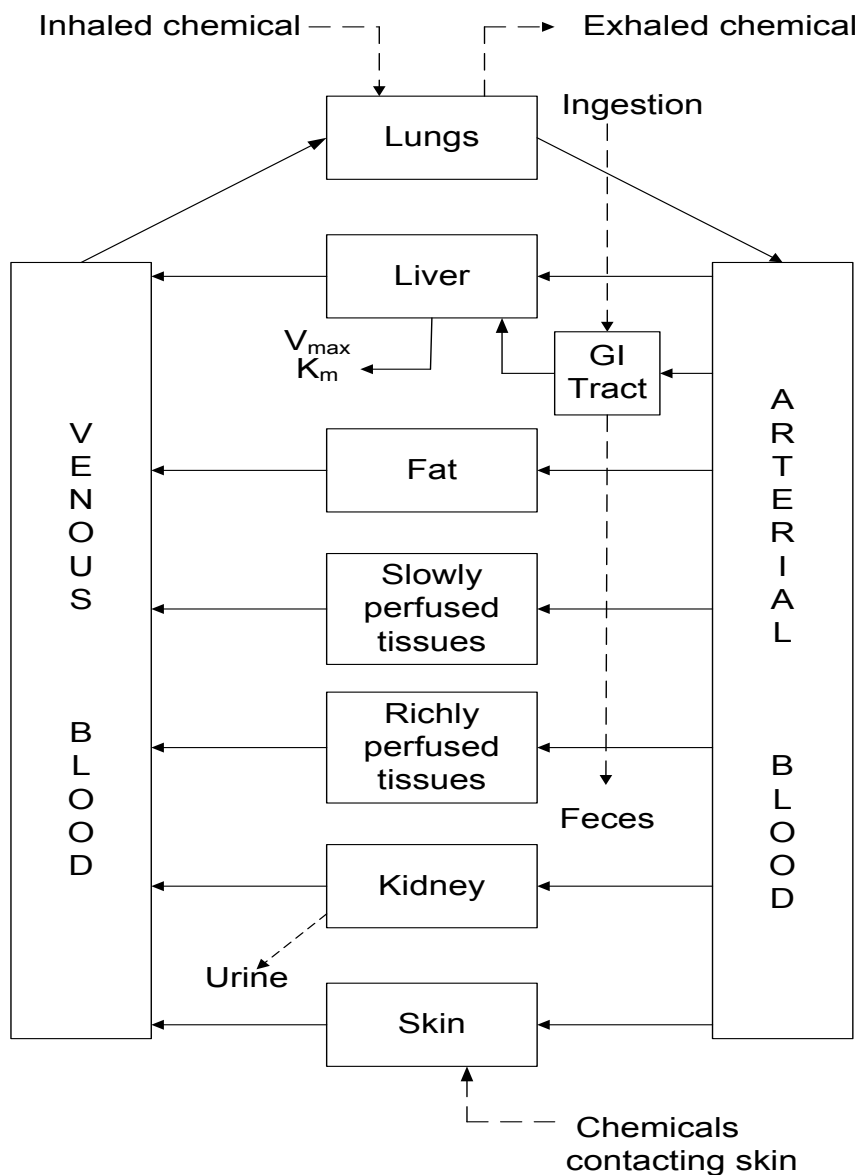
Literature on PBPK modeling of 1,3-butadiene is extensive (Beaudouin et al. 2010; Bois et al. 1999; Bond et al. 1996; Brochot et al. 2007; Csanady et al. 1996; Evelo et al. 1993; Filser et al. 1993; Johanson and Filser 1993, 1996; Kohn 1997; Kohn and Melnick 1993, 1996, 2000, 2001; Leavens and Bond 1996, Péry and Bois 2009; Seilken et al. 1996; Smith et al. 2001; Sweeney et al. 1996, 1997, 2001). Models have been developed to simulate 1,3-butadiene kinetics in mice (Bond et al. 1996; Csanady et al. 1996; Johanson and Filser 1993, 1996; Kohn and Melnick 1993, 1996, 2000, 2001; Leavens and Bond 1996; Sweeney et al. 1996, 1997, 2001), rats (Bond et al. 1996; Csanady et al. 1996; Johanson and Filser 1993, 1996; Kohn and Melnick 1993, 1996, 2000, 2001; Sweeney et al. 1996, 1997, 2001), and humans (Beaudouin et al. 2010; Bond et al. 1996; Brochot et al. 2007; Csanady et al. 1996; Evelo et al. 1993; Johanson and Filser 1996; Péry and Bois 2009). Model structures differ with respect to the number of physiological compartments simulated, the extent to which secondary and tertiary metabolites are simulated, and in which tissue compartments metabolism is assumed to occur. Selected examples are described in greater detail in the sections that follow.

### Johanson and Filser 1996

**Description of the Model.** The Johanson and Filser model (Filser et al. 1993; Johanson and Filser 1993, 1996) model simulates absorption and disposition of 1,3-butadiene and the metabolite, 3,4-epoxy-1-butene, in the mouse, rat, and human. The hepatic conjugation of 3,4-epoxy-1-butene to GSH is also simulated. Tissue compartments include the blood/lung, liver, fat, and muscle/richly-perfused tissues.

## 3. HEALTH EFFECTS

**Figure 3-3. Conceptual Representation of a Physiologically Based Pharmacokinetic (PBPK) Model for a Hypothetical Chemical Substance**



Note: This is a conceptual representation of a physiologically based pharmacokinetic (PBPK) model for a hypothetical chemical substance. The chemical substance is shown to be absorbed via the skin, by inhalation, or by ingestion, metabolized in the liver, and excreted in the urine or by exhalation.

Source: Adapted from Krishnan and Andersen 1994

## 3. HEALTH EFFECTS

Model parameters are presented in [Table 3-4](#). Both the exchange of 1,3-butadiene and 3,4-epoxy-1-butene between blood and tissue or lung air is assumed to be first-order and flow-limited. *In vitro* derivation of tissue:air and tissue:blood partition coefficients was performed by the model authors and reported in the study. Michaelis-Menten expressions were included for 1,3-butadiene oxidation, 3,4-epoxy-1-butene hydrolysis, and 3,4-epoxy-1-butene conjugation to GSH, all occurring in the liver compartment. Values for physiological (alveolar, pulmonary, and tissue perfusion rates, organ weights) and metabolic parameters ( $V_{\max}$ ,  $K_m$ , and GSH content and elimination rates) were taken from the literature, except for the affinity constant ( $K_m$ ) for 1,3-butadiene oxidation, which was fit to chamber air 1,3-butadiene timecourse data from rat and mouse closed chamber experiments. Elimination of 1,3-butadiene and 3,4-epoxy-1-butene was represented as either metabolism or as exchange passage back to the lung air.

**Risk Assessment.** This model has not been used in risk assessment. The model predicts that steady-state blood concentrations of 3,4-epoxy-1-butene resulting from continuous inhalation exposures to 1,3-butadiene would be higher in mice compared to rats or humans. At non-saturating conditions (e.g., exposures <1,000 ppm), the ratio of blood 3,4-epoxy-1-butene concentrations predicted from the model were: 1.6:1.0:0.3 for mouse:rat:human (Johanson and Filser 1996).

**Validation of the Model.** The  $K_m$  for 1,3-butadiene oxidation and 3,4-epoxy-1-butene hydrolysis were the only parameters optimized against close-chamber gas uptake data for 1,3-butadiene or 3,4-epoxy-1-butene (1,000–5,000 ppm) in rats (Filser and Bolt 1984) and mice (Kreiling et al. 1987). Model predictions were evaluated against the concentration of 3,4-epoxy-1-butene appearing in the chambers (due to metabolism and exhalation from the animals) during the 1,3-butadiene exposures, and were found to predict 3,4-epoxy-1-butene levels that were similar to observations.

**Target Tissues.** The model simulates concentrations of 1,3-butadiene and 3,4-epoxy-1-butene in liver, a target tissue for 1,3-butadiene metabolites, as well as in blood, fat, and a lumped compartment for muscle and richly-perfused tissues.

**Species Extrapolation.** The model has been developed for simulations of rats, mice, and humans. Extrapolation to other species would require species-specific physiological and metabolism parameter values and blood-tissue partition coefficients.

## 3. HEALTH EFFECTS

**Table 3-4. Physiological and Chemical Parameters Used in the Johanson and Filser (1993) PBPK Model for 1,3-Butadiene**

|                                                                 |                                                                              |  | Mouse                      | Rat                        |
|-----------------------------------------------------------------|------------------------------------------------------------------------------|--|----------------------------|----------------------------|
| Physiological data                                              |                                                                              |  |                            |                            |
| Body weight (bw)<br>(g)                                         | Standard animal                                                              |  | 25                         | 250                        |
|                                                                 | Simulations                                                                  |  | 27.5                       | 157.5–217.5                |
| Alveolar ventilation<br>(mL/minute)                             | Standard animal                                                              |  | 15                         | 70.2                       |
|                                                                 | Simulations                                                                  |  | proportional to $bw^{2/3}$ | proportional to $bw^{2/3}$ |
| Cardiac output                                                  | Standard animal                                                              |  | 17                         | 83                         |
|                                                                 | Simulations                                                                  |  | proportional to $bw^{2/3}$ | proportional to $bw^{2/3}$ |
| Blood flows<br>(percent of cardiac<br>output)                   | Muscle and vessel-rich group<br>(VRG)                                        |  | 66                         | 66                         |
|                                                                 | Fat                                                                          |  | 9                          | 9                          |
|                                                                 | Liver                                                                        |  | 25                         | 25                         |
|                                                                 | Lung and arterial                                                            |  | 1                          | 1                          |
| Compartment<br>volumes <sup>a</sup> (percent<br>of body weight) | Muscle and VRG                                                               |  | 75                         | 80                         |
|                                                                 | Fat                                                                          |  | 10                         | 7                          |
|                                                                 | Liver                                                                        |  | 5.5                        | 4                          |
|                                                                 |                                                                              |  |                            |                            |
| Tissue:air partition coefficients                               |                                                                              |  |                            |                            |
| Butadiene                                                       | Lung and arterial, muscle and<br>VRG, liver                                  |  | 0.76                       | 0.76                       |
|                                                                 | Fat                                                                          |  | 21.9                       | 21.9                       |
|                                                                 | Blood                                                                        |  | 3.03                       | 3.03                       |
| Epoxybutene                                                     | Lung and arterial, muscle and<br>VRG, liver                                  |  | 58.9                       | 58.9                       |
|                                                                 | Fat                                                                          |  | 155                        | 155                        |
|                                                                 | Blood                                                                        |  | 83.4                       | 83.4                       |
|                                                                 | Water                                                                        |  | 43.0                       | 43.0                       |
| Metabolic constants                                             |                                                                              |  |                            |                            |
| Butadiene oxidation                                             | Microsomal protein (mg/g liver)                                              |  | 30                         | 30                         |
|                                                                 | $V_{max}$ (nmol·minute <sup>-1</sup> ·mg <sup>-1</sup> )                     |  | 3.22                       | 2.17                       |
|                                                                 | $K_m$ (μmol/L air)                                                           |  | 5                          | 5                          |
| Epoxybutene<br>hydrolysis                                       | Microsomal protein (mg/g liver)                                              |  | 30                         | 30                         |
|                                                                 | $V_{max}$ (nmol·minute <sup>-1</sup> ·mg <sup>-1</sup> )                     |  | 19                         | 17                         |
|                                                                 | Apparent $K_m$ (mmol/L)                                                      |  | 1.5                        | 0.7                        |
|                                                                 | Intrinsic $K_m$ (percent of apparent<br>$K_m$ )                              |  | 20%                        | 20%                        |
| Epoxybutene<br>conjugation                                      | Cytosolic protein (mg/g liver)                                               |  | 95                         | 95                         |
|                                                                 | $V_{max}/K_m$ of epoxybutene<br>(μL·minute <sup>-1</sup> ·mg <sup>-1</sup> ) |  | 15                         | 11                         |
|                                                                 | $K_m$ towards epoxybutene<br>(mmol/L)                                        |  | 100                        | 100                        |
|                                                                 | $K_m$ towards glutathione (mmol/L)                                           |  | 0.1                        | 0.1                        |
| Glutathione kinetics                                            | Initial steady-state concentration<br>(mmol/L)                               |  | 5.5                        | 4.2                        |
|                                                                 | Elimination rate constant (hour <sup>-1</sup> )                              |  | 0.15                       | 0.15                       |

<sup>a</sup>Density was set to 1 for all organs

Source: Johanson and Filser 1993 (although simulations from a human model were reported in Johanson and Filser 1996, parameter values were not reported)

## 3. HEALTH EFFECTS

**High-low Dose Extrapolation.** The model has been evaluated for simulating inhalation exposures in mice and rats ranging from 500 to 5,000 ppm.

**Interroute Extrapolation.** The model simulates inhalation exposures only and would require additional parameterization to simulate exposures by other routes.

**Strengths and Limitations.** Strengths of the model are that it simulates disposition and clearance of inhaled 1,3-butadiene, as well as production and clearance of 3,4-epoxy-1-butene, the major oxidative metabolite formed in rodents. Limitations include: (1) the model has not been evaluated for inhalation exposures below 500 ppm; (2) the model does not simulate the appearance and disposition of other metabolites, such as the diepoxide, diols, and GSH-conjugate products eliminated in the urine; and (3) the model does not simulate 1,3-butadiene disposition in humans.

### Kohn and Melnick 2001

**Description of the Model.** The Kohn and Melnick model (Kohn and Melnick 1993, 1996, 2000, 2001) simulates absorption of 1,3-butadiene and the disposition of 1,3-butadiene and the metabolite, 3,4-epoxy-1-butene, in the mouse and rat. Model parameters are presented in [Tables 3-5, 3-6, and 3-7](#). The body is represented by discrete compartments for venous and arterial blood, lung, liver, kidney, fat, the gastrointestinal tract, and lumped compartments for richly- and poorly perfused tissues (viscera and muscle, respectively). Values for physiological flow rates, tissue volumes, and tissue:blood partition coefficients were taken from the literature. 1,3-Butadiene is eliminated by exhalation to lung air or oxidative metabolism to 3,4-epoxy-1-butene and its oxidation metabolites (3,4-epoxy-1-butene, DEB, 3,4-epoxy-1,2-diol, 3-butene-1,2-diol) and glutathione conjugates. All of these pathways are described as saturable Michaelis-Menten processes, with  $V_{\max}$  and  $K_m$  values optimized against published closed-chamber 1,3-butadiene and 3,4-epoxy-1-butene uptake data (Bolt et al. 1984; Filser and Bolt 1984; Kreiling et al. 1986b, 1987). Epoxide hydrolysis was modeled such that dissociation of the diepoxide from P450 in the microsome is followed by preferential binding of the epoxide hydrolase, replicating the so-called privileged access model for epoxide hydrolysis. The model simulates production and utilization of glutathione in kidney, liver, and lung, with the assumption that glutathione production is limited by availability of cysteine for the first step in glutathione synthesis ( $\gamma$ -glutamylcysteine synthetase).

**Risk Assessment.** This model has not been used in risk assessment.

## 3. HEALTH EFFECTS

**Table 3-5. Physiological Parameter Values Used in the Kohn and Melnick (2001) PBPK Model for 1,3-Butadiene**

|                                                 | Mouse (percent) | Rat (percent) |
|-------------------------------------------------|-----------------|---------------|
| Tissue compartment volumes, percent body weight |                 |               |
| Liver                                           | 5.5             | 3.7           |
| Lung                                            | 0.6             | 0.52          |
| Alveolar                                        | 0.5             | 0.515         |
| Kidney                                          | 1.67            | 1.48          |
| Gastrointestinal tract                          | 7.5             | 7.5           |
| Viscera                                         | 3.93            | 14.3          |
| Fat                                             | 6               | 7             |
| Muscle and skin                                 | 64.5            | 54.2          |
| Blood                                           | 6               | 5.4           |
| Capillary blood volume, percent tissue volume   |                 |               |
| Liver                                           | 11              | 13.8          |
| Lung                                            | 11              | 18            |
| Kidney                                          | 10.2            | 16            |
| Gastrointestinal tract                          | 2.9             | 2.65          |
| Viscera                                         | 7.1             | 7.1           |
| Fat                                             | 3               | 2             |
| Muscle and skin                                 | 1.3             | 2             |
| Blood flow rate, percent cardiac output         |                 |               |
| Liver (hepatic artery only)                     | 4.4             | 3.9           |
| Kidney                                          | 16.3            | 13.3          |
| Gastrointestinal tract                          | 18.1            | 18.1          |
| Viscera                                         | 22.4            | 24.8          |
| Fat                                             | 5               | 6.5           |
| Muscle and skin                                 | 33.8            | 33.4          |

Source: Kohn and Melnick 2001

## 3. HEALTH EFFECTS

**Table 3-6. Chemical Partition Coefficients Parameter Values Used in the Kohn and Melnick (2001) PBPK Model for 1,3-Butadiene**

|                        | Butadiene | Epoxybutane | Butenediol | Epoxybutanediol | Diepoxybutane |
|------------------------|-----------|-------------|------------|-----------------|---------------|
| Blood:air              | 1.95      | 56.8        | —          | —               | —             |
| Liver                  | 0.595     | 0.984       | 1.04       | 0.903           | 1.41          |
| Lung                   | 0.615     | 0.977       | 1.107      | 0.958           | 1.41          |
| Kidney                 | 0.472     | 0.842       | 0.962      | 0.833           | 1.54          |
| Gastrointestinal tract | 0.446     | 0.908       | 1.22       | 1.06            | 1.41          |
| Viscera                | 0.446     | 0.908       | 1.22       | 1.06            | 1.41          |
| Fat                    | 10.8      | 2.25        | 0.573      | 0.496           | 2.19          |
| Muscle and skin        | 0.564     | 0.736       | 1.139      | 0.986           | 1.82          |

Source: Kohn and Melnick 2001

## 3. HEALTH EFFECTS

**Table 3-7. Chemical Metabolism Parameter Values Used in the Kohn and Melnick (2001) PBPK Model for 1,3-Butadiene<sup>a</sup>**

|                                   | Liver                 | Lung                    | Kidney                  |
|-----------------------------------|-----------------------|-------------------------|-------------------------|
| <b>Butadiene metabolism</b>       |                       |                         |                         |
| $V_{\max}^{P450}$                 | 155, <b>130</b>       | 139, <b>9.6</b>         | 1,430, <b>30</b>        |
| $K_m^{P450}$                      | 0.002, <b>0.00375</b> | 0.00501, <b>0.00775</b> | 0.00501, <b>0.00216</b> |
| <b>Epoxybutene metabolism</b>     |                       |                         |                         |
| $V_{\max}^{P450}$                 | 45.1, <b>24.3</b>     | 10.2, <b>9.84</b>       | 48.6, <b>12.6</b>       |
| $K_m^{P450}$                      | 0.0156, <b>0.145</b>  | 0.0156, <b>0.145</b>    | 0.0156, <b>0.145</b>    |
| $V_m^{EH}$                        | 347, <b>584</b>       | 34.8, <b>42.8</b>       | 113, <b>14.7</b>        |
| $K_m^{EH}$                        | 1.59, <b>0.26</b>     | 1.59, <b>0.7</b>        | 1.59, <b>0.7</b>        |
| $V_{\max}^{GST}$                  | 6,420, <b>4,260</b>   | 720, <b>196</b>         | 960, <b>494</b>         |
| Adjusted $K_{mx}^{GST}$           | 3.59, <b>2.59</b>     | 3.59, <b>4.94</b>       | 3.59, <b>4.39</b>       |
| <b>Butenediol metabolism</b>      |                       |                         |                         |
| $V_{\max}^{P450}$                 | 16.3, 67.1            | 1.0, 31.5               | 1.0, <b>85.0</b>        |
| $K_m^{P450}$                      | 0.0156, <b>0.145</b>  | 0.0156, <b>0.145</b>    | 0.0156, <b>0.145</b>    |
| $V_{\max}^{GST}$                  | 3,480, 1,230          | 491, 276                | 1,070, <b>658</b>       |
| $K_{mx}^{GST}$                    | 34, <b>34</b>         | 34, <b>34</b>           | 34, <b>34</b>           |
| <b>Epoxybutanediol metabolism</b> |                       |                         |                         |
| $V_{\max}^{EH}$                   | 363, 1,150            | 69.5, <b>169</b>        | 10.0, <b>152</b>        |
| $K_m^{EH}$                        | 8.1, <b>2.76</b>      | 7.5, <b>7.1</b>         | 7.5, <b>7.1</b>         |
| $V_{\max}^{GST}$                  | 2,260, <b>271</b>     | 50.0, <b>100</b>        | 50.0, <b>138</b>        |
| $K_{mx}^{GST}$                    | 6.40, <b>4.17</b>     | 6.40, <b>4.17</b>       | 6.40, <b>4.17</b>       |
| <b>Diepoxybutane metabolism</b>   |                       |                         |                         |
| $V_{\max}^{EH}$                   | 1,920, <b>3,170</b>   | 10.0, <b>1,160</b>      | 35.2, <b>1,000</b>      |
| $K_m^{EH}$                        | 8.1, <b>2.76</b>      | 7.5, <b>7.1</b>         | 7.5, <b>7.1</b>         |
| $V_{\max}^{GST}$                  | 9,720, <b>1,940</b>   | 100, 100                | 100, 100                |
| Adjusted $K_{mx}^{GST}$           | 6.40, <b>4.17</b>     | 6.40, <b>4.17</b>       | 6.40, <b>4.17</b>       |
| <b>Cysteine metabolism</b>        |                       |                         |                         |
| Tissue cysteine                   | 0.193, <b>0.195</b>   | 0.171, <b>0.127</b>     | 0.280, <b>0.326</b>     |
| $V_{\max}^{\gamma\text{-GCS}}$    | 420, <b>396</b>       | 54, <b>50</b>           | 7,920, <b>6,080</b>     |

EH = epoxide hydrolase;  $\gamma$ -GCS = gamma-glutamylcysteine synthetase; GST = glutathione S-transferase

<sup>a</sup>Non-bold values for mouse; bold values for rat; values in italics were estimated by formal optimization; entries in italics indicate optimized parameter values;  $V_{\max}$  values in nmol/hour/mg of protein;  $K_m$  values and tissue cysteine concentrations in mM.

Source: Kohn and Melnick 2001



## 3. HEALTH EFFECTS

**Validation of the Model.** The model predicted similar profiles of 1,3-butadiene and 3,4-epoxy-1-butene uptake data (100–4,000 ppm) in mice and rats against which it was optimized. Further, it predicted single time point concentrations of 1,3-butadiene in mice and rats similar to observations from nose-only exposures to 7–1,250 ppm (Bond et al. 1986; Himmelstein et al. 1994). Additionally, it predicted similar percentages of GSH depletion in mouse and rat lung and liver observed following 7-hour 1,3-butadiene exposures of 50–2,000 ppm (Deutschmann and Laib 1989).

**Target Tissues.** The model simulates concentrations of 1,3-butadiene and 3,4-epoxy-1-butene in lung, liver, and kidney, all target tissues for 1,3-butadiene metabolite intoxication, as well as in blood, fat, gastrointestinal tract, and lumped compartments for muscle and viscera.

**Species Extrapolation.** The model has been developed for simulations of rats and mice. Extrapolation of predictions to humans would require additional species-specific values for physiology and metabolism, as well as human data to verify the accuracy of the predictions.

**High-low Dose Extrapolation.** The model has been evaluated for simulating inhalation exposures ranging from 7 to 4,000 ppm.

**Interroute Extrapolation.** The model simulates inhalation exposures only and would require additional parameterization to simulate exposures by other routes.

**Strengths and Limitations.** Strengths of the model are that it simulates disposition and clearance of inhaled 1,3-butadiene, as well as production and clearance of the major oxidative metabolites of 1,3-butadiene in kidney, liver, and lung, including 3,4-epoxy-1-butene, 1,2:3,4-diepoxymethane, 3,4-epoxy-1,2-diol, and 3-butene-1,2-diol. It also simulates production and utilization of GSH in the kidney, lung, and liver, which allows prediction of glutathione depletion resulting from 1,3-butadiene metabolism. Limitations include: (1) the model has not been evaluated against data for inhalation exposures in humans, and (2) the model does not simulate the appearance and disposition of other metabolites, such as the diepoxide, diols, and GSH-conjugate products eliminated in the urine.

## 3. HEALTH EFFECTS

**Brochot et al. 2007**

**Description of the Model.** The Brochot model (Brochot and Bois 2005; Brochot et al. 2007) simulates absorption of 1,3-butadiene and the disposition of 1,3-butadiene, 1,2-epoxy-3-butene, and 1,2:3,4-diepoxibutane in the blood, fat, and lumped compartments for richly- and poorly-perfused tissues in humans. Model parameters are presented in [Table 3-8](#). In addition, the disposition and clearance of 3-butene-1,2-diol and 3,4-epoxy-1,2-butanediol was modeled for the blood and richly- and poorly-perfused tissues. Tissue:blood partition coefficients were taken from the literature. 1,3-Butadiene is eliminated by exhalation to lung air or oxidative metabolism, epoxide hydrolysis, or GSH conjugation in the richly-perfused tissues to mono- and diepoxide and the two diols. Since the model was intended to direct further study design for low-ppm human exposures, all of the metabolic steps are described as 1<sup>st</sup>-order processes, governed by a rate constant for each pathway. The metabolic rate constants and physiological parameters were optimized using Bayesian techniques against 133 datasets from individual subjects inhaling 2 ppm 1,3-butadiene for 20 minutes (Lin et al. 2001). Several extensions of the Brochot et al. (2007) model have been reported. A 23-compartment model was developed for simulating 1,3-butadiene and 1,2-epoxy-3-butene kinetics in humans (Péry and Bois 2009). The model simulates flow-limited distribution to each tissue with partitioning assumed to occur predominantly into tissue fat (estimated from the fat:blood partition coefficient and fat content of each tissue). Beaudouin et al. (2010) further extended this model to a generic human lifetime PBPK model that included growth and transplacental transfer to the fetus.

**Risk Assessment.** This model has not been used in risk assessment.

**Validation of the Model.** The model fit well against the human data from which it was calibrated. Its performance has not been evaluated against other independent human inhalation data.

**Target Tissues.** The model predicts parent compound, mono- and diepoxide, and vicinal thiol (including the epoxydiol) concentrations in the blood, but not in other tissues.

**Species Extrapolation.** The model was optimized for humans. Extrapolation of predictions to animals would require additional species-specific values for physiology and metabolism, as well as animal data (available in the literature) to verify the accuracy of the predictions.

## 3. HEALTH EFFECTS

**Table 3-8. Physiological and Chemical Parameters Used in the Brochot et al. 2007 PBPK Model for 1,3-Butadiene Humans**

| Parameter                                         | Mean <sup>a</sup> | Standard deviation <sup>a</sup> |
|---------------------------------------------------|-------------------|---------------------------------|
| Body weight (kg)                                  | 70                |                                 |
| Sex                                               | M                 |                                 |
| Metabolic activity in liver (mg protein/kg liver) |                   |                                 |
| Microsomal protein                                | 14,500            |                                 |
| Cytosolic protein                                 | 45,000            |                                 |
| Relative weight (percent of body weight)          |                   |                                 |
| Liver                                             | 0.026             |                                 |
| Relative volumes (percent of body weight)         |                   |                                 |
| Well-perfused tissues                             | 0.10              | 0.02                            |
| Fat                                               | 0.21              | 0.05                            |
| Relative flows (percent of total blood flow)      |                   |                                 |
| Poorly perfused tissues                           | 0.26              | 0.06                            |
| Fat                                               | 0.05              | 0.01                            |
| Pulmonary characteristics                         |                   |                                 |
| Minute volume (L/minute)                          | 7.5               | 1.87                            |
| Ventilation perfusion ratio                       | 1.0               | 0.25                            |
| Dead space fraction                               | 0.33              | 0.08                            |
| Partition coefficients for BD                     |                   |                                 |
| Blood:air                                         | 1.22              | 0.30                            |
| Poorly perfused tissues:blood                     | 0.72              | 0.18                            |
| Well-perfused tissues:blood                       | 0.69              | 0.17                            |
| Fat:blood                                         | 18.4              | 4.6                             |
| Partition coefficients for EB                     |                   |                                 |
| Blood:air                                         | 93.3              | 23.3                            |
| Poorly perfused tissues:blood                     | 0.49              | 0.12                            |
| Well-perfused tissues:blood                       | 0.59              | 0.15                            |
| Fat:blood                                         | 1.80              | 0.45                            |
| Partition coefficients for DEB                    |                   |                                 |
| Poorly perfused tissues:blood                     | 1.98              | 0.49                            |
| Well-perfused tissues:blood                       | 1.53              | 0.38                            |
| Fat:blood                                         | 2.20              | 0.55                            |
| Partition coefficients for BDD                    |                   |                                 |
| Poorly perfused tissues:blood                     | 1.00              | 0.25                            |
| Well-perfused tissues:blood                       | 1.00              | 0.25                            |
| Partition coefficients for EBD                    |                   |                                 |
| Poorly perfused tissues:blood                     | 1.00              | 0.25                            |
| Well-perfused tissues:blood                       | 1.00              | 0.25                            |
| Metabolic constants (minute <sup>-1</sup> )       |                   |                                 |
| BD → EB                                           | 0.119             | 0.06                            |

## 3. HEALTH EFFECTS

**Table 3-8. Physiological and Chemical Parameters Used in the Brochot et al. 2007 PBPK Model for 1,3-Butadiene Humans**

| Parameter                                 | Mean <sup>a</sup> | Standard deviation <sup>a</sup> |
|-------------------------------------------|-------------------|---------------------------------|
| Metabolic constants                       |                   |                                 |
| EB → DEB                                  | 0.020             | 0.01                            |
| EB → BDD                                  | 0.511             | 0.25                            |
| DEB → EBD                                 | 0.471             | 0.23                            |
| BDD → EBD                                 | 0.008             | 0.01                            |
| Other transformations (L/kg liver/minute) |                   |                                 |
| EB by GSH                                 | 0.195             | 0.10                            |
| DEB by GSH                                | 0.113             | 0.06                            |
| EBD by GSH                                | 0.056             | 0.03                            |
| EBD by hydrolysis                         | 0.235             | 0.12                            |
| BDD by ADH                                | 0.045             | 0.02                            |

<sup>a</sup>Mean and standard deviation of lognormal distributions, reflecting variability in the human population.

ADH = alcohol dehydrogenase; BD = 1,3-butadiene; BDD = 3-butene-1,2-diol; DEB = 1,2:3,4-diepoxybutane; EB = 1,2-epoxy-3-butene; EBD = 3,4-epoxy-1,2-butanediol; GSH = glutathione

Source: Brochot et al. 2007

## 3. HEALTH EFFECTS

**High-low Dose Extrapolation.** The model was calibrated for low (2 ppm) exposures in humans. Extrapolation to higher doses will require that the metabolic expression be modified to account for saturation of the oxidative, hydrolytic, and conjugating pathways described.

**Interroute Extrapolation.** The model was designed to simulate inhalation exposures. Additional parameters and absorption expressions must be added (and optimized with oral or dermal data) in order to extrapolate internal dosimetry from inhalation exposures across other routes of exposure. Parameters for gastrointestinal absorption of 1,3-butadiene were reported for use in a generic lifetime model (Beaudouin et al. 2010).

**Strengths and Limitations.** The model is the first PBTK model to simultaneously predict blood levels of 1,3-butadiene and its epoxide and vicinal diol metabolites in humans. Limitations include: (1) the model has not been evaluated against data for inhalation exposures in animals, and (2) the model does not account for saturation of metabolic pathways that may occur in humans (and have been observed in rodents) exposed to higher inhalation concentrations of 1,3-butadiene.

**Sweeney et al. 2001**

**Description of the Model.** The Sweeney et al. (1996, 1997, 2001) model simulates inhalation absorption of 1,3-butadiene and the disposition of 1,3-butadiene, 1,2-epoxy-3-butene, and 1,2:2,3 diepoxybutane in mice, rats, and humans. Blood, fat, liver, and lumped compartments for richly- and poorly-perfused tissues are simulated. Model parameters are presented in [Table 3-9](#), [3-10](#), [3-11](#), [3-12](#), [3-13](#), [3-14](#), and [3-15](#). 1,3-Butadiene is eliminated by exhalation to lung air or oxidative metabolism to 1,3-epoxide-3-butene or to other products (e.g., aldehydes) in liver. The monoepoxide (1,3-epoxide-3-butene) undergoes further oxidative metabolism to the diepoxide (DEB) and both the mono- and diepoxide undergo epoxide hydrolysis, GSH conjugation, or nonenzymatic degradation. Enzymatic reactions are simulated as saturable reactions ( $K_m$ ,  $V_{max}$ ) and non-enzymatic elimination reactions are simulated as first-order reactions. Production and utilization of glutathione in liver are simulated, which allows prediction of glutathione depletion resulting from 3,4-epoxy-1-butene metabolism.

**Risk Assessment.** This model has not been used in risk assessment.

## 3. HEALTH EFFECTS

**Table 3-9. Physiological Parameters Used in Sweeney et al. (1997) 1,3-Butadiene PBPK Model**

| Parameter                                                                                | Rat         | Mouse       |
|------------------------------------------------------------------------------------------|-------------|-------------|
| Alveolar ventilation <sup>a</sup> ( $Q_{pu}$ ) (L/hour/kg)                               | 17          | 41          |
| Cardiac output <sup>a</sup> ( $Q_t$ ) (L/hour/kg)                                        | 17          | 41          |
| Body weight (BW) (kg)                                                                    | 0.215–0.475 | 0.028–0.035 |
| Blood flow <sup>b</sup> ( $Q_i$ ) (fraction of cardiac output) ( $F_i$ ) (dimensionless) |             |             |
| Lung                                                                                     | 1.0         | 1.0         |
| Fat                                                                                      | 0.09        | 0.09        |
| Slowly perfused tissues                                                                  | 0.15        | 0.15        |
| Richly perfused tissues                                                                  | 0.51        | 0.51        |
| Liver                                                                                    | 0.25        | 0.25        |
| Organ volumes <sup>c</sup> ( $V_i$ ) (fraction of body weight) (dimensionless)           |             |             |
| Lung                                                                                     | 0.0053      | 0.005       |
| Fat                                                                                      | 0.09        | 0.10        |
| Slowly perfused tissues                                                                  | 0.71        | 0.7         |
| Richly perfused tissues                                                                  | 0.0347      | 0.0226      |
| Liver                                                                                    | 0.05        | 0.0624      |

<sup>a</sup>Alveolar ventilation and cardiac output are given for a hypothetical 1-kg animal. In the model simulations, the parameter is multiplied by the body weight of the animal (in kg) to calculate the ventilation rate and cardiac output (in L/hour) for that individual animal.

<sup>b</sup>Tissue blood flows are calculated by multiplying the total cardiac output by the fractional flow:  $Q_i = F_i \times Q_t$ .

<sup>c</sup>Tissue volumes are calculated by multiplying the body weight by the fractional volume:  $V_i = F_i \times BW$ .

Source: Sweeney et al. 1997

## 3. HEALTH EFFECTS

**Table 3-10. Partition Coefficients Used in Sweeney et al. (1997) PBPK Model for 1,3-Butadiene**

| Tissue | Butadiene <sup>a</sup> |       | Epoxybutene <sup>a</sup> |       | Diepoxybutane <sup>b</sup> |
|--------|------------------------|-------|--------------------------|-------|----------------------------|
|        | Rat                    | Mouse | Rat                      | Mouse | Mouse                      |
| Blood  | 1.49                   | 1.34  | 50.4                     | 36.6  | 0.437                      |
| Liver  | 1.19                   | 1.35  | 72.0                     | 42.1  | 0.615                      |
| Lung   | 0.92                   | 1.47  | 54.7                     | 56.3  | ND                         |
| Kidney | ND                     | ND    | ND                       | ND    | ND                         |
| Muscle | 1.47                   | 4.01  | 19.8                     | 23.6  | 0.795                      |
| Fat    | 22.2                   | 19.2  | 138.0                    | 91.2  | 0.959                      |
| Saline | 0.088                  |       | 44.3                     |       | 0.723                      |
| Oil    | 23.2                   |       | 164                      |       | ND                         |

<sup>a</sup>Tissue:air partition coefficients.<sup>b</sup>Tissue:hexane partition coefficient.

ND = not determined

Source: Sweeney et al. 1997

## 3. HEALTH EFFECTS

**Table 3-11. Nonenzymatic Reaction Rate Constants Used in Sweeney et al. (1997)  
PBPK Model for 1,3-Butadiene**

| Tissue | Epoxybutene |       | Diepoxybutane |       |
|--------|-------------|-------|---------------|-------|
|        | Rat         | Mouse | Rat           | Mouse |
| Blood  | 0.582       | 0.558 | ND            | 0.189 |
| Liver  | 4.94        | 4.14  | ND            | 3.15  |
| Lung   | 6.07        | 2.70  | ND            | 4.1   |
| Fat    | 1.72        | 1.56  | ND            | 2.8   |
| Muscle | 0           | 0     | ND            | 0     |

ND = not determined

Source: Sweeney et al. 1997



## 3. HEALTH EFFECTS

**Table 3-12. Metabolism Rate Constants Used in the Sweeney et al. (1997) PBPK Model for 1,3-Butadiene**

| Substrate     | Tissue | Pathway                 | Units        | Parameter value |         |
|---------------|--------|-------------------------|--------------|-----------------|---------|
|               |        |                         |              | Rat             | Mouse   |
| Butadiene     | Liver  | Oxidation               | μmol/kg/hour | 62              | 338     |
|               |        | (to all products)       | μmol/L       | 3.75            | 2.0     |
|               |        | Oxidation               | μmol/kg/hour | 8.2             | 97      |
|               |        | (to epoxybutene only)   | μmol/L       | 1.54            | 0.88    |
|               |        | Oxidation               | μmol/kg/hour | 54              | 243     |
|               |        | (to other volatiles)    | μmol/L       | 4.36            | 2.72    |
|               | Lung   | Oxidation               | μmol/kg/hour | 1.01            | 21.6    |
|               |        | (to all products)       | μmol/L       | 7.75            | 5.01    |
|               |        | Oxidation               | μmol/kg/hour | 0.13            | 6.4     |
|               |        | (to epoxybutene only)   | μmol/L       | 3.18            | 1.6     |
|               |        | Oxidation               | μmol/kg/hour | 0.88            | 16.1    |
|               |        | (to other volatiles)    | μmol/L       | 9.14            | 9.5     |
| Epoxybutene   | Liver  | Oxidation               | μmol/kg/hour | 57.1            | 176.6   |
|               |        | (one enzyme)            | μmol/L       | 141             | 145     |
|               |        | Oxidation               | μmol/kg/hour | 10              | 32.5    |
|               |        | (two enzymes)           | μmol/L       | 141             | 15.6    |
|               |        |                         | μmol/kg/hour | 47.1            | 144.1   |
|               |        |                         | μmol/L       | 141             | 145     |
|               |        | Hydrolysis              | μmol/kg/hour | 260             | 754     |
|               |        |                         | μmol/L       | 260             | 1,590   |
|               |        | Glutathione conjugation | μmol/kg/hour | 78,100          | 154,000 |
|               |        |                         | μmol/L       | 13,800          | 35,300  |
|               |        |                         | μmol/L       | 100             | 100     |
|               |        | Glutathione conjugation | μmol/kg/hour | 819             | 4,088   |
| Diepoxybutane | Liver  |                         | μmol/L       | 17,400          | 36,500  |
|               |        | Hydrolysis              | μmol/kg/hour | 5,555           | 4,193   |
|               |        |                         | μmol/L       | 2,700           | 8,100   |
|               |        | Glutathione conjugation | μmol/kg/hour | 60,264          | 50,342  |
|               |        |                         | μmol/L       | 24,000          | 6,400   |
|               |        |                         |              |                 |         |
|               | Lung   | Hydrolysis              | μmol/kg/hour | 122.7           | 466.1   |
|               |        |                         | μmol/L       | 7,100           | 7,500   |
|               |        | Glutathione conjugation | μmol/kg/hour | 332             | 577     |
|               |        |                         | μmol/L       | 4,170           | 1,700   |

Source: Sweeney et al. 1997

## 3. HEALTH EFFECTS

**Table 3-13. Physiological Parameters Used in the Sweeney et al. (2010) PBPK Model for 1,3-Butadiene in Humans**

| Parameter                           | Value  | Units                 | Comment           |
|-------------------------------------|--------|-----------------------|-------------------|
| Alveolar ventilation rate (QPC)     | 4.3    | L/hour/kg body weight | QP = QPC x BW     |
| Cardiac output (QCC)                | 4.5    | L/hour/kg body weight | QC = QCC x BW     |
| Fractional blood flow to liver      | 0.227  | Dimensionless         | QR = 0.76 x QC-QL |
| Fractional blood flow to fat        | 0.052  | Dimensionless         | QS = 0.24 x QC-QF |
| Fractional weight of liver          | 0.027  | Dimensionless         |                   |
| Fractional weight of lung           | 0.0076 | Dimensionless         | VR = 0.09-VL-VLU  |
| Fractional weight of fat            | 0.2142 | Dimensionless         | VS = 0.81-VF      |
| Body weight                         | 70     | kg                    |                   |
| Cytosolic protein content of liver  | 89,000 | mg protein/kg liver   | Rat value         |
| Microsomal protein content of liver | 77,000 | mg protein/kg liver   | Human value       |

Source: Sweeney et al. 2001

## 3. HEALTH EFFECTS

**Table 3-14. Chemical Partition Coefficients Used in the Sweeney et al. (2010) PBPK Model for 1,3-Butadiene in Humans**

| Parameter                    | Value | Comment                                                                                                 |
|------------------------------|-------|---------------------------------------------------------------------------------------------------------|
| <b>1,3-Butadiene</b>         |       |                                                                                                         |
| Blood:air                    | 1.22  | Average of individual values for humans                                                                 |
| Liver:air                    | 0.68  | Human tissue                                                                                            |
| Fat:air                      | 22.5  | Human tissue                                                                                            |
| Lung:air                     | 0.48  | Human tissue                                                                                            |
| Slowly perfused tissue:air   | 0.88  | Value for human muscle                                                                                  |
| Richly perfused tissue:air   | 0.84  | Values for human kidney, brain, and liver weighted by contribution to body weight                       |
| <b>Butadiene monoepoxide</b> |       |                                                                                                         |
| Blood:air                    | 93.3  | Human tissue                                                                                            |
| Liver:air                    | 55.3  | Human tissue                                                                                            |
| Fat:air                      | 168   | Human tissue                                                                                            |
| Lung:air                     | 55.3  | Value measured for human liver                                                                          |
| Slowly perfused tissue:air   | 45.8  | Value measured for human muscle                                                                         |
| Richly perfused tissue:air   | 55.3  | Value measured for human liver                                                                          |
| <b>Butene diol</b>           |       |                                                                                                         |
| Liver:blood                  | 1     | Volume of distribution=0.87 L/kg in mouse; mouse model has perfused tissues=0.9 kg/kg total body weight |
| Rest of body:blood           | 1     | Volume of distribution=0.87 L/kg in mouse; mouse model has perfused tissues=0.9 kg/kg total body weight |
| <b>Butadiene diepoxide</b>   |       |                                                                                                         |
| Liver:blood                  | 1.53  | Rat tissue                                                                                              |
| Fat:blood                    | 2.2   | Rat tissue                                                                                              |
| Lung:blood                   | 1.53  | Rat liver value                                                                                         |
| Slowly perfused tissue:air   | 1.82  | Rat muscle value                                                                                        |
| Richly perfused tissue:air   | 1.41  | Rat kidney value                                                                                        |
| <b>Epoxybutane diol</b>      |       |                                                                                                         |
| Liver:blood                  | 1     | Assumption based on butene diol and butadiene diepoxide partition coefficients                          |
| Rest of body:blood           | 1     | Assumption based on butene diol and butadiene diepoxide partition coefficients                          |

Source: Sweeney et al. 2001

## 3. HEALTH EFFECTS

**Table 3-15. Chemical Metabolism Parameters Used in the Sweeney et al. (2010) PBPK Model for 1,3-Butadiene in Humans**

| Parameter (units)                                                                                                               | Baseline value <sup>a</sup> |
|---------------------------------------------------------------------------------------------------------------------------------|-----------------------------|
| $V_{\max}$ for epoxidation of 1,3-butadiene to butadiene monoepoxide ( $\mu\text{mol}/\text{mg}$ protein/hour)                  | 0.0132                      |
| $K_m$ for epoxidation of 1,3-butadiene to butadiene monoepoxide ( $\mu\text{M}$ )                                               | 0.7                         |
| $V_{\max}$ for epoxidation of butadiene monoepoxide to butadiene diepoxide ( $\mu\text{mol}/\text{mg}$ microsomal protein/hour) | 0.031                       |
| $K_m$ for epoxidation of butadiene monoepoxide to butadiene diepoxide ( $\mu\text{M}$ )                                         | 880                         |
| $V_{\max}$ for hydrolysis of butadiene monoepoxide to butene diol                                                               | 1.4                         |
| $K_m$ for hydrolysis of butadiene monoepoxide to butene diol                                                                    | 540                         |
| $V_{\max}$ for conjugation of butadiene monoepoxide and glutathione ( $\mu\text{mol}/\text{mg}$ cytosolic protein/hour)         | 2.7                         |
| $K_m$ for conjugation of butadiene monoepoxide and glutathione ( $\mu\text{M}$ )                                                | 10,400                      |
| $V_{\max}$ for conjugation of butadiene diepoxide and glutathione ( $\mu\text{mol}/\text{mg}$ cytosolic protein/hour)           | 0.4                         |
| $K_m$ for conjugation of butadiene diepoxide and glutathione ( $\mu\text{M}$ )                                                  | 3,390                       |
| $V_{\max}$ for epoxidation of butene diol to epoxybutane diol ( $\mu\text{mol}/\text{mg}$ microsomal protein/hour)              | 0.031                       |
| $K_m$ for epoxidation of butene diol to epoxybutane diol ( $\mu\text{M}$ )                                                      | 880                         |
| $V_{\max}$ for hydrolysis of butadiene diepoxide to epoxybutane diol ( $\mu\text{mol}/\text{mg}$ microsomal protein/hour)       | 9.2                         |
| $K_m$ for hydrolysis of butadiene diepoxide to epoxybutane diol ( $\mu\text{M}$ )                                               | 4,605                       |
| $V_{\max}$ for metabolism of butene diol by alcohol dehydrogenase ( $\mu\text{mol}/\text{mg}$ cytosolic protein/hour)           | 0.64                        |
| $K_m$ for metabolism of butene diol by alcohol dehydrogenase ( $\mu\text{M}$ )                                                  | 10,600                      |
| $V_{\max}$ for hydrolysis of epoxybutane diol to erythritol ( $\mu\text{mol}/\text{mg}$ microsomal protein/hour)                | 4.6                         |
| $K_m$ for hydrolysis of epoxybutane diol to erythritol ( $\mu\text{M}$ )                                                        | 4,605                       |
| $V_{\max}$ for conjugation of epoxybutane diol and glutathione ( $\mu\text{mol}/\text{mg}$ cytosolic protein/hour)              | 0.2                         |
| $K_m$ for conjugation of epoxybutane diol and glutathione ( $\mu\text{M}$ )                                                     | 3,390                       |

<sup>a</sup>Median or average parameter values.

Source: Sweeney et al. 2001

### 3. HEALTH EFFECTS

**Validation of the Model.** The model was calibrated and evaluated against data from intravenous studies in rats and inhalation exposures of mice and rats (Sweeney et al. 1997) and humans (Sweeney et al. 2001).

**Target Tissues.** The model has been used to predict parent compound, monoepoxide, and diepoxide concentrations in the blood.

**Species Extrapolation.** Mouse, rat, and human models have been developed. Extrapolation of predictions to animals would require additional species-specific values for physiology and metabolism, as well as animal data (available in the literature) to verify the accuracy of the predictions.

**High-low Dose Extrapolation.** The model has been evaluated for simulating inhalation exposures in mice and rats ranging from 60 to 1,250 ppm in rodents and 5 ppm in humans.

**Interroute Extrapolation.** The model was designed to simulate inhalation exposures. Additional parameters and absorption expressions must be added (and optimized with oral or dermal data) in order to extrapolate internal dosimetry from inhalation exposures across other routes of exposure.

**Strengths and Limitations.** Strengths of this model include simulation of an alternative oxidative pathway for 1,3-butadiene (other than leading to 1,2-epoxy-3-butene); simulations of both enzymatic and nonenzymatic elimination of 1,3-butadiene metabolites; and simulation of production and utilization of glutathione.

## 3.5 MECHANISMS OF ACTION

### 3.5.1 Pharmacokinetic Mechanisms

**Formation of Reactive Metabolites.** The role of metabolism of 1,3-butadiene to reactive metabolites and the importance of species differences in metabolism of 1,3-butadiene to human health risk assessment were recently reviewed by Kirmam et al. (2010a). As discussed in Section 3.4.3 (Toxicokinetics/Metabolism; also see [Figure 3-2](#)), 1,3-butadiene is metabolized by oxidation, hydrolysis, and conjugation reactions, with oxidation and hydrolysis reactions leading to the formation of several reactive epoxide intermediates. Of the reactive intermediates formed, EB (formed by oxidation of 1,3-butadiene), DEB (formed by oxidation of EB), and EBD (formed by hydrolysis reactions of DEB) are reactive electrophilic

## 3. HEALTH EFFECTS

compounds that have been shown to interact with DNA. The order of genotoxic potency of the epoxide metabolites is DEB >> EB > EBD. The higher genotoxic potency of DEB may be due to its ability to bind to two molecules or two places in the molecule at the same time (e.g., DNA-protein cross-links or DNA cross-links) (see discussion below in Section 3.5.2 on mechanisms of genotoxicity) (Albertini et al. 2010). Thus, the formation of reactive metabolites is critical to the genotoxic activity of 1,3-butadiene. Although little information was identified regarding the role of reactive metabolites in the development of other adverse effects of 1,3-butadiene (e.g., nongenotoxic, noncancer), given their reactive nature, it is likely that reactive epoxide metabolites play an important role in the development of other adverse effects.

**Species Differences in Metabolism.** Metabolism of 1,3-butadiene appears to follow the same enzymatic pathways in all species, including humans, with production of the same reactive intermediates. However, as discussed below, important species differences exist in the rates of formation and detoxification of reactive metabolites (Bond et al. 1993; Csanády et al. 1992; Dahl et al. 1991; Filser et al. 2001, 2007, 2010; Himmelstein et al. 1997; Henderson et al. 1996, 2001; Kirman et al. 2010a; Krause and Elfarra 1997; Schmidt and Loeser 1985; Thornton-Manning et al. 1995a). As a result, rodents, particularly mice, have much higher tissue levels of reactive metabolites than nonhuman primates and humans. Therefore, based on the assumption that the same mechanism of action is involved in the development of 1,3-butadiene-induced toxicity (i.e., interaction of the reactive metabolites with DNA and other cellular macromolecules) is the same for all species, mice are expected to be much more sensitive to 1,3-butadiene than other rats, nonhuman primates, and humans.

As reviewed by Kirman et al. (2010a), evidence for species differences in metabolism of 1,3-butadiene is available from *in vitro* studies, studies using isolated perfused livers, and *in vivo* studies measuring tissue and urine metabolite levels and blood hemoglobin adduct levels. Results of *in vitro* studies using hepatic microsomal fractions isolated from mice, rats, and humans show that conversion of EB to DEB in mice is 3.3-fold greater than in rats and 2.4–61-fold greater than in humans. Studies in isolated perfused livers show differences in metabolism of 1,3-butadiene in mice and rats. In livers perfused with 1,3-butadiene, three epoxide metabolites (EB, DEB, and EBD) were identified in perfusion effluent in mice, whereas only one epoxide metabolite (EB) was identified in rats. Effluent concentrations of EB in mice were 8.5-fold greater than in rats. For perfusion studies with EB (which is oxidized to form DEB), DEB formation was greater in mice than in rats. Metabolite levels in tissues following inhalation exposure of mice and rats to 1,3-butadiene also provide evidence of species differences. Compared to rats, EB and DEB levels in blood and tissues of mice were from approximately 2–15- and >100-fold higher,

## 3. HEALTH EFFECTS

respectively, with levels exhibiting dose- and time-dependence (see discussion below on nonlinear kinetics) (Filser et al. 2007). Based on evaluation of hemoglobin adduct biomarkers (adducts formed by interaction of 1,3-butadiene metabolites with hemoglobin), mice appear to have higher DEB levels than rats and much higher levels than humans (Swenberg et al. 2011). However, quantitative measurements of differences in hemoglobin adduct profiles show considerable variability, possibly due to differences in exposure conditions. Comparison of urinary excretion profiles of 1,3-butadiene metabolites in mice, rats, and humans also shows that species differences exist in the detoxification pathways. Findings suggest that humans and rats are more “efficient” at detoxification of reactive metabolites than mice. Taken together, results of *in vitro* and *in vivo* studies showing that mice have higher levels of reactive metabolites, particularly the highly reactive DEB, than other species suggest that mice may be uniquely sensitive to toxic effects of 1,3-butadiene and, therefore, may not be an appropriate animal model for use in human health risk assessment of 1,3-butadiene.

***Nonlinear Toxicokinetics (Metabolism).*** Metabolism of 1,3-butadiene exhibits nonlinear kinetics (Kirman et al. 2010a), with both dose- and duration-dependent effects (see discussion in Section 3.4.3 Toxicokinetics/Metabolism). Because the formation of reactive metabolites are critical in the development of 1,3-butadiene toxicity, nonlinearity in metabolic processes has the potential to affect dose-response extrapolation from animals to humans. Several processes have been proposed as sources of nonlinear kinetics; these include inhibition, induction and saturation of various metabolizing enzymes and depletion of glutathione (as reviewed by Kirman et al. 2010a).

### 3.5.2 Mechanisms of Toxicity

***Genotoxicity.*** The genotoxicity of 1,3-butadiene and its electrophilic metabolites have been extensively studied. A comprehensive review of the genotoxicity of 1,3-butadiene metabolites, focusing primarily on EB, DEB, and EDB, was recently published (Albertini et al. 2010). The weight of evidence strongly suggests that 1,3-butadiene metabolites, rather than 1,3-butadiene itself, are responsible for genotoxic effects, due to their highly reactive nature. Of these metabolites, the order of potency for mutagenicity is DEB >> EB > EDB. Results of *in vitro* studies in bacterial and mammalian cells (including human cells) show that the electrophilic metabolites of 1,3-butadiene form DNA adducts, induce DNA strand breaks, increase unscheduled DNA synthesis and DNA excision repair, induce sister-chromatid exchange, induce micronucleus formation, and produce mutations, chromosome aberrations (including breaks), and aneuploidy (as reviewed by Albertini et al. 2010). Results of *in vivo* studies in animals show that 1,3-butadiene metabolites form adducts with DNA (Koturbash et al. 2011a, 2011b). Other studies evaluating genotoxicity following inhalation exposure to 1,3-butadiene provide indirect evidence of

## 3. HEALTH EFFECTS

genotoxicity of 1,3-butadiene metabolites (Cunninham et al. 1986; Jauhar et al. 1988; Lovreglio et al. 2006; Sharief et al. 1986; Sram et al. 1998; Tice et al. 1987).

**Carcinogenicity.** As discussed in Section 3.2.1.7, chronic exposure to 1,3-butadiene is associated with an increased risk of mortality due to leukemia in styrene-butadiene workers and the development of multisite cancers in laboratory rodents. The mode of action for carcinogenicity of 1,3-butadiene was recently assessed by Kirman et al. (2010b). As discussed above (Mechanism of Toxicity, Genotoxicity), the genotoxicity of electrophilic metabolites of 1,3-butadiene (DEB, EB, and EBD) have been extensively studied. Results show that DEB, EB, and EBD react with DNA and are mutagenic. Based on the weight of evidence for genotoxicity, it is likely that the carcinogenic mode of action of 1,3-butadiene is mutagenic activity of the electrophilic 1,3-butadiene metabolites.

**Ovarian atrophy.** Intraperitoneal studies in mice suggest that 1,3-butadiene metabolites are the causative agent for the ovarian effects observed in intermediate- and chronic-duration inhalation studies (NTP 1993). Dose-related decreases in ovarian and uterine weights, number of small (primordial) ovarian follicles, and number of growing (primary to pre-antral) ovarian follicles were observed following a 30-day intraperitoneal exposure to EB or DEB (Doerr et al. 1996). The ED<sub>50</sub> (i.e., the effective dose that reduces the number of follicles to 50% of controls) values for small and growing follicles were 0.29 and 0.40 mmol/kg, respectively, for EB and 0.10 and 0.14 mmol/kg, respectively, for DEB. Similarly, decreases in ovarian or uterine weight and the number of ovarian follicles were also observed in rats similarly exposed to DEB (Doerr et al. 1996). However, no alterations were observed in rats administered EB at doses as high as 1.43 mmol/kg. These data strongly suggest that DEB is the causative agent of the ovarian atrophy.

Although similar effects were observed in rats and mice administered DEB, mice appear to be more sensitive to its toxicity than rats. Administration of 0.14 mmol/kg resulted in 83 and 52% depletion of small and growing follicles, respectively, in mice and only 31 and 40% reductions in rats. When the dose-response plot for ovarian weight is based on area under the blood EB or DEB concentration-time curve (estimated using PBPK modeling), the curves are similar for both species (Sweeney et al. 2001). Sweeney et al. (2001) also used PBPK modeling to estimate the blood area under the DEB concentration-response curve using the data from the NTP (1993) study, which found ovarian effects in mice exposed to  $\geq 6.25$  ppm, and from the Owen study (Owen and Glaister 1990; Owen et al. 1987), which did not find ovarian effects in rats exposed to  $\leq 8,000$  ppm. In mice, the blood area under the DEB curve was consistent with the results of the Doerr et al. 1996) intraperitoneal study. The blood DEB area under the



## 3. HEALTH EFFECTS

curve for rats exposed to 8,000 ppm 6 hours/day, 5 days/week for 105 weeks would be 189  $\mu\text{M}$ -hour; this value lies between the predicted NOAEL and LOAEL for ovarian effects in rats administered DEB via intraperitoneal injection.

### 3.5.3 Animal-to-Human Extrapolations

Comparison of rat and mouse data identify large differences in sensitivity to 1,3-butadiene, which are due to metabolic differences between species. Humans, rats, and mice metabolize 1,3-butadiene using the same enzymatic pathways resulting in the production of the same reactive metabolites, in particular, EB, DEB, and EBD. However, quantitative differences in the rate of formation and detoxification of reactive metabolites have been found that result in higher tissue levels of reactive metabolites in rodents, particularly mice, than in humans (Bond et al. 1993; Csanády et al. 1992; Dahl et al. 1991; Filser et al. 2001, 2007, 2010; Henderson et al. 1996, 2001; Himmelstein et al. 1997; Kirman et al. 2010a; Krause and Elfarra 1997; Schmidt and Loeser 1985; Thornton-Manning et al. 1995b). *In vitro* and perfusion data show that mice are more efficient than rats at oxidizing 1,3-butadiene to form EB, and the conversion of EB to DEB in mice is 3.3-fold greater than in rats and 2.4–61-fold greater than in humans (Kirman et al. 2010a). In addition, mice have a higher ratio of 1,3-butadiene activation to detoxification than rats or humans; the ratio of activation to detoxification was 74:1 in mouse, 6:1 in rat, and 6:1 in human liver tissues (Bond et al. 1993).

Following inhalation exposure to 1,3-butadiene, blood and tissue levels of EB and DEB were 2–15- and >100-fold higher, respectively, in mice as compared to rats (Filser et al. 2007). At equivalent inhalation concentrations, the total amount of 1,3-butadiene metabolites were 5–50 times lower in cynomolgus monkeys than in mice and 4–14 times lower in monkeys compared to rats (Dahl et al. 1991). Swenberg et al. (2011) estimated DEB blood levels measured 1,3-butadiene-derived hemoglobin adducts levels in rats, mice, and humans exposed to approximately 1 ppm 1,3-butadiene. The estimated DEB doses were 0.02, 0.42, and 24 nM-hour/ppm-hour in humans, rats, and mice, respectively. Thus, the extrapolation of rodent data to humans would require the use of an internal dose metric to account for these species differences in the metabolism of 1,3-butadiene. Although PBPK models have been developed in rodents (Johanson and Filser 1993; Kohn and Melnick 1993, 1996, 2000) and a preliminary model has been developed in humans (Brochot et al. 2007), the models are limited in their ability to predict internal doses for key metabolites (Kirman and Grant 2012). An alternative approach to using PBPK models would be to use a biomarker of exposure to the reactive metabolites. Several biomarkers of exposure have been identified for reactive 1,3-butadiene metabolites including MHB-Val hemoglobin adducts, *N*-(2,3,4-tri-

## 3. HEALTH EFFECTS

hydroxybutyl)valine (THB-Val) hemoglobin adducts, and *pyr*-Val hemoglobin adducts, which have been shown to be good surrogate biomarkers for EB, EBD, and DEB, respectively (Georgieva et al. 2010; Slikker et al. 2004).

### 3.6 TOXICITIES MEDIATED THROUGH THE NEUROENDOCRINE AXIS

Recently, attention has focused on the potential hazardous effects of certain chemicals on the endocrine system because of the ability of these chemicals to mimic or block endogenous hormones. Chemicals with this type of activity are most commonly referred to as *endocrine disruptors*. However, appropriate terminology to describe such effects remains controversial. The terminology *endocrine disruptors*, initially used by Thomas and Colborn (1992), was also used in 1996 when Congress mandated the EPA to develop a screening program for “...certain substances [which] may have an effect produced by a naturally occurring estrogen, or other such endocrine effect[s]...”. To meet this mandate, EPA convened a panel called the Endocrine Disruptors Screening and Testing Advisory Committee (EDSTAC), and in 1998, the EDSTAC completed its deliberations and made recommendations to EPA concerning *endocrine disruptors*. In 1999, the National Academy of Sciences released a report that referred to these same types of chemicals as *hormonally active agents*. The terminology *endocrine modulators* has also been used to convey the fact that effects caused by such chemicals may not necessarily be adverse. Many scientists agree that chemicals with the ability to disrupt or modulate the endocrine system are a potential threat to the health of humans, aquatic animals, and wildlife. However, others think that endocrine-active chemicals do not pose a significant health risk, particularly in view of the fact that hormone mimics exist in the natural environment. Examples of natural hormone mimics are the isoflavonoid phytoestrogens (Adlercreutz 1995; Livingston 1978; Mayr et al. 1992). These chemicals are derived from plants and are similar in structure and action to endogenous estrogen. Although the public health significance and descriptive terminology of substances capable of affecting the endocrine system remains controversial, scientists agree that these chemicals may affect the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body responsible for maintaining homeostasis, reproduction, development, and/or behavior (EPA 1997). Stated differently, such compounds may cause toxicities that are mediated through the neuroendocrine axis. As a result, these chemicals may play a role in altering, for example, metabolic, sexual, immune, and neurobehavioral function. Such chemicals are also thought to be involved in inducing breast, testicular, and prostate cancers, as well as endometriosis (Berger 1994; Giwercman et al. 1993; Hoel et al. 1992).

## 3. HEALTH EFFECTS

No *in vivo* or *in vitro* studies were located regarding endocrine disruption in humans and/or animals after exposure to 1,3-butadiene.

**3.7 CHILDREN'S SUSCEPTIBILITY**

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans, when all biological systems will have fully developed. Potential effects on offspring resulting from exposures of parental germ cells are considered, as well as any indirect effects on the fetus and neonate resulting from maternal exposure during gestation and lactation. Relevant animal and *in vitro* models are also discussed.

Children are not small adults. They differ from adults in their exposures and may differ in their susceptibility to hazardous chemicals. Children's unique physiology and behavior can influence the extent of their exposure. Exposures of children are discussed in Section 6.6, Exposures of Children.

Children sometimes differ from adults in their susceptibility to hazardous chemicals, but whether there is a difference depends on the chemical (Guzelian et al. 1992; NRC 1993). Children may be more or less susceptible than adults to health effects, and the relationship may change with developmental age (Guzelian et al. 1992; NRC 1993). Vulnerability often depends on developmental stage. There are critical periods of structural and functional development during both prenatal and postnatal life, and a particular structure or function will be most sensitive to disruption during its critical period(s). Damage may not be evident until a later stage of development. There are often differences in pharmacokinetics and metabolism between children and adults. For example, absorption may be different in neonates because of the immaturity of their gastrointestinal tract and their larger skin surface area in proportion to body weight (Morselli et al. 1980; NRC 1993); the gastrointestinal absorption of lead is greatest in infants and young children (Ziegler et al. 1978). Distribution of xenobiotics may be different; for example, infants have a larger proportion of their bodies as extracellular water, and their brains and livers are proportionately larger (Altman and Dittmer 1974; Fomon 1966; Fomon et al. 1982; Owen and Brozek 1966; Widdowson and Dickerson 1964). The infant also has an immature blood-brain barrier (Adinolfi 1985; Johanson 1980) and probably an immature blood-testis barrier (Setchell and Waites 1975). Many xenobiotic metabolizing enzymes have distinctive developmental patterns. At various stages of growth and development, levels of particular enzymes may be higher or lower than those of adults, and sometimes unique enzymes may exist at particular developmental stages (Komori et al. 1990; Leeder and Kearns 1997; NRC 1993; Vieira et al. 1996). Whether differences in xenobiotic metabolism make the

### 3. HEALTH EFFECTS

child more or less susceptible also depends on whether the relevant enzymes are involved in activation of the parent compound to its toxic form or in detoxification. There may also be differences in excretion, particularly in newborns who all have a low glomerular filtration rate and have not developed efficient tubular secretion and resorption capacities (Altman and Dittmer 1974; NRC 1993; West et al. 1948). Children and adults may differ in their capacity to repair damage from chemical insults. Children also have a longer remaining lifetime in which to express damage from chemicals; this potential is particularly relevant to cancer.

Certain characteristics of the developing human may increase exposure or susceptibility, whereas others may decrease susceptibility to the same chemical. For example, although infants breathe more air per kilogram of body weight than adults breathe, this difference might be somewhat counterbalanced by their alveoli being less developed, which results in a disproportionately smaller surface area for alveolar absorption (NRC 1993).

No human data are available to determine whether children are more sensitive than adults to 1,3-butadiene toxicity. Mechanistic data in animals suggest that the ratio of 1,3-butadiene oxidation:epoxide hydrolysis may be a significant determinant of 1,3-butadiene sensitivity (see Sections 3.5.1 and 3.5.2). It is not known if the ratio of 1,3-butadiene oxidation:epoxide hydrolysis is different in children than adults. Unborn children may be more sensitive to 1,3-butadiene toxicity than adults, as changes in fetal body weight and developmental effects have been identified at the lowest LOAELs in mice exposed to acute- and intermediate-duration inhalation exposures (Anderson et al. 1996; DOE/NTP 1987b). Several studies have associated the development of childhood leukemia to close proximity of birthplace to industrial point sources of 1,3-butadiene (and other high-volume industrial chemicals, including benzene) (Knox et al. 2005, 2006; Reynolds et al. 2003; Whitworth et al. 2008). Although these study authors suggest that *in utero* exposure to 1,3-butadiene may have significantly contributed to cancer risks in these populations, there are no estimates of actual prenatal or postnatal exposures of mothers or children, respectively.

#### 3.8 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as markers of exposure, markers of effect, and markers of susceptibility (NAS/NRC 1989).

## 3. HEALTH EFFECTS

A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself, substance-specific metabolites in readily obtainable body fluid(s), or excreta. However, several factors can confound the use and interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposures from more than one source. The substance being measured may be a metabolite of another xenobiotic substance (e.g., high urinary levels of phenol can result from exposure to several different aromatic compounds). Depending on the properties of the substance (e.g., biologic half-life) and environmental conditions (e.g., duration and route of exposure), the substance and all of its metabolites may have left the body by the time samples can be taken. It may be difficult to identify individuals exposed to hazardous substances that are commonly found in body tissues and fluids (e.g., essential mineral nutrients such as copper, zinc, and selenium). Biomarkers of exposure to 1,3-butadiene are discussed in Section 3.8.1.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that, depending on magnitude, can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effects caused by 1,3-butadiene are discussed in Section 3.8.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 3.10, Populations That Are Unusually Susceptible.

### 3.8.1 Biomarkers Used to Identify or Quantify Exposure to 1,3-Butadiene

Two urinary metabolites of 1,3-butadiene have been identified in tollbooth workers. Sapkota et al. (2006) measured 258–378 ng/mL 1,2-dihydroxy-4-(N-acetylcysteinyl)-butane and 6–9.7 ng/mL of the isomeric mixture of 1-hydroxy-2-(N-acetylcysteinyl)-3-butene and 1-(N-acetylcysteinyl)-2-hydroxy-3-butene in

## 3. HEALTH EFFECTS

workers' urine following average ambient 1,3-butadiene exposures of 0.4–1 ppb. These biomarkers are quite specific to 1,3-butadiene exposure, but the measured levels were not significantly associated with exposure, although this may have been due to the small sample size used or the low (0.0005 ppm) exposure levels studied. N-acetyl-S-((1-hydroxymethyl)-2-propenyl)cysteine and N-acetyl-S-((2-hydroxymethyl)-3-propenyl)cysteine, the isomeric mixture known as MHBMA (or M2), are the GST conjugation products of EB found in human urine following 1,3-butadiene exposure. Another urinary metabolite, N-acetyl-S-(3,4-dihydroxybutyl)cysteine, or DHBMA (M1), is formed by EBdiol conjugation with glutathione via GST (Boogaard et al. 2001a; McDonald et al. 2004). These urinary metabolites have been used as biomarkers of 1,3-butadiene exposures in several human studies (Albertini et al. 2001, 2007; Ammenheuser et al. 2001; Boogaard et al. 2001a; Fustinoni et al. 2004).

Protein adducts have been widely used to monitor the formation of alkylating metabolites and can be used as dose metrics to compare species differences in metabolism. Hemoglobin adducts accumulate over the lifespan of the erythrocyte and they represent cumulative exposure since they are not removed by enzymatic repair systems (Georgieva et al. 2010). Three N-terminal valine hemoglobin adducts have been identified for 1,3-butadiene: MHB-Val, THB-Val, and *pyr*-Val. MHB-Val, THB-Val, and *pyr*-Val are formed when EB, DEB, and EB-diol, respectively, react with hemoglobin (Georgieva et al. 2010; Slikker et al. 2004). A linear accumulation of MHB-Val and THB-Val was observed in B6C3F1 mice and Sprague-Dawley rats after intraperitoneal (Sun et al. 1989b) and inhalation (HEI 2000) exposures. Species differences have been detected in the amount of adducts formed at a given 1,3-butadiene concentration. Higher levels of MHB-Val and *pyr*-Val adducts were found in mice compared to rats (Albrecht et al. 1993; Boysen et al. 2004). Concentration-response studies have shown that the formation of *pyr*-Val hemoglobin adducts is saturable. In rats, the formation of *pyr*-Val adducts plateaus at inhalation exposures of  $\geq 200$  ppm (Georgieva et al. 2010). In mice, the formation of *pyr*-Val adducts did not plateau; however, the rate of formation decreased below 1.5 ppm (Georgieva et al. 2010). MHB-Val and THB-Val hemoglobin adduct levels were well correlated with 1,3-butadiene exposure in 1,3-butadiene monomer workers (Albertini et al. 2001, 2007; Begemann et al. 2001a, 2001b; Osterman-Golkar et al. 1996) and Chinese polymer workers (Hayes et al. 2000; HEI 2000). *Pyr*-Val was not detected in blood of male and female Czech workers exposed to 0.2–0.4 ppm (Albertini et al. 2007). *Pyr*-Val adducts were detected in the blood of workers not occupationally exposed to 1,3-butadiene, in monomer workers and polymerization workers (Boysen et al. 2012); the levels in the polymerization workers (mean 1,3-butadiene exposure level of 0.81 ppm) were significantly higher than in the control and monomer workers (mean 1,3-butadiene exposure levels of 0.01 and 0.29 ppm, respectively). When the three groups of workers were combined, a significant association between *pyr*-Val adduct levels and

## 3. HEALTH EFFECTS

individual 1,3-butadiene exposure levels was found. Using levels of these hemoglobin adducts, Swenberg et al. (2011) estimated metabolite levels in rats, mice, and humans, which allowed for a species comparison of reactive metabolite levels at a given 1,3-butadiene concentration.

Zhao et al. (2000) found a significant linear relationship of DNA adduction and 1,3-butadiene exposure between 1,3-butadiene workers and controls. The levels of N1-(2,3,4-trihydroxybutyl)adenine adduct in lymphocytes of 1,3-butadiene workers (mean exposure: 0.3 ppm; range: <0.005–7.7 ppm) were 5-fold higher than controls (mean exposure: 0.01 ppm; range: <0.001–0.07 ppm). 1,3-Butadiene-specific urinary metabolites and hemoglobin and DNA adducts have also been observed in animals. Excretion of 1,3-butadiene metabolites was reported to be high in the urine of exposed monkeys (Dahl et al. 1990). DNA adducts were detected in the livers of mice and rats exposed to radiolabeled 1,3-butadiene (Kreiling et al. 1986b). In mice exposed to 1,3-butadiene by nose-only inhalation, the N7-guanine adduct (N7-(1-(hydroxymethyl)-2,3-dihydroxypropyl)guanine) from interaction with 3,4-epoxy-1,2-diol, was the major DNA adduct measured (Boogaard et al. 2001b).

1,3-Butadiene has been measured in expired air of forestry workers living in mountain villages (Perbellini et al. 2003); however, the levels measured (median of 1.2 ng/L) were not correlated with any exposure to 1,3-butadiene.

### 3.8.2 Biomarkers Used to Characterize Effects Caused by 1,3-Butadiene

Dermal, ocular, and/or upper respiratory irritation can occur following 1,3-butadiene exposure (NIOSH 2005) and may alert the exposed individual. However, the effects are not specific for 1,3-butadiene exposure and may be caused by several other chemicals.

Given the genotoxic (Section 3.3) and carcinogenic (Section 3.2.1.7) activity of 1,3-butadiene, a useful biomarker of effect would correlate a quantifiable measure of genetic mutation with 1,3-butadiene exposure. 1,4-Bis-(guan-7-yl)-2,3-butanediol (bis-N7G-BD) and 1-(guan-7-yl)-4-(aden-1-yl)-2,3-butanediol (N7G-N1A-BD) are DEB-specific DNA-DNA cross-links identified in rats and mice inhaling up to 625 ppm 1,3-butadiene (Goggin et al. 2009, 2011). Mice exhibited 4–10-fold higher levels than rats of these cross-links. Further, higher levels of bis-N7G-BD were measured in females, compared to males. The sensitivity of female mice to these biomarkers and to 1,3-butadiene-induced carcinogenicity suggests that: (1) DEB is the putative carcinogenic metabolite of 1,3-butadiene, and

## 3. HEALTH EFFECTS

(2) bis-N7G-BD and N7G-N1A-BD levels may quantitatively inform on genotoxicity leading to tumor development.

Another biomarker of genetic change is the mutation frequency of the *hprt* gene locus in human peripheral lymphocytes, which has been used in multiple studies of 1,3-butadiene SBR, monomer, and polymer workers in the United States, China, and Czech Republic. These studies are discussed in detail in Section 3.3. Several of these studies demonstrated good correlation between the increase in *hprt* mutation frequency and 1,3-butadiene exposure (Abdel-Rahman et al. 2001, 2003, 2005; Ammenheuser et al. 2001; Ma et al. 2000; Ward et al. 1994, 1996, 2001), as well as correlation with urinary and hematological biomarkers of exposure. Others did not find a significant correlation between exposure and mutation frequency (Albertini et al. 2001, 2007; Hayes et al. 1996, 2000; HEI 2003; Liu et al. 2008; Tates et al. 1996). The reasons for the differences in sensitivity of the *hprt* mutation as a biomarker may include the very low exposures that were studied and differences in assay procedures (as discussed in Section 3.3). It is unclear at this time which *hprt* mutation frequency assay is most adequate for risk assessment.

Two biomarkers for carcinogenic effect have been consistently found in multiple tumors sites in rodent chronic bioassays. A number of malignant gliomas and neuroblastomas in mice chronically inhaling 1,3-butadiene exhibited mutations of the p53 gene and H- and K-*ras* oncogenes (Kim et al. 2005), which have also been observed in forestomach tumors (Sills et al. 2001), hemangiosarcomas (Hong et al. 2000), and lymphomas (Zhuang et al. 1997) of chronically exposed mice.

### 3.9 INTERACTIONS WITH OTHER CHEMICALS

In addition to 1,3-butadiene, workers in the rubber industry are exposed to other chemicals, including styrene and its mutagenic metabolite, styrene oxide (Loprieno et al. 1978; Norppa et al. 1980; Pohlova et al. 1985; Watabe et al. 1978), as well as dithiocarbamates (Irons and Pyatt 1998). It is unclear whether these other chemicals or their active metabolites have a synergistic harmful effect in humans, but a multivariate analysis of an SBR worker cohort (HEI 2006) did not detect interactive effects of co-exposure to 1,3-butadiene, styrene, and dimethyldithiocarbamate (DMDTC). Animal studies have found that DMDTC can qualitatively and quantitatively effect the metabolism of 1,3-butadiene. *In vitro* studies show that DMDTC treatment decreases the metabolism of 1,3-butadiene to EB and the metabolism of epoxybutene to DEB in rats and mice (Green et al. 2001). Styrene has been shown to inhibit the metabolism of 1,3-butadiene in rats simultaneously exposed to both compounds (Laib et al. 1992;



## 3. HEALTH EFFECTS

Leavens et al. 1996). The inhibition was only observed at 1,000 ppm 1,3-butadiene concentration and not at 100 ppm (Leavens et al. 1996). However, blood levels of EB increased and the blood levels of DEB were unaffected by styrene co-exposure, as compared to exposure to 1,3-butadiene only (Leavens et al. 1996). Thus, co-exposure to styrene may not affect the toxicity of 1,3-butadiene. Inhalation exposure to 1,3-butadiene and styrene did not affect the genotoxic potential of 1,3-butadiene in mice (Leavens et al. 1997).

**3.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE**

A susceptible population will exhibit a different or enhanced response to 1,3-butadiene than will most persons exposed to the same level of 1,3-butadiene in the environment. Reasons may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters result in reduced detoxification or excretion of 1,3-butadiene, or compromised function of organs affected by 1,3-butadiene. Populations who are at greater risk due to their unusually high exposure to 1,3-butadiene are discussed in Section 6.7, Populations with Potentially High Exposures.

The human and animal data do not identify a gender-specific susceptibility to 1,3-butadiene. Human *in vivo* data do not identify specific populations that may be sensitive to the effects of 1,3-butadiene. Polymorphisms in metabolic enzymes may affect the toxicokinetics of 1,3-butadiene and render some individuals more sensitive to toxicity, based on increased sensitivity to genetic changes seen in these groups. Lymphocytes from GSTT1-null 1,3-butadiene workers in Texas had higher induction of sister chromatid exchange following *in vitro* DEB exposure (Kelsey et al. 1995), while GSTT1-null Czech workers exhibited higher rates of chromosomal aberrations (Sorsa et al. 1996). However, no such effects were observed in other Czech (Sram et al. 1998) or Chinese (Hayes et al. 2000) workers that were GSTT1 or GSTM1 deficient. Increased *hprt* mutation frequencies have been reported in U.S. 1,3-butadiene workers with various polymorphisms in EH (Abdel-Rahman et al. 2001, 2003, 2005). In *in vitro* studies with human lymphocytes, EB induced higher levels of sister chromatid exchanges in GSTM1-null samples, as compared to GSTM1 samples (Uusküla et al. 1995), suggesting that the clastogenic change may be the result of less EB being detoxified via GSTM1-mediated glutathione conjugation. Similarly, EB induced higher levels of sister chromatid exchanges in lymphocytes from GSTT1-null individuals, as compared to GSTT1-positive individual (Bernardini et al. 1998).

The relationship between polymorphisms and the urinary excretion of M1 and M2 metabolites has been exposed in studies of Czech 1,3-butadiene workers. GST polymorphisms resulted in shifts in the mean

### 3. HEALTH EFFECTS

ratio of  $M2/(M1 + M2)$  (indicative of the activity of EB glutathione conjugation pathway), which was significantly lower in GSTM1-null workers, as compared to GSTM1-positive workers (HEI 2003); a lower ratio was also found in the GSTT1-null workers, but it was not significantly different from the GSTT1-positive workers. No significant alterations in M1 or M2 concentrations or the ratio were found for workers genotyped for CYP2E1, EH, and ADH polymorphisms. Regression analysis revealed that the slopes of the 1,3-butadiene concentration-M2 levels and concentration-M2/(M1+M2) was significantly different in CYP2E1 D/D intron 6 polymorphism compared to workers who were heterozygous for C/D (HEI 2003). In a subsequent study by this group, a significant difference in M2/(M1+M2) ratio was observed in the GSTT1-null workers (Albertini et al. 2007). When different EH genotypes were combined into high, intermediate, and low activity phenotypes, significantly higher M2 levels were observed in the low activity genotype group (Albertini et al. 2007).

Animals studies indicate that the predominant factor in species sensitivity is related to the toxicokinetics of 1,3-butadiene, specifically, the ratio of P450-mediated oxidation:epoxide hydrolase activity (see Section 3.4.3). Human populations that have a higher ratio of 1,3-butadiene oxidation:hydrolysis metabolism may also be more sensitive, although such populations have not been identified. In terms of absorption capacity, Asian volunteers had about 20% greater fractional absorption of inhaled 1,3-butadiene than did Caucasians, African-Americans, or Hispanics (Lin et al. 2001). However, it is not known if this results in higher internal doses of the putative epoxide toxicants.

#### 3.11 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to 1,3-butadiene. However, because some of the treatments discussed may be experimental and unproven, this section should not be used as a guide for treatment of exposures to 1,3-butadiene. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice.

##### 3.11.1 Reducing Peak Absorption Following Exposure

No specific antidotes for 1,3-butadiene are available; however, recommendations have been made for general treatment of intoxicated persons (Bronstein and Currance 1988; Stutz and Janusz 1988). First, the exposed individual should be removed from the contaminated area and contaminated clothing should be taken away (Currance et al. 2007; Leikin and Paloucek 2002). It has been suggested that exposed skin should be washed with soapy water and contaminated eyes should be flushed with water. Inhalation

### 3. HEALTH EFFECTS

exposure to high 1,3-butadiene concentrations may result in narcosis leading to respiratory paralysis and death. Data have shown that neurological effects, including anesthesia have been observed in animals exposed to 250,000 ppm (Carpenter et al. 1944). Therefore, administration of oxygen has been used and ventilation has been assisted as needed in cases of 1,3-butadiene poisoning.

#### **3.11.2 Reducing Body Burden**

No information is available regarding displacing or removing absorbed 1,3-butadiene prior to metabolism to reactive metabolites.

#### **3.11.3 Interfering with the Mechanism of Action for Toxic Effects**

Toxicity studies found mice to be extremely sensitive (DOE/NTP 1987b; Melnick et al. 1989, 1990b; NTP 1993). Studies on the metabolism of 1,3-butadiene demonstrated that the chemical is converted to its epoxy derivatives by P450 isoforms in lung, liver, kidney (see Section 3.4.3), and possibly other tissues. The epoxides may be responsible for most toxic and carcinogenic effects caused by 1,3-butadiene exposure. The epoxides are detoxified by hydrolysis or conjugation with glutathione (Kreiling et al. 1988). A higher rate of epoxide formation and a greater depletion of hepatic nonprotein sulfhydryl content in mice is probably responsible for their higher susceptibility to 1,3-butadiene toxicity. Since the macromolecular covalent binding is enhanced only after a substantial decrease of glutathione levels (Kreiling et al. 1988), sufficient availability of glutathione should mitigate the effects of 1,3-butadiene exposure.

Interference with the oxidative metabolism of 1,3-butadiene may also be effective in allowing pulmonary clearance of the parent compound to occur before reactive epoxides are formed. In mice, a 30–56% reduction in induction of erythrocyte micronuclei was observed in mice pretreated with various P450 inhibitors (Jackson et al. 2000b). However, the side effects of systemically inhibiting P450s in humans are unknown.

### **3.12 ADEQUACY OF THE DATABASE**

Section 104(I)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of 1,3-butadiene is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (NTP), is required to assure

## 3. HEALTH EFFECTS

the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of 1,3-butadiene.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

### 3.12.1 Existing Information on Health Effects of 1,3-Butadiene

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to 1,3-butadiene are summarized in [Figure 3-4](#). The purpose of this figure is to illustrate the existing information concerning the health effects of 1,3-butadiene. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not necessarily imply anything about the quality of the study or studies, nor should missing information in this figure be interpreted as a “data need”. A data need, as defined in ATSDR’s *Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles* (Agency for Toxic Substances and Disease Registry 1989), is substance-specific information necessary to conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

As seen from [Figure 3-4](#), information regarding acute systemic effects (respiratory tract irritation and narcotic effect), chronic systemic effects (respiratory irritation), genotoxicity, and cancer exists for inhalation exposure in humans. No information was located regarding oral or dermal exposure of humans to 1,3-butadiene.

Inhalation studies in animals provide data on death, systemic effects, immunologic effects, neurologic effects, reproductive and developmental effects, genotoxicity, and carcinogenicity. No information was located regarding effects in animals after oral or dermal exposure to 1,3-butadiene.

### 3.12.2 Identification of Data Needs

No studies were located regarding effects following oral or dermal exposure, and no pharmacokinetic studies by the oral or dermal routes were located; therefore, it is not possible to predict if effects

## 3. HEALTH EFFECTS

**Figure 3-4. Existing Information on Health Effects of 1,3-Butadiene**

|            | Systemic |       |              |         |                         |            |              |               |           |        |
|------------|----------|-------|--------------|---------|-------------------------|------------|--------------|---------------|-----------|--------|
|            | Death    | Acute | Intermediate | Chronic | Immunologic/Lymphoretic | Neurologic | Reproductive | Developmental | Genotoxic | Cancer |
| Inhalation | ●        |       | ●            |         | ●                       |            |              | ●             | ●         |        |
| Oral       |          |       |              |         |                         |            |              |               |           |        |
| Dermal     |          |       |              |         |                         |            |              |               |           |        |

**Human**

|            | Systemic |       |              |         |                         |            |              |               |           |        |
|------------|----------|-------|--------------|---------|-------------------------|------------|--------------|---------------|-----------|--------|
|            | Death    | Acute | Intermediate | Chronic | Immunologic/Lymphoretic | Neurologic | Reproductive | Developmental | Genotoxic | Cancer |
| Inhalation | ●        | ●     | ●            | ●       | ●                       | ●          | ●            | ●             | ●         | ●      |
| Oral       |          |       |              |         |                         |            |              |               |           |        |
| Dermal     |          |       |              |         |                         |            |              |               |           |        |

**Animal**

● Existing Studies

## 3. HEALTH EFFECTS

following oral or dermal exposure would be similar to those observed after inhalation exposure. Because 1,3-butadiene exists primarily as a gas and has been detected in soil off-gases at hazardous waste sites, inhalation exposure appears to be the greatest concern. However, it is not known if 1,3-butadiene is present in groundwater or soil at these hazardous waste sites because it is difficult to analyze these media for the compound. 1,3-Butadiene has been detected in industrial waste water and drinking water, and is poorly soluble in water (735 ppm). Therefore, oral and dermal routes of exposure cannot be ruled out. Information concerning 1,3-butadiene toxicity by these routes of exposure would be useful.

**Acute-Duration Exposure.** Acute inhalation exposure to very high concentrations (>25,000 ppm) of 1,3-butadiene may lead to narcosis and death by respiratory paralysis in animals (Carpenter et al. 1944). Data in humans are limited to a study of two men exposed to various concentrations of 1,3-butadiene for 6–8 hours that examined clinical signs and psychomotor function (Carpenter et al. 1944). No studies were located that correlated the level of exposure with the first signs of toxicity in humans or animals. Developmental effects were seen in mice after exposure to concentrations as low as 40 ppm (DOE/NTP 1987b; Irvine 1981). Although the available animal data identify critical targets of toxicity following acute-duration inhalation exposure, the database lacks adequate toxicokinetic and PBPK modeling data, which could be used to account for species differences in the metabolism of 1,3-butadiene; thus, an acute-duration MRL was not derived. Additionally, there are limited data on the potential effects of the parent compound, such as neurotoxicity, which may be a more sensitive target of toxicity in humans. Because people living at or near these hazardous waste sites may be exposed for brief periods of time, more dose-response data for acute exposures by oral and inhalation routes is considered to be important.

**Intermediate-Duration Exposure.** No information is available regarding effects of 1,3-butadiene during intermediate-duration exposure in humans. No studies were located regarding effects in humans or animals following oral or dermal exposure to 1,3-butadiene, and pharmacokinetic data for these routes of exposure are insufficient to predict whether the disposition or toxicity of 1,3-butadiene following oral or dermal exposure would be similar to that following inhalation exposure. Therefore, information regarding the toxicity of 1,3-butadiene by the oral route of exposure would be useful. Several studies on intermediate-duration inhalation exposure to 1,3-butadiene have been conducted in animals (Anderson et al. 1996, 1998; Crouch et al. 1979; Irons et al. 1986a; NTP 1984, 1993; Thurmond et al. 1986). Atrophy of reproductive organs, anemia, precancerous hyperplasia in multiple organs, and cancer occurred in mice exposed to  $\geq 200$  ppm (Irons et al. 1986a; NTP 1984, 1993). The observed hematological changes (macrocytic megaloblastic anemia) were similar to those found in human preleukemic syndrome (Biemer 1983), suggesting that 1,3-butadiene exposure might interfere with normal bone marrow cell

## 3. HEALTH EFFECTS

development. Further investigation of this topic could be valuable since epidemiological studies in humans indicate that hematopoietic tissue may be a possible target for 1,3-butadiene toxicity (Checkoway and Williams 1982). Several studies have identified potential targets of toxicity following intermediate-duration exposure; however, the available toxicokinetic and PBPK modeling data do not allow for adequate adjustment for species differences in the metabolism of 1,3-butadiene. Thus, intermediate-duration inhalation MRLs were not derived.

**Chronic-Duration Exposure and Cancer.** Possible risk for hematological disorders was reported in humans after chronic inhalation exposure to 1,3-butadiene in occupational settings, but exposure levels are lacking, and exposure to other chemicals occurs in these settings (Checkoway and Williams 1982). However, other studies (Cowles et al. 1994; Tsai et al. 2005) that monitored 1,3-butadiene levels have not found hematological alterations. Well-conducted inhalation studies identified respiratory effects, liver necrosis, gonadal atrophy, multi-site cancer, and increased mortality in mice at exposures as low as 6.25 ppm (Melnick et al. 1989, 1990a; NTP 1984, 1993), while renal pathology, cancer, and increased mortality were observed in rats exposed to  $\geq 1,000$  ppm (Owen et al. 1987). Because a serious LOAEL of severe ovarian atrophy (with complete destruction of oocytes, follicles, and corpora lutea) was found at 6.25 ppm, with no associated NOAEL, no chronic MRL has been derived. Chronic-duration studies are needed that identify a NOAEL in mice for gonadal atrophy. Oral studies are lacking, and toxicokinetic data are insufficient to predict toxicity across routes of exposure. Therefore, information concerning the possible toxicity of 1,3-butadiene by this route would be useful to identify the target organs and the thresholds for toxic effects.

Epidemiological studies in humans indicate a possible increase in risk of leukemia from occupational exposure to 1,3-butadiene (Cheng et al. 2007; Delzell et al. 1996; Divine 1990; Divine and Hartman 2001; Divine et al. 1993; Downs et al. 1987; Macaluso et al. 1996; Matanoski and Schwartz 1987; Matanoski et al. 1982, 1989a, 1989b, 1990; McMichael et al. 1974, 1975, 1976; Meinhardt et al. 1982; Ward et al. 1995). This is supported by the information about mutagenic activity of 1,3-butadiene metabolites (de Meester 1988) and by well-conducted chronic inhalation studies that provide information on carcinogenic effects of 1,3-butadiene in mice and rats (Melnick et al. 1989; NTP 1984, 1993; Owen et al. 1987). IARC (2009) and EPA (EPA 2002; IRIS 2012) concluded that there is sufficient evidence for the carcinogenicity of 1,3-butadiene in animals. IARC has classified 1,3-butadiene in group 1, carcinogenic to humans. EPA has classified 1,3-butadiene as a human carcinogen. The Department of Health and Human Services (NTP 2011) also identified 1,3-butadiene as a “known human carcinogen”. Further epidemiological investigations that examined additional potential targets of 1,3-butadiene toxicity

## 3. HEALTH EFFECTS

would be useful. Also, data are needed on 1,3-butadiene exposure to urban populations living in close proximity to major roadways and intersections, as well as on long-term follow-up of health effects, particularly the detection of diminishment in reproductive capability and prevalence of lympho-hematopoietic cancers.

No chronic oral or dermal carcinogenicity studies in animals were located, and pharmacokinetic data are insufficient to predict a carcinogenic potential of 1,3-butadiene by these routes.

**Genotoxicity.** Studies of chromosomal aberrations, sister chromatid exchanges, and *hprt* mutation frequencies among petrochemical and 1,3-butadiene monomer workers exposed to low levels ( $\leq 2$  ppm) provide conflicting results (Albertini et al. 2001, 2007; Ammenheuser et al. 2001; Hayes et al. 1996, 2000; HEI 2003; Lovreglio et al. 2006; Sram et al. 1998; Tates et al. 1996; Ward et al. 1996, 2001; Zhou et al. 1986). 1,3-Butadiene has caused increases in micronuclei induction, chromosomal aberration frequency, and mutations of proto-oncogenes in *in vivo* studies of rats and mice following inhalation exposure (Autio et al. 1994; Cochrane and Skopek 1993; Cunningham et al. 1986; Irons et al. 1987b; Jauhar et al. 1988; Meng et al. 1999, 2000, 2004, 2007; Recio et al. 1992; Sills et al. 2001; Tice et al. 1987; Vodicka et al. 2006). Information on the genotoxic effects of 1,3-butadiene was also obtained from *in vitro* studies in prokaryotic (Arce et al. 1989; de Meester 1988, de Meester et al. 1980; Victorin and Stahlberg 1988) and eukaryotic (Cochrane and Skopek 1993; Sasiadek et al. 1991) organisms. These data sufficiently characterize the mutagenic potential of 1,3-butadiene metabolites.

**Reproductive Toxicity.** The atrophy of gonads in mice after chronic inhalation exposures as low as 6.25 ppm 1,3-butadiene was reported (Melnick et al. 1989; NTP 1984, 1993). The fertility of rats, guinea pigs, or rabbits was reported to be unaltered by acute- and intermediate-duration inhalation exposure to 1,3-butadiene (Anderson et al. 1996, 1998; Carpenter et al. 1944). Sperm head abnormalities were found in male mice exposed to 1,3-butadiene by inhalation (DOE/NTP 1988b). Further information regarding the reproductive effects of 1,3-butadiene in animals such as multigeneration studies would be useful to estimate the possible risk for reproductive effects in humans. An epidemiological study among exposed populations concentrating on reproductive effects would be useful.

No studies were located regarding reproductive toxicity of 1,3-butadiene by the oral or dermal routes in humans or animals, and pharmacokinetic data were insufficient to suggest the potential for 1,3-butadiene to cause reproductive effects by these routes of exposure. The potential for exposure of humans by the oral and dermal routes, however, is not known.



## 3. HEALTH EFFECTS

**Developmental Toxicity.** No information on developmental toxicity in humans was located. A developmental study by the inhalation route indicated growth retardation in rat fetuses and an increase in major skeletal abnormalities at a concentration of 1,000 ppm of 1,3-butadiene (Irvine 1981). Furthermore, fetotoxicity was observed in mice at acute-duration exposures of 40 ppm and intermediate-duration exposures of 12.5 ppm 1,3-butadiene (DOE/NTP 1987b). More data on developmental toxicity in other species (at least one of them nonrodent) would be useful to identify the possible developmental risk for humans. The developmental effects following other routes of exposure have not been studied, and pharmacokinetic data are insufficient to predict that responses would be similar to those by the inhalation route. Therefore, studies of oral exposures in animals to determine the possible developmental effects of 1,3-butadiene and the thresholds for these effects would be useful.

**Immunotoxicity.** Reduction in thymus weight and lymphoid histopathology was seen after the intermediate-duration exposure of mice to  $\geq 625$  ppm 1,3-butadiene (NTP 1993; Thurmond et al. 1986). The indications of disturbances in hemato- and lymphatopoietic stem cell regulations were observed after inhalation exposure of mice to 1,3-butadiene (Liederman et al. 1986). The high incidence of lymphoma among mice after the chronic exposure (NTP 1984, 1993) also indicates that the immune system is a target. A battery of immune function tests has not been performed in humans or animals. More data regarding humans and animals would be useful for determining potential human immunotoxicity of 1,3-butadiene. Studies regarding skin sensitization with 1,3-butadiene are lacking.

**Neurotoxicity.** Narcosis has been demonstrated in animals after acute inhalation exposure to very high levels of 1,3-butadiene (250,000 ppm) (Carpenter et al. 1944). No reliable information was located regarding neurotoxicity due to chronic inhalation exposure or to oral or dermal exposure for any duration. Information regarding early, subtle signs of possible neurological effects with correlation to the exposure levels is lacking. A battery of neurological and neurobehavioral tests would be useful to better define the neurological end points.

**Epidemiological and Human Dosimetry Studies.** Several epidemiological studies on health effects of 1,3-butadiene have been conducted (Case and Hosker 1954; Fox et al. 1974; Matanoski and Schwartz 1987; Matanoski et al. 1989a, 1989b; McMichael et al. 1974, 1975, 1976; Meinhardt et al. 1982). The limitation of these studies is that the cohorts of exposed workers were recruited from the rubber industry, in which the people were exposed to a mixture of various chemicals. Some genotoxicity studies have been conducted among 1,3-butadiene manufacturing workers and petrochemical workers

## 3. HEALTH EFFECTS

exposed to low ( $\leq 2$  ppm) exposures, but have reported conflicting results (Albertini et al. 2001, 2007; Ammenheuser et al. 2001; Hayes et al. 1996, 2000; HEI 2003; Kelsey et al. 1995; Lovreglio et al. 2006; Tates et al. 1996; Ward et al. 1996, 2001; Wickliffe et al. 2009; Zhou et al. 1986). Reliable dosimetry data on the exposed populations would be useful for good epidemiological comparisons. Efforts to improve estimates of past exposures and to more accurately define current exposure levels to 1,3-butadiene would be valuable. Epidemiological studies should concentrate on the possible carcinogenic effect of 1,3-butadiene in humans and on changes in hemato- and lymphatopoietic systems as possible targets for 1,3-butadiene induced toxicity. The data obtained from workers exposed occupationally to low concentrations of 1,3-butadiene could possibly be extrapolated to populations living near hazardous waste sites.

**Biomarkers of Exposure and Effect.**

**Exposure.** The determination of 1,3-butadiene-derived urinary metabolites (Albertini et al. 2001, 2007; Ammenheuser et al. 2001; Bechtold et al. 1994; Dahl et al. 1990; Hayes et al. 1996, 2000; Sapkota et al. 2006; Ward et al. 1994, 1996), DNA adducts (Kreiling et al. 1986b; Sun et al. 1989b; Zhao et al. 2001), and hemoglobin adducts (Albertini et al. 2001, 2007; Begemann et al. 2001a, 2001b; Boogaard et al. 2001b; Hayes et al. 2000; HEI 2000; Osterman-Golkar et al. 1996) of rats, mice, and humans exposed to 1,3-butadiene has been performed. Data are needed that accurately correlate the level of biomarkers measured in the body, particularly for *pyr*-Val hemoglobin adducts with the exposure to 1,3-butadiene, as well as the variability in this correlation between sexes and ethnicity.

**Effect.** Conflicting data exist for the sensitivity of human lymphocyte *hprt* mutation frequencies resulting from occupational exposures (Albertini et al. 2001, 2007; Ammenheuser et al. 2001; Hayes et al. 1996, 2000; HEI 2003; Kelsey et al. 1995; Lovreglio et al. 2006; Tates et al. 1996; Ward et al. 1996, 2001; Wickliffe et al. 2009; Zhou et al. 1986). In mice and rats, levels of DEB-specific DNA-DNA cross-links correlated well with 1,3-butadiene inhalation and relative sensitivity of female mice to toxicity (Goggin et al. 2009). Two biomarkers for carcinogenic effect have been consistently found in multiple tumors sites in rodent chronic bioassays. A number of malignant gliomas and neuroblastomas in mice chronically inhaling 1,3-butadiene exhibited mutations of the p53 gene and H- and K-ras oncogenes (Kim et al. 2005), which have also been observed in forestomach tumors (Sills et al. 2001), hemangiosarcomas (Hong et al. 2000), and lymphomas (Zhuang et al. 1997) of chronically exposed mice. Data for reliable and specific biomarkers indicating onset of developmental effects in animals would be useful to determine if comparable exposure may lead to these effects in humans.

## 3. HEALTH EFFECTS

**Absorption, Distribution, Metabolism, and Excretion.** The timecourse of 1,3-butadiene and its primary metabolite, EB, in human blood has been investigated in volunteers inhaling 2 ppm for 20 minutes (Lin et al. 2001). *In vitro* studies have characterized some of the metabolism dynamics of 1,3-butadiene in animals (Bolt et al. 1983; Csanady et al. 1992; Duescher and Elfarra 1994; Elfarra et al. 1996, 2001; Himmelstein et al. 1994, 1995; Jackson et al. 2000a; Kreiling et al. 1987; Laib et al. 1990; Malvoisin and Roberfroid 1982; Malvoisin et al. 1979; Schmidt and Loeser 1985, 1986; Thornton-Manning et al. 1995b, 1997). Several toxicokinetic studies on 1,3-butadiene metabolism *in vivo* have been conducted in rats and mice following inhalation exposure (Bolt et al. 1983; Bond et al. 1987; Himmelstein et al. 1994; Kohn and Melnick 2001; Kreiling et al. 1986b; Shugaev 1969), but not following exposure by other routes. Thus, further studies in animals by the oral route to determine possible target organs by this route could be useful. Ethical considerations limit the testing of humans, but the development of methods to determine urinary and breath excretion of 1,3-butadiene and its metabolites by humans with known exposure to 1,3-butadiene may provide a means of monitoring humans for exposure.

**Comparative Toxicokinetics.** The study by Schmidt and Loeser (1985) indicated that there is a difference between the capability of mouse and rat liver postmitochondrial fractions to produce 1,2-epoxybutene-3 after incubation with 1,3-butadiene. Furthermore, monkey and human postmitochondrial liver preparations catalyzed the formation of only a small amount of the epoxide. Higher levels of the toxic epoxides were found in blood of mice following 1,3-butadiene exposure as compared to monkeys (Bolt et al. 1983; Csanady et al. 1992; Dahl et al. 1990; Duescher and Elfarra 1994; Elfarra et al. 1996, 2001; Jackson et al. 2000a; Sun et al. 1989a). Species differences in the toxicokinetics of a chemical may account for differences in toxic responses. Analysis of the blood, breath, and urine of humans exposed to 1,3-butadiene for parent compound and metabolites over time would provide a greater knowledge of the human metabolic pathways. Qualitative and quantitative comparison of human metabolites with those of animals could help identify the most appropriate species to serve as a model for predicting toxic effects and mechanisms of action in humans.

**Methods for Reducing Toxic Effects.**

**Children's Susceptibility.** Data needs relating to both prenatal and childhood exposures, and developmental effects expressed either prenatally or during childhood, are discussed in detail in the Developmental Toxicity subsection above.

## 3. HEALTH EFFECTS

The sensitivity of children to 1,3-butadiene toxicity, if any, is unknown. In mice, acute- and intermediate-duration inhalation exposures resulted in *in utero* fetal effects at exposure levels of 21.5–40 ppm (Anderson et al. 1996; DOE/NTP 1987b). No information is available for interfering with effects of 1,3-butadiene toxicity *in utero*.

Child health data needs relating to exposure are discussed in Section 6.8.1, Identification of Data Needs: Exposures of Children.

### 3.12.3 Ongoing Studies

The following ongoing studies were identified in the Federal Research in Progress database (FEDRIP 2009).

E. Struble is being funded by EPA National Health and Environmental Effects Research Laboratory (HEERL) to use environmental irradiation chambers (smog chambers) to induce the natural photochemically stimulated transformations of environmental pollutants. The synthetic urban smog mixture is composed of 55 hydrocarbon species representative of the ambient air of an average city in the United States. In this study, A549 cells were exposed simultaneously to irradiated and non-irradiated chamber mixtures for 5 hours. Post exposure, adverse health effects were determined by measures of increased cellular stress cytokine release and cytotoxicity. Exposure to the photochemically-generated products of 1,3-butadiene, toluene, and methanol induced increases in both cytotoxicity and IL-8 gene expression compared to 1,3-butadiene, toluene, or methanol alone. The exposure design was used to investigate the toxicity of chemicals after photochemical reactions and interactions with the urban atmosphere on healthy and susceptible individuals using representative *in vitro* samples. This research informs the toxicity from exposures to multiple environmental pollutants found in urban settings.

James A. Swenberg is being funded by the National Institute of Environmental Health Sciences (NIEHS) to examine the molecular dose of previously unexplored DNA adducts in rodents exposed to 1,3-butadiene and 3-butene-1,2-diol (BD-diol). The data will be compared with mutation frequencies and mutational spectra to determine (1) if a particular adduct could be used as a mutagenic indicator, and (2) to determine the effects of exposure on gene expression. First, it will be determined if hydroxymethylvinyl ketone (HMVK) is formed *in vivo* during exposure to 1,3-butadiene and BD-diol in a sex-, species-, and exposure concentration-dependent manner, resulting in mutagenicity. Secondly,

## 3. HEALTH EFFECTS

promutagenic N1 adenine adducts will be observed to see if they are converted to more stable inosine adducts, which may accumulate in tissues during chronic exposures. Several specific aims will be accomplished while addressing these questions. Specific Aim 1 is to examine the formation of potentially mutagenic DNA adducts (specifically 1, N2-propanodeoxyguanosine) by HMVK *in vivo*. Specific Aim 2 is to determine the utility of the N-terminal valine adduct of HMVK (HMK-Val) as a biomarker of HMVK formation by BD and BD-diol. Specific Aim 3 is to develop methods for detecting N1- inosine, N1- and N6-adenine adducts derived from BD metabolites *in vivo*. Specific Aim 4 is to determine the mutagenic responses induced by BD exposures and characterize the impact of BD-diol-derived metabolites on the spectra of mutations induced by BD exposure in the B6C3F1 mouse and F344 rat to identify which adducts studied in Aims 1 and 3 are quantitative indicators of mutagenesis. Specific Aim 5 will examine the effects of exposure to BD and BD-diol on gene expression and DNA repair pathways. Collectively, these experiments have been designed to inform on adduct formation, DNA repair, mutagenicity, and genomic alterations in rodents exposed to 1,3-butadiene and BD-diol, as well as the impact of glutathione depletion and DNA repair deficiency.

Elaine Symanski is being funded by the National Cancer Institute (NCI) to evaluate an association between lymphohematopoietic (LH) cancer incidence and air pollution in the Houston metropolitan area, with particular emphasis on three compounds: benzene, 1,3-butadiene, and styrene. Data from the Texas Cancer Registry (TCR) from 1995 to 2005 and the Texas Commission on Environmental Quality (TCEQ) from 1992 to 2003 will be analyzed to: (1) investigate the spatial and temporal distribution of LH cancer incidence in Harris and surrounding counties; (2) evaluate the association between distance from industrial sources and LH cancer incidence; (3) identify optimal methods for assessing ambient levels of benzene, 1,3-butadiene, and styrene using existing TCEQ monitoring data; and (4) evaluate the association between ambient levels of benzene, styrene, and 1,3-butadiene and LH cancer incidence using Poisson regression in single and multi-pollutant models. This will be the first study to examine this association in Harris and seven surrounding counties (Texas) and is among the first to utilize monitored levels of HAPs to assess increased risks of LH cancer associated with air pollution. This work will also correlate cancer rates with proximity to industrial facilities as well as ambient levels of benzene, styrene, and 1,3-butadiene. This study will address a gap in the literature by examining the association between HAPs and cancer incidence in Harris County, Texas and will further explore innovative methods to evaluate this association utilizing existing data sources.

Natalia Y. Tretyakova is being funded by the National Cancer Institute to evaluate the role of DNA-DNA cross-linking in the genotoxicity of diepoxybutane and 1,3-butadiene. DEB-DNA cross-links will be

## 3. HEALTH EFFECTS

structurally characterized and formation sequence preferences will be determined. The hydrolytic stability of DEB-DNA cross-links will be evaluated for their recognition by the *E. coli* UvrABC repair complex. DEB-DNA cross-links in rodent tissues will be quantified by capillary high performance liquid chromatography-electrospray tandem mass spectrometry (HPLC-ESI-MS/MS) methods. This research will provide valuable information on the molecular mechanisms underlying the genotoxic activity of diepoxybutane.

## 4. CHEMICAL AND PHYSICAL INFORMATION

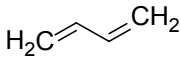
### 4.1 CHEMICAL IDENTITY

Information regarding the chemical identity of 1,3-butadiene is located in [Table 4-1](#). This information includes synonyms, chemical formula and structure, and identification numbers.

### 4.2 PHYSICAL AND CHEMICAL PROPERTIES

Information regarding the physical and chemical properties of 1,3-butadiene is located in [Table 4-2](#).

**Table 4-1. Chemical Identity of 1,3-Butadiene<sup>a</sup>**

| Characteristic           | Information                                                                                                                         |
|--------------------------|-------------------------------------------------------------------------------------------------------------------------------------|
| Chemical name            | 1,3-Butadiene                                                                                                                       |
| Synonyms and trade names | Butadiene; buta-1,3-diene; biethylene; bivinyl; divinyl; vinylethylene; erythrene; alpha,-gamma-butadiene; pyrrolylene <sup>b</sup> |
| Chemical formula         | C <sub>4</sub> H <sub>6</sub>                                                                                                       |
| Chemical structure       |                                                    |
| Identification numbers:  |                                                                                                                                     |
| CAS registry             | 106-99-0                                                                                                                            |
| NIOSH RTECS              | EI9275000 <sup>c</sup>                                                                                                              |
| EPA hazardous waste      | R0377-0754 <sup>d</sup>                                                                                                             |
| DOT/UN/NA/IMDG shipping  | 1010                                                                                                                                |
| EINECS                   | 203-450-8                                                                                                                           |
| HSDB                     | 181                                                                                                                                 |
| NCI                      | C50602                                                                                                                              |

<sup>a</sup>All information obtained from HSDB 2009 and ChemID Plus Advanced 2009 except where noted.

<sup>b</sup>O'Neil et al. 2006.

<sup>c</sup>NIOSH 2005.

<sup>d</sup>Miller 1978.

CAS = Chemical Abstracts Service; DOT/UN/NA/IMDG = Department of Transportation/United Nations/North America/International Maritime Dangerous Goods Code; EINECS = European Inventory of Existing Commercial chemical Substances; EPA = Environmental Protection Agency; HSDB = Hazardous Substances Data Bank; NCI = National Cancer Institute; NIOSH = National Institute for Occupational Safety and Health; RTECS = Registry of Toxic Effects of Chemical Substances



**Table 4-2. Physical and Chemical Properties of 1,3-Butadiene**

| Property                      | 1,3-Butadiene                                                         | Reference               |
|-------------------------------|-----------------------------------------------------------------------|-------------------------|
| Molecular weight              | 54.09                                                                 | O'Neil et al. 2006      |
| Color                         | Colorless                                                             | Lewis 2007              |
| Physical state                | Gas                                                                   | Lewis 2007              |
| Melting point                 | -108.966 °C                                                           | O'Neil et al. 2006      |
| Boiling point                 | -4.5 °C                                                               | O'Neil et al. 2006      |
| Density:                      |                                                                       |                         |
| at 25 °C (g/cm <sup>3</sup> ) | 0.6149                                                                | Lide 2008               |
| Vapor density                 | 1.88 (air=1)                                                          | NIOSH 2005              |
| Odor                          | Mildly aromatic; gasoline-like                                        | Lewis 2007              |
| Water                         | Not applicable <sup>a</sup>                                           | Amoore and Hautala 1983 |
| Air                           | 1.6 ppm                                                               | Amoore and Hautala 1983 |
| Odor threshold                |                                                                       |                         |
| Solubility:                   |                                                                       |                         |
| Water at 25 °C                | 735 mg/L                                                              | McAuliffe 1966          |
| Organic solvent(s)            | Soluble in ether, ethanol and benzene; very soluble in acetone        | Lide 2008               |
| Partition coefficients:       |                                                                       |                         |
| Log K <sub>ow</sub>           | 1.99                                                                  | Hansch et al. 1995      |
| K <sub>oc</sub>               | 288 (estimated) <sup>b</sup>                                          | HSDB 2009               |
| Vapor pressure at 25 °C       | 2.11x10 <sup>3</sup> mm Hg                                            | AIChE 2000              |
| Henry's law constant at 25 °C | 7.4x10 <sup>-2</sup> atm-m <sup>3</sup> /mol (estimated) <sup>c</sup> | HSDB 2009               |
| Autoignition temperature      | 414 °C                                                                | Lewis 2007              |
| Flashpoint                    | -76 °C                                                                | Lewis 2007              |
| Explosive limits              | 2.0-11.5%                                                             | O'Neil et al. 2006      |
| Conversion factors            | 1 ppm=2.21 mg/m <sup>3</sup><br>1 mg/m <sup>3</sup> =0.452 ppm        | NIOSH 2005              |

<sup>a</sup>Amoore and Hautala (1983) reported an odor threshold of 0.0014 ppm for 1,3-butadiene in water; however, these authors state that this solution lacks enough persistence for this value to be used for reference purposes.

<sup>b</sup>This K<sub>oc</sub> value was estimated using the measured log K<sub>ow</sub> value (1.99) and a regression derived equation.

<sup>c</sup>This Henry's Law constant value was calculated from the measured vapor pressure (2.11x10<sup>3</sup> mm Hg at 25 °C) and water solubility (735 mg/L).

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## 5. PRODUCTION, IMPORT/EXPORT, USE, AND DISPOSAL

### 5.1 PRODUCTION

1,3-Butadiene was discovered in the nineteenth century and its use in the development of rubber-like polymers was explored during the early 1900s (Grub and Loser 2005; Sun and Wristers 2002). Large volume production of 1,3-butadiene in the United States began during World War II as a result of the nation's synthetic rubber program (American Chemical Society 2007; Dolnick and Potash 1948). U.S. production rose to 2.7 billion pounds in 1965 as new plants were started to meet the increasing butadiene-rubber demand of the auto industry (Chemical Market Reporter 2006; Grub and Loser 2005; Kirshenbaum 1978). Production reached 3.7 billion pounds (1,674 metric tons) by 1974 and then fluctuated through the 1980s as a response to market pressures (Grub and Loser 2005; Kirshenbaum 1978; Sun and Wristers 2002). Production growth resumed during the 1990s, reaching 4.5 billion pounds (2,020 metric tons) in 1998 (Grub and Loser 2005). Actual production volumes of 1,3-butadiene manufactured in the United States during more recent years are not available; however, the annual U.S. production capacity was reported to be 6 billion pounds (2,800 metric tons) during 2008 (Chemical Week 2008; SRI 2008).

The 1,3-butadiene market is heavily dependent on the synthetic rubber demand of the auto industry, which accounts for approximately 60% of the total consumption of this substance (Chemical Market Reporter 2006). Also, since most 1,3-butadiene is produced through steam cracking, the supply of this chemical has been largely influenced by the demand for ethylene, the primary product from steam cracking (Sun and Wristers 2002).

The companies that produced 1,3-butadiene in the United States, their production sites, and their annual capacities during 2008 (the most recent year for which figures are available) are shown in [Table 5-1](#) (SRI 2008). [Table 5-2](#) summarizes the number of facilities in each state that manufactured or processed 1,3-butadiene in 2007, the ranges of maximum amounts on site, if reported, and the activities and uses as reported in the Toxics Release Inventory (TRI) (TRI09 2011). The data listed in this table should be used with caution since only certain types of facilities are required to report. This is not an exhaustive list.

Except for a small amount of 1,3-butadiene produced by the oxydehydrogenation of n-butene, all the 1,3-butadiene produced in the United States is a co-product of ethylene manufacture (Chemical Market Reporter 2006; Sun and Wristers 2002). In this process, feed streams ranging from light hydrocarbons to heavy gas oils are cracked in the presence of steam at 700–900 °C (Grub and Loser 2005; Kirshenbaum

## 5. PRODUCTION, IMPORT/EXPORT, USE, AND DISPOSAL

**Table 5-1. Companies that Produce 1,3-Butadiene in the United States and Annual Capacities During 2008**

| Company                      | Location               | Capacity (million pounds/year) | Capacity (metric tons) |
|------------------------------|------------------------|--------------------------------|------------------------|
| Equistar Chemicals, LP       | Alvin, Texas           | 150                            | 68,060                 |
|                              | Channelview, Texas     | 865                            | 392,500                |
|                              | Corpus Christi, Texas  | 200                            | 90,740                 |
| ExxonMobil Chemical Company  | Baton Rouge, Louisiana | 385                            |                        |
|                              | Baytown, Texas         | 325                            | 147,500                |
| INEOS Americas, LLC          |                        |                                |                        |
| INEOS Olefins & Polymers USA | Alvin, Texas           | 235                            | 106,600                |
| Sabina Petrochemicals LLC    | Port Arthur, Texas     | 900                            |                        |
| Shell Chemical LP            | Deer Park, Texas       | 360                            | 163,300                |
|                              | Norco, Louisiana       | 575                            | 260,900                |
| Texas Petrochemicals, Inc.   | Houston, Texas         | 1,080                          | 490,000                |
|                              | Port Neches, Texas     | 925                            | 419,700                |
| Total                        |                        | 6,000                          | 2,722,000              |

Source: SRI 2008

## 5. PRODUCTION, IMPORT/EXPORT, USE, AND DISPOSAL

**Table 5-2. Facilities that Produce, Process, or Use 1,3-Butadiene**

| State <sup>a</sup> | Number of facilities | Minimum amount on site in pounds <sup>b</sup> | Maximum amount on site in pounds <sup>b</sup> | Activities and uses <sup>c</sup>              |
|--------------------|----------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| AL                 | 8                    | 0                                             | 9,999,999                                     | 1, 3, 5, 6, 8, 13, 14                         |
| AR                 | 6                    | 1,000                                         | 999,999                                       | 1, 2, 3, 6, 9, 12                             |
| AZ                 | 1                    | 10,000                                        | 99,999                                        | 9                                             |
| CA                 | 73                   | 0                                             | 999,999,999                                   | 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14     |
| CO                 | 13                   | 0                                             | 9,999,999                                     | 1, 3, 5, 6, 8, 12, 13                         |
| CT                 | 3                    | 1,000,000                                     | 9,999,999                                     | 2, 3, 6                                       |
| DE                 | 11                   | 1,000                                         | 9,999,999                                     | 1, 2, 3, 5, 6, 7, 9, 12, 13                   |
| GA                 | 4                    | 100,000                                       | 9,999,999                                     | 1, 5, 6                                       |
| HI                 | 7                    | 1,000                                         | 99,999                                        | 1, 3, 5, 6, 13, 14                            |
| IA                 | 5                    | 100,000                                       | 9,999,999                                     | 1, 3, 4, 5, 6                                 |
| IL                 | 39                   | 0                                             | 49,999,999                                    | 1, 2, 3, 5, 6, 7, 8, 9, 11, 12, 13, 14        |
| IN                 | 24                   | 0                                             | 9,999,999                                     | 1, 3, 4, 5, 6, 7, 8, 12, 13, 14               |
| KS                 | 11                   | 0                                             | 499,999,999                                   | 1, 3, 6, 10, 13                               |
| KY                 | 16                   | 10,000                                        | 9,999,999                                     | 1, 3, 4, 5, 6, 7, 9, 12, 13                   |
| LA                 | 106                  | 0                                             | 10,000,000,000                                | 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14     |
| MI                 | 28                   | 0                                             | 49,999,999                                    | 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13         |
| MN                 | 10                   | 0                                             | 9,999,999                                     | 1, 2, 3, 5, 6, 7, 12, 13                      |
| MO                 | 6                    | 0                                             | 9,999                                         | 1, 5, 7, 12, 14                               |
| MS                 | 12                   | 1,000                                         | 499,999,999                                   | 1, 2, 3, 4, 5, 6, 7, 9, 13, 14                |
| MT                 | 19                   | 100                                           | 999,999                                       | 1, 3, 5, 6, 8, 12, 13, 14                     |
| NC                 | 9                    | 0                                             | 49,999,999                                    | 2, 3, 6, 7, 8, 10                             |
| ND                 | 4                    | 0                                             | 9,999                                         | 1, 2, 3, 4, 6                                 |
| NE                 | 2                    | 0                                             | 9,999                                         | 3, 8, 11, 12                                  |
| NH                 | 1                    | 1,000                                         | 9,999                                         | 6                                             |
| NJ                 | 16                   | 0                                             | 99,999                                        | 1, 3, 4, 5, 6, 7, 12, 13                      |
| NM                 | 2                    | 0                                             | 9,999                                         | 1, 3, 5, 7, 11                                |
| NY                 | 7                    | 10,000                                        | 999,999                                       | 2, 3, 4, 6, 8, 11                             |
| OH                 | 33                   | 0                                             | 49,999,999                                    | 1, 2, 3, 4, 5, 6, 7, 8, 9, 12, 13, 14         |
| OK                 | 21                   | 0                                             | 9,999,999                                     | 1, 3, 5, 6, 7, 8, 9, 10, 11, 12, 13           |
| PA                 | 20                   | 0                                             | 9,999,999                                     | 1, 2, 3, 5, 6, 7, 8, 12, 13, 14               |
| PR                 | 1                    | 10,000,000                                    | 49,999,999                                    | 1, 5, 7                                       |
| SC                 | 3                    | 0                                             | 99,999                                        | 1, 5, 6, 13, 14                               |
| TN                 | 21                   | 0                                             | 9,999,999                                     | 1, 2, 3, 5, 6, 7, 8, 9, 10, 12, 13, 14        |
| TX                 | 234                  | 0                                             | 10,000,000,000                                | 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 |
| UT                 | 15                   | 100                                           | 999,999                                       | 1, 3, 4, 5, 6, 7, 8, 9, 12                    |
| VA                 | 7                    | 0                                             | 9,999                                         | 1, 3, 4, 5, 6, 13                             |
| VI                 | 5                    | 100                                           | 99,999                                        | 1, 2, 3, 6, 13                                |
| WA                 | 27                   | 0                                             | 9,999,999                                     | 1, 2, 3, 4, 5, 6, 7, 11, 12, 13, 14           |

## 5. PRODUCTION, IMPORT/EXPORT, USE, AND DISPOSAL

**Table 5-2. Facilities that Produce, Process, or Use 1,3-Butadiene**

| State <sup>a</sup> | Number of facilities | Minimum amount on site in pounds <sup>b</sup> | Maximum amount on site in pounds <sup>b</sup> | Activities and uses <sup>c</sup> |
|--------------------|----------------------|-----------------------------------------------|-----------------------------------------------|----------------------------------|
| WI                 | 2                    | 100,000                                       | 9,999,999                                     | 6                                |
| WV                 | 10                   | 0                                             | 99,999,999                                    | 1, 5, 6, 7, 8, 12                |
| WY                 | 5                    | 0                                             | 99,999                                        | 1, 3, 4, 6                       |

<sup>a</sup>Post office state abbreviations used.<sup>b</sup>Amounts on site reported by facilities in each state.<sup>c</sup>Activities/Uses:

- |                          |                          |                             |
|--------------------------|--------------------------|-----------------------------|
| 1. Produce               | 6. Impurity              | 11. Chemical Processing Aid |
| 2. Import                | 7. Reactant              | 12. Manufacturing Aid       |
| 3. Onsite use/processing | 8. Formulation Component | 13. Ancillary/Other Uses    |
| 4. Sale/Distribution     | 9. Article Component     | 14. Process Impurity        |
| 5. Byproduct             | 10. Repackaging          |                             |

Source: TRI09 2011 (Data are from 2009)

## 5. PRODUCTION, IMPORT/EXPORT, USE, AND DISPOSAL

1978; Sun and Wristers 2002). The fraction of 1,3-butadiene produced by this process varies widely with the type of feedstock used and is lowest with low-boiling input streams (Grub and Loser 2005; Kirshenbaum 1978).

Purification of the crude C4 stream resulting from the steam cracking process cannot be achieved by a simple distillation due to the close boiling point of the various products and the fact that 1,3-butadiene forms an azeotrope with butane (Grub and Loser 2005; Kirshenbaum 1978). 1,3-Butadiene can be removed from the hydrocarbon stream by liquid-liquid extraction or extractive distillation. The selective solvents used in these processes include aqueous cupric ammonium acetate, acetonitrile, furfural, dimethylformamide, N,N-dimethylacetamide, and N-methylpyrrolidinone (Grub and Loser 2005; Sun and Wristers 2002).

The oxidative dehydrogenation of n-butene, used in the production of 1,3-butadiene, is a highly selective, irreversible process that involves heating the starting material, air, and a suitable catalyst together at 400–450 °C (Grub and Loser 2005; Kirshenbaum 1978; Sun and Wristers 2002). The hydrogen released in the dehydrogenation step combines with oxygen, producing large amounts of heat, which makes this process energy-efficient (Grub and Loser 2005; Kirshenbaum 1978; Sun and Wristers 2002). As indicated above, oxidative dehydrogenation of n-butene has not been competitive with the steam cracking process for manufacture of 1,3-butadiene (Chemical Market Reporter 2006; Grub and Loser 2005). Rather, this method has been used on a campaign basis when there is a large enough differential between feedstock and 1,3-butadiene prices (Grub and Loser 2005).

## 5.2 IMPORT/EXPORT

Large amounts of 1,3-butadiene are imported into the United States as consumption typically exceeds production (Chemical Market Reporter 2006; Chemical Week 2008; Sun and Wristers 2002). U.S. imports of 1,3-butadiene in 2005 were approximately 600 million pounds (Chemical Market Reporter 2006). More recent import data are not available. U.S. Exports of 1,3-butadiene are considered to be negligible (Chemical Market Reporter 2006).

## 5.3 USE

1,3-Butadiene is used as a monomer in the production of rubber and plastics; approximately 60% of the 1,3-butadiene consumed in the United States is used in the production of synthetic rubbers (Chemical Market Reporter 2006). 1,3-Butadiene uses can be broken down into the following categories: synthetic

## 5. PRODUCTION, IMPORT/EXPORT, USE, AND DISPOSAL

rubber, 61% (styrene butadiene rubber [SBR], 30%; polybutadiene elastomer, 25%; polychloroprene elastomer, 4%; nitrile elastomer, 2%) adiponitrile/hexamethylene diamine (HMDA), 11%; styrene-butadiene latex, 12%; ABS resins, 5%; and other uses, 11% (Chemical Market Reporter 2006).

Automobile tires are the major end-use product (Chemical Market Reporter 2006). Both styrene butadiene rubber and polybutadiene are used heavily for this purpose. 1,3-Butadiene is also used for other automotive applications such as high-impact polystyrene and ABS resin plastics (Chemical Market Reporter 2006). 1,3-Butadiene is a precursor in the production of adiponitrile (used to make nylon) and chloroprene (used to make neoprene) (Chemical Market Reporter 2006).

#### 5.4 DISPOSAL

The recommended method of disposal of 1,3-butadiene is by incineration within a suitable combustion chamber or in a safe area (HSDB 2009). Gaseous 1,3-butadiene can be burned directly; however, liquefied 1,3-butadiene (in a compressed cylinder) must be atomized before burning (HSDB 2009). Kennedy et al. (2009) report that burning off 1,3-butadiene, also referred to as flaring, is commonly practiced at facilities where excess amounts of this substance need to be destroyed (Kennedy et al. 2009).



## 6. POTENTIAL FOR HUMAN EXPOSURE

### 6.1 OVERVIEW

1,3-Butadiene has been identified in at least 13 of the 1,699 hazardous waste sites that have been proposed for inclusion on the EPA National Priorities List (NPL) (HazDat 2007). However, the number of sites evaluated for 1,3-butadiene is not known. The frequency of these sites can be seen in [Figure 6-1](#).

1,3-Butadiene is a high-volume, volatile hydrocarbon used in the production of commercial plastics and synthetic rubbers (Chemical Market Reporter 2006). The chemical reactivity of this monomer is utilized in its transformation into polymeric materials (Sigsby et al. 1987).

1,3-Butadiene may be released to the environment as an intentional or fugitive emission during its production, use, storage, transport, or disposal. Large amounts (1.17 million pounds) of this hydrocarbon are released to the atmosphere from commercial processes (TRI09 2011). Data on the detection of 1,3-butadiene in soil and water are scarce. In the past, it has been qualitatively detected in drinking water (EPA 1978; Kraybill 1980); however, more recent measurements are not available.

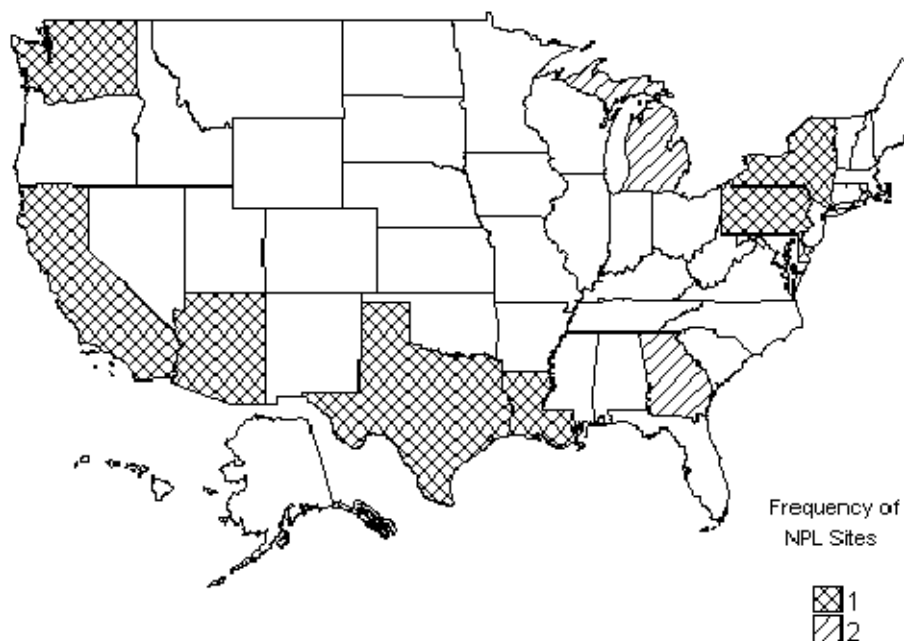
1,3-Butadiene is a highly volatile gas; therefore, it is expected to partition predominantly to the atmosphere. In the atmosphere, 1,3-butadiene is expected to undergo rapid destruction, primarily by photo-initiated reactions. The reaction with photochemically produced hydroxyl radicals has a calculated half-life of approximately 6 hours and is expected to be the dominant pathway for atmospheric removal (Atkinson 1989). Destruction of atmospheric 1,3-butadiene by the gas-phase reaction with tropospheric ozone and by the night-time reaction with nitrate radicals in urban areas is also expected to be significant (Atkinson and Carter 1984; Atkinson et al. 1984).

Limited data have been located on the fate of 1,3-butadiene in soil or water. Based on its physical properties, rapid volatilization of 1,3-butadiene from either soil or water to the atmosphere is expected to dominate over all other potential environmental processes. Based on estimated soil adsorption coefficient values, 1,3-butadiene is not expected to adsorb significantly to soil or sediment, nor is it expected to bioconcentrate in fish or aquatic organisms based on estimated bioconcentration and bioaccumulation factors.

Although 1,3-butadiene undergoes rapid photooxidation in the atmosphere, it is almost always present at very low concentrations in urban and suburban areas (Curren et al. 2006; Grant et al. 2007; Oguz et al.

## 6. POTENTIAL FOR HUMAN EXPOSURE

**Figure 6-1. Frequency of NPL Sites with 1,3-Butadiene Contamination**



Derived from HazDat 2007

## 6. POTENTIAL FOR HUMAN EXPOSURE

2003; Reiss 2006; Reiss and Griffin 2004; Sax et al. 2004). Automobile exhaust is a constant source of 1,3-butadiene release to the atmosphere. Because of the compound's ubiquity in the urban/suburban atmosphere, the general population is exposed to low ppb levels of 1,3-butadiene through inhalation (Higashino et al. 2007; Hughes et al. 2003). Exposure to 1,3-butadiene may also occur from the inhalation of cigarette smoke or the smoke from wood fires (Adam et al. 2006; Bartle et al. 1969; Blomberg and Widmark 1975; Brunnemann et al. 1990; Carmella et al. 2009; Counts et al. 2006; Gustafson et al. 2007; Lofroth et al. 1989; Pankow et al. 2004, 2007; Penn and Snyder 2007; Stump et al. 1989; Thweatt et al. 2007; Vainiotalo et al. 2008). Ingestion of contaminated food or drinking water may also lead to low levels of exposure, although current levels of this compound in food and water samples are not known, nor is there a good understanding of their frequency of detection (EPA 1978; Hughes et al. 2003; Kraybill 1980; Leber 2001; McNeal and Breder 1987; Startin and Gilbert 1984). The levels of 1,3-butadiene in soil are not known. Elevated levels of exposure for the general population may occur for those near its site of manufacture or facilities where it is made into polymeric materials.

Occupational exposure to 1,3-butadiene is expected to be limited to those working at facilities that manufacture 1,3-butadiene or convert it into commercial polymers (Anttinen-Klemetti et al. 2006; Begemann et al. 2001a; Fustinoni et al. 2004; Jones and Harris 1983; Lovreglio et al. 2006; Meinhardt et al. 1982; Sathiakumar et al. 2007; Tsai et al. 2001, 2005; Ward et al. 2001). Exposure by inhalation is expected to be the dominant pathway for exposure. Dermal exposure to liquified 1,3-butadiene could occur during an explosion of a pressurized storage tank or some other catastrophic event.

## 6.2 RELEASES TO THE ENVIRONMENT

The Toxics Release Inventory (TRI) data should be used with caution because only certain types of facilities are required to report (EPA 2005). This is not an exhaustive list. Manufacturing and processing facilities are required to report information to the TRI only if they employ 10 or more full-time employees; if their facility is included in Standard Industrial Classification (SIC) Codes 10 (except 1011, 1081, and 1094), 12 (except 1241), 20–39, 4911 (limited to facilities that combust coal and/or oil for the purpose of generating electricity for distribution in commerce), 4931 (limited to facilities that combust coal and/or oil for the purpose of generating electricity for distribution in commerce), 4939 (limited to facilities that combust coal and/or oil for the purpose of generating electricity for distribution in commerce), 4953 (limited to facilities regulated under RCRA Subtitle C, 42 U.S.C. section 6921 et seq.), 5169, 5171, and 7389 (limited S.C. section 6921 et seq.), 5169, 5171, and 7389 (limited to facilities primarily engaged in solvents recovery services on a contract or fee basis); and if their facility produces,

## 6. POTENTIAL FOR HUMAN EXPOSURE

imports, or processes  $\geq 25,000$  pounds of any TRI chemical or otherwise uses  $>10,000$  pounds of a TRI chemical in a calendar year (EPA 2005).

**6.2.1 Air**

Estimated releases of 1.17 million pounds (530 metric tons) of 1,3-butadiene to the atmosphere from 193 domestic manufacturing and processing facilities in 2009 accounted for about 99% of the estimated total environmental releases from facilities required to report to the TRI (TRI09 2011). These releases are summarized in [Table 6-1](#).

The dominant sources for the release of 1,3-butadiene to the atmosphere are fugitive or accidental emissions during its manufacture, use, transport, and storage. Low levels of 1,3-butadiene are continuously emitted to the atmosphere from many sources including exhaust from motor vehicle engines using petroleum-based fuels.

EPA's National Emission Inventory database (NEI) contains detailed information about sources that emit criteria air pollutants and their precursors, and hazardous air pollutants for the 50 United States, Washington DC, Puerto Rico, and the U.S. Virgin Islands. Emission data for 1,3-butadiene in 2005 is presented in [Table 6-2](#) and suggest that automobile usage accounts for approximately one-third of all emissions.

1,3-Butadiene was measured in the exhaust of typical automobiles and light trucks using both winter and summer gasoline formulations and accounted for up to 0.12% of total hydrocarbon emissions (Stump et al. 1989). An earlier study determined that the concentration of 1,3-butadiene in automobile exhaust was 20–60 ppb (Neligan 1962). 1,3-Butadiene has also been detected in the exhaust of diesel engines (Hayano et al. 1985) and high-altitude jet aircraft engines operating under simulated conditions (Katzman and Libby 1975).

There are several minor sources for the release of 1,3-butadiene to the atmosphere, all of which involve the thermal breakdown of other materials. 1,3-Butadiene has been detected as a component of the sidestream smoke from cigarettes (Adam et al. 2006; Bartle et al. 1969; Blomberg and Widmark, 1975; Carmella et al. 2009; Penn and Snyder 2007; Vainiotalo et al. 2008). The average amount of 1,3-butadiene in sidestream cigarette smoke is 205–361  $\mu\text{g}/\text{cigarette}$  (Brunnemann et al. 1990), with an average airborne yield of 400  $\mu\text{g}/\text{cigarette}$  (Lofroth et al. 1989).

## 6. POTENTIAL FOR HUMAN EXPOSURE

**Table 6-1. Releases to the Environment from Facilities that Produce, Process, or Use 1,3-Butadiene<sup>a</sup>**

| State <sup>c</sup> | RF <sup>d</sup> | Reported amounts released in pounds per year <sup>b</sup> |                    |                 |                   |                    |                      |                       |                  |
|--------------------|-----------------|-----------------------------------------------------------|--------------------|-----------------|-------------------|--------------------|----------------------|-----------------------|------------------|
|                    |                 | Air <sup>e</sup>                                          | Water <sup>f</sup> | UI <sup>g</sup> | Land <sup>h</sup> | Other <sup>i</sup> | Total release        |                       |                  |
|                    |                 |                                                           |                    |                 |                   |                    | On-site <sup>j</sup> | Off-site <sup>k</sup> | On- and off-site |
| AL                 | 2               | 3,069                                                     | 0                  | 0               | 47                | 0                  | 3,069                | 47                    | 3,116            |
| AR                 | 1               | 1,140                                                     | 0                  | 0               | 0                 | 0                  | 1,140                | 0                     | 1,140            |
| AZ                 | 1               | 1,460                                                     | 0                  | 0               | 0                 | 0                  | 1,460                | 0                     | 1,460            |
| CA                 | 17              | 6,116                                                     | 0                  | 0               | 8                 | 0                  | 6,116                | 8                     | 6,124            |
| CO                 | 3               | 776                                                       | 0                  | 0               | 0                 | 0                  | 776                  | 0                     | 776              |
| CT                 | 1               | 32                                                        | 0                  | 0               | 0                 | 0                  | 32                   | 0                     | 32               |
| DE                 | 1               | 534                                                       | 0                  | 0               | 0                 | 0                  | 534                  | 0                     | 534              |
| GA                 | 2               | 3,732                                                     | 0                  | 0               | 0                 | 0                  | 3,732                | 0                     | 3,732            |
| HI                 | 1               | 401                                                       | 0                  | 0               | 0                 | 0                  | 401                  | 0                     | 401              |
| IA                 | 1               | 4,766                                                     | 0                  | 0               | 0                 | 0                  | 4,766                | 0                     | 4,766            |
| IL                 | 7               | 43,415                                                    | 10                 | 0               | 44                | 0                  | 43,425               | 44                    | 43,469           |
| IN                 | 5               | 3,859                                                     | 5                  | 0               | 0                 | 0                  | 3,864                | 0                     | 3,864            |
| KS                 | 3               | 120                                                       | 0                  | 0               | 0                 | 0                  | 120                  | 0                     | 120              |
| KY                 | 5               | 15,246                                                    | 0                  | 0               | 726               | 0                  | 15,246               | 726                   | 15,972           |
| LA                 | 23              | 110,511                                                   | 574                | 0               | 65                | 0                  | 111,085              | 65                    | 111,150          |
| MI                 | 2               | 3,585                                                     | 0                  | 0               | 0                 | 0                  | 3,585                | 0                     | 3,585            |
| MN                 | 1               | 3,983                                                     | 2                  | 0               | 0                 | 0                  | 3,985                | 0                     | 3,985            |
| MS                 | 1               | 809                                                       | 0                  | 0               | 0                 | 0                  | 809                  | 0                     | 809              |
| MT                 | 3               | 903                                                       | 0                  | 0               | 0                 | 0                  | 903                  | 0                     | 903              |
| NC                 | 3               | 858                                                       | 0                  | 0               | 0                 | 0                  | 858                  | 0                     | 858              |
| ND                 | 1               | 89                                                        | 0                  | 0               | 0                 | 0                  | 89                   | 0                     | 89               |
| NJ                 | 4               | 102                                                       | 0                  | 0               | 0                 | 0                  | 102                  | 0                     | 102              |
| OH                 | 13              | 35,145                                                    | 6                  | 0               | 4,161             | 5                  | 39,311               | 6                     | 39,317           |
| OK                 | 4               | 6,981                                                     | 0                  | 0               | 0                 | 0                  | 6,981                | 0                     | 6,981            |
| PA                 | 4               | 2,258                                                     | 0                  | 0               | 0                 | 0                  | 2,258                | 0                     | 2,258            |
| SC                 | 1               | 5,520                                                     | 0                  | 0               | 0                 | 0                  | 5,520                | .                     | 5,520            |
| TN                 | 4               | 4,648                                                     | 0                  | 0               | 0                 | 0                  | 4,648                | 0                     | 4,648            |
| TX                 | 67              | 902,132                                                   | 49                 | 2,266           | 10                | 6                  | 904,446              | 17                    | 904,463          |
| UT                 | 2               | 1,069                                                     | 0                  | 0               | 0                 | 0                  | 1,069                | 0                     | 1,069            |
| VA                 | 1               | 962                                                       | 0                  | 0               | 0                 | 0                  | 962                  | 0                     | 962              |
| VI                 | 1               | 1,039                                                     | 0                  | 0               | 0                 | 0                  | 1,039                | 0                     | 1,039            |
| WA                 | 4               | 292                                                       | 0                  | 0               | 0                 | 0                  | 292                  | 0                     | 292              |
| WI                 | 1               | 156                                                       | 0                  | 0               | 0                 | 0                  | 156                  | 0                     | 156              |
| WV                 | 1               | 2,100                                                     | 0                  | 0               | 13                | 0                  | 2,113                | 0                     | 2,113            |

## 6. POTENTIAL FOR HUMAN EXPOSURE

**Table 6-1. Releases to the Environment from Facilities that Produce, Process, or Use 1,3-Butadiene<sup>a</sup>**

| Reported amounts released in pounds per year <sup>b</sup> |                 |                  |                    |                 |                   |                    |                      |                       |                  |
|-----------------------------------------------------------|-----------------|------------------|--------------------|-----------------|-------------------|--------------------|----------------------|-----------------------|------------------|
| State <sup>c</sup>                                        | RF <sup>d</sup> | Air <sup>e</sup> | Water <sup>f</sup> | UI <sup>g</sup> | Land <sup>h</sup> | Other <sup>i</sup> | Total release        |                       |                  |
|                                                           |                 |                  |                    |                 |                   |                    | On-site <sup>j</sup> | Off-site <sup>k</sup> | On- and off-site |
| WY                                                        | 2               | 500              | 0                  | 0               | 0                 | 0                  | 500                  | 0                     | 500              |
| Total                                                     | 193             | 1,168,307        | 646                | 2,266           | 5,074             | 11                 | 1,175,391            | 913                   | 1,176,304        |

<sup>a</sup>The TRI data should be used with caution since only certain types of facilities are required to report. This is not an exhaustive list. Data are rounded to nearest whole number.

<sup>b</sup>Data in TRI are maximum amounts released by each facility.

<sup>c</sup>Post office state abbreviations are used.

<sup>d</sup>Number of reporting facilities.

<sup>e</sup>The sum of fugitive and point source releases are included in releases to air by a given facility.

<sup>f</sup>Surface water discharges, waste water treatment-(metals only), and publicly owned treatment works (POTWs) (metal and metal compounds).

<sup>g</sup>Class I wells, Class II-V wells, and underground injection.

<sup>h</sup>Resource Conservation and Recovery Act (RCRA) subtitle C landfills; other onsite landfills, land treatment, surface impoundments, other land disposal, other landfills.

<sup>i</sup>Storage only, solidification/stabilization (metals only), other off-site management, transfers to waste broker for disposal, unknown

<sup>j</sup>The sum of all releases of the chemical to air, land, water, and underground injection wells.

<sup>k</sup>Total amount of chemical transferred off-site, including to POTWs.

RF = reporting facilities; UI = underground injection

Source: TRI09 2011 (Data are from 2009)

## 6. POTENTIAL FOR HUMAN EXPOSURE

**Table 6-2. 1,3-Butadiene Emission Data for 2005<sup>a</sup>**

| Category name                              | Type     | Annual emissions (tons) | Percentage |
|--------------------------------------------|----------|-------------------------|------------|
| Fuel comb, residential fireplaces          | Nonpoint | 3,352.17                | 6.35       |
| On-road vehicles, gasoline                 | Onroad   | 16,008.77               | 30.32      |
| Miscellaneous sources                      | Nonpoint | 17,627.65               | 33.39      |
| On-road vehicles, diesel                   | Onroad   | 1,058.86                | 2.01       |
| Agricultural field burning                 | Nonpoint | 339.31                  | 0.64       |
| Fuel comb, residential woodstoves          | Nonpoint | 315.31                  | 0.60       |
| Graphic arts                               | Point    | 0.00                    | 0.00       |
| Gas stations                               | Point    | 0.01                    | 0.00       |
| Wildfires                                  | Nonpoint | 3,340.53                | 6.33       |
| Bulk gasoline terminals                    | Point    | 5.11                    | 0.01       |
| Fuel comb, commercial/institutional        | Nonpoint | 0.07                    | 0.00       |
| Planes, trains, and ships                  | Nonroad  | 109.07                  | 0.21       |
| Prescribed fires                           | Nonpoint | 164.95                  | 0.31       |
| Industrial process, storage and transfer   | Nonpoint | 2.61                    | 0.00       |
| Industrial process, NEC                    | Point    | 129.88                  | 0.25       |
| Surface coating, industrial                | Point    | 16.81                   | 0.03       |
| Non-road equipment, diesel                 | Nonroad  | 364.74                  | 0.69       |
| Non-road equipment, gasoline               | Nonroad  | 8,062.72                | 15.27      |
| Industrial process, petroleum refineries   | Nonpoint | 1.30                    | 0.00       |
| Industrial process, petroleum refineries   | Point    | 22.15                   | 0.04       |
| Industrial process, cement manufacturing   | Point    | 64.37                   | 0.12       |
| Logging slash burning                      | Nonpoint | 211.34                  | 0.40       |
| Fuel comb, electric utility                | Point    | 2.30                    | 0.00       |
| Waste disposal, open burning               | Point    | 0.03                    | 0.00       |
| Waste disposal, open burning               | Nonpoint | 123.52                  | 0.23       |
| Fuel comb, commercial/institutional        | Point    | 0.78                    | 0.00       |
| Industrial process, oil and gas production | Point    | 7.71                    | 0.01       |
| Waste disposal                             | Nonpoint | 1.52                    | 0.00       |
| Industrial process, chemical manufacturing | Point    | 603.65                  | 1.14       |
| Degreasing                                 | Point    | 0.01                    | 0.00       |
| Industrial process, chemical manufacturing | Nonpoint | 1.44                    | 0.00       |
| Solvent, NEC                               | Point    | 0.24                    | 0.00       |
| Planes, trains, and ships                  | Point    | 323.90                  | 0.61       |
| Fuel comb, industrial boilers, ICEs        | Nonpoint | 2.86                    | 0.01       |
| Fuel comb, industrial boilers, ICEs        | Point    | 111.72                  | 0.21       |
| Waste disposal                             | Point    | 37.16                   | 0.07       |
| Industrial process, oil and gas production | Nonpoint | 0.18                    | 0.00       |
| Industrial process, storage and transfer   | Point    | 217.59                  | 0.41       |

## 6. POTENTIAL FOR HUMAN EXPOSURE

**Table 6-2. 1,3-Butadiene Emission Data for 2005<sup>a</sup>**

| Category name                      | Type  | Annual emissions (tons) | Percentage |
|------------------------------------|-------|-------------------------|------------|
| Industrial process, pulp and paper | Point | 0.30                    | 0.00       |
| Industrial process, metals         | Point | 161.81                  | 0.31       |

<sup>a</sup>Emission estimates are subject to updates for subsequent revised versions of the 2005 National Emissions Inventory data. These numbers may be different than values published for previous versions of the 2005 data and may also be different than values for subsequent revisions of the data as generated by EPA.

ICEs = internal combustion engines; NEC = not elsewhere classified

Source: EPA 2008



## 6. POTENTIAL FOR HUMAN EXPOSURE

The burning of plastics or rubber has been shown to release small amounts of 1,3-butadiene (Miller 1978). In a test designed to simulate a real-life electrical overload condition, 1,3-butadiene was detected when polyurethane coated wire was heated to 250 °C for 40 minutes (Rigby 1981). 1,3-Butadiene has also been measured as a component of the smoke from brush fire (Stephens and Burleson 1969), and as a stack emission from waste incinerators (Junk and Ford 1980). The concentrations of 1,3-butadiene were not presented in these studies. The mean, minimum, and maximum concentrations of 1,3-butadiene measured in the air of nine municipal structural fires were 1.03, 0.03, and 4.84 ppm, respectively (Austin et al. 2001). The sources of 1,3-butadiene were considered to be both the combustion of wood and the thermal degradation of polymeric materials. Forest fires are considered to be a natural source of 1,3-butadiene in the atmosphere (Curren et al. 2006). Detection of 1,3-butadiene while heating rapeseed oil indicates that the heating of cooking oils may be a source of 1,3-butadiene in indoor air (Pellizzari et al. 1995).

1,3-Butadiene has been identified in air samples collected at 8 of the 13 NPL hazardous waste sites where it was detected in some environmental media (HazDat 2007).

**6.2.2 Water**

Estimated releases of 646 pounds (0.29 metric tons) of 1,3-butadiene to surface water from 193 domestic manufacturing and processing facilities in 2009, accounted for about 0.05% of the estimated total environmental releases from facilities required to report to the TRI (TRI09 2011). An additional 913 pounds (0.08 metric tons) were transferred off-site which includes releases to publicly owned treatment works (POTWs) (TRI09 2011). These releases are summarized in [Table 6-1](#).

Additional information regarding the release of 1,3-butadiene to water was not located in the available literature. 1,3-Butadiene has been identified in groundwater samples collected at 1 of the 13 NPL hazardous waste sites where it was detected in some environmental media (HazDat 2007). It was not identified in surface water at any of the NPL sites.

**6.2.3 Soil**

Estimated releases of 5,074 pounds (2.3 metric tons) of 1,3-butadiene to soils from 193 domestic manufacturing and processing facilities in 2009, accounted for about 0.43% of the estimated total environmental releases from facilities required to report to the TRI (TRI09 2011). An additional

## 6. POTENTIAL FOR HUMAN EXPOSURE

2,266 pounds (1.03 metric tons), constituting about 0.19% of the total environmental emissions, were released via underground injection (TRI09 2011). These releases are summarized in [Table 6-1](#).

Additional information regarding the release of 1,3-butadiene to soil was not located in the available literature. 1,3-Butadiene has been identified in soil samples collected at 2 of the 13 NPL hazardous waste sites where it was detected in some environmental media (HazDat 2007). It was not identified in sediment at any of the NPL sites.

### 6.3 ENVIRONMENTAL FATE

#### 6.3.1 Transport and Partitioning

1,3-Butadiene's high volatility suggests that it will partition predominantly to the atmospheric compartment, where it is not expected to be adsorbed to particulate matter to any significant extent (Eisenreich et al. 1981).

Based on the calculated Henry's Law constant of  $7.4 \times 10^{-2}$  atm-m<sup>3</sup>/mol, the half-life for volatilization of 1,3-butadiene is 2.2 hours from a model river (1 m deep, flowing at 1 m/second, with a wind velocity of 3 m/second) and 2.9 days from a model lake (1 m deep, flowing at 0.05 m/second, with a wind velocity of 0.5 m/second) (Lyman et al. 1990). Based on an experimental log octanol/water partition coefficient of 1.99 (Hansch et al. 1995), a calculated soil adsorption coefficient of 288 (Lyman et al. 1990) suggests that adsorption to sediment and suspended organic matter will not be a significant fate process. From the log octanol/water partition coefficient, a calculated bioconcentration factor of 19 (Lyman et al. 1990) indicates that 1,3-butadiene will not bioconcentrate in fish and aquatic organisms to any significant extent. However, no experimental data have been located to verify these theoretical values.

If released to soil, 1,3-butadiene is expected to volatilize rapidly from either moist or dry soil to the atmosphere. This follows from the estimated lack of any appreciable adsorption to soil, and consideration of 1,3-butadiene's calculated Henry's law constant for moist soil or its vapor pressure, 2,100 mm Hg at 25 °C (AIChE 2000), for dry soil. Both values suggest a rapid rate of volatilization from their respective media.

The calculated soil adsorption coefficient of 288 (Hansch et al. 1995; Lyman et al. 1990) suggests that 1,3-butadiene may display moderate mobility in soil (Swann et al. 1983). However, the expected rapid rate of volatilization and the possibility of rapid degradation in soil suggest that there is little potential for

## 6. POTENTIAL FOR HUMAN EXPOSURE

1,3-butadiene to leach into groundwater. But until adequate groundwater monitoring for 1,3-butadiene has been performed, the partitioning of 1,3-butadiene in soil cannot be adequately addressed.

## 6.3.2 Transformation and Degradation

### 6.3.2.1 Air

Butadiene is a reactive, electron-rich chemical that is expected to undergo rapid reactions with the electrophilic oxidants typically present in the atmosphere: ozone, photochemically produced hydroxyl radicals, nitrate radicals, and molecular oxygen. Among these, the most rapid reaction in the atmosphere is with photochemically produced hydroxyl radicals.

The atmospheric degradation of 1,3-butadiene by photo-initiated processes has been established empirically by early studies. These studies typically involved irradiating urban air samples in atmospheric chambers of varying complexity and monitoring the disappearance of each constituent. Using this technique, 1,3-butadiene, at an average concentration of 12.4 ppb, disappeared in 6 hours when irradiated with natural sunlight during October (Kopczynski et al. 1972). In another study, a half-life of 2 hours was determined for the atmospheric removal of 1,3-butadiene using natural sunlight in October or November (Altshuller et al. 1970). In smog chamber studies, the sunlight oxidation of 1,3-butadiene led to the formation of fairly potent eye irritants, suggesting destruction of this compound with concomitant formation of oxygenated species (Dimitriades et al. 1975; Heuss and Glasson 1968). It is believed that the destruction of 1,3-butadiene occurs by photo-initiated bimolecular processes rather than direct photochemical degradation (Kopczynski et al. 1972). It is important to note that the rate of destruction of 1,3-butadiene when it was irradiated with natural light depended on the time of day in which the irradiation occurred. Furthermore, these studies were performed in October and November, when the amount and the intensity of available sunlight is diminished over that of summer months; thus, these values probably represent the high end of the compound's atmospheric lifetime. The individual processes responsible for the destruction of 1,3-butadiene in the atmosphere are discussed below.

Numerous studies have determined the rate constant for the gas-phase reaction of 1,3-butadiene with photochemically produced hydroxyl radicals (Atkinson 1985; Atkinson and Aschmann 1984; Atkinson et al. 1977, 1979; Maldotti et al. 1980). The experimental rate constant  $6.85 \times 10^{-11}$  cm<sup>3</sup>/molecule-second at 26 °C (Atkinson et al. 1977) is representative. Given an average hydroxyl radical concentration of  $5 \times 10^5$  molecule/cm<sup>3</sup> (Atkinson 1985), the half-life for this second-order process is 5.6 hours. Major products of this reaction include acrolein and formaldehyde (Baker et al. 2005).

## 6. POTENTIAL FOR HUMAN EXPOSURE

Gas-phase 1,3-butadiene also reacts with ozone in the atmosphere. Rate constants ranging from  $6.7 \times 10^{-18}$  to  $8.4 \times 10^{-18}$  cm<sup>3</sup>/molecule-second at 25 °C have been published in the literature (Atkinson and Carter 1984; Jaspar et al. 1974). Using an average atmospheric ozone concentration of  $7 \times 10^{11}$  molecules/cm<sup>3</sup> (Atkinson and Carter 1984), half-lives ranging from 1.4 to 1.7 days can be calculated for this second-order process. Therefore, the reaction of 1,3-butadiene with ozone is expected to contribute to the overall destruction of atmospheric 1,3-butadiene. The initial products from the reaction of 1,3-butadiene with ozone are acrolein, formaldehyde, acetylene, ethylene, and formic anhydride (Niki et al. 1983). All of these products are susceptible to secondary reactions with ozone and other atmospheric oxidants.

The night-time degradation of 1,3-butadiene is also expected to occur via the gas-phase reaction with nitrate radicals; this tends to be significant in urban areas, where the concentration of this oxidant is typically higher than in rural areas (Altshuller and Cohen 1964; Gay and Bulfalini 1971; Maldotti et al. 1980). A rate constant of  $5.4 \times 10^{-14}$  cm<sup>3</sup>/molecule-second at 22 °C has been determined for this reaction. This corresponds to a half-life of 14.9 hours using an average atmospheric nitrate radical concentration of  $2.4 \times 10^8$  molecule/cm<sup>3</sup> (Atkinson et al. 1984), typical of mildly polluted urban centers. Acrolein has been identified as a primary product of this reaction.

In summary, there are three gas-phase pathways that degrade 1,3-butadiene in the troposphere. Depending on local conditions, any one or all of these reactions may occur. Destruction of atmospheric 1,3-butadiene by the gas-phase reaction with photochemically produced hydroxyl radicals is expected to be the dominant photo-initiated pathway. Degradation via nitrate radicals is expected to be a significant night-time process in urban areas.

### 6.3.2.2 Water

Data on the degradation of 1,3-butadiene in aquatic systems are limited. Experimental data are restricted to microbial degradation studies performed under aerobic conditions. The bulk of these data were obtained from isolated bacterial strains (pure cultures), not with mixed microbial populations typically found in natural systems, and are not considered to be representative of the biodegradation of 1,3-butadiene in the environment. However, results from these studies suggest that biodegradation of 1,3-butadiene proceeds through oxidation to form 3,4-epoxybutene (Hou et al. 1979, 1980, 1983; Patel et al. 1982a; Watkinson and Somerville 1976). Watkinson and Somerville (1976) reported further degradation.

## 6. POTENTIAL FOR HUMAN EXPOSURE

**6.3.2.3 Sediment and Soil**

As is the case for the degradation of 1,3-butadiene in water, very limited data on the destruction of this compound in soil could be located in the available literature. Results from pure culture studies suggest a similar aerobic biodegradation pathway for 1,3-butadiene in soil compared to the pathway in water (Hou et al. 1979; Patel et al. 1979, 1982a, 1982b; VanGinkel et al. 1987; Watkinson and Somerville 1976).

**6.3.2.4 Other Media**

Specific information regarding the transformation or degradation of 1,3-butadiene in other environmental media was not located.

**6.4 LEVELS MONITORED OR ESTIMATED IN THE ENVIRONMENT**

Reliable evaluation of the potential for human exposure to 1,3-butadiene depends in part on the reliability of supporting analytical data from environmental samples and biological specimens. Concentrations of 1,3-butadiene in unpolluted atmospheres and in pristine surface waters are often so low as to be near the limits of current analytical methods. In reviewing data on 1,3-butadiene levels monitored or estimated in the environment, it should also be noted that the amount of chemical identified analytically is not necessarily equivalent to the amount that is bioavailable. The analytical methods available for monitoring 1,3-butadiene in a variety of environmental media are detailed in Chapter 7.

**6.4.1 Air**

1,3-Butadiene is widely detected at low ppb levels in urban air samples. Reported average concentrations range from 0.1 to 2  $\mu\text{g}/\text{m}^3$  (0.04–0.9 ppb) (Curren et al. 2006; Grant et al. 2007; Oguz et al. 2003; Reiss 2006; Reiss and Griffin 2004; Sax et al. 2004). A major source of 1,3-butadiene in urban air is automobile engine exhaust (Broderick and Marnane 2002; Sigsby et al. 1987; Stump and Dropkin 1985; Stump et al. 1989). Atmospheric emissions from industrial facilities that produce or use 1,3-butadiene are another major source of the compound in areas located near these facilities. Concentrations as high as 40  $\mu\text{g}/\text{m}^3$  (18 ppb) have been measured in the air near industrial sites (highest 24-hour level measured downwind from a major industrial source) (Grant et al. 2007). Curren et al. (2006) analyzed 3,267 air samples collected at eight rural locations in Canada and reported a mean 1,3-butadiene concentration of 0.02  $\mu\text{g}/\text{m}^3$  (0.009 ppb) at these sites. EPA (2003a) estimated that the average background concentration of 1,3-butadiene in the air of the United States is 0.13  $\mu\text{g}/\text{m}^3$  (0.058 ppb). McCarthy et al. (2006)

## 6. POTENTIAL FOR HUMAN EXPOSURE

provided a lower estimate ( $<0.02 \mu\text{g}/\text{m}^3$ ; 0.009 ppb) of 1,3-butadiene in the background air of North America. [Table 6-3](#) lists reported concentrations of 1,3-butadiene measured in outdoor air.

1,3-Butadiene has also been detected in indoor air samples. Mean concentrations of 1,3-butadiene measured in indoor air of homes in New York were  $1.0 \mu\text{g}/\text{m}^3$  (0.5 ppb) during the winter and  $1.2 \mu\text{g}/\text{m}^3$  (0.54 ppb) during the summer (Sax et al. 2004). In Los Angeles, mean concentrations were  $0.5 \mu\text{g}/\text{m}^3$  (0.2 ppb) during the winter and  $0.2 \mu\text{g}/\text{m}^3$  (0.09 ppb) during the fall. The concentrations of 1,3-butadiene measured in a tavern were 11 and  $19 \mu\text{g}/\text{m}^3$  (5.0 and 8.6 ppb) in two separate studies, while the outside air concentration was  $\leq 1 \mu\text{g}/\text{m}^3$  (0.5 ppb) at the same time (Lofroth et al. 1989). The difference in the indoor versus outdoor concentration may be ascribed to the presence of 1,3-butadiene in cigarette smoke. The concentration of 1,3-butadiene in a smoke-filled bar was  $2.7\text{--}4.5 \mu\text{g}/\text{m}^3$  (1.2–2.0 ppb) (Brunnemann et al. 1990). Higher 1,3-butadiene levels have been observed in air samples collected from the smoking areas of restaurants and pubs than in air samples collected from the nonsmoking areas of these buildings (Kim et al. 2001; Vainiotalo et al. 2008). Mean 1,3-butadiene concentrations measured in the indoor air of 32 smoking homes and 32 nonsmoking homes were 1.7 and  $0.5 \mu\text{g}/\text{m}^3$  (0.77 and 0.23 ppb), respectively (Kim et al. 2001). Mean 1,3-butadiene levels were 0.31–0.38  $\mu\text{g}/\text{m}^3$  (0.14–0.17 ppb) in the air of wood burning homes and  $0.114 \mu\text{g}/\text{m}^3$  (0.0515 ppb) in non-wood burning homes in Hagfors, Sweden (Gustafson et al. 2007). [Table 6-4](#) lists reported concentrations of 1,3-butadiene measured in indoor air.

#### 6.4.2 Water

No current information on the occurrence of 1,3-butadiene in water was located in the available literature.

1,3-Butadiene was found in 1 of 204 water samples taken in 1975–1976 from surface waters near known industrialized areas across the United States. The single positive sample was obtained in the Carquinez Strait, Contra Costa, California, at an approximate concentration of 2 ppb (Ewing et al. 1977).

No specific data on its presence in drinking water, such as monitoring dates or concentration, were located; however, 1,3-butadiene has been qualitatively detected in U.S. drinking water in the past (EPA 1978; Kraybill 1980).

#### 6.4.3 Sediment and Soil

No data on the occurrence of 1,3-butadiene in soil were located in the available literature.

## 6. POTENTIAL FOR HUMAN EXPOSURE

**Table 6-3. 1,3-Butadiene Concentrations in Outdoor Air**

| Location                      | Concentration (µg/m <sup>3</sup> ) |         |                   |       |                      |         | Reference                   |
|-------------------------------|------------------------------------|---------|-------------------|-------|----------------------|---------|-----------------------------|
|                               | n                                  | >LOD    | LOD               | Mean  | Median               | Maximum |                             |
| Urban                         |                                    |         |                   |       |                      |         |                             |
| New York City                 |                                    |         |                   |       |                      |         | Sax et al. 2004             |
| Winter                        | 31                                 | 14%     | 0.06              | 0.1   | ND                   | 0.7     |                             |
| Summer                        | 27                                 | 11%     | 0.06              | 0.1   | ND                   | 2.0     |                             |
| Los Angeles                   |                                    |         |                   |       |                      |         | Sax et al. 2004             |
| Winter                        | 35                                 | 24%     | 0.06              | 0.2   | ND                   | 1.7     |                             |
| Fall                          | 32                                 | 3%      | 0.06              | 0.01  | ND                   | 0.3     |                             |
| Houston                       |                                    |         |                   |       |                      |         | Reiss 2006                  |
| 16 Locations                  | 4,374                              | 61%     | 0.02 <sup>a</sup> | 1.3   | —                    | 7.2     |                             |
| Texas                         |                                    |         |                   |       |                      |         | Grant et al. 2007           |
| 47 Urban/<br>industrial sites | —                                  | <30–74% | 0.61              | 0.5   | —                    | 40      |                             |
| Baltimore                     |                                    |         |                   |       |                      |         | Sapkota and<br>Buckley 2003 |
| Toll booth                    | 56                                 | 100%    | 0.46              | —     | 2–13.5 <sup>b</sup>  | 19      |                             |
| Baltimore                     |                                    |         |                   |       |                      |         | Kim et al. 2007             |
| Parking garage                | 24 <sup>c</sup>                    | —       | —                 | —     | 0.2–0.5 <sup>d</sup> | 8       |                             |
| Canada                        |                                    |         |                   |       |                      |         | Curren et al.<br>2006       |
| 30 Locations                  | 5,160                              | —       | 0.001–0.02        | 0.22  | 0.17                 | 2.58    |                             |
| Rural                         |                                    |         |                   |       |                      |         |                             |
| Canada                        |                                    |         |                   |       |                      |         | Curren et al.<br>2006       |
| 8 Locations                   | 3,267                              | —       | 0.001–0.02        | 0.02  | —                    | —       |                             |
| Background estimate           |                                    |         |                   |       |                      |         |                             |
| United States                 | —                                  | —       | —                 | 0.13  | 0.10                 | 2.2     | EPA 2003a                   |
| North America                 | —                                  | —       | —                 | <0.02 | <0.02                | <0.02   | McCarthy et al.<br>2006     |

<sup>a</sup>Value is referred to as the reporting limit.<sup>b</sup>Lower and upper median values correspond to measurements taken during 12–3 a.m. and 6–9 a.m., respectively.<sup>c</sup>Seven-hour samples were collected on 18 week days and 6 weekend days.<sup>d</sup>Lower and upper median values correspond to measurements taken on weekends and weekdays, respectively.

LOD = limit of detection; n = number of samples; ND = not detected

## 6. POTENTIAL FOR HUMAN EXPOSURE

**Table 6-4. 1,3-Butadiene Concentrations in Indoor Air**

| Location                       | Concentration ( $\mu\text{g}/\text{m}^3$ ) |       |           |           |           |                        | Reference              |
|--------------------------------|--------------------------------------------|-------|-----------|-----------|-----------|------------------------|------------------------|
|                                | n                                          | >LOD  | LOD       | Mean      | Median    | Maximum                |                        |
| New York City                  |                                            |       |           |           |           |                        | Sax et al. 2004        |
| Winter                         | 36                                         | 64%   | 0.06      | 1.0       | 0.7       | 5.8                    |                        |
| Summer                         | 30                                         | 44%   | 0.06      | 1.2       | ND        | 12                     |                        |
| Los Angeles                    |                                            |       |           |           |           |                        | Sax et al. 2004        |
| Winter                         | 40                                         | 60%   | 0.06      | 0.5       | 0.5       | 1.8                    |                        |
| Fall                           | 32                                         | 38%   | 0.06      | 0.2       | ND        | 1.5                    |                        |
| Arizona NHEXAS                 |                                            |       |           |           |           |                        | Gordon et al. 1999     |
| Home indoor                    | 24                                         | 4%    | 0.38      | ND        | ND        | 0.6                    |                        |
| Home outdoor                   | 14                                         | 0%    | 0.38      | ND        | ND        | ND                     |                        |
| Ottawa, Canada                 |                                            |       |           |           |           |                        | Graham et al. 2004     |
| House background               | 17                                         | 65%   | 0.1       | 0.51      | 0.36      | 1.63                   |                        |
| Cold-start house <sup>a</sup>  | 17                                         | 100%  | 0.1       | 5.76      | 2.69      | 28.6                   |                        |
| Cold-start garage <sup>a</sup> | 17                                         | 100%  | 0.1       | 82.8      | 84.7      | 166                    |                        |
| Birmingham, United Kingdom     |                                            |       |           |           |           |                        | Kim et al. 2001        |
| 6 smoking homes                | 32                                         | 100%  | 0.11      | 1.7       | 0.7       | 10.8                   |                        |
| 6 nonsmoking homes             | 32                                         | <100% | 0.11      | 0.5       | 0.4       | 1.1                    |                        |
| Restaurants                    | 6                                          | —     | 0.11      | 1.5       | —         | —                      |                        |
| Pubs                           | 6                                          | —     | 0.11      | 3.0       | —         | —                      |                        |
| Other indoor <sup>b</sup>      | 43                                         | —     | 0.11      | 0.2–0.9   | —         | —                      |                        |
| Helsinki, Finland              |                                            |       |           |           |           |                        | Vainiotalo et al. 2008 |
| 10 Restaurants                 |                                            |       |           |           |           |                        |                        |
| Smoking area                   | 20                                         | 100%  | 0.02      | 4.3       | —         | 10.1                   |                        |
| Non-smoking area               | 20                                         | 100%  | 0.02      | 1.1       | —         | 3.9                    |                        |
| Hagfors, Sweden                |                                            |       |           |           |           |                        | Gustafson et al. 2007  |
| Wood burning homes             | 14                                         | NR    | 0.03–0.15 | 0.31–0.38 | 0.20–0.23 | 0.90–1.54 <sup>c</sup> |                        |
| Reference homes                | 10                                         | NR    | 0.03–0.15 | 0.11      | 0.10–0.11 | 0.18–0.24 <sup>c</sup> |                        |
| Area outdoor                   | 9                                          | NR    | 0.03–0.15 | 0.12      | 0.11      | —                      |                        |

<sup>a</sup>Concentrations measured in the house and garage after a cold vehicle engine was started in the attached garage.

<sup>b</sup>Includes offices, department stores, cinemas, perfume shop, libraries, and laboratories.

<sup>c</sup>90<sup>th</sup> percentile values.

LOD = limit of detection; n = number of samples; ND = not detected; NHEXAS = National Human Exposure Assessment Survey; NR = not reported



## 6. POTENTIAL FOR HUMAN EXPOSURE

**6.4.4 Other Environmental Media**

1,3-Butadiene is used to manufacture synthetic rubber and plastics that are frequently used for food packaging. Because residual 1,3-butadiene may be present in the polymers used to make the containers, both the packaging and the food contained therein have been analyzed. In one study, 1,3-butadiene at a concentration of 8–9 ng/g (ppb) was detected in three of three brands of olive oil packaged in 1,3-butadiene rubber-modified acrylonitrile-acrylic bottles (McNeal and Breder 1987). Analysis of the bottles themselves found 1,3-butadiene residues as high as 6,600 ng/g (ppb). Soft-plastic packaging tubs used as containers for potato salad, cottage cheese, and yogurt had residual 1,3-butadiene levels in the range of 21–1,700 ng/g (ppb). However, no 1,3-butadiene was detected in any of the food packed in these containers (detection limit 1 ppb). Chewing gum made with a 1,3-butadiene rubber base did not show residual traces of this diene (McNeal and Breder 1987). Soft-plastic margarine tubs from five major name brands in the United Kingdom contained 1,3-butadiene residues ranging from 5 to 310 µg/kg (ppb), but none of the monomer was detected in the margarine samples themselves (detection limit 0.2 µg/kg) (Startin and Gilbert 1984). The authors concluded that migration of the 1,3-butadiene monomer from plastic packaging to food is unlikely to present a problem. Residual levels of 1,3-butadiene in food containers are closely regulated by the Food and Drug Administration.

Pellizzari et al. (1995) measured 0.1 mg of 1,3-butadiene in rapeseed oil emissions during 20 minutes of heating the oil in a wok at 260 °C. The presence of 1,3-butadiene was attributed to the pyrolytic decomposition of unsaturated fatty acids in the oil.

**6.5 GENERAL POPULATION AND OCCUPATIONAL EXPOSURE**

1,3-Butadiene is almost always present in the air at low levels due to its emission from motor vehicles. Therefore, the general population is probably routinely exposed to ppb levels of this compound. Exposure to 1,3-butadiene by the general population is expected to be dominated by inhalation (Higashino et al. 2007; Hughes et al. 2003). Reported mean concentrations of 1,3-butadiene measured in urban air generally range from 0.1 to 2 µg/m<sup>3</sup> (0.4–0.9 ppb) (Curren et al. 2006; Grant et al. 2007; Oguz et al. 2003; Reiss 2006; Reiss and Griffin 2004; Sax et al. 2004). Sapkota et al. (2006) measured mean 1,3-butadiene personal air exposure levels of 1.22 µg/m<sup>3</sup> (0.55 ppb) for suburban-weekend exposure, 1.47 µg/m<sup>3</sup> (0.66 ppb) for urban-week day exposure, and 2.88 µg/m<sup>3</sup> (1.3 ppb) for tollbooth worker exposure in the Baltimore, Maryland area.

## 6. POTENTIAL FOR HUMAN EXPOSURE

Inhalation of 1,3-butadiene by the general population may also occur due to other sources. 1,3-Butadiene has been identified in cigarette smoke; therefore, smokers and those nearby are exposed to this compound (Adam et al. 2006; Bartle et al. 1969; Blomberg and Widmark 1975; Brunnemann et al. 1990; Carmella et al. 2009; Counts et al. 2006; Lofroth et al. 1989; Pankow et al. 2004, 2007; Penn and Snyder 2007; Thweatt et al. 2007; Vainiotalo et al. 2008). Reported delivery levels of 1,3-butadiene in the mainstream smoke of cigarettes range from 1.3 to 100  $\mu\text{g}/\text{cigarette}$  (Pankow et al. 2004; Thweatt et al. 2007). Nazaroff and Singer (2004) calculated a 1,3-butadiene inhalation intake of 16–37  $\mu\text{g}/\text{day}$  for nonsmokers who live with a smoker. This value was based on an exposure relevant emission factor of 515  $\mu\text{g}$  1,3-butadiene/cigarette and a 1,3-butadiene exposure concentration of 1.4–3.1  $\mu\text{g}/\text{m}^3$  (Nazaroff and Singer 2004). Counts et al. (2006) measured 1,3-butadiene concentrations ranging from 11.2 to 59.3  $\mu\text{g}/\text{cigarette}$  in the mainstream smoke of 26 different commercial cigarettes sold in the United States.

1,3-Butadiene is present in the smoke from brush fires, wood fires, and municipal structural fires (Austin et al. 2001; Gustafson et al. 2007; Stephens and Burleson 1969), suggesting that inhalation of the smoke from wood fires will lead to low-level exposure to 1,3-butadiene. Gustafson et al. (2007) reported a mean 1,3-butadiene concentration of 0.33  $\mu\text{g}/\text{m}^3$  (0.15 ppb) in the personal air of individuals living in wood-burning homes and 0.14  $\mu\text{g}/\text{m}^3$  (0.063 ppb) in the personal air of a reference group. Its presence in waste incinerator emissions (Junk and Ford 1980) suggests that exposure to the general population may occur for those living nearby. Small amounts of 1,3-butadiene are produced by the thermal degradation of polyurethane-coated wire, an event that may occur during an electrical overload (Rigby 1981). The thermal degradation of other 1,3-butadiene-based plastics or rubbers may produce 1,3-butadiene (Miller 1978), also leading to low-level exposure of the general population by inhalation.

If the mean daily urban air concentration of 1,3-butadiene is 0.29 ppb (0.64  $\mu\text{g}/\text{m}^3$ ), as determined in an analysis and compilation of experimental reports of ambient monitoring data obtained from 1970 to 1987 (Shah and Heyerdahl 1988), a nonoccupational daily intake of 12.8  $\mu\text{g}$  per person can be obtained based on an average human intake of 20  $\text{m}^3$  air/day. Marshall et al. (2006) calculated a 1,3-butadiene intake rate of 7.3  $\mu\text{g}/\text{day}$  based on an exposure concentration of 0.55  $\mu\text{g}/\text{m}^3$  and a mean breathing rate of 13.1  $\text{m}^3/\text{day}$ . Kim et al. (2002) measured mean personal air exposure concentrations of 1.1  $\mu\text{g}/\text{m}^3$  (0.50 ppb) during the night time and 0.8  $\mu\text{g}/\text{m}^3$  (0.36 ppb) during the day time for 12 residents of Birmingham, United Kingdom who were nonsmokers.

No data are available that quantify general population exposure to 1,3-butadiene by other routes of intake, such as ingestion of contaminated drinking water. Low-level exposure by ingestion of contaminated

## 6. POTENTIAL FOR HUMAN EXPOSURE

drinking water may occur as 1,3-butadiene has been qualitatively detected in U.S. drinking water supplies (EPA 1978; Kraybill 1980). Given that residues of 1,3-butadiene have been found in plastic and rubber food containers and in a few samples of the food contained in these containers (McNeal and Breder 1987), very low-level exposure to the general population may occur by ingestion of contaminated foods packaged in these containers (Hughes et al. 2003; Leber 2001). Leber (2001) estimated an upper-bound 1,3-butadiene intake of 44 ng/day from food contact sources which include styrene butadiene rubber-containing chewing gum, polymeric coatings, closures with sealing gaskets, and other indirect additives.

According to the National Occupational Exposure Survey (NOES) conducted by NIOSH between 1980 and 1983, 9,456 workers, of which 286 are women, were estimated to be exposed to 1,3-butadiene (NIOSH 1989). The NOES database does not contain information on the frequency, concentration, or duration of exposure of workers to any chemicals listed therein. These surveys provide only an estimate of the number of workers potentially exposed to chemicals in the workplace. During a study involving 13,130 men who had been employed for at least 1 year at any of eight synthetic rubber plants in the United States or one in Canada between 1943 and 1992, it was estimated that 10,429 (79%) of these individuals had occupational exposure to 1,3-butadiene (Delzell et al. 2001; Macaluso et al. 2004). Occupational exposure to 1,3-butadiene is expected to be limited to inhalation of this compound, although dermal contact with liquified 1,3-butadiene may occur during a large spill, tank explosion, pipeline rupture, or similar catastrophic event. Specific industrial classifications or job descriptions involving exposure to 1,3-butadiene are provided below.

Levels of 1,3-butadiene measured in the air at styrene-butadiene rubber (SBR) plants have been reported (Anttinen-Klemetti et al. 2006; Jones and Harris 1983; Meinhardt et al. 1982; Sathiakumar et al. 2007; Ward et al. 2001). [Table 6-5](#) lists 1,3-butadiene air concentrations associated with typical SBR operations. These concentrations were measured in air samples collected at different times between 1977 and 1991 at a synthetic rubber plant in Canada (Sathiakumar et al. 2007). These data show that individuals directly involved in the production of styrene-butadiene rubber have the greatest exposure to 1,3-butadiene at these facilities, although high concentrations were also associated with some equipment maintenance and control technician operations as well. Air concentrations associated with tank farm and transfer pumphouse operations (mean, 103 mg/m<sup>3</sup>; 56.6 ppm) were at least an order of magnitude greater than those of any of the other operations. Maximum concentrations measured at this facility were as high as 1,490 mg/m<sup>3</sup> (673 ppm).

## 6. POTENTIAL FOR HUMAN EXPOSURE

**Table 6-5. Air Concentrations of 1,3-Butadiene Corresponding to Typical Operations Within a Styrene-Butadiene Rubber (SBR) Plant<sup>a</sup>**

| Operation                                                               | n   | Concentration (mg/m <sup>3</sup> ) |        |         |         |
|-------------------------------------------------------------------------|-----|------------------------------------|--------|---------|---------|
|                                                                         |     | Mean                               | Median | Minimum | Maximum |
| SBR production                                                          |     |                                    |        |         |         |
| Polymerization                                                          |     |                                    |        |         |         |
| Tank farm, transfer pumphouse                                           | 231 | 103                                | 33.1   | 0.13    | 1,490   |
| Reactor, blowdown, panel board                                          | 261 | 9.9                                | 2.2    | 0.04    | 146     |
| Recovery, compressor house, high solids recovery                        | 333 | 22.5                               | 3.5    | 0.04    | 1,200   |
| Unspecified operative                                                   | 35  | 2.4                                | 0.91   | 0.00    | 34.3    |
| Coagulation, blending, solutions prep                                   | 314 | 2.1                                | 0.44   | 0.04    | 42.0    |
| Finishing                                                               |     |                                    |        |         |         |
| Baler, packager, reclaim                                                | 111 | 0.64                               | 0.33   | 0.07    | 4.2     |
| Dryer, baler dryer                                                      | 134 | 2.9                                | 0.44   | 0.11    | 271     |
| Unspecified operative                                                   | 39  | 0.29                               | 0.15   | 0.04    | 1.5     |
| Maintenance, production, maintenance field                              |     |                                    |        |         |         |
| Foreman, engineer                                                       | 15  | 0.44                               | 0.40   | 0.04    | 1.5     |
| Instrument worker, meter person, electrician, maintenance inspector     | 56  | 4.9                                | 0.42   | 0.04    | 108     |
| Pipefitter, oiler, mechanic, blacksmith, boilermaker, outside machinist | 250 | 9.7                                | 1.8    | 0.04    | 234     |
| Cleanup crew, laborer, work pool, utility person                        | 74  | 3.1                                | 1.1    | 0.11    | 40.0    |
| Technical/lab                                                           |     |                                    |        |         |         |
| Rubber control, technician                                              | 210 | 30.1                               | 2.1    | 0.00    | 1,150   |
| Butadiene control, hydrocarbon control, technician                      | 41  | 4.9                                | 0.15   | 0.00    | 67.4    |
| Shipping/distribution                                                   |     |                                    |        |         |         |
| Labor, utility person, service person                                   | 24  | 0.20                               | 0.15   | 0.00    | 0.66    |
| Utilities                                                               |     |                                    |        |         |         |
| Copolymer effluent operative                                            | 103 | 1.3                                | 0.88   | 0.00    | 17.5    |
| Unspecified/miscellaneous operative                                     | 114 | 0.42                               | 0.22   | 0.00    | 4.2     |

<sup>a</sup>Air samples were collected at different times at a Canadian synthetic rubber plant between 1977 and 1991.

Source: Sathiakumar et al. 2007

## 6. POTENTIAL FOR HUMAN EXPOSURE

1,3-Butadiene has been measured in the air of petrochemical facilities (Begemann et al. 2001a; Fustinoni et al. 2004; Lovreglio et al. 2006; Tsai et al. 2001, 2005). Mean time-weighted average concentrations of 1,3-butadiene were  $10.23 \text{ mg/m}^3$  (4.55 ppm) between 1979 and 1996 and  $0.56 \text{ mg/m}^3$  (0.25 ppm) between 1997 and 2003 at the Deer Park and Norco Manufacturing complexes owned by Shell Oil Company (Tsai et al. 2005). Short-term exposures to this substance were as high as  $987 \text{ mg/m}^3$  (439 ppm) in 1979–1996 and  $337 \text{ mg/m}^3$  (150 ppm) in 1997–2003. The decline in 1,3-butadiene levels in air at these facilities in recent years is attributed to the implementation of new health standards in 1996 (Tsai et al. 2001, 2005). A walk-through survey of 11 monomers, 17 polymers, and 2 end-user plants found that personal exposures ranged from  $<0.006 \text{ ppm}$  to  $374 \text{ ppm}$  ( $0.013\text{--}827 \text{ mg/m}^3$ ) (Fasen et al. 1990).

Two studies have reported levels of 1,3-butadiene measured in human blood, breath, and urine. Perbellini et al. (2003) measured average 1,3-butadiene concentrations of 1.0, 1.9, and 1.0 ng/L in the alveolar air, blood, and urine, respectively, of 46 forestry workers who were nonsmokers and 3.6, 11.4, and 3.9 ng/L in the alveolar air, blood, and urine, respectively, of 15 forestry workers who were smokers. The individuals had not been involved in forestry work for 2 months; therefore, occupational exposure was not considered to be a contributing factor. Fustinoni et al. (2004) measured end-shift 1,3-butadiene levels of 2.4, 3.8, and 4.3 ng/L in the exhaled air, urine, and blood, respectively, of 42 workers with a mean personal exposure of  $11.5 \text{ }\mu\text{g/m}^3$  (5.20 ppb) and levels of 2.3, 3.1, and 5.9 ng/L in the exhaled air, urine, and blood, respectively, of 43 workers with a mean personal exposure of  $0.9 \text{ }\mu\text{g/m}^3$  (0.4 ppb). Pre-shift levels in exhaled air and urine were 2.4 and 3.8 ng/L, respectively, for the higher exposed workers and below detection for the lower-exposed workers.

Other occupations where exposures to 1,3-butadiene may occur include petroleum refinery workers, professional bus, truck, and taxi drivers, parking garage attendants, tollbooth workers, and employees working in areas where smoking is permitted.

## 6.6 EXPOSURES OF CHILDREN

This section focuses on exposures from conception to maturity at 18 years in humans. Differences from adults in susceptibility to hazardous substances are discussed in Section 3.7, Children's Susceptibility.

Children are not small adults. A child's exposure may differ from an adult's exposure in many ways. Children drink more fluids, eat more food, breathe more air per kilogram of body weight, and have a larger skin surface in proportion to their body volume. A child's diet often differs from that of adults.

## 6. POTENTIAL FOR HUMAN EXPOSURE

The developing human's source of nutrition changes with age: from placental nourishment to breast milk or formula to the diet of older children who eat more of certain types of foods than adults. A child's behavior and lifestyle also influence exposure. Children crawl on the floor, put things in their mouths, sometimes eat inappropriate things (such as dirt or paint chips), and spend more time outdoors. Children also are closer to the ground, and they do not use the judgment of adults to avoid hazards (NRC 1993).

Children are expected to be exposed to 1,3-butadiene primarily through inhalation of low levels in air. Children who live near areas of heavy vehicle traffic or near industrial facilities where 1,3-butadiene is produced or used may be exposed to higher levels of 1,3-butadiene. The available data indicate that exposure to 1,3-butadiene through ingestion of food and drinking water is expected to be low relative to inhalation exposure. Biomonitoring data for children, including levels of 1,3-butadiene and its metabolites measured in breast milk, neonatal blood, cord blood, and meconium fluid have not been located in the available literature.

**6.7 POPULATIONS WITH POTENTIALLY HIGH EXPOSURES**

High levels of exposure to 1,3-butadiene are likely to be limited to those resulting from an occupationally related use of this compound. Inhalation is the most likely route of high exposure to 1,3-butadiene. 1,3-Butadiene is stored and transported in pressurized tanks, and it is possible that high levels of exposure by inhalation or dermal contact with the liquified gas may occur during the loading and unloading of these tanks, or by the accidental rupture of these tanks. Occupations where potentially high exposures to 1,3-butadiene may occur include those in styrene-butadiene rubber facilities, those in petroleum refineries, professional bus, truck, and taxi drivers, parking garage attendants, tollbooth workers, and employees working in areas where smoking is permitted. Individuals who live near facilities where 1,3-butadiene is produced or used have the potential for high exposure to this substance. Individuals who are frequently exposed to smoke from combustion sources, such as firefighters, may have high exposures to 1,3-butadiene (Austin et al. 2001). Individuals living very close to high traffic roads may be exposed to higher levels of butadiene.

**6.8 ADEQUACY OF THE DATABASE**

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of 1,3-butadiene is available. Where adequate information is not available, ATSDR, in conjunction with NTP is required to assure the initiation of a program of

## 6. POTENTIAL FOR HUMAN EXPOSURE

research designed to determine the health effects (and techniques for developing methods to determine such health effects) of 1,3-butadiene.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

### 6.8.1 Identification of Data Needs

**Physical and Chemical Properties.** The physical and chemical properties of 1,3-butadiene are well documented (Amoore and Hautala 1983; Hansch et al. 1995; HSDB 2009; Lewis 2007; Lide 2008; McAuliffe 1966; O'Neil et al. 2006; NIOSH 2005), and its environmental fate can be estimated from these properties (Lyman et al. 1982). No data needs are identified.

**Production, Import/Export, Use, Release, and Disposal.** According to the Emergency Planning and Community Right-to-Know Act of 1986, 42 U.S.C. Section 11023, industries are required to submit substance release and off-site transfer information to the EPA. The TRI, which contains this information for 2009, became available in February of 2011. This database is updated yearly and should provide a list of industrial production facilities and emissions.

The trends in the production and use of 1,3-butadiene are well documented (Chemical Market Reporter 2006; Chemical Week 2008; Grub and Loser 2005; Kirshenbaum 1978; SRI 2008; Sun and Wristers 2002), and there do not appear to be any critical information gaps. 1,3-Butadiene monomer does not occur in most products used in the home, although residues of this compound in commercial packages, especially food containers (McNeal and Breder 1987), are not well described. It is clear that the majority of 1,3-butadiene is released to the atmosphere (TRI09 2011). The disposal of 1,3-butadiene appears to be a straightforward process (HSDB 2009). No data needs are identified.

**Environmental Fate.** The fate of 1,3-butadiene in the atmosphere is well understood (Atkinson 1985; Atkinson and Carter 1984; Atkinson et al. 1984; Kopczynski et al. 1972). The fate of 1,3-butadiene in soil and water is not well understood, and partitioning from these media has to be determined from the physical and chemical properties of this compound (Lyman et al. 1982). A reliable method capable of

## 6. POTENTIAL FOR HUMAN EXPOSURE

detecting 1,3-butadiene in soil and water was not located in the available literature, and it is not clear whether 1,3-butadiene is absent from these media or simply not yet detected. The persistence of 1,3-butadiene in soil and water is not known, and the degree of partitioning from one environmental compartment to another can only be estimated. Exposure via ingestion or dermal contact to populations surrounding hazardous waste sites cannot, therefore, be accurately determined. Experimental data that address the partitioning of 1,3-butadiene in the environment, its potential to enter drinking water supplies, and its lifetime in soil and water are necessary to completely characterize the environmental fate of this compound.

**Bioavailability from Environmental Media.** Numerous toxicokinetic and toxicity studies in humans and animals have demonstrated the bioavailability of 1,3-butadiene from air. No data on the bioavailability of 1,3-butadiene from other sources (water or soil, for example) were located in the available literature. In conjunction with the data needs for determining 1,3-butadiene in environmental media, bioavailability studies from environmental media would be useful.

**Food Chain Bioaccumulation.** In theory, 1,3-butadiene is not believed to bioconcentrate significantly in fish and aquatic organisms; thus, it is not expected to biomagnify in the food chain (Hansch and Leo 1995; Lyman et al. 1982). No data addressing this point, however, were located in the available literature. Validation of these theories by valid experimental studies will aid in establishing a quantitative determination of 1,3-butadiene exposure to the general public.

**Exposure Levels in Environmental Media.** Reliable monitoring data for the levels of 1,3-butadiene in contaminated media at hazardous waste sites are needed so that the information obtained on levels of 1,3-butadiene in the environment can be used in combination with the known body burden of 1,3-butadiene to assess the potential risk of adverse health effects in populations living in the vicinity of hazardous waste sites.

Data on the levels of 1,3-butadiene in environmental media are limited. Extensive data on the occurrence of 1,3-butadiene in ambient air samples are available (Curren et al. 2006; Grant et al. 2007; Oguz et al. 2003; Reiss 2006; Reiss and Griffin 2004; Sax et al. 2004), but more data would be helpful. Data on the occurrence of 1,3-butadiene in water samples are very limited (Ewing et al. 1979). The presence of 1,3-butadiene in drinking water has been noted in the literature, but no concentrations or frequency of detection are available (EPA 1978; Kraybill 1980). The development of reliable analytical techniques for



## 6. POTENTIAL FOR HUMAN EXPOSURE

the analysis of 1,3-butadiene in soil and water will establish unambiguously the levels at which this compound is found in environmental media.

**Exposure Levels in Humans.** Limited data on levels of occupational exposure to 1,3-butadiene were available in the literature; however, occupational exposure to this compound appears to be limited to a readily definable group of industrial classifications (NIOSH 1989; Sathiakumar et al. 2007). Exposure levels for the general population are not well defined. Studies that correlate personal exposure with daily activities are necessary to adequately establish exposure levels for 1,3-butadiene. Biological monitoring studies cannot be performed until acceptable experimental techniques are developed. Exposure levels for those living near hazardous waste sites are not available and should be established.

This information is necessary for assessing the need to conduct health studies on these populations.

**Exposures of Children.** No information regarding exposures of children to 1,3-butadiene are currently available. Biomonitoring data for children, including levels of 1,3-butadiene and its metabolites measured in breast milk, neonatal blood, cord blood, and meconium fluid have not been located in the available literature. Studies are needed to help determine if there are differences between childhood and adult exposure to 1,3-butadiene.

Child health data needs relating to susceptibility are discussed in Section 3.12.2, Identification of Data Needs: Children's Susceptibility.

**Exposure Registries.**

No exposure registries for 1,3-butadiene were located. This substance is not currently one of the compounds for which a sub-registry has been established in the National Exposure Registry. The substance will be considered in the future when chemical selection is made for sub-registries to be established. The information that is amassed in the National Exposure Registry facilitates the epidemiological research needed to assess adverse health outcomes that may be related to exposure to this substance.

**6.8.2 Ongoing Studies**

Ongoing studies related to the potential for human exposure to 1,3-butadiene were not located.

## 6. POTENTIAL FOR HUMAN EXPOSURE

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## 7. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting, measuring, and/or monitoring 1,3-butadiene, its metabolites, and other biomarkers of exposure and effect to 1,3-butadiene. The intent is not to provide an exhaustive list of analytical methods. Rather, the intention is to identify well-established methods that are used as the standard methods of analysis. Many of the analytical methods used for environmental samples are the methods approved by federal agencies and organizations such as EPA and the National Institute for Occupational Safety and Health (NIOSH). Other methods presented in this chapter are those that are approved by groups such as the Association of Official Analytical Chemists (AOAC) and the American Public Health Association (APHA). Additionally, analytical methods are included that modify previously used methods to obtain lower detection limits and/or to improve accuracy and precision.

### 7.1 BIOLOGICAL MATERIALS

No standardized method to test for the presence of 1,3-butadiene in biological materials presently exists. Only a limited number of techniques have been employed to measure this compound in biological materials.

Perbellini et al. (2003) have developed a method to measure unmetabolized 1,3-butadiene concentrations in human blood, urine, and exhaled air. Breath samples were collected by expiration into headspace vials. Venous blood samples with EDTA added as an anticoagulant or urine samples were injected into a glass tube. Samples were analyzed using gas chromatography-mass spectrometry (GC-MS). Reported detection limits for 1,3-butadiene were 0.5 ng/L in blood, 1 ng/L in urine, and 0.8 ng/L in alveolar air.

A technique for the determination of 1,3-butadiene in margarine samples was reported by Startin and Gilbert (1984). The margarine sample is placed in a vial, sealed, and heated to 70 °C where it is allowed to equilibrate for 1 hour. The amount of 1,3-butadiene in the sample is determined by withdrawing a headspace sample, and injecting it directly into a GC equipped with a MS detection system. Quantitation is obtained by comparison of the peak height to that of a standard of known concentration. The sensitivity of this method allows quantitation down to 0.001 mg/kg (1 ppb). A similar headspace technique was used to test for the presence of butadiene in olive oil, vegetable oil, and yogurt samples (McNeal and Breder 1987).

## 7.2 ENVIRONMENTAL SAMPLES

Standardized methods for determining 1,3-butadiene in environmental samples are limited to air samples, as no methodology has been described for analyzing this compound in water or soil samples (EPA 1982, 1986). A representative list of the methods available for the determination of 1,3-butadiene in air samples can be found in [Table 7-1](#). The determination of 1,3-butadiene in personal air can be obtained using the procedures outlined in NIOSH Method 1024 (NIOSH 1994) and OSHA Method 56 (OSHA 2009a) or EPA Methods TO-14A and TO-15 (EPA 1999a, 1999b).

For NIOSH Method 1024, the air sample is obtained by passing a known volume of air (5–25 L) through a set of tandem coconut charcoal tubes, which adsorb 1,3-butadiene and remove it from the air stream (NIOSH 1994). The collected 1,3-butadiene is then removed from the adsorption tube by extraction with methylene chloride. Injection of the methylene chloride solution into a GC equipped with a flame ionization detector (FID) separates 1,3-butadiene from any interfering compounds that may be present. The choice of chromatography column for this determination is not crucial, as long as it cleanly separates 1,3-butadiene from other compounds.

The estimated quantitation limit (LOQ) of this method is 0.02 ppm, with an applicable range of 0.04–220 µg per sample (approximately 0.04–100 ppm) for a 25 L sample. The precision of this method appears to change as a function of the concentration being measured, due to desorption efficiencies changing as a function of sample concentration. With increasing concentration, the preparation of a standard becomes more difficult. A limitation of this study is the relatively high LOQ (20 ppb), since concentrations observed in environmental settings are often <1 ppb.

In NIOSH Method 1024, quantitation of 1,3-butadiene is accomplished by comparing the area under the sample's response signal to that of a known amount of 1,3-butadiene. The preparation and injection of a gaseous 1,3-butadiene standard is a difficult procedure; it must be performed carefully or erroneous results will occur. Sample storage appears to dramatically affect the results of the measurement. Samples stored at -4 °C displayed an average recovery of 93–98% over a 21-day period, while samples stored at room temperature ranged from 61 to 95%.

OSHA Method 56 for analyzing 1,3-butadiene in air samples is similar to the NIOSH method described above. Air is drawn through sampling tubes containing charcoal absorbent coated with 4-*tert*-butylcatechol. The samples are then desorbed with carbon disulfide and analyzed using gas chromatograph-

## 7. ANALYTICAL METHODS

**Table 7-1. Analytical Methods For Determining 1,3-Butadiene in Environmental Samples**

| Sample air                 | Preparation method                                                                                                    | Analytical method | Sample detection limit          | Percent recovery | Reference                    |
|----------------------------|-----------------------------------------------------------------------------------------------------------------------|-------------------|---------------------------------|------------------|------------------------------|
| Personal air (Method 1024) | Pass air through charcoal tube followed by desorption with methylene chloride                                         | GC/FID            | 0.04–220 µg/sample (25L sample) | 61–98            | NIOSH 1994                   |
| Personal air (Method 56)   | Pass air through charcoal tube coated with 4- <i>tert</i> -butylcatechol followed by desorption with carbon disulfide | GC/FID            | 200 µg/m <sup>3</sup> (90 ppb)  | 77–94            | OSHA 2009a                   |
| Air (Method TO-14A)        | Pressurized container sampling followed by cryogenic concentration                                                    | GC/MS/SIM or SCAN | No data                         | No data          | EPA 1999a                    |
| Air (Method TO-15)         | Pressurized container sampling followed by multisorbent concentration and thermal desorption                          | GC/MS/SIM or SCAN | No data                         | No data          | EPA 1999b                    |
| Air                        | Collect air in Tedlar bag concentrate on Tenax cartridge, thermal desorption                                          | GC/FID            | No data                         | No data          | Stump and Dropkin 1985       |
| Air                        | Pass air through charcoal tube solvent desorption                                                                     | GC/MS             | No data                         | No data          | Texas Air Control Board 1990 |
| Air (real time)            | Draw air into 12-foot sampling loop, direct injection                                                                 | GS/MS             | No data                         | No data          | Texas Air Control Board 1990 |
| Air                        | Pass air through sorbent tubes containing Carbopack B/Carbosieve SIII followed by thermal desorption                  | GC/MS             | 0.11–0.16 µg/m <sup>3</sup>     | >95%             | Kim et al. 1999              |

FID = flame ionization detector; GC = gas chromatography; MS = mass spectrometry; SCAN = wide range of mass to charge ratio scanning; SIM = select ion monitoring

## 7. ANALYTICAL METHODS

flame ionization detector (GC-FID). The recommended air volume and sampling rate is 3 L at 0.05 L/minute. The detection limit is 200  $\mu\text{g}/\text{m}^3$  and the quantitation limit is 343  $\mu\text{g}/\text{m}^3$ . As with NIOSH Method 1024, the usefulness of OSHA Method 56 for analysis of 1,3-butadiene in environmental media is limited since the reported detection and quantitation limits are much higher than levels often observed in environmental settings.

1,3-Butadiene, along with other volatile hydrocarbons, has been found in ambient air samples by a technique that uses cryogenic concentration before GC analysis. This technique is performed by collecting a large volume of air in a specially designed bag or other sampling container and concentrating the volatile components by condensation at low temperatures. The sample is separated into its components by GC and quantified with an internal standard. Numerous variations of this method were found in the literature (Curren 2006; Graham et al. 2004; Lonneman et al. 1979; Neligan 1962; Stephens and Burleson 1967, 1969; Stump and Dropkin 1985).

EPA Methods TO-14A and TO-15 describe procedures for the analysis of volatile organic compounds (VOCs) in air (EPA 1999a, 1999b). Method TO-14A calls for cryogenic concentration of the air sample as described above. Method TO-15 calls for pressurized air sampling using a stainless steel canister. The sample is then passed through a solid multisorbent concentrator and the concentrator is finally dry-purged with helium. The sample is thermally desorbed prior to analysis. For both of these methods, analysis is performed using GC followed by either a specific or nonspecific detector. However, the use of a specific detector is recommended, such as linear quadrupole mass spectrometer operating in either select ion monitoring (SIM) mode or a mode that scans a wide range of mass to charge ratios (SCAN). Precision and recovery data for 1,3-butadiene are not specified in these methods. Kim et al. (1999) developed an improved method by using a combination Carboxen 1000/Carbosieve SIII sorbent as a collection material. Sample collection was followed by thermal desorption and GC/MS analysis. These authors reported a precision of 2.4–13%, recoveries of >95%, and a detection limit of 0.11–0.16  $\mu\text{g}/\text{m}^3$  for this method. Therefore, this method is useful for measuring 1,3-butadiene concentrations in the low ppb ( $\mu\text{g}/\text{m}^3$ ) range in environmental air samples.

### 7.3 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of 1,3-butadiene is available. Where adequate information is

## 7. ANALYTICAL METHODS

not available, ATSDR, in conjunction with NTP is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of 1,3-butadiene.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

### 7.3.1 Identification of Data Needs

**Methods for Determining Biomarkers of Exposure and Effect.** No standardized method for the determination of biomarkers of exposure and effect for 1,3-butadiene was located.

**Exposure.** Biomarkers that have been proposed as indicators of exposure to 1,3-butadiene include the urinary metabolites 1,2-dihydroxybutyl mercapturic acid (DHBMA or M1) and 1- and 2-monohydroxy-3-butenyl mercapturic acid (MHBMA or M2) and the hemoglobin adducts 1- and 2-hydroxy-3-butenyl valine (MHB-Val) and N-(2,3,4-trihydroxy-butyl)valine (THB-Val) (Albertini et al. 2001; Boogaard et al. 2001a; Carrieri et al. 2009; McDonald et al. 2004; Sapkota et al. 2006; Schettgen et al. 2009; Shen et al. 2009). Measurement of unmetabolized 1,3-butadiene in human blood and urine may be preferable for assessing very low levels of exposure to this substance ( $0.4 \mu\text{g}/\text{m}^3$  median concentration in personal air) (Fustinoni et al. 2004; Perbellini et al. 2003; Schettgen et al. 2009).

Both the urinary metabolites and the hemoglobin adducts have been well correlated with exposure to 1,3-butadiene (Albertini et al. 2001; Preston 2007). Methods developed to measure these biomarkers have utilized either HPLC or GC followed by tandem MS (Boogaard et al. 2001a; Carrieri et al. 2009; Sapkota et al. 2006; Schettgen et al. 2009). Shen et al. (2009) has developed a method based on liquid chromatography/electrospray ionization-mass spectrometry to measure 3-butene-1,2-diol, a 1,3-butadiene urinary metabolite intermediate.

**Effect.** Biomarkers of effect resulting from 1,3-butadiene exposure, such as gene mutation and chromosomal changes, have been explored; however, no clear associations have been observed (Albertini et al. 2001; Preston 2007).

## 7. ANALYTICAL METHODS

**Methods for Determining Parent Compounds and Degradation Products in Environmental**

**Media.** Data on the determination of 1,3-butadiene in environmental media were limited. 1,3-Butadiene in air samples has been detected by techniques routinely used for detecting volatile hydrocarbons (Kim et al. 1999; Stump and Dropkin 1985; Texas Air Control Board 1990; EPA 1999a, 1999b).

Improvements to these methods have made possible the detection of 1,3 butadiene concentrations in the low ppb ( $\mu\text{g}/\text{m}^3$ ) range in environmental air samples (Kim et al. 1999). Procedures accepted for the determination of volatile hydrocarbons in other environmental media (soil, water, sediment, plants, etc.) may also be suitable for 1,3-butadiene. This question can be answered only by the data obtained from properly designed experiments. The information will assist in determining the prevalence of this compound in the environment and aid in a quantitative determination of human exposure to 1,3-butadiene.

**7.3.2 Ongoing Studies**

Ongoing studies related to the development of analytical methods for 1,3-butadiene were not located.



## 8. REGULATIONS, ADVISORIES, AND GUIDELINES

EPA (IRIS 2012) has established an inhalation reference concentration (RfC) for 1,3-butadiene of 0.9 ppb based on a BMCL<sub>10</sub> of 0.88 ppm for ovarian atrophy in female B6C3F1 mice exposed to 1,3-butadiene by inhalation for 6 hours/day, 5 days/week for up to 103 weeks.

EPA has not established an oral reference dose (RfD) for 1,3-butadiene (IRIS 2012).

OSHA has required employers of workers who are occupationally exposed to 1,3-butadiene to institute engineering controls and work practices to reduce and maintain employee exposure at or below permissible exposure limits (PELs) (OSHA 2009b). The employer must use engineering and work practice controls to reduce exposures to not exceed 1 ppm for 1,3-butadiene at any time (OSHA 2009b).

EPA has designated 1,3-butadiene as a hazardous air pollutant (HAP) under the Clean Air Act (CAA) (EPA 2009b). 1,3-Butadiene is on the list of chemicals appearing in “Toxic Chemicals Subject to Section 313 of the Emergency Planning and Community Right-to-Know Act of 1986” and has been assigned a reportable quantity (RQ) limit of 100 pounds (EPA 2009d). The RQ represents the amount of a designated hazardous substance which, when released to the environment, must be reported to the appropriate authority.

The international and national regulations, advisories, and guidelines regarding 1,3-butadiene in air, water, and other media are summarized in [Table 8-1](#).

## 8. REGULATIONS, ADVISORIES, AND GUIDELINES

**Table 8-1. Regulations, Advisories, and Guidelines Applicable to 1,3-Butadiene**

| Agency                      | Description                       | Information                                     | Reference                |
|-----------------------------|-----------------------------------|-------------------------------------------------|--------------------------|
| <u>INTERNATIONAL</u>        |                                   |                                                 |                          |
| Guidelines:                 |                                   |                                                 |                          |
| IARC                        | Carcinogenicity classification    | Group 1 <sup>a</sup>                            | IARC 2009                |
| WHO                         | Air quality guidelines            | No guideline value is recommended at this time  | WHO 2000                 |
|                             | Drinking water quality guidelines | No                                              | WHO 2006                 |
| <u>NATIONAL</u>             |                                   |                                                 |                          |
| Regulations and Guidelines: |                                   |                                                 |                          |
| a. Air                      |                                   |                                                 |                          |
| ACGIH                       | TLV (8-hour TWA)                  | 2 ppm                                           | ACGIH 2008               |
| AIHA                        | ERPG-1 <sup>b</sup>               | 10 ppm                                          | AIHA 2008                |
|                             | ERPG-2 <sup>b</sup>               | 200 ppm                                         |                          |
|                             | ERPG-3 <sup>b</sup>               | 5,000 ppm                                       |                          |
| EPA                         | RfC                               | 0.9 ppb                                         | IRIS 2012                |
|                             | Inhalation unit risk              | $3 \times 10^{-5}$ per $\mu\text{g}/\text{m}^3$ |                          |
| EPA                         | AEGL-1 <sup>c</sup>               |                                                 | EPA 2009a                |
|                             | 10 minutes                        | 670 ppm                                         |                          |
|                             | 30 minutes                        | 670 ppm                                         |                          |
|                             | 60 minutes                        | 670 ppm                                         |                          |
|                             | 4 hours                           | 670 ppm                                         |                          |
|                             | 8 hours                           | 670 ppm                                         |                          |
|                             | AEGL-2 <sup>c</sup>               |                                                 |                          |
|                             | 10 minutes                        | 6,700 ppm                                       |                          |
|                             | 30 minutes                        | 6,700 ppm                                       |                          |
|                             | 60 minutes                        | 5,300 ppm                                       |                          |
|                             | 4 hours                           | 3,400 ppm                                       |                          |
|                             | 8 hours                           | 2,700 ppm                                       |                          |
|                             | AEGL-3 <sup>c</sup>               |                                                 |                          |
|                             | 10 minutes                        | 27,000 ppm                                      |                          |
|                             | 30 minutes                        | 27,000 ppm                                      |                          |
|                             | 60 minutes                        | 22,000 ppm                                      |                          |
|                             | 4 hours                           | 14,000 ppm                                      |                          |
|                             | 8 hours                           | 6,800 ppm                                       |                          |
|                             | Level of distinct odor awareness  | 3.7 ppm                                         |                          |
|                             | Hazardous air pollutant           | Yes                                             | EPA 2009b<br>42 USC 7412 |

## 8. REGULATIONS, ADVISORIES, AND GUIDELINES

**Table 8-1. Regulations, Advisories, and Guidelines Applicable to 1,3-Butadiene**

| Agency                  | Description                                                                                            | Information                                                               | Reference                      |
|-------------------------|--------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------|--------------------------------|
| <u>NATIONAL</u> (cont.) |                                                                                                        |                                                                           |                                |
|                         | Regulated flammable substances and threshold quantities for accidental release prevention <sup>d</sup> | 10,000 pounds                                                             | EPA 2009c<br>40 CFR 68.130     |
| NIOSH                   | REL (10-hour TWA)                                                                                      | Potential occupational carcinogens                                        | NIOSH 2005                     |
|                         | IDLH (10% LEL)                                                                                         | 2,000 ppm                                                                 |                                |
|                         | Target organs                                                                                          | Eyes, respiratory system, central nervous system, and reproductive system |                                |
| OSHA                    | PEL (8-hour TWA) for general industry                                                                  | 1 ppm                                                                     | OSHA 2009b<br>29 CFR 1910.1051 |
|                         | STEL (15-minutes)                                                                                      | 5 ppm                                                                     |                                |
| b. Water                |                                                                                                        |                                                                           |                                |
| EPA                     | Drinking water standards and health advisories                                                         | No                                                                        | EPA 2006a                      |
|                         | National primary drinking water standards                                                              | No                                                                        | EPA 2003b                      |
|                         | National recommended water quality criteria                                                            | No                                                                        | EPA 2006b                      |
| c. Food                 |                                                                                                        |                                                                           |                                |
| FDA                     | EAFUS <sup>e</sup>                                                                                     | No                                                                        | FDA 2008                       |
| d. Other                |                                                                                                        |                                                                           |                                |
| ACGIH                   | Carcinogenicity classification                                                                         | A2 <sup>f</sup>                                                           | ACGIH 2008                     |

## 8. REGULATIONS, ADVISORIES, AND GUIDELINES

**Table 8-1. Regulations, Advisories, and Guidelines Applicable to 1,3-Butadiene**

| Agency | Description                                                | Information                          | Reference                  |
|--------|------------------------------------------------------------|--------------------------------------|----------------------------|
| EPA    | Carcinogenicity classification                             | Carcinogenic to humans by inhalation | IRIS 2012                  |
|        | RfD                                                        | No data                              |                            |
|        | Superfund, emergency planning, and community right-to-know |                                      |                            |
|        | Designated CERCLA hazardous substance                      | Yes <sup>g</sup>                     | EPA 2009d<br>40 CFR 302.4  |
|        | Reportable quantity                                        | 100 pounds                           |                            |
|        | Effective date of toxic chemical release reporting         | 01/01/1987                           | EPA 2009e<br>40 CFR 372.65 |
| NTP    | Carcinogenicity classification                             | Known to be a human carcinogen       | NTP 2005                   |

<sup>a</sup>Group 1: carcinogenic to humans

<sup>b</sup>ERPG-1 is the maximum airborne concentration below which nearly all individuals could be exposed for up to 1 hour without experiencing other than mild, transient health effects; ERPG-2 is the maximum airborne concentration below which nearly all individuals could be exposed for up to 1 hour without experiencing irreversible or other serious adverse effects; and ERPG-3 is the maximum airborne concentration below which nearly all individuals could be exposed for up to 1 hour without life-threatening health effects (AIHA 2008).

<sup>c</sup>AEGL-1 is the airborne concentration of a substance above which it is predicted that the general population, including susceptible individuals, could experience notable discomfort, irritation, or certain asymptomatic nonsensory effects, however, the effects are not disabling and are transient and reversible upon cessation of exposure; AEGL-2 is the airborne concentration of a substance above which it is predicted that the general population, including susceptible individuals, could experience irreversible or other serious, long-lasting adverse health effects or an impaired ability to escape; and AEGL-3 is the airborne concentration of a substance above which it is predicted that the general population, including susceptible individuals, could experience life-threatening health effects or death (EPA 2009a).

<sup>d</sup>Basis for listing: flammable gas

<sup>e</sup>The EAFUS list of substances contains ingredients added directly to food that FDA has either approved as food additives or listed or affirmed as GRAS.

<sup>f</sup>A2: suspected human carcinogen

<sup>g</sup>Designated CERCLA hazardous substance pursuant to Section 112 of the Clean Air Act

ACGIH = American Conference of Governmental Industrial Hygienists; AEGL = acute exposure guideline levels; AIHA = American Industrial Hygiene Association; CERCLA = Comprehensive Environmental Response, Compensation, and Liability Act; CFR = Code of Federal Regulations; EAFUS = Everything Added to Food in the United States; EPA = Environmental Protection Agency; ERPG = emergency response planning guidelines; FDA = Food and Drug Administration; GRAS = Generally Recognized As Safe; IARC = International Agency for Research on Cancer; IDLH = immediately dangerous to life or health; IRIS = Integrated Risk Information System; LEL = lower explosive limit; NIOSH = National Institute for Occupational Safety and Health; NTP = National Toxicology Program; OSHA = Occupational Safety and Health Administration; PEL = permissible exposure limit; REL = recommended exposure limit; RfC = inhalation reference concentration; RfD = oral reference dose; STEL = short-term exposure limit; TLV = threshold limit values; TWA = time-weighted average; USC = United States Code; WHO = World Health Organization

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## 10. GLOSSARY

**Absorption**—The taking up of liquids by solids, or of gases by solids or liquids.

**Acute Exposure**—Exposure to a chemical for a duration of 14 days or less, as specified in the Toxicological Profiles.

**Adsorption**—The adhesion in an extremely thin layer of molecules (as of gases, solutes, or liquids) to the surfaces of solid bodies or liquids with which they are in contact.

**Adsorption Coefficient ( $K_{oc}$ )**—The ratio of the amount of a chemical adsorbed per unit weight of organic carbon in the soil or sediment to the concentration of the chemical in solution at equilibrium.

**Adsorption Ratio ( $K_d$ )**—The amount of a chemical adsorbed by sediment or soil (i.e., the solid phase) divided by the amount of chemical in the solution phase, which is in equilibrium with the solid phase, at a fixed solid/solution ratio. It is generally expressed in micrograms of chemical sorbed per gram of soil or sediment.

**Benchmark Dose (BMD)**—Usually defined as the lower confidence limit on the dose that produces a specified magnitude of changes in a specified adverse response. For example, a  $BMD_{10}$  would be the dose at the 95% lower confidence limit on a 10% response, and the benchmark response (BMR) would be 10%. The BMD is determined by modeling the dose response curve in the region of the dose response relationship where biologically observable data are feasible.

**Benchmark Dose Model**—A statistical dose-response model applied to either experimental toxicological or epidemiological data to calculate a BMD.

**Bioconcentration Factor (BCF)**—The quotient of the concentration of a chemical in aquatic organisms at a specific time or during a discrete time period of exposure divided by the concentration in the surrounding water at the same time or during the same period.

**Biomarkers**—Broadly defined as indicators signaling events in biologic systems or samples. They have been classified as markers of exposure, markers of effect, and markers of susceptibility.

**Cancer Effect Level (CEL)**—The lowest dose of chemical in a study, or group of studies, that produces significant increases in the incidence of cancer (or tumors) between the exposed population and its appropriate control.

**Carcinogen**—A chemical capable of inducing cancer.

**Case-Control Study**—A type of epidemiological study that examines the relationship between a particular outcome (disease or condition) and a variety of potential causative agents (such as toxic chemicals). In a case-controlled study, a group of people with a specified and well-defined outcome is identified and compared to a similar group of people without outcome.

**Case Report**—Describes a single individual with a particular disease or exposure. These may suggest some potential topics for scientific research, but are not actual research studies.

**Case Series**—Describes the experience of a small number of individuals with the same disease or exposure. These may suggest potential topics for scientific research, but are not actual research studies.

## 10. GLOSSARY

**Ceiling Value**—A concentration of a substance that should not be exceeded, even instantaneously.

**Chronic Exposure**—Exposure to a chemical for 365 days or more, as specified in the Toxicological Profiles.

**Cohort Study**—A type of epidemiological study of a specific group or groups of people who have had a common insult (e.g., exposure to an agent suspected of causing disease or a common disease) and are followed forward from exposure to outcome. At least one exposed group is compared to one unexposed group.

**Cross-sectional Study**—A type of epidemiological study of a group or groups of people that examines the relationship between exposure and outcome to a chemical or to chemicals at one point in time.

**Data Needs**—Substance-specific informational needs that if met would reduce the uncertainties of human health assessment.

**Developmental Toxicity**—The occurrence of adverse effects on the developing organism that may result from exposure to a chemical prior to conception (either parent), during prenatal development, or postnatally to the time of sexual maturation. Adverse developmental effects may be detected at any point in the life span of the organism.

**Dose-Response Relationship**—The quantitative relationship between the amount of exposure to a toxicant and the incidence of the adverse effects.

**Embryotoxicity and Fetotoxicity**—Any toxic effect on the conceptus as a result of prenatal exposure to a chemical; the distinguishing feature between the two terms is the stage of development during which the insult occurs. The terms, as used here, include malformations and variations, altered growth, and *in utero* death.

**Environmental Protection Agency (EPA) Health Advisory**—An estimate of acceptable drinking water levels for a chemical substance based on health effects information. A health advisory is not a legally enforceable federal standard, but serves as technical guidance to assist federal, state, and local officials.

**Epidemiology**—Refers to the investigation of factors that determine the frequency and distribution of disease or other health-related conditions within a defined human population during a specified period.

**Genotoxicity**—A specific adverse effect on the genome of living cells that, upon the duplication of affected cells, can be expressed as a mutagenic, clastogenic, or carcinogenic event because of specific alteration of the molecular structure of the genome.

**Half-life**—A measure of rate for the time required to eliminate one half of a quantity of a chemical from the body or environmental media.

**Immediately Dangerous to Life or Health (IDLH)**—The maximum environmental concentration of a contaminant from which one could escape within 30 minutes without any escape-impairing symptoms or irreversible health effects.

**Immunologic Toxicity**—The occurrence of adverse effects on the immune system that may result from exposure to environmental agents such as chemicals.

## 10. GLOSSARY

**Immunological Effects**—Functional changes in the immune response.

**Incidence**—The ratio of individuals in a population who develop a specified condition to the total number of individuals in that population who could have developed that condition in a specified time period.

**Intermediate Exposure**—Exposure to a chemical for a duration of 15–364 days, as specified in the Toxicological Profiles.

**In Vitro**—Isolated from the living organism and artificially maintained, as in a test tube.

**In Vivo**—Occurring within the living organism.

**Lethal Concentration<sub>(LO)</sub> (LC<sub>LO</sub>)**—The lowest concentration of a chemical in air that has been reported to have caused death in humans or animals.

**Lethal Concentration<sub>(50)</sub> (LC<sub>50</sub>)**—A calculated concentration of a chemical in air to which exposure for a specific length of time is expected to cause death in 50% of a defined experimental animal population.

**Lethal Dose<sub>(LO)</sub> (LD<sub>LO</sub>)**—The lowest dose of a chemical introduced by a route other than inhalation that has been reported to have caused death in humans or animals.

**Lethal Dose<sub>(50)</sub> (LD<sub>50</sub>)**—The dose of a chemical that has been calculated to cause death in 50% of a defined experimental animal population.

**Lethal Time<sub>(50)</sub> (LT<sub>50</sub>)**—A calculated period of time within which a specific concentration of a chemical is expected to cause death in 50% of a defined experimental animal population.

**Lowest-Observed-Adverse-Effect Level (LOAEL)**—The lowest exposure level of chemical in a study, or group of studies, that produces statistically or biologically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control.

**Lymphoreticular Effects**—Represent morphological effects involving lymphatic tissues such as the lymph nodes, spleen, and thymus.

**Malformations**—Permanent structural changes that may adversely affect survival, development, or function.

**Minimal Risk Level (MRL)**—An estimate of daily human exposure to a hazardous substance that is likely to be without an appreciable risk of adverse noncancer health effects over a specified route and duration of exposure.

**Modifying Factor (MF)**—A value (greater than zero) that is applied to the derivation of a Minimal Risk Level (MRL) to reflect additional concerns about the database that are not covered by the uncertainty factors. The default value for a MF is 1.

**Morbidity**—State of being diseased; morbidity rate is the incidence or prevalence of disease in a specific population.

**Mortality**—Death; mortality rate is a measure of the number of deaths in a population during a specified interval of time.

## 10. GLOSSARY

**Mutagen**—A substance that causes mutations. A mutation is a change in the DNA sequence of a cell's DNA. Mutations can lead to birth defects, miscarriages, or cancer.

**Necropsy**—The gross examination of the organs and tissues of a dead body to determine the cause of death or pathological conditions.

**Neurotoxicity**—The occurrence of adverse effects on the nervous system following exposure to a chemical.

**No-Observed-Adverse-Effect Level (NOAEL)**—The dose of a chemical at which there were no statistically or biologically significant increases in frequency or severity of adverse effects seen between the exposed population and its appropriate control. Effects may be produced at this dose, but they are not considered to be adverse.

**Octanol-Water Partition Coefficient ( $K_{ow}$ )**—The equilibrium ratio of the concentrations of a chemical in *n*-octanol and water, in dilute solution.

**Odds Ratio (OR)**—A means of measuring the association between an exposure (such as toxic substances and a disease or condition) that represents the best estimate of relative risk (risk as a ratio of the incidence among subjects exposed to a particular risk factor divided by the incidence among subjects who were not exposed to the risk factor). An OR of greater than 1 is considered to indicate greater risk of disease in the exposed group compared to the unexposed group.

**Organophosphate or Organophosphorus Compound**—A phosphorus-containing organic compound and especially a pesticide that acts by inhibiting cholinesterase.

**Permissible Exposure Limit (PEL)**—An Occupational Safety and Health Administration (OSHA) allowable exposure level in workplace air averaged over an 8-hour shift of a 40-hour workweek.

**Pesticide**—General classification of chemicals specifically developed and produced for use in the control of agricultural and public health pests.

**Pharmacokinetics**—The dynamic behavior of a material in the body, used to predict the fate (disposition) of an exogenous substance in an organism. Utilizing computational techniques, it provides the means of studying the absorption, distribution, metabolism, and excretion of chemicals by the body.

**Pharmacokinetic Model**—A set of equations that can be used to describe the time course of a parent chemical or metabolite in an animal system. There are two types of pharmacokinetic models: data-based and physiologically-based. A data-based model divides the animal system into a series of compartments, which, in general, do not represent real, identifiable anatomic regions of the body, whereas the physiologically-based model compartments represent real anatomic regions of the body.

**Physiologically Based Pharmacodynamic (PBPD) Model**—A type of physiologically based dose-response model that quantitatively describes the relationship between target tissue dose and toxic end points. These models advance the importance of physiologically based models in that they clearly describe the biological effect (response) produced by the system following exposure to an exogenous substance.

**Physiologically Based Pharmacokinetic (PBPK) Model**—Comprised of a series of compartments representing organs or tissue groups with realistic weights and blood flows. These models require a

## 10. GLOSSARY

variety of physiological information: tissue volumes, blood flow rates to tissues, cardiac output, alveolar ventilation rates, and possibly membrane permeabilities. The models also utilize biochemical information, such as air/blood partition coefficients, and metabolic parameters. PBPK models are also called biologically based tissue dosimetry models.

**Prevalence**—The number of cases of a disease or condition in a population at one point in time.

**Prospective Study**—A type of cohort study in which the pertinent observations are made on events occurring after the start of the study. A group is followed over time.

**$q_1^*$** —The upper-bound estimate of the low-dose slope of the dose-response curve as determined by the multistage procedure. The  $q_1^*$  can be used to calculate an estimate of carcinogenic potency, the incremental excess cancer risk per unit of exposure (usually  $\mu\text{g/L}$  for water,  $\text{mg/kg/day}$  for food, and  $\mu\text{g/m}^3$  for air).

**Recommended Exposure Limit (REL)**—A National Institute for Occupational Safety and Health (NIOSH) time-weighted average (TWA) concentration for up to a 10-hour workday during a 40-hour workweek.

**Reference Concentration (RfC)**—An estimate (with uncertainty spanning perhaps an order of magnitude) of a continuous inhalation exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious noncancer health effects during a lifetime. The inhalation reference concentration is for continuous inhalation exposures and is appropriately expressed in units of  $\text{mg/m}^3$  or ppm.

**Reference Dose (RfD)**—An estimate (with uncertainty spanning perhaps an order of magnitude) of the daily exposure of the human population to a potential hazard that is likely to be without risk of deleterious effects during a lifetime. The RfD is operationally derived from the no-observed-adverse-effect level (NOAEL, from animal and human studies) by a consistent application of uncertainty factors that reflect various types of data used to estimate RfDs and an additional modifying factor, which is based on a professional judgment of the entire database on the chemical. The RfDs are not applicable to nonthreshold effects such as cancer.

**Reportable Quantity (RQ)**—The quantity of a hazardous substance that is considered reportable under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA). Reportable quantities are (1) 1 pound or greater or (2) for selected substances, an amount established by regulation either under CERCLA or under Section 311 of the Clean Water Act. Quantities are measured over a 24-hour period.

**Reproductive Toxicity**—The occurrence of adverse effects on the reproductive system that may result from exposure to a chemical. The toxicity may be directed to the reproductive organs and/or the related endocrine system. The manifestation of such toxicity may be noted as alterations in sexual behavior, fertility, pregnancy outcomes, or modifications in other functions that are dependent on the integrity of this system.

**Retrospective Study**—A type of cohort study based on a group of persons known to have been exposed at some time in the past. Data are collected from routinely recorded events, up to the time the study is undertaken. Retrospective studies are limited to causal factors that can be ascertained from existing records and/or examining survivors of the cohort.

**Risk**—The possibility or chance that some adverse effect will result from a given exposure to a chemical.

## 10. GLOSSARY

**Risk Factor**—An aspect of personal behavior or lifestyle, an environmental exposure, or an inborn or inherited characteristic that is associated with an increased occurrence of disease or other health-related event or condition.

**Risk Ratio**—The ratio of the risk among persons with specific risk factors compared to the risk among persons without risk factors. A risk ratio greater than 1 indicates greater risk of disease in the exposed group compared to the unexposed group.

**Short-Term Exposure Limit (STEL)**—The American Conference of Governmental Industrial Hygienists (ACGIH) maximum concentration to which workers can be exposed for up to 15 minutes continually. No more than four excursions are allowed per day, and there must be at least 60 minutes between exposure periods. The daily Threshold Limit Value-Time Weighted Average (TLV-TWA) may not be exceeded.

**Standardized Mortality Ratio (SMR)**—A ratio of the observed number of deaths and the expected number of deaths in a specific standard population.

**Target Organ Toxicity**—This term covers a broad range of adverse effects on target organs or physiological systems (e.g., renal, cardiovascular) extending from those arising through a single limited exposure to those assumed over a lifetime of exposure to a chemical.

**Teratogen**—A chemical that causes structural defects that affect the development of an organism.

**Threshold Limit Value (TLV)**—An American Conference of Governmental Industrial Hygienists (ACGIH) concentration of a substance to which most workers can be exposed without adverse effect. The TLV may be expressed as a Time Weighted Average (TWA), as a Short-Term Exposure Limit (STEL), or as a ceiling limit (CL).

**Time-Weighted Average (TWA)**—An allowable exposure concentration averaged over a normal 8-hour workday or 40-hour workweek.

**Toxic Dose<sub>(50)</sub> (TD<sub>50</sub>)**—A calculated dose of a chemical, introduced by a route other than inhalation, which is expected to cause a specific toxic effect in 50% of a defined experimental animal population.

**Toxicokinetic**—The absorption, distribution, and elimination of toxic compounds in the living organism.

**Uncertainty Factor (UF)**—A factor used in operationally deriving the Minimal Risk Level (MRL) or Reference Dose (RfD) or Reference Concentration (RfC) from experimental data. UFs are intended to account for (1) the variation in sensitivity among the members of the human population, (2) the uncertainty in extrapolating animal data to the case of human, (3) the uncertainty in extrapolating from data obtained in a study that is of less than lifetime exposure, and (4) the uncertainty in using lowest-observed-adverse-effect level (LOAEL) data rather than no-observed-adverse-effect level (NOAEL) data. A default for each individual UF is 10; if complete certainty in data exists, a value of 1 can be used; however, a reduced UF of 3 may be used on a case-by-case basis, 3 being the approximate logarithmic average of 10 and 1.

**Xenobiotic**—Any chemical that is foreign to the biological system.

## APPENDIX A. ATSDR MINIMAL RISK LEVELS AND WORKSHEETS

The Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) [42 U.S.C. 9601 et seq.], as amended by the Superfund Amendments and Reauthorization Act (SARA) [Pub. L. 99–499], requires that the Agency for Toxic Substances and Disease Registry (ATSDR) develop jointly with the U.S. Environmental Protection Agency (EPA), in order of priority, a list of hazardous substances most commonly found at facilities on the CERCLA National Priorities List (NPL); prepare toxicological profiles for each substance included on the priority list of hazardous substances; and assure the initiation of a research program to fill identified data needs associated with the substances.

The toxicological profiles include an examination, summary, and interpretation of available toxicological information and epidemiologic evaluations of a hazardous substance. During the development of toxicological profiles, Minimal Risk Levels (MRLs) are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration for a given route of exposure. An MRL is an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse noncancer health effects over a specified duration of exposure. MRLs are based on noncancer health effects only and are not based on a consideration of cancer effects. These substance-specific estimates, which are intended to serve as screening levels, are used by ATSDR health assessors to identify contaminants and potential health effects that may be of concern at hazardous waste sites. It is important to note that MRLs are not intended to define clean-up or action levels.

MRLs are derived for hazardous substances using the no-observed-adverse-effect level/uncertainty factor approach. They are below levels that might cause adverse health effects in the people most sensitive to such chemical-induced effects. MRLs are derived for acute (1–14 days), intermediate (15–364 days), and chronic (365 days and longer) durations and for the oral and inhalation routes of exposure. Currently, MRLs for the dermal route of exposure are not derived because ATSDR has not yet identified a method suitable for this route of exposure. MRLs are generally based on the most sensitive chemical-induced end point considered to be of relevance to humans. Serious health effects (such as irreparable damage to the liver or kidneys, or birth defects) are not used as a basis for establishing MRLs. Exposure to a level above the MRL does not mean that adverse health effects will occur.

MRLs are intended only to serve as a screening tool to help public health professionals decide where to look more closely. They may also be viewed as a mechanism to identify those hazardous waste sites that

## APPENDIX A

are not expected to cause adverse health effects. Most MRLs contain a degree of uncertainty because of the lack of precise toxicological information on the people who might be most sensitive (e.g., infants, elderly, nutritionally or immunologically compromised) to the effects of hazardous substances. ATSDR uses a conservative (i.e., protective) approach to address this uncertainty consistent with the public health principle of prevention. Although human data are preferred, MRLs often must be based on animal studies because relevant human studies are lacking. In the absence of evidence to the contrary, ATSDR assumes that humans are more sensitive to the effects of hazardous substance than animals and that certain persons may be particularly sensitive. Thus, the resulting MRL may be as much as 100-fold below levels that have been shown to be nontoxic in laboratory animals.

Proposed MRLs undergo a rigorous review process: Health Effects/MRL Workgroup reviews within the Division of Toxicology and Human Health Sciences (proposed), expert panel peer reviews, and agency-wide MRL Workgroup reviews, with participation from other federal agencies and comments from the public. They are subject to change as new information becomes available concomitant with updating the toxicological profiles. Thus, MRLs in the most recent toxicological profiles supersede previously published levels. For additional information regarding MRLs, please contact the Division of Toxicology and Human Health Sciences (proposed), Agency for Toxic Substances and Disease Registry, 1600 Clifton Road NE, Mailstop F-62, Atlanta, Georgia 30333.



## APPENDIX B. USER'S GUIDE

### Chapter 1

#### Public Health Statement

This chapter of the profile is a health effects summary written in non-technical language. Its intended audience is the general public, especially people living in the vicinity of a hazardous waste site or chemical release. If the Public Health Statement were removed from the rest of the document, it would still communicate to the lay public essential information about the chemical.

The major headings in the Public Health Statement are useful to find specific topics of concern. The topics are written in a question and answer format. The answer to each question includes a sentence that will direct the reader to chapters in the profile that will provide more information on the given topic.

### Chapter 2

#### Relevance to Public Health

This chapter provides a health effects summary based on evaluations of existing toxicologic, epidemiologic, and toxicokinetic information. This summary is designed to present interpretive, weight-of-evidence discussions for human health end points by addressing the following questions:

1. What effects are known to occur in humans?
2. What effects observed in animals are likely to be of concern to humans?
3. What exposure conditions are likely to be of concern to humans, especially around hazardous waste sites?

The chapter covers end points in the same order that they appear within the Discussion of Health Effects by Route of Exposure section, by route (inhalation, oral, and dermal) and within route by effect. Human data are presented first, then animal data. Both are organized by duration (acute, intermediate, chronic). *In vitro* data and data from parenteral routes (intramuscular, intravenous, subcutaneous, etc.) are also considered in this chapter.

The carcinogenic potential of the profiled substance is qualitatively evaluated, when appropriate, using existing toxicokinetic, genotoxic, and carcinogenic data. ATSDR does not currently assess cancer potency or perform cancer risk assessments. Minimal Risk Levels (MRLs) for noncancer end points (if derived) and the end points from which they were derived are indicated and discussed.

Limitations to existing scientific literature that prevent a satisfactory evaluation of the relevance to public health are identified in the Chapter 3 Data Needs section.

#### Interpretation of Minimal Risk Levels

Where sufficient toxicologic information is available, ATSDR has derived MRLs for inhalation and oral routes of entry at each duration of exposure (acute, intermediate, and chronic). These MRLs are not meant to support regulatory action, but to acquaint health professionals with exposure levels at which adverse health effects are not expected to occur in humans.

## APPENDIX B

MRLs should help physicians and public health officials determine the safety of a community living near a chemical emission, given the concentration of a contaminant in air or the estimated daily dose in water. MRLs are based largely on toxicological studies in animals and on reports of human occupational exposure.

MRL users should be familiar with the toxicologic information on which the number is based. Chapter 2, "Relevance to Public Health," contains basic information known about the substance. Other sections such as Chapter 3 Section 3.9, "Interactions with Other Substances," and Section 3.10, "Populations that are Unusually Susceptible" provide important supplemental information.

MRL users should also understand the MRL derivation methodology. MRLs are derived using a modified version of the risk assessment methodology that the Environmental Protection Agency (EPA) provides (Barnes and Dourson 1988) to determine reference doses (RfDs) for lifetime exposure.

To derive an MRL, ATSDR generally selects the most sensitive end point which, in its best judgement, represents the most sensitive human health effect for a given exposure route and duration. ATSDR cannot make this judgement or derive an MRL unless information (quantitative or qualitative) is available for all potential systemic, neurological, and developmental effects. If this information and reliable quantitative data on the chosen end point are available, ATSDR derives an MRL using the most sensitive species (when information from multiple species is available) with the highest no-observed-adverse-effect level (NOAEL) that does not exceed any adverse effect levels. When a NOAEL is not available, a lowest-observed-adverse-effect level (LOAEL) can be used to derive an MRL, and an uncertainty factor (UF) of 10 must be employed. Additional uncertainty factors of 10 must be used both for human variability to protect sensitive subpopulations (people who are most susceptible to the health effects caused by the substance) and for interspecies variability (extrapolation from animals to humans). In deriving an MRL, these individual uncertainty factors are multiplied together. The product is then divided into the inhalation concentration or oral dosage selected from the study. Uncertainty factors used in developing a substance-specific MRL are provided in the footnotes of the levels of significant exposure (LSE) tables.

## **Chapter 3**

### **Health Effects**

#### **Tables and Figures for Levels of Significant Exposure (LSE)**

Tables and figures are used to summarize health effects and illustrate graphically levels of exposure associated with those effects. These levels cover health effects observed at increasing dose concentrations and durations, differences in response by species, MRLs to humans for noncancer end points, and EPA's estimated range associated with an upper-bound individual lifetime cancer risk of 1 in 10,000 to 1 in 10,000,000. Use the LSE tables and figures for a quick review of the health effects and to locate data for a specific exposure scenario. The LSE tables and figures should always be used in conjunction with the text. All entries in these tables and figures represent studies that provide reliable, quantitative estimates of NOAELs, LOAELs, or Cancer Effect Levels (CELs).

The legends presented below demonstrate the application of these tables and figures. Representative examples of LSE Table 3-1 and Figure 3-1 are shown. The numbers in the left column of the legends correspond to the numbers in the example table and figure.

## APPENDIX B

**LEGEND****See Sample LSE Table 3-1 (page B-6)**

- (1) Route of Exposure. One of the first considerations when reviewing the toxicity of a substance using these tables and figures should be the relevant and appropriate route of exposure. Typically when sufficient data exist, three LSE tables and two LSE figures are presented in the document. The three LSE tables present data on the three principal routes of exposure, i.e., inhalation, oral, and dermal (LSE Tables 3-1, 3-2, and 3-3, respectively). LSE figures are limited to the inhalation (LSE Figure 3-1) and oral (LSE Figure 3-2) routes. Not all substances will have data on each route of exposure and will not, therefore, have all five of the tables and figures.
- (2) Exposure Period. Three exposure periods—acute (less than 15 days), intermediate (15–364 days), and chronic (365 days or more)—are presented within each relevant route of exposure. In this example, an inhalation study of intermediate exposure duration is reported. For quick reference to health effects occurring from a known length of exposure, locate the applicable exposure period within the LSE table and figure.
- (3) Health Effect. The major categories of health effects included in LSE tables and figures are death, systemic, immunological, neurological, developmental, reproductive, and cancer. NOAELs and LOAELs can be reported in the tables and figures for all effects but cancer. Systemic effects are further defined in the "System" column of the LSE table (see key number 18).
- (4) Key to Figure. Each key number in the LSE table links study information to one or more data points using the same key number in the corresponding LSE figure. In this example, the study represented by key number 18 has been used to derive a NOAEL and a Less Serious LOAEL (also see the two "18r" data points in sample Figure 3-1).
- (5) Species. The test species, whether animal or human, are identified in this column. Chapter 2, "Relevance to Public Health," covers the relevance of animal data to human toxicity and Section 3.4, "Toxicokinetics," contains any available information on comparative toxicokinetics. Although NOAELs and LOAELs are species specific, the levels are extrapolated to equivalent human doses to derive an MRL.
- (6) Exposure Frequency/Duration. The duration of the study and the weekly and daily exposure regimens are provided in this column. This permits comparison of NOAELs and LOAELs from different studies. In this case (key number 18), rats were exposed to "Chemical x" via inhalation for 6 hours/day, 5 days/week, for 13 weeks. For a more complete review of the dosing regimen, refer to the appropriate sections of the text or the original reference paper (i.e., Nitschke et al. 1981).
- (7) System. This column further defines the systemic effects. These systems include respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, and dermal/ocular. "Other" refers to any systemic effect (e.g., a decrease in body weight) not covered in these systems. In the example of key number 18, one systemic effect (respiratory) was investigated.
- (8) NOAEL. A NOAEL is the highest exposure level at which no harmful effects were seen in the organ system studied. Key number 18 reports a NOAEL of 3 ppm for the respiratory system, which was used to derive an intermediate exposure, inhalation MRL of 0.005 ppm (see footnote "b").

## APPENDIX B

- (9) LOAEL. A LOAEL is the lowest dose used in the study that caused a harmful health effect. LOAELs have been classified into "Less Serious" and "Serious" effects. These distinctions help readers identify the levels of exposure at which adverse health effects first appear and the gradation of effects with increasing dose. A brief description of the specific end point used to quantify the adverse effect accompanies the LOAEL. The respiratory effect reported in key number 18 (hyperplasia) is a Less Serious LOAEL of 10 ppm. MRLs are not derived from Serious LOAELs.
- (10) Reference. The complete reference citation is given in Chapter 9 of the profile.
- (11) CEL. A CEL is the lowest exposure level associated with the onset of carcinogenesis in experimental or epidemiologic studies. CELs are always considered serious effects. The LSE tables and figures do not contain NOAELs for cancer, but the text may report doses not causing measurable cancer increases.
- (12) Footnotes. Explanations of abbreviations or reference notes for data in the LSE tables are found in the footnotes. Footnote "b" indicates that the NOAEL of 3 ppm in key number 18 was used to derive an MRL of 0.005 ppm.

**LEGEND****See Sample Figure 3-1 (page B-7)**

LSE figures graphically illustrate the data presented in the corresponding LSE tables. Figures help the reader quickly compare health effects according to exposure concentrations for particular exposure periods.

- (13) Exposure Period. The same exposure periods appear as in the LSE table. In this example, health effects observed within the acute and intermediate exposure periods are illustrated.
- (14) Health Effect. These are the categories of health effects for which reliable quantitative data exists. The same health effects appear in the LSE table.
- (15) Levels of Exposure. Concentrations or doses for each health effect in the LSE tables are graphically displayed in the LSE figures. Exposure concentration or dose is measured on the log scale "y" axis. Inhalation exposure is reported in mg/m<sup>3</sup> or ppm and oral exposure is reported in mg/kg/day.
- (16) NOAEL. In this example, the open circle designated 18r identifies a NOAEL critical end point in the rat upon which an intermediate inhalation exposure MRL is based. The key number 18 corresponds to the entry in the LSE table. The dashed descending arrow indicates the extrapolation from the exposure level of 3 ppm (see entry 18 in the table) to the MRL of 0.005 ppm (see footnote "b" in the LSE table).
- (17) CEL. Key number 38m is one of three studies for which CELs were derived. The diamond symbol refers to a CEL for the test species-mouse. The number 38 corresponds to the entry in the LSE table.

## APPENDIX B

- (18) Estimated Upper-Bound Human Cancer Risk Levels. This is the range associated with the upper-bound for lifetime cancer risk of 1 in 10,000 to 1 in 10,000,000. These risk levels are derived from the EPA's Human Health Assessment Group's upper-bound estimates of the slope of the cancer dose response curve at low dose levels ( $q_1^*$ ).
- (19) Key to LSE Figure. The Key explains the abbreviations and symbols used in the figure.

## SAMPLE

1 →

Table 3-1. Levels of Significant Exposure to [Chemical x] – Inhalation

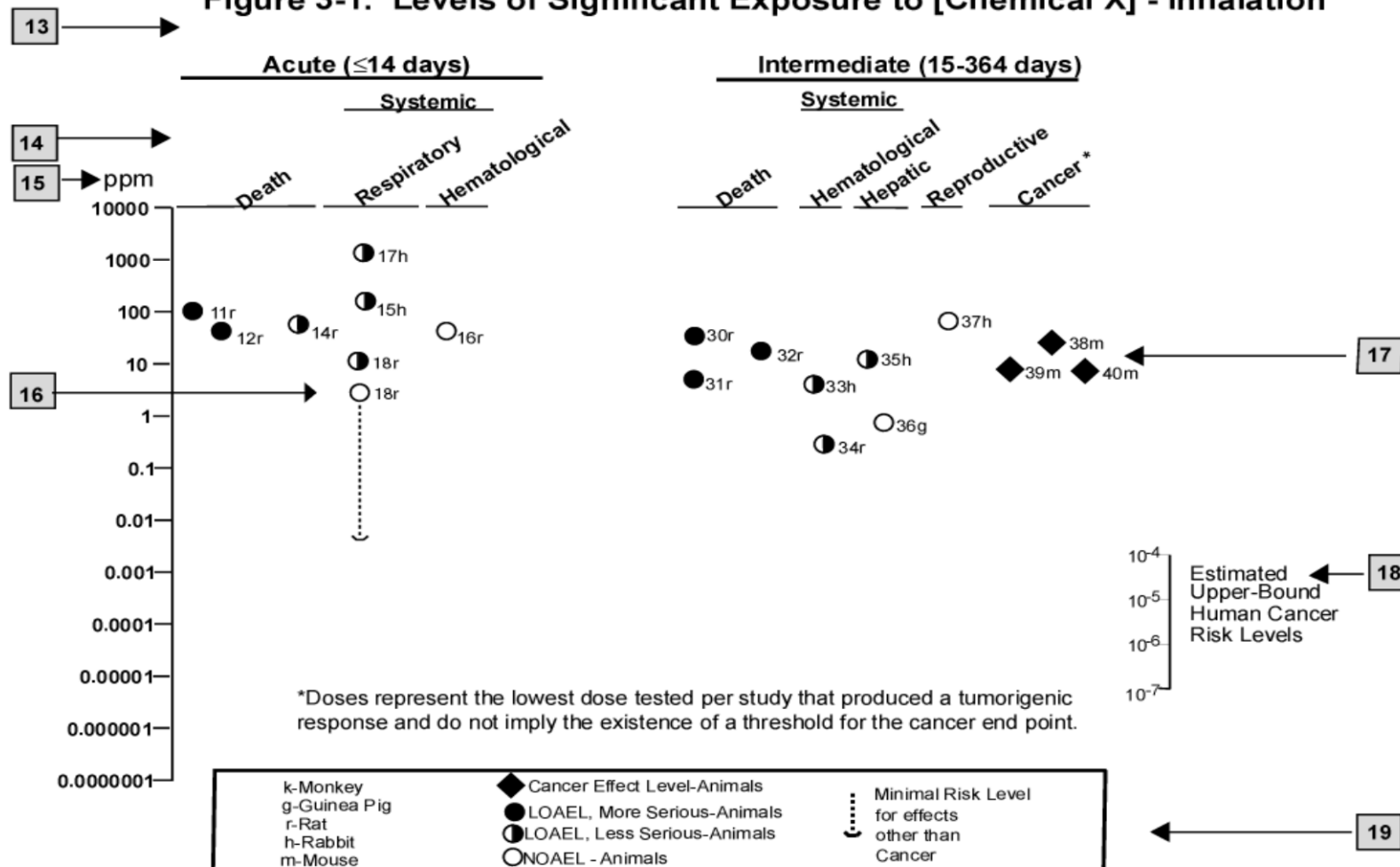
|   | Key to figure <sup>a</sup> | Species               | Exposure frequency/<br>duration | System                        | NOAEL (ppm) | LOAEL (effect)     |                  | Reference                            |                  |
|---|----------------------------|-----------------------|---------------------------------|-------------------------------|-------------|--------------------|------------------|--------------------------------------|------------------|
|   |                            |                       |                                 |                               |             | Less serious (ppm) | Serious (ppm)    |                                      |                  |
| 2 | →                          | INTERMEDIATE EXPOSURE |                                 |                               |             |                    |                  |                                      |                  |
|   |                            | 5                     | 6                               | 7                             | 8           | 9                  |                  | 10                                   |                  |
| 3 | →                          | Systemic              | ↓                               | ↓                             | ↓           | ↓                  |                  | ↓                                    |                  |
| 4 | →                          | 18                    | Rat                             | 13 wk<br>5 d/wk<br>6 hr/d     | Resp        | 3 <sup>b</sup>     | 10 (hyperplasia) | Nitschke et al. 1981                 |                  |
|   |                            | CHRONIC EXPOSURE      |                                 |                               |             |                    |                  |                                      |                  |
|   |                            | Cancer                |                                 |                               |             |                    | 11               |                                      |                  |
|   |                            |                       |                                 |                               |             | ↓                  |                  |                                      |                  |
|   |                            | 38                    | Rat                             | 18 mo<br>5 d/wk<br>7 hr/d     |             |                    | 20               | (CEL, multiple organs)               | Wong et al. 1982 |
|   |                            | 39                    | Rat                             | 89–104 wk<br>5 d/wk<br>6 hr/d |             |                    | 10               | (CEL, lung tumors, nasal tumors)     | NTP 1982         |
|   |                            | 40                    | Mouse                           | 79–103 wk<br>5 d/wk<br>6 hr/d |             |                    | 10               | (CEL, lung tumors, hemangiosarcomas) | NTP 1982         |

12 →

<sup>a</sup> The number corresponds to entries in Figure 3-1.<sup>b</sup> Used to derive an intermediate inhalation Minimal Risk Level (MRL) of  $5 \times 10^{-3}$  ppm; dose adjusted for intermittent exposure and divided by an uncertainty factor of 100 (10 for extrapolation from animal to humans, 10 for human variability).

# SAMPLE

Figure 3-1. Levels of Significant Exposure to [Chemical X] - Inhalation



## APPENDIX B

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**APPENDIX C. ACRONYMS, ABBREVIATIONS, AND SYMBOLS**

|                   |                                                                       |
|-------------------|-----------------------------------------------------------------------|
| ACGIH             | American Conference of Governmental Industrial Hygienists             |
| ACOEM             | American College of Occupational and Environmental Medicine           |
| ADI               | acceptable daily intake                                               |
| ADME              | absorption, distribution, metabolism, and excretion                   |
| AED               | atomic emission detection                                             |
| AFID              | alkali flame ionization detector                                      |
| AFOSH             | Air Force Office of Safety and Health                                 |
| ALT               | alanine aminotransferase                                              |
| AML               | acute myeloid leukemia                                                |
| AOAC              | Association of Official Analytical Chemists                           |
| AOEC              | Association of Occupational and Environmental Clinics                 |
| AP                | alkaline phosphatase                                                  |
| APHA              | American Public Health Association                                    |
| AST               | aspartate aminotransferase                                            |
| atm               | atmosphere                                                            |
| ATSDR             | Agency for Toxic Substances and Disease Registry                      |
| AWQC              | Ambient Water Quality Criteria                                        |
| BAT               | best available technology                                             |
| BCF               | bioconcentration factor                                               |
| BEI               | Biological Exposure Index                                             |
| BMD/C             | benchmark dose or benchmark concentration                             |
| BMD <sub>x</sub>  | dose that produces a X% change in response rate of an adverse effect  |
| BMDL <sub>x</sub> | 95% lower confidence limit on the BMD <sub>x</sub>                    |
| BMDS              | Benchmark Dose Software                                               |
| BMR               | benchmark response                                                    |
| BSC               | Board of Scientific Counselors                                        |
| C                 | centigrade                                                            |
| CAA               | Clean Air Act                                                         |
| CAG               | Cancer Assessment Group of the U.S. Environmental Protection Agency   |
| CAS               | Chemical Abstract Services                                            |
| CDC               | Centers for Disease Control and Prevention                            |
| CEL               | cancer effect level                                                   |
| CELDS             | Computer-Environmental Legislative Data System                        |
| CERCLA            | Comprehensive Environmental Response, Compensation, and Liability Act |
| CFR               | Code of Federal Regulations                                           |
| Ci                | curie                                                                 |
| CI                | confidence interval                                                   |
| CL                | ceiling limit value                                                   |
| CLP               | Contract Laboratory Program                                           |
| cm                | centimeter                                                            |
| CML               | chronic myeloid leukemia                                              |
| CPSC              | Consumer Products Safety Commission                                   |
| CWA               | Clean Water Act                                                       |
| DHEW              | Department of Health, Education, and Welfare                          |
| DHHS              | Department of Health and Human Services                               |
| DNA               | deoxyribonucleic acid                                                 |
| DOD               | Department of Defense                                                 |
| DOE               | Department of Energy                                                  |
| DOL               | Department of Labor                                                   |

## APPENDIX C

|                  |                                                               |
|------------------|---------------------------------------------------------------|
| DOT              | Department of Transportation                                  |
| DOT/UN/          | Department of Transportation/United Nations/                  |
| NA/IMDG          | North America/Intergovernmental Maritime Dangerous Goods Code |
| DWEL             | drinking water exposure level                                 |
| ECD              | electron capture detection                                    |
| ECG/EKG          | electrocardiogram                                             |
| EEG              | electroencephalogram                                          |
| EEGL             | Emergency Exposure Guidance Level                             |
| EPA              | Environmental Protection Agency                               |
| F                | Fahrenheit                                                    |
| F <sub>1</sub>   | first-filial generation                                       |
| FAO              | Food and Agricultural Organization of the United Nations      |
| FDA              | Food and Drug Administration                                  |
| FEMA             | Federal Emergency Management Agency                           |
| FIFRA            | Federal Insecticide, Fungicide, and Rodenticide Act           |
| FPD              | flame photometric detection                                   |
| fpm              | feet per minute                                               |
| FR               | Federal Register                                              |
| FSH              | follicle stimulating hormone                                  |
| g                | gram                                                          |
| GC               | gas chromatography                                            |
| gd               | gestational day                                               |
| GLC              | gas liquid chromatography                                     |
| GPC              | gel permeation chromatography                                 |
| HPLC             | high-performance liquid chromatography                        |
| HRGC             | high resolution gas chromatography                            |
| HSDB             | Hazardous Substance Data Bank                                 |
| IARC             | International Agency for Research on Cancer                   |
| IDLH             | immediately dangerous to life and health                      |
| ILO              | International Labor Organization                              |
| IRIS             | Integrated Risk Information System                            |
| K <sub>d</sub>   | adsorption ratio                                              |
| kg               | kilogram                                                      |
| kkg              | metric ton                                                    |
| K <sub>oc</sub>  | organic carbon partition coefficient                          |
| K <sub>ow</sub>  | octanol-water partition coefficient                           |
| L                | liter                                                         |
| LC               | liquid chromatography                                         |
| LC <sub>50</sub> | lethal concentration, 50% kill                                |
| LC <sub>Lo</sub> | lethal concentration, low                                     |
| LD <sub>50</sub> | lethal dose, 50% kill                                         |
| LD <sub>Lo</sub> | lethal dose, low                                              |
| LDH              | lactic dehydrogenase                                          |
| LH               | lutinizing hormone                                            |
| LOAEL            | lowest-observed-adverse-effect level                          |
| LSE              | Levels of Significant Exposure                                |
| LT <sub>50</sub> | lethal time, 50% kill                                         |
| m                | meter                                                         |
| MA               | <i>trans,trans</i> -muconic acid                              |
| MAL              | maximum allowable level                                       |
| mCi              | millicurie                                                    |

## APPENDIX C

|          |                                                              |
|----------|--------------------------------------------------------------|
| MCL      | maximum contaminant level                                    |
| MCLG     | maximum contaminant level goal                               |
| MF       | modifying factor                                             |
| MFO      | mixed function oxidase                                       |
| mg       | milligram                                                    |
| mL       | milliliter                                                   |
| mm       | millimeter                                                   |
| mmHg     | millimeters of mercury                                       |
| mmol     | millimole                                                    |
| mppcf    | millions of particles per cubic foot                         |
| MRL      | Minimal Risk Level                                           |
| MS       | mass spectrometry                                            |
| NAAQS    | National Ambient Air Quality Standard                        |
| NAS      | National Academy of Science                                  |
| NATICH   | National Air Toxics Information Clearinghouse                |
| NATO     | North Atlantic Treaty Organization                           |
| NCE      | normochromatic erythrocytes                                  |
| NCEH     | National Center for Environmental Health                     |
| NCI      | National Cancer Institute                                    |
| ND       | not detected                                                 |
| NFPA     | National Fire Protection Association                         |
| ng       | nanogram                                                     |
| NHANES   | National Health and Nutrition Examination Survey             |
| NIEHS    | National Institute of Environmental Health Sciences          |
| NIOSH    | National Institute for Occupational Safety and Health        |
| NIOSHTIC | NIOSH's Computerized Information Retrieval System            |
| NLM      | National Library of Medicine                                 |
| nm       | nanometer                                                    |
| nmol     | nanomole                                                     |
| NOAEL    | no-observed-adverse-effect level                             |
| NOES     | National Occupational Exposure Survey                        |
| NOHS     | National Occupational Hazard Survey                          |
| NPD      | nitrogen phosphorus detection                                |
| NPDES    | National Pollutant Discharge Elimination System              |
| NPL      | National Priorities List                                     |
| NR       | not reported                                                 |
| NRC      | National Research Council                                    |
| NS       | not specified                                                |
| NSPS     | New Source Performance Standards                             |
| NTIS     | National Technical Information Service                       |
| NTP      | National Toxicology Program                                  |
| ODW      | Office of Drinking Water, EPA                                |
| OERR     | Office of Emergency and Remedial Response, EPA               |
| OHM/TADS | Oil and Hazardous Materials/Technical Assistance Data System |
| OPP      | Office of Pesticide Programs, EPA                            |
| OPPT     | Office of Pollution Prevention and Toxics, EPA               |
| OPPTS    | Office of Prevention, Pesticides and Toxic Substances, EPA   |
| OR       | odds ratio                                                   |
| OSHA     | Occupational Safety and Health Administration                |
| OSW      | Office of Solid Waste, EPA                                   |
| OTS      | Office of Toxic Substances                                   |

## APPENDIX C

|                  |                                                  |
|------------------|--------------------------------------------------|
| OW               | Office of Water                                  |
| OWRS             | Office of Water Regulations and Standards, EPA   |
| PAH              | polycyclic aromatic hydrocarbon                  |
| PBPD             | physiologically based pharmacodynamic            |
| PBPK             | physiologically based pharmacokinetic            |
| PCE              | polychromatic erythrocytes                       |
| PEL              | permissible exposure limit                       |
| pg               | picogram                                         |
| PHS              | Public Health Service                            |
| PID              | photo ionization detector                        |
| pmol             | picomole                                         |
| PMR              | proportionate mortality ratio                    |
| ppb              | parts per billion                                |
| ppm              | parts per million                                |
| ppt              | parts per trillion                               |
| PSNS             | pretreatment standards for new sources           |
| RBC              | red blood cell                                   |
| REL              | recommended exposure level/limit                 |
| RfC              | reference concentration                          |
| RfD              | reference dose                                   |
| RNA              | ribonucleic acid                                 |
| RQ               | reportable quantity                              |
| RTECS            | Registry of Toxic Effects of Chemical Substances |
| SARA             | Superfund Amendments and Reauthorization Act     |
| SCE              | sister chromatid exchange                        |
| SGOT             | serum glutamic oxaloacetic transaminase          |
| SGPT             | serum glutamic pyruvic transaminase              |
| SIC              | standard industrial classification               |
| SIM              | selected ion monitoring                          |
| SMCL             | secondary maximum contaminant level              |
| SMR              | standardized mortality ratio                     |
| SNARL            | suggested no adverse response level              |
| SPEGL            | Short-Term Public Emergency Guidance Level       |
| STEL             | short term exposure limit                        |
| STORET           | Storage and Retrieval                            |
| TD <sub>50</sub> | toxic dose, 50% specific toxic effect            |
| TLV              | threshold limit value                            |
| TOC              | total organic carbon                             |
| TPQ              | threshold planning quantity                      |
| TRI              | Toxics Release Inventory                         |
| TSCA             | Toxic Substances Control Act                     |
| TWA              | time-weighted average                            |
| UF               | uncertainty factor                               |
| U.S.             | United States                                    |
| USDA             | United States Department of Agriculture          |
| USGS             | United States Geological Survey                  |
| VOC              | volatile organic compound                        |
| WBC              | white blood cell                                 |
| WHO              | World Health Organization                        |

## APPENDIX C

|               |                          |
|---------------|--------------------------|
| $>$           | greater than             |
| $\geq$        | greater than or equal to |
| $=$           | equal to                 |
| $<$           | less than                |
| $\leq$        | less than or equal to    |
| $\%$          | percent                  |
| $\alpha$      | alpha                    |
| $\beta$       | beta                     |
| $\gamma$      | gamma                    |
| $\delta$      | delta                    |
| $\mu\text{m}$ | micrometer               |
| $\mu\text{g}$ | microgram                |
| $q_1^*$       | cancer slope factor      |
| $-$           | negative                 |
| $+$           | positive                 |
| $(+)$         | weakly positive result   |
| $(-)$         | weakly negative result   |

## APPENDIX C

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## APPENDIX D. INDEX

|                                      |                                                                          |
|--------------------------------------|--------------------------------------------------------------------------|
| absorbed dose.....                   | 93                                                                       |
| adenocarcinoma .....                 | 46                                                                       |
| adsorbed .....                       | 130                                                                      |
| adsorption.....                      | 121, 130, 148                                                            |
| aerobic.....                         | 132, 133                                                                 |
| ambient air .....                    | 108, 144, 150                                                            |
| anemia.....                          | 9, 10, 13, 37, 102                                                       |
| bioaccumulation.....                 | 121                                                                      |
| bioavailability .....                | 144                                                                      |
| bioconcentration factor .....        | 130                                                                      |
| biodegradation.....                  | 132, 133                                                                 |
| biomarker .....                      | 7, 8, 11, 16, 17, 58, 61, 62, 87, 89, 93, 94, 95, 96, 106, 109, 147, 151 |
| blood cell count.....                | 9, 10, 37                                                                |
| body weight effects.....             | 39                                                                       |
| breast milk.....                     | 142, 145                                                                 |
| cancer .....                         | 4, 8, 9, 10, 13, 14, 20, 21, 43, 44, 45, 46, 92, 100, 102, 103, 109      |
| carcinogen .....                     | 4, 46, 103, 156                                                          |
| carcinogenic .....                   | 10, 19, 20, 46, 88, 95, 96, 99, 103, 104, 106, 156                       |
| carcinogenicity.....                 | 8, 10, 61, 88, 95, 100, 103, 104                                         |
| carcinoma.....                       | 46                                                                       |
| cardiovascular .....                 | 36                                                                       |
| cardiovascular effects.....          | 36                                                                       |
| cholinesterase.....                  | 40                                                                       |
| chromosomal aberrations.....         | 52, 53, 54, 97, 104                                                      |
| clearance .....                      | 61, 63, 69, 73, 74, 99                                                   |
| death.....                           | 8, 9, 10, 13, 19, 21, 40, 41, 99, 100, 102, 156                          |
| deoxyribonucleic acid (see DNA)..... | 5                                                                        |
| dermal effects.....                  | 38                                                                       |
| developmental effects .....          | 8, 9, 10, 12, 13, 42, 92, 100, 105, 106, 107                             |
| DNA (see deoxyribonucleic acid)..... | 5, 49, 52, 54, 86, 87, 88, 93, 95, 106, 108, 109                         |
| elimination rate .....               | 63, 67                                                                   |
| endocrine.....                       | 90, 91                                                                   |
| fetus.....                           | 74, 91                                                                   |
| fractional absorption .....          | 98                                                                       |
| gastrointestinal effects .....       | 36                                                                       |
| general population.....              | 7, 8, 123, 137, 138, 145, 156                                            |
| genotoxic.....                       | 8, 19, 47, 54, 86, 87, 95, 97, 104, 110                                  |
| genotoxicity.....                    | 47, 55, 86, 87, 88, 96, 100, 105, 109                                    |
| groundwater .....                    | 102, 129, 131                                                            |
| growth retardation.....              | 105                                                                      |
| half-life.....                       | 7, 93, 121, 130, 131, 132                                                |
| hematological effects .....          | 36                                                                       |
| hematopoietic.....                   | 8, 39, 45, 49, 103, 104                                                  |
| hepatic effects .....                | 38                                                                       |
| hydrolysis.....                      | 51, 57, 61, 62, 67, 68, 69, 74, 76, 77, 84, 85, 92, 98, 99               |
| hydroxyl radical .....               | 121, 131, 132                                                            |
| immune system .....                  | 105                                                                      |
| immunological .....                  | 19, 39, 40                                                               |
| immunological effects.....           | 39, 40                                                                   |

## APPENDIX D

|                               |                                                |
|-------------------------------|------------------------------------------------|
| K <sub>ow</sub> .....         | 113                                            |
| leukemia .....                | 37, 39, 43, 44, 45, 46, 88, 92, 103            |
| lymphatic .....               | 4, 43                                          |
| lymphopoietic .....           | 8, 10                                          |
| lymphoreticular .....         | 8, 10                                          |
| micronuclei .....             | 49, 50, 52, 99, 104                            |
| musculoskeletal effects ..... | 38                                             |
| neonatal .....                | 142, 145                                       |
| neoplasm .....                | 46                                             |
| neoplastic .....              | 9, 10, 11, 38                                  |
| neurobehavioral .....         | 90, 105                                        |
| neurological effects .....    | 3, 12, 40, 99, 105                             |
| non-Hodgkin's lymphoma .....  | 44                                             |
| ocular effects .....          | 39                                             |
| odds ratio .....              | 43                                             |
| partition coefficients .....  | 15, 55, 56, 67, 68, 69, 74, 79, 83             |
| pharmacodynamic .....         | 64                                             |
| pharmacokinetic .....         | 11, 63, 64, 65, 66, 91, 100, 102, 104, 105     |
| rate constant .....           | 17, 68, 74, 131, 132                           |
| renal effects .....           | 38                                             |
| reproductive effects .....    | 10, 40, 104                                    |
| respiratory effects .....     | 21, 103                                        |
| sarcoma .....                 | 44, 46                                         |
| solubility .....              | 113                                            |
| spermatozoa .....             | 12                                             |
| systemic effects .....        | 13, 21, 100                                    |
| T3 .....                      | 22                                             |
| thyroid .....                 | 10, 46, 57                                     |
| toxicokinetic .....           | 11, 15, 16, 17, 19, 97, 98, 102, 103, 107, 144 |
| tumors .....                  | 10, 13, 40, 45, 96, 106                        |
| vapor pressure .....          | 113, 130                                       |
| volatility .....              | 130                                            |
| volatilization .....          | 121, 130                                       |





# TOBACCO SMOKING

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Tobacco smoking was considered by previous IARC Working Groups in 1986, 1987 and 2002 ([IARC, 1986, 1987, 2004a](#)). Since that time, new data have become available, these have been incorporated into the *Monograph*, and taken into consideration in the present evaluation.

## 1. Exposure Data

### 1.1 Smoked tobacco products

Smoked forms of tobacco include various kinds of cigarettes (manufactured, hand-rolled, filtered, un-filtered and flavoured), cigars and pipes. While cigarette smoking, particularly manufactured cigarettes, is by far the main form of tobacco smoked globally, in some countries other forms of smoked tobacco are dominant ([IARC, 2004a](#)). In India, for example, bidis (made of coarse and uncured tobacco) account for about 60% of smoked tobacco products whereas cigarettes account for 20% ([Ray & Gupta, 2009](#); [IIPS, 2010](#)). Water pipes, another form of smoked tobacco known by other various names such as gaza, hookah, narghile, shisha, hubble-bubble, are commonly smoked in the Eastern Mediterranean region, in some parts of Asia including India, and in North Africa ([Asma et al., 2009](#)).

### 1.2 Chemical composition of tobacco smoke

#### 1.2.1 Smoke from cigarettes

One cubic cm of fresh, un-aged cigarette mainstream smoke [the smoke emerging from the mouth end of a cigarette during smoking] has about  $4 \times 10^9$  particles with a mean diameter of about  $0.2 \mu\text{m}$  ([Borgerding & Klus, 2005](#)). The size of the particles increases as the smoke ages. Temperatures in the burning cone of the cigarette are about  $800^\circ\text{C}$  during the smoulder period between puffs and increase to  $910\text{--}920^\circ\text{C}$  at the periphery of the cone during puffing ([Borgerding & Klus, 2005](#)). Hydrogen is generated in the glowing cone, resulting in an oxygen deficient reducing atmosphere ([Borgerding & Klus, 2005](#)). The approximate composition of mainstream smoke of a plain cigarette is summarized in [Table 1.1](#) ([Borgerding & Klus, 2005](#)). The total particulate matter, after subtraction of the amounts of nicotine and water, is referred to as 'tar'.

Over 5300 compounds have been identified in tobacco smoke ([Rodgman & Perfetti, 2009](#)). Classes of compounds include but are not limited to neutral gases, carbon and nitrogen oxides, amides, imides, lactams, carboxylic acids, lactones, esters, aldehydes, ketones,

**Table 1.1 Approximate chemical composition of mainstream smoke generated by a plain cigarette**

| Compound or class of components | Relative amount w/w (%) |
|---------------------------------|-------------------------|
| Nitrogen                        | 58                      |
| Oxygen                          | 12                      |
| Carbon dioxide                  | 13                      |
| Carbon monoxide                 | 3.5                     |
| Hydrogen, argon                 | 0.5                     |
| Water                           | 1                       |
| Volatile organic substances     | 5                       |
| Particulate phase               | 8                       |

From [Borgerding & Klus \(2005\)](#)

alcohols, phenols, amines, *N*-nitrosamines, *N*-heterocyclics, aliphatic hydrocarbons, monocyclic and polycyclic aromatic hydrocarbons (PAHs), nitriles, anhydrides, carbohydrates, ethers, nitro compounds and metals ([Rodgman & Perfetti, 2009](#)).

The addictive properties of tobacco smoke are attributed to nicotine, the principal tobacco alkaloid in smoke ([Hukkanen et al., 2005](#)). Minor tobacco alkaloids include nornicotine, anatabine and anabasine ([Hukkanen et al., 2005](#)). The tobacco alkaloids are not generally considered carcinogenic, but are accompanied by carcinogens in each puff of smoke.

There are over 70 carcinogens in tobacco smoke that have been evaluated by the *IARC Monographs* programme as having sufficient evidence for carcinogenicity in either laboratory animals or humans ([IARC, 2004a](#)). The different chemical classes of carcinogens and representatives of each are presented in [Table 1.2](#) ([IARC, 2004a](#)). Sixteen of these – benzo[*a*]pyrene (BaP), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and *N'*-nitrosonornicotine (NNN), 2-naphthylamine, 4-aminobiphenyl, formaldehyde, 1,3-butadiene, benzene, vinyl chloride, ethylene oxide, arsenic, beryllium, nickel compounds, chromium VI, cadmium, and polonium-210 – are classified as carcinogenic to

humans (Group 1). Structures of some representative carcinogens in cigarette smoke are shown in Fig. 1.1. There are other likely carcinogens in cigarette smoke that have not been evaluated by the *IARC Monographs* programme. These include, for example, PAHs with incompletely characterized occurrence levels and carcinogenic activities; over 500 PAHs have been identified ([Rodgman & Perfetti, 2006](#)).

PAHs, tobacco-specific *N*-nitrosamines, aromatic amines, aldehydes and certain volatile organics likely contribute significantly to the carcinogenic activity of tobacco smoke ([Hecht, 2003](#)).

In the early part of the 20<sup>th</sup> century, PAHs were identified as carcinogenic constituents of coal tar ([Phillips, 1983](#)). They are products of incomplete combustion of all organic matter and occur, always as complex mixtures, in tars, soots, broiled foods, vehicle engine exhaust and tobacco smoke. PAHs are generally locally acting carcinogens, and some, such as the prototypic compound BaP, have strong carcinogenic activity on mouse skin and in rodent lung. Heterocyclic analogues of PAHs also occur in cigarette smoke. Concentrations of individual PAHs in mainstream cigarette smoke are generally in the range of 1–50 ng per cigarette ([IARC, 2004a](#)).

Among the carcinogenic *N*-nitrosamines in tobacco smoke are tobacco-specific *N*-nitrosamines, which are derived from, and structurally related to, the tobacco alkaloids. Two of the most important of these are NNK and NNN ([Hecht & Hoffmann, 1988](#)). Levels of NNK and NNN in cigarette smoke vary depending on tobacco type and other factors, but are frequently in the range of 50–200 ng per cigarette ([IARC, 2004a](#)).

Aromatic amines were first identified as human carcinogens from industrial exposures in the dye industry in the early part of the 20<sup>th</sup> century. They include the well known human bladder carcinogens 2-naphthylamine and 4-aminobiphenyl which, along with other

**Table 1.2 Tobacco smoke carcinogens evaluated in the IARC Monographs**

| Chemical Class                                                           | Number of Carcinogens | Representative Carcinogens                                                         |
|--------------------------------------------------------------------------|-----------------------|------------------------------------------------------------------------------------|
| Polycyclic aromatic hydrocarbons (PAHs) and their heterocyclic analogues | 15                    | Benzo[a]pyrene (BaP)<br>Dibenz[a,h]anthracene                                      |
| N-Nitrosamines                                                           | 8                     | 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)<br>N'-Nitrosoornicotine (NNN) |
| Aromatic amines                                                          | 12                    | 4-Aminobiphenyl<br>2-Naphthylamine                                                 |
| Aldehydes                                                                | 2                     | Formaldehyde<br>Acetaldehyde                                                       |
| Phenols                                                                  | 2                     | Catechol<br>Caffeic acid                                                           |
| Volatile hydrocarbons                                                    | 3                     | Benzene<br>1,3-Butadiene<br>Isoprene                                               |
| Other organics                                                           | 12                    | Ethylene oxide<br>Acrylonitrile                                                    |
| Inorganic compounds                                                      | 8                     | Cadmium<br>Polonium-210                                                            |

There are many other carcinogens in cigarette smoke that have not been evaluated in an *IARC Monograph*.  
From [IARC \(2004a\)](#)

isomers, are found in cigarette smoke, but their levels are generally quite low (1–20 ng per cigarette) ([IARC, 2004a](#)).

Aldehydes such as formaldehyde and acetaldehyde occur widely in the human environment and are also found in human blood. Concentrations of acetaldehyde and formaldehyde in cigarette smoke are far higher than those of PAHs, N-nitrosamines or aromatic amines but their carcinogenic activities are weak ([Hecht, 2003](#)). Cigarette mainstream smoke typically contains 10–30 µg formaldehyde/cigarette and 800–900 µg acetaldehyde/cigarette ([IARC, 2004a](#)).

Volatile hydrocarbons in cigarette smoke include 1,3-butadiene, a powerful multi-organ carcinogen in the mouse, and benzene, a known human leukaemogen. 1,3-Butadiene (20–40 µg/cigarette) and benzene (12–50 µg/cigarette) are two of the most prevalent strong carcinogens in cigarette smoke ([IARC, 2004a](#)).

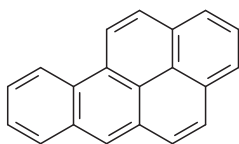
In summary, cigarette smoke is an exceedingly complex mixture which contains over 5300

compounds including multiple toxicants and carcinogens.

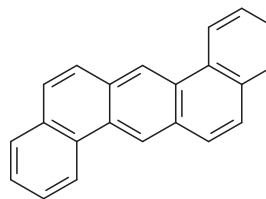
### 1.2.2 Smoke from other tobacco products

Some constituents have been measured in roll-your-own cigarettes, and their levels are comparable to or higher than those in commercial brands. Carcinogen and toxicant levels expressed per unit are higher in cigars than in cigarettes because of their larger size, and in some instances are also higher per litre of smoke. Levels of nicotine and tobacco-specific nitrosamines were comparable in bidis and commercial Indian cigarettes; bidis also contain high levels of eugenol, as do kreteks. Levels of NNK and NNN in chuttas were considerably higher than in standard cigarettes ([IARC, 2004a](#)).

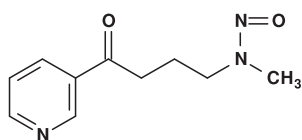
**Fig. 1.1 Structures of some representative tobacco smoke carcinogens**



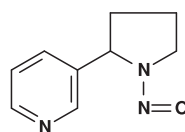
**benzo[a]pyrene (BaP)**



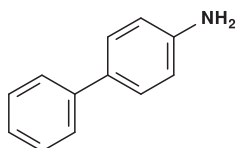
**dibenz[a,h]anthracene**



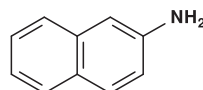
**4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)**



**N'-nitrososornicotine (NNN)**



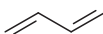
**4-aminobiphenyl**



**2-naphtylamine**



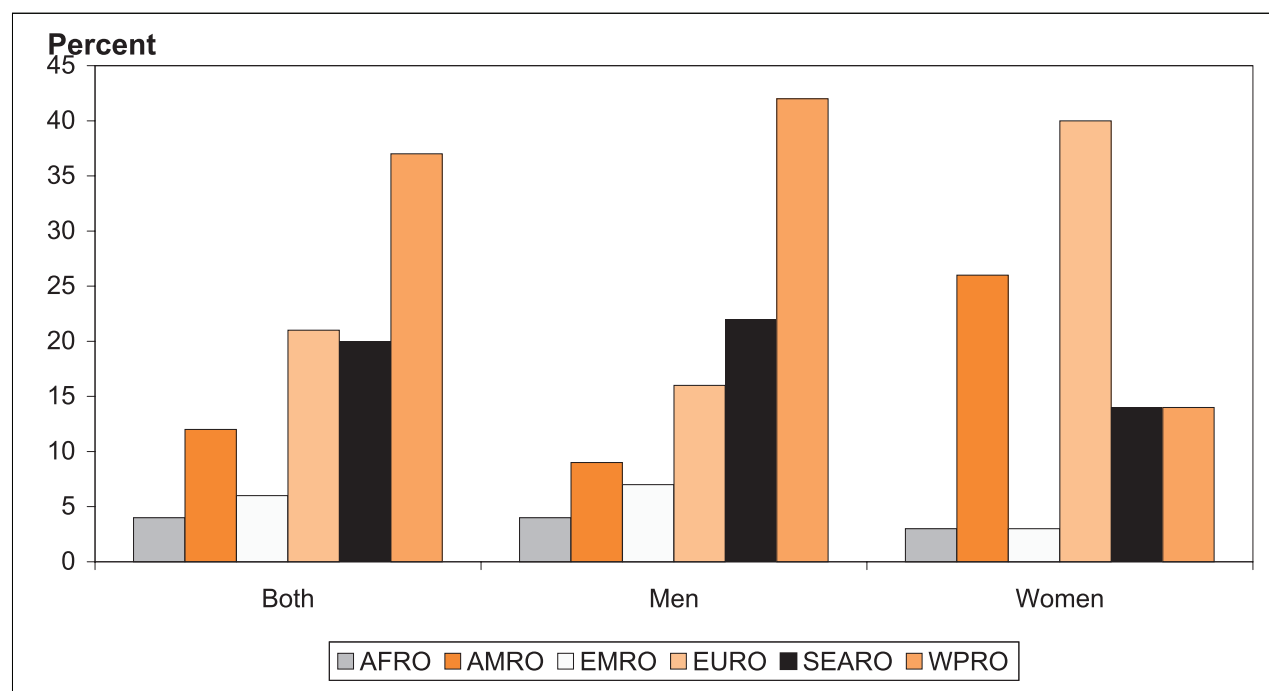
**benzene**



**1,3-butadiene**



**ethylene oxide**

**Fig. 1.2 Proportion of adult smokers by WHO region in 2009**

From [WHO \(2011\)](#)

## 1.3 Prevalence of tobacco smoking

### 1.3.1 Data collection and methods

Data on smoking tobacco are available from WHO's Global Infobase ([www.who.int/infobase](http://www.who.int/infobase)) and the WHO Global Health Observatory ([www.who.int/gho/en](http://www.who.int/gho/en)) – repositories of information on tobacco use and other risk factors in young people (13–15 years old) and adults (aged 15 years and over). The data span several years and are acquired from government reports, journals and unpublished sources. WHO has in the recent past used and modelled these data to produce estimates of tobacco smoking prevalence, published in the WHO Reports on the Global Tobacco Epidemic. For a complete explanation of methods used, the reader is referred to the Technical Note on Prevalence in the 3<sup>rd</sup> WHO Report on the Global Tobacco Epidemic ([WHO, 2011](http://www.who.int/2011)). The six WHO regions are: EMRO, Eastern Mediterranean Region; EURO, European

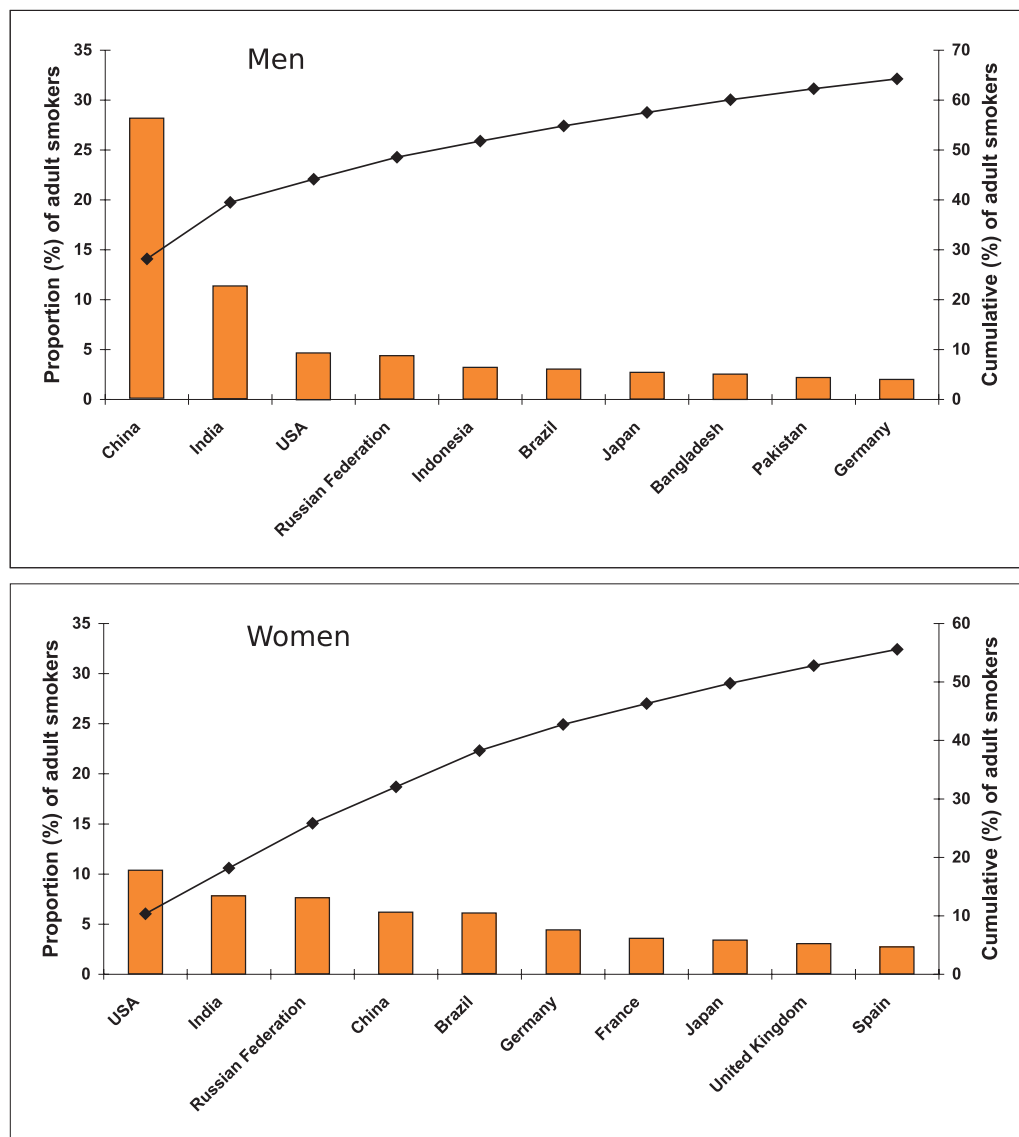
Region; AFRO, African Region; WPRO, Western Pacific Region; SEARO, South East Asian Region; AMRO, Region of the Americas. A listing of the countries in each region can be viewed at <http://www.who.int/about/structure/en/index.html>.

### 1.3.2 Distribution of smokers by WHO region and country

WHO estimates that in 2009, there was about 1.1 billion adult smokers worldwide, representing nearly a quarter (22%) of the global adult population ([WHO, 2011](#)). A disaggregation by the six WHO regions (Fig. 1.2) shows that over a third of smokers worldwide live in WPRO (highly influenced by the People's Republic of China), followed by SEARO, which has around a fifth of the world's smokers (influenced by India and Indonesia).

The number of smokers in any country is a function of both the prevalence of smoking and the size of the population. A further

**Fig. 1.3 Proportion and cumulative percentage of smokers in high-burden countries, in men (A) and women (B) in 2009**



From [WHO \(2011\)](#)

disaggregation of the regions by country shows that a few countries account for a large proportion of tobacco smokers. Ranked in descending order of the number of smokers, the five countries of China, India, United States of America (USA), Russian Federation and Indonesia account for about 52% of adult smokers in the world, with China and India alone accounting for 40% (Fig. 1.3). Furthermore, nearly two-thirds of the

world's smokers live in only ten countries of the world.

### 1.3.3 Distribution of smokers by sex

With a global average smoking prevalence of 36%, men account for just over 80% of all smokers. The male adult prevalence is 4–5 times that for women, at 8%. This difference varies across WHO

regions. Smoking among men, concentrated in the five countries of China, India, Indonesia, Russian Federation and USA (Fig. 1.3), accounts for about 56% of global smoking among men. Women smokers are mostly concentrated in EURO and AMRO. These two regions account for 40% and 26% of all women smokers globally, respectively. The prevalences for women in these two regions are about half of those in men, whereas the difference is substantially greater in the other regions. Just as men smoke more than women everywhere, so too among young people, boys generally smoke more than girls. There is an increasing concern, however, that the gap may diminish, not because of a reduction in boys prevalence but because of an increase in the proportion of girls who are taking up smoking ([Warren et al., 2006](#)).

### 1.3.4 The four stage smoking model

#### (a) The four stages of tobacco use

[Lopez et al. \(1994\)](#) used trend data on smoking prevalence and tobacco attributable mortality to show the evolution of tobacco use in a country. Four stages of smoking and attributable mortality have been identified to represent the growth and eventual decline of smoking among men and women (Fig. 1.4).

Stage 1 is characterized by low smoking prevalence in men (less than 15%) and very low in women (less than 10%). Death and disease from smoking are not apparent in this phase, as nearly all health effects from smoking are related to past smoking habits and their cumulative effects rather than current smoking. In Stage 2, smoking prevalence in men rapidly increases while it increases more slowly in women. Towards the end of this stage, smoking prevalence in men typically peaks to lie at 50–60%, with 10% of deaths in men attributable to smoking; deaths in women are comparatively fewer. After a protracted period of high smoking prevalence, Stage 3 shows a decline in smoking prevalence in men to around 40%.

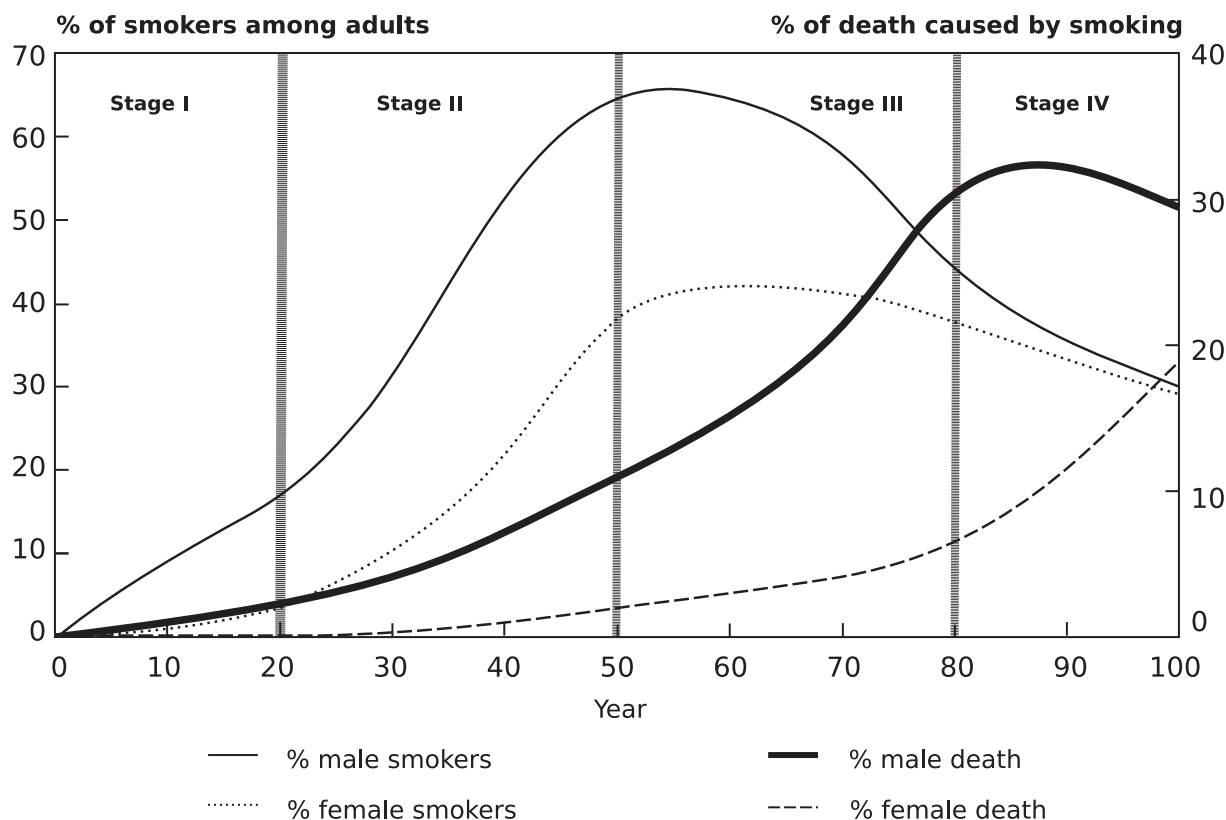
Smoking prevalence in women peaks and then begins to decline; towards the end of this stage the gap between men's and women's prevalence starts to narrow. However, smoking attributable deaths in men increase from around 10% to 25–30% within a span of three decades; in women the deaths are increasing but are still quite low. In the final Stage 4, smoking prevalences in both men and women continue to decline albeit relatively slowly in comparison with Stage 3, with the gap substantially narrowing to lie at around five percentage points, and as little as one percentage point in some countries. In Stage 4, smoking mortality in men peaks to between 30–35% and then declines to below 30% at the end of this period. In women, the health effects from past smoking persist, with increasing mortality, but remain lower than in men, and recently have begun to decline in some countries.

#### (b) Smoking prevalence worldwide

Using prevalence data for men and women collected in 2006 for 140 countries, WHO determined at which stage of the tobacco epidemic countries are in the model of [Lopez et al. \(1994\)](#). In Fig. 1.5, countries have been ranked by smoking prevalence in men in ascending order for Stages 1 and 2, and then in descending order for Stages 3 and 4. (Smoking prevalence in men is almost always higher than in women, with a few exceptions observed in the fourth stage.) While most countries fit the classification, there are a few exceptions, most of which in the last stage. Prevalence between Stage 3 and Stage 4 is discontinuous in both sexes. This is due to the classification followed, which puts countries with a relatively narrow difference in prevalence between men and women in Stage 4 even though their prevalence is largely comparable with those in Stage 3.

Most African countries fall in the first stage of the smoking model, characterized by low smoking prevalence in men and very low prevalence in women. Three of the five high burden



**Fig. 1.4 The four stages of the tobacco epidemic**

From [Lopez et al. \(1994\)](#)

countries fall in stage 2 (India, Indonesia and China), with the rest comprising a combination of countries from Africa, South East Asia, eastern Europe and the Middle East. At this stage smoking prevalences in women continue to remain very low, most countries having a prevalence in adult women of less than 10%.

Stage 3 includes the fourth high burden country (Russian Federation), along with countries in eastern Europe, South America and western Europe, which fall at the end of Stage 3. Stage 4 is populated entirely by the developed countries of western Europe, North America and Oceania. The USA, the fifth high burden country, fall in the last stage as a result of the relatively small difference in the smoking prevalence between men and women compared to the

other intermediate stages. As mentioned before, Stage 4 includes countries where the smoking prevalence is higher in women than in men, with a small (< 8%) difference.

### (c) Age-specific prevalence

Age-specific prevalence for men and women aged 15 years or older is presented for six representative countries for current smoking (Fig. 1.6). There are wide variations in age-specific prevalence between these countries. In men, prevalence varies from less than 10% to 75% in the 15–19 years age range to lie between 10% and 55% in the oldest age range. Prevalence among women varies from less than 1% to as high as 45% in young adults (15–19 years). Unlike men, prevalence in women tends to converge after age

Fig. 1.5 Prevalence of smoking in 140 countries in 2009, staged according to the model by Lopez

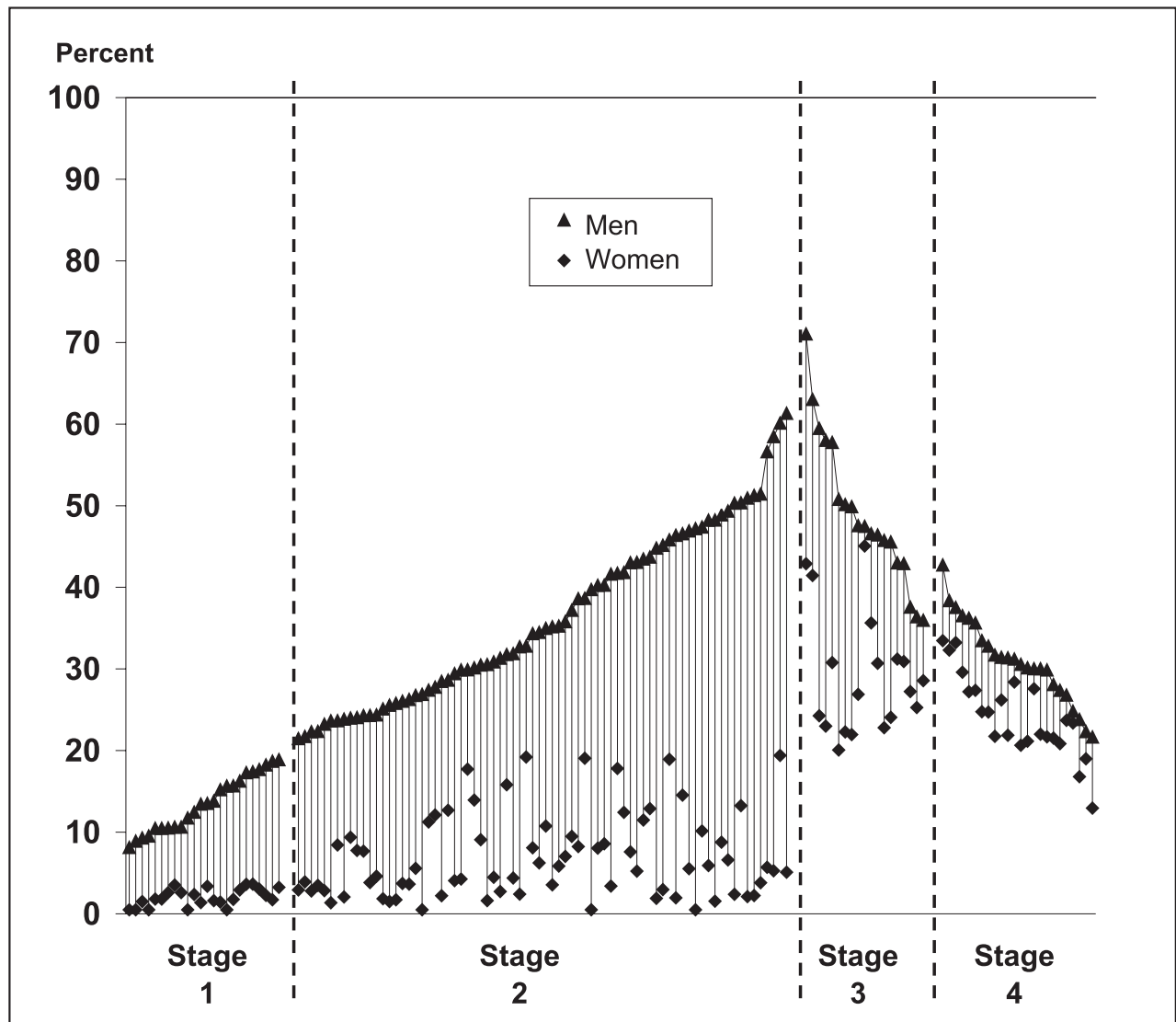
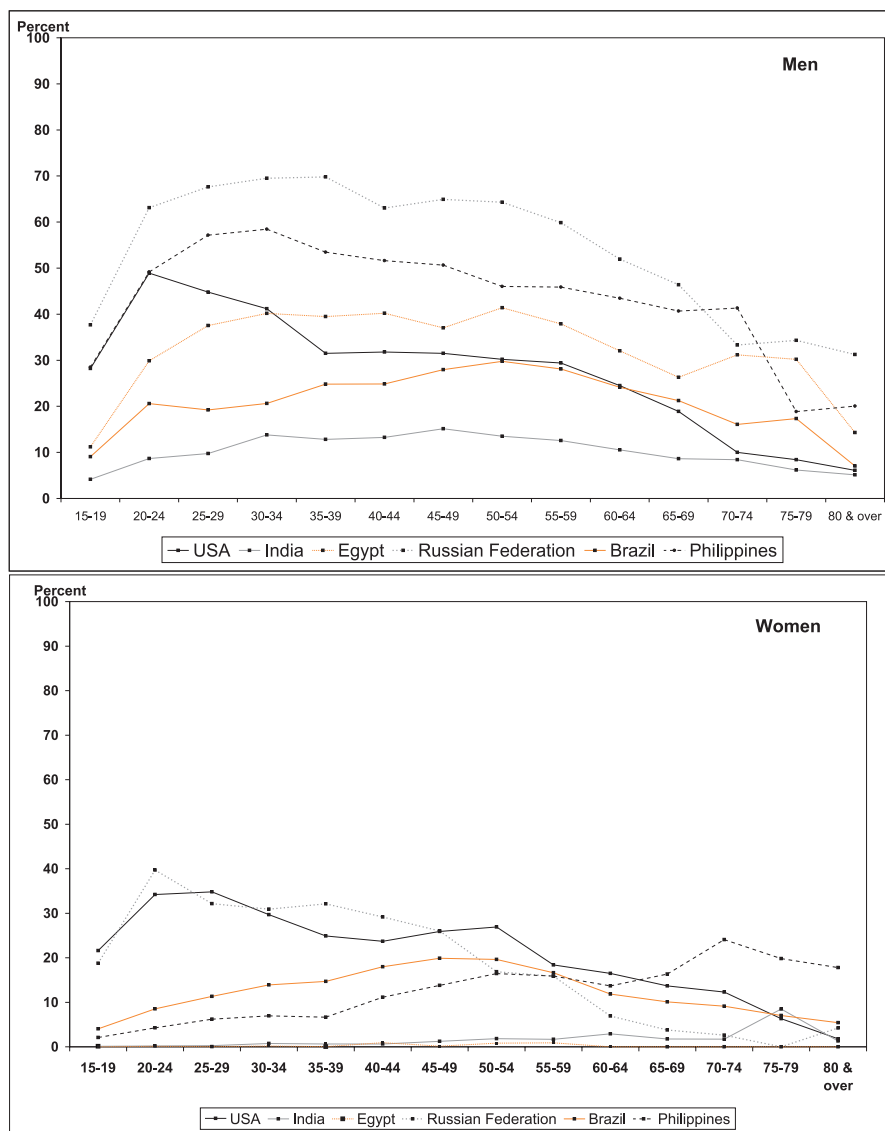


**Fig. 1.6 Age-specific rates of smoking prevalence, in men and women in 2009**

50, lying within 15 percentage points. Prevalence in women is almost always lower than in men in all age groups.

Initiation of smoking is shifting, and is taking place at earlier ages in both developed and developing countries. In developed countries, quitting smoking is also shifting to occur at a younger age, whereas in developing countries there is no such evidence.

#### (d) *Smoking in youth*

Information on smoking habits in youth are collected from a variety of youth surveys that include the Global Youth Tobacco Survey (GYTS), Global school-based Student Health Survey (GSHS) and Health Behaviour in School Aged Children (HBSC). Some countries have their own youth surveys, or have them as part of a general health or household survey, such as the Student Survey in Argentina, the Youth Smoking Survey in Canada, and New Zealand's Tobacco Survey.

The GYTS is a school-based survey designed to monitor tobacco use among youths aged 13 to 15 years. The GYTS uses a standard set of questions and sampling methods in over 160 countries. The survey has core questions that span seven thematic areas pertinent to tobacco. In addition to these, countries can include country-specific questions that allow assessment of tobacco use unique to the country. To assess prevalence of smoking, students are asked to report their smoking habits for both cigarettes and other tobacco products that they may have consumed over the past 30 days. Since its inception in 1999, the GYTS has covered over 2 million students. Although most GYTS are national surveys, in some countries they are limited to subnational locations. Further, countries conduct the GYTS in different years, rendering comparison for the same year difficult.

Prevalence of current tobacco use [including smokeless tobacco] in youth in 2004–09 for fourteen high burden low and middle income

countries is shown in Fig. 1.7. The Russian Federation has the highest prevalence of current tobacco use among the high burden countries for which national data are available. Further, in the Americas and Europe the difference in prevalence between boys and girls is smaller than in other regions. In contrast, in Egypt, India and Thailand, prevalences in boys are significantly higher than in girls.

Fig. 1.8 shows the range of current tobacco use by WHO region for boys and for girls and for both sexes combined. There are wide variations in current tobacco use within each region. The largest variations are observed in EMRO and SEARO irrespective of sex, reflecting potentially disparate initiation rates in countries within the region. In AFRO, the range of current tobacco use between boys and girls is virtually the same. In some countries (e.g. Argentina, Peru, Sierra Leone, Bulgaria, Croatia, Cook Islands, New Zealand), tobacco use in girls exceeds that in boys; but overall boys and girls show remarkably similar propensity to take up tobacco use.

[Warren \*et al.\* \(2006\)](#) present global estimates and regional averages for current tobacco smoking in youth using GYTS data spanning 1999–2005. Their estimates show that one in five boys and one in seven girls currently smoke tobacco. Prevalence of current smoking for both boys and girls combined was highest in AMRO (22.2%) and lowest in WPRO (11.4%). AMRO have the highest average for current tobacco smoking for boys (24%) and for girls (20.4%) whereas the lowest prevalence was in WPRO among boys (15%) and in SEARO among girls (7.1%).

## 1.4 Regulations and policies: the WHO Framework Convention on Tobacco Control

The WHO Framework Convention on Tobacco Control (WHO FCTC) – the first multi-lateral evidence-based treaty on tobacco control

Fig. 1.7 Prevalence of current tobacco use in youth for selected countries, 2005–2009

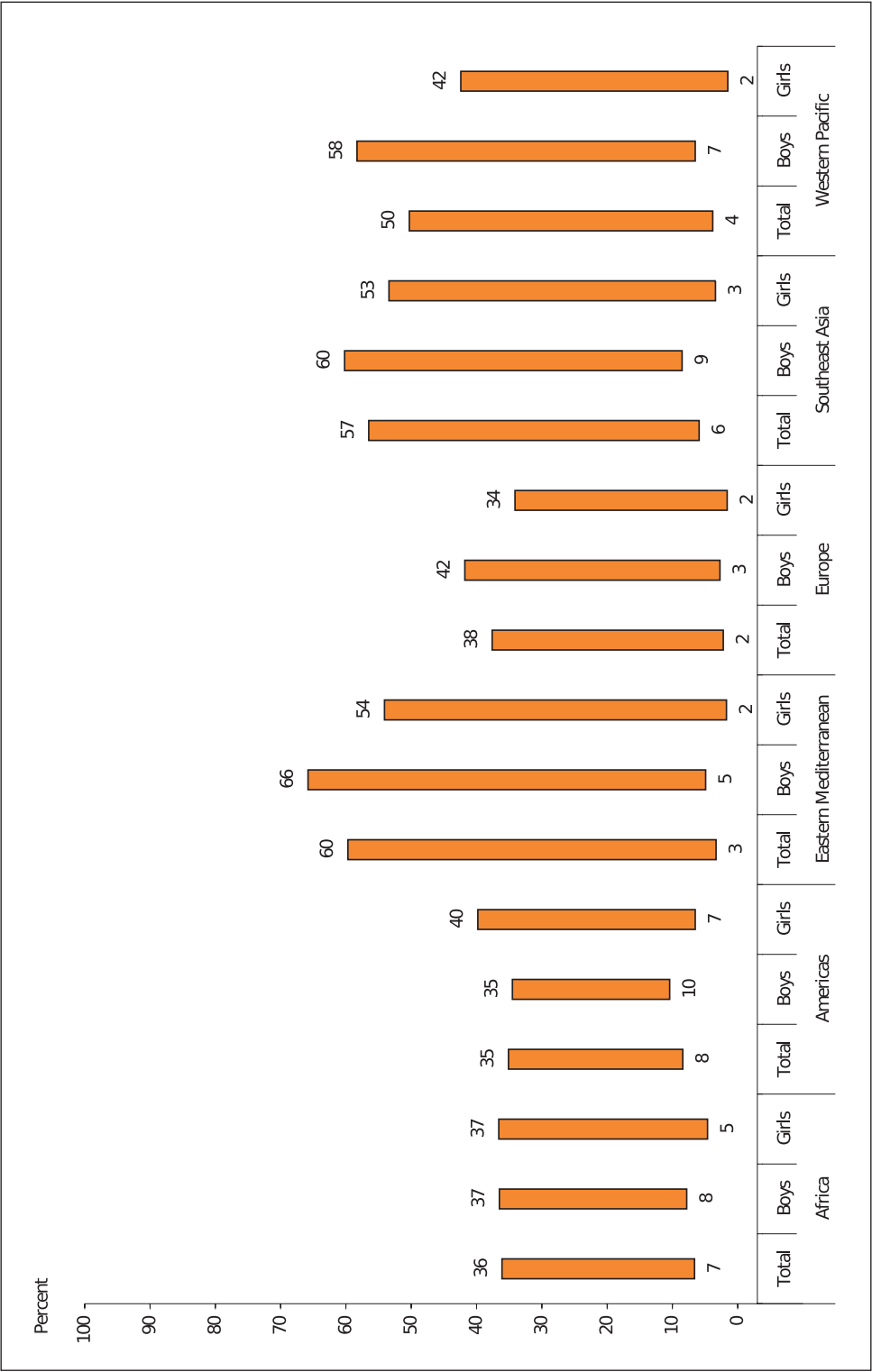
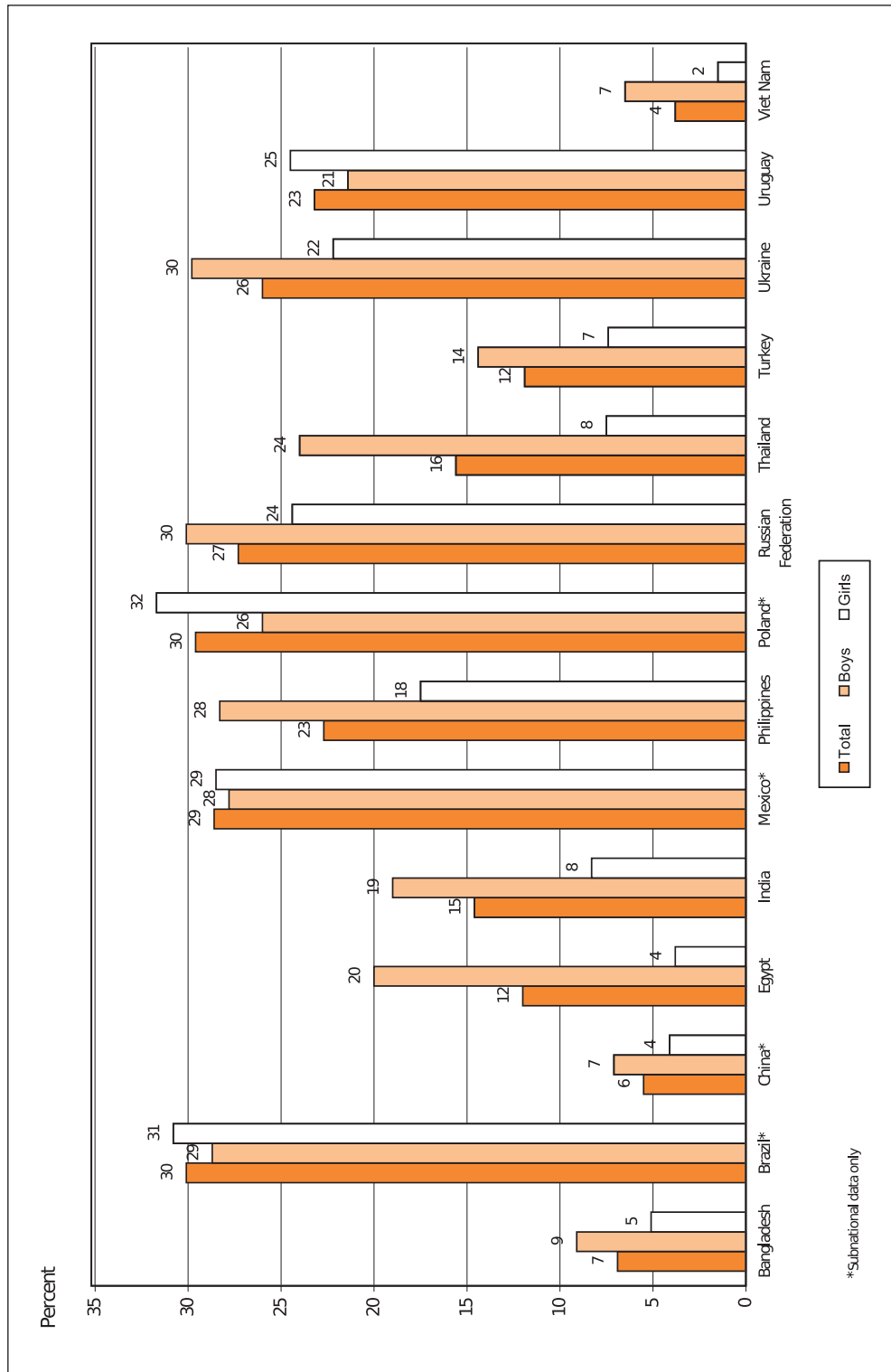


**Fig. 1.8 Range of prevalence of current tobacco use in youth, 2005–2009, by WHO region**



Figures have been rounded off and show prevalences in countries with national and subnational jurisdiction.

– articulates tobacco control measures available to countries to counter the growing tobacco epidemic. This treaty, which entered into force in 2005, represents one of the most universal treaties in the United Nations history. In 2008, the WHO launched MPOWER, a technical assistance package comprised of six strategies that reflects one or more of the WHO FCTC measures and helps countries meet their commitments to the WHO FCTC.

## 2. Cancer in Humans

### 2.1 Introduction

The available knowledge on the relationship between tobacco smoking and a variety of human cancers is based primarily on epidemiological evidence. An immense amount of such evidence has been obtained, and only a small proportion can be referred to here. The cancers considered to be causally related to tobacco smoking in the previous *IARC Monograph* on tobacco smoking ([IARC, 2004a](#)) included lung, oral cavity, nasal cavity and paranasal sinuses, nasopharynx, oropharynx, hypopharynx, larynx, oesophagus (adenocarcinoma and squamous cell carcinoma), upper aerodigestive tract combined, stomach, pancreas, liver, kidney (body and pelvis), ureter, urinary bladder, cervix and myeloid leukaemia. In addition, it was concluded that there was evidence suggesting lack of carcinogenicity for cancers of the breast and of the endometrium.

Since 2002, there have been additional cohort and case-control studies on the relationship of tobacco smoking in different forms to these and other cancers in many countries. A large body of evidence has been obtained from cohort studies with respect to different cancer sites and types of tobacco product. These cohort studies are described briefly in Table 2.1 (available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.1.pdf>), listed by country.

Case-control studies are described in the sections pertaining to cancer sites. More studies are now available from countries and populations that are still at an early stage of the tobacco epidemic. These studies are prone to underestimate the true strengths of the association between tobacco smoking and any specific cancer as the full effect of duration of smoking cannot be evaluated.

## 2.2 Cancer of the lung

### 2.2.1 Overview of studies

The main cause of lung cancer in humans is tobacco smoking and most information establishing this fact comes from epidemiological studies in which the assessment of exposure was based on self-reported information on personal smoking habits via self-administered questionnaire or in-person interviews. Since the previous *IARC Monograph* ([IARC, 2004a](#)), numerous studies have been published on the issues of tobacco smoking and sex and racial/ethnic susceptibility, ‘tar’ yields as measured by machine smoking, the relationship between histological changes and the design of cigarettes, dose-response association, genetic susceptibilities and interactions.

### 2.2.2 Factors affecting risk

Recent epidemiological studies incorporating measures of smoking metabolites in serum or urine are helping to refine our understanding of exposure-response relationships with tobacco smoke. Dose-response evidence has been obtained from three cohort studies ([Flanders et al., 2003](#); [Boffetta et al., 2006](#); [Yuan et al., 2009](#); Table 2.2 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.2.pdf>) and four pooled analyses ([Lubin & Caporaso, 2006](#); [Lubin et al., 2007a, b, 2008](#); Table 2.3 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.3.pdf>)

since the previous *IARC Monograph* ([IARC, 2004a](#)).

The US American Cancer Society Cancer Prevention Study-II (CPS-II) is the largest cohort study on smoking and lung cancer risk using questionnaire assessment of exposure ([Flanders et al., 2003](#)). In this study cigarette smoking duration is a much stronger predictor of lung cancer mortality than is cigarette smoking intensity, regardless of age in both men and women. These results are qualitatively similar to those reported by [Doll & Peto \(1978\)](#) and are consistent with [IARC \(2004a\)](#).

In a questionnaire-based assessment of the association of tobacco smoking with lung cancer risk, smokers at higher smoking intensities seem to experience a “reduced potency” per pack such that for equal total exposure, the excess odds ratio per pack-year decreases with intensity ([Lubin et al. 2008](#)). Below 15–20 cigarettes/day, the excess odds ratio/pack-year increases with intensity ([Lubin & Caporaso, 2006](#); [Lubin et al., 2007a](#)) while above 20 cigarettes/day, there is an ‘inverse-exposure-rate’ effect ([Lubin et al., 2007a](#)) suggesting a greater risk for total exposure delivered at lower intensity (or a longer duration) than the equivalent exposure delivered at a higher intensity. The intensity effects were also statistically homogeneous across diverse cancer types, indicating that after accounting for risk from total pack-years, intensity patterns were comparable for cancer of the lung, bladder, oral cavity, pancreas and oesophagus. These analyses suggest that the risk of lung cancer increases with increasing tobacco exposure at all dose levels, but there is some levelling-off effect at the highest intensity of tobacco smoking.

However, when serum cotinine was used as a measure of exposure to tobacco smoking, rather than questionnaire-based data, the odds ratio of lung cancer increased linearly over the full range of exposure from  $\leq 5$  ng/mL through  $\geq 378$  ng/mL, with an odds ratio of 55.1 (95% confidence interval (CI): 35.7–85.0) in the

highest exposure group. These results suggest that the decreased rate of lung cancer risk at high intensity of tobacco smoke previously described is a statistical artefact. Such an effect may be due to an inaccurate assessment of total tobacco smoke exposure from questionnaire-based studies at high exposure levels ([Boffetta et al., 2006](#)). Somewhat similar results were obtained when both 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) and total cotinine in urine were measured in subjects of two large cohort studies from Shanghai men and Singapore men and women ([Yuan et al., 2009](#)). Among smokers with comparable smoking histories (as noted in questionnaire data) there is a 9-fold variation in subsequent risk of lung cancer between those with high and those with low levels of total urinary NNAL and cotinine. Thus measurements of urinary cotinine and total NNAL at a single point in time in a smoker could substantially improve the predictive power of a lung cancer assessment model based solely on self-reported smoking history (number of cigarettes/day, number of years of regular smoking). A positive NNAL-lung cancer association of comparable magnitude was observed in both Shanghai and Singapore subjects despite differences in the NNK content of tobacco smoked. The independent association between total urinary cotinine and lung cancer risk, after adjustment for total urinary NNAL and smoking history, suggests that tobacco smoke compounds other than NNK play a role in lung cancer development in smokers. Further, a single measurement of urinary NNAL may closely predict the average level of NNAL measured over a much longer period of time.

### 2.2.3 Types of tobacco or of cigarette

#### (a) Tar levels

In a previous *IARC Monograph* ([IARC, 1986](#)), it was concluded on the basis of the case-control, cohort studies and ecological evaluations



available at the time that prolonged use of ‘high-tar’ and unfiltered cigarettes is associated with greater risks than prolonged use of filter-tipped and ‘low-tar’ cigarettes. More recently ([IARC, 2004a](#)), it has been recognized that the actual quantitative impact of reduced ‘tar’ and filter-tipped cigarettes is difficult to assess because of, respectively, the concomitant increase in tobacco-specific nitrosamines that accompanies the greater use of blend tobacco and the compensatory changes in smoking behaviour by smokers attempting to maintain their accustomed level of nicotine intake. Nevertheless, it was concluded that changes in cigarette types since the 1950s have probably tended to reduce the risk for lung cancer associated with tobacco smoking.

Additional refinement in assessing the health effects associated with smoking cigarettes of various tar content has been possible since the publication of the earlier reports. Compared with smokers of medium tar (15–21 mg) filtered cigarettes risk was higher among men and women who smoked high tar ( $\geq 22$  mg) non-filtered brands but there was no difference in risk among men and women who smoked ‘very low tar’ or ‘low tar’ brands compared with those who smoked ‘medium tar’ brands ([Harris et al., 2004](#)). Regardless of tar content of their cigarettes, all current smokers had a far greater risk for lung cancer than people who had stopped smoking or had never smoked ([Harris et al., 2004](#)).

#### (b) *Mentholated cigarettes*

In the previous *IARC Monograph* ([IARC, 2004a](#)) the conclusion was drawn that there is no additional risk associated with smoking mentholated cigarettes when total consumption (pack-years) was controlled versus non-mentholated ones. Recent evidence supports that conclusion.

Mentholated cigarettes first appeared in the 1920s, but were not widely used until the mid-1950s ([Bogen, 1929](#); [Federal Trade Commission, 2001](#)). Since the early 1970s, menthol varieties have accounted for 25–60% of all cigarettes

sold in the USA ([Federal Trade Commission, 2001](#)). There are strong ethnic differences in the use of menthol cigarettes; more than 60% of Black smokers of both sexes use menthol brands compared to fewer than 25% of White smokers ([Royce et al., 1993](#); [Hymowitz et al., 1995](#)). Studies have generally not demonstrated an increased risk of lung cancer for mentholated cigarettes versus non-mentholated cigarettes ([Kabat & Hebert, 1994](#); [Carpenter et al., 1999](#); [Brooks et al., 2003](#); [Stellman et al., 2003](#)). Recent evidence also suggests that users of mentholated cigarettes smoke fewer pack-years than those of non-mentholated cigarettes.

The higher incidence of lung cancer among Blacks is an important public health concern but the causes remain unclear. Mentholated cigarette use does not appear to explain the racial disparity observed in lung cancer risk among those having the same total tobacco consumption.

#### 2.2.4 *Histology*

Compiled databases from IARC and other sources indicated that squamous cell carcinoma rates [per 100000 person-years] among men declined by 30% or more in North America and some European countries between 1980–82 and 1995–97, while changing less dramatically in other areas; small cell carcinoma rates decreased less rapidly. In contrast, the proportion of adenocarcinoma cases rose among men and women in virtually all areas, with the increases among men exceeding 50% in many areas of Europe ([Devesa et al., 2005](#)).

Based on a comparison of two large cohort studies initiated by the American Cancer Society (ACS) (CPS-I and CPS-II) in 1960 and 1980, respectively, a stronger association between smoking and adenocarcinoma was observed in recent compared to earlier follow-up periods ([Thun & Heath, 1997](#)). Additionally, an association between cigarette smoking and bronchioloalveolar carcinoma was also found in several

studies ([Falk et al., 1992](#); [Morabia & Wynder, 1992](#)).

A meta-analysis of 8 cohort and 14 case-control studies conducted in Japan among active smokers indicated significant excess lung cancer risks among men for both squamous cell carcinoma (relative risk (RR), 11.7) and adenocarcinoma (RR, 2.30). Among women the risks were 11.3 for squamous cell carcinoma and 1.37 for adenocarcinoma ([Wakai et al., 2006](#)).

### 2.2.5 Population characteristics

#### (a) Sex

Meta-analyses on sex-specific susceptibility to lung cancer associated with tobacco smoking are presented in Table 2.4 (available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.4.pdf>) and cohort studies in Table 2.5 (available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.5.pdf>).

In the 1990s, two case-control studies indicated that relative risks for lung cancer associated with specific amounts and duration of cigarette smoking may be higher among women than among men ([Risch et al., 1993](#); [Zang & Wynder, 1996](#)).

In the large NIH-AARP [National Institutes of Health-American Association of Retired People] cohort ([Freedman et al., 2008](#)), smoking was associated with lung cancer risk in both men and women. Age-standardized incidence rates for lung cancer tended to be higher in men than in women with comparable smoking histories (for current smokers and for quitters of less than 10 years), and in cases with squamous cell tumours. However, lung cancer risk was generally similar between men and women.

In a joint analysis, results from the Nurses' Health Study of women and the Health Professionals Follow-up Study in men ([Bain et al., 2004](#)) suggest little difference in lung cancer susceptibility between men and women given equal smoking exposure. The hazard ratio

in women ever smokers compared with men was 1.11 (95%CI: 0.95–1.31).

Serum cotinine levels were analysed in lung cancer cases and controls ([Boffetta et al., 2006](#)). The lung cancer odds ratios (ORs) estimated for men and women were very similar for those with comparable serum cotinine levels. Other studies that have carefully quantified tobacco exposure via self-administered questionnaire or interview provide additional evidence of a comparable increase in lung cancer risk in the two sexes ([Kreuzer et al., 2000](#); [Flanders et al., 2003](#); [Bain et al., 2004](#)).

In a meta-analysis of observational studies on cigarette smoking and cancer from 1961–2003 (conducted on 177 case-control studies, 75 cohort studies and two nested case-control studies), dose-response estimates were available in 44 studies: 19 with estimates for men only, 11 with estimates for women only and 14 with separate estimates for men and women ([Gandini et al., 2008](#)). Overall, the risk of lung cancer for men and women increased by 7% for each additional cigarette smoked per day (RR, 1.07; 95%CI: 1.06–1.08). The increased risk appears to be slightly higher in women (RR, 1.08; 95%CI: 1.07–1.10) than in men (RR, 1.07; 95%CI: 1.05–1.08) ( $P < 0.001$ ; adjusting for study type).

#### (b) Ethnicity

It has been postulated that susceptibility to lung cancer from tobacco smoking may differ by race and ethnicity ([Schwartz & Swanson, 1997](#); [Peto et al., 1999](#); [Stellman et al., 2001](#); [Kiyohara et al., 2004, 2005, 2006](#); [Pinsky, 2006](#); [Wakai et al., 2006](#); [Vineis et al., 2007](#); [Takahashi et al., 2008](#); Table 2.6 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.6.pdf>). Lung cancer incidence rates vary considerable across racial/ethnic groups in the USA and elsewhere. Black men have higher rates than white men, while Hispanics, Asians and American Indians of both sexes have lower rates than whites ([Stellman et al., 2003](#); [SEER, 2004](#)).

Nutritional habits, smoking patterns, type of tobacco smoked and genetic factors may play a role in such differences between racial and ethnic groups.

The association of tobacco smoking and lung cancer does not appear to be as strong among Japanese as among populations of North America or Europe ([Wakai et al., 2006](#)). In a meta-analysis of 8 cohort studies and 14 case-control studies conducted in Japan, the excess lung cancer risks observed for both men (RR, 4.39; 95%CI: 3.92–4.92) and women (RR, 2.79; 95%CI: 2.44–3.20) in both case-control and cohort studies were lower than would have been expected from studies in North America and Europe. The lower lifetime consumption of cigarettes in Japanese, due in part to a later initiation of smoking and a lower consumption per day has been suggested to explain this. Other differences that may have etiological significance include tobacco ingredients, different filters on cigarettes, lifestyle factors including diet, and possibly differences in genetic susceptibility. [The Working Group noted that North American or European populations were not directly included in any of these studies.]

Data from the Asian Pacific Cohort Studies Collaboration, 31 studies involving 480125 persons, evaluated the risk of death from lung cancer associated with smoking habits in Australia, New Zealand and Asia ([Huxley et al., 2007b](#)). Among Asian men the hazard ratio was 2.48 versus 9.87 in men in Australia and New Zealand. Among women, the corresponding estimates were 2.35 and 19.33, respectively. [In these studies, Asian populations smoked fewer cigarettes for a shorter period of time compared to those in Australia and New Zealand.]

Based on data from the National Cancer Institute's Surveillance, Epidemiology, and End Results program (SEER), Chinese women residing in the USA have a fourfold increased risk of lung cancer, and Filipino women a twofold increased risk, compared to that expected based on rates in

non-Hispanic whites in the USA with a similar amount of cigarettes smoked ([Epplein et al., 2005](#)). Among Chinese women, the increased risk was largely restricted to adenocarcinoma and large cell undifferentiated carcinoma. Chinese females residents of the western US mainland have a much higher risk of lung cancer than would be expected from their tobacco use patterns, just as they do in Asia ([Peto et al., 1999](#); [Epplein et al., 2005](#)), the reason for these difference have not been identified. [Controlling for potential confounding factors was limited using aggregate data from SEER.]

Age, sex and race-specific risks of lung cancer mortality among lifetime non-smokers were compared in the two large ACS Cancer Prevention Study cohorts (CPS-I; CPS-II). The mortality rate was higher among African American women than among white women in CPS-II (hazard ratio (HR), 1.43; 95%CI: 1.11–1.36) ([Thun et al., 2006](#)). This suggests an inherent susceptibility difference between white and black women but it could also be explained by access to care, diet, or exposure to environmental carcinogens.

The risk for lung cancer associated with cigarette smoking in 183813 African-American, Japanese-American, Latino, native Hawaiian and white men and women was examined in the Multiethnic Cohort Study in the USA ([Haiman et al., 2006](#)). Information on demographic factors, smoking status, cigarettes/day smoked, years of smoking, years since quitting, diet, occupations, educational level and racial and ethnic group were collected for all subjects through a self-administered questionnaire at enrolment. Information about age of smoking initiation and cessation rates were collected on a subgroup of 5090 study subjects. Incident lung cancer cases were identified by linkage to the SEER cancer registries covering California and Hawaii. Among those who smoked no more than 10 cigarettes/day and those who smoked 11–20 cigarettes/day, relative risks ranged from 0.21 to 0.39 ( $P < 0.001$ ) among Japanese Americans and Latinos and from 0.45

to 0.57 ( $P < 0.001$ ) among whites as compared with African Americans. However, at levels exceeding 30 cigarettes/day, differences between racial/ethnic groups were no longer significant. The differences in lung cancer risk by racial group associated with smoking were observed for both men and women and for all histological types of lung cancer. These findings could not be explained by differences between populations in other known or suspected risk factors, including diet, occupation, and education level or by age at starting smoking or cessation of smoking.

Polymorphisms in glutathione-S-transferase (GST), GSTM1, GSTT1 and GSTP1 genes in humans are associated with reduction of enzymatic activity towards several substrates, including those found in tobacco smoke. In a population based case-control study involving early-onset lung cancer, African Americans carrying at least one G allele at the GSTP1 locus were more likely to have lung cancer compared with African Americans without a G allele after adjustment for age, sex, pack-years of smoking and a history of lung cancer in a first degree relative (OR, 2.9; 95%CI: 1.29–6.20). African Americans with either one or two risk genotypes at the GSTM1 (i.e. null genotype) and GSTP1 loci were at increased risk of having lung cancer compared with those having fully functional GSTM1 and GSTP1 genes (one risk genotype: OR, 2.8; 95%CI: 1.1–7.2 and two risk genotypes: OR, 4.0; 95%CI: 1.3–12.2). No significant single gene associations between GSTM1, GSTT1 and GSTP1 and early-onset lung cancer were observed in Caucasians, after adjusting for age, sex, pack-years and a family history of lung cancer ([Cote et al., 2005](#)).

The cytochrome P450 (CYP) superfamily of enzymes catalyses one of the first steps in the metabolism of carcinogens such as polycyclic aromatic hydrocarbons, nitroaromatics and arylamines. A population-based case-control study of lung cancer in the metropolitan Detroit area found that neither CYP1A1 MspI nor CYP1A1

Ile<sup>462</sup>Val was associated with lung cancer susceptibility among Caucasians or African Americans. Among Caucasians, however, CYP1B1 Leu<sup>432</sup> Val was significantly associated with lung cancer susceptibility (OR for at least one Val allele, 2.87; 95%CI: 1.63–5.07). Individuals with both this polymorphism and exposure to second-hand tobacco smoke were at particularly high risk for lung cancer. Combinations of particular CYP1B1 polymorphisms appeared to increase risk, although no combination differed significantly from the risk associated with CYP1B1 Leu<sup>432</sup> Val alone ([Cote et al., 2005](#); [Wenzlaff et al., 2005](#)).

The hypothesis that polymorphisms in TP53 may modulate the risk for lung cancer associated with tobacco smoke was evaluated in a case-control study of lung cancer in Baltimore, Maryland. African-Americans with Pro-T-A-G-G haplotype (combining the polymorphisms TP53\_01 (rs1042522), TP53\_65 (rs9895829), TP53\_66 (rs2909430), TP53\_16 (rs1625895), and TP\_11 (rs12951053)) had both an increased risk for lung cancer (HR, 2.32; 95%CI: 1.38–4.10) and a worsened lung cancer prognosis (HR, 2.38; 95%CI: 0.38–4.10) compared with those having the Arg-T-A-G-T haplotype. No association of TP53 polymorphisms with lung cancer was observed in Caucasians ([Mechanic et al., 2007](#)). Common genetic variation in TP53 could modulate lung cancer pathways in African Americans. Differences in lung cancer susceptibility may exist based on race, tobacco exposure and selected genetic polymorphisms ([Mechanic et al., 2007](#)).

## 2.2.6 Interactions

### (a) Diet and exercise

Antioxidant vitamins, carotenoids, isothiocyanates, total dietary vegetables and fruit, and physical exercise have been associated with a decreased risk for cancer in some studies but the overall protective effect of diet and exercise account for only a small fraction of the total risk associated with tobacco smoking.



The association of fruit and vegetable with lung cancer incidence among both smokers and non-smokers was evaluated in the European Prospective Investigation into Cancer and Nutrition (EPIC). In current smokers lung cancer risk was significantly decreased with higher vegetable consumption, the hazard ratio being 0.78 (95%CI: 0.62–0.98) per 100 g increase in daily vegetable consumption, and 0.90 (95%CI: 0.81–0.99) per 100 g fruit ([Linseisen et al., 2007](#)). While overall consumption of fruits and vegetables was not found to be protective of lung cancer in the NIH-AARP Diet and Health Study, higher consumption of several botanical subgroups (i.e. rosaceae, convolvulaceae, and umbelliferae) was significantly inversely associated with risk, but only in men ([Wright et al., 2008](#)).

Cruciferous vegetables (i.e. broccoli, cabbage, cauliflower, Brussels sprouts, kale) are rich in isothiocyanates and have been hypothesized to have anticancer properties that may contribute to reduced risk for lung cancer. Isothiocyanates may inhibit the bioactivation of procarcinogens found in tobacco smoke such as polycyclic aromatic hydrocarbons and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone ([Hecht, 2000](#)). Isothiocyanates may also enhance excretion of carcinogenic metabolites before they can damage DNA ([Gasper et al., 2005](#)). Furthermore, sulforaphane, a major isothiocyanate found in broccoli, can induce cell cycle arrest and apoptosis ([Seow et al., 2005](#)). GSTM1 and GSTT1 encode isoenzymes that play an important role in xenobiotic metabolism ([Hecht, 2000](#)). Individuals with homozygous deletion of GSTM1 and GSTT1, or both may metabolize isothiocyanates less efficiently and may be more intensely exposed to isothiocyanates after consumption of cruciferous vegetables. Epidemiological evidence from 30 studies on the association between lung cancer and either total cruciferous vegetable consumption (6 cohort and 12 case–control studies) or specific cruciferous vegetables (1 cohort and 11 case–control studies) was recently evaluated

([Lam et al., 2009](#)). The risk for lung cancer among those in the highest category of total cruciferous vegetable intake was 22% lower in case–control studies (pooled OR, 0.78; 95%CI: 0.70–0.88) and 17% lower in cohort studies (pooled RR, 0.83; 95%CI: 0.62–1.08). The strongest inverse association of total cruciferous vegetable intake with lung cancer was seen among individuals with GSTM1 and GSTT1 double null genotypes (OR, 0.41; 95%CI: 0.26–0.65; *p* for interaction = 0.01). The inverse association was observed in both smokers and non-smokers.

The potential role of vitamin A in the development of lung cancer attracted early research interest ([Bjelke, 1975](#)). Carotenoids were thought to have anti-cancer properties and early evidence from case–control studies tended to support an inverse association of lung cancer incidence with  $\beta$ -carotene intake and with serum concentrations of  $\beta$ -carotene. However, the case–control design is not ideal for assessing the effect of serum carotenoids as a risk factor for lung cancer risk since the disease is likely to effect serum levels. In a meta-analysis of six randomized clinical trials and 25 prospective observational studies, [Gallicchio et al. \(2008\)](#) computed a pooled relative risk for studies comparing  $\beta$ -carotene supplements with placebo of 1.10 (95%CI: 0.89–1.36). Among observational studies, the pooled relative risk for total carotenoid dietary intake from six studies was 0.86 (95%CI: 0.75–0.99) among current smokers. For dietary intake of  $\beta$ -cryptoxanthin, data from six studies gave a pooled relative risk among smokers of 0.75 (95%CI: 0.58–0.96). No other carotenoids significantly reduced the risk in current, former or never smokers.

Based on a review of the literature, antioxidant vitamins show no clear protective effect on lung cancer risk in smokers or non-smokers, although there was some, albeit inconsistent, evidence pointing to a protective role for vitamin C and E. No clear protective role was observed for vitamin A ([Ruano-Ravina et al., 2006](#)).

Increased physical activity has been associated with a reduction in the incidence and mortality from all-site cancer and some site-specific cancers in studies of non-smokers, but less is known about whether physical activity is associated with similar risk reduction in smokers. Several early studies suggested that physical activity is associated with decreased risk of lung cancer in men and women after adjusting for smoking, with risk reductions estimated from 18% (Peterson *et al.*, 2001) to 62% (Kubík *et al.*, 2001). The effect of physical activity on lung cancer risk was assessed in a sample drawn from participants in the Beta-Carotene and Retinol Efficacy Trial. The results suggested that physical activity may play a small role in reducing cancer risk and mortality among those with significant tobacco exposure. The incidence of lung cancer and of all cancer sites combined seemed to be more attenuated by exercise in men than in women, while the attenuation in lung cancer mortality was greater in women than in men. These effects may be more pronounced for younger people and may differ inconsistently by pack-years of smoking (Alfano *et al.*, 2004).

#### (b) Radon

In a pooled analysis of data from 13 case-control studies of residential radon and lung cancer from nine European countries (7148 cases of lung cancer and 14208 controls), the dose-response relation seemed to be linear with no threshold and remained significant in analyses limited to individuals from homes with measured radon < 200 Bq/m<sup>3</sup>. The absolute risks of lung cancer by age 75 years at radon concentrations of 0, 100, and 400 Bq/m<sup>3</sup> would be about 0.4%, 0.5% and 0.7%, respectively, for lifelong non-smokers, and about 25 times greater (10%, 12% and 16%) for cigarette smokers. These studies show appreciable hazards from residential radon, particularly for smokers and recent ex-smokers (Darby *et al.*, 2005). Similar risks were identified in a

pooling project of North American case-control studies (Krewski *et al.*, 2005).

#### (c) Asbestos

Exposure to asbestos and tobacco smoking are both known causes of lung cancer in humans (Doll & Peto, 1978; de Klerk *et al.*, 1996). Some studies suggest a multiplicative effect [where the effect of asbestos exposure is a multiple of the effect of smoking] (Hammond *et al.*, 1979; Doll & Peto, 1985), and meta-analyses have suggested that the additive model [where asbestos exposure and smoking are independent of each other] is unsound (Lee, 2001; Liddell, 2001). In a recent study of 2935 asbestos miners, persons exposed to asbestos and tobacco who subsequently quit smoking remained at a 90% increased risk of lung cancer up to 20 years after smoking cessation, compared to never-smoker asbestos workers (Reid *et al.*, 2006a).

#### (d) Genetic polymorphisms

Lung cancer is plausibly caused by the interplay between environmental factors and several low-risk alleles. Attempts in identifying specific single nucleotide polymorphisms (SNPs) responsible for modulating lung cancer risk have yielded few conclusive results. Recent studies have focused on mechanistically plausible polymorphisms in genes coding for enzymes involved in the activation, detoxification and repair of chemical damage caused by tobacco smoke. Genetic association studies indicate that several inherited genetic polymorphisms may be associated with lung cancer risk, but the data from individual studies with low statistical power are conflicting. Evidence from pooled or meta-analyses, along with some individual studies, is briefly summarized below.

##### (i) Metabolic genes

Most of the 70 carcinogens in tobacco smoke are procarcinogens that must be activated by phase I enzymes and may then be deactivated by

phase II enzymes. Polymorphisms that alter the function of the genes involved in the activation or detoxification of tobacco smoke carcinogens can potentially influence an individual's risk of developing a tobacco-related cancer.

Meta and pooled analyses of 34 case-control, genotype-based studies were conducted to assess the effect of GSTT1 genotypes and smoking on lung cancer risk. No significant interaction was observed ([Raimondi et al., 2006](#)). A pooled analysis of 21 case-control studies from the International Collaborative study of Genetic Susceptibility to Environmental Carcinogens showed no evidence of increased risk for lung cancer among carriers of the GSTM1 null genotype and there was no evidence of interaction between GSTM1 genotype and either smoking status or cumulative tobacco consumption ([Benhamou et al., 2002](#)). Similarly, in another pooled analysis the summary OR indicated the slow acetylator genotype of N-acetyltransferase 2 (NAT2) detoxification enzyme was not associated with lung cancer risk among Caucasians ([Borlak & Reamon-Buettner, 2006](#)). In a pooled analysis to test the hypothesis of interaction among genetic variants in increasing the individual risk for cancer, the cumulative effect of variants in three metabolic genes, CYP1A1, GSTM1 and GSTT1 was assessed. The risk for lung cancer was increased with the combination of CYP1A1\*2B or CYP1A1\*4 alleles and the double deletion of both GSTM1 and GSTT1 up to an OR of 8.25 (95%CI: 2.29–29.77). The combination including CYP1A1\*4 among never smokers was associated with an OR of 16.19 (95%CI: 1.90–137). These estimates did not change after adjustment by the number of cigarettes smoked and duration of smoking. The results were consistent across ethnicities and were approximately the same for adenocarcinoma and squamous cell carcinoma ([Vineis et al., 2007](#)).

Microsomal epoxide hydrolase 1 (EPHX1) plays an important role in both the activation and detoxification of tobacco-derived carcinogens.

Polymorphisms at exons 3 and 4 of the EPHX1 gene have been reported to be associated with variations in EPHX1 activity. In a meta-analysis of 13 case-control studies the low-activity (variant) genotype of EPHX1 polymorphism at exon 3 was associated with decreased risk for lung cancer (OR, 0.65; 95%CI: 0.44–0.96) among whites. In white-populations, the high activity (variant) genotype of EPHX1 polymorphism at exon 4 was associated with a modest increased risk of lung cancer (OR, 1.22; 95%CI: 0.79–1.90) and the predicted low activity was associated with a modest decrease in risk (OR, 0.72; 95%CI: 0.43–1.22) ([Kiyohara et al., 2006](#)).

#### (ii) DNA repair and cell cycle pathways

Data from 14 studies of lung cancer were used in a pooled analysis focusing on 18 sequence variants in 12 DNA repair genes, including APEX1, OGG1, XRCC1, XRCC2, XRCC3, ERCC1, XPD, XPF, XPG, XPA, MGMT and TP53 ([Hung et al., 2008a](#)). None of the variants appeared to have a large effect on lung cancer risk. In a recent meta-analysis the X-ray repair cross-complementing protein group 3 (XRCC3) and the xeroderma pigmentosum group D (XPD)/excision repair cross-complementing group 2 (ERCC2) genes were evaluated ([Manuguerra et al., 2006](#)). The authors found no association between these genes and the cancer sites investigated (skin, breast and lung). A significant association was identified for XPD/ERCC2 single nucleotide polymorphisms (codons 312 and 751) and lung cancer.

#### (iii) Nicotine acetylcholine receptor genes

A series of large genome-wide association studies for lung cancer have identified susceptibility loci for lung cancer in chromosome arms 5p, 6p and 15q ([Landi et al., 2009](#)). In particular, the susceptibility locus at chromosome region 15q25 includes several genes, including three that encode nicotinic acetylcholine receptor subunits (CHRNA5, CHRNA3 and CHRNB4). Nicotinic acetylcholine receptor subunit genes

code for proteins that form receptors present in neuronal and other tissue, in particular alveolar epithelial cells, pulmonary neuroendocrine cells, and lung cancer cell lines ([Wang et al., 2001](#); [Minna, 2003](#)) and bind to nicotine and nicotine derivatives including NNN. An association of CHRNA3 and CHRNA5 variants with nicotine dependence has been reported ([Saccone et al., 2007](#); [Berrettini et al., 2008](#)). These genes may act, at least partially, upon cigarette smoking. Current smokers with one or two copies of the susceptibility variant are likely to smoke between one and two cigarettes more a day ([Spitz et al., 2008](#)). Evidence for an effect of the 15q25 locus among never smokers is conflicting, with an association found in one study in Europe ([Hung et al., 2008b](#)) and one in Asia ([Wu et al., 2009a](#)), but not in others. Whether genes in the 15q25 locus have an effect on lung cancer beyond their propensity to increase numbers of cigarettes smoked is unclear.

Three genome-wide association studies identified genetic factors that modified disease risk. The first was a genome-wide association analysis to identify genetic polymorphisms associated with lung cancer risk in 1154 lung cancer patients of European ancestry who were current or former smokers and 1137 control subjects who were frequency matched to the lung cancer patients by age, sex, race and smoking status. Two SNPs, rs105173 and rs803419, which mapped to a region of strong linkage disequilibrium within 15q25.1, were strongly associated with risk of lung cancer, with an odds ratio for rs105173 of 1.31 ( $P = 9.84 \times 10^{-6}$ ). This finding was replicated with an additional 711 case subjects and 632 control subjects from Texas ( $P = 0.00042$ ) and in 2013 case subjects and 3062 control subjects in the United Kingdom ( $P = 2.33 \times 10^{-10}$ ). The region of interest encompasses the nicotinic acetylcholine receptor subunit genes CHRNA3 and CHRNA5 (as well as CHRNB4) ([Spitz et al., 2008](#)). A second genome-wide association study conducted among 1989 lung cancer cases and

2625 controls from six central European countries confirm these results ([Hung et al., 2008a](#)). In a third genome-wide association study of 665 Icelandic, 269 Spanish and 90 Dutch lung cancer cases and 32244 controls a common variant in the nicotinic acetylcholine receptor gene cluster [chromosome region 15q24] was significantly associated with lung cancer risk (OR, 1.31; 95%CI: 0.1.19–1.44). The variant was observed to have a significant effect on the number of cigarettes smoked per day ([Thorgeirsson et al., 2008](#)). These studies have all shown a link between this variant and lung cancer risk either through a mechanism involving nicotine dependence or a direct role in downstream signalling pathways that promote carcinogens. Together these results provide compelling evidence of a locus at 15q25 and 15q24 predisposing to lung cancer.

#### (iv) *Alpha(1)-antitrypsin*

Alpha(1)-antitrypsin deficiency ( $\alpha(1)$ ATD) is one of the most common genetic disorders, especially among European descendants. Recent results suggest that  $\alpha(1)$ ATD carriers are at a 70–100% increased risk of lung cancer, accounting for 11% to 12% of patients with lung cancer ([Yang et al., 2008](#)). [The specific effect by smoking status was not evaluated.]

#### (v) *Other genes*

Mutations in the checkpoint CHEK2 gene have been associated with increased risk of breast, prostate and colon cancer and a decreased risk of lung cancer among those with the I157T missense variant of the CHEK2 gene. In a large Polish case–control study CHEK2 mutations were protective against lung cancer (OR, 0.3; 95%CI: 0.2–0.5) ([Cybulski et al., 2008](#)).

The Swedish Family-Cancer Database was used to compare the rate of lung cancers among persons without family history of lung cancer to those with a family history ([Li & Hemminki, 2004](#)). A high risk by family history in adenocarcinoma (standardized incidence ratio (SIR),



2.03) and large cell carcinoma (SIR, 2.14) was found, a slightly lower risk among patients with squamous cell carcinoma (SIR, 1.63) and small cell carcinoma (SIR, 1.55). Among siblings, an increased risk was shown for concordant adenocarcinoma and small cell carcinoma at all ages and for all histological types when cancer was diagnosed before age 50. At young age, risks between siblings were higher than those between offspring and parents. These data suggest that a large proportion of lung cancers before age 50 are heritable and due to a high-penetrant recessive gene or genes that predispose to tobacco carcinogen susceptibility.

#### (e) *Viral infection*

Data are limited regarding lung cancer risk in human immunodeficiency virus (HIV)-infected persons with modest immune suppression, before the onset of acquired immunodeficiency syndrome (AIDS). Among 57350 HIV-infected persons registered in the USA during 1991–2002 (median CD4 counts 491 cells/mm<sup>3</sup>), 871 cancers occurred. Risk was elevated for several non-AIDS defining malignancies, including cancer of the lung (SIR, 2.6 [ $n = 109$ ]) (Engels *et al.*, 2008). [Specific evaluation with smoking status was not performed.]

## 2.3 Cancers of the upper aerodigestive tract

Evidence relating to cancers of the upper aerodigestive tract obtained from relevant cohort and case-control studies on specific sites is described in Sections 2.3.1 to 2.3.6; studies that looked at several subsites combined are described in Section 2.3.7. The major potential confounders for the relationship between smoking and cancers of the upper aerodigestive tract are alcohol consumption and use of any form of smokeless tobacco, and for some sites infection with human papillomavirus (HPV) (especially HPV16). In

general, the studies examined by the Working Group had adjusted for these two confounders when appropriate. Some studies also adjusted for dietary intake, especially of fruits and vegetables, although few reported stratified relative risks.

### 2.3.1 *Cancer of the oral cavity*

Tobacco smoking was found to be causally related to oral cancer (IARC, 1986, 2004a). New studies on the relationship between oral cancer and cigarette smoking published since the most recent IARC Monograph (IARC, 2004a) include four cohort studies (Table 2.7 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.7.pdf>), and eight case-control studies (Tables 2.8–2.11 online; see below).

#### (a) *Intensity and duration of smoking*

Intensity of smoking was measured in almost all cohort (Table 2.7 online) and case-control studies (IARC 2004a; Table 2.8 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.8.pdf> and Table 2.9 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.9.pdf>). In addition to the number of cigarettes or amount of tobacco smoked daily, cumulative exposure to cigarette smoke was also measured in terms of pack-years, tobacco-years or lifetime tobacco consumption. The link between duration of cigarette consumption and oral cancer was examined in 15 case-control studies. Seven case-control studies also considered age at starting smoking.

One cohort study (McLaughlin *et al.*, 1995) and 14 case-control studies reported a dose-dependent increase in risk with increasing number of cigarettes smoked daily or increasing daily tobacco consumption (Franceschi *et al.*, 1990, 1992, 1999; Nandakumar *et al.*, 1990; Zheng *et al.*, 1990; Choi & Kahyo, 1991; Oreggia *et al.*, 1991; Bundgaard *et al.*, 1995; Zheng *et al.*, 1997; De Stefani *et al.*, 1998; Hayes *et al.*, 1999; De Stefani

*et al.*, 2007; Subapriya *et al.*, 2007; Muwonge *et al.* 2008). Whenever analysed, the trend was always statistically significant (Franceschi *et al.*, 1990, 1992; Oreggia *et al.*, 1991; Bundgaard *et al.*, 1995; McLaughlin *et al.* 1995; Hayes *et al.*, 1999; Subapriya *et al.*, 2007), except in the study of Muwonge *et al.* (2008) which also included bidi smokers.

Bundgaard *et al.* (1995) used lifetime tobacco consumption divided into four categories and reported a positive, significant trend after adjustment for life-time consumption of alcohol and other risk factors. A positive trend was also found in all studies that have analysed consumption in pack-years or tobacco-years (Zheng *et al.*, 1990; Maier *et al.*, 1992a; Macfarlane *et al.*, 1995; Hung *et al.*, 1997; Zheng *et al.*, 1997; De Stefani *et al.*, 1998, 2007; Applebaum *et al.*, 2007; Muwonge *et al.*, 2008), except Muwonge *et al.* (2008).

Ten studies (Franceschi *et al.*, 1990, 1992; Nandakumar *et al.*, 1990; Zheng *et al.*, 1990; Choi & Kahyo, 1991; Oreggia *et al.*, 1991; Zheng *et al.*, 1997; De Stefani *et al.*, 1998, 2007; Znaor *et al.*, 2003; Subapriya *et al.*, 2007; Muwonge *et al.*, 2008) classified the duration of smoking in up to four categories, and all but one (Nandakumar *et al.*, 1990) reported increased relative risks and a positive trend.

Of six studies that considered age at start of smoking (Franceschi *et al.*, 1990, 1992; Choi & Kahyo, 1991; Oreggia *et al.*, 1991; Zheng *et al.*, 1997; Balaram *et al.*, 2002) two reported a statistically significant trend of increasing risk with decreasing age at starting (Franceschi *et al.*, 1990, 1992).

#### (b) Cessation of smoking

Three cohort studies (McLaughlin *et al.*, 1995; Freedman *et al.*, 2007a; Friborg *et al.* 2007) and nine case-control studies (Zheng *et al.*, 1990; Choi & Kahyo, 1991; Oreggia *et al.*, 1991; Franceschi *et al.*, 1992; Ko *et al.*, 1995; Zheng *et al.*, 1997; De Stefani *et al.*, 1998, 2007; Schildt *et al.*, 1998; Balaram *et al.*, 2002; Pacella-Norman

*et al.*, 2002; Muwonge *et al.* 2008) estimated risks for former smokers which were always lower than those for current smokers and in five studies almost reached unity (Zheng *et al.*, 1990; Choi & Kahyo, 1991; Zheng *et al.*, 1997; Schildt *et al.*, 1998; Muwonge *et al.*, 2008). Twelve case-control studies examined the risk by years since quitting and all reported a negative trend, with relative risks compared with those in non-smokers decreasing to near unity after 10 or more years (Franceschi *et al.*, 1990, 1992; De Stefani *et al.*, 1998, 2007; Schlecht *et al.*, 1999a; Table 2.7 online; Table 2.10 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.10.pdf>).

#### (c) Type of cigarette

The effect of the type of cigarette smoked was examined in several case-control studies (Table 2.11 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.11.pdf>). The characteristics of the cigarettes included the presence of a filter, the type of tobacco, the tar content and whether the product was manufactured or hand-rolled. Two studies reported a statistically significantly higher risk for black than for blond tobacco (Oreggia *et al.*, 1991; De Stefani *et al.*, 1998, 2007). Similarly, a much higher risk was found for hand-rolled cigarettes than for manufactured cigarettes, and plain cigarettes had a much higher risk than filter-tipped cigarettes (De Stefani *et al.*, 1998, 2007). In one study the differences between black and blond tobacco and between hand-rolled and manufactured cigarettes persisted after stratification by duration of smoking (De Stefani *et al.*, 1998). Smoking cigarettes with a high-tar content led to higher risks than smoking cigarettes with a low-tar content (Franceschi *et al.*, 1992) and the same trend was observed for cigarettes without filter compared to cigarettes with filter (De Stefani *et al.*, 2007).

*(d) Sex*

Sex-specific effects were examined in two case-control studies ([Zheng et al., 1990](#); [Hayes et al., 1999](#)). In both studies, the relative risks for all categories of intensity, duration of smoking and pack-years were higher for women than for men. [The Working Group noted that the background risk of oral cancer is considerably lower in women than men. Thus, the higher relative risk estimates in women than men indicate a higher proportionate contribution from smoking in women than men, rather than higher absolute risk.]

*2.3.2 Cancer of the pharynx*

Tobacco smoking was considered to be an important cause of oropharyngeal and hypopharyngeal cancers in the previous *IARC Monographs* on tobacco smoking ([IARC, 1986, 2004a](#)). Since then, results available from three cohort (Table 2.12 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.12.pdf>) and seven case-control studies (Table 2.13 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.13.pdf> and Table 2.14 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.14.pdf>) provide further support for the association. Many studies, however, combine cancers of the oral cavity and pharynx (see Section 2.3.7). This section summarizes the evidence from all eight cohort and 21 case-control studies that reported results specifically on oropharyngeal and hypopharyngeal cancer, or on pharyngeal cancer in general; the latter may include data on nasopharyngeal cancer.

The risk for pharyngeal cancer was significantly increased in smokers in four cohort studies ([Doll et al., 2005](#); [McLaughlin et al., 1995](#); [Freedman et al., 2007a](#); [Friborg et al., 2007](#)) and all but one of the case-control studies ([Rao et al., 1999](#)). The trend of increasing risk associated with increasing daily or cumulative consumption

of cigarettes was evident from all these studies, particularly those from Europe ([Brugere et al., 1986](#); [Tuyns et al., 1988](#); [Franceschi et al., 1990, 1999](#); [Maier et al., 1994](#); [Escribano Uzcudun et al., 2002](#); [Vlajinac et al., 2006](#)), India ([Znaor et al., 2003](#); [Sapkota et al., 2007](#)), Uruguay ([De Stefani et al., 1998, 2007](#)) and the USA ([McLaughlin et al., 1995](#); [Applebaum et al., 2007](#)), and less strongly so in studies from Canada ([Elwood et al., 1984](#)) and the Republic of Korea ([Choi & Kahyo, 1991](#)). The multicentre study in Europe, North and South America of [Hashibe et al. \(2007c\)](#) showed increased risks according to frequency (cigarettes/day) and duration (years) in never drinkers. [Applebaum et al. \(2007\)](#) found a relationship between increasing risk of pharyngeal cancer and increased pack-years of smoking in subjects with negative HPV16 serology but not in those with positive HPV16 serology (p value for interaction = 0.007).

In two case-control studies the risk increased with decreasing age at starting smoking ([Franceschi et al., 1990](#); [Choi & Kahyo, 1991](#)), but adjustment was not made for duration and intensity of smoking. In a case-control study from Spain ([Escribano Uzcudun et al., 2002](#)) the risk increased with the age of starting smoking.

Former smokers had consistently lower relative risks than did current smokers in both cohort ([McLaughlin et al., 1995](#); [Freedman et al., 2007a](#)) and case-control studies ([Choi & Kahyo, 1991](#); [De Stefani et al., 1998](#); [Vlajinac et al., 2006](#)). In comparison with non-smokers, the relative risks for former smokers who had quit smoking for more than 10 years were between 2 and 4 ([Franceschi et al., 1990](#); [De Stefani et al., 1998](#); [La Vecchia et al., 1999](#)), whereas the relative risks for current smokers in these studies were 10–14. In one study in Brazil ([Schlecht et al., 1999a](#)), relative risks for former smokers who had stopped smoking for more than 10 years approached 1, whereas that for current smokers was just below 6. Consumption of black tobacco, hand-rolled cigarettes or plain cigarettes resulted in a higher

risk for pharyngeal cancer than consumption of blond tobacco, manufactured cigarettes or filter-tipped cigarettes (De Stefani *et al.*, 1998; 2007).

### 2.3.3 Cancer of the nasal cavity and accessory sinuses

In the Life Span Study in Japan (Akiba, 1994) the association of tobacco use with sinonasal cancer was examined. A total of 26 cases of sino-nasal cancer were identified among 61505 adults during follow-up. Relative risk estimates, adjusted for sex, location, population group, atomic bomb exposure, year of birth and attained age, were 2.9 (95%CI: 0.5–) and 4.0 (95%CI: 1.2–) for former and current smokers, respectively, when compared with non-smokers [upper confidence limits were not reported]. The cohort of 34439 British doctors followed up to 50 years (Doll *et al.*, 2005) showed increased risk for current smokers and smokers of more than 25 cigarettes per day, but only six deaths from nasal cavity and sinuses cancers were observed (Table 2.15 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.15.pdf>).

A total of nine case-control studies of nasal cavity and sinus cancers have been conducted. When histological types were combined, all studies found an increased risk associated with cigarette smoking, but only one was statistically significant (Caplan *et al.*, 2000). In seven studies, dose-response in terms of intensity of smoking (cigarettes/day), duration of smoking or pack-years was considered. A positive significant trend was found in five studies (Brinton *et al.*, 1984; Hayes *et al.*, 1987; Fukuda & Shibata, 1990; Zheng *et al.*, 1993; Caplan *et al.*, 2000) and suggested in the other two (Strader *et al.*, 1988; Zheng *et al.*, 1992c).

One study (Zheng *et al.*, 1993a) found a significant decrease in risk for sinonasal cancer associated with increasing number of years since cessation of smoking. In a previous study, the

same authors had found a negative, non-significant association (Zheng *et al.*, 1992c).

Five studies analysed squamous-cell carcinomas and adenocarcinomas separately (Brinton *et al.*, 1984; Hayes *et al.*, 1987; Strader *et al.*, 1988; Zheng *et al.*, 1992c; 't Mannetje *et al.*, 1999). In all studies, there was a significantly increased risk for squamous-cell carcinomas, whereas the risk was generally not increased for adenocarcinomas.

### 2.3.4 Cancer of the nasopharynx

#### (a) Cohort studies

The risk for nasopharyngeal carcinoma has been examined in relation to tobacco use in six cohort studies, three of them reported since the last evaluation (IARC 2004a; Table 2.16 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.16.pdf>). In one study, conducted in a low-risk area (Chow *et al.*, 1993a), a significant increase in risk among smokers and suggestive positive dose-response relationships by duration of smoking and age at starting smoking were found. In another study, conducted in Province of Taiwan, China, an area in which nasopharyngeal cancer area is endemic, a similarly increased risk was found, but it was not statistically significant (Liaw & Chen, 1998). Doll *et al.* (2005) identified a risk only for smokers of more than 25 cigarettes per day, however, this result was based on only four deaths. Friberg *et al.* (2007) in Singapore found statistically significant increased risk of nasopharyngeal cancer only for those smoking for 40 years or more. Hsu *et al.* (2009) in Taiwan, China observed increased statistically significant risks only for those smoking for 30 years or more and those with cumulative exposure of 30 pack-years or more.

#### (b) Case-control studies

The study designs and the results of the case-control studies on the association of nasopharyngeal carcinoma with cigarette smoking



reported since the previous *IARC Monograph* (IARC, 2004a) are given in Table 2.17 (available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.17.pdf>) and Table 2.18 (available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.18.pdf>), one being a nested case–control analysis within a cohort study (Marsh *et al.*, 2007).

In total, 14 informative case–control studies were available. In almost all of these, the risk for nasopharyngeal carcinoma was higher in smokers than in non-smokers. In Taiwan, China (Cheng *et al.*, 1999) high risks were statistically significant only for duration of smoking of 20 years or more. In the five studies conducted in the USA (Mabuchi *et al.*, 1985; Nam *et al.*, 1992; Zhu *et al.*, 1995; Vaughan *et al.*, 1996; Marsh *et al.*, 2007), where the incidence of nasopharyngeal carcinoma is low, the relative risks for current smokers ranged between 2 and 4, but were not statistically significant in the two studies (Mabuchi *et al.*, 1985; Marsh *et al.*, 2007). In a study conducted in Shanghai, an area of China in which nasopharyngeal carcinoma is not endemic (Yuan *et al.*, 2000), the relative risk was just below 2. In one study from the Philippines there was a sevenfold increase in risk after more than 30 years of smoking (West *et al.*, 1993). The four studies (Lin *et al.*, 1973; Yu *et al.*, 1990; Ye *et al.*, 1995; Cao *et al.*, 2000) conducted in areas of China in which nasopharyngeal carcinoma is endemic (Taiwan, China, Guangzhou, and Sihui) found relative risks for ever smoking ranging between 2 and 5. In the study from the North of Africa (Feng *et al.*, 2009) the only statistically significant increased risk was found for differentiated nasopharyngeal cancer in those that had smoked more than 22 cigarettes/day. [The result, based only on three cases, is very unstable (RR, 313; 95%CI: 1.94–50336).]

A statistically significant dose–response relationship was detected in seven studies that evaluated the effects of daily or cumulative exposure to tobacco smoke (Yu *et al.*, 1990; Nam *et al.*, 1992;

Zhu *et al.*, 1995; Vaughan *et al.*, 1996; Cao *et al.*, 2000; Yuan *et al.*, 2000; Feng *et al.*, 2009) and was suggestive in two others (Lin *et al.*, 1973; West *et al.*, 1993). In two studies the risk of nasopharyngeal carcinoma decreased with increasing time since quitting smoking (Nam *et al.*, 1992; Vaughan *et al.*, 1996).

In the remaining studies, six from areas in which nasopharyngeal carcinoma is endemic (Ng, 1986; Yu *et al.*, 1986; Sriamporn *et al.*, 1992; Zheng *et al.*, 1994; Cheng *et al.*, 1999; Feng *et al.*, 2009; Guo *et al.*, 2009) and seven from areas in which it was not endemic (Henderson *et al.*, 1976; Lanier *et al.*, 1980; Mabuchi *et al.*, 1985; Ning *et al.*, 1990; Armstrong *et al.*, 2000; Marsh *et al.*, 2007), the relative risks for nasopharyngeal carcinoma for ever smoking were not significantly increased (Lanier *et al.*, 1980; Mabuchi *et al.*, 1985; Cheng *et al.*, 1999) or were close to 1.0 (Henderson *et al.*, 1976; Ng, 1986; Yu *et al.*, 1986; Ning *et al.*, 1990; Sriamporn *et al.*, 1992; Zheng *et al.*, 1994; Guo *et al.*, 2009).

In the two studies that distinguished between different histological types, relative risks were higher for keratinized (squamous-cell) carcinoma than for unkeratinized carcinoma (Zhu *et al.*, 1995; Vaughan *et al.*, 1996).

In the three studies in which men and women were analysed separately (Lin *et al.*, 1973; Nam *et al.*, 1992; Yuan *et al.*, 2000), the relative risks were found to increase similarly in both sexes in two studies (Nam *et al.*, 1992; Yuan *et al.*, 2000) and were higher among women in the study of Lin *et al.* (1973).

### 2.3.5 Cancer of the oesophagus

In the previous *IARC Monograph* (IARC, 2004a), both histological subtypes of oesophageal cancer (squamous-cell carcinoma and adenocarcinoma) were considered to be causally related to cigarette smoking. Many more epidemiological studies have since been conducted, and results of these studies further support this conclusion.

(a) *Squamous cell carcinoma and unspecified cancer of the oesophagus*

Since the previous *IARC Monograph* (IARC, 2004a), there have been reports on 9 cohort studies (Table 2.19 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.19.pdf>) and 22 case-control studies (Tables 2.20–2.23; see below), making 30 cohort and 55 case-control studies in all. All showed that the risk of oesophageal squamous cell carcinoma was associated with cigarette smoking. In one study (Li *et al.*, 1989), the elevated risk was observed only in an area with a relatively low incidence of oesophageal cancer. However, two later studies in the same area, Lin County, China, found a twofold increase in risk for oesophageal cancer among smokers (Gao *et al.*, 1994; Lu *et al.*, 2000).

In most cohort studies and in most case-control studies with relatively large sample sizes (IARC, 2004a; Table 2.19 online; Table 2.20 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.20.pdf>; Table 2.21 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.21.pdf>), the risk for oesophageal cancer was shown to increase with increasing duration of smoking (11 cohort and 32 case-control studies) or number of cigarettes smoked daily (18 cohort and 31 case-control studies), and to decrease with increasing age at starting smoking (12 case-control studies). In comparison with pharyngeal and laryngeal cancers, relative risks for oesophageal cancer estimated by duration and by intensity of smoking were somewhat lower (see Sections 2.3.2 and 2.3.6, respectively).

Ten cohort and 20 case-control studies (IARC, 2004a; Table 2.19 online; Table 2.22 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.22.pdf>) investigated the effect of smoking cessation on risk of oesophageal cancer. Although not all studies analysed the trend, all found a decreasing

relative risk with increasing number of years since quitting. In some studies, the risk first started to decrease after 10 years of cessation (Brown *et al.*, 1988; Rolón *et al.*, 1995; Gammon *et al.*, 1997; Castellsagué *et al.*, 1999; Freedman *et al.*, 2007b; Bosetti *et al.*, 2008) or after 30 years of cessation (Pandeya *et al.*, 2008).

When comparing the types of tobacco smoked (Table 2.23 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.23.pdf>), consumption of black tobacco resulted in a higher risk for oesophageal cancer than did consumption of blond tobacco (De Stefani *et al.*, 1990; Rolón *et al.*, 1995; Castellsagué *et al.*, 1999; Launoy *et al.*, 2000; Vioque *et al.*, 2008). Similarly, smoking untipped cigarettes generally resulted in a higher risk than smoking filter-tipped cigarettes (Vaughan *et al.*, 1995; Gammon *et al.*, 1997; Castellsagué *et al.*, 1999).

Two studies from the USA reported risks separately for blacks and whites. After adjustment for alcohol consumption, age and income, risks were very similar for former and current smokers and for the number of cigarettes smoked per day and duration of smoking (Brown *et al.*, 1994a; Brown *et al.*, 2001).

(b) *Adenocarcinoma of the oesophagus*

Two decades ago it was noted that incidence rates for adenocarcinoma of the oesophagus and gastric cardia had increased steadily in the USA, whereas the incidence rate for squamous-cell carcinoma of the oesophagus had remained relatively stable (Blot *et al.*, 1991). An increase in the incidence of adenocarcinoma of the distal oesophagus and cardia was also noted in the United Kingdom (Powell & McConkey, 1990), and in several other countries. Since 1990, several studies have focused on the risk factors for adenocarcinoma of the oesophagus. Since the last evaluation (IARC, 2004a) one cohort study (Freedman *et al.*, 2007b) and three case-control studies (Table 2.24 available at <http://monographs.iarc.fr/ENG/Monographs/>

[vol100E/100E-01-Table2.24.pdf](#); Table 2.25 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.25.pdf>) have been reported, totaling 13 case-control studies on the association of cigarette smoking and adenocarcinoma of the oesophagus.

(i) *Intensity and duration of smoking*

Ten studies, three that included only cases of adenocarcinoma of the oesophagus ([Menke-Pluymers et al., 1993](#); [Gammon et al., 1997](#); [Wu et al., 2001](#)), three that included cases of adenocarcinoma of the oesophagus, gastro-oesophageal junction and gastric cardia combined ([Kabat et al., 1993](#); [Brown et al., 1994b](#); [Vaughan et al., 1995](#)), and four that stratified by histology ([Lindblad et al., 2005](#); [Freedman et al., 2007b](#); [Hashibe et al., 2007a](#); [Pandeya et al., 2008](#)), showed a significant positive association of adenocarcinoma of the oesophagus with cigarette smoking. The relative risks were somewhat lower than those for squamous cell carcinoma of the oesophagus. Three studies, one in China ([Gao et al., 1994](#)), one in Sweden ([Lagergren et al., 2000](#)), and one in the USA ([Zhang et al., 1996](#)), reported similarly elevated relative risks, but some of these risks were not statistically significant, probably because of relatively small numbers of cases.

Of those studies that reported risks adjusted for alcohol consumption, a positive, significant dose-response relationship was found with intensity of smoking ([Kabat et al., 1993](#); [Brown et al., 1994b](#); [Gammon et al., 1997](#); [Hashibe et al., 2007a](#)), duration of smoking ([Gammon et al., 1997](#); [Pandeya et al., 2008](#)) and/or pack-years ([Vaughan et al., 1995](#); [Zhang et al., 1996](#); [Gammon et al., 1997](#); [Pandeya et al., 2008](#)).

(ii) *Cessation of smoking*

Ten studies provided point estimates for former smokers. In eight, relative risks were lower in former smokers than in current smokers, although they remained elevated ([Kabat et al.,](#)

[1993](#); [Gao et al., 1994](#); [Vaughan et al., 1995](#); [Gammon et al., 1997](#); [Wu et al., 2001](#); [Lindblad et al., 2005](#); [Freedman et al., 2007b](#); [Pandeya et al., 2008](#)), and were increased in the other studies ([Lagergren et al., 2000](#); [Hashibe et al., 2007a](#)). The decrease in relative risk associated with years since cessation was weak, but a significant trend was found in two out of six studies ([Gammon et al., 1997](#); [Wu et al., 2001](#)).

(iii) *Confounding*

With the exception of two studies ([Levi et al., 1990](#); [Wu et al., 2001](#)), all studies adjusted for alcohol intake as a potential confounder. Three more recent studies also adjusted for fruit and vegetables intake ([Freedman et al., 2007b](#); [Hashibe et al., 2007a](#); [Pandeya et al., 2008](#)). Ten of these studies were conducted in the USA ([Kabat et al., 1993](#); [Brown et al., 1994b](#); [Vaughan et al., 1995](#); [Zhang et al., 1996](#); [Gammon et al., 1997](#); [Freedman et al., 2007b](#)) the Netherlands ([Menke-Pluymers et al., 1993](#)), the United Kingdom ([Lindblad et al., 2005](#)), central and eastern Europe ([Hashibe et al., 2007a](#)) and Australia ([Pandeya et al., 2008](#)), where chewing of betel quid with tobacco or use of other forms of smokeless tobacco are not likely confounders. One study conducted in Sweden was adjusted for snuff use ([Lagergren et al., 2000](#)).

(iv) *Sex*

[Kabat et al. \(1993\)](#) examined risks for men and women separately and observed similar patterns in both sexes, although risks among current smokers and heavy smokers were somewhat higher for women than for men. [Lindblad et al. \(2005\)](#) also found higher risks in women than in men, but they were not statistically significant.

### 2.3.6 Cancer of the larynx

Laryngeal cancer is one of the cancers most strongly associated with cigarette smoking ([IARC, 1986, 2004a](#)). Since the previous IARC

*Monograph*, more epidemiological evidence has become available to strengthen this conclusion.

#### (a) Potential confounders

Other causes of laryngeal cancer include alcohol consumption, some occupational exposures (e.g. sulphuric acid; [IARC, 2012a](#)) and possibly some dietary habits. In investigating associations between smoking and laryngeal cancer, potential confounding by alcohol consumption has been considered in most of the studies.

#### (b) Intensity and duration of smoking

Cohort and case-control studies have been carried out in Asia, Europe, North and South America, and South Africa. In all, the risk for laryngeal cancer was consistently higher in smokers, and a positive significant trend was observed with increasing duration and intensity of smoking ([IARC, 2004a](#); Table 2.26 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.26.pdf>; Table 2.27 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.27.pdf>; Table 2.28 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.28.pdf>).

In most case-control studies, the relative risks for laryngeal cancer were near to or greater than 10 for smokers who had smoked for longer than 40 years ([Falk et al., 1989](#); [Zheng et al., 1992b](#)) or had smoked more than 20 cigarettes per day ([Tuyns et al., 1988](#); [Falk et al., 1989](#); [Choi & Kahyo, 1991](#); [Zatonski et al., 1991](#); [Muscat & Wynder, 1992](#); [Zheng et al., 1992b](#); [Hedberg et al., 1994](#); [Sokić et al., 1994](#); [Talamini et al., 2002](#)). Cancer of the larynx in non-smokers is so rare that several studies used as the reference category light smokers ([Herity et al., 1982](#); [Olsen et al., 1985a](#); [De Stefani et al., 1987](#); [Zatonski et al., 1991](#); [López-Abente et al., 1992](#); [Maier & Tisch, 1997](#)), or former smokers ([Hashibe et al., 2007b](#)). Consequently, relative risks were lower

in these studies, although the increases were still statistically significant.

Three case-control studies reported odds ratios for cancer of the larynx that increased with decreasing age of starting smoking ([Franceschi et al., 1990](#); [Zatonski et al., 1991](#); [Talamini et al., 2002](#)).

#### (c) Cessation of smoking

The risk for cancer of the larynx declines rather rapidly after cessation of smoking ([IARC, 2004a](#); Table 2.29 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.29.pdf>). No detectable higher risk compared with never-smokers was seen among subjects who had quit smoking for at least 10 years ([Franceschi et al., 1990](#); [Ahrens et al., 1991](#); [Schlecht et al., 1999a, b](#); [Bosetti et al., 2006](#); [Hashibe et al., 2007b](#)).

#### (d) Types of tobacco or of cigarette

Some investigators considered the role of type of tobacco ([IARC, 2004a](#); Table 2.30 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.30.pdf>). An average 2.5-fold higher risk was observed in smokers of black tobacco compared to smokers of blond tobacco ([De Stefani et al., 1987](#); [Tuyns et al., 1988](#); [López-Abente et al., 1992](#)). Smoking untipped cigarettes also led to a higher risk than smoking filter-tipped cigarettes ([Wynder & Stellman, 1979](#); [Tuyns et al., 1988](#); [Falk et al., 1989](#)). Those that smoke cigarettes only had higher risks of larynx cancer than those that smoke cigars only ([Hashibe et al., 2007b](#)).

#### (e) Subsites

Six studies investigated the risk for glottic and supraglottic cancer separately ([Olsen et al., 1985a](#); [Tuyns et al., 1988](#); [López-Abente et al., 1992](#); [Maier et al., 1992b](#); [Muscat & Wynder, 1992](#); [Sapkota et al., 2007](#)). The cancer risk increased with increasing amount smoked per



day and with cumulative exposure for both subsites (IARC, 2004a; Table 2.28 online). In addition, the observed relative risks were higher for supraglottic cancer than for glottic cancer (Maier *et al.*, 1992b; Sapkota *et al.*, 2007).

#### (f) Sex

Few studies investigated sex-specific effects. In one cohort study (Raitiola & Pukander, 1997) similar risks were found for men and women, whereas in two case-control studies (Zheng *et al.*, 1992b; Tavani *et al.*, 1994), the relative risks for women were up to 10-fold higher than for the corresponding categories in men, though a small number of cases were involved. However, Freedman *et al.* (2007a) observed higher relative risks in men than women (Table 2.26 online). One study looked at women only and found higher risks of laryngeal cancer in former and current smokers relative to non-smokers, and also according to the number of cigarettes per day with a clear dose-response effect ( $P < 0.001$ ) (Gallus *et al.*, 2003b).

### 2.3.7 Cancer of the upper aerodigestive tract combined

In epidemiological studies, especially in cohort studies in which there are few cases at some sites, investigators often combine cancers of the oral cavity, pharynx, larynx and oesophagus and term these ‘cancer of the upper aerodigestive tract’. This section summarizes the data from 19 cohort studies (IARC, 2004a; Table 2.31 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.31.pdf>), and 40 case-control studies (IARC, 2004a; Tables 2.32–2.35; see below).

#### (a) Intensity and duration of smoking

In all but two cohort studies from Japan (Kono *et al.*, 1987; Akiba, 1994), the risk for cancer of the upper aerodigestive tract was strongly associated with cigarette smoking. Relative risks increased

with increasing daily cigarette consumption (Hammond & Horn, 1958; Doll *et al.*, 1980, 1994; Akiba & Hirayama, 1990; Kuller *et al.*, 1991; Chyou *et al.*, 1995; Engeland *et al.*, 1996; Murata *et al.*, 1996; Yuan *et al.*, 1996; Kjaerheim *et al.*, 1998; Liaw & Chen, 1998; Yun *et al.*, 2005; Freedman *et al.*, 2007a), duration of smoking (Chyou *et al.*, 1995; Yun *et al.*, 2005; Friberg *et al.*, 2007) or pack-years (Liaw & Chen, 1998; Freedman *et al.*, 2007a).

The main characteristics and results of the case-control studies are presented in IARC (2004a), and in Table 2.32 (available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.32.pdf>) and Table 2.33 (available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.33.pdf>), respectively. Intensity of smoking was measured in most of these studies. The link between duration of smoking and cancer of the upper aerodigestive tract was examined in 20 case-control studies (Blot *et al.*, 1988; Merletti *et al.*, 1989; Barra *et al.*, 1991; De Stefani *et al.*, 1992, 2007; Franceschi *et al.*, 1992; Day *et al.*, 1993; Mashberg *et al.*, 1993; Kabat *et al.*, 1994; Lewin *et al.*, 1998; Bosetti *et al.*, 2000a; Garrote *et al.*, 2001; Gallus *et al.*, 2003a; Lissowska *et al.*, 2003; Znaor *et al.*, 2003; Castellsagué *et al.*, 2004; Menvielle *et al.*, 2004a, b; Rodriguez *et al.*, 2004; Hashibe *et al.*, 2007c; Sapkota *et al.*, 2007). Nine also considered age at starting smoking (Blot *et al.*, 1988; Merletti *et al.*, 1989; Barra *et al.*, 1991; Franceschi *et al.*, 1992; Day *et al.*, 1993; Lewin *et al.*, 1998; Garrote *et al.*, 2001; Lissowska *et al.*, 2003; Menvielle *et al.*, 2004a).

In all but one study (Rao *et al.*, 1999) there was an increased risk for cancer of the upper aerodigestive tract associated with cigarette smoking. A clear dose-response relationship was seen with increasing daily tobacco consumption and duration of smoking as well as with decreasing age at starting smoking in most of the studies examined.

*(b) Cessation of smoking*

Twelve cohort studies ([Doll et al., 1980, 1994](#); [Tomita et al., 1991](#); [Akiba, 1994](#); [Chyou et al., 1995](#); [Engeland et al., 1996](#); [Nordlund et al., 1997](#); [Kjaerheim et al., 1998](#); [Yun et al., 2005](#); [Freedman et al., 2007a](#); [Friborg et al., 2007](#); [Ide et al., 2008](#)) provided point estimates for former smokers ([IARC 2004a](#); Table 2.31 online). The relative risks for former smokers were always lower than those for current smokers.

In 16 case-control studies the relative risk by years since quitting was examined and generally a statistically significant negative trend was found (Table 2.34 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.34.pdf>).

*(c) Types of cigarette*

The characteristics studied in several case-control studies included the use of a filter, the type of tobacco, the tar content and whether the product was manufactured or hand-rolled ([IARC, 2004a](#); Table 2.35 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.35.pdf>). Consumption of black tobacco, cigars, untipped cigarettes, hand-rolled cigarettes, or cigarettes with a high-tar yield generally resulted in a higher risk than consumption of blond tobacco ([Merletti et al., 1989](#); [Castellsagué et al., 2004](#); [De Stefani et al., 2007](#)), filter-tipped cigarettes ([Merletti et al., 1989](#); [Mashberg et al., 1993](#); [Kabat et al., 1994](#); [Lissowska et al., 2003](#); [De Stefani et al., 2007](#)), manufactured cigarettes ([De Stefani et al., 1992, 2007](#)) or low-tar cigarettes ([Franceschi et al., 1992](#)). Two studies from India ([Znaor et al., 2003](#); [Sapkota et al., 2007](#)) revealed higher risks of *bidi* smoking related to cigarettes smoking.

*(d) Sex*

Sex-specific effects were analysed in four cohort studies ([IARC 2004a](#); Table 2.31 online). In three cohort studies ([Hammond & Seidman,](#)

[1980](#); [Akiba & Hirayama, 1990](#); [Freedman et al., 2007a](#)) a higher relative risk was found for male smokers than for female smokers; however, [Ide et al. \(2008\)](#) detected a higher risk among women in a study with a small number of cases.

In three case-control studies ([Blot et al., 1988](#); [Kabat et al., 1994](#); [Muscat et al., 1996](#)) the relative risks were higher for women than for men in all categories of intensity of smoking (number of cigarettes per day), cumulative exposure (cumulative tar consumption, pack-years, duration of smoking) and age at starting smoking, as well as for former smokers. However, the trends in men were always in the same direction and of the same order of magnitude. An exception to the pattern was that in one study ([Merletti et al., 1989](#)) the relative risk for smoking filter-tipped cigarettes was higher than that for smoking untipped cigarettes for women.

Overall, the strength of association by sex was generally similar, especially when taking into account the fact that women generally under-report levels of smoking and that most studies included many fewer women than men.

*(e) Ethnicity*

Relative risks were reported separately for blacks and whites in a large case-control study from the USA ([Day et al., 1993](#)). Relative risks adjusted for alcohol consumption, sex and other relevant variables were very similar for the number of cigarettes smoked per day, years of cigarette smoking, age at starting smoking and number of years since stopping smoking.

## 2.4 Cancer of the stomach

### 2.4.1 Overview of studies

In the previous *IARC Monograph* ([IARC, 2004a](#)) it was concluded that there was *sufficient evidence* that tobacco smoking causes cancer of the stomach. Three meta-analyses have since examined the evidence for gastric cancer in 42

independent cohort studies published between 1958 and July 2007 ([Ladeiras-Lopes et al., 2008](#)), in 46 case–control studies published between 1997 and June 2006 ([La Torre et al., 2009](#)), and in 10 cohort and 16 case–control studies conducted in Japanese populations published between 1966 and March 2005 ([Nishino et al., 2006](#); Table 2.36 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.36.pdf>). For current smokers compared to never smokers, the risk for stomach cancer was found to be statistically significantly increased by 53% ([Ladeiras-Lopes et al., 2008](#)), 56% ([Nishino et al., 2006](#)), and 57% when considering high quality case–control studies ([La Torre et al., 2009](#)), with moderate to high heterogeneity.

Since the previous *IARC Monograph* ([IARC, 2004a](#)), the association between cigarette smoking and stomach cancer risk (15 studies) and mortality (4 studies) has been examined in 19 cohort studies (Table 2.37 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.37.pdf>). Eleven of these were conducted in Asia ([Sasazuki et al., 2002](#); [Jee et al., 2004](#); [Koizumi et al., 2004](#); [Wen et al., 2004](#); [Fujino et al., 2005](#); [Sauvaget et al., 2005](#); [Tran et al., 2005](#); [Kurosawa et al., 2006](#); [Kim et al., 2007](#); [Sung et al., 2007](#); [Shikata et al., 2008](#)), seven in Europe ([Simán et al., 2001](#); [González et al., 2003](#); [Doll et al., 2005](#); [Lindblad et al., 2005](#); [Sjödahl et al., 2007](#); [Batty et al., 2008](#); [Zendehdel et al., 2008](#)) and one in the USA ([Freedman et al., 2007a](#)). Only the updated British Doctors' study ([Doll et al., 2005](#)) and the most recent studies ([Shikata et al., 2008](#); [Zendehdel et al., 2008](#)) were not included in the meta-analysis of cohort studies ([Ladeiras-Lopes et al., 2008](#)). Elevated risks in current smokers were found in all studies. The reported association of current smoking with mortality in the four cohort studies conducted in Taiwan, China ([Wen et al., 2004](#)), Japan ([Kurosawa et al., 2006](#)) and the United Kingdom ([Doll et al., 2005](#); [Batty et al., 2008](#)) was comparable to that with incidence.

In addition, the association between smoking and stomach cancer risk has been reported in 37 case–control studies since the previous *IARC Monograph*, of which 22 are hospital-based and 15 population-based. With the exception of three studies ([Campos et al., 2006](#); [García-González et al., 2007](#); [Suwanrungruang et al., 2008](#); Table 2.38 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.38.pdf>), all these studies were included in the meta-analysis conducted by ([La Torre et al., 2009](#)).

## 2.4.2 Factors affecting risk

### (a) Intensity and duration

Clear evidence has been provided by the meta-analyses as well as by the additional cohort studies that the risk for stomach cancer increases significantly with increasing daily cigarette consumption, duration or pack-years of smoking, although individual studies did not always find statistically significant dose–response relationships. In one meta-analysis based on 21 cohort studies, the risk for stomach cancer increased statistically significantly by 53% with consumption of approximately 20 cigarettes per day ([Ladeiras-Lopes et al., 2008](#)). Using trend estimation analysis as proposed by [Greenland & Longnecker \(1992\)](#), the authors found an increase in relative risk from 1.3 for the lowest consumption to 1.7 for smoking 30 cigarettes per day.

### (b) Cessation of smoking

Risk for stomach cancer has been generally found to be lower in former smokers than in current smokers. In six of the cohort studies decreasing risk with increasing years since stopping smoking was found although none found statistically significant dose–response relationships ([González et al., 2003](#); [Koizumi et al., 2004](#); [Sauvaget et al., 2005](#); [Freedman et al., 2007a](#); [Kim et al., 2007](#); [Zendehdel et al., 2008](#)). Risk in former smokers was comparable to never smokers after quitting for 5 years ([Kim et al.,](#)

2007), 10 years ([González et al., 2003](#); [Sauvaget et al., 2005](#); [Freedman et al., 2007a](#)) or 15 years ([Koizumi et al., 2004](#)).

### 2.4.3 Subsites

The effect of current smoking on the risk for stomach cancer by subsite was assessed in ten cohort studies. Elevated risks were found for both cardia and non-cardia cancers. In six studies higher risks were found for cancer of the gastric cardia than for cancer of the distal stomach ([Simán et al. 2001](#); [González et al., 2003](#); [Freedman et al., 2007a](#); [Sung et al., 2007](#); [Shikata et al., 2008](#); [Zendehdel et al., 2008](#)), three studies found no difference ([Sasazuki et al., 2002](#); [Lindblad et al., 2005](#); [Tran et al., 2005](#)), and in one study higher risk for cancer in the antrum rather than the body or the cardia was found ([Koizumi et al., 2004](#)). A meta-analysis yielded statistically significant summary relative risks of 1.87 for cardia cancers and 1.60 for non-cardia cancers based on nine cohort studies ([Ladeiras-Lopes et al., 2008](#)). However, there was substantial heterogeneity across studies for cardia cancers. For case–controls studies, the corresponding odds ratios were 2.05 (95%CI: 1.50–2.81) and 2.04 (95%CI: 1.66–2.50), respectively, with greater heterogeneity for non-cardia cancers. Criteria for the classification by subsite were not always described ([Simán et al., 2001](#); [Koizumi et al., 2004](#); [Lindblad et al., 2005](#); [Tran et al., 2005](#)) and some studies included tumours located in the upper third of the stomach in the group of cardia cancer ([Sasazuki et al., 2002](#); [Sung et al., 2007](#); [Shikata et al. 2008](#)).

In three studies risk estimates for smoking associated stomach cancer were estimated by histological type ([Sasazuki et al., 2002](#); [Koizumi et al., 2004](#); [Shikata et al., 2008](#)). The relative risks were 2.1 (95%CI: 1.2–3.6), 1.6 (95%CI: 1.1–2.3) and 2.3 (95%CI: 1.3–4.1) for the differentiated type, respectively, and 0.6 (95%CI: 0.3–1.1), 2.1

(95%CI: 1.1–4.1), and 1.3 (95%CI: 0.5–3.5) for the non-differentiated type, respectively.

### 2.4.4 Population characteristics

In four of the additional cohort studies risk was reported separately for men and women ([González et al., 2003](#); [Jee et al., 2004](#); [Fujino et al., 2005](#); [Kim et al., 2007](#)), in three studies only for men ([Koizumi et al., 2004](#); [Tran et al., 2005](#); [Sung et al., 2007](#)) and in one mortality study for men as well as for women ([Wen et al., 2004](#)). Generally, the relative risks were smaller in women than in men. For all stomach cancers, risk in current smokers compared to never smokers was found to be significantly increased by 62% in men (based on 18 studies) and by 20% in women (based on nine studies) in the meta-analysis of cohort studies ([Ladeiras-Lopes et al., 2008](#)). The men–women differences were independent of exposure level but could be explained by the sex difference in the distribution by histological type and other factors associated with socioeconomic status.

Ethnicity does not appear to modify the effect of smoking on stomach cancer risk. In the meta-analysis of case–control studies risk in current smokers was increased by 78% in Caucasians and by 48% in Asians ([La Torre et al., 2009](#)). The summary risk based on the cohort studies increased by 46% and 47% in Caucasian and Asian studies, respectively. In a meta-regression analysis including the variables sex, population, and fruit and vegetable consumption, sex but not origin of the population showed significant differences in risk estimates ([Ladeiras-Lopes et al., 2008](#)).

### 2.4.5 Bias and confounding

Generally, most cohort studies have relied on baseline information and did not update the exposure information, possibly leading to misclassification of smoking status. Most of



the recent cohort studies have accounted for confounding by alcohol consumption ([Fujino et al., 2005](#); [Lindblad et al., 2005](#); [Sjödahl et al., 2007](#); [Sung et al., 2007](#)) as well as fruit and vegetable consumption ([González et al., 2003](#); [Koizumi et al., 2004](#); [Freedman et al., 2007a](#)) and still observed significantly increased risk of stomach cancer in current smokers.

#### 2.4.6 *Helicobacter pylori* infection

The association between tobacco smoking and stomach cancer could be confounded or modified by the effect of *H. pylori* infection, an established risk factor for stomach cancer. In three case-control studies ([Zaridze et al., 2000](#); [Brenner et al., 2002](#); [Wu et al., 2003](#)), and two cohort studies ([Simán et al., 2001](#); [Shikata et al., 2008](#)) the joint effects and possible interaction between *H. pylori* status and smoking in relation to risk for stomach cancer was investigated. Among subjects who had *H. pylori* infection, the risk for stomach cancer was higher in current smokers than in non-smokers by 1.6 to 2.7 fold, providing evidence for a causal effect of tobacco smoking independently of *H. pylori* infection. Smoking was associated with risk elevations of the same order of magnitude among subjects without *H. pylori* infection. Smoking and *H. pylori* therefore may act synergistically, leading to very high risks in current smokers with *H. pylori* infection compared to non-smokers without *H. pylori* infection. In one study that examined risk by subsite an effect of smoking independent of *H. pylori* infection for gastric cardia as well as distal gastric cancer was found ([Wu et al., 2003](#)). In none of the studies was there statistically significant evidence for interaction.

## 2.5 Cancer of the pancreas

### 2.5.1 Overview of studies

Previous *IARC Monographs* ([IARC, 1986, 2004a](#)) concluded that exposure to tobacco smoke caused cancer of the pancreas. Additional evidence has come from a pooled analysis of eight cohort studies with almost 1500 incident cases of pancreatic cancer and an equal number of controls ([Lynch et al., 2009](#)) as well as a meta-analysis of 82 independent studies (42 case-control studies, 40 cohort studies) published between 1950 and 2007 ([Iodice et al., 2008](#); Table 2.39 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.39.pdf>). In the meta-analysis 74% and 20% significant increased risks for current and former smokers, respectively, were found with significant heterogeneity of effect regarding current smoking across studies. Adjustment for confounders explained some of the heterogeneity ([Iodice et al., 2008](#)). A similar significant risk elevation of 77% for current smokers was found in the pooled analysis, without study heterogeneity ([Lynch et al., 2009](#)). For former smokers, risk was increased non-significantly by 9%.

Since the previous *IARC Monograph* ([IARC, 2004a](#)), a total of 15 cohort studies have reported on the association between cigarette smoking and pancreatic cancer incidence (8 studies) and mortality (5 studies) or both (one study) (Table 2.40 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.40.pdf>), two of which were included in the pooled analysis ([Coughlin et al., 2000](#); [Vrieling et al., 2009](#)). Excluding case-control studies that did not report odds ratios for current smokers, there were three additional case-control studies ([Duell et al., 2002](#); [Inoue et al., 2003](#); [Alguacil & Silverman, 2004](#); Table 2.41 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.41.pdf>). The effect of cigar and pipe smoking on pancreatic cancer was

also examined in the ACS Cancer Prevention Study II regarding mortality ([Shapiro et al., 2000](#); [Henley et al., 2004](#)) and in the Kaiser Permanente Medical Care Program regarding incidence ([Iribarren et al., 1999](#)). All the additional studies showed an increased risk for pancreatic cancer associated with tobacco smoking, generally higher in current than in former smokers. The reported risk estimates were not always statistically significant, predominantly due to the small size of some studies and therefore lack of statistical precision.

### 2.5.2 Factors affecting risks

#### (a) Intensity and duration

Clear evidence has been provided by the meta-analysis, the pooled analysis as well as the additional studies that the risk for cancer of the pancreas increases significantly with increasing daily cigarette consumption, duration and pack-years of smoking ([Coughlin et al., 2000](#); [Gapstur et al., 2000](#); [Nilsen & Vatten, 2000](#); [Nilsson et al., 2001](#); [Isaksson et al., 2002](#); [Doll et al., 2005](#); [Yun et al., 2005](#); [Ansary-Moghaddam et al., 2006](#); [Gallicchio et al., 2006](#); [Vrieling et al., 2009](#)). In the meta-analysis risk of pancreatic cancer increased significantly by 62% with an increase of 20 cigarettes per day (based on 45 studies) and by 16% with a 10-year increase in smoking duration (based on 16 studies), but with significant study heterogeneity. In the pooled analysis, the excess odds ratio per pack-years generally declined with increasing smoking intensity ([Lynch et al., 2009](#)).

#### (b) Cessation of smoking

A reduction in risk in former smokers who had stopped smoking for at least 10 years was found in the meta-analysis ([Iodice et al., 2008](#)) and the pooled study ([Lynch et al., 2009](#)). In some cohort studies risk was already comparable to never smokers five years after quitting ([Boyle et al., 1996](#); [Fuchs et al., 1996](#); [Nilsen & Vatten, 2000](#); [Vrieling et al., 2009](#)).

#### (c) Types of tobacco

In non-cigarette smokers, mortality from pancreatic cancer was increased although not statistically significantly so in cigar smokers in the CPS-II cohort study ([Shapiro et al., 2000](#)) as well as a large case-control study ([Alguacil & Silverman, 2004](#)) but was less clearly elevated in the smaller Kaiser Permanente cohort study ([Iribarren et al., 1999](#)). There was a significantly increased mortality for current cigar smokers who reported inhaling cigar smoke ([Shapiro et al., 2000](#)). Pipe smoking was also found to be associated with an increased risk of cancer of the pancreas, which was stronger in those who reported that they inhaled the smoke ([Henley et al., 2004](#)). A limitation of the cohort studies is that smoking habits were reported only at baseline, misclassification of smoking exposure is likely to underestimate the associated risks. In the meta-analysis there was a significant increase in risk of 47% associated with current cigar and/or pipe smoking (18 studies) and a non-significant risk elevation of 29% with former cigar and/or pipe smoking (5 studies) ([Iodice et al., 2008](#)).

### 2.5.3 Population characteristics

The effect of sex on pancreatic cancer risk was investigated in two cohort studies ([Nilsen & Vatten, 2000](#); [Larsson et al., 2005](#)) and on pancreatic cancer mortality in four cohort studies ([Coughlin et al., 2000](#); [Gapstur et al., 2000](#); [Nilsson et al., 2001](#); [Lin et al., 2002a](#)). The relative risks were comparable between men and women and no consistent evidence for an effect modification by sex was observed.

Ethnicity does not appear to modify the association of smoking with pancreatic cancer risk. The roughly twofold elevated risk in current smokers compared to never smokers was observed both in studies of Caucasians ([Lynch et al., 2009](#)) and of Asians ([Lin et al., 2002a](#); [Jee et al., 2004](#); [Yun et al., 2005](#); [Li et al., 2006](#)). In populations of the Asia-Pacific Region, there

was also no difference in the strength of association between Asia and Australia/New Zealand ([Ansary-Moghaddam et al., 2006](#)).

#### 2.5.4 Confounding factors

In two large cohort studies the risk estimates for pancreatic cancer associated with cigarette smoking were not substantially influenced by adjustment for further potential confounding factors, including diabetes, body mass index (BMI), alcohol and dietary intake ([Coughlin et al., 2000](#); [Vrieling et al., 2009](#)).

## 2.6 Cancer of the colorectum

### 2.6.1 Overview of studies

In the previous *IARC Monograph* ([IARC, 2004a](#)) it was not possible to conclude that the association between tobacco smoking and colorectal cancer is casual, principally because of concern about confounding by other risk factors. That evaluation was based on a total of 60 epidemiologic studies, although only few were specifically designed to study the effects of smoking. Studies have however shown consistently that cigarette smoking is a risk factor for colorectal adenomatous polyps, which are recognized precursor lesions of colorectal cancer ([Hill, 1978](#)). To explain this discrepancy, [Giovannucci et al. \(1994\)](#) hypothesized that a long induction period is required for tobacco to play a role in colorectal carcinogenesis, which would not be captured by studies with shorter follow-up time.

Four recent meta-analyses consistently showed a strong association between cigarette smoking and colorectal cancer ([Botteri et al., 2008a](#); [Liang et al., 2009](#); [Huxley et al., 2009](#); [Tsoi et al., 2009](#)).

### 2.6.2 Cohort studies

Since the previous *IARC Monograph* ([IARC, 2004a](#)), 22 additional cohort studies have investigated the association between tobacco smoke and colorectal cancer (Table 2.42 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.42.pdf>). [Studies that did not provide point estimates of risk ([Andersen et al., 2009](#); [Hansen et al., 2009](#); [Murphy et al., 2009](#)) and included prevalent colorectal cancer in patients with other diagnosis ([Chan et al., 2007](#)) are excluded from this review]. Seven of the studies were conducted in Europe, nine in Asia and five in the USA. In eleven studies, risk estimates were reported solely for colorectal cancer ([Tiemersma et al., 2002a](#); [Limburg et al., 2003](#); [Otani et al., 2003](#); [Colangelo et al., 2004](#); [Sanjoaquin et al., 2004](#); [Lüchtenborg et al., 2005a](#); [Kim et al., 2006](#); [Akhter et al., 2007](#); [Huxley, 2007a](#); [Kenfield et al., 2008](#); [Hannan et al., 2009](#)), five studies separately for colon cancer and rectal cancer ([Shimizu et al., 2003](#); [Wakai et al., 2003](#); [Jee et al., 2004](#); [Yun et al., 2005](#); [Batty et al., 2008](#)) and five studies both for colorectal cancer as well as for colon and rectal cancers ([Terry et al., 2002a](#); [van der Hel et al., 2003a](#); [Doll et al., 2005](#); [Paskett et al., 2007](#); [Tsong et al., 2007](#); [Gram et al., 2009](#)). Six studies were restricted to women ([Terry et al., 2002a](#); [Limburg et al., 2003](#); [van der Hel et al., 2003a](#); [Paskett et al., 2007](#); [Kenfield et al., 2008](#); [Gram et al., 2009](#)), and two studies to men ([Doll et al., 2005](#); [Yun et al., 2005](#); [Akhter et al., 2007](#)). One study reported both colorectal incidence and mortality ([Limburg et al., 2003](#)) and three studies only reported colorectal cancer mortality ([Doll et al., 2005](#); [Huxley, 2007a](#); [Batty et al., 2008](#); [Kenfield et al., 2008](#)).

#### (a) Smoking status

Virtually all studies reported elevated risk associated with smoking, although results were not always statistically significant. The largest meta-analysis based on 36 prospective studies

with data from a total of 3007002 subjects found that compared to never smokers, current smokers had a 15% significantly higher risk of developing colorectal cancer and 27% significantly higher risk of colorectal cancer mortality (Liang *et al.*, 2009; Table 2.43 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.43.pdf>). In former smokers, colorectal cancer risk was also significantly elevated by 20% whereas colorectal cancer mortality was non-significantly increased by 20%. The risk estimates were not significantly different between colon and rectal cancer for current smokers (RR, 1.10 versus 1.19) and for former smokers (RR, 1.10 versus 1.20). There was no heterogeneity among colorectal cancer studies and no evidence for publication bias. Comparable risk elevations in current and former smokers were found in the other meta-analyses. For current smokers, the risk for colorectal cancer increased significantly by 16% when using data from 22 cohort studies (Huxley *et al.*, 2009), by 20% based on 28 cohort studies (Tsoi *et al.*, 2009), and by 7% based on data from 45 cohort and case-control studies (Botteri *et al.*, 2008a). In the latter meta-analysis a 17% significantly higher risk of colorectal cancer in former smokers was found.

#### (b) Intensity of smoking

All but three of the recent 21 cohort studies (van der Hel *et al.*, 2003a; Jee *et al.*, 2004; Sanjoaquin *et al.*, 2004) investigated dose-response relationships, using at least one of number of cigarettes smoked, duration of smoking, pack-years of smoking, age at smoking initiation, time since smoking cessation. In two further studies (Tiemersma *et al.*, 2002a; Batty *et al.*, 2008) these parameters were examined separately in current and former smokers, as by Chao *et al.* (2000). Statistically significant dose-response trends with amount smoked daily were reported for colorectal cancer (Lüchtenborg *et al.*, 2005a; Akhter *et al.*, 2007; Paskett *et al.*, 2007; Kenfield *et al.*, 2008), for colon cancer (Paskett

*et al.*, 2007), and for rectal cancer (Paskett *et al.*, 2007; Tsong *et al.*, 2007). The dose-response of daily cigarette consumption and colorectal cancer was assessed in two meta-analyses (Liang *et al.*, 2009; Tsoi *et al.*, 2009) and both found statistically significant relationships. Based on eleven studies, Liang *et al.* (2009) found that risk for colorectal cancer increased significantly by 17% with an increase of 20 cigarettes/day and by 38% with an increase of 40 cigarettes/day, while colorectal cancer mortality increased by 41% and 98%, respectively (Table 2.43 online). The risk elevation associated with an increase of 20 cigarettes/day was greater for rectal than for colon cancer (13% versus 3%) but this difference was not statistically significant.

#### (c) Duration of smoking

In addition to two previously reported studies (Hsing *et al.*, 1998; Chao *et al.*, 2000), thirteen studies have examined duration of smoking and colorectal cancer risk. A statistically significant trend of increasing risk with increasing duration was found for colorectal (Limburg *et al.*, 2003; Kim *et al.*, 2006; Paskett *et al.*, 2007; Gram *et al.*, 2009), for colon cancer (Paskett *et al.*, 2007) and for rectal cancer (Terry *et al.*, 2002a; Paskett *et al.*, 2007; Tsong *et al.*, 2007). In one study, increasing duration of smoking was significantly associated with risk for colorectal cancer solely in former smokers (Tiemersma *et al.*, 2002a). Based on eight studies (Terry *et al.*, 2002a; Tiemersma *et al.*, 2002a; Limburg *et al.*, 2003; Lüchtenborg *et al.*, 2005a; Kim *et al.*, 2006; Akhter *et al.*, 2007; Paskett *et al.*, 2007; Tsong *et al.*, 2007), a meta-analysis for duration of smoking and colorectal cancer incidence yielded highly significant results (Liang *et al.*, 2009). Risk was increased by 9.4% with a 20-year increase in smoking duration and 19.7% with a 40-year increase. Smoking duration was also significantly associated with risk for rectal cancer but not for colon cancer. In another meta-analysis where dose-response relationship was modelled, a nonlinear increase in risk with



increasing duration was observed ([Botteri et al., 2008a](#)). The risk started to increase after approximately 10 years of smoking and reached statistical significance after 30 years.

#### (d) *Pack-years*

Since the previous *IARC Monograph*, the association of colorectal cancer with pack-years of cigarette smoking has been evaluated in six studies ([Limburg et al., 2003](#); [Otani et al., 2003](#); [Shimizu et al., 2003](#); [Wakai et al., 2003](#); [Kim et al., 2006](#); [Gram et al., 2009](#)). In addition to the previously reported significant results ([Giovannucci et al., 1994](#); [Heineman et al., 1994](#); [Chao et al., 2000](#); [Stürmer et al., 2000](#)), a statistically significant trend of increasing risk with increasing pack-years was found for colorectal cancer in two studies ([Limburg et al., 2003](#); [Gram et al., 2009](#)), and for colon cancer in one study ([Gram et al., 2009](#)). In their dose-response analysis of pack-years and colorectal incidence, [Liang et al. \(2009\)](#) included five studies ([Giovannucci et al., 1994](#); [Stürmer et al., 2000](#); [Limburg et al., 2003](#); [Otani et al., 2003](#); [Kim et al., 2006](#)) and found a statistically significant trend of increasing risk with increasing pack-years of smoking for colorectal cancer but not specifically for colon or rectal cancer. Risk for colorectal cancer increased by 27% for an increase of 35 pack-years and by 50% for an increase of 60 pack-years.

#### (e) *Age at initiation*

In nine of the cohort studies the age at smoking initiation in relation to colorectal cancer (eight studies) or colon and rectal cancer (four studies) was investigated. In four studies a statistically significant trend of increasing risk with decreasing age at initiation of smoking for colorectal cancer was found ([Limburg et al., 2003](#); [Kim et al., 2006](#); [Akhter et al., 2007](#); [Gram et al., 2009](#)) and for colon cancer ([Gram et al., 2009](#)) and rectal cancer ([Tsong et al., 2007](#)). In one meta-analysis ([Liang et al., 2009](#)), a highly significant association was found for age at

smoking initiation and colorectal cancer incidence based on six studies ([Limburg et al., 2003](#); [Kim et al., 2006](#); [Akhter et al., 2007](#); [Paskett et al., 2007](#); [Tsong et al., 2007](#); [Gram et al., 2009](#)). Risk for colorectal cancer was reduced by 2.2% for a 5-year delay in smoking initiation and by 4.4% for a 10-year delay.

#### (f) *Smoking cessation*

The effect of smoking cessation by years since stopping was assessed in seven studies, six for colorectal cancer ([Tiemersma et al., 2002a](#); [Lüchtenborg et al., 2005a, 2007](#); [Paskett et al., 2007](#); [Kenfield et al., 2008](#); [Gram et al., 2009](#); [Hannan et al., 2009](#)) and three for colon and/or rectal cancer ([Wakai et al., 2003](#); [Paskett et al., 2007](#); [Gram et al., 2009](#)). In one study a statistically significant trend in risk reduction with years since quitting was found both overall as well as separately for men and for women ([Hannan et al., 2009](#)).

#### (g) *Population characteristics*

It has been suggested that the association between smoking and colorectal cancer may be stronger in men than in women. In the three recent cohort studies reporting sex-specific results ([Shimizu et al., 2003](#); [Wakai et al., 2003](#); [Colangelo et al., 2004](#)), this was only observed in studies in Japan ([Shimizu et al., 2003](#); [Wakai et al., 2003](#)), but could be attributed to the very low prevalence of smoking in women. The studies restricted to women have generally shown associations with cigarette smoking that were of comparable magnitude to those observed in men ([Terry et al., 2002a](#); [Limburg et al., 2003](#); [van der Hel et al., 2003a](#); [Paskett et al., 2007](#); [Kenfield et al., 2008](#); [Gram et al., 2009](#)).

Recent studies have been carried out either in Europe and in USA, with predominantly Caucasian study subjects, or in Asia, mostly in Japan and in the Republic of Korea. The results from these studies suggest no differences in the association between tobacco smoking and

colorectal cancer between different ethnic groups.

#### (h) *Subsites*

Smoking and risks for colon cancer and for rectal cancer were investigated in eleven of the 21 additional studies. Risk patterns are generally consistent between colon and rectal cancer ([Otani et al., 2003](#); [van der Hel et al., 2003a](#); [Wakai et al., 2003](#); [Jee et al., 2004](#); [Yun et al., 2005](#); [Batty et al., 2008](#)). In some studies, dose-response relationships were stronger for rectal cancer than for colon cancer ([Terry et al., 2002a](#); [Paskett et al., 2007](#)) or were statistically significant only for rectal cancer ([Shimizu et al., 2003](#); [Doll et al., 2005](#); [Tsong et al., 2007](#)). In a meta-analysis ([Liang et al., 2009](#)) the association was stronger for rectal cancer than for colon cancer in the subset of cohort studies that differentiated cancer by site. Most dose-response variables were not associated with colon cancer incidence whereas associations were stronger for rectal cancer incidence and statistically significant with longer duration of smoking, albeit based only on a small number of studies. In one cohort study the increased risk associated with smoking was more apparent for proximal than for distal colon cancer ([Lüchtenborg et al., 2005a](#)), which was not found in an earlier study ([Heineman et al., 1994](#)).

#### (i) *Confounding and effect modification*

Smokers have been shown to be more likely than non-smokers to be physically inactive, to use alcohol, to have lower consumption of fruits and vegetables and higher consumption of fat and meat, and they are less likely to be screened for colorectal cancer ([Le Marchand et al., 1997](#); [Ghadirian et al., 1998](#); [Nkondjock & Ghadirian, 2004](#); [Reid et al., 2006b](#); [Mutch et al., 2009](#)).

Few potential confounders were considered in the cohort studies evaluated in the previous IARC Monograph ([IARC, 2004a](#)). Of the cohort studies published since, all except three ([van der Hel et al., 2003a](#); [Jee et al., 2004](#); [Doll et al., 2005](#))

considered two or more potential confounders. In eleven of the recent studies adjustments were made for physical activity, alcohol consumption, overweight/obesity ([Terry et al., 2002a](#); [Limburg et al., 2003](#); [Otani et al., 2003](#); [Wakai et al., 2003](#); [Yun et al., 2005](#); [Akhter et al., 2007](#); [Ashktorab et al., 2007](#); [Paskett et al., 2007](#); [Tsong et al., 2007](#); [Kenfield et al., 2008](#); [Hannan et al., 2009](#)), and seven also adjusted for dietary habits (e.g. intake of fruits and vegetables, dietary fibres, fat, red meat). Among the studies with the latter adjustments, eight ([Giovannucci et al., 1994](#); [Chao et al., 2000](#); [Stürmer et al., 2000](#); [Limburg et al., 2003](#); [Yun et al., 2005](#); [Akhter et al., 2007](#); [Paskett et al., 2007](#); [Hannan et al., 2009](#)) found significant dose-response relationships with at least one of the smoking variables. In two studies a significant association of smoking with colorectal cancer risk was observed after accounting for history of colonoscopy ([Paskett et al., 2007](#); [Hannan et al., 2009](#)). Risk factors in multivariable analyses in several studies were level of education, use of menopausal hormone therapy, family history and regular aspirin use. The association between smoking and colorectal cancer was not modified by these other characteristics, or by alcohol consumption in two studies ([Otani et al., 2003](#); [Tsong et al., 2007](#)). Therefore, confounding factors do not seem to affect the observed significant increase in risk for colorectal cancer associated with tobacco smoking and the dose-response relationships with smoking variables.

When considering other types of smoking, it is generally found that cigar and pipe smoking are less associated with socioeconomic class and other life-style habits than cigarette smoking. Therefore, it is logical to assume that, for these types of smoking, risk associations derived from epidemiologic studies may be less prone to potential confounding. In all the cohort studies reviewed in the previous IARC Monograph ([IARC, 2004a](#)) an elevated, though not always statistically significant, risk was consistently reported for cancers of the colon and the rectum

associated with exclusive pipe and/or cigar smoking.

Infection with JC virus has been proposed as a potential risk factor for colon cancer ([Rollison et al., 2009](#)) but results still need further validation.

Three cohort studies assessed possible modifying effects by genetic susceptibility. Rapid acetylator phenotype (as determined by polymorphisms of the *NAT2* gene involved in metabolism of heterocyclic aromatic amines) was found to increase the risk for colorectal cancer in smokers, in one ([van der Hel et al., 2003a](#)) but not in another study ([Tiemersma et al., 2002a](#)). For genes involved in the metabolism of polycyclic aromatic hydrocarbons such as *GSTM1* or *GSTT1*, no statistical contribution to the risk of colorectal cancer associated with smoking was observed ([Tiemersma et al., 2002a](#); [Lüchtenborg et al., 2005a](#)).

### 2.6.3 Case-control studies

Thirty-one case-control studies were included in the previous *IARC Monograph* ([IARC, 2004a](#)). Although results were inconsistent with respect to risk association in ever versus former and current smokers, a dose-response relationship with smoking variables was found in some studies. Since then, seventeen case-control studies investigating the association between tobacco smoke and colorectal cancer risk have been published, seven carried out in Asia, four in Europe, five in North America and one in Hawaii (Table 2.44 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.44.pdf>). Six studies reported solely for colorectal cancer ([Ateş et al., 2005](#); [Chia et al., 2006](#); [Verla-Tebit et al., 2006](#); [Lüchtenborg et al., 2007](#); [Steinmetz et al., 2007](#); [Wu et al., 2009b](#)), four separately for colon and rectal cancer ([Ji et al., 2002](#); [Sharpe et al., 2002](#); [Minami & Tateno, 2003](#); [Goy et al., 2008](#)), two for colorectal cancer as well as for colon and rectal cancer ([Ho et al., 2004](#); [Gao](#)

[et al., 2007](#); [Wei et al., 2009](#)), three for colon cancer only ([Diergaarde et al., 2003](#); [Kim et al., 2003](#); [Hu et al., 2007](#)) and one for rectal cancer only ([Slattery et al., 2003](#)). Nine of the studies reported risk estimates separately for men and for women.

#### (a) Smoking status

Most case-control studies considered the effects of current and former smoking separately. A positive association between smoking and colorectal cancer was found in virtually all the studies, although the results were generally not statistically significant. Statistically significant increased risk was reported in current smokers for colorectal cancer ([Chia et al., 2006](#); [Wu et al., 2009b](#)), for rectal cancer ([Slattery et al., 2003](#); [Ho et al., 2004](#)), and in former smokers for colorectal cancer both in men and women combined ([Chia et al., 2006](#)) and in women only ([Lüchtenborg et al., 2007](#)). Five studies, which did not focus on the main effects of smoking, only evaluated risks for ever smoking ([Diergaarde et al., 2003](#); [Kim et al., 2003](#); [Ateş et al., 2005](#); [Gao et al., 2007](#); [Hu et al., 2007](#)); none of these reported significant risk estimates.

#### (b) Intensity of smoking

Nine case-control studies investigated dose-response relationships considering at least one smoking variable. Number of cigarettes smoked daily was evaluated in seven studies, three for colorectal cancer ([Verla-Tebit et al., 2006](#); [Lüchtenborg et al., 2007](#); [Wu et al., 2009b](#)), two for colon and rectal cancer ([Ji et al., 2002](#); [Minami & Tateno, 2003](#)), one for rectal cancer ([Slattery et al., 2003](#)) and one for colorectal cancer and both subsites ([Ho et al., 2004](#)). Statistically significant positive trends of increasing risk with increasing number of cigarettes smoked daily were found for colorectal cancer in only one study ([Wu et al., 2009b](#)).

(c) *Duration of smoking, pack-years, age at initiation, smoking cessation*

Duration of smoking was examined in several studies in relation to colorectal cancer ([Ho et al., 2004](#); [Chia et al., 2006](#); [Verla-Tebit et al., 2006](#); [Lüchtenborg et al., 2007](#); [Wu et al., 2009b](#)) and/or to colorectal cancer by subsite ([Ji et al., 2002](#); [Minami & Tateno, 2003](#); [Ho et al., 2004](#)). A statistically significant trend with increasing number of years smoked was found in two of the five studies of colorectal cancer ([Chia et al., 2006](#); [Wu et al., 2009b](#)). In one study, increasing duration of smoking was significantly associated with risk for rectal cancer in ever smokers but not in current smokers ([Ho et al., 2004](#)). In only one earlier case-control study was a significant association in ever smokers with increasing number of years of smoking for colon as well as rectal cancer found ([Newcomb et al., 1995](#)).

Duration of smoking exposure was assessed by pack-years of smoking in seven studies ([Ji et al., 2002](#); [Slattery et al., 2003](#); [Chia et al., 2006](#); [Verla-Tebit et al., 2006](#); [Lüchtenborg et al., 2007](#); [Goy et al., 2008](#); [Wu et al., 2009b](#)) and by age at smoking initiation in three studies ([Ji et al., 2002](#); [Slattery et al., 2003](#); [Wu et al., 2009b](#)). All four studies that evaluated pack-years of smoking with respect to colorectal cancer risk found statistically significant associations. Two studies found a significant association with increasing pack-years in men and women combined; when investigated separately, the increasing trend was statistically significant only in women ([Verla-Tebit et al., 2006](#)) or only in men ([Wu et al., 2009b](#)). In one study a statistically significant trend with pack-years of smoking in both men and women was found only with non-filtered cigarettes ([Lüchtenborg et al., 2007](#)); the relative risk was significant for colon as well as rectal cancer and was greater for rectal cancer.

In two studies a non-significant trend of decreasing risk with increasing time since

stopped smoking was found ([Verla-Tebit et al., 2006](#); [Lüchtenborg et al., 2007](#)).

(d) *Subsites and molecular subtypes*

A stronger association between tobacco smoking and rectal cancer compared with colon cancer has generally been observed in the studies that reported risk estimates by cancer site. In a recent meta-analysis including both cohort and case-control studies, higher smoking-related risk estimates for rectal cancer were found than for proximal and distal colon cancer ([Botteri et al., 2008a](#)). Stronger relative risk in ever smokers, but not in current smokers, was found for proximal compared to distal tumours in one recent study ([Hu et al., 2007](#)).

Colorectal cancer is a multipathway disease. A molecular approach to its classification utilizes: (1) the type of genetic instability, specifically microsatellite instability, and (2) the presence of DNA methylation or the CpG island methylator phenotype (CIMP) ([Jass, 2007](#)). Smoking has been associated with microsatellite instability in sporadic colon cancer. Higher risk for microsatellite-unstable than for microsatellite-stable tumours was found in four studies ([Slattery et al., 2000](#); [Yang et al., 2000](#); [Chia et al., 2006](#); [Campbell et al., 2009](#)). The observed twofold risk elevation for colorectal cancer showing microsatellite instability is similar in order of magnitude to that found for colorectal polyps. In only one small study similar risk estimates for stable and unstable tumours were found ([Diergaarde et al., 2003](#)). Microsatellite instability is characteristic of hereditary nonpolyposis colorectal cancer syndrome and smoking has been associated with colorectal cancer in patients with this syndrome ([Watson et al., 2004](#); [Diergaarde et al., 2007](#)). Among sporadic colorectal tumours with microsatellite instability, about 11–28% carry somatic genetic mutations. In addition, the association of colon cancer with smoking was increased two to threefold when widespread CIMP and/or *BRAF* mutation, irrespective of microsatellite instability



status, was present ([Samowitz et al., 2006](#)). These data indicate that the association with MSI-high tumours may be attributed to the association of smoking with CIMP and *BRAF* mutation.

#### (e) Effect modification

Effect modification by genetic polymorphisms in enzymes metabolizing tobacco smoke constituents could provide further evidence for a causal association between smoking and colorectal cancer. Most studies that have investigated modification of colorectal cancer risk associated with smoking by genetic polymorphisms of xenobiotic enzymes were too small to be informative ([Inoue et al., 2000](#); [Smits et al., 2003](#); [Jin et al., 2005](#); [Tranah et al., 2005](#); [van den Donk et al., 2005](#); [Tijhuis et al., 2008](#)). Studies on the possible differential effect by acetylation status have reported stronger association of tobacco smoking (in terms of pack-years) with colorectal cancer risk in slow acetylators phenotypes ([Lilla et al., 2006](#)), and with rectal cancer in rapid acetylators phenotypes ([Curtin et al., 2009](#)). Furthermore, *CYP1A1* and *GSTM1* variant alleles were found to greatly affect colon cancer or rectal cancer risk in smokers ([Slattery et al., 2004](#)).

### 2.6.4 Colorectal polyps

Colorectal adenomas and possibly some hyperplastic polyps are considered precursors of colorectal cancer. The epidemiologic evidence on the relationship between cigarette smoking and colorectal polyps has been generally consistent. Since the previous *IARC Monograph* ([IARC, 2004a](#)), twelve further independent studies have investigated this association (Table 2.45 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.45.pdf>). All studies found a significantly increased risk for polyps in association with one or more smoking variables. A recent meta-analysis including 42 studies reported a statistically significant positive association between smoking and colorectal adenomas

([Botteri et al., 2008b](#)). The meta-analysis, which included several studies that did not explicitly report relative risks for tobacco smoking ([Cardoso et al., 2002](#); [Voskuil et al., 2002](#); [Sparks et al., 2004](#); [Gong et al., 2005](#); [Jiang et al., 2005](#); [Kim et al., 2005](#); [Mitrou et al., 2006](#); [Otani et al., 2006](#); [Skjelbred et al., 2006](#)), found a twofold risk elevation for colorectal adenomas in current smokers and a 50% increase in former smokers. The association had been previously found to be equally strong in men and women. In one of two recent studies, there was no difference in the results for men and women separately ([Tranah et al., 2004](#)) but significantly greater effects in women were found in the other ([Hermann et al., 2009](#)).

Significant positive trends with number of cigarettes per day were found in four ([Ji et al., 2006](#); [Larsen et al., 2006](#); [Stern et al., 2006](#); [Shrubsole et al., 2008](#)) of five studies ([Tiemersma et al., 2004](#)). Dose-response with duration of smoking was assessed in four studies ([Ji et al., 2002](#); [Tiemersma et al., 2004](#); [Stern et al., 2006](#); [Shrubsole et al., 2008](#)) and with pack-years of smoking in five studies ([Hoshiyama et al., 2000](#); [Ulrich et al., 2001](#); [Tranah et al., 2004](#); [Ji et al., 2006](#); [Shrubsole et al., 2008](#); [Omata et al., 2009](#)). All nine studies found statistically significant trends, which were consistent with those for adenomas and hyperplastic polyps when reported separately ([Ulrich et al., 2001](#); [Ji et al., 2006](#); [Shrubsole et al., 2008](#)). Ever smokers were estimated to have a 13% (95%CI: 9–18%) increasing risk of presenting with adenomatous polyps for every additional 10 pack-years smoked in comparison to never smokers, based on data from 19 studies ([Botteri et al., 2008b](#)).

Decreasing risks with years since quitting smoking were found in four studies ([Ulrich et al., 2001](#); [Tiemersma et al., 2004](#); [Ji et al., 2006](#); [Shrubsole et al., 2008](#)), statistically significant so in the latter three studies. In comparison to never smokers, former smokers retained moderately elevated risk for colorectal polyps even 20

years after quitting smoking. One study examined both dose metrics (cigarettes per day, duration, and pack-years) and recency of tobacco use: in subjects who had quit smoking for at least 20 years, only the heaviest users of tobacco still had modest excess risks ([Ji et al., 2006](#)).

It has been proposed that the association between cigarette smoking and polyps may be stronger with non-progressing adenomas, such as those that are smaller and less villous but the hypothesis is not supported in most studies ([Anderson et al., 2003](#); [Toyomura et al., 2004](#); [Ji et al., 2006](#); [Skjelbred et al., 2006](#)). In one study a clearly higher risk for large and multiple adenomas in every anatomic site of the colon was found in a dose-response manner ([Toyomura et al., 2004](#)). A meta-analysis found that the combined risk estimate for high-risk adenomas associated with smoking was greater than that for low-risk adenomas and that the difference was statistically significant for current smokers but not former smokers ([Botteri et al., 2008b](#)). In addition, a stronger association of smoking with hyperplastic polyps than with adenomas was found in some studies ([Ulrich et al., 2001](#); [Ji et al., 2006](#); [Shrubsole et al., 2008](#)) but not in another ([Erhardt et al., 2002](#)). The risk associated with smoking may be even higher in subjects presenting with concurrent benign hyperplastic and adenomatous polyps ([Ji et al., 2006](#); [Shrubsole et al., 2008](#)).

Relative risk estimates for tobacco smoking and polyps generally range between 2 and 3 whereas those for colorectal cancer range between 1.2 and 1.4. One possible explanation is the effect dilution due to the inclusion of a high proportion of individuals with precursor lesions in the unscreened control groups in most colorectal cancer studies ([Terry & Neugut, 1998](#)). Some indirect evidence for this hypothesis is provided by the meta-analysis of colorectal adenomas, which showed that the smoking-associated risk for adenomas was significantly higher in studies including subjects who had undergone complete

colonoscopy in comparison to those in which some or all controls had undergone incomplete examination (i.e. only sigmoidoscopy) ([Abrams et al., 2008](#); [Botteri et al., 2008b](#)).

It is also possible that smoking is associated with a subset of colorectal cancers so that relative risk estimates for colorectal cancer as a whole are diluted. The pattern of risk observed for colorectal cancer by microsatellite instability status and for type of colorectal polyps suggests that the traditional (non-serrated) adenoma-carcinoma sequence may proceed through a hyperplastic polyps-mixed polyps-serrated adenoma progression and that smoking may be more strongly related to the development of these subtypes ([Jass et al., 2000](#); [Hawkins & Ward, 2001](#)). More recently, a *BRAF* mutation was shown to be a specific marker for the serrated polyp neoplasia pathway originating from a hyperplastic polyp, in which the CIMP-high develops early and the microsatellite instability carcinoma develops late ([O'Brien et al., 2006](#)). The findings of strong associations between smoking and colon cancer with CIMP and/or *BRAF* mutation, irrespective of microsatellite status, are compatible with this observation ([Samowitz et al., 2006](#)).

## 2.7 Hepatocellular carcinoma

### 2.7.1 Overview of studies

In the previous *IARC Monograph* ([IARC, 2004a](#)), a causal relationship between liver cancer (hepatocellular carcinoma) and smoking was established. Two case-control and one cohort studies have been published since (Table 2.46 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.46.pdf>). Overall, most cohort studies and the largest case-control studies, most notably those that included community controls, showed a moderate association between tobacco smoking and risk for hepatocellular carcinoma.

Confounding from alcohol has been addressed in the best studies. The association between alcohol drinking and hepatocellular carcinoma is strong, and alcohol intake is frequently misclassified, leading to potential residual confounding. However an association with smoking has been demonstrated also among non-drinkers.

A meta-analysis was based on 38 cohort studies and 58 case-control studies ([Lee et al., 2009](#)). Compared to never smokers, the meta-relative risks adjusted for appropriate confounders were 1.51 (95%CI: 1.37–1.67) for current smokers and 1.12 (0.78–1.60) for former smokers. The increased liver cancer risk among current smokers appeared to be consistent in strata of different regions, study designs, study sample sizes, and publication periods. The association with smoking was observed in non-alcohol-drinkers (RR, 1.34; 95%CI: 0.92–1.94 in men and 1.31; 95%CI: 0.70–2.44 in women). Further supportive evidence is provided by the association between smoking and liver cancer observed among Chinese women and Japanese women, in whom alcohol drinking is extremely rare ([Li et al., 2011](#)). One difficulty is that sometimes studies do not specify the histology of liver cancer (hepatocellular versus intra-hepatic biliary tract).

In the update of the Whitehall study ([Batty et al., 2008](#)) (a cohort of 17363 government employees in London, followed-up for 38 years), the hazard ratio for death from liver cancer was 1.03 (0.49–2.16) in former smokers and 1.43 (0.69–2.95) in current smokers (based on 57 deaths). In the 50-year follow-up of the British doctors cohort ([Doll et al., 2005](#)), there were 74 deaths from liver cancer. Death rates per 100000 per year were 4.4 in never smokers, 10.7 in smokers of 1–14 cigarettes/day, 2.6 in smokers of 15–24 cigarettes/day, and 31.3 in smokers of  $\geq 25$  cigarettes/day.

## 2.7.2 Factors affecting risks

### (a) Dose-response relationship

Most studies, including the recent ones (Table 2.46 online), show a dose-response relationship with the number of cigarettes smoked and with smoking duration, with exceptions such as [Franceschi et al. \(2006\)](#) and some older studies from Asia. Relative risk estimates increased to 2.0 after 20 years of smoking.

### (b) Cessation

Though former smokers tend to have lower relative risks than current smokers, there were no consistent patterns of risks after cessation, including in the recent studies (Table 2.46 online).

## 2.7.3 Interaction with hepatitis B or C

Infection with hepatitis B virus (HBV) is one of the major causes of liver cancer worldwide, whereas hepatitis C virus (HCV) infection causes a large fraction of liver cancer in Japan, Northern Africa and southern Europe. While many studies, most notably from Asia, have found no attenuation of the association between smoking and liver cancer after adjustment/stratification for markers of HBV or HCV infection, an apparent interaction between smoking and HBV or HCV infection has been reported. The increase in risk for liver cancer associated with cigarette smoking appears to be greater among HBV carriers than among uninfected persons in some studies ([Tu et al., 1985](#)), but not in others ([Kuper et al., 2000a](#)). Two recent reports ([Franceschi et al., 2006](#); [Hassan et al., 2008a](#)) studied possible interactions between smoking and hepatitis virus infection and both reported an apparent interaction between smoking and hepatitis C infection. Interactions between smoking and hepatitis B infection were not found among men in one study ([Hassan et al., 2008a](#)) and the rarity of HBsAg prevented the evaluation of HBV and smoking in the other ([Franceschi et al., 2006](#);

Table 2.46 online). In the meta-analysis by [Lee et al. \(2009\)](#) adjustment for HBV reduced the relative risks in both men and women, while adjustment for HCV did not change the risk in women and increased it in men.

## 2.8 Renal cell carcinoma

### 2.8.1 Overview of studies

The previous *IARC Monograph* ([IARC, 2004a](#)) concluded that renal-cell carcinoma is associated with tobacco smoking in both men and women. Four case-control studies and no cohort studies have become available since then (Table 2.47 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.47.pdf>). Overall these confirm the previous evidence, though with some conflicting results. In particular, both the study by [Hu et al. \(2005\)](#) in Canada and the multicentre European study by [Brennan et al. \(2008\)](#) do not show a clear effect of smoking. In contrast, the study by [Theis et al. \(2008\)](#) shows an increased risk with smoking duration (irregular, levelling-off after 40 years) and a statistically significant dose-response relationship with pack-years.

In the update of the Whitehall study ([Batty et al., 2008](#)) (a cohort of 17363 government employees in London, followed for 38 years), the hazard ratio for deaths from kidney cancer was 0.64 (0.32–1.26) for former smokers, and 1.29 (0.69–2.41) for current smokers (based on 68 deaths). In the 50-year follow-up of the British doctor cohort ([Doll et al., 2005](#)) there were 140 deaths from kidney cancer. Mortality rates per 100000 per year were 9.3 in never smokers, 16.4 in smokers of 1–14 cigarettes/day, 16.6 in smokers of 15–24 cigarettes/day, and 15.5 in smokers of  $\geq 25$  cigarettes/day (age-adjusted).

[Hunt et al. \(2005\)](#) performed a meta-analysis based on 19 case-control studies and 5 cohort studies (total 8032 cases in case-control and 1326 in cohort studies). The relative risk for smoking

men was 1.54 (1.42–1.68), and for smoking women was 1.22 (1.09–1.36). A dose-response relationship was found in both men and women. The association observed was more convincing in population-based compared to hospital-based studies.

### 2.8.2 Confounding

Hypertension is a well established risk factor for kidney cancer but the association with smoking is only indirect. Potential confounding from hypertension was considered only by [Brennan et al. \(2008\)](#).

Other potential confounders such as BMI have been appropriately addressed in most studies.

### 2.8.3 Cessation

Most studies reviewed in the previous *Monograph* showed a lower risk for former smokers compared to current smokers, with a significant negative trend with increasing number of years since quitting ([IARC, 2004a](#)). In case-control study on smoking cessation and renal-cell carcinoma, the decrease in risk became significant only after 30 years of quitting ([Parker et al., 2003](#)). In the meta-analysis ([Hunt et al., 2005](#)), former smokers were at reduced risk after 10 years or more of quitting. A clear decline in risk after cessation was also reported by [Theis et al. \(2008\)](#). [The Working Group noted the poor quality of the study, considering the low response rate among controls.]



## 2.9 Cancer of the lower urinary tract (including cancer of the bladder, ureter, and renal pelvis)

### 2.9.1 Overview of studies

The previous *IARC Monograph* ([IARC, 2004a](#)) clearly identified a causal relationship of smoking with transitional-cell carcinomas and squamous-cell carcinomas of the bladder, ureter and renal pelvis both in men and women. Two new case-control studies ([Cao et al., 2005](#); [Samanic et al., 2006](#); Table 2.48 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.48.pdf>) and two cohort studies ([Bjerregaard et al., 2006](#); [Alberg et al., 2007](#); Table 2.49 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.49.pdf>) have been reported since then in addition to updates of cohort studies with longer follow-up.

In the update of the Whitehall study ([Batty et al., 2008](#)) (a cohort of 17363 government employees in London, followed-up for 38 years), the hazard ratio for death from bladder cancer was 0.98 (0.62–1.54) in former smokers and 1.66 (1.06–2.59) in current smokers (based on 164 deaths). In the 50-year follow-up of the British doctors cohort ([Doll et al., 2005](#)), there were 220 deaths from bladder cancer. Death rates per 100000 per year were 13.7 in never smokers, 37.7 in smokers of 1–14 cigarettes/day, 31.8 in smokers of 15–24 cigarettes/day, and 51.4 in smokers of  $\geq 25$  cigarettes/day. All the new studies confirm the existence of a dose-response relationship with the number of cigarettes smoked and with duration, and a decline in relative risk with time since quitting smoking, compared to non-quitters.

### 2.9.2 Types of tobacco

The risk of lower urinary tract cancer was more strongly associated with smoking air-cured (black) tobacco than smoking flue-cured

(blond) tobacco in several studies ([IARC, 2004a](#)). The stronger association with air-cured (black) than blond tobacco among current smokers has not been clearly confirmed in a re-analysis of the Spanish multicentre case-control study ([Samanic et al., 2006](#)). Relative risks in current smokers were 7.3 (4.9–10.9) in black tobacco smokers and 5.8 (3.4–10.0) in blond tobacco smokers; in former smokers, 4.2 (2.9–6.0) for black tobacco and 1.8 (1.0–3.2) for blond tobacco (Table 2.48 online). The effect of cessation was more pronounced in blond tobacco smokers than in black tobacco smokers, suggesting potentially different mechanisms of action of the two types of tobacco. Air-cured (black) tobacco is richer in arylamines.

### 2.9.3 Gene-environment interactions

A large number of studies have considered gene-environment interactions between tobacco smoking and genetic polymorphisms, including DNA repair genes ([Vineis et al., 2009](#)) and genes involved in carcinogen metabolism ([Malats, 2008](#); [Dong et al., 2008](#)). Overall, there is evidence that the slow acetylator variant of the *NAT2* gene is involved in bladder carcinogenesis and may interact with smoking. The meta-relative risk for *NAT2* slow acetylator and bladder cancer was 1.46 (95%CI: 1.26–1.68;  $P = 2.5 \times 10^{-7}$ ), based on 36 studies and 5747 cases ([Dong et al., 2008](#)). Similar but weaker evidence has been provided for *GSTM1* ([Malats, 2008](#)).

The extent of interaction between *NAT2* variants and smoking is still unclear. In one study the *NAT2* acetylation status was found to modulate the association of bladder cancer and cigarette smoking through smoking intensity and not smoking duration ([Lubin et al., 2007](#)). Studies are not consistent concerning the three-way association between smoking intensity, *NAT2* and bladder cancer. Some studies found greater effects at a lower level of exposure and others the opposite ([Malats, 2008](#)). Genome-wide

association studies have indicated 8q24 as a region that may confer high risk for bladder cancer ([Kiemenev et al., 2008](#)).

## 2.10 Myeloid leukaemia (acute and chronic)

Myeloid leukaemia in adults was observed to be causally related to cigarette smoking in the previous *IARC Monograph* ([IARC, 2004a](#)). Risk increased with amount of tobacco smoked in a substantial number of adequate studies, with evidence of a dose–response relationship. Biological plausibility for a causal relationship of smoking with myeloid leukaemia is provided by the finding of known leukaemogens in tobacco smoke, one of which (benzene) is present in relatively large amounts. No evidence was found for an association with acute lymphocytic leukaemia.

One recently published cohort study included information on acute and chronic myeloid leukaemias ([Fernberg et al., 2007](#)), based on 372 incident cases. A weak association was found between acute myeloid leukaemia and intensity of smoking, and a statistically significant association with current smoking (RR, 1.5; 95%CI: 1.06–2.11). No association was found with chronic myeloid leukaemia.

In the update of the Whitehall study ([Batty et al., 2008](#)) (a cohort of 17363 government employees in London, followed-up for 38 years), the hazard ratio for mortality from myeloid leukaemias (acute plus chronic) was 5.08 (95%CI: 1.78–14.5) for current smokers, and 3.84 (95%CI: 1.35–11.0) for former smokers (based on 66 deaths). In the 50-year follow-up of the British doctors cohort ([Doll et al., 2005](#)), there were 100 deaths from myeloid leukaemias. The mortality rates per 100000 per year were 6.3 in never smokers, 2.8 in smokers of 1–14 cigarettes/day, 14.0 in smokers of 15–24, and 18.3 in smokers of  $\geq 25$  cigarettes/day (age-adjusted).

## 2.11 Other leukaemias and lymphomas

### 2.11.1 Non-Hodgkin lymphoma

Six cohort studies have been published on the association between non-Hodgkin lymphoma and smoking, all reviewed in the previous *IARC Monograph* ([IARC, 2004a](#)). In five of these, no increased risk among smokers was evident ([Doll et al., 1994](#); [McLaughlin et al., 1995](#); [Adami et al., 1998](#); [Herrinton & Friedman, 1998](#); [Parker et al., 2000](#)). However, in one study, men who had ever smoked cigarettes had a twofold increase in risk for non-Hodgkin lymphoma, and the risk was still higher among the heaviest smokers ([Linnet et al., 1992](#)). Data from case–control studies generally also fail to support an effect of smoking on the incidence of non-Hodgkin lymphoma ([Peach & Barnett, 2001](#); [Stagnaro et al., 2001](#); [Schöllkopf et al., 2005](#); [Bracci & Holly, 2005](#); Table 2.50 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.50.pdf>). Reanalysis of data of an Italian study ([Stagnaro et al., 2004](#)) found a statistically significant association (OR, 1.4; 95%CI: 1.1–1.7) for blond tobacco exposure and non-Hodgkin lymphoma risk.

Three studies and a pooled analysis have examined histological subtypes of non-Hodgkin lymphoma. In one cohort study in women, smoking was associated with increased risk for follicular non-Hodgkin lymphoma ([Parker et al., 2000](#)). Similarly, two other studies reported a weak positive association between smoking and risk for follicular lymphoma, but no effect for other histological types ([Herrinton & Friedman, 1998](#); [Stagnaro et al., 2001](#)). A large pooled analysis based on nine North-American and European case–control studies found an overall odds ratio of 1.07 (95%CI: 1.0–1.15) for smokers; the association was particularly strong for follicular lymphoma (OR, 1.31; 95%CI: 1.12–1.52) ([Morton et al., 2005](#)).

### 2.11.2 Hodgkin lymphoma

In the previous *IARC Monograph* ([IARC, 2004a](#)) seven studies on the association between Hodgkin lymphoma and smoking were examined and null or weakly positive associations were noted. Among studies published since, a positive association was observed in two case-control ([Willett et al., 2007](#); [Kanda et al., 2009](#)) and three cohort studies ([Nieters et al., 2006](#); [Lim et al., 2007](#); [Nieters et al., 2008](#)), while one study found no clear association ([Monnereau et al., 2008](#)). Several other recent studies also reported a positive association, but with some internal inconsistencies. In a European multicentre case-control study, no association was observed between tobacco and Hodgkin lymphoma for subjects below age 35 years, whereas for older subjects, ever-smokers experienced a doubled risk of Hodgkin lymphoma as compared to never smokers ([Besson et al., 2006](#)). In contrast, a positive association was observed in young adults participating in the International Twin Study ([Cozen et al., 2009](#)). A positive association was observed in a Scandinavian case-control study, but without a clear dose-response ([Hjalgrim et al., 2007](#)). In a case-control study addressing infectious precursors, particularly Epstein-Barr virus (EBV), an increased risk for EBV-positive Hodgkin lymphoma was found among current smokers ([Glaser et al., 2004](#); Table 2.50 online).

Several of the above studies found positive associations for Hodgkin lymphoma while also demonstrating null or inverse associations with non-Hodgkin lymphoma ([Nieters et al., 2006](#); [Lim et al., 2007](#); [Nieters et al., 2008](#); [Kanda et al., 2009](#)).

### 2.11.3 Multiple myeloma

In the previous *IARC Monograph* ([IARC, 2004a](#)), the large majority of studies on tobacco smoking and risk for multiple myeloma evaluated showed no clear association. More recently,

two case-control studies found a positive association ([Vlajinac et al., 2003](#); [Nieters et al., 2006](#)), whereas no clear association was observed in another case-control study ([Monnereau et al., 2008](#)) or in a cohort study in Sweden ([Fernberg et al., 2007](#); Table 2.51 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.51.pdf>).

## 2.12 Cancer of the breast

Approximately 150 epidemiological studies have been published on the relationship between breast cancer and active and passive smoking. The results from these studies have been comprehensively examined in peer-reviewed literature ([Palmer & Rosenberg, 1993](#); [Terry et al., 2002a](#); [Johnson et al., 2002](#); [Johnson, 2005](#); [Terry & Goodman, 2006](#); [Miller et al., 2007](#)). The previous *IARC Monograph* ([IARC, 2004a](#)) considered studies conducted through June 2002 and concluded that there is evidence suggesting lack of carcinogenicity of tobacco smoking in humans for cancers of the female breast.

Other consensus reviews have drawn different conclusions, based partly on the availability of new data, and partly on differences in interpretation:

- The 2001 US Surgeon General Report on Women and Smoking (Department of Health & Human Services, 2001) concluded that tobacco smoking does not appear to appreciably affect breast cancer risk overall. However, several issues were not entirely resolved, including whether starting to smoke at an early age increases risk, whether certain subgroups defined by genetic polymorphisms are differentially affected by smoking, and whether exposure to second-hand smoke affects risk.
- The 2004 US Surgeon General report on “The Health Consequences of Smoking” (Department of Health & Human

Services, 2004) concluded the evidence is suggestive of no causal relationship between tobacco smoking and breast cancer.

- The 2009 Canadian Expert Panel on Tobacco Smoke and Breast Cancer Risk ([Collishaw et al., 2009](#)) concludes that based on the weight of evidence from epidemiological and toxicological studies and understanding of biological mechanisms, the associations between tobacco smoking and both pre- and post-menopausal breast cancer are consistent with causality.

The lack of agreement in the conclusions from these groups is not surprising, given that the observed associations are weaker and less consistent for breast cancer than for other tobacco-related cancers. Furthermore, several methodological considerations could either obscure a small increase in risk caused by tobacco smoking, or alternatively introduce a spurious association where no causal relationship exists.

### 2.12.1 Methodological and related issues

The principal concerns about studies of tobacco smoking and breast cancer are the following: timing of exposure, the relevant disease endpoint, the potential for confounding by factors associated with both smoking and the occurrence/detection of breast cancer, the hypothesis that tobacco smoking may have opposing effects on breast cancer risk (protective and detrimental), and the hypothesis that some women may be genetically more susceptible to develop breast cancer from smoking, and that increased risk in these subgroups may be obscured in analyses of average risk in the population.

#### (a) Misclassification of exposure

Self-reported information on tobacco smoking is generally considered more reliable than questionnaire information on exposure to second-hand tobacco smoke. However, studies of tobacco smoking have not uniformly considered the duration of smoking (years), the average amount smoked (cigarettes/day), or the timing of initiation in relation to first full-term pregnancy. Only one ([Al-Delaimy et al., 2004](#)) of the seven available cohort studies updated the information on smoking behaviour during follow-up. Whereas some exposure variables, such as age at initiation and age at first full-term pregnancy remain constant over time, others, such as smoking status, duration and age at cessation do not. Furthermore, the average age at initiation and duration of smoking are highly correlated with birth cohort and attained age. While the number of years of smoking before first full term pregnancy has been proposed as a potentially relevant measure of exposure, the range of this variable is constrained except among women whose first pregnancy occurs at an older age, which is itself an independent risk factor for breast cancer.

#### (b) Specificity of disease endpoints

Breast cancer is not a single disease. Accordingly, some researchers have postulated that exposure to tobacco smoke (from tobacco smoking or second-hand tobacco smoke) could differentially affect certain clinical subtypes of breast such as pre- or post-menopausal cancers or tumours with or without hormonal receptors. It is also possible that smoking might affect the survival of women with breast cancer, whether or not it affects incidence rates. Most published studies have measured incidence rates as the endpoint, although some have measured mortality rates or effects on survival.



*(c) Confounding*

Alcohol consumption is positively correlated with tobacco smoking ([Marshall et al., 1999](#)) and is an established cause of breast cancer ([IARC, 2010a](#); *Monograph on Consumption of Alcoholic Beverages* in this Volume). Most epidemiologic studies attempt to control for alcohol consumption using questionnaire information on usual drinking patterns. This approach is vulnerable to residual confounding, because self-reported data on lifetime alcohol consumption leave room for misclassification. Potential confounding by alcohol consumption is of greater concern for current than for former smokers, since, on average, current smokers drink more than former smokers ([Reynolds et al., 2004a, b](#)). One study by the Collaborative Group on Hormonal Factors and Breast Cancer ([Hamajima et al., 2002](#)) controlled rigorously for alcohol consumption by restricting the analysis of smoking and breast cancer to women who reported drinking no alcohol.

Conversely, mammography screening can be a negative confounder in studies of tobacco smoking and breast cancer incidence. Few studies of tobacco smoking in relation to breast cancer have controlled for mammography screening. Current smokers report a lower frequency of mammographic screening than never-smokers, whereas health conscious former smokers report higher screening rates ([Gross et al., 2006](#)). Mammography screening affects the detection rather than the occurrence of breast cancer; it detects some tumours that might otherwise never have been recognized and allows earlier diagnosis of others, thereby increasing breast cancer incidence in the short-term. The consequence of uncontrolled confounding by mammography screening would be to underestimate an association between current smoking and breast cancer incidence, and to overestimate the association in former smokers. Confounding by screening

would be expected to have the opposite effect in studies of breast cancer mortality.

Other correlates of tobacco smoking might also confound a potential association between tobacco smoking and breast cancer, although their net effect is likely to be smaller and harder to predict than confounding by alcohol and mammography screening. Women who smoke undergo menopause about two to three years earlier than never-smokers ([Baron et al., 1990](#)). The effect of this may be partly or wholly offset by the greater likelihood of girls who experience early menarche to initiate smoking in early adolescence ([Jean et al., 2011](#)). There is no documentation that smokers and never-smokers differ with respect to average years of ovulation. Tobacco smoking also has a complex relationship to body mass index. Post-menopausal women who smoke are less likely to be overweight or obese than former or never smokers, but overweight adolescent girls are more likely to begin smoking for weight control ([Fine et al., 2004](#)). Similarly complex relationships exist between smoking and physical activity. Current smokers report less physical activity than either former or never smokers ([Kaczynski et al., 2008](#); [Trost et al., 2002](#)), but only a small proportion of the population engages in the vigorous physical activity that is needed to protect against breast cancer. The socioeconomic correlates of smoking have changed over time. Women who attended college during the 1960s and 1970s were more likely to initiate smoking than less educated women, but subsequently college-educated women have been more likely to quit. Thus, the potential for confounding by reproductive patterns and use of post-menopausal hormone treatment varies by birth cohort and differs for current and former smokers.

Most epidemiological studies have attempted to control for factors that might confound the relationship between breast cancer and tobacco smoking using questionnaire information collected on these factors. None of the published

studies have been able to control for all of the potential confounders, however. Most studies lack data on screening behaviour and have limited information on alcohol consumption, use of post-menopausal hormones, and physical activity.

(d) *Potential anti-estrogenic effects of tobacco smoking*

Indirect evidence suggests that tobacco smoking may have anti-estrogenic effects that might offset the adverse effects of tobacco smoke carcinogens on breast cancer risk. [Baron et al. \(1990\)](#) pointed to observations suggesting lower estrogen activity levels in women who smoke compared to those who do not. Smokers have lower risk of endometrial cancer (Department of Health & Human Services, 2004), higher risk of osteoporosis ([Jensen et al., 1985](#); [Jensen & Christiansen, 1988](#)), earlier age at natural menopause ([Baron et al., 1990](#)) and lower mammography density ([Roubidoux et al., 2003](#)) than women who do not smoke. Smoking also attenuates the effects of hormone replacement therapy (HRT) on lipid profiles ([Jensen & Christiansen, 1988](#)) and serum estrone ([McDivitt et al., 2008](#)). No difference in serum concentrations of estradiol and estrone between post-menopausal smokers and non-smokers have been reported in several studies ([Cassidenti et al., 1992](#); [Khaw et al., 1988](#); [Berta et al., 1991](#); [Longcope et al., 1986](#); [Berta et al., 1992](#); [Cauley et al., 1989](#); [Friedman et al., 1987](#); [Key et al., 1991](#)). However, smokers have been observed to have higher levels of androgens ([Cassidenti et al., 1992](#)) (specifically androstenedione) ([Khaw et al., 1988](#); [Cauley et al., 1989](#); [Friedman et al., 1987](#); [Key et al., 1991](#)), prolactin ([Berta et al., 1991](#)), and unbound serum estradiol ([Cassidenti et al., 1992](#)).

(e) *Genetically susceptible subgroups*

Certain subgroups of women may have greater risk of breast cancer when exposed to tobacco smoke because of genetic or other factors

affecting cancer susceptibility. Potential interactions between inherited polymorphisms and tobacco smoking have been studied for selected candidate genes that affect carcinogen metabolism, modulation of oxidative damage, immune responses, and DNA repair (see Sections 2.12.4b and 4.2).

## 2.12.2 Analytical studies

Over 130 epidemiological studies on tobacco smoking and breast cancer were reviewed.

(a) *Incidence in current and former smokers*

Since the previous IARC Monograph ([IARC, 2004a](#)), seven reports on cohort studies ([Al-Delaimy et al., 2004](#); [Reynolds et al., 2004a](#); [Gram et al., 2005](#); [Hanaoka et al., 2005](#); [Olson et al., 2005](#); [Cui et al., 2006](#); [Ha et al., 2007](#)) have been published on breast cancer incidence in relation to tobacco smoking (Table 2.52 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.52.pdf>). Breast cancer incidence was significantly associated with current tobacco smoking in three studies ([Reynolds et al., 2004a](#); [Olson et al., 2005](#); [Cui et al., 2006](#)), with relative risk estimates among the larger studies ranging from 1.12 (95%CI: 0.92–1.37) ([Al-Delaimy et al., 2004](#)) to 1.32 (95%CI: 1.10–1.57) ([Reynolds et al., 2004a](#)). Former smoking was significantly associated with risk in only one cohort ([Al-Delaimy et al., 2004](#)), with relative risk estimates across all of the cohorts ranging from 1.00 (95%CI: 0.93–1.08) ([Cui et al., 2006](#)) to 1.18 (95%CI: 1.02–1.36) ([Al-Delaimy et al., 2004](#)). The association with breast cancer is stronger in current than in former smokers in four of the seven cohort studies ([Reynolds et al., 2004a](#); [Hanaoka et al., 2005](#); [Olson et al., 2005](#); [Cui et al., 2006](#)), although the confidence intervals overlap widely in all but one ([Cui et al., 2006](#)). [The Working group noted that three cohort studies ([Gram et al., 2005](#); [Hanaoka et al., 2005](#); [Olson et al., 2005](#)) provided data on both

the age-adjusted and the multivariate-adjusted risk estimates for current and former smoking. None of these showed attenuation of the estimate associated with current smoking, and two (Hanaoka *et al.*, 2005; Olson *et al.*, 2005) reported somewhat stronger estimates when adjusted for established risk factors besides age. None of the studies adjusted for the frequency of mammography screening. Residual confounding by screening and incomplete control for other risk factors would be expected to cause underestimation of the association with current smoking, and overestimation of the association with former smoking.]

Since the previous *IARC Monograph* (IARC, 2004a), a total of 12 case-control studies on tobacco smoking and breast cancer incidence have been published (Table 2.53 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.53.pdf>). Results from the case-control studies are less consistent than those from the cohort studies. Six studies (Li *et al.*, 2004; Mechanic *et al.*, 2006; Magnusson *et al.*, 2007; Prescott *et al.*, 2007; Roddam *et al.*, 2007; Slattery *et al.*, 2008) differentiated between current and former smokers, while the six other reports (Band *et al.*, 2002; Lash & Aschengrau, 2002; Gammon *et al.*, 2004; Rollison *et al.*, 2008; Ahern *et al.*, 2009; Young *et al.*, 2009) specify only ever or never smokers. Only one study (Li *et al.*, 2004) reported a borderline significant increase in risk associated with current smoking, and two studies (Band *et al.*, 2002; Rollison *et al.*, 2008) with ever smoking.

None of the six case-control studies that presented data on breast cancer incidence separately for current and former smokers found a significant difference in risk between the two smoking categories; the relative risk estimates were higher for former than for current smokers in four of the studies (Mechanic *et al.*, 2006; Prescott *et al.*, 2007; Roddam *et al.*, 2007; Slattery *et al.*, 2008) and identical in the fifth (Magnusson *et al.*, 2007).

#### (b) *Years of cessation*

When the relative risk for breast cancer incidence in former smokers is examined by years since cessation in cohort studies (Table 2.54 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.54.pdf>), the point estimates do not consistently decrease with longer time since cessation. In none of the four cohort studies (London *et al.*, 1989b; Egan *et al.*, 2002; Reynolds *et al.*, 2004a; Cui *et al.*, 2006) and in only one (Li *et al.*, 2005) of the five case-control studies (Chu *et al.*, 1990; Gammon *et al.*, 1998; Johnson *et al.*, 2000; Kropp & Chang-Claude, 2002; Li *et al.*, 2005) that formally tested for trend (Table 2.55 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.55.pdf>) was there a statistically significant decrease in relative risk observed with longer time since cessation. Only one study has reported data on breast cancer mortality in relation to years since quitting or age at cessation (Calle *et al.*, 1994). A statistically significant inverse trend in the relative risk estimates was reported with both years since quitting ( $p$  trend = 0.04) and younger age at cessation ( $p$  trend = 0.02). [The Working Group noted that the inverse trends in the relative risk of dying from breast cancer observed in this study are weaker than those observed with most other cancers designated as causally associated with smoking.]

#### (c) *Duration of smoking and age at initiation*

Tables 2.56–2.61 (see below for links) list the published epidemiologic studies that relate breast cancer incidence to duration of tobacco smoking, age at initiation and/or timing relative to first full term pregnancy.

Longer duration of smoking is associated with higher breast cancer incidence in five of seven cohort studies (Table 2.56 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.56.pdf>). A similar trend is seen inconsistently among the 33 case-control



studies that report relative risk estimates by duration of smoking (Table 2.57 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.57.pdf>). Among the 18 studies that reported a formal test of trend, eight studies (Gammon *et al.*, 1998; Johnson *et al.*, 2000; Reynolds *et al.*, 2004a; Gram *et al.*, 2005; Li *et al.*, 2005; van der Hel *et al.*, 2005; Cui *et al.*, 2006; Mechanic *et al.*, 2006) reported a statistically significant or borderline increase in the relative risk of incident breast cancer with the duration of smoking; seven studies (Ewertz, 1990; Palmer *et al.*, 1991; Egan *et al.*, 2002; Al-Delaimy *et al.*, 2004; Lissowska *et al.*, 2006; Magnusson *et al.*, 2007; Prescott *et al.*, 2007) reported no trend, and one study (Brinton *et al.*, 1986) reported an inverse relationship.

Thirty studies, including cohort (Tables 2.58 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.58.pdf>) and case-control studies (Table 2.59 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.59.pdf>) related breast cancer incidence to age at smoking initiation. Fifteen of these (Chu *et al.*, 1990; Ewertz, 1990; Palmer *et al.*, 1991; Nordlund *et al.*, 1997; Gammon *et al.*, 1998; Johnson *et al.*, 2000; Egan *et al.*, 2002; Kropp & Chang-Claude, 2002; Gram *et al.*, 2005; Cui *et al.*, 2006; Lissowska *et al.*, 2006; Ha *et al.*, 2007; Lissowska *et al.*, 2007; Magnusson *et al.*, 2007; Prescott *et al.*, 2007; Slattery *et al.*, 2008) reported a formal test of trend. Among these, only two (Gram *et al.*, 2005; Ha *et al.*, 2007) found a statistically significant or borderline significantly higher risk in women who began smoking at a younger ages; twelve studies (Chu *et al.*, 1990; Ewertz, 1990; Palmer *et al.*, 1991; Nordlund *et al.*, 1997; Gammon *et al.*, 1998; Johnson *et al.*, 2000; Egan *et al.*, 2002; Cui *et al.*, 2006; Lissowska *et al.*, 2006; Magnusson *et al.*, 2007; Prescott *et al.*, 2007; Slattery *et al.*, 2008) found no relationship with age at initiation, and one (Kropp & Chang-Claude, 2002) reported higher risk among women who began

smoking later. [The Working Group noted that at least two studies (Cui *et al.*, 2006; Slattery *et al.*, 2008) appear to have included never-smokers in the tests of trend and that the categories that define age at initiation differ across studies.]

The relative risk of incident breast cancer according to the timing of smoking initiation relative to first full-term pregnancy was reported in 21 studies, of cohort (Table 2.60 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.60.pdf>) and case-control (Table 2.61 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.61.pdf>) design. For nine studies (Hunter *et al.*, 1997; Egan *et al.*, 2002; Al-Delaimy *et al.*, 2004; Reynolds *et al.*, 2004a; Li *et al.*, 2005; Cui *et al.*, 2006; Prescott *et al.*, 2007; Rollison *et al.*, 2008; Young *et al.*, 2009) categorical data on years of smoking before first pregnancy are presented, whereas for 12 (Lash & Aschengrau, 1999; Innes & Byers, 2001; Band *et al.*, 2002; Kropp & Chang-Claude, 2002; Lash & Aschengrau, 2002; Fink & Lash, 2003; Lawlor *et al.*, 2004; Gram *et al.*, 2005; Olson *et al.*, 2005; Lissowska *et al.*, 2006; Magnusson *et al.*, 2007; Slattery *et al.*, 2008) whether smoking was initiated before or after the initial pregnancy was considered. Breast cancer incidence is consistently higher when smoking began before or during first pregnancy in most (Hunter *et al.*, 1997; Lash & Aschengrau, 1999; Innes & Byers, 2001; Band *et al.*, 2002; Egan *et al.*, 2002; Al-Delaimy *et al.*, 2004; Reynolds *et al.*, 2004a; Gram *et al.*, 2005; Li *et al.*, 2005; Olson *et al.*, 2005; Cui *et al.*, 2006; Slattery *et al.*, 2008; Young *et al.*, 2009) but not all (Kropp & Chang-Claude, 2002; Lash & Aschengrau, 2002; Fink & Lash, 2003; Prescott *et al.*, 2007) studies that tested this. [The Working Group noted that the number of years of smoking before first pregnancy is highly correlated with age at first full-term pregnancy, which is itself an independent risk factor for breast cancer.]

It has been argued that some studies, and especially cohort studies, may underestimate



the true association between tobacco smoking and breast cancer risk by ignoring or underestimating lifetime exposure to second-hand tobacco smoke of those in the referent group ([California Environmental Protection Agency, 2005](#); [Johnson, 2005](#); [Collishaw \*et al.\*, 2009](#)). This criticism is based on the hypothesis that exposure to second-hand smoke may confer almost the same degree of breast cancer risk as tobacco smoking. Under this hypothesis, the inclusion of women exposed to second-hand smoke in the referent group dilutes the contrast between exposed and unexposed women in studies of tobacco smoking, and causes underestimation of the association between tobacco smoking and breast cancer. In several case-control studies the association between breast cancer and tobacco smoking strengthened when the referent group was defined as women with “never active, never-passive” exposure to tobacco smoke ([Morabia \*et al.\*, 1996](#); [Lash & Aschengrau, 1999](#); [Johnson \*et al.\*, 2000](#); [Kropp & Chang-Claude, 2002](#)). In contrast, a stronger association between tobacco smoking and breast cancer risk, when women exposed only to second-hand smoke are excluded from the referent group, has not been observed in cohort studies ([Egan \*et al.\*, 2002](#); [Reynolds \*et al.\*, 2004a](#)). Debate continues over whether the case-control studies should be considered “of highest quality” because they provide “lifetime exposure assessment” ([Collishaw \*et al.\*, 2009](#)) or whether the cohort studies are more credible, because prospectively-collected exposure data are not susceptible to the recall bias that can affect retrospective studies.

#### (d) *Survival and mortality from breast cancer*

The relationship between smoking and the natural history of breast cancer has been examined in several studies ([Daniell, 1988](#); [Ewertz \*et al.\*, 1991](#); [Daniell \*et al.\*, 1993](#); [Scanlon \*et al.\*, 1995](#); [Yu \*et al.\*, 1997](#); [Manjer \*et al.\*, 2000](#); [Murin & Inciardi, 2001](#); [Holmes \*et al.\*, 2007](#)). In cross-sectional analyses, [Daniell \*et al.\* \(1993\)](#) found that

smokers with breast cancer had more and larger lymph node metastases than non-smokers, after controlling for primary tumour size and other variables. Further, a case-control study ([Murin & Inciardi, 2001](#)) and a retrospective cohort study ([Scanlon \*et al.\*, 1995](#)) found smoking to be associated with an increased risk of developing pulmonary metastases from breast cancer. However, these studies could not definitively distinguish lung metastases from primary lung cancers.

Five cohort studies have focused specifically upon the association of tobacco smoking with either breast cancer survival ([Ewertz \*et al.\*, 1991](#); [Yu \*et al.\*, 1997](#); [Manjer \*et al.\*, 2000](#); [Holmes \*et al.\*, 2007](#)) or breast cancer death rates ([Calle \*et al.\*, 1994](#)). A study of 1774 Danish women showed no association between smoking and breast cancer survival ([Ewertz \*et al.\*, 1991](#)), as did a study of 5056 women with breast cancer in the Nurse’s Health Study ([Holmes \*et al.\*, 2007](#)). In contrast, follow-up of 792 women with in situ or invasive breast cancer detected in a screening study in Malmö, Sweden found a crude relative risk for smokers and ex-smokers, compared to never smokers, of 1.44 (95%CI: 1.01–2.06) and of 1.13 (95%CI: 0.66–1.94), respectively ([Manjer \*et al.\*, 2000](#)). The relative risk associated with smoking remained significant after adjustment for age and stage at diagnosis (RR, 2.14; 95%CI: 1.47–3.10). A study based on the ACS Cancer Prevention Study II reported an association between current smoking and increased breast cancer death rates after six years of follow-up (Table 2.56 online; [Calle \*et al.\*, 1994](#)). Risk of death attributed to breast cancer was positively and significantly related to the duration of current smoking reported at the time of enrolment. However, the authors acknowledge that mortality studies cannot exclude biases arising from the effect of smoking on overall death rates, which could increase the potential for prevalent breast cancer to be coded as the underlying cause of death on the death certificate ([Calle \*et al.\*, 1994](#)).

### 2.12.3 Subtypes

#### (a) Pre- versus post-menopausal

Since the previous *IARC Monograph* ([IARC, 2004a](#)), 19 case-control studies have published data on tobacco smoking in relation to pre- and post-menopausal breast cancer (Table 2.62 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.62.pdf>). The results are inconsistent. Of the 12 studies that provide information separately for current smokers ([Schechter et al., 1985](#); [Brinton et al., 1986](#); [Rohan & Baron, 1989](#); [Ewertz, 1990](#); [Baron et al., 1996](#); [Gammon et al., 1998](#); [Millikan et al., 1998](#); [Johnson et al., 2000](#); [Zheng et al., 2002](#); [Magnusson et al., 2007](#); [Slattery et al., 2008](#)), only five ([Schechter et al., 1985](#); [Johnson et al., 2000](#); [Magnusson et al., 2007](#); [Slattery et al., 2008](#)) found a stronger association with pre- than with post-menopausal breast cancer. The other analyses show either similar associations ([Brinton et al., 1986](#); [Ewertz, 1990](#); [Baron et al., 1996](#); [Gammon et al., 1998](#); [Millikan et al., 1998](#); [Zheng et al., 2002](#)) or a stronger association with post-menopausal breast cancer ([Rohan & Baron, 1989](#); [Millikan et al., 1998](#); [Johnson et al., 2000](#); [Zheng et al., 2002](#)).

#### (b) Hormone receptor status

Two cohort studies ([London et al., 1989a](#); [Manjer et al., 2001](#)), one case-control study ([Morabia et al., 1998](#)) and a case series ([Yoo et al., 1997](#)) have examined the association between quantitative measures of cigarette smoking and breast cancer risk according to estrogen receptor (ER) status. In one of the cohort studies ([Manjer et al., 2001](#)), a statistically significant increased risk (RR, 1.6) of ER negative tumours associated with current smoking was found but no clear association between smoking and ER positive tumours, and no difference in the association with progesterone receptor (PR)-positive and PR-negative tumours. In the other three studies

there was no clear difference in the association related to ER or PR receptor status.

### 2.12.4 Susceptible populations

More than 30 studies and meta-analyses ([Alberg et al., 2004](#); [Terry & Goodman, 2006](#); [Ambrosone et al., 2008](#); [Collishaw et al., 2009](#)) have evaluated whether a family history of breast cancer and/or inherited polymorphisms in various genes may confer greater susceptibility to develop breast cancer from exposure to tobacco smoke. These are described below in relation to the measure indicating potential susceptibility.

#### (a) Family history

In two studies, whether a family history of breast cancer modifies susceptibility to develop breast cancer from tobacco smoking has been examined. [Couch et al. \(2001\)](#) measured breast cancer incidence among female family members in a cohort of breast cancer cases diagnosed between 1944 and 1952 at the University of Minnesota. Sisters and daughters in families with at least three breast and/or ovarian cancers were at 2.4 fold higher risk for breast cancer (95%CI: 1.2–5.1) if they smoked compared to never-smokers. No dose-response was observed in relation to pack-years of smoking.

[Suzuki et al. \(2007\)](#) reported a statistically significant interaction between family history of breast cancer and smoking history in a hospital-based case-control study of 3861 breast cancer cases treated at a large cancer centre in Japan between 1988 and 2000. A family history of breast cancer in the absence of smoking was associated with a relative risk of 1.44 (95%CI: 1.21–1.71); the relative risk estimate was 1.95 (95%CI: 1.36–2.81) in women who reported < 30 pack-years of tobacco smoking, and 4.33 (95%CI: 1.65–11.40) in women who reported > 30 pack-years of smoking.

[The Working group noted that Japanese women who smoked during this time period

may have differed from never-smokers in other characteristics related to breast cancer. Besides its strong correlation with female smoking, “Westernization” might be associated with delayed childbearing, smaller families, higher body mass index, and greater use of post-menopausal hormones.]

### (b) Genetic polymorphisms

Studies of breast cancer, smoking and low penetrance genetic polymorphisms are summarized in Table 2.63 (available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.63.pdf>). The candidate genes in these studies are involved in carcinogen metabolism [*N*-acetyltransferases (*NAT1*, *NAT2*), cytochrome P450s (*CYP1A1*, *CYP1B1*, *CYP2E2*), GSTs], host responses to oxidative stress (superoxide dismutase) or to infectious organisms (myeloperoxidase and immunoglobulin binding protein) and DNA repair (*O*<sup>6</sup>-methylguanine DNA methyltransferase, nucleotide excision repair).

The most consistent associations with breast cancer risk have been observed among long-term smokers with the *NAT2* slow acetylation genotype (Terry & Goodman, 2006). *NAT2* slow acetylation genotype is thought to confer less capability to detoxify tobacco smoke carcinogens and is associated with an increase in breast cancer risk (Ambrosone *et al.*, 1996, 2008). Approximately 50–60% of Caucasian women are reported to be slow acetylators.

Table 2.63 (online) lists 15 studies of polymorphisms in *NAT2*, of which 9 were included in a pooled analysis and 13 in a meta-analysis (Ambrosone *et al.*, 2008). [The study by Delfino *et al.* (2000) was excluded from these analyses because cases included women with benign breast disease; the study by Lilla *et al.* (2005) was not considered because it is based on the same population as that by Chang-Claude *et al.* (2002).] The meta-analysis found a statistically significant association between ever tobacco

smoking and breast cancer risk among women with the *NAT2* slow acetylator genotype (meta-RR, 1.27; 95%CI: 1.16–1.40) but not in those with rapid acetylator genotype (meta-RR, 1.05; 95%CI: 0.95–1.17). Pack-years of tobacco smoking was significantly associated with increasing breast cancer risk among women with *NAT2* slow acetylator genotype (meta-RR for ever smokers, 1.44; 95%CI: 1.23–1.68, for > 20 pack-years versus never smokers), but not among rapid acetylators (Ambrosone *et al.*, 2008). No main effect was seen between *NAT2* status and breast cancer risk (meta-RR, 1.0; 95%CI: 0.93–1.07). In contrast to an earlier meta-analysis (Alberg *et al.*, 2004), this study observed no difference in risk for pre- or post-menopausal breast cancer. The pooled analysis of nine studies (Ambrosone *et al.*, 2008) reported pooled risk estimates for pre- and post-menopausal women of 1.49 (95%CI: 1.08–2.04) and 1.42 (95%CI: 1.16–1.74), respectively, among women with slow *NAT2* genotype and at least 20 pack-years of smoking compared to never-smokers. The corresponding values for women with rapid acetylator genotype were 1.29 (95%CI: 0.89–1.86) and 0.88 (95%CI: 0.69–1.13). A statistically significant interaction was observed between pack-years of smoking as a continuous variable and *NAT2* genotype (*p* interaction = 0.03).

A population-based case-control study published after the meta-analysis by Ambrosone *et al.* compared the prevalence of the *NAT2* genotypes and their joint effect with smoking on breast cancer risk in Hispanic and non-Hispanic white women (Baumgartner *et al.*, 2009). Non-Hispanic white women were more likely (*P* < 0.001) than Hispanics to have a slow (41.7% versus 33.5%) or very slow (19.0% versus 11.1%) *NAT2* acetylator status. Breast cancer risk was significantly increased in non-Hispanic smoking white women with a very slow acetylator genotype (RR, 2.46; 95%CI: 1.07–5.65 for current versus never).

[The Working Group noted that publication bias remains a concern in the studies of *NAT2* published to date. All of the studies included in the meta-analysis by Ambrosone *et al.* were published between 1996 and 2006; some among them (Morabia *et al.*, 2000; Sillanpää *et al.*, 2007) reported very strong associations that seem inconsistent with the rest of the data. Because genetic studies often examine multiple genes, it is plausible that studies that find no main effect with *NAT2* have not examined this association or that null results for smoking have not been published.]

Fewer studies with less consistent findings have been published on polymorphisms in other genes such as *NAT1*, *CYP1A1*, *GST*, *NOS3*, *MPO*, *MnSOD2* and various DNA repair genes (Table 2.63 online).

#### 2.12.5 High penetrance genes & prognosis

At least seven studies have examined the hypothesis that tobacco smoking may modify breast cancer risk among women who carry *BRCA1* and *BRCA2* mutations (Brunet *et al.*, 1998; Ghadirian *et al.*, 2004; Colilla *et al.*, 2006; Gronwald *et al.*, 2006; Nkondjock *et al.*, 2006; Breast Cancer Family Registry, 2008; Ginsburg *et al.*, 2009). The results have been inconsistent. A recent case-control study of women under age 50 years who were carriers of mutations in *BRCA1* or *BRCA2* reported increased risk for breast cancer associated with as little as five pack-years of smoking. Compared to non-smokers, the risk associated with five or more pack-years of smoking was 2.3 (95%CI: 1.6–3.5) for *BRCA1* mutation carriers and 2.6 (95%CI: 1.8–3.9) for *BRCA2* mutation carriers (Breast Cancer Family Registry, 2008). In contrast, six other studies reported no increased risk among *BRCA1* or *BRCA2* carriers who smoke. The Canadian Panel review (Collishaw *et al.*, 2009) postulated that the five previous studies (Brunet *et al.*, 1998; Ghadirian *et al.*, 2004; Colilla *et al.*,

2006; Gronwald *et al.*, 2006; Nkondjock *et al.*, 2006) may have failed to observe a relationship because they included prevalent cases. However, a sixth study published since the Canadian panel review is also negative (Ginsburg *et al.*, 2009).

## 2.13 Cancer of the cervix

The association between smoking and cervical cancer has been examined in many epidemiological studies over the past few decades.

Since the previous *IARC Monograph* (IARC, 2004a), additional epidemiological studies have been published. Study design and results of the case-control studies restricted to HPV positive women or that adjusted for HPV status are presented in Table 2.64 (available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.64.pdf>) and Table 2.65 (available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.65.pdf>). Cohort studies and pooled analyses are presented in Table 2.66 (available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.66.pdf>) and Table 2.67 (available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.67.pdf>), respectively. Table 2.68 (available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.68.pdf>) and Table 2.69 (available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.69.pdf>) present additional cohort studies and pooled analyses on tobacco smoking and cervical, cervical intraepithelia neoplasia and carcinoma in situ, with or without controlling for HPV status, respectively.

### 2.13.1 Dose-response relationship

A positive association between smoking and incidence of cervical squamous-cell carcinoma, which account for approximately 90% of all cervical cancers, has been shown consistently over several decades in many epidemiological studies



of various designs conducted across different geographic regions. Dose–response associations with smoking intensity and duration were noted in many of the studies where such associations were examined ([Berrington de González et al., 2004](#); [Appleby et al., 2006](#)). Conversely, no clear association was found among former smokers. For adenocarcinoma of the cervix, which usually account for less than 10% of the total of all types of cervical cancer, there appears to be no clear association with smoking ([Berrington de González et al., 2004](#)).

### 2.13.2 Interaction with HPV positivity

Epidemiological studies of smoking and cervical cancer increasingly have considered the effects of HPV infection, which is recognized as the main etiological factor for invasive and pre-invasive cervical neoplasia worldwide ([IARC, 1995, 2012b](#)). HPV infection has been considered not only with respect to possible effect modification ([Hellberg & Stendahl, 2005](#); [Gunnell et al., 2006](#)), but also to confounding, as both HPV infection and smoking habits are directly associated with number of sexual partners and other indications of high-risk sexual behaviours ([Sikström et al., 1995](#); [Wang et al., 2004](#); [Hellberg & Stendahl, 2005](#); [McIntyre-Seltman et al., 2005](#); [Syrjänen et al., 2007](#)). Although there have been exceptions ([Syrjänen et al., 2007](#)), recent studies have generally continued to show that statistical adjustment for the potential confounding effects of HPV infection, or restricting studies to women with high risk HPV infection ([Plummer et al., 2003](#)), does not appreciably alter the finding of a positive association or its magnitude ([McIntyre-Seltman et al., 2005](#); [Appleby et al., 2006](#); [Tolstrup et al., 2006](#); [Tsai et al., 2007](#); [Nishino et al., 2008](#); [Kapeu et al., 2009](#)).

Statistical adjustment for the potentially confounding effect of HPV infection was usually based on the measured presence of HPV DNA in cervical cells or anti-HPV serum antibodies

in multivariate analytical models; as noted above, studies have also restricted their analyses to HPV-positive cases and controls. As there is currently no reliable marker of persistent HPV infection, case–control studies based on a cross-sectional measurement of HPV cannot distinguish between transient and persistent infections ([Franco et al., 1999](#)). Tobacco smoking is suspected to facilitate acquisition or persistence of an HPV infection through a reduced number of Langerhans cells and CD4 lymphocytes, which are markers of local immune response in the cervix ([Vaccarella et al., 2008](#)). In addition, smoking may affect innate immunity ([Ferson et al., 1979](#)). Current smokers have been shown to have a slightly higher HPV prevalence than non-smokers in a broad range of world populations after adjustment for life-time number of sexual partners (OR, 1.18; 95%CI: 1.01–1.39) ([Vaccarella et al., 2008](#)). Studies have evaluated the effect of smoking on HPV persistence. One study shows lower probability of HPV clearance among ever smokers ([Giuliano et al., 2002](#)) but a few others found no relationship ([Molano et al., 2003](#); [Richardson et al., 2005](#)).

## 2.14 Cancer of the endometrium

### 2.14.1 Overview of studies

To date, at least 42 epidemiological studies have examined the association between smoking and endometrial cancer, 25 reviewed in the previous *IARC Monograph* ([IARC, 2004a](#)) and 17 published since then ([Petridou et al., 2002](#); [Folsom et al., 2003](#); [Furberg & Thune, 2003](#); [Newcomb & Trentham-Dietz, 2003](#); [Beral et al., 2005](#); [Matthews et al., 2005](#); [Viswanathan et al., 2005](#); [Okamura et al., 2006](#); [Strom et al., 2006](#); [Trentham-Dietz et al., 2006](#); [Weiss et al., 2006a](#); [Al-Zoughool et al., 2007](#); [Bjørge et al., 2007](#); [Lacey et al., 2007](#); [Loerbroks et al., 2007](#); [Setiawan et al., 2007](#); [Lindemann et al., 2008](#)). Study design and results of the additional studies

are presented separately for the case-control studies (Table 2.70 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.70.pdf> and Table 2.71 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.71.pdf>, respectively) and for the cohort studies (Table 2.72 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.72.pdf> and Table 2.73 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.73.pdf>, respectively).

#### (a) Cohort studies

The majority of the 13 cohort studies (Engeland *et al.*, 1996; Terry *et al.*, 1999, 2002b; Folsom *et al.*, 2003; Furberg & Thune 2003; Beral *et al.*, 2005; Viswanathan *et al.*, 2005; Al-Zoughool *et al.*, 2007; Bjørge *et al.*, 2007; Lacey *et al.*, 2007; Loerbroks *et al.*, 2007; Setiawan *et al.*, 2007; Lindemann *et al.*, 2008) suggest a decreased risk among current smokers, including the largest study with over 9000 cases (Bjørge *et al.*, 2007). In five of these studies quantitative smoking measures have been examined in relation to endometrial cancer risk (Terry *et al.*, 1999, 2002b; Viswanathan *et al.*, 2005; Al-Zoughool *et al.*, 2007; Loerbroks *et al.*, 2007). Of these, one (Terry *et al.*, 1999) found a 50% reduced risk among current smokers in the highest level of intensity (11 cigarettes per day or more) compared with non-smokers, but the number of cases was low and the confidence intervals correspondingly wide. A more recent and larger cohort study (Terry *et al.*, 2002b) found a statistically significant 40% reduced risk among current smokers of more than 20 cigarettes per day, but showed somewhat weaker and statistically non-significant reductions in risk with smoking of long duration or high cumulative consumption (i.e. pack-years). In contrast, the risk among former smokers was similar to that among never smokers. The largest of these studies generally showed decreasing risk of endometrial cancer with increasing

smoking intensity, duration, and pack-years of consumption (Viswanathan *et al.*, 2005). Three studies examined the association between time since smoking cessation and endometrial cancer risk. Two of these studies suggested a positive association with time since quitting (compared with non-smokers) (Viswanathan *et al.*, 2005; Loerbroks *et al.*, 2007), whereas one found no association (Terry *et al.*, 2002b).

#### (b) Case-control studies

The results of 17 population-based case-control studies (Smith *et al.*, 1984; Tyler *et al.*, 1985; Franks *et al.*, 1987; Elliott *et al.*, 1990; Rubin *et al.*, 1990; Brinton *et al.*, 1993; Goodman *et al.*, 1997; Shields *et al.*, 1999; Jain *et al.*, 2000; McCann *et al.*, 2000; Newcomer *et al.*, 2001; Weiderpass & Baron, 2001; Newcomb & Trentham-Dietz, 2003; Matthews *et al.*, 2005; Strom *et al.*, 2006; Trentham-Dietz *et al.*, 2006; Weiss *et al.*, 2006a), that have included between 46 and 1304 endometrial cancer cases, generally have shown reductions in risk among current smokers compared with never smokers (although the magnitude of the reduction in risk has varied somewhat); results among former smokers compared with never smokers were equally variable, albeit somewhat weaker overall. The results of eight hospital-based case-control studies (Kelsey *et al.*, 1982; Lesko *et al.*, 1985; Levi *et al.*, 1987; Stockwell & Lyman, 1987; Koumantaki *et al.*, 1989; Austin *et al.*, 1993; Petridou *et al.*, 2002; Okamura *et al.*, 2006), which included between 83 and 1374 endometrial cancer cases, are somewhat consistent with those of population-based studies. They showed moderate (e.g. 30–40%) reduction in risks among current compared with never smokers, and unaltered risks (or perhaps a small 10–20% reduction in risk) in former compared with never smokers. The largest of the hospital-based studies (Stockwell & Lyman, 1987), with 1374 cases and 3921 controls, found both former and current smokers to be at moderately (approximately 30%) reduced risk

of endometrial cancer. To date, six population-based case-control studies ([Tyler et al., 1985](#); [Lawrence et al., 1987, 1989](#); [Brinton et al., 1993](#); [Newcomer et al., 2001](#); [Weiderpass & Baron, 2001](#)) have examined quantitative measures of smoking in relation to endometrial cancer risk, generally showing inverse associations to be strongest among current smokers of high intensity or long duration.

### 2.14.2 Confounders

Whereas the majority of these studies adjusted their relative risk estimates for potentially confounding variables, such as BMI, HRT, parity, diabetes, and age at menopause, studies that did not adjust for these variables tended to show similar inverse associations. Within individual studies, statistical adjustment for the effects of BMI and other covariates often made little difference, although some attenuation of relative risk estimates has been noted ([Weiderpass & Baron, 2001](#); [Terry et al., 2002c](#)).

### 2.14.3 Effect modification

The association between smoking and endometrial cancer risk according to factors that are known determinants of endogenous hormone concentrations, and which may counteract or augment possible tobacco-related hormonal changes, have been examined in several studies. These factors include menopausal status, HRT and BMI. Effect modification can reflect true underlying differences in the association across strata (for example, if cigarette smoking acts to reduce or modify estrogen concentrations differently in one group compared with another), but can also reflect methodological factors, such as differences that occur by chance or through the varying prevalence of confounding variables.

#### (a) Menopausal status

Although endometrial cancer is rare among pre-menopausal women, several studies have examined the association between cigarette smoking and endometrial cancer risk according to menopausal status, because the effect of smoking (if any) might vary according to the underlying hormonal milieu. The studies have included two cohort studies ([Terry et al., 2002b](#); [Al-Zoughool et al., 2007](#)), five population-based case-control studies ([Smith et al., 1984](#); [Franks et al., 1987](#); [Lawrence et al., 1987](#); [Brinton et al., 1993](#); [Weiderpass & Baron, 2001](#)), and four hospital-based case-control studies ([Lesko et al., 1985](#); [Levi et al., 1987](#); [Stockwell & Lyman, 1987](#); [Koumantaki et al., 1989](#)). In all but one of these studies, a study of early stage endometrial cancer ([Lawrence et al., 1987](#)), the inverse association was (to varying degrees) stronger among post-menopausal than pre-menopausal women. Among pre-menopausal women, the relative risk estimates for cigarette smoking have been inconsistent, sometimes showing increased risks with certain measures of cigarette smoking ([Smith et al., 1984](#); [Stockwell & Lyman, 1987](#); [Koumantaki et al., 1989](#); [Brinton et al., 1993](#); [Al-Zoughool et al., 2007](#)), sometimes showing decreased risks ([Lawrence et al., 1987](#); [Levi et al., 1987](#); [Brinton et al., 1993](#); [Terry et al., 2002b](#)), and sometimes showing practically no association ([Lesko et al., 1985](#); [Weiderpass & Baron, 2001](#); [Al-Zoughool et al., 2007](#)). In analyses limited to post-menopausal women, on the other hand, all showed between 10% and 80% reduced risks of endometrial cancer with the various smoking measures.

#### (b) Hormone replacement therapy

Given the possibility that cigarette smoking affects hormone concentrations mostly among women who are taking HRT ([Jensen et al., 1985](#); [Jensen & Christiansen, 1988](#); [Cassidenti et al., 1990](#)), the inverse association between tobacco

smoking and endometrial cancer risk might be stronger among HRT users than among non-users. However, the results of studies that have examined the association between smoking and endometrial cancer risk according to HRT use have been equivocal ([Weiss \*et al.\*, 1980](#); [Franks \*et al.\*, 1987](#); [Lawrence \*et al.\*, 1987](#); [Levi \*et al.\*, 1987](#); [Terry \*et al.\*, 2002b](#); [Beral \*et al.\*, 2005](#)). Whereas in two studies ([Franks \*et al.\*, 1987](#); [Levi \*et al.\*, 1987](#)) a larger reduction in risk among smokers taking HRT than among smokers not taking HRT was observed, in two other studies ([Lawrence \*et al.\*, 1987](#); [Terry \*et al.\*, 2002b](#)) there was no difference in the association according to HRT status. A cohort study that examined associations only among women using HRT showed no clear association among users of continuous combined HRT and cyclic combined HRT, but some suggestion of increased risk among smokers who used tibolone (perhaps more clearly among former smokers) ([Beral \*et al.\*, 2005](#)). Thus, although effect modification by HRT status is biologically plausible, the available epidemiological evidence is equivocal.

#### (c) *Relative body weight*

Obesity is an established risk factor for endometrial cancer ([IARC, 2002](#)). Smokers tend to have a lower BMI than non-smokers, although former smokers tend to have a higher BMI than current or never smokers ([Baron \*et al.\*, 1990](#)). Two case-control studies have examined the association between cigarette smoking and endometrial cancer risk according to BMI, one population-based ([Elliott \*et al.\*, 1990](#)) and one hospital-based ([Levi \*et al.\*, 1987](#)). Neither of these studies found clear differences in the association between smoking and endometrial cancer risk according to BMI. In a population-based case-control study of early stage endometrial cancer ([Lawrence \*et al.\*, 1987](#)), the inverse association with cigarette smoking tended to become stronger with increasing absolute rather than relative body weight.

#### 2.14.4 *Gene polymorphisms*

Cigarette smoking and estrogen are both thought to influence cancer risk through pathways that are under the control of specific genes, such as those involved in the formation of bulky DNA adducts by estrogen metabolites ([Cavalieri \*et al.\*, 2000](#)) and both bulky and non-bulky adducts formed by carcinogens in tobacco smoke ([Terry & Rohan, 2002](#)). Therefore, studies have been conducted to examine the association between smoking and endometrial cancer risk according to genes that repair these types of DNA damage. In a moderately-sized population-based case-control study no clear effect modification according to certain polymorphisms in the *XPA* and *XPC* genes, both of which are involved in the nucleotide excision repair of bulky DNA adducts and may influence endometrial cancer risk, were found ([Weiss \*et al.\*, 2005, 2006b](#)). A nested case-control study also showed no clear effect modification according to three polymorphisms in *CYP1A1* ([McGrath \*et al.\*, 2007](#)), a gene that encodes microsomal CYP1A1, which contributes to aryl hydrocarbon hydroxylase activity, catalysing the metabolism of PAHs and other carcinogens found in tobacco smoke ([Masson \*et al.\*, 2005](#)). In another nested case-control study some evidence was found that the association between smoking and endometrial cancer may vary according to a polymorphism (Ile<sup>143</sup>Val) in *O*<sup>6</sup>-methylguanine DNA methyltransferase (*MGMT*). Overall, studies that address the association between smoking and endometrial cancer risk according to genotype are scarce.

### 2.15 Cancer of the prostate

Many epidemiological studies have examined the association between cigarette smoking and prostate cancer risk, and most have shown no consistent association ([Hickey \*et al.\*, 2001](#); [Levi & La Vecchia, 2001](#); [Batty \*et al.\*, 2008](#); [Butler](#)



*et al.*, 2009; *Huncharek et al.*, 2010; Table 2.74 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.74.pdf>; Table 2.75 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.75.pdf>). However, questions remain regarding whether smoking may alter risk in various population subgroups, for example, those defined by certain genotypes, and whether any association with smoking may be stronger for, or limited to, advanced tumours or prostate cancer mortality. Regarding this latter issue, the majority of epidemiological studies, including several large, long-term cohort studies, have reported a positive association between smoking and prostate cancer mortality (*Rohrmann et al.*, 2007; *Zu & Giovannucci*, 2009). Several studies that examined smoking in relation to both prostate cancer incidence and mortality tend to show positive results only for the latter (*Rohrmann et al.*, 2007; *Zu & Giovannucci*, 2009). Given the largely null results with respect to prostate cancer incidence, the latter findings suggest that smoking is less likely to be a causal agent in prostate cancer initiation than an agent that acts on existing tumours to promote their progression (*Zu & Giovannucci*, 2009).

A recent review of smoking and prostate cancer that focused specifically on aggressive and fatal tumours, considered the findings from 14 cohort studies (*Zu & Giovannucci*, 2009). Nine of these studies showed statistically significant increased risk with at least one smoking measure, and five showed increased risks that were not statistically significant for any measure. Only one study showed no association with any measure of tobacco consumption. Seven studies of various designs examined smoking with respect to indicators of cancer aggressive behaviour at the time of diagnosis. In these studies smoking was associated positively with tumour grade, risk of regional, distant, extraprostatic or metastatic disease, Gleason score, and biochemical outcome (failure) after prostate brachytherapy

and in several dose–response associations with the respective endpoint were demonstrated. In one study smoking cessation was associated with a decline in risk compared with that among current smokers.

The association between smoking and prostate cancer risk according to genotype and other potentially effect-modifying factors have been examined in several studies. For example, in a population-based case–control study tobacco use was a risk factor for prostate cancer primarily among men with high BMI (*Sharpe & Siemiatycki*, 2001). The results of a cohort study in Switzerland suggest that risk of prostate cancer mortality is increased in smokers, particularly those with low plasma vitamin E levels (*Eichholzer et al.*, 1999). These latter associations, as well as those regarding several genotypes that may modify the association (*Mao et al.*, 2004; *Nock et al.*, 2006; *Quiñones et al.*, 2006; *Yang et al.*, 2006; *Iguchi et al.*, 2009; *Kesarwani et al.*, 2009), have yet to be fully clarified.

[The Working Group noted that several of the studies of smoking and prostate cancer mortality did not demonstrate clear dose–response associations with risk, and noted the possibility of bias due to confounding by screening behaviour. However, in the Health Professionals Follow-up Study, screening behaviour was not found to differ appreciably between smokers and non-smokers. In an analysis limited to men with a negative digital rectal examination in the prior two years, stronger associations were found between smoking and metastatic prostate cancer risk among high intensity smokers (RR, 4.2; 95%CI: 1.6–10.9) (*Zu & Giovannucci*, 2009). This finding was evidence against bias from screening behaviour.]

## 2.16 Cancer of the ovary

### 2.16.1 Overview of studies

A total of over 30 epidemiological studies have investigated the association between tobacco smoking and ovarian cancer risk. Of these, 24 were case-control studies (IARC, 2004a; Table 2.76 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.76.pdf>; Table 2.77 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.77.pdf>) and six were cohort studies (IARC, 2004a; Table 2.78 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.78.pdf>; Table 2.79 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.79.pdf>). Most studies showed no statistically significant association between a measure of smoking and risk for ovarian cancer overall (Newhouse *et al.*, 1977; Smith *et al.*, 1984; Tzonou *et al.*, 1984; Baron *et al.*, 1986; Stockwell & Lyman, 1987; Whittemore *et al.*, 1988; Hartge *et al.*, 1989; Polychronopoulou *et al.*, 1993; Engeland *et al.*, 1996; Goodman *et al.*, 2001; Goodman & Tung, 2003; Pan *et al.*, 2004; Zhang *et al.*, 2004; Kurian *et al.*, 2005; Niwa *et al.*, 2005; Baker *et al.*, 2006; Huusom *et al.*, 2006; Fujita *et al.*, 2008; Lurie *et al.*, 2008; Nagle *et al.*, 2008; Tworoger *et al.*, 2008); some showed positive associations (Doll *et al.*, 1980; Tverdal *et al.*, 1993; Kuper *et al.*, 2000b; Marchbanks *et al.*, 2000; Green *et al.*, 2001; Modugno *et al.*, 2002; Gram *et al.*, 2008; Rossing *et al.*, 2008) and one (Riman *et al.*, 2004) showed an inverse association.

### 2.16.2 Histological subtypes

Differences in ovarian cancer risk factor profiles have been observed according to histological type, on the basis of which it has been suggested that mucinous and non-mucinous tumours are etiologically distinct diseases (Risch *et al.*, 1996). Epidemiological studies that have considered histological type tend to support a

positive association primarily between cigarette smoking and mucinous ovarian tumours (Kuper *et al.*, 2000b; Marchbanks *et al.*, 2000; Green *et al.*, 2001; Modugno *et al.*, 2002; Pan *et al.*, 2004; Zhang *et al.*, 2004; Kurian *et al.*, 2005; Tworoger *et al.*, 2008). In contrast, two studies showed no clear association between smoking and risk of mucinous or non-mucinous ovarian tumours (Riman *et al.*, 2004; Baker *et al.*, 2006). In addition, one early case-control study (Newhouse *et al.*, 1977), with 300 ovarian cancer cases and with both population and hospital controls, found no clear association with “ever” compared with “never” smoking, and reported no differences according to histological type.

A pooled analysis of 10 case-control studies (Kurian *et al.*, 2005) with 254 cases of mucinous and 1580 non-mucinous tumours found an increased risk of mucinous tumours among current smokers (RR, 2.4; 95%CI: 1.5–3.8), a positive association that was not observed with other histological types. Former smokers in that analysis did not have an increased risk of any histological type of ovarian cancer. This type of dose-response, whereby current smokers have a higher risk than former smokers, was observed in most, but not all, studies of mucinous ovarian cancer (Tables 2.77 and 2.79 online). Overall, the positive association between cigarette smoking and risk of mucinous ovarian tumours is generally consistent across both case-control and cohort studies conducted among various populations. In contrast, associations with smoking have been mostly null with respect to non-mucinous ovarian tumours, suggesting that recall bias is unlikely to explain the association with mucinous tumours.

[The Working Group considered the possibility that women who smoke may come to medical attention more frequently. This raises the possibility of detection bias, because mucinous tumours, benign or malignant, tend to be quite large and could be more easily detected on routine physical exam or testing. However, the

Working Group felt that detection bias would not account for the association entirely.

## 2.17 Cancer of the thyroid

The previous *IARC Monograph* ([IARC, 2004a](#)) noted inconsistent associations between smoking and thyroid cancer risk. In 2003, a pooled analysis of 14 case–control studies showed that smoking was inversely associated with thyroid cancer risk ([Mack et al., 2003](#); Table 2.80 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.80.pdf>; Table 2.81 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.81.pdf>). The sample consisted of 2725 thyroid cancer cases (2247 women, 478 men) and 4776 controls (3699 women, 1077 men). The inverse association was stronger among current smokers (RR, 0.6; 9% CI: 0.6–0.7) than former smokers (RR, 0.9; 9% CI: 0.8–1.1) and were similar in both men and women, for both papillary and follicular thyroid cancers, as well as by age and region. An inverse association between smoking and thyroid cancer risk was also found in a subsequent case–control study ([Nagano et al., 2007](#)). In contrast, two case–control studies ([Zivaljevic et al., 2004](#); [Bufalo et al., 2006](#)) reported no clear association between smoking and thyroid cancer risk (no risk ratio estimates were reported; hence, data are not shown in the tables) and a cohort study with 169 incident cases of thyroid cancer, also found no clear association with any qualitative or quantitative smoking measure ([Navarro Silvera et al., 2005](#); Table 2.82 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.82.pdf>; Table 2.83 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.83.pdf>).

## 2.18 Other cancers

The cancers reviewed in this section generally have low incidence and mortality rates and are not considered to be strongly associated with cigarette smoking. This raises the possibility of preferential reporting of positive associations in epidemiological studies.

### 2.18.1 Cancer of the salivary gland

Studies of smoking and cancers of the salivary gland reviewed in the previous *IARC Monograph* ([IARC, 2004a](#)) were sparse and their results were inconsistent ([Spitz et al., 1990](#); [Swanson & Burns, 1997](#); [Hayes et al., 1999](#)). A few additional studies also show inconsistent results ([Kotwall, 1992](#); [Pinkston & Cole, 1996](#); [Horn-Ross et al., 1997](#); [Vories & Ramirez, 1997](#); [Muscat & Wynder, 1998](#)). Studies that focused specifically on Warthin's tumour [papillary cystadenoma lymphomatosum or adenolymphoma, a benign tumour of the parotid gland] tend to show strong positive associations with smoking ([Kotwall, 1992](#); [Pinkston & Cole, 1996](#); [Vories & Ramirez, 1997](#)). One study ([Pinkston & Cole, 1996](#)) compared the risk for Warthin's tumour with that for other salivary gland tumours and found that smoking increased risk significantly only for Warthin's tumour.

### 2.18.2 Cancer of the small intestine

Epidemiological studies (all of case–control design) reviewed in the previous *IARC Monograph* ([IARC, 2004a](#)) have been inconsistent in showing a positive association between smoking and cancers of the small intestine ([Chow et al., 1993b](#); [Chen et al., 1994](#); [Wu et al., 1997](#); [Negri et al., 1999](#); [Kaerlev et al., 2002](#)). A more recent study showed no clear association ([Hassan et al., 2008b](#)).

### 2.18.3 Cancers of the gallbladder and extra-hepatic bile ducts

Epidemiological studies of smoking and risk of cancers of the gallbladder and extra-hepatic bile ducts reviewed in the previous *IARC Monograph* ([IARC, 2004a](#)) tended to show null, weak, or moderately strong positive associations. More recent studies also tend to show either no clear association with biliary tract carcinoma/extra-hepatic cholangiocarcinoma ([Shaib et al., 2007](#); [Welzel et al., 2007](#)) or suggest positive associations with gallbladder/biliary cancers ([Pandey & Shukla, 2003](#); [Yagyu et al., 2008](#); [Grainge et al., 2009](#)). Attention should be paid to potential confounders, especially BMI, when considering the results of epidemiological studies of risk of cancers of the gallbladder and extra-hepatic bile ducts. Recent studies that statistically adjusted for BMI, on gallbladder disease risk ([Grainge et al., 2009](#)) or on extrahepatic biliary tract carcinoma risk ([Ahrens et al., 2007](#)), showed a positive and null association with smoking, respectively. To date, there are too few studies with adequate control for potentially confounding factors to determine any clear pattern.

### 2.18.4 Soft-tissue sarcoma

As reported in the previous *IARC Monograph* ([IARC, 2004a](#)), one cohort study found an association between cigarette smoking and mortality from soft-tissue sarcoma after 26 years of follow-up but no dose-response relationship with the number of cigarettes/day, duration of smoking or pack-years ([Zahm et al., 1992](#)). No effect of cigarette smoking was detected in an Italian hospital-based case-control study ([Franceschi & Serraino, 1992](#)).

### 2.18.5 Cancer of the skin

#### (a) Melanoma

Several case-control studies found no difference in the prevalence of tobacco smoking between patients with malignant melanoma and controls, and one study found an inverse association ([IARC, 2004a](#)). An inverse association with smoking was also found in the US Radiologic Technologists cohort Study ([Freedman et al., 2003a](#)). In that study, smoking for at least 30 years compared with never smoking was inversely related to melanoma risk (RR, 0.6; 95%CI: 0.3–1.3), though risk was not associated with number of cigarettes/day. An inverse association was also observed in a cohort of Swedish construction workers ([Odenbro et al., 2007](#)). In this study, the risk for malignant melanoma was reduced in a dose-dependant manner for both cigarette and pipe smokers. The possibility that smoking may reduce the risk for melanoma should, therefore, be considered.

#### (b) Non-melanoma skin cancer

Four studies showed a positive association between smoking and non-melanoma skin cancer risk ([De Stefani et al., 1995](#); [Wojno, 1999](#); [Smith & Randle, 2001](#); [Boyd et al., 2002](#)), and two found no clear association ([van Dam et al., 1999](#); [Corona et al., 2001](#)). When distinguishing between histological subtypes, tobacco smoking was linked to the incidence of squamous-cell carcinoma of the skin in most studies, whereas the results for basal cell carcinoma remain inconsistent ([Zak-Prelich et al., 2004](#)). No clear association between smoking and risk for basal cell carcinoma was found in a cohort study ([Freedman et al., 2003b](#)).

### 2.18.6 Cancer of the penis

Case-control studies of smoking and penile cancer ([Hellberg et al., 1987](#); [Daling et al., 1992, 2005](#); [Maden et al., 1993](#); [Harish & Ravi, 1995](#);



Table 2.84 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.84.pdf>; Table 2.85 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.85.pdf>) and reviews of studies of smoking and penile cancer and population surveys (Dillner *et al.*, 2000; Favorito *et al.*, 2008; Bleeker *et al.*, 2009; Table 2.86 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.86.pdf>; Table 2.87 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.87.pdf>) consistently showed a positive association. In most studies there was a dose–response relationship, with higher risks among those with increased smoking intensity and/or duration. A study in Brazil showed a positive correlation with penile tumour grade (Favorito *et al.*, 2008). Based on the two reviews (Dillner *et al.*, 2000; Bleeker *et al.*, 2009), relative risks were generally increased twofold to fivefold among smokers.

Most studies did not adjust for HPV infection. In one case–control study (Daling *et al.*, 2005), current smoking was associated with a 160% increased risk of HPV-positive penile cancer ( $n = 75$ ), and a 180% increased risk of HPV-negative penile cancer ( $n = 19$ ), suggesting no important effect modification.

### 2.18.7 Cancer of the testis

Studies reviewed in the previous IARC Monograph (IARC, 2004a) showed no association between cigarette smoking and risk for testicular cancer. More recently, two case–control studies showed positive associations with smoking, one in Canada (Srivastava & Kreiger, 2004) and one in the Czech Republic (Dusek *et al.*, 2008).

### 2.18.8 Cancer of the central nervous system

A recent meta-analysis was conducted on smoking in relation to glioma risk (Mandelzweig *et al.*, 2009), which included 17 epidemiological

studies (6 cohort and 11 case–control). It was concluded that smoking is not associated with risk of glioma, despite a small significant increased risk seen in cohort studies. A recent cohort study found no association between smoking and carcinoma of the brain (Batty *et al.*, 2008). There have been no consistent associations of smoking with other CNS tumours (IARC, 2004a). In a population-based case–control study in the USA, smoking was associated with increased risk of intracranial meningioma in men (OR, 2.1; 95%CI: 1.1–4.2) but not in women (Phillips *et al.*, 2005).

### 2.18.9 Cancer of the adrenal gland

Data on risk factors for adrenal carcinoma are sparse. In the US Veterans' Study there was a fivefold increase in risk among current cigarette smokers during 26 years of follow-up, with risk being particularly high among those who smoked most intensely (Chow *et al.*, 1996). Other forms of tobacco use were associated with a statistically non-significant increase in risk. A case–control study in the USA found a twofold increase in risk for adrenal cancer among heavy smokers in men, but not in women (Hsing *et al.*, 1996).

## 2.19 Bidi smoking

### 2.19.1 Cancer of the oral cavity

#### (a) Overview of studies

The association between cancers of oral cavity and bidi smoking has been examined in 10 case–control studies conducted in India (Sankaranarayanan *et al.*, 1989a, b, 1990a; Rao *et al.*, 1994; Rao & Desai, 1998; Dikshit & Kanhere, 2000; Balaram *et al.*, 2002; Znaor *et al.*, 2003; Subapriya *et al.*, 2007; Muwonge *et al.*, 2008; Table 2.88 available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.88.pdf>). In these studies both cases and controls were interviewed and analyses were restricted

to men, except for the studies by [Balaram \*et al.\* \(2002\)](#) and [Subapriya \*et al.\* \(2007\)](#), because very few women smoked among study subjects.

Three hospital-based case-control studies considered cancers of subsites of the oral cavity (gingiva, tongue and floor of the mouth, buccal and labial mucosa) ([Sankaranarayanan \*et al.\*, 1989a, b, 1990a](#)). All three studies showed a higher oral cancer risk for bidi smoking. In one early study an unadjusted relative risk of 1.6 (95%CI: 1.3–2.0) for oral cancer in bidi smokers was reported ([Rao \*et al.\*, 1994](#)). [The Working Group noted that the study had several deficiencies, particularly in the selection of controls that resulted in cigarette smoking apparently being protective for oral cancer.] In another early study ([Rao & Desai, 1998](#)) relative risks were estimated after stratification by age and place of residence. Bidi smoking was a significant risk factor for cancer of the base of the tongue (RR, 5.9; 95%CI: 4.2–8.2) but not significant for cancer of the anterior tongue. Relative risk for bidi smoking adjusted for alcohol drinking, illiteracy, non-vegetarian diet and tobacco chewing showed significant risk for cancer of the base of the tongue (RR, 4.7; 95%CI: 3.5–6.3) but not for cancer of the anterior tongue. In a population-based case-control study a relative risk of 1.5 (95%CI: 0.9–2.4), adjusted for age and tobacco quid chewing for smokers (bidis and/or cigarettes), was found ([Dikshit & Kanhere, 2000](#)).

Two hospital-based multi centre case-control studies on cancer of the oral cavity were conducted in southern India. One included 309 cases and 292 controls ([Balaram \*et al.\*, 2002](#)). The risk for oral cavity cancer among those who smoked < 20 bidis per day was 2.0 (95%CI: 1.1–3.8) and 2.5 (95%CI: 1.4–4.4) for ≥ 20 per day. The second study included 1563 cases and 3638 controls and found a risk for bidi smoking only of 2.2 (95%CI: 1.75–2.63) compared to never smokers, adjusted for age, centre, level of education, alcohol consumption and chewing ([Znaor \*et al.\*, 2003](#)).

In a hospital-based case-control study with 388 oral squamous cell carcinoma cases (202 men and 186 women) and an equal number of age and sex-matched controls the effect of life-style factors (tobacco chewing, smoking and alcohol drinking, diet and dental care) on the risk of oral cancer was evaluated ([Subapriya \*et al.\*, 2007](#)). Both cases and controls were interviewed using a structured questionnaire. The risk estimate for bidi smoking based on 22 cases (84 cases included in the model) and 22 controls was 4.6 (95%CI not given).

Data from a randomized control trial conducted between 1996 and 2004 in Trivandrum, southern India were used in a nested case-control analysis with 282 (163 men and 119 women) incident oral cancer cases and 1410 matched population controls aged 35 years and over ([Muwonge \*et al.\*, 2008](#)). Oral cancer risk among men, adjusted for education and religion, was 1.9 (95%CI: 1.1–3.2) for bidi smokers only compared to never smokers. No association was found between mixed smoking of bidi and cigarette and risk of oral cancer.

[Rahman \*et al.\* \(2003\)](#) performed a meta-analysis to investigate the relationship between bidi smoking and oral cancer. They identified 12 case-control studies published in English during 1996–2002 with quantitative information on bidi smoking and oral cancer. Of these, ten studies were conducted in India, one in Sri Lanka and one in Pakistan. All cases were confirmed histologically and exposure data were collected by direct interview. In these studies ORs were not adjusted for tobacco chewing or alcohol drinking. The OR for bidi smokers compared to never smokers based on random effects model was 3.1 (95%CI: 2.0–5.0). The ORs ranged from 2.0 to 3.6 in different regions of India: studies conducted in Mumbai had an OR of 3.6 (95%CI: 1.6–7.9), in central India 2.7 (95%CI: 1.6–4.6), in Kerala 2.0 (95%CI: 1.5–2.9) and in Bangalore 2.0 (95%CI: 1.1–3.7).

*(b) Dose-response evidence*

The trends in relative risks by intensity and duration of bidi smoking were both statistically significant in two studies ([Rao et al., 1994](#); [Rao & Desai, 1998](#)). A meta-analysis based on three studies on duration of bidi smoking and on five studies on number of bidi sticks per day, showed a dose-response relationship for duration of bidi smoking but not for number of sticks used per day ([Rahman et al., 2003](#)).

In a nested case-control analysis ([Muwonge et al., 2008](#)) a dose-response relationship was observed for duration of bidi smoking ( $P = 0.045$ ). [It is not clear if the analysis was restricted to bidi smokers only ( $n = 40$  men) and if smokers with combined smoking habits (bidi and cigarette) were excluded. Moreover, ORs for the dose-response analysis were not reported.]

*2.19.2 Cancer of the pharynx*

Five case-control studies, two hospital-based ([Wasnik et al., 1998](#); [Rao et al., 1999](#)), one population-based ([Dikshit & Kanhere, 2000](#)) and two multicentric studies ([Znaor et al., 2003](#); [Sapkota et al., 2007](#)) were conducted on cancers of oropharynx and hypopharynx in India (Table 2.88 online). In all these studies, analyses were restricted to men because very few women smoked among study subjects.

[Wasnik et al. \(1998\)](#) conducted a case-control study on oropharyngeal cancers with cases and controls were matched on age and sex. Odds ratios for tobacco smoking, predominantly in the form of bidi and/or chillum, were 2.3 (95%CI: 1.2–3.7) after adjustment for tobacco chewing and outdoor occupation. [The Working Group noted some problems with the data analysis.]

[Rao et al. \(1999\)](#) reported a relative risk for bidi smoking adjusted for alcohol, illiteracy, diet and tobacco chewing of 4.7 (3.6–6.3) for oropharyngeal cancer and of 2.8 (2.1–3.7) for cancer of the hypopharynx. [Dikshit & Kanhere \(2000\)](#) found

an odds ratio for oropharyngeal cancer among bidi smokers only of 7.9 (95%CI: 5.1–12.4).

[Znaor et al. \(2003\)](#) reported a risk for bidi smoking only for pharyngeal cancer of 4.7 (95%CI: 3.5–6.3) and for combined bidi and cigarette smoking of 3.6 (95%CI: 2.55–4.98). [Sapkota et al. \(2007\)](#) reported an odds ratio for hypopharyngeal cancer of 6.8 (95%CI: 4.6–10.0) for bidi smokers compared to never smokers.

A dose-response relationship was observed for intensity and duration of bidi smoking for both cancers of oropharynx and hypopharynx ([Rao et al., 1999](#); [Dikshit & Kanhere, 2000](#); [Sapkota et al., 2007](#)).

*2.19.3 Cancer of the lung*

One cohort study ([Jayalekshmy et al., 2008](#)), one population-based case-control study ([Dikshit & Kanhere, 2000](#)) and two hospital-based case-control studies ([Gupta et al., 2001](#); [Gajalakshmi et al., 2003](#)) in India (Table 2.88 online) have investigated the relationship between bidi smoking and lung cancer. In all these studies both cases and controls were interviewed and analyses were restricted to men because very few women smoked among study subjects. One hospital-based case-control study in Chiang Mai, Thailand, looked at the association between lung cancer and *khii yoo*, hand-rolled cigars. The risk for lung cancer for *khii yoo* smoking was 1.2 in men and 1.5 in women,  $P > 0.05$  ([Simarak et al., 1977](#)).

In the population based case-control study by [Dikshit & Kanhere \(2000\)](#) the age-adjusted relative risk for lung cancer among bidi smokers only was 11.6 (95%CI: 6.4–21.3).

[Gupta et al. \(2001\)](#) reported an odds ratio for bidi smoking of 5.8 (95%CI: 3.4–9.7) from a hospital-based case-control study of lung cancer conducted in Chandigarh. [Gajalakshmi et al. \(2003\)](#) conducted a case-control study in two centres in which all subjects were interviewed by trained social investigators with standard

questionnaires. Odds ratios were adjusted for age, educational level, centre, chewing and alcohol habit. The odds ratios of lung cancer for former and current bidi smokers were 3.4 (95%CI: 2.1–5.4) and 5.3 (95%CI: 3.8–7.3), respectively. Odds ratios for former and current smokers of cigarette and bidi combined were 4.0 (95%CI: 2.5–6.6) and 9.1 (95%CI: 6.2–13.2), respectively.

Baseline data of a cohort of 359 619 residents in Kerala, India was collected by direct interview using standardized questionnaires during 1990–97 ([Jayalekshmy et al., 2008](#)). After excluding rare earth workers, those who died, were diagnosed with cancer before 1997 or died within three years of interview, there were 65 829 bidi-smoking men aged 30–84 years old. Two hundred and twelve lung cancer cases were identified by the Karunagappally Cancer Registry between 1997 and 2004. The relative risk for lung cancer for current compared to never bidi smokers calculated by Poisson regression analysis and adjusted for age, religion and education was 3.9 (95%CI: 2.6–6.0;  $P < 0.001$ ). The risk was lower among former than among current smokers.

#### (a) Dose–response evidence

Lung cancer risks increased with increasing bidi smoking intensities. The highest odds ratio was found for 9 pack-years (3.9; 95%CI: 2.1–7.1) ([Gupta et al., 2001](#)). In a cohort study [Jayalekshmy et al. \(2008\)](#) found increased lung cancer incidence with increasing number of bidi sticks smoked per day ( $P < 0.001$ ) and with increasing duration of bidi smoking ( $P < 0.001$ ). [The number of lung cancer cases was small in each category, resulting in wide confidence intervals.] [Gajalakshmi et al. \(2003\)](#) also reported increased risk with duration and intensity of bidi smoking.

#### (b) Cessation of smoking

In two case–control studies ([Gupta et al., 2001](#); [Gajalakshmi et al., 2003](#)) there was a clear decreasing trend in risk for years since quitting.

[Gajalakshmi et al. \(2003\)](#) reported that lung cancer risk of former bidi smokers fell to 0.4 (0.1–1.2) after quitting for more than 15 years. The cohort study conducted in Kerala did not have the power to assess the risk associated with stopping bidi smoking ([Jayalekshmy et al., 2008](#)).

#### 2.19.4 Cancer of the larynx

Two hospital based case–control studies ([Sankaranarayanan et al., 1990b](#); [Rao et al., 1999](#)) showed a higher risk for bidi smokers (Table 2.88 online). The relative risk was adjusted for age and religion in [Sankaranarayanan et al. \(1990b\)](#) study and for alcohol use, illiteracy, vegetarian/non-vegetarian diet and tobacco chewing in [Rao et al. \(1999\)](#) study. A multicentre case–control study on laryngeal cancer was conducted in four Indian centres using standardized questionnaires adjusting risks for centre, age, socio-economic status, alcohol consumption, tobacco snuffing and tobacco chewing ([Sapkota et al., 2007](#)). Compared to never smokers bidi smokers had a higher risk for cancers of the supraglottis (OR, 7.5; 95%CI: 3.8–14.7), glottis (OR, 5.3; 95%CI: 3.2–8.9) and rest of larynx (OR, 9.6; 95%CI: 5.6–16.4).

All levels of intensity and duration of bidi smoking were associated with significant relative risk estimates and dose–response for laryngeal cancer ([Sankaranarayanan et al., 1990b](#); [Rao et al., 1999](#)). A strong dose–response relationship was observed for duration and frequency of bidi smoking for cancers of supraglottis, glottis and rest of larynx ([Sapkota et al., 2007](#)).

#### 2.19.5 Cancer of the oesophagus

Three hospital-based case–control studies and one multicentre study ([Sankaranarayanan et al., 1991](#); [Nandakumar et al., 1996](#); [Nayar et al., 2000](#); [Znaor et al., 2003](#)) showed increased risk for oesophageal cancer among bidi smokers in India (Table 2.88 online). A significantly elevated



risk for all three segments of the oesophagus was reported ([Nandakumar et al., 1996](#)). One study ([Nayar et al., 2000](#)) adjusted for chewing of betel leaf with tobacco and low consumption of vegetables other than leafy vegetables. The multicentre case–control study conducted in two centres in South India found an increased risk for oesophageal cancer for bidi smoking only (OR, 3.3; 95%CI: 2.45–4.39) ([Znaor et al., 2003](#)). Odds ratios were adjusted for age, centre, level of education, alcohol consumption and chewing. Only men were analysed in all the above studies.

Significant effects were noted in men for all levels of intensity and for duration of more than 20 years of bidi smoking ([Sankaranarayanan et al., 1991](#)).

### 2.19.6 Cancer of the stomach

In a hospital-based case–control study the association between stomach cancer and bidi smoking was analysed as part of a multicentre study ([Gajalakshmi & Shanta, 1996](#)). Cases and controls were matched on age, sex, religion and mother tongue. The odds ratio for stomach cancer for current bidi smokers only was 3.2 (95%CI: 1.8–5.7) and for current smokers of any type of tobacco was 2.7 (95%CI: 1.8–4.1).

Table 2.88 (online) summarizes the studies published since the last *IARC Monograph* ([IARC, 2004a](#)). A hospital-based case–control study of stomach cancer included 170 stomach cancer cases (121 men and 49 women) and 2184 controls (1309 men and 875 women) aged 30–75 years ([Rao et al., 2002](#)). The association between bidi smoking and stomach cancer was not significant (RR, 0.8; 95%CI: 0.5–1.2) in a univariate analysis. The risk increased with increase in lifetime exposure to bidi smoking and was highly significant ( $P < 0.001$ ).

One study investigated stomach cancer risk in association with smoking of *meiziol*, a local cigarette in Mizoram, India ([Phukan et al., 2005](#)). Statistically significant higher risks were seen for

smokers of combined users of tobacco (cigarette and *meiziol*), with an odds ratio of 3.1 (95%CI: 2.0–11.1). Among users of a single type of tobacco, higher risks were seen for *meiziol* smokers (OR, 2.2; 95%CI: 1.3–9.3) in the multivariate model in comparison to cigarette smokers. Overall, the excess risk was limited to smokers of  $> 10$  *meiziols* per day.

## 2.20 Synergistic effects of tobacco smoking and alcohol drinking

This section addresses the combined effects of smoking and alcohol consumption on cancers of oral cavity, pharynx, larynx and oesophagus, which have been examined extensively. For the purposes of this report interdependence of effects is termed *effect modification*, and *synergism* and *antagonism* are used to describe the consequences of the interdependence of disease risk when both risk factors are present ([Rothman & Greenland, 1998](#)). The studies varied in their methods and in the approaches used to assess effect modification, which ranged from descriptive to formal estimation of interaction terms in multivariate models. Study designs of the case–control and cohort studies are presented in Table 2.89 (available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.89.pdf>) and Table 2.90 (available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.90.pdf>), respectively; and the results for both study designs are presented in Table 2.91 (available at <http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.91.pdf>).

### 2.20.1 Cancers of the upper aerodigestive tract

It was noted in the previous *IARC Monograph* ([IARC, 2004a](#)) with relatively large numbers of cases and controls that the pattern of increasing cancer risk with increasing alcohol consumption is strong ([Mashberg et al., 1993](#); [Kabat et al., 1994](#)).

For cancers of the oral cavity, recent evidence comes from seven case-control studies and one cohort study. The pattern of odds ratios for smoking, across categories of alcohol consumption, is consistent with synergism. In four case-control studies with relatively large numbers of cases and controls (more than 200 cases and equivalent number of controls), the pattern of increasing cancer risks with increasing alcohol consumption was strong ([Schlecht \*et al.\*, 1999b](#); [Znaor \*et al.\*, 2003](#); [Castellsagué \*et al.\*, 2004](#); [Hashibe \*et al.\*, 2009](#)). In the cohort study from Taiwan, China ([Yen \*et al.\*, 2008](#)) similar strong risks were also observed. In all four case-control studies in which the estimate of formal statistical interaction was examined, the tests were statistically significant ([Schlecht \*et al.\*, 1999b](#); [Znaor \*et al.\*, 2003](#); [Castellsagué \*et al.\*, 2004](#); [Hashibe \*et al.\*, 2009](#)). In two case-control studies from India ([Znaor \*et al.\*, 2003](#); [Muwonge \*et al.\*, 2008](#)) and in the cohort study from Taiwan, China ([Yen \*et al.\*, 2008](#)) the interaction of tobacco smoking, alcohol and betel quid chewing was examined. In general, the results suggested increasing risks when betel quid chewing was included in the model.

Five case-control studies and one cohort study examined the effect of interaction between tobacco and alcohol in pharyngeal cancer. The results from case-control studies were similar to those observed for oral cancer ([Olsen \*et al.\*, 1985b](#); [Choi & Kahyo, 1991](#); [Schlecht \*et al.\*, 1999b](#); [Znaor \*et al.\*, 2003](#); [Hashibe \*et al.\*, 2009](#)). In a Singapore cohort study ([Friborg \*et al.\*, 2007](#)) the pattern of odds ratios for smoking across categories of alcohol consumption was consistent with synergism for oropharyngeal but not for nasopharyngeal cancer.

Two cohort and fourteen case-control studies reported on joint effects of tobacco smoking and alcohol drinking on the risk for oesophageal cancer. Since multiple logistic regression models were used for analysing most of these studies, some of them tested likelihood ratio test

for departure from multiplicativity of the individual effects of tobacco and alcohol. Generally, the positive results were stronger for squamous cell carcinoma. However, these tests for interaction are inadequate to assess synergy. Four studies from India and Taiwan, China, included betel quid chewing to the joint effect analysis of tobacco smoking and alcohol consumption and the results suggested increasing risks of oesophageal cancer.

Most of the twenty case-control studies of laryngeal cancer provided strong evidence for synergism of tobacco smoking and alcohol consumption. Only [Zheng \*et al.\* \(1992\)](#) did not find consistent evidence with synergism. In several studies, tests for interaction were carried out and reported to be 'non significant.' These were tests for departure from the multiplicative models, typically multiple logistic regression models, used to analyse the case-control data, and not tests for departure from additive model.

Several studies (14 case-control, 3 cohort) reported on cancer of the 'mixed upper aerodigestive tract', comprising studies on squamous cell carcinomas, regardless of specific sites. These studies also provided strong evidence for synergism.

The Working Group considers that there is strong evidence of tobacco smoking and alcohol consumption interaction on the incidence of upper aerodigestive tract cancers, as well as with regard to cancer of specific subsites of this anatomical region.

## 2.21 Synthesis

### 2.21.1 Lung

Tobacco smoking is the major cause of lung cancer, primarily from cigarettes. Duration of smoking is the strongest determinant of lung cancer in smokers. Risk also increases in proportion to the number of cigarettes smoked. The strong dose- and duration-response

relationships between lung cancer and tobacco smoking have been confirmed more recently in both questionnaire-based and biomarker-based studies. Tobacco smoking increases the risk of all histological types of lung cancer.

Differences in the intensity and/or duration of tobacco smoking may explain, in part, the lower lung cancer risks in Asian populations relative to whites. However, several studies of genetic polymorphisms among African-American and Caucasian populations provide some preliminary evidence supporting the hypothesis of a racial/ethnic disparity in susceptibility.

The results from observational studies do not provide strong support that a higher intake or a greater circulating concentration of carotenoids reduce lung cancer risk, particular in light of the elevated risk of lung cancer observed in the randomized trials of  $\beta$ -carotene supplementation. Residual confounding from smoking and the possibility that carotenoid measurements are serving as markers for a diet rich in total fruit and vegetables mitigate the likelihood of any protective role for total carotenoids or  $\beta$ -cryptoxanthins.

The specific genes that are responsible for enhanced lung cancer risk remain poorly understood, in spite of hundreds of candidate gene studies. Single-gene studies conducted to date have several limitations which contribute to inconclusive results, including small sample size and associated low power to detect moderate risks when allele frequencies are low.

### 2.21.2 Upper aerodigestive tract

#### (a) Oral cavity

Tobacco smoking is causally associated with cancer of the oral cavity in both men and women. Since the previous *Monograph*, additional evidence has accumulated that further confirms the association. Risk increases with duration and intensity of smoking, and decreases after quitting.

#### (b) Pharynx

Tobacco smoking is an important cause of oropharyngeal and hypopharyngeal cancers. The risk increases with increasing duration and intensity of smoking and decreases with increasing time since quitting.

#### (c) Nasal cavity and accessory sinuses

The evidence of an association between tobacco smoking and sinonasal cancer is based on the results from case-control studies, each of which may be subject to different sources of bias. However, presence of a dose-response relationship in most studies, the decrease in risk associated with time since quitting, the consistently higher risks for squamous-cell carcinoma than for adenocarcinoma and the lack of potential confounders support the existence of a causal association.

#### (d) Nasopharynx

Although the interpretation of the results is complicated by small sample sizes in several studies, by different criteria used for the selection of controls and by the control groups in some studies including smoking-related diseases, the combined evidence shows an association between tobacco smoking and nasopharyngeal carcinoma in both endemic and non-endemic areas. Most studies that adjusted for known and suspected causes of nasopharyngeal carcinoma such as intake of Chinese-style salted fish, other dietary factors, alcohol drinking and family history of nasopharyngeal carcinoma, suggested only a limited confounding effect of these factors. Adjustment for infection with Epstein-Barr virus (human herpes virus 4), a major cause of nasopharyngeal carcinoma worldwide, was possible in just one of the available studies. However, it is unlikely that confounding by infection with Epstein-Barr virus would explain the observed association between tobacco smoking and risk for nasopharyngeal carcinoma.

*(e) Oesophagus*

Several well conducted case-control studies found a statistically significant higher risk for adenocarcinoma of the oesophagus in smokers than in nonsmokers. Positive dose-response relationships obtained using various indicators of amount smoked support a causal association, which is further corroborated by the findings of decreasing risks after smoking cessation. Several of these studies reported relative risks adjusted for alcohol consumption and other potential confounders. Further risk factors, such as chewing betel quid with tobacco or use of other forms of smokeless tobacco, have not been considered in these populations, but are not likely to be strong confounders. Studies from Australia, China and Europe also found increased risks for smokers.

*(f) Larynx*

Laryngeal cancer is one of the cancers most strongly associated with cigarette smoking. Recent epidemiological evidence strengthens this conclusion.

*2.21.3 Stomach*

The additional epidemiologic data showing a consistent association of stomach cancer with tobacco smoking in both men and women greatly strengthens the previous conclusion of a causal association. There was insufficient evidence for differential risks between cardia and non-cardia stomach cancer. Confounding and effect modification by *H. pylori* has not been found.

*2.21.4 Pancreas*

The additional data supports the previous evaluation that cancer of the pancreas is causally associated with tobacco smoking. The risk increases with increasing daily consumption levels and duration of smoking and decreases with increasing time since cessation of smoking.

The risk remains elevated after accounting for potential confounding factors.

*2.21.5 Colorectum*

At the previous evaluation, there was already some evidence from prospective cohort and case-control studies that the risk of colorectal cancer is increased among tobacco smokers. However, inadequate adjustment for various potential confounders was considered to possibly account for some of the small increase in risk that appears to be associated with smoking. Since then, an appreciable amount of data has accumulated to support a causal association with smoking. In virtually all the cohort studies published since elevated risk associated with smoking was found, although not always statistically significant. More than half of the cohort studies that assessed dose-response relationships found statistically significant increasing risks with increasing daily cigarette consumption, duration of smoking and/or pack-years of smoking. Risk of colorectal cancer decreased with increasing delay in smoking initiation and years since cessation of smoking. A meta-analysis based on 36 cohort studies with data from a total of 3 million subjects found a significantly 15% increased risk of colorectal cancer and 27% higher risk of colorectal cancer mortality in current smokers compared to never smokers. A stronger association with smoking for rectal cancer than for colon cancer was found in the meta-analysis of the subset of cohort studies that differentiated colorectal cancer by site. Risk for colorectal cancer increased significantly by 17% and by 38% with 20 cigarettes and 40 cigarettes/day, respectively, and was elevated by 9.4% and by 19.7% with a 20-year and a 40-year duration of smoking, respectively. While these results are persuasive, this meta-analysis could not correct for the potential confounders in the individual studies. Convincing evidence has been provided by three large cohort studies that adjusted for at



least four important potential confounders (i.e. physical activity, alcohol consumption, body mass index and dietary intake of fruits and vegetables and/or meat); two studies also adjusted for history of colonoscopy. Significant dose–response relationships were found with one or more of the smoking variables, for risk of colorectal cancer and/or colon cancer and/or rectal cancer. Earlier cohort studies may not have been able to establish the association because of insufficient follow-up time and a limited number of cases. Updated results of several large cohort studies, which now show clearly significant increased risk of colorectal cancer associated with smoking, provide support for the lag-time hypothesis for smoking and colorectal risk.

Recent evidence suggests that smoking may be associated with the subtype of colorectal cancer characterized by microsatellite instability, and by CIMP status and BRAF mutation. For this subtype, the magnitude of risk associated with smoking reaches the twofold risk elevation consistently observed for colorectal adenomas and supported by a recent meta-analysis. Smoking has been associated with a stronger risk for hyperplastic polyps than for adenomas. Also, CIMP positivity and BRAF mutations have been associated with hyperplastic polyps, particularly serrated polyps. These data suggest that smoking may be associated primarily with a subtype of colorectal cancer that develops through a hyperplastic (serrated) polyp progression. The association with smoking may therefore be diluted when considering colon cancers overall.

#### 2.21.6 Liver

Recent studies on smoking and hepatocellular carcinoma supports the established causal relationship. Supporting evidence comes from the consistency of the findings across regions (with the best evidence coming from Asian studies), and the observations of an association among non-drinkers and after controlling for hepatitis B or C virus infection.

#### 2.21.7 Kidney

Recent evidence supports a causal association between kidney cancer and smoking. After adjustment for body mass index and hypertension, current and former smokers still had a greater risk for renal-cell cancer. A dose–response relationship with the number of cigarettes smoked has been noted in most studies, and a few also noted a reduction in risk after cessation.

#### 2.21.8 Urinary bladder

Tobacco smoking is causally associated to bladder cancer, based on a large number of case–control and cohort studies that showed statistically significant associations not explained by confounding or bias. Risk increased with the duration of smoking and the number of cigarettes smoked. Also, stopping smoking at any age avoids the further increase in risk incurred by continued smoking. The evidence supporting a modulating role by *NAT2* polymorphisms is convincing.

#### 2.21.9 Myeloid leukaemia

There is evidence for a causal association of tobacco smoking with myeloid leukaemia.

#### 2.21.10 Breast

New evidence from cohort and case–control studies and from meta-analyses of genetic polymorphisms has become available since the previous *IARC Monograph* ([IARC, 2004a](#)). Results from seven new cohort studies consistently show a small overall association between current smoking and breast cancer incidence, with relative risk estimates ranging from 1.1–1.3 in studies with at least 100 exposed cases. The overall association is weaker than that observed with other cancers that have been designated as causally related to smoking, and the dose–response relationships (with years of smoking,

cigarettes smoked per day, age at initiation) are correspondingly small.

Emerging evidence from case-control studies suggests that inherited polymorphisms in the *NAT2* gene, which encode the slow acetylator phenotype, may modify (increase) the association between smoking and breast cancer. The p-value for interaction with pack-years of smoking as a continuous variable is statistically significant ( $P = 0.03$ ) and another small study published since this meta-analysis supports the conclusion. The potential for publication bias remains of concern.

It is biologically plausible that tobacco smoke could be causally related to breast cancer risk. There are multiple chemicals in tobacco smoke that are known to cause mammary cancer in rodents. These substances reach the breast in humans; some are stored in adipose tissue, and some can be detected in nipple aspirate and DNA adducts.

Hypotheses have been proposed to explain why numerous well conducted epidemiological studies have generally not observed strong or consistent associations between tobacco smoking and breast cancer. Underlying all of these is the theory that tobacco smoking may have both protective and detrimental effects on breast cancer risk, which cancel each other out and which could explain the atypical dose-response relationship that has been reported between tobacco smoke and breast cancer from some studies.

### 2.21.11 Cervix

The largely positive findings observed in studies of cohort design, the relatively high consistency of positive associations found for squamous-cell carcinoma of the cervix (but not adenocarcinomas) across all epidemiological studies, including those with adjustment for a wide range of potentially confounding variables, and the positive associations observed in studies

restricted to HPV-positive individuals, all argue against the observed positive association being due to recall or selection bias or confounding.

### 2.21.12 Endometrium

The results of epidemiological studies to date, including recent studies, largely show inverse associations of smoking with risk of postmenopausal endometrial cancer. However, the Working Group noted the few studies of premenopausal cancer that were less consistent, as well as indications of an increased risk among smokers in a recent multicentre European study.

### 2.21.13 Prostate

Many epidemiological studies have examined the association between cigarette smoking and prostate cancer risk, and most have shown no consistent association. The question remains whether smoking may alter risk in various population subgroups.

### 2.21.14 Ovary

A causal association between cigarette smoking and risk for mucinous ovarian tumours is indicated by 1) the consistency of the positive association across the large majority of ten pooled case-control studies and ten additional independent epidemiological studies of both case-control and cohort design, 2) the relatively strong magnitude of the association (typically greater than a doubling of risk among current smokers), 3) the tendency to show dose-response associations with risk, such that current smokers generally have higher risk than former smokers and the dose-response observed with measures of smoking intensity in some (but not all) studies, and 4) the specificity of the positive association with the mucinous histological type, which argues against recall bias as an explanation of the findings.

### 2.21.15 Thyroid

A pooled analysis of 14 case–control studies showed that smoking was inversely associated with thyroid cancer risk. Similar inverse associations were also observed in two subsequent case–control studies.

### 2.21.16 Other sites

There is inconsistent or sparse evidence for an association between tobacco smoking and other cancer sites that were considered by the Working Group.

### 2.21.17 Bidi smoking

Overall, bidi smoking increases the risk for cancers of the oral cavity, oropharynx, hypopharynx, larynx, lung, oesophagus and stomach.

## 3. Cancer in Experimental Animals

### 3.1 Mainstream tobacco smoke

#### 3.1.1 Mouse

There have been multiple studies of the carcinogenic potential of tobacco smoke in mice ([Table 3.1](#)). Lifetime exposure of several mouse strains to cigarette smoke failed to result in the production of lung tumours ([Harris & Negroni, 1967](#); [Otto & Elmenhorst, 1967](#); [Henry & Kouri, 1986](#)). However, studies involving lifetime exposure of C57BL mice to a mixture of flue-cured or air-cured cigarette smoke or to the gas phase of flue-cured cigarette smoke led to significant increases in the number of lung tumours (adenomas) ([Harris \*et al.\*, 1974](#)). Similarly, lifetime exposure of Snell's mice to the gas phase of cigarette smoke led to an increased incidence of lung adenocarcinomas ([Leuchtenberger & Leuchtenberger, 1970](#)). Exposure of B6C3F<sub>1</sub>

female mice to smoke for lifetime led to increased incidence of lung adenomas, bronchiolar papillomas and lung adenocarcinomas in smoke-exposed mice. In addition, the occurrence of squamous cell carcinomas of the nasal cavity in smoke-exposed mice was increased ([Hutt \*et al.\*, 2005](#)). In a recent study, Swiss mice were exposed whole-body to cigarette smoke for 120 days, starting within 12 hours of the birth. Smoke-exposed mice developed microscopic lung tumours beginning only 75 days after birth and reaching an overall incidence of 78.3% after 181–230 days. The mean lung tumour multiplicity was 6.1 and 13.6 tumours per mouse in males and females, respectively. In addition, malignant tumours, some of which may have had a metastatic origin, were detected in the urinary tract of smoke-exposed mice ([Balansky \*et al.\*, 2007](#)).

#### 3.1.2 Rat

Several studies have evaluated the carcinogenic potential of mainstream tobacco smoke in rats ([Table 3.1](#)). Exposure of Wistar rats to cigarette smoke for lifetime did not increase the lung tumour incidence ([Davis \*et al.\*, 1975](#)). In contrast, exposure of Fischer 344 rats to a mixture of non-filter cigarette smoke for 128 weeks resulted in an increased incidence of nasal and lung tumours. There was also an increase in subcutaneous sarcomas at forelimb ulceration sites ([Dalbey \*et al.\*, 1980](#)). CDF rats were exposed to low-dose cigarette smoke (LCS) or high-dose cigarette smoke (HCS) for 126 weeks. The incidence of lung tumours was significantly higher only in female rats that received HCS ([Finch \*et al.\*, 1995](#)). In a recent study, Fischer 344 rats received whole body exposure to smoke containing either 100 mg (LCS) or 250 mg (HCS) total particulate matter/m<sup>3</sup> for 30 months. This led to significant increases in the incidence of lung and nasal cavity tumours in male rats treated with HCS but not with LCS. In female rats, there were significant increases in the incidence of lung adenomas

in animals treated with HCS and of all lung tumours in animals treated with both LCS and HCS. There was also a significant increase in the occurrence of nasal cavity tumours in female rats treated with HCS ([Mauderly et al., 2004](#)).

### 3.1.3 Hamster

Four studies have evaluated the ability of mainstream tobacco smoke to induce tumours in hamsters ([Table 3.1](#)). Syrian golden hamsters were exposed to either a mixture of German reference cigarette smoke or of dark air-cured cigarette smoke for lifetime. There were increases in the incidence of laryngeal carcinomas in hamsters exposed to both smoke preparations ([Dontenwill et al., 1973](#)). In a subsequent study, hamsters were exposed to a mixture of German reference cigarette smoke containing 1.5 mg nicotine, 0.173 mg phenol and 12.7 mL carbon monoxide/cigarette for lifetime. The incidence of laryngeal tumours in smoke-exposed hamsters was higher than in controls ([Dontenwill et al., 1977](#)). BIO male hamsters exposed to a mixture of US reference smoke for 100 weeks developed laryngeal and nasopharyngeal tumours ([Bernfeld et al., 1974](#)). In a subsequent study, male BIO hamsters exposed to smoke from commercial British filter cigarettes developed higher incidence of laryngeal tumours than controls ([Bernfeld et al., 1979](#)).

## 3.2 Co-administration of tobacco smoke with known carcinogens and other agents

Study design and results of the studies on co-administration of tobacco smoke with known carcinogens and other agents are summarized in [Table 3.2](#).

### 3.2.1 Rat

#### (a) Benzo[a]pyrene

Wistar rats received a single intratracheal instillation of 2 mg benzo[a]pyrene followed by lifetime exposure to cigarette smoke. This treatment led to a low incidence of lung tumours that was not significantly higher than in controls ([Davis et al., 1975](#)). In another study Wistar rats were given intratracheal instillations of benzo[a]pyrene mixed with ferric oxide and exposed to cigarette smoke either during initiation and post-initiation or only after treatment with benzo[a]pyrene/ferric oxide (post-initiation). Inhalation of cigarette smoke during the initiation and post-initiation phases of carcinogenesis resulted in a higher lung tumour (squamous-cell carcinoma) multiplicity than that seen in rats exposed during the post-initiation phase only ([Gupta et al., 1990](#)).

#### (b) Radon progeny

Sprague-Dawley rats were exposed to radon progeny at cumulative doses of 4000, 500 or 100 work-level-months (WLM), with or without concurrent exposure to cigarette smoke by inhalation for one year. Rats exposed to 4000 WLM radon progeny, without exposure to smoke, developed lung carcinomas (17/50). Thirty four carcinomas were seen in 50 rats exposed to radon and cigarette smoke. The 500 WLM radon progeny group exposed to radon only had 2/28 lung carcinomas as compared with 8/30 rats exposed to radon and cigarette smoke. No tumours were observed in rats treated with 100 WLM radon and one carcinoma was seen among 30 rats exposed to 100 WLM radon and cigarette smoke. Seventy five percent of the lung tumours were squamous-cell carcinomas, 20% were adenocarcinomas, and the remainder were undifferentiated carcinomas ([Chameaud et al., 1982](#)).



**Table 3.1 Carcinogenicity in response to mainstream tobacco smoke in animals**

| Species, strain (sex)<br>Reference                                                                           | Animals/group at start<br>Dosing regimen<br>Duration                                                                                                                          | Lung burden                              | Results<br>Target organ<br>Incidence and/or multiplicity<br>of tumours (%)                                                                                                                                            | Significance                                                                 | Comments                                                                       |
|--------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------|--------------------------------------------------------------------------------|
| Mice, C57BL (M, F)<br><a href="#">Harris &amp; Negroni (1967)</a>                                            | 100 animals/sex/group<br>Nose-only, mixture of fresh non-filter cigarette smoke/<br>air (1/39, v/v), nicotine, 0.1 mg/mL; CO, 0.064% (v/v),<br>12 min/every other d; lifetime | Nicotine,<br>14–17 µg                    | Alveolar adenocarcinomas:<br>M–4/100 (alveolar adenocarcinoma)<br>Controls–0/100<br>F–4/100 (alveolar adenocarcinoma)<br>Controls–0/100                                                                               | $P = 0.06$<br>$P = 0.06$                                                     |                                                                                |
| Mice, C57BL and BLH (sex,<br>NR)<br><a href="#">Otto &amp; Elmenhorst (1967)</a>                             | 126 animals/group<br>Whole-body, gas phase of 12 cigarettes puffed 2 sec/<br>min, concentration (NR), 90 min/d; lifetime (~27 mo)                                             | NR                                       | Lung (adenomas):<br>C57BL–7/126 (5.5%)<br>Controls–3/90 (3%)<br>BLH–40/126 (32%)<br>Controls–19/60 (32%)                                                                                                              | NS<br>NS                                                                     |                                                                                |
| Mice, (C57BL/Cum × C3H/<br>Anf Cum) <sub>F<sub>1</sub></sub> (F)<br><a href="#">Henry &amp; Kouri (1986)</a> | 2053, 1 014 sham<br>Nose-only, 10% smoke from US reference cigarettes,<br>concentration (NR), smoke 20 sec/min, 6–8 min/d,<br>5 d/wk for 110 wk; 116 wk                       | Particulate<br>deposition,<br>125–200 µg | Alveolar adenocarcinomas:<br>19/978 (2%)<br>Sham-exposed controls–7/651<br>(1%)                                                                                                                                       | $P = 0.10$                                                                   | Shorter latency of<br>tumour occurrence<br>in smoke-exposed<br>group suggested |
| Mice, C57BL (M, F)<br><a href="#">Harris et al. (1974)</a>                                                   | 100 animals/sex/group<br>Nose-only, mixture of fresh flue-cured or air-cured<br>cigarette smoke/air (1/39, v/v), concentration (NR), 12<br>min/d on alternate d; lifetime     | NR                                       | M: 9/162 <sup>a</sup> (5%, flue-cured),<br>7/189 <sup>a</sup> (4%, air-cured)<br>Controls–3/160 <sup>a</sup> (2%)<br>F: 7/164 <sup>a</sup> (4%, flue-cured), 0/173<br>(air-cured)<br>Controls–1/159 <sup>a</sup> (1%) | $P = 0.07$ , flue-cured<br>$P > 0.05$ , air-cured<br>$P = 0.04$ , flue-cured |                                                                                |
|                                                                                                              | Nose-only, gas phase of flue-cured cigarette smoke,<br>concentration (NR), 12 min/d on alternate d; lifetime                                                                  | NR                                       | M: 3/8 <sup>a</sup> (37%)<br>Controls–3/160 <sup>a</sup> (2%)<br>F: 2/88 <sup>a</sup> (2%)<br>Controls–1/159 <sup>a</sup> (1%)                                                                                        | $P > 0.05$<br>$P > 0.05$                                                     |                                                                                |

Table 3.1 (continued)

| Species, strain (sex)<br>Reference                                               | Animals/group at start<br>Dosing regimen<br>Duration                                                                                                                                  | Lung burden    | Results<br>Target organ<br>Incidence and/or multiplicity<br>of tumours (%) | Significance                 | Comments |
|----------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------|----------------------------------------------------------------------------|------------------------------|----------|
| Mice, Snell (M, F)<br><a href="#">Leuchtenberger &amp; Leuchtenberger (1970)</a> | 160 M, 118 F<br>Whole-body, whole fresh cigarette smoke, concentration (NR), 2 puffs, 1 × /d, lifetime (26 mo)                                                                        | Nicotine, 5 µg | M:<br>Lung A-7/107 (6.5%)<br>Controls-8/106 (7.5%)                         | NS                           |          |
|                                                                                  |                                                                                                                                                                                       |                | Lung AdC-11/107 (10%)<br>Controls-5/106 (4.7%)                             | P = 0.15                     |          |
|                                                                                  |                                                                                                                                                                                       |                | F:<br>Lung A-2/65 (3%)<br>Controls-1/78 (1.2%)                             | P = 0.475                    |          |
|                                                                                  |                                                                                                                                                                                       |                | Lung AdC-5/65 (7.7%)<br>Controls-3/78 (3.8%)                               | P = 0.035                    |          |
|                                                                                  |                                                                                                                                                                                       | NR             | M:<br>Lung A-1/44 (2%)<br>Controls-8/106 (7%)                              | NS                           |          |
|                                                                                  |                                                                                                                                                                                       |                | Lung AdC-10/44 (23%)<br>Controls-5/106 (5%)                                | P = 0.005                    |          |
|                                                                                  |                                                                                                                                                                                       |                | F:<br>Lung A-3/44 (7%)<br>Controls-1/78 (1%)                               | P = 0.15                     |          |
|                                                                                  |                                                                                                                                                                                       |                | Lung AdC-5/44 (11%)<br>Controls-3/78 (4%)                                  | P = 0.15                     |          |
|                                                                                  |                                                                                                                                                                                       |                | Lung A: 93/330 (28%)<br>Sham-exposed controls-22/326 (7%)                  | P < 0.001                    |          |
|                                                                                  |                                                                                                                                                                                       |                | Bronchiolar papillomas: 15/330 (4%)<br>Controls-0/326                      | P < 0.001                    |          |
| Mice, B6C3F1 (F)<br><a href="#">Hutt et al. (2005)</a>                           | 330, 326 controls<br>Whole-body, smoke from Kentucky 2R1 unfiltered reference cigarettes, 250 mg total particulate matter/m <sup>3</sup> , 6 h/d, 5 d/wk for 30 mo; 30 mo or lifetime | NR             | Lung AdC: 67/330 (20%)<br>Controls-9/326                                   | P < 0.001                    |          |
|                                                                                  |                                                                                                                                                                                       |                | All lung tumours: 148/330 (45%)<br>Controls-31/326                         | P < 0.001 <sup>b</sup>       |          |
|                                                                                  |                                                                                                                                                                                       |                | Nasal cavity tumours: 20/330 (6%)<br>Controls-0/326                        | P = 0.002, one-tailed Fisher |          |
|                                                                                  |                                                                                                                                                                                       |                | Squamous-cell carcinomas: 9/330 (3%)<br>Controls-0/326                     |                              |          |
|                                                                                  |                                                                                                                                                                                       |                |                                                                            |                              |          |
|                                                                                  |                                                                                                                                                                                       |                |                                                                            |                              |          |

**Table 3.1 (continued)**

| Species, strain (sex)<br>Reference                                             | Animals/group at start<br>Dosing regimen<br>Duration                                                                                                                                                                   | Lung burden                             | Results<br>Target organ<br>Incidence and/or multiplicity<br>of tumours (%)                                                                                                                                  | Significance                                    | Comments |
|--------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------|----------|
| Mice, Swiss (M, F)<br><a href="#">Balansky <i>et al.</i> (2007)</a>            | 38, 36 controls (neonatal mice, 21 h of age)<br>Whole-body, cigarette smoke/air, 818 mg total<br>particulate matter/m <sup>3</sup> , 65 min/d for 120 d                                                                | NR                                      | Lung A: 15/38 (19%)<br>Sham-exposed controls–0/36<br>Lung AdC: 7/38 (18%)<br>Controls–0/38<br>Kidney A: 6/16 (16%) (F only)<br>Controls–0/21<br>Liver carcinomas: 2/16 (5%) (F<br>only)<br>Controls–0/21    | $P < 0.001$<br><br>$P < 0.01$<br><br>$P < 0.01$ |          |
| Rats, Wistar (F)<br><a href="#">Davis <i>et al.</i> (1975)</a>                 | 408, 102 untreated, 102 sham<br>Nose-only, mixture of cigarette smoke/air (1/5),<br>concentration (NR), 15 sec/min, 2 × 11 min/d, 5 d/wk,<br>lifetime                                                                  | NR                                      | 4/408 (1%) (1 lung C and 3<br>lung neoplasms of uncertain<br>malignancy)<br>Controls–0/102<br>Sham treated controls–0/102                                                                                   | NS                                              |          |
| Rats, F344 (F)<br><a href="#">Dalbey <i>et al.</i> (1980)</a>                  | 80, 63 untreated, 30 sham<br>Nose-only, mixture of non-filter cigarette smoke/<br>air (1/10), 18.4 mg smoke particulate and 0.89 mg<br>nicotine/cigarette, 1 cigarette/h, 7 cigarettes/d, 5 d/wk<br>for 128 wk; 160 wk | Particulate<br>deposition,<br>1.75 mg/d | 10/80 (12%) (1 nasal AdC, 1<br>nasal C, 5 pulmonary A, 1<br>pulmonary C, 2 alveoleogenic C)<br>Controls–3/93 (3%)<br>Subcutaneous sarcomas at<br>forelimb ulceration sites: 21/80<br>(26%)<br>Controls–0/93 | $P < 0.05$<br><br>$P < 0.05$                    |          |
| Rats, CDF (F344)/CrIBR<br>(M, F)<br><a href="#">Finch <i>et al.</i> (1995)</a> | 2165 animals<br>Whole-body, cigarette smoke/air, 100 mg (LCS) or<br>250 mg (HCS) total particulate matter/m <sup>3</sup> , 6 h/d, 5 d/<br>wk for 30 mo; lifetime                                                       | NR                                      | Lung tumours: M:<br>LCS 3/173 (2%)<br>HCS 7/78 (9%)<br>Filtered air 3/119 (2%)<br>F:<br>LCS–4/145 (3%)<br>HCS–6/83 (7%)<br>Filtered air–0/113                                                               | $P < 0.05$<br><br>$P < 0.05$                    |          |

Table 3.1 (continued)

| Species, strain (sex)<br>Reference                          | Animals/group at start<br>Dosing regimen<br>Duration                                                                                                                                                                               | Lung burden | Results<br>Target organ<br>Incidence and/or multiplicity<br>of tumours (%)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | Significance                                                                                                                                                                                                                                                                                                                                    | Comments |
|-------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------|
| Rats, F344 (M, F)<br><a href="#">Mauderly et al. (2004)</a> | M: 178 LCS, 82 HCS<br>F: 175 LCS, 81 HCS<br>Whole-body, smoke from Kentucky 1R3 unfiltered<br>reference cigarettes, 100 mg (LCS) or 250 mg (HCS)<br>total particulate matter/m <sup>3</sup> , 6 h/d, 5 d/wk for 30 mo;<br>lifetime | NR          | <p>M:<br/>Lung A–<br/>LCS 4/178 (2%)<br/>HCS 2/82 (2%)<br/>Sham-exposed controls 1/118<br/>(1%)</p> <p>Lung AdC–<br/>LCS 1/178 (1%)<br/>HCS 5/82 (6%)<br/>Controls 3/118 (3%)</p> <p>All lung tumours–<br/>LCS 4/178 (2%)<br/>HCS 7/82 (8%)<br/>Controls 4/118</p> <p>Nasal cavity (all tumour<br/>types)–<br/>LCS 1/178 (1%)<br/>HCS 5/82 (6%)<br/>Controls 1/118 (1%)</p> <p>F: Lung A–<br/>LCS 7/175 (6%)<br/>HCS 7/81 (9%)<br/>Sham-exposed controls 0/119</p> <p>AdC–<br/>LCS 4/175 (2%)<br/>HCS 4/81 (5%)<br/>Controls 0/119</p> <p>All lung tumours–<br/>LCS 10/175 (6%)<br/>HCS 11/81 (13%)<br/>Controls 0/119</p> <p>Nasal cavity (all tumour<br/>types)–<br/>LCS 0/175<br/>HCS 3/81<br/>Controls 0/119</p> | <p>NS</p> <p>NS</p> <p>NS; trend, <math>P = 0.055</math></p> <p><math>P = 0.032</math> (HCS);<br/>trend, <math>P = 0.010</math></p> <p>NS (LCS); <math>P &lt; 0.001</math><br/>(HCS)</p> <p>NS</p> <p><math>P = 0.023</math> (LCS);<br/><math>P = 0.001</math> (HCS)</p> <p><math>P = 0.020</math> (HCS);<br/>trend, <math>P = 0.003</math></p> |          |

Table 3.1 (continued)

| Species, strain (sex)<br>Reference                                            | Animals/group at start<br>Dosing regimen<br>Duration                                                                                                                                                                              | Lung burden | Results<br>Target organ<br>Incidence and/or multiplicity<br>of tumours (%)                                                                                                                                                                 | Significance | Comments |
|-------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------|----------|
| Hamsters, Syrian golden<br>(M, F)<br><a href="#">Dontenwill et al. (1972)</a> | 80 animals/sex/group<br>Whole-body, mixture of German reference cigarette<br>smoke/air (1/15), concentration (NR), smoke of<br>30 cigarettes for 7–10 min; 1, 2 or 3 × /d, 5 d/wk,<br>lifetime                                    | NR          | Laryngeal carcinomas: 1/160<br>(1%), 17/160 (11%) and 11/160<br>(7%)<br>Controls–0/80                                                                                                                                                      |              |          |
|                                                                               | Whole-body, mixture of dark air-cured cigarette<br>smoke/air (1/15), concentration (NR), Smoke of<br>30 cigarettes for 7–10 min; twice/d, 5 d/wk, lifetime                                                                        | NR          | Laryngeal carcinomas: 2/160<br>(1%)<br>Controls–0/80                                                                                                                                                                                       |              |          |
| Hamsters, Syrian golden<br>(M, F)<br><a href="#">Dontenwill et al. (1972)</a> | 80 animals/group<br>Whole-body, mixture of German reference cigarette<br>smoke/air (1/15), 1.5 mg nicotine, 0.173 mg phenol and<br>12.7 mL CO/cigarette, smoke of 30 cigarettes for 7–10<br>min; 1, 2 or 3 × /d, 5 d/wk, lifetime | NR          | M:<br>Laryngeal C–0, 4, 6 and 11%,<br>Controls–0%<br>F:<br>Laryngeal C–0, 1, 2 and 7%<br>Controls–0%                                                                                                                                       |              |          |
|                                                                               | 102 animals/group<br>Whole-body, mixture of US reference cigarette smoke/<br>air (1/5), concentration (NR), duration (NR)                                                                                                         |             | Inbred BIO 15.16:<br>Laryngeal tumours–9/84 (10%)<br>Nasopharyngeal tumours–2/84<br>(2%)<br>Sham-exposed controls 0/42<br>Controls 0/40<br>Inbred BIO 87.20:<br>Laryngeal tumours–2/87 (2%)<br>Sham-exposed controls 0/44<br>Controls 0/48 |              |          |
| Rats, Inbred BIO 15.16 (M)<br><a href="#">Bernfield et al. (1979)</a>         | Number at start (NR)<br>Whole-body, 11 or 22% smoke from commercial<br>British filter cigarettes, concentration (NR), 2 × 12<br>min/d, 7 d/wk for 35–42 wk; up to 74–80 wk                                                        | NR          | Laryngeal carcinomas:<br>11% smoke–3/44 (7%)<br>22% smoke–27/57 (47%)<br>Sham-exposed controls 0/36;<br>Controls 0/50                                                                                                                      |              |          |

<sup>a</sup> Most of these lung tumours are adenomas<sup>b</sup> Nasal cavity tumours included 14 squamous cell carcinomas (5 in situ), 5 hemangiomas, and 1 respiratory papilloma  
A, adenoma; AdC, adenocarcinoma; C, carcinoma; CO, carbon monoxide; d, day or days; F, female; h, hour or hours; HCS, high cigarette smoke; LCS, low cigarette smoke; M, male; min, minute or minutes; mo, month or months; NR, not reported; NS, not significant; sec, second or seconds; wk, week or weeks; yr, year or years

**Table 3.2 Carcinogenicity in response to exposure to mainstream tobacco smoke in conjunction with exposure to known carcinogens or other agents in animals**

| Species, strain (sex) Reference                                     | Animals/group at start Dosing regimen Duration                                                                                                                                                                                                         | Results Target organ Incidence and/or multiplicity of tumours (%)                                                                                                                                                                                                            | Significance |
|---------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------|
| Rats, Wistar (F) <a href="#">Davis et al. (1975)</a>                | 84 or 408 animals/group<br>A single intratracheal instillation of benzo[a]pyrene (2 mg) + infusine + carbon black followed by British reference cigarette smoke/air (1/5); 1 cigarette, twice/d, 5 d/wk, lifetime                                      | 3/84 (4%, lung C), 1/84 (1%, lung C; benzo[a]pyrene alone), 4/408 (1%, 3 A + 1 malignant neoplasm; cigarette smoke only), 0/204 (controls + sham-exposed controls)                                                                                                           | NS           |
| Rats, Wistar (M) <a href="#">Gupta et al. (1990)</a>                | 35 animals/group<br>Cigarette smoke; 5 cigarettes/8.2 L air; 1 h/d during 2nd–24th wk or 10th–24th wk of the study<br>Benzo[a]pyrene (20 mg) + Fe <sub>2</sub> O <sub>3</sub> ; intratracheally (3 weekly doses) during 6th–8th wk of the study; 24 wk | Conventional diet: 2nd–24th wk, 2.14 lung C/animal; 10th–24th wk, 1.33 lung C/animal; benzo[a]pyrene control, 1.22 lung C/animal.<br>Vitamin A-deficient diet: 2nd–24th wk, 2.86 lung C/animal; 10th–24th wk, 1.67 lung C/animal; benzo[a]pyrene control, 1.83 lung C/animal |              |
| Rats, Sprague-Dawley, sex NR <a href="#">Chameaud et al. (1982)</a> | 28–50 animals/group<br>French reference cigarette smoke (9 cigarettes/500 L air); 10–15 min session, 4 d/wk for 1 yr<br>Radon progeny (4 000, 500 or 100 WLM) Lifetime                                                                                 | 4000 WLM: 34/50 (68%, lung C); 17/50 (34%, lung C; radon progeny alone)<br>500 WLM: 8/30 (27%, lung C); 2/28 (7%, lung C; radon progeny alone)<br>100 WLM: 1/30 (3%, lung C); 0/50 (radon progeny alone)                                                                     | P = 0.0015   |
| CDF (F344)/CrIBR (M, F) <a href="#">Finch et al. (1995)</a>         | Number at start (NR)<br>Cigarette smoke/air, 100 mg (LCS) or 250 mg (HCS) total particulate matter/m <sup>3</sup> ; 6 h/d, 5 d/wk for 126 wk<br><sup>239</sup> PuO <sub>2</sub> aerosol, 1 wk (6th wk of the study); > 52 wk                           | 49–61% (lung tumours, LCS + <sup>239</sup> PuO <sub>2</sub> )<br>72–74% (HCS + <sup>239</sup> PuO <sub>2</sub> )<br>20–33% ( <sup>239</sup> PuO <sub>2</sub> )<br>2–3% (LCS)<br>7–8% (HCS)                                                                                   |              |
| Syrian golden (M, F) <a href="#">Dontenwill et al. (1973)</a>       | 80 animals/sex/group<br>German reference cigarette smoke/air (1/15); Smoke of 30 cigarettes for 7–10 min; twice/d, 5 d/wk, lifetime<br>DMBA (0.5 mg); intratracheally 10 d before the beginning of smoke exposure                                      | 32/160 (20%, laryngeal C), 17/160 (11%, laryngeal C; smoke only), 0/160 (DMBA alone)                                                                                                                                                                                         |              |

**Table 3.2 (continued)**

| Species, strain (sex)<br>Reference                                       | Animals/group at start<br>Dosing regimen<br>Duration                                                                                                                         | Results<br>Target organ<br>Incidence and/or multiplicity of tumours (%)                                                                        | Significance                       |
|--------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------|
| Syrian golden, (sex NR)<br><a href="#">Hoffmann <i>et al.</i> (1979)</a> | 20 or 40 animals/group<br>Cigarette smoke/air (1/7); Cigarette smoke 2 × 10 min/d, 5 d/wk, 48 wk<br>DMBA (0.24 mg); intralaryngeally                                         | 3/40 (7%, laryngeal C), 0/20 (smoke only), 0/20 (DMBA alone)                                                                                   |                                    |
| Syrian golden (M)<br><a href="#">Takahashi <i>et al.</i> (1992)</a>      | 10 or 30 animals/group<br>Cigarette smoke/air (1/7); Smoke of 30 cigarettes for 9 min; twice/d, 5 d/wk, 12 wk<br>NDEA (100 mg/kg bw); subcutaneously                         | Non-filter cigarettes (2.10 ± 1.74 P+H/animal) and filter cigarettes (1.93 ± 1.55 P + H/animal) versus sham-exposed (0.97 ± 1.03 P + H/animal) | <i>P</i> < 0.01<br><i>P</i> < 0.01 |
| Syrian golden (M)<br><a href="#">Harada <i>et al.</i> (1985)</a>         | 30 animals/group<br>Non-filter cigarette smoke/air (1/7); Smoke of 30 cigarettes for 6 min: twice/d, 5 d/wk, 58 wk<br>NDEA (10 mg/hamster); subcutaneously (12 weekly doses) | Nasal cavity tumours<br>14/30 (47%, smoke + NDEA), 5/30 (17%, NDEA alone)                                                                      | <i>P</i> < 0.05                    |

A, adenoma; bw, body weight; C, carcinoma; d, day or days; DMBA, 7,12-dimethylbenz[*a*]anthracene; F, female; h, hour or hours; HCS, high cigarette smoke; LCS, low cigarette smoke; M, male; min, minute or minutes; mo, month or months; NDEA, *N*-nitrosodiethylamine; NR, not reported; NS, not significant; P + H, epithelial hyperplasias and papillomas; sec, second or seconds; wk, week or weeks; WLM, work-level-months; yr, year or years

### (c) *Plutonium oxide*

CDF®/CrlBR rats were exposed to either filtered air or mainstream cigarette smoke at concentrations of either 100 or 250 mg total particulate matter/m<sup>3</sup> (LCS and HCS groups, respectively). At 12 weeks, rats were removed from smoke chambers and exposed nose-only to plutonium oxide (<sup>239</sup>PuO<sub>2</sub>) then returned to the smoke chambers one week later for 30 months of continuous exposure to either filtered air or cigarette smoke. The incidence and multiplicity of lung tumours (adenocarcinomas, squamous-cell carcinomas, adenosquamous carcinomas) in animals exposed to both concentrations of cigarette smoke and <sup>239</sup>PuO<sub>2</sub> were higher than those in animals exposed to <sup>239</sup>PuO<sub>2</sub>, LCS or HCS alone (Finch *et al.*, 1995).

### 3.2.2 Hamster

#### (a) 7,12-Dimethylbenz[*a*]anthracene

Groups of 160 Syrian golden hamsters received 7,12-dimethylbenz[*a*]anthracene (DMBA) intratracheally, followed by cigarette smoke for life, or treated with cigarette smoke or DMBA only. A total of 32 squamous-cell carcinomas of the larynx were observed in animals treated with both DMBA and cigarette smoke, in comparison with 17 in hamsters exposed to cigarette smoke only and none in hamsters treated with DMBA alone (Dontenwill *et al.*, 1973). Similar results were reported from other experiments in which Syrian golden hamsters were exposed to DMBA and cigarette smoke (Hoffmann *et al.*, 1979).

#### (b) *N*-Nitrosodiethylamine

Groups of hamsters received a single subcutaneous injection of *N*-nitrosodiethylamine (NDEA) and then were exposed to smoke from unfiltered cigarettes, filtered cigarettes and sham smoke. Controls were exposed to either unfiltered cigarette smoke, filtered cigarette smoke or sham smoke. In the NDEA-smoke-treated

groups, epithelial hyperplasias and/or papillomas of the larynx were induced at higher frequency than in controls (Takahashi *et al.*, 1992). Hamsters exposed to cigarette smoke in air also received 12 weekly subcutaneous injections of NDEA (total dose, 10 mg/hamster). Treatment with NDEA only resulted in both benign and malignant tumours of the respiratory tract, and co-exposure to cigarette smoke potentiated the development of tumours in the nasal cavity (Harada *et al.*, 1985).

## 3.3 Smoke condensates

Study design and results of the studies on administration of tobacco smoke condensates are summarized in Table 3.3.

### 3.3.1 Skin application

#### (a) Mouse

Cigarette-smoke condensate produces both benign and malignant tumours on mouse skin. The carcinogenic potency of the cigarette-smoke condensate depends upon tobacco variety, composition of cigarette paper and the presence of additives (Wynder *et al.*, 1957; Gargus *et al.*, 1976; Gori, 1976).

#### (b) Rabbit

Cigarette-smoke condensate induced skin papillomas and carcinomas when applied to the ears of rabbits for lifetime (Graham *et al.*, 1957).

### 3.3.2 Intrapulmonary administration

Injection of 24 mg cigarette-smoke condensate into the lungs of female Osborne Mendel rats led to the development of squamous cell carcinomas (Stanton *et al.*, 1972). These observations were confirmed by Dagle *et al.* (1978) who observed a dose-dependent incidence of lung carcinomas when cigarette-smoke condensate prepared from two types of cigarettes were given.



**Table 3.3 Carcinogenicity in response to exposure to cigarette-smoke condensate in animals**

| Species, strain (sex) Reference                                     | Animals/group at start Dosing regimen Duration                                                                                                                                                                                                                                                                                 | Results Target organ Incidence and/or multiplicity of tumours (%)                                                                                                                                                                                                                                                                                  | Significance |
|---------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------|
| Mice CAF1 (M, F) <a href="#">Wynder et al. (1953)</a>               | 112, 44 controls<br>Skin painting (dorsal) of CSC, CSC/acetone solution (40 mg CSC/ application), 3 × /wk, lifetime                                                                                                                                                                                                            | 36/81 (44%, skin epidermoid C), 0/30 (acetone controls)                                                                                                                                                                                                                                                                                            |              |
| Mice ICR Swiss (F) <a href="#">Gargus et al. (1976)</a>             | 5200<br>Skin painting (dorsal) of CSC, CSC/acetone solution (150 mg or 300 mg CSC/wk), 6 × /wk, 78 wk                                                                                                                                                                                                                          | 482/5200 (9%, skin C), 3/800 (0.4%, acetone controls) <sup>a</sup>                                                                                                                                                                                                                                                                                 |              |
| Mice ICR Swiss (F) <a href="#">Gori (1976)</a>                      | 4900<br>Skin painting (dorsal) of CSC, CSC/acetone solution (25 mg or 50 mg CSC/application), 6 × /wk, 78 wk                                                                                                                                                                                                                   | 1157/4900 (24%, skin C), 0/800 (acetone controls)                                                                                                                                                                                                                                                                                                  |              |
| Mice, ICR/Ha Swiss (F) <a href="#">Hoffmann &amp; Wynder (1971)</a> | 30 animals/group<br>Skin painting (dorsal) with CSC active fraction with or without subsequent painting of the skin with croton oil, CSC active fraction/acetone (2.5 mg of 0.6% CSC/ application), 10 times on alternate d<br>Croton oil (2.5%), 3 × /wk, up to 12 mo, 10 d after last CSC active fraction application; 15 mo | After 12 and 15 mo: 4/30 (13%, skin C), 0/65 (croton oil controls)                                                                                                                                                                                                                                                                                 |              |
| Mice, Swiss (F) <a href="#">Wynder &amp; Hoffmann (1961)</a>        | 30–50 animals/group<br>Skin painting (dorsal) of CSC with or without initiation by DMBA application; DMBA (75 µg); CSC/acetone (75 mg CSC/application, start: 1 wk after DMBA application), 2–3 × /wk, 12 mo; 15 mo                                                                                                            | DMBA: 2/30 (7%, skin C)<br>2 × CSC: 1/40 (3%, skin C)<br>DMBA + 2 × CSC: 8/30 (27%, skin C)<br>3 × CSC: 11/50 (22%, skin C)<br>DMBA + 3 × CSC: 11/30 (37%, skin C)                                                                                                                                                                                 |              |
| Mice, SENCAR (F) <a href="#">Meckley et al. (2004a)</a>             | 40 animals/group<br>Skin painting (dorsal) of CSC from Kentucky IR4F reference cigarettes, with or without initiation by DMBA application; DMBA (75 µg) or acetone, 1x. Then starting 1 wk after DMBA or acetone: CSC in acetone, 0, 10, 20 or 40 mg/application, 3 × /wk, 29 wk; 31 wk                                        | Mean mice with tumours/mice per group at 31 wk:<br>No DMBA: 0/40 acetone-acetone, 9/40 (22%) acetone-CSC 40 mg/treatment<br>DMBA/CSC: 0/40, 3/40 (1.0), 16/40 (75 tumours/16 mice = 4.7), 32/40 (200 tumours/32 mice = 6.3)                                                                                                                        |              |
| Mice, SENCAR (F) <a href="#">Meckley et al. (2004b)</a>             | 40 animals/group<br>Skin painting (dorsal) of CSC from Kentucky IR4F reference cigarettes or ECLIPSE (non-burned) cigarettes, with or without initiation by DMBA application; DMBA (75 µg) or acetone, 1 × . Then starting 1 wk after DMBA or acetone: CSC in acetone, 0, 10, 20 or 40 mg/application, 3 × /wk, 29 wk; 31 wk   | No DMBA: acetone/acetone 0/40 (0); acetone/1R4F CSC 40 mg, 9/40 (1.8); acetone/ECLIPSE CSC 40 mg, 0/40 (0)<br>DMBA/CSC: acetone, 0/40 (0); 1R4F CSC 10 mg, 6/40 (1.8); 1R4F CSC 12 mg, 28/40 (6.6); 1R4F CSC 40 mg, 36/40 (6.8); ECLIPSE CSC, 0/40 (0); ECLIPSE CSC 10 mg, 1/40 (1); ECLIPSE CSC 20 mg, 2/40 (5.5); ECLIPSE CSC 40 mg, 12/40 (2.6) |              |

Table 3.3 (continued)

| Species, strain (sex)<br>Reference                                         | Animals/group at start<br>Dosing regimen<br>Duration                                                                                                                                                                                                                                                                                          | Results<br>Target organ<br>Incidence and/or multiplicity of tumours (%)                                                                                                                                                                                                                                    | Significance                           |
|----------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------|
| Mice, SENCAR (F)<br><a href="#">Hayes et al. (2007)</a>                    | 40 or 50 animals/group<br>Skin painting (dorsal) of CSC from heat-exchanged flue cured tobacco (HE; low TSNA) or direct-fire (DF) cured tobacco, with or without initiation by DMBA application; DMBA (75 µg) or acetone, 1 × . Then starting 1 wk after DMBA or acetone: CSC/acetone, 0, 9, 18, or 36 mg/ application, 3 × /wk, 29 wk; 31 wk | No DMBA: acetone/acetone 0/40; acetone/DF CSC 36 mg, 5/50 (1.4); acetone/HE CSC 36 mg, 8/50 (1.3)<br>DMBA/CSC: DF CSC, 0/40, DF CSC 9 mg, 15/40 (5.5); DF CSC 18 mg, 30/40 (10.0); DF CSC 36 mg, 43/50 (8.2); HE CSC, 0/40, HE CSC 9 mg, 17/40 (4.8); HE CSC 18 mg, 32/40 (7.3); HE CSC 36 mg, 42/50 (8.5) | $P < 0.05$<br>$P < 0.05$<br>$P < 0.05$ |
| Mice, Swiss albino (M)<br><a href="#">Pakhale et al. (1988)</a>            | 20 animals/group<br>Oral gavage of Indian bidi smoke condensate; 1 mg bidi smoke condensate/0.1 mg DMSO, 5 d/wk, 55 wk; 90 wk                                                                                                                                                                                                                 | 4/15 (27%, hepatic haemangiomas); 1/15 (7%, stomach papilloma); 1/15 (7%, stomach carcinoma); 1/15 (7%, oesophageal carcinoma); 0/15 (untreated or DMSO-treated controls)                                                                                                                                  |                                        |
| Rats, Osborne Mendel (F)<br><a href="#">Stanton et al. (1972)</a>          | Number/group at start (NR)<br>Intrapulmonary administration of CSC pellet; CSC/ beeswax:tricaprylin (24 mg CSC/injection), up to 107 wk after implantation                                                                                                                                                                                    | 14/40 <sup>c</sup> (35%, lung squamous-cell C), 0/63 <sup>c</sup> (beeswax:tricaprylin controls)                                                                                                                                                                                                           |                                        |
| Rats, OM/NCR (F)<br><a href="#">Dagle et al. (1978)</a>                    | 120 <sup>d</sup><br>Intrapulmonary administration of CSC pellet; CSC/ beeswax:tricaprylin (5, 10, 20 or 67 mg CSC/injection), 120 wk after implantation                                                                                                                                                                                       | 4, 10, 20 and 42% pulmonary C prevalence; 0% C prevalence for 3 control groups of about 190 rats each                                                                                                                                                                                                      |                                        |
| Rabbits, Albino New Zealand (M, F)<br><a href="#">Graham et al. (1957)</a> | 38, 7 controls<br>Skin painting of CSC (both ears); CSC/acetone solution (100 mg CSC/ application/ear), 5 × /wk, lifetime (4–6 yr)                                                                                                                                                                                                            | 4/38 (11%, 2 skin C + 1 skin liposarcoma + 1 skin fibrosarcoma), 0/7 (acetone controls)                                                                                                                                                                                                                    |                                        |

<sup>a</sup> Skin papillomas<sup>b</sup> Mostly adenomas<sup>c</sup> Incidence in animals that died 43–107 weeks after injection<sup>d</sup> 4 × 10 rats/group terminated before 120 weeks<sup>e</sup> Total visually identified and histologically confirmed skin tumours included mostly squamous papillomas and carcinomas [Tumour incidences and multiplicities estimated from graphs]C, carcinoma; CSC, cigarette-smoke condensate; d, day or days; DMBA, 7,12-dimethylbenz[*a*]anthracene; DMSO, dimethyl sulfoxide; F, female; M, male; NR, not reported; TSNA, tobacco-specific *N*-nitrosamines; wk, week or weeks

### 3.3.3 Initiation-promotion skin painting studies

Cigarette-smoke condensate and its fractions can act as skin co-carcinogens in Swiss and SENCAR mice when tested in conjunction with croton oil ([Hoffmann & Wynder, 1971](#)) or DMBA ([Wynder & Hoffmann, 1961](#); [Meckley et al., 2004a, b](#); [Hayes et al., 2007](#)).

### 3.3.4 Bidi smoke

Swiss albino mice administered 1 mg bidi smoke condensate in dimethyl sulfoxide (DMSO) by oral gavage developed haemangiomas (4/15), stomach carcinoma (1/15), and esophageal carcinoma (1/15), whereas no tumours were observed in controls ([Pakhale et al., 1988](#)).

## 3.4 Synthesis

Mainstream tobacco smoke induced lung tumours in mice, lung and nasal cavity tumours in rats and laryngeal carcinomas in hamsters.

Co-administration of tobacco smoke with benzo[*a*]pyrene, radon progeny and plutonium resulted in higher lung tumour responses in rats than administration of either agent alone. Hamsters exposed to cigarette smoke and either DMBA or NDEA had higher lung tumour responses compared to cigarette smoke, DMBA or NDEA alone.

Topical application of cigarette-smoke condensate led to the development of skin tumours in mice and rabbits; intrapulmonary administration of cigarette-smoke condensate induced squamous cell carcinomas in rat lung.

## 4. Other Relevant Data

### 4.1 Overview of the mechanistic evidence for the carcinogenicity of tobacco

#### 4.1.1 Conceptual model of the carcinogenesis of tobacco and tobacco smoke

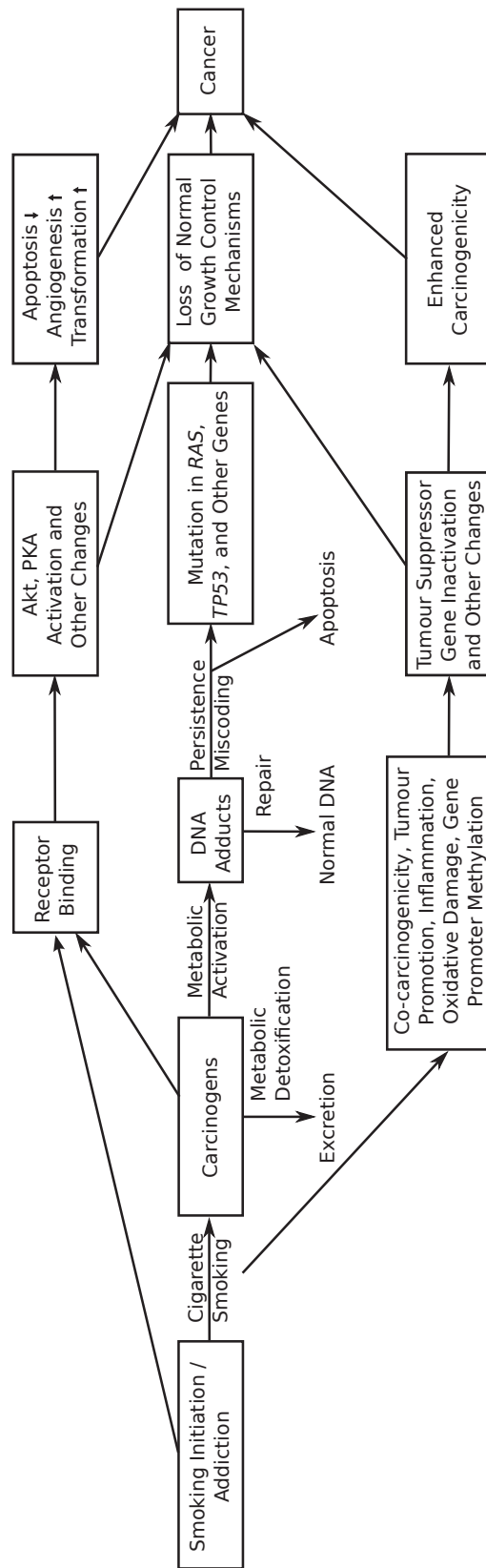
A conceptual model for understanding mechanisms by which tobacco smoke causes cancer is shown in Fig. 4.1 ([Hecht, 1999, 2003](#)). This model also applies to smokeless tobacco and other forms of smoked tobacco and, in theory, to second-hand tobacco smoke since it contains all of the same carcinogens and toxicants as mainstream cigarette smoke, although at lower doses.

The major accepted mechanistic pathway is summarized in the central track of Fig. 4.1. Smokers inhale carcinogens which, either directly or after metabolism, covalently bind to DNA, forming DNA adducts. DNA adducts are central to chemical carcinogenesis because they can cause miscoding and permanent mutations. If these mutations occur in critical regions of oncogenes and tumour suppressor genes, which are essential in growth control, the result can be loss of normal cellular proliferation mechanisms, genomic instability, and cancer. A study that sequenced 623 cancer-related genes in 188 human lung adenocarcinomas validated this premise by finding multiple somatic mutations in critical growth control genes, consistent with the chronic bombardment of cellular DNA by tobacco smoke carcinogens and their metabolically activated forms ([Ding et al., 2008](#)).

Each step of this conceptual model is considered in detail below.

Most people begin smoking cigarettes when they are teenagers, and become addicted to nicotine. Nicotine is not generally considered to be a carcinogen ([Schuller, 2009](#)), but it is accompanied in each puff of each cigarette by a complex

**Fig. 4.1 Conceptual model for understanding mechanisms of tobacco carcinogenesis**



There may be overlap between these three tracks: e.g. mutation in *TP53* leading to tumour suppressor gene inactivation.

mixture of carcinogens and toxicants. There are over 60 carcinogens in cigarette smoke that have been evaluated in the previous *IARC Monograph* as having *sufficient evidence* for carcinogenicity in laboratory animals ([IARC, 2004a](#)), sixteen of which are considered to be *carcinogenic to humans* (Group 1). There are also many other carcinogens and potential carcinogens in cigarette smoke that have not been evaluated ([Rodgman & Perfetti, 2006](#); see Section 1.1). Structures of tobacco smoke constituents and biomarkers discussed here are presented in Fig. 4.2.

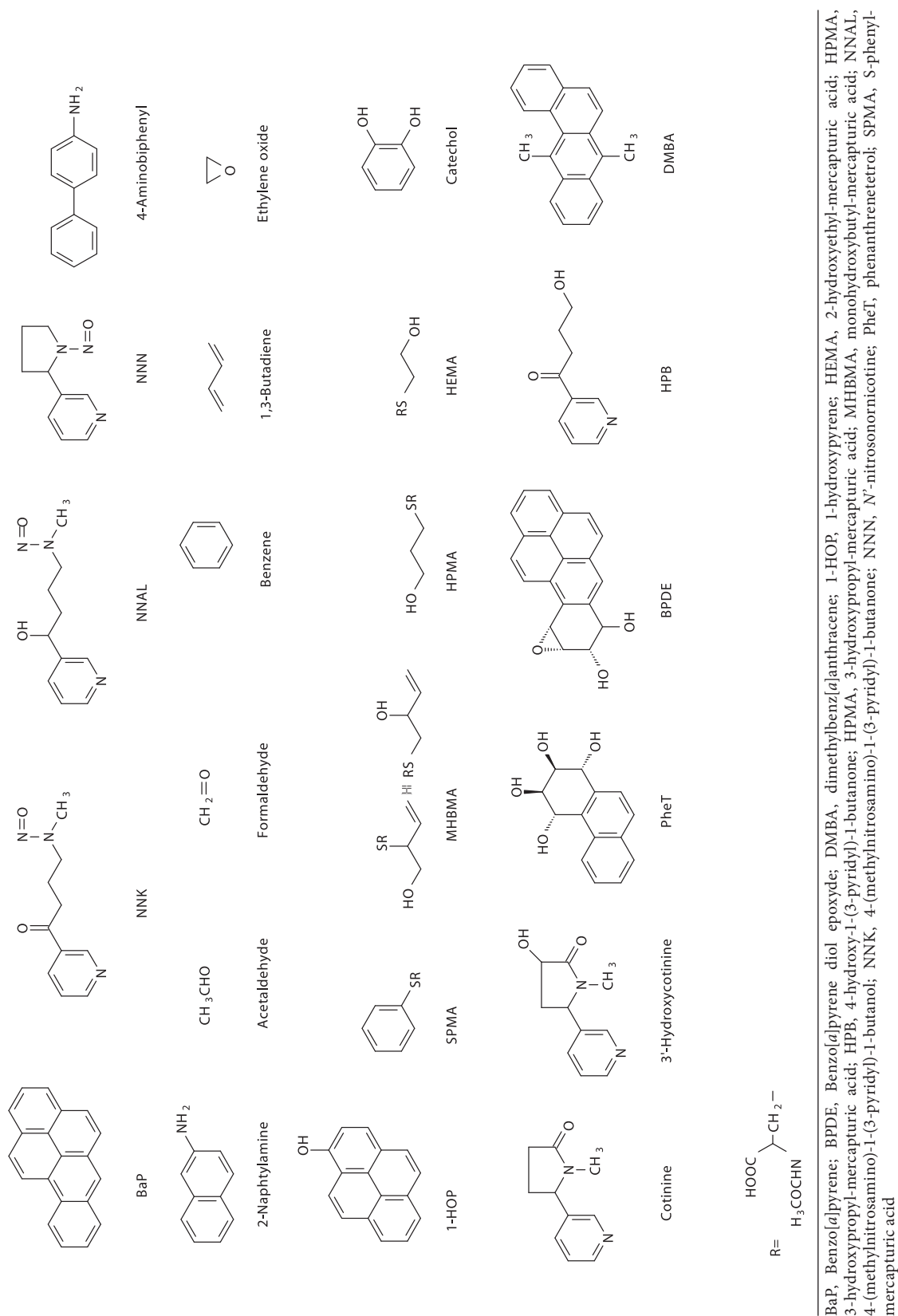
Numerous studies demonstrate the uptake of tobacco smoke carcinogens and toxicants by smokers, and showed higher levels of their metabolites in urine and blood of smokers than non-smokers (Sections 4.1.1 and 4.1.2). There are substantial differences in carcinogen exposure among people because of the number and types of cigarettes they smoke and the ways in which they smoke them. These differences can be monitored in part by biomarkers of exposure such as urinary metabolites or haemoglobin adducts (Section 4.1.2). Haemoglobin adducts of multiple aromatic amines and volatile carcinogens have been demonstrably related to tobacco ([Hatsukami et al., 2006a](#)). There may also be differences in carcinogen exposure due to genetic variations (Section 4.2).

The body's response to cigarette smoke constituents is similar to its response to pharmaceutical agents and other foreign compounds. Drug metabolizing enzymes, most frequently CYPs, convert these compounds to more water soluble forms, facilitating excretion. During this natural protective attempt, some reactive intermediates are formed. These intermediates are frequently electrophilic (electron seeking, or bearing a partial or full positive charge). Electrophilic intermediates may react with water, generally resulting in detoxification, or may covalently bind to nucleophilic (electron rich) sites in DNA, forming DNA adducts ([Guengerich, 2001](#); [Jalas et al., 2005](#)), which are

critical in the carcinogenic process (see Section 4.1.3c). CYP1A1 and CYP1B1, repeatedly shown to be inducible by cigarette smoke via interactions of smoke compounds with the aryl hydrocarbon receptor (AhR), are particularly important in the metabolic activation of PAHs, while CYP2A13 is critical for the metabolism of NNK ([Nebert et al., 2004](#); [Jalas et al., 2005](#)). The inducibility of certain CYPs may be a critical aspect of cancer susceptibility in smokers ([Nebert et al., 2004](#)). CYP1A2, CYP2A6, CYP2E1 and CYP3A4 are also important in the metabolism of cigarette smoke carcinogens to DNA binding intermediates ([Jalas et al., 2005](#)), and aldo-keto reductase enzymes, also induced by tobacco smoke ([Quinn et al., 2008](#)), are involved in the metabolism of NNK, BaP and other tobacco smoke carcinogens. Competing with this process of “metabolic activation” resulting in DNA binding is the intended metabolic detoxification, which leads to harmless excretion of carcinogen metabolites, and is also catalysed by CYPs and a variety of other enzymes including GSTs, uridine diphosphate-glucuronosyl transferases (UGTs), and arylsulfatases. The relative amounts of carcinogen metabolic activation and detoxification differ among individuals. It is widely hypothesized that this balance will affect cancer risk with those having higher activation and lower detoxification capacity being the most susceptible. This premise is supported in part by molecular epidemiologic studies of polymorphisms, or variants in more than 1% of the population, in certain genes coding for these enzymes ([Vineis et al., 2003](#); [Carlsten et al., 2008](#)).

DNA adducts are thought to be a critical lesion in carcinogenesis. Many investigations demonstrate the presence of DNA adducts in human tissues, and some of these are summarized in Section 4.1.2c. There is massive evidence, particularly from studies which use relatively non-specific DNA adduct measurement methods, that DNA adduct levels in the lung and other tissues of smokers are higher than in non-smokers, and some epidemiologic data link

Fig. 4.2 Structures of compounds discussed in the text





these higher adduct levels to increased cancer risk (IARC, 2004b; Veglia *et al.*, 2008). However, there is much more limited evidence from studies using specific carcinogen-derived DNA adducts as biomarkers (Pfeifer *et al.*, 2002). Oxidative DNA damage has also been observed, and this may result partially from exposure to metals in cigarette smoke (Stavrides, 2006).

Cellular DNA repair systems can excise DNA adducts and restore normal DNA structure (Christmann *et al.*, 2003). These complex multiple systems include direct base repair by alkyltransferases, removal of DNA damage by base and nucleotide excision repair, mismatch repair, and double strand repair. If these DNA repair systems are unsuccessful in fixing the damage, then the DNA adducts can persist, increasing the probability of a permanent mutation. There are polymorphisms in genes coding for some DNA repair enzymes. If these variants lead to deficient DNA repair, the probability of cancer development can increase (Vineis *et al.*, 2009).

DNA adducts can cause miscoding during replication when DNA polymerase enzymes misread the DNA adduct and consequently insert the wrong base opposite to it. There is some specificity in the relationship between specific DNA adducts formed from cigarette smoke carcinogens and the types of mutations which they cause. G to T and G to A mutations have often been observed (Section 4.1.3) (Hecht, 1999). Extensive studies have characterized the mutations which occur because of specific carcinogen-DNA adducts (Delaney & Essigmann, 2008). Mutations have been reported in the *KRAS* oncogene in lung cancer and in the *TP53* tumour suppressor gene in a variety of cigarette smoke-induced cancers (Ahrendt *et al.*, 2001; Pfeifer *et al.*, 2002; Ding *et al.*, 2008). The cancer causing role of these genes has been firmly established in animal studies (Lubet *et al.*, 2000; Johnson *et al.*, 2001). A selection and promotion process may also play a role in the final mutation spectrum seen in genes

in smoking-associated tumours (Rodin & Rodin, 2005; Sudo *et al.*, 2008).

Urinary mutagenicity, sister chromatid exchanges, micronuclei in buccal cells, and other genetic effects have been consistently observed in smokers at higher levels than in non-smokers (IARC, 2004a; Proia *et al.*, 2006). In addition to mutations, numerous cytogenetic changes are observed in lung cancer, and chromosome damage throughout the field of the aerodigestive tract is strongly associated with cigarette smoke exposure. Mutations resulting from DNA adducts can cause loss of normal cellular growth control functions, via a complex process of signal transduction pathways, ultimately resulting in genomic instability, cellular proliferation and cancer (Ding *et al.*, 2008). Apoptosis, or programmed cell death, is a protective process, and can remove cells which have DNA damage, thus serving as a counterbalance to these mutational events. The balance between apoptotic mechanisms and those suppressing apoptosis will have a major impact on tumour growth.

While the central track of Fig. 4.1 is the major pathway by which tobacco smoke carcinogens cause cancer, other mechanisms also contribute, as indicated in the top and bottom tracks (Hecht, 2003). Nicotine, NNK, and NNN bind to nicotinic and other cellular receptors, resulting in activation of serine/threonine kinase Akt (also known as protein kinase B), protein kinase A, and other changes. Nicotine and NNK increase expression of survivin, an inhibitor of apoptosis in normal human bronchial epithelial cells, and survivin mRNA is detected in bronchial brush samples from heavy smokers (Jin *et al.*, 2008). This can cause decreased apoptosis, increased angiogenesis, and increased transformation (Heeschen *et al.*, 2001; West *et al.*, 2003). Thus, although nicotine is not carcinogenic, it may enhance carcinogenicity in various ways (Schuller, 2009). Cigarette smoke also contains well established oxidants, co-carcinogens, tumour promoting fractions, and inflammatory agents, as well as

cilia-toxic compounds such as acrolein, which impede clearance. Many studies demonstrate the co-carcinogenic and cytotoxic effects of catechol, an important constituent of cigarette smoke. An epigenetic pathway frequently observed in tobacco-induced cancers is enzymatic methylation of promoter regions of genes such as *p16* and *FHIT* [fragile histidine triad gene, a gene coding for a dinucleoside 5', 5'''- P<sup>1</sup>, P<sup>3</sup>-triphosphate hydrolase, a putative tumour suppressor protein] resulting in gene silencing, which are also strongly implicated in tobacco-induced lung cancer (D'Agostini *et al.*, 2006; Bhutani *et al.*, 2008). When this occurs in tumour suppressor genes, the result can be unregulated proliferation (Belinsky, 2005). Inflammation due to smoking is associated with tumour promotion and activation of factors such as NFκB. Inflammation also plays a role in chronic obstructive pulmonary disease (COPD), which in turn is an independent risk factor for lung cancer (Smith *et al.*, 2006; Turner *et al.*, 2007; Lee *et al.*, 2008a).

This conceptual model can be applied to smokeless tobacco products. Smokeless tobacco products have much lower levels of carcinogens and toxicants that result from combustion, so the effects of these agents are not seen to a significant extent. The most prevalent strong carcinogens in smokeless tobacco are the tobacco-specific nitrosamines; other nitrosamines, PAHs, aldehydes and metals are also present, and there are large amounts of some inorganic salts that may contribute to inflammation (IARC, 2007a; Stepanov *et al.*, 2008). An additional factor in carcinogenesis by betel quid with tobacco is the basic pH resulting from addition of slaked lime to the quid, leading to oxidative damage and inflammation (IARC, 2004b).

Multiple studies demonstrate that tobacco-specific nitrosamines are absorbed and metabolised in smokeless tobacco users (IARC, 2007a).

There is evidence for DNA adduct formation in oral tissues of smokeless tobacco users, and sister chromatid exchanges, chromosomal

aberrations, and micronuclei – consequences of DNA adduct formation – have been reported (Proia *et al.*, 2006; Warnakulasuriya & Ralhan, 2007). Many studies have demonstrated RAS and TP53 mutations in smokeless tobacco users (Warnakulasuriya & Ralhan, 2007) consistent with the conceptual framework.

Oxidative stress and reactive oxygen species could play a significant role in cancer induction in smokeless tobacco users, particularly at high pH (Boffetta *et al.*, 2008). Chronic local inflammation and irritation induced by smokeless tobacco and its constituents could have a tumour promoting or co-carcinogenic effect (Boffetta *et al.*, 2008). Upregulation of cyclooxygenase-2, involved in prostaglandin synthesis and inflammation, has been observed in animal studies upon exposure to smokeless tobacco (Boffetta *et al.*, 2008). Smokeless tobacco products have relatively high levels of sodium chloride (NaCl), which could contribute to inflammation, tumour promotion, and co-carcinogenesis. Cancer of the oral cavity is strongly associated with tobacco smoking (IARC, 2004a) or chewing (IARC, 2007a) and alcoholic beverage drinking (IARC, 2010a). However only a fraction of exposed subjects develop tumours, which suggests that other exposures such as HPV may be independently involved or act as cofactors. HPV is known to infect the oral cavity of healthy individuals and several HPV-related lesions have been characterized (IARC, 2007b). Herpes simplex virus has also been shown to enhance the carcinogenicity of smokeless tobacco products in animal studies (Park *et al.*, 1986). These factors may contribute significantly to the local carcinogenic effects characteristic of smokeless tobacco use.

#### 4.1.2 Absorption, distribution, metabolism and excretion

There are examples of toxicant and carcinogen metabolism and excretion for representatives of virtually every major class of compounds;



some of these are summarized in [Table 4.1](#). Nicotine and five of its urinary metabolites – cotinine, 3'-hydroxycotinine and their glucuronides, and nicotine glucuronide – comprise about 73–96% of the nicotine dose ([Hukkanen et al., 2005](#)), and are found in blood, sweat, hair and toenails ([Al Delaimy, 2002](#); [Hukkanen et al., 2005](#); [Stepanov et al., 2007](#); [Al Delaimy & Willett, 2008](#)). Metabolites of various polycyclic aromatic hydrocarbons including pyrene, phenanthrene, fluorene, and benzo[a]pyrene have been quantified in human urine and are higher in smokers than in non-smokers ([Hecht, 2002](#); [Hecht et al., 2005a](#); [Jacob et al., 2007](#); [Hansen et al., 2008](#)). Metabolites of tobacco-specific nitrosamines – NNAL and its glucuronides (total NNAL) from NNK; and NNN and its glucuronides (total NNN) from NNN – are present in human urine ([Hecht, 2002](#); [Stepanov & Hecht, 2005](#); [Hecht et al., 2008a](#); [Stepanov et al., 2008](#)). Total NNAL has also been quantified in blood and toenails ([Hecht et al., 2002](#); [Stepanov et al., 2007](#)). Aromatic amine-haemoglobin adducts have been frequently measured in human blood, and their levels increase with smoking ([Hecht, 2002](#); [Hatsukami et al., 2006a](#)). Mercapturic acids of several tobacco smoke compounds such as benzene, 1,3-butadiene, acrolein, and ethylene oxide are present in human urine and are related to smoking ([Carmella et al., 2009](#)). Haemoglobin adducts of acrylonitrile and related compounds are elevated in smokers' blood, and levels of metals such as Cd are increased in smokers' urine ([Carmella et al., 2002](#); [IARC, 2004b](#)).

All of the metabolites listed in [Table 4.1](#) are elevated in cigarette smokers; in studies of second-hand smoke exposure, only nicotine metabolites and urinary total NNAL are consistently increased in exposed versus non-exposed subjects, although one very large study also observed an increase in PAH metabolites ([Pirkle et al., 2006](#); [Hecht, 2008](#); [Suwan-ampai et al., 2009](#)). Smokeless tobacco users have significantly raised levels of nicotine metabolites

and tobacco-specific nitrosamine metabolites compared to non-tobacco users ([Hecht et al., 2007](#)).

#### 4.1.3 Biomarkers

Tobacco carcinogen biomarkers are quantifiable entities that can be *specifically* related to tobacco carcinogens. Specificity to a given carcinogen is critical because tobacco carcinogens vary widely in their potency and target organs.

Considering the mechanistic framework outlined in Fig. 4.1, one could visualize various types of biomarkers. Currently, biomarkers of carcinogen/toxicant dose, reflecting the second box of the central track of Fig. 4.1, are by far the most extensively used and validated. The second most common are measurements of DNA adducts (or protein adducts as their surrogates), but fewer of these have both practical utility and validation with respect to tobacco carcinogen specificity.

The use of tobacco carcinogen biomarkers bypasses many uncertainties in estimation of dose. The most commonly used estimation of dose is self-reported number of cigarettes/day, but this is not a very good marker. It may not be reported accurately and it provides no information on the way in which the cigarettes were smoked, which is critical when one considers the common phenomenon of smoker's compensation. Brand information together with machine smoking measurements of specific components is another way of obtaining a measure of dose. However, machine smoking measurements are known to have limitations and the application of a given machine smoking protocol to a given smoker requires smoking topography measurements for that smoker. A disadvantage of tobacco carcinogen biomarkers is that they are affected to some extent by individual differences in metabolism, which may complicate interpretation of dose.

**Table 4.1 Examples of toxicant or carcinogen metabolites in tobacco users**

| Toxicant or carcinogen                  | Examples of metabolites in tobacco users                                                                             | References                                                                                                                                                              |
|-----------------------------------------|----------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Nicotine                                | Cotinine, 3'-hydroxycotinine and their glucuronides in urine, blood or saliva; nicotine and cotinine in toenails     | <a href="#">Al Delaimy (2002)</a> , <a href="#">Hukkanen et al. (2005)</a> , <a href="#">Al Delaimy &amp; Willett (2008)</a> , <a href="#">Stepanov et al. (2007)</a>   |
| Polycyclic Aromatic Hydrocarbons (PAHs) | 1-hydroxypyrene, phenanthrols, phenanthrene tetraols, fluorenols, benzo[a]pyrenols, benzo[a]pyrene tetraols in urine | <a href="#">Hecht (2002)</a> , <a href="#">Hecht et al. (2005a)</a> , <a href="#">Hansen et al. (2008)</a> , <a href="#">Jacob et al. (2007)</a>                        |
| Tobacco-specific nitrosamines           | NNAL and its glucuronides (total NNAL) in urine or blood, total NNN in urine; NNAL and NNN in toenails               | <a href="#">Hecht (2002)</a> , <a href="#">Hecht et al. (2002, 2008a)</a> , <a href="#">Stepanov &amp; Hecht (2005)</a> , <a href="#">Stepanov et al., (2007, 2008)</a> |
| Aromatic amines                         | Parent amines in urine and haemoglobin adducts in blood                                                              | <a href="#">Hecht (2002)</a> , <a href="#">Hatsukami et al. (2006a)</a>                                                                                                 |
| Volatile hydrocarbons                   |                                                                                                                      |                                                                                                                                                                         |
| Benzene<br>1,3-Butadiene                | Muconic acid and S-phenyl-mercapturic acid (SPMA) in urine; Monohydroxybutyl-mercapturic acid (MHBMA) in urine       | <a href="#">Hecht (2002)</a> , <a href="#">Carmella et al. (2009)</a>                                                                                                   |
| Acrolein                                | 3-hydroxypropyl-mercapturic acid (HPMA) in urine                                                                     | <a href="#">Carmella et al. (2009)</a>                                                                                                                                  |
| Ethylene oxide                          | 2-hydroxyethyl-mercapturic acid (HEMA) in urine, haemoglobin adducts in blood                                        | <a href="#">Bono et al. (2002)</a> , <a href="#">Carmella et al. (2009)</a>                                                                                             |
| Acrylonitrile                           | Haemoglobin adducts in blood                                                                                         | <a href="#">Carmella et al. (2002)</a>                                                                                                                                  |
| Metals                                  | Cadmium in urine                                                                                                     | <a href="#">IARC (2004a)</a>                                                                                                                                            |

**(a) Urinary biomarkers**

Probably the most practical and, to date, the most extensively applied tobacco carcinogen biomarkers are urinary metabolites of tobacco carcinogens, and these have been comprehensively reviewed ([Hecht, 2002](#); [IARC, 2004a](#)). Advantages include the ready availability of samples, and concentrations in urine that are easily quantifiable using modern analytical chemistry methods, most frequently liquid chromatography-tandem mass spectrometry (LC-MS/MS). The urinary metabolites listed in [Table 4.1](#) have all been used as biomarkers and all are validated with respect to exposure in cigarette smokers ([Carmella et al., 2009](#)). Total nicotine equivalents (the sum of nicotine and the five metabolites in [Table 4.1](#)) is a particularly effective way of estimating nicotine dose from tobacco products.

Total NNAL, the sum of NNAL and its glucuronides, is a highly useful biomarker of NNK exposure ([Hecht, 2002, 2003](#); [Hatsukami et al., 2006a](#)). The tobacco-specificity of NNK, and therefore total NNAL, is a key feature of this biomarker because studies in which it is applied are not confounded by other environmental or dietary exposures. It also has a considerably longer half-life than cotinine and several other urinary biomarkers. Total NNAL has been used in numerous studies that estimated uptake of NNK in smokers under varying circumstances. In one example, smokers reduced their number of cigarettes smoked per day, but there was not a corresponding decrease in NNK uptake due to compensation ([Hecht et al., 2004](#)). In another study, NNK and PAH uptake, estimated by total NNAL and 1-hydroxypyrene, respectively, were compared in smokers of regular, light, and ultra-light cigarettes, and found to be similar, consistent with epidemiologic studies that

demonstrate no protection against lung cancer in smokers of light compared to regular cigarettes (Hecht *et al.*, 2005b). Other studies evaluated NNK uptake in smokers who switched from their current cigarette brand to products advertised as being less hazardous, but the results generally did not support these claims (Hatsukami *et al.*, 2004). One of the most useful applications of total NNAL has been in studies of non-smokers exposed to second-hand tobacco smoke (Hecht, 2003). The sensitivity and specificity of this biomarker are ideal for such studies, and it is the most commonly elevated tobacco carcinogen biomarker in non-smokers exposed to second-hand smoke. Total NNAL has also found utility in establishing NNK uptake in smokeless tobacco users (Hecht *et al.*, 2002, 2007, 2008a, b; Hecht, 2008).

The relationship of urinary total NNAL to lung cancer was demonstrated in a study of stored urine samples collected years before diagnosis of lung cancer from smokers in Shanghai, China and Singapore (Yuan *et al.*, 2009). There was a significant relationship between total NNAL and lung cancer incidence, after correction for numbers of cigarettes smoked per day and duration of smoking. An 8.5 fold increased risk for lung cancer was observed for those smokers in the highest tertile of total NNAL and cotinine, relative to smokers with the same smoking history but in the lowest tertiles of total NNAL and cotinine. Urinary biomarkers were also used to demonstrate higher uptake of nicotine and NNK per cigarette in smokers with polymorphisms in the nicotinic acetylcholine genes associated with lung cancer in genome-wide association studies (see Section 4.2; Le Marchand *et al.*, 2008). Collectively, these results indicate that urinary total NNAL is not only a biomarker of exposure, but also a biomarker of risk for lung cancer.

### (b) Serum and saliva metabolites

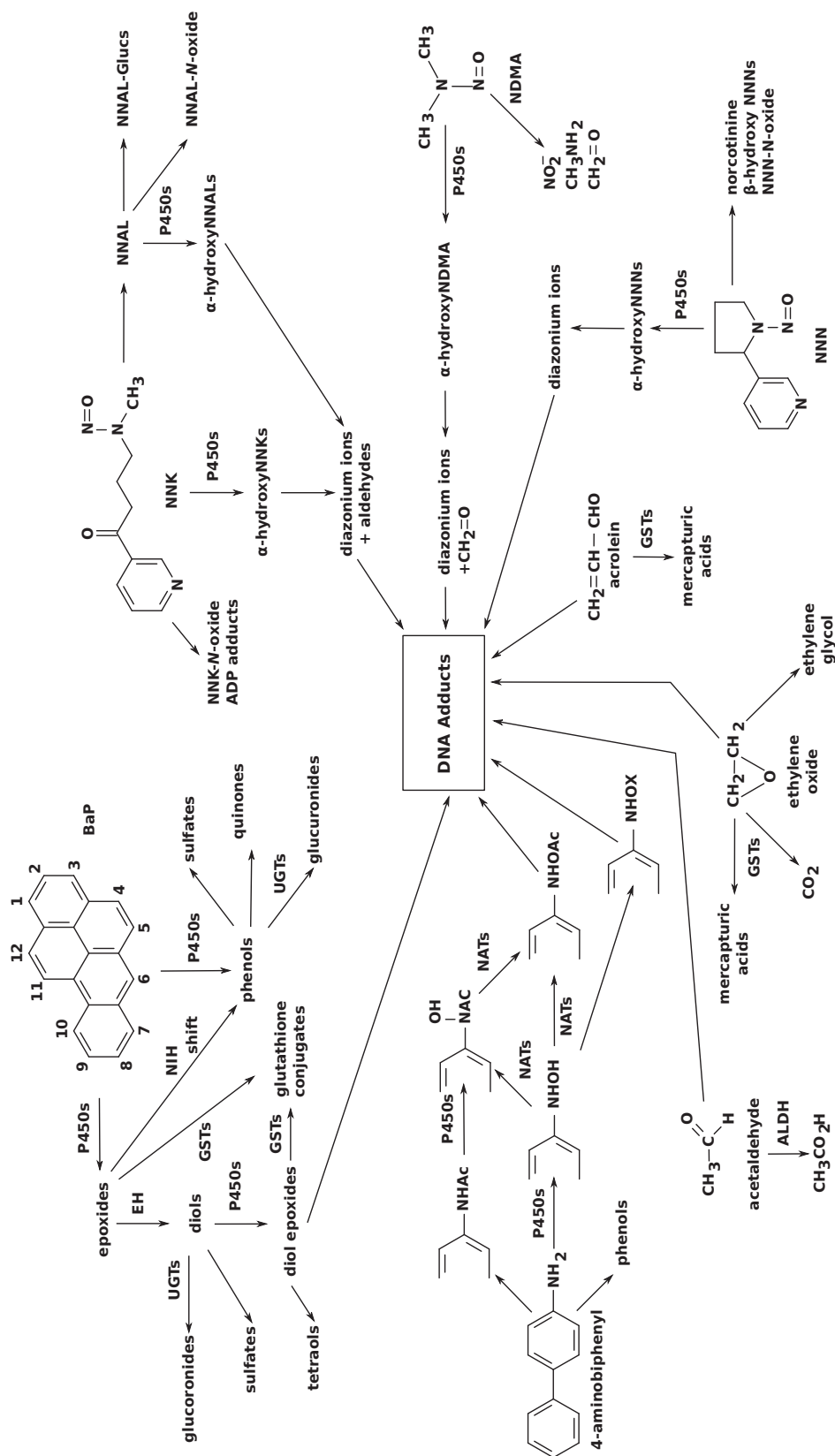
Serum and saliva metabolites have been used as biomarkers much less often than urine metabolites. The most frequently measured tobacco smoke toxicant in serum and saliva is cotinine, documented as a useful biomarker of cigarette smoking in many studies (Lee, 1999; Hukkanen *et al.*, 2005). Total NNAL can be readily quantified in serum and its levels remain relatively constant in a given smoker sampled at bimonthly intervals over a one year period. Consistent with the results described above, one study showed a significant relationship between total NNAL in prospectively collected serum samples from smokers and lung cancer risk (Church *et al.*, 2009). Other biomarkers that have been measured in serum include cadmium, benzene, styrene and *r*-1,*t*-2,3,*c*-4-tetrahydroxy-1,2,3,4-tetrahydrophenanthrene (PheT) (IARC, 2004a; Church *et al.*, 2009).

### (c) DNA adducts

Fig. 4.3 presents an overview of metabolism and DNA adduct formation from eight tobacco smoke compounds (clockwise from top left): BaP, NNK, *N*-nitrosodimethylamine (NDMA), NNN, acrolein, ethylene oxide, acetaldehyde and 4-aminobiphenyl. Evidence exists for DNA adduct formation from each of these carcinogens in smokers, based on studies carried out with tissues or blood cells. DNA adduct biomarkers have been applied mainly in studies of smokers, and there is far less evidence from studies of second-hand tobacco smoke or smokeless tobacco use.

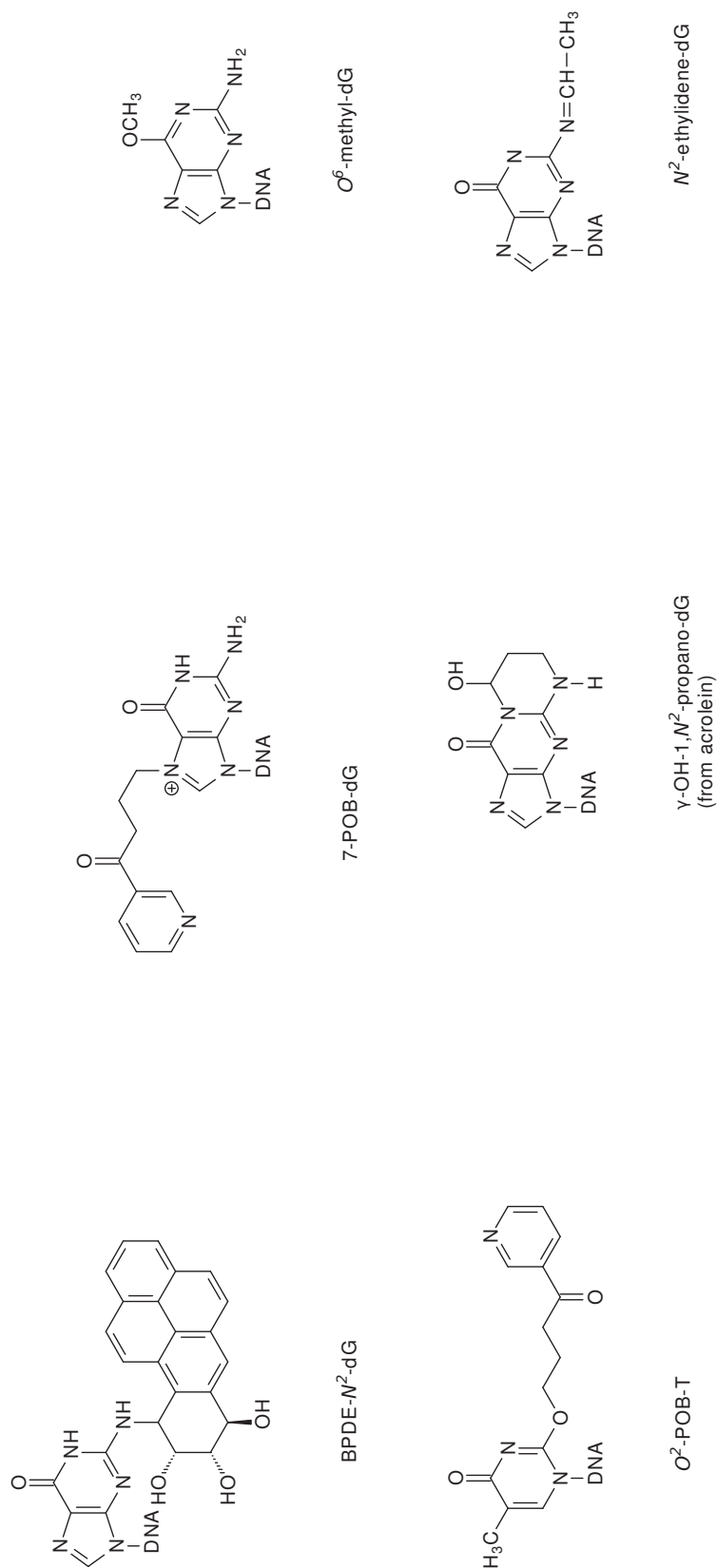
The structures of DNA adducts of tobacco smoke carcinogens have been characterized in detail, but a complete description of these structures is beyond the scope of this section. Selected DNA adduct structures are shown in Fig. 4.4. A major DNA adduct of BaP results from *trans*- addition of the benzo[*a*]pyrene diol epoxide (BPDE) to the *N*<sup>2</sup>-position of dG (Szeliga

Fig. 4.3 Overview of metabolism and DNA adduct formation from eight tobacco smoke constituents



4-ABP, 4-aminobiphenyl; AC, acetyl; ADP, adenosine diphosphate; ALDH, aldehyde dehydrogenase; AKR, aldo-ketoreductase; B[a]P, benzo[a]pyrene; EH, epoxide hydrolase; Gluc, glucuronide; GSTs, glutathione S-transferases; NATs, N-acetyltransferases; NDMA, N-nitrosodimethylamine; NIH shift, phenomenon of hydroxylation-induced intramolecular migration; NNAL, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN, N'-nitrosomethyl-1-butanone; NNN-N-oxide, NNN-N-oxide; UGTs, uridine-5'-diphosphate-glucuronosyl transferases  
Adapted from Cooper *et al.* (1983); Preussmann & Stewart (1984); Kadlubar & Beland (1985); Hecht (1998, 1999); Penning & Drury (2007); IARC (2008, 2010b).

Fig. 4.4 Structures of some DNA adducts of tobacco smoke constituents



BPDE-*N*<sup>2</sup>-dG, benzo[*a*]pyrene diol epoxide-*N*<sup>2</sup>-deoxyguanosine; 7-POB-dG, pyridyloxobutyl-deoxyguanosine; *O*<sup>2</sup>-POB-T, *O*<sup>2</sup>-pyridyloxobutyl-thymidine



& Dipple, 1998). Pyridyloxobutyl (POB)-DNA adducts of NNK and NNN are formed at the 7- and  $O^6$ -positions of deoxyguanosine dG, the  $O^2$ -position of thymidine, and the  $O^2$ -position of deoxycytidine (Hecht, 2008). They can be measured in part as 4-hydroxy-1-(3-pyridyl)-1-butanone (HPB) released upon hydrolysis. Metabolic activation of NNK also leads to 7-methyl-dG and  $O^6$ -methyl-dG, identical to the DNA adducts formed from NDMA and other DNA methylating agents (Hecht, 2008). Ethylating agents and ethylene oxide in cigarette smoke also alkylate dG (Zhao *et al.*, 1999; Singh *et al.*, 2005). Acrolein and crotonaldehyde react with DNA to produce exocyclic 1, $N^2$ -dG adducts, while acetaldehyde forms a Schiff base adduct with the exocyclic  $N^2$  amino group of dG. There is evidence for the presence of all these DNA adducts in tissues or blood cells of smokers, but there are also many studies in which these specific adducts have been sought but not found (Boysen & Hecht, 2003).

Measurement of these DNA adducts as biomarkers potentially can provide the most direct link between cellular exposure and cancer, because DNA adducts are so critical in carcinogenesis. However, it is challenging because their levels are extremely low, frequently ranging from 1 per  $10^6$  to 1 per  $10^8$  normal bases, and the tissue or blood samples containing them are usually available in only small quantities. Fortunately, the routine detection ofamol levels [attomole, equivalent to 10 moles] of DNA adducts by conventional LC-MS/MS techniques is now feasible (Singh & Farmer, 2006). There are still relatively few examples of quantitation of *specific* DNA adducts of tobacco carcinogens in tissues of smokers using mass spectrometry, high pressure liquid chromatography (HPLC)-fluorescence, HPLC with electrochemical detection, or postlabelling techniques (Pfeifer *et al.*, 2002). A much larger body of work has used the highly sensitive, but relatively non-specific  $^{32}\text{P}$ -postlabelling and immunoassay methods of

DNA adduct detection. Although the adducts detected using  $^{32}\text{P}$ -postlabelling are often referred to as “aromatic DNA adducts,” there is strong evidence that they are not related to PAHs (Arif *et al.*, 2006). Adduct levels are generally higher in lung tissues of smokers than non-smokers while studies using blood DNA have produced varied results. Adducts have also been detected in the larynx, oral and nasal mucosa, bladder, cervix, breast, pancreas, stomach, placenta, foetal tissue, cardiovascular tissues, sputum, and sperm of smokers (IARC, 2004a). A meta-analysis of the relationship of DNA adduct levels in smokers to cancer, as determined by  $^{32}\text{P}$ -postlabelling in the majority of studies or enzyme linked immunosorbent assay (ELISA), demonstrated a positive relationship in current smokers (Veglia *et al.*, 2003; 2008).

#### (d) Protein adducts

Carcinogen-haemoglobin (Hb) and serum albumin adducts are regarded as surrogates for DNA adduct measurements. Although these proteins are not targets for carcinogenesis, virtually all carcinogens that react with DNA will also react with protein. Advantages of haemoglobin adducts include the ready availability of haemoglobin from blood and the relatively long lifetime of the erythrocyte in humans – 120 days –, which provides an opportunity for adducts to accumulate. Studies on protein adducts in smokers have been comprehensively reviewed (IARC, 2004a).

Haemoglobin adducts of aromatic amines are a highly informative type of carcinogen biomarker, with levels that are consistently higher in smokers than non-smokers, particularly for 3-aminobiphenyl and 4-aminobiphenyl-Hb adducts. Haemoglobin binds aromatic amines efficiently because heme accelerates the rate of nitrosoarene formation from the hydroxylamine, which is produced metabolically from the aromatic amine by CYP1A2 (Fig. 4.3; Skipper & Tannenbaum, 1990). Binding of the nitrosoarene occurs at the  $\beta$ -93 cysteine residue of human

haemoglobin; the adduct is hydrolysed releasing the free amine, which is quantified by GC-MS (Skipper & Tannenbaum, 1990). Adduct levels are clearly related to cigarette smoking (Skipper & Tannenbaum, 1990). Adducts that form at the terminal valine of haemoglobin are also useful biomarkers: examples include those derived from ethylene oxide, acrylonitrile and acrylamide (Bergmark, 1997; Fennell *et al.*, 2000). Ethylated N-terminal valine of haemoglobin is also higher in smokers than in non-smokers (Carmella *et al.*, 2002).

HPB-releasing Hb adducts of NNK and NNN have been quantified in studies of smokers and smokeless tobacco users (IARC, 2004a, 2007a). These adducts are thought to be tobacco-specific, but some studies report their presence in non-smokers (Falter *et al.*, 1994; Schlöbe *et al.*, 2008).

#### 4.1.4 Genetic and related effects

##### (a) Mutagenicity and cytogenetic effects

Tobacco smoke and its condensates are mutagenic in a wide variety of test systems from bacteria to mammalian cells in culture to rodents and humans (DeMarini, 2004; IARC, 2004a; Husgafvel-Pursiainen, 2004). In bacterial systems, the heterocyclic amines and aromatic amines in condensates account for much of the frameshift mutagenicity, whereas the PAHs and nitrosamines may account for some of the base-substitution mutagenicity (DeMarini *et al.*, 1995). G to T is the predominant class of base-substitution mutation induced by condensates in experimental systems and found in oncogenes and tumour-suppressor genes in smoking-associated lung tumours (IARC, 2004a). The genotoxic potencies of a variety of condensates in several genotoxicity assays likely have only qualitative value with regard to health risk assessment (DeMarini *et al.*, 2008). This is consistent with findings that smokers of low- or high-tar cigarettes have similar urinary levels of lung carcinogens (Hecht *et al.*, 2005b; Hatsukami *et al.*,

2006b) and similar risks for lung cancer (Harris *et al.*, 2004).

In rodents, cigarette smoke induces sister chromatid exchange and micronuclei in bone marrow and lung cells. Human newborns of smoking mothers have increased frequencies of *HPRT* mutations, chromosomal translocations, and DNA strand breaks. Sperm of smokers has increased frequencies of aneuploidy, DNA adducts, strand breaks, and oxidative damage. Cigarette smoke also causes germ-cell mutations in mice (Yauk *et al.*, 2007). Collectively, these data suggest that smoking is likely a germ-cell mutagen in humans. Smoking produces mutagenic urine and somatic-cell mutations in humans, including *HPRT* mutations, sister chromatid exchange, microsatellite instability and DNA damage in a variety of tissues. Genotoxic effects have been found in eight organ sites at which tobacco smoke causes cancer in humans (DeMarini, 2004; IARC, 2004a).

##### (b) Mutations in *TP53*, *KRAS* and related genes

Gene mutation data from a variety of databases, including the IARC Cancer *TP53* Mutation Database (<http://www-p53.iarc.fr/>), have been collated in the Genetic Alterations in Cancer (GAC) database (<http://dir-apps.niehs.nih.gov/gac/>) so that mutations in a variety of genes in various cancerous tissues can be compared. An assessment of the Gene Alterations in Cancer database showed that at least three genes were mutated more frequently in lung tumours from smokers than non-smokers (Lea *et al.*, 2007): *TP53* (39 versus 26%), *K-RAS* (20 versus 3%), and loss of heterozygosity at *FHIT* (57 versus 27%). Thus, genes in the cell cycle (*TP53*), cell signalling (*KRAS*) and apoptotic (*FHIT*) pathways are mutated more frequently in smoking- rather than in nonsmoking-associated lung tumours. Genomic sequencing of lung tumours has identified other mutated genes that are associated with smoking; ten times more genes are mutated in

lung tumours from smokers compared to non-smokers ([Ding et al., 2008](#)).

GC to TA transversions were the predominant class of base-substitution mutation found in *TP53* and *KRAS* genes in lung tumours from smokers, with the frequency of this mutation in *TP53* being 30% in smokers versus 22% in non-smokers. In smoking-associated oral cancers, the percentage of GC to TA mutations in *TP53* was 15% versus 2%, respectively. This mutation spectrum is consistent with that produced by a variety of known carcinogens present in tobacco smoke ([IARC, 2004a](#)). At the codon level, the most frequently mutated codons in *TP53* in lung tumours of smokers were 157, 175, 245, 248, and 273, all of which occur in the DNA-binding domain of the protein; among these codons, only 273 was mutated in lung tumours from non-smokers. Only three of these codons (157, 245 and 273) were mutated in smoking-associated larynx tumours, and only codon 157 was mutated in smoking-associated oral tumours. Thus, the mutational specificity at *TP53* is different among smoking- and nonsmoking-associated tumours and among smoking-associated tumours at various organs ([Lea et al., 2007](#)). Thus, different pathways are involved in the development of different types of tumours ([Le Calvez et al., 2005](#); [Mounawar et al., 2007](#); [Subramanian & Govindan, 2008](#)).

#### 4.1.5 Effects on gene expression profile

As indicated in a review by [Sen et al. \(2007\)](#) involving microarray analysis of 18 studies in human smokers, 7 in smoke-exposed rodents, and 3 in condensate-exposed mammalian cells, smoking generally upregulated a wide variety of genes, especially those involved in the stress response, phase I metabolism, and immune response. Genes that were consistently expressed differentially in smokers (as assessed in alveolar macrophages, lung cells or peripheral lymphocytes) included metallothioneins, heat-shock

proteins, superoxide dismutase, glutathione transferase, heme oxygenase, *CYP* genes (*1A2*, *1A1* and *1B1*), interleukins and chemokines.

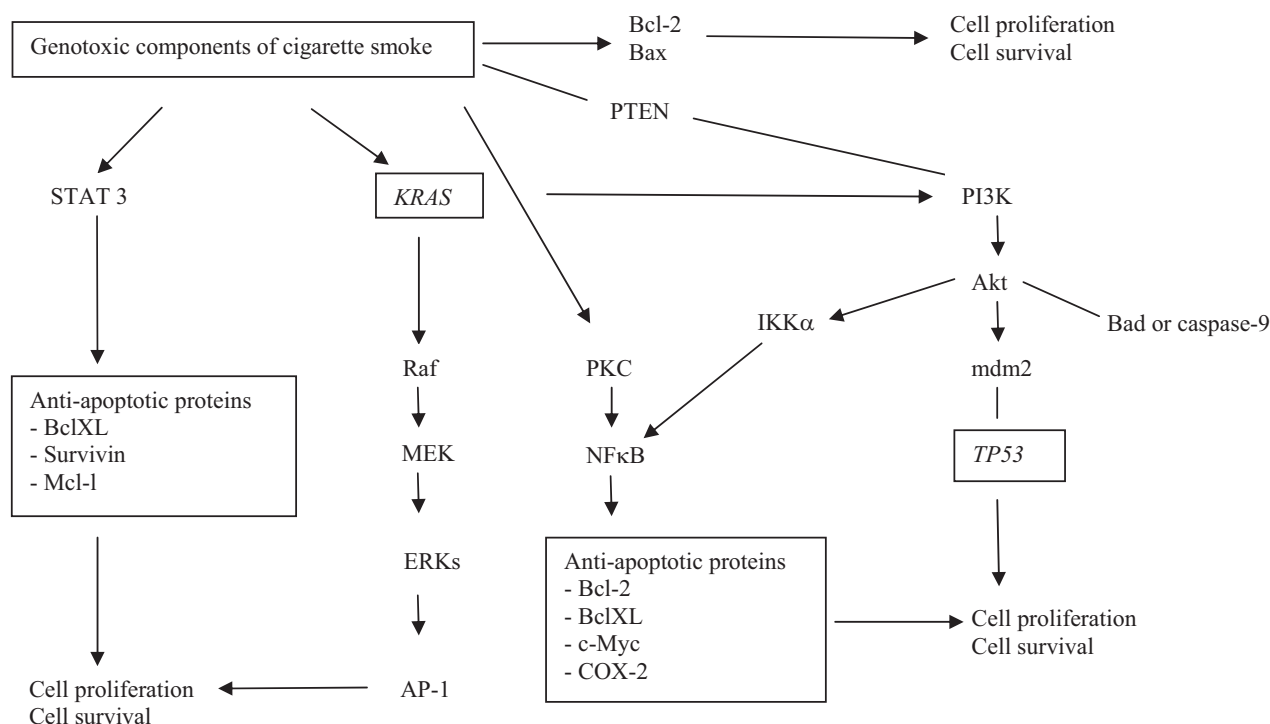
[Spira et al. \(2004\)](#) analysed global gene expression in bronchial epithelial cells and found that the expression levels of metabolizing and antioxidant genes had reverted to control levels after two years of smoking cessation. However, expression of potential oncogenes and tumour-suppressor genes never reverted to never-smoker levels even after years of smoking cessation. Consistently, expression of microRNAs is generally downregulated by cigarette smoke ([Izzotti et al., 2009](#)). As discussed below, smoking also altered methylation patterns and gene expression in smoking-associated tumours.

#### 4.1.6 Other effects associated with carcinogenesis

##### (a) Proliferation, differentiation, apoptosis, and inflammation

As noted above, the signal-transduction pathways in lung tumours from smokers are distinctly different from those of non-smokers ([Mountzios et al., 2008](#)). Fig 4.5 shows details of signalling pathways that are deregulated by tobacco smoke. The involvement of high frequencies of mutated *K-RAS* and *TP53* genes in smoking-associated lung tumours results in altered regulation of cell proliferation, differentiation, cytoskeletal organization and protein trafficking. Cigarette smoking activates *NF- $\kappa$ B*, which induces pro-inflammatory cytokine expression and induces growth factors and proliferative signals ([Mountzios et al., 2008](#)). This gene also influences the expression of the anti-apoptotic gene *BCL2* and pro-apoptotic gene *BAX*. Smoking produces chronic inflammation, which promotes cancer ([Walser et al., 2008](#)). Smoking results in high levels of reactive oxygen species, which damage epithelial and endothelial cells and impair their function. In smoking-associated lung cancer, elevated levels of cyclooxygenase-2 (COX-2) and



**Fig. 4.5 General scheme of some cell-signalling pathways that are deregulated by tobacco smoke in lung carcinogenesis**

Akt, serine/threonine protein kinase; ERKs, extracellular regulated kinases; MEK, mitogen-activated protein kinase; Bad, Bcl2-associated agonist of cell death; PI3K, phosphatidylinositol 3-kinase; PKC, protein kinase C; NF-κB, nuclear factor κB; IKKα, inhibitor of nuclear factor κ-B kinase; PTEN, phosphatase and tensin homologue; STAT3, signal transducer and activator of transcription, COX-2, cyclooxygenase-2

prostaglandin (PGE<sub>2</sub>) indicate apoptosis resistance, proliferation, immunosuppression, angiogenesis, invasion, and epithelial-mesenchymal transition (Walser *et al.*, 2008).

#### (b) Endogenous nitrosation

Intragastric formation of *N*-nitroso compounds, measured using urinary nitrosamino acids excreted in urine, was increased in smokers compared to non-smokers (Hoffmann & Brunnemann, 1983). Two recent studies demonstrated that NNN forms endogenously in some users of nicotine replacement therapy products (Stepanov *et al.*, 2009a, b).

#### (c) Hormonal changes

These are described in Section 4.3.2a.

## 4.2 Polymorphisms in carcinogen-metabolizing genes

### 4.2.1 Introduction

It has been long proposed that the known variation among individuals in their capacity to activate and detoxify carcinogens may be associated with increased susceptibility to cancer, and that polymorphisms of carcinogen-metabolising genes may play a significant role. The most intensively studied genes involved in the metabolism of carcinogens include the various *CYP* genes, the *GST* genes and the *NAT* genes. Other relevant xenobiotic-metabolising genes, such as *EPHX*, sulfotransferase (*SULT*), *UGT*, myeloperoxidase (*MPO*), and NAD(P)H quinone oxidoreductase-1 (*NQO1*) genes, have also been studied. Recently, extensive pooled studies and

reviews have been published on polymorphisms of carcinogen-metabolising genes and their role in cancer susceptibility, especially in tobacco-related lung cancer and cancers at other sites. Similarly, various biomarkers of exposure and genotoxicity that are presumed to provide a mechanistic basis for such associations have been comprehensively investigated in relation to these polymorphisms. A brief overview based largely on reviews and the meta- and pooled analyses is presented here.

#### 4.2.2 Genetic polymorphisms of carcinogen metabolism: some central genes

##### (a) CYP genes

CYPs comprise the principal enzyme system catalysing various phase I oxidation reactions, including metabolic activation and detoxification of many carcinogenic substances in tobacco smoke such as PAHs. Of the various CYP enzymes expressed in humans, many of those belonging to CYP1 to CYP3 families play a role in carcinogen metabolism, producing highly reactive DNA-damaging metabolites as well as detoxified metabolites ([Guengerich & Shimada, 1998](#); [Lang & Pelkonen, 1999](#); [Ingelman-Sundberg, 2004](#)). CYPs have evolved into a wide superfamily with close to 60 different active genes currently identified; most of these genes exhibit polymorphism ([www.cypalleles.ki.se](http://www.cypalleles.ki.se)).

##### (i) CYP1A1

Several allelic variants of the human *CYP1A1* gene are currently known ([www.cypalleles.ki.se](http://www.cypalleles.ki.se)). The major variant forms of the *CYP1A1* gene (wildtype allele *CYP1A1\*1*) mostly frequently studied for association to cancer susceptibility include the following two alleles: (i) *CYP1A1\*2A* allele (m1 allele; *Msp* I) and (ii) *CYP1A1\*2B* ([Cascorbi et al., 1996](#)) or *CYP1A1\*2C* ([www.cypalleles.ki.se](http://www.cypalleles.ki.se)) allele (m2 allele; Ile<sup>462</sup>Val). Importantly, the *CYP1A1* m1 allele and m2 allele are in complete linkage disequilibrium in

Caucasians ([Kawajiri, 1999](#); [Bartsch et al., 2000](#)). In addition, *CYP1A1\*4* allele (m4; Thr<sup>461</sup>Asn) ([Cascorbi et al., 1996](#)), and *CYP1A1\*3* (m3) allele found in African-Americans but not in Caucasians or Asians ([Crofts et al., 1993](#)) are included in some studies ([Bartsch et al., 2000](#)).

In smoking-related lung cancer, the various *CYP1A1* polymorphisms as well as the differences in the frequencies of the rare variant alleles between ethnicities contribute to the differences in findings. There are collective analyses of data predominantly indicating an overall mild to moderate effect of *CYP1A1* polymorphisms on lung cancer risk ([Kawajiri, 1999](#); [Bartsch et al., 2000](#); [Houlston, 2000](#); [Le Marchand et al., 2003](#); [Vineis et al., 2003](#); [Vineis et al., 2004](#); [Lee et al., 2008a](#); [Shi et al., 2008](#)). In many reviews and meta- or pooled analyses the increased risk associated with *CYP1A1* polymorphism has most clearly been seen in Asian populations ([Kawajiri, 1999](#); [Le Marchand et al., 2003](#); [Vineis et al., 2003](#); [Lee et al., 2008a](#); [Shi et al., 2008](#)).

Multiple studies have also analysed the gene-gene interactions between *CYP1A1*, *GSTM1* and *GSTT1* polymorphisms and lung cancer ([d'Errico et al., 1999](#); [Houlston, 1999](#); [Benhamou et al., 2002](#); [Bolt & Thier, 2006](#); [Raimondi et al., 2006](#); [Ye et al., 2006](#); [Carlsten et al., 2008](#)). Some of the analyses have indicated that the elevated risk for lung cancer may be more pronounced for some *CYP1A1/GSTM1* null genotype combinations ([Le Marchand et al., 1998](#); [Bartsch et al., 2000](#); [Vineis et al., 2004, 2007](#); [Lee et al., 2008a](#); [Shi et al., 2008](#)).

##### (ii) CYP1A2

*CYP1A2* is highly inducible and metabolises, including deacetylation reactions, many tobacco smoke carcinogens such as aromatic and heterocyclic amines and nitro-aromatic compounds, and tobacco-specific nitrosamines such as NNK ([Nebert et al., 2004](#); [Jalas et al., 2005](#); [IARC, 2007a](#)). A few major variant alleles have been described ([www.cypalleles.ki.se](http://www.cypalleles.ki.se)), some of which

may have been reported to influence inducibility ([Nakajima et al., 1999](#); [Ingelman-Sundberg et al., 2007](#)). Overall, the phenotype-genotype relations have not been well established for *CYP1A2*, although current evidence points towards contribution of genetic variation ([Murayama et al., 2004](#); [Ingelman-Sundberg et al., 2007](#)); data on possible associations with tobacco related cancer are sparse ([Agundez, 2004](#); [Nebert & Dalton, 2006](#)).

#### (iii) *CYP2A6*

Several aspects of smoking behaviour are likely to be influenced by *CYP2A6* genetic variation, which influences nicotine metabolism ([Malaiyandi et al., 2005](#); [Mwenifumbo & Tyndale, 2007](#)). The most important functionally altered allele is *CYP2A6\*4* (gene deletion), which confers a poor-metabolizer phenotype in homozygous individuals ([Malaiyandi et al., 2005](#); [Ingelman-Sundberg et al., 2007](#); [Mwenifumbo & Tyndale, 2007](#)). In some studies, polymorphic variants of *CYP2A6* gene have been implicated in susceptibility to smoking-related cancers ([Gambier et al., 2005](#); [Malaiyandi et al., 2005](#); [Nakajima, 2007](#)). In line with this, the accumulated data have suggested that *CYP2A6* polymorphism may affect cancer risk in smokers but not in non-smokers ([Tan et al., 2001](#); [Kamatani et al., 2005](#); [Malaiyandi et al., 2005](#); [Canova et al., 2009](#)).

#### (iv) *CYP2A13*

From human CYPs, *CYP2A13* is the primary form involved in the metabolic activation of the tobacco-specific nitrosamines NNK and NNN ([Jalas et al., 2005](#); [IARC, 2007a](#)). The *CYP2A13* gene exhibits polymorphism in humans ([Zhang et al., 2002](#); [Jalas et al., 2005](#)), and experimental studies suggest that some of the polymorphisms may affect the hydroxylation of NNN and NNK ([Jalas et al., 2005](#); [Schlicht et al., 2007](#)). However, the data on possible effects of these polymorphisms on the risk of tobacco-related cancers in

humans are still limited ([Wang et al., 2003](#); [Song et al., 2009](#); [Timofeeva et al., 2009](#)).

#### (v) *CYP2D6*

The *CYP2D6* gene shows high variability in expression. The enzyme is not inducible, and therefore genetic variation largely contributes to the interindividual variation in enzyme activity. Currently, more than 100 different functional *CYP2D6* gene variants have been described, and these are divided into alleles causing abolished, decreased, normal, and ultrarapid enzyme activity ([Ingelman-Sundberg, 2005](#); [Ingelman-Sundberg et al., 2007](#)). The most important null alleles leading to poor-metabolizer phenotype are *CYP2D6\*4* (splice defect) and *CYP2D6\*5* (gene deletion) ([Ingelman-Sundberg, 2005](#); [Ingelman-Sundberg et al., 2007](#)).

A large series of studies have been carried out over the past 20 years on the association between *CYP2D6* polymorphism and susceptibility to lung cancer and to some other tobacco-related cancers ([Wolf & Smith, 1999](#)). Despite some indication of an association between *CYP2D6* poor-metabolizer and decreased risk for lung cancer, no major role for *CYP2D6* in carcinogen metabolism or a molecular basis for such an association have been discovered ([Wolf & Smith, 1999](#); [Ingelman-Sundberg, 2005](#)).

#### (vi) *Other CYP genes*

*CYP1B1* allelic variants that affect the catalytic activity have been described but they have been studied to a lesser extent for the association with susceptibility to smoking-related cancers ([Thier et al., 2003](#)). Some positive findings have been reported on head and neck cancer ([Ko et al., 2001](#)), and lung cancer ([Zienolddiny et al., 2008](#)).

Several polymorphisms have been characterized in the *CYP2E1* gene and several positive associations with the risk of different cancers have been reported, in particular for cancers of the upper aerodigestive tract, lung and gastrointestinal tract (Section 2.19). *CYP2E1* may also

play an important role in the interaction of the carcinogenic effects of alcohol and tobacco (Section 4.4).

From the human *CYP3A* locus (*CYP3A4*, *CYP3A5* and *CYP3A7*), the *CYP3A4\*1B* allele has been associated with lung cancer and prostate cancer in some studies but not in all ([Dally et al., 2003](#); [Rodriguez-Antona & Ingelman-Sundberg, 2006](#)). However, the role of these variants in relation to tobacco smoking is unknown.

#### (b) *GSTM1* and other *GST* genes

Polymorphic *GST* genes have long been proposed to modify susceptibility to lung cancer ([Seidegård et al., 1986](#); [Ketterer et al., 1992](#)). The polymorphic genes encoding the various classes of cytosolic *GST* enzymes include the *GSTM1* and *GSTM3* genes (mu class), the *GSTP1* gene (pi class), and the *GSTT1* gene (theta class). The gene deletion (null) allele of the *GSTM1* gene (*GSTM1\*0*) and of the *GSTT1* gene (*GSTT1\*0*) have been the most intensively studied polymorphisms in relation to increased susceptibility to cancer ([Strange et al., 2001](#); [Bolt & Thier, 2006](#); [McIlwain et al., 2006](#)). For the *GSTP1* gene, the form most abundantly present in lung tissue, genetic variation in exon 5 (*GSTP1\*2*; *Ile<sup>105</sup>Val*), in exon 6 (*Ala<sup>114</sup>Val*), as well as a combination of these, are the variations most frequently studied for cancer susceptibility ([Watson et al., 1998](#); [Cote et al., 2009](#)).

Numerous reviews, meta- and pooled analyses have been published over the past 15 years or so for the *GST* genes with systematic assessments covering altogether tens of thousands of cases and controls. For the *GSTM1* null genotype, such analyses have largely provided negative, suggestive or at most moderately positive results for an association with an increased risk for lung cancer ([d'Errico et al., 1999](#); [Houlston, 1999](#); [Benhamou et al., 2002](#); [Ye et al., 2006](#); [Carlsten et al., 2008](#)). The larger the studies, the less significant the estimates for the role of *GSTM1* emerge in systematic analysis ([Ye et al., 2006](#); [Carlsten](#)

[et al., 2008](#)). Also the varying allele frequencies related to ethnic background affect the findings for *GSTM1* as well as for many other genes ([Garte et al., 2001](#); [Ye et al., 2006](#); [Carlsten et al., 2008](#); [Lee et al., 2008a](#)).

In a meta-analysis of the association between the *GSTT1* gene polymorphism and lung cancer no association between *GSTT1* null genotype and risk for lung cancer in Caucasians was observed, but a positive association was found for Asians ([Raimondi et al., 2006](#)). A significant association for either Caucasians or Asians was also not found in a pooled analysis ([Raimondi et al., 2006](#)). A meta-analysis found no significant association between lung cancer risk and the *GSTP1 Ile<sup>105</sup>Val* polymorphism; but the pooled analysis suggested an overall statistically significant mild association between lung cancer and homozygosity or heterozygosity for the *Val<sup>105</sup>* allele ([Cote et al., 2009](#)).

A recent body of epidemiologic data suggests an inverse association between cruciferous vegetables/isothiocyanates intake and cancers of the colorectum, lung and breast; the studies also provide evidence that this protective effect is greater among individuals who possess the *GSTM1* or *T1* null genotype, who would be expected to accumulate higher levels of isothiocyanates at the target tissue level, a pre-requisite for their enzyme-inducing effects ([Seow et al., 2005](#)). The association between isothiocyanates and cancer, and its modification by *GSTM1* and *GSTT1* status, is most consistent for lung cancer and appears to be strongest among current smokers who possess the combined *GSTM1* and *GSTT1* null genotypes ([London et al., 2000a](#); [Spitz et al., 2000](#); [Zhao et al., 2001](#); [Brennan et al., 2005](#); [Seow et al., 2005](#)).

#### (c) *NAT1* and *NAT2* genes

The pooled and meta-analyses carried out on *NAT1* and *NAT2* polymorphisms and bladder cancer risk have consistently reported significantly increased risk for *NAT2* slow acetylators



([Dong et al., 2008](#); [Malats, 2008](#); see also Section 2.9). Data on *NAT1* fast acetylators are inconsistent, as are the studies suggesting an increased risk for *NAT2* rapid acetylator status. Additionally, genotypes for other genes, specially *GSTM1*, have also been implicated ([Vineis et al. 2001](#); [García-Closas et al., 2005](#); [Hein, 2006](#); [Sanderson et al., 2007](#); [Dong et al., 2008](#); [Malats, 2008](#)).

In a recent large study on tobacco-related lung cancer and upper aerodigestive cancers, the *NAT* genes, in particular *NAT\*10* haplotype, emerged from a set of 16 genes as involved in the risk ([McKay et al., 2008](#)). When more than one hundred single nucleotide polymorphisms for 31 genes involved in phase I or phase II metabolism or in antioxidant defence were investigated, only four of the previously reported polymorphisms of the *GSTP1*, *EPHX1* and superoxide dismutase *SOD2* genes and the *NAT1* fast acetylator phenotype remained significantly associated with risk of non-small cell lung cancer after correction for multiple testing ([Zienolddiny et al., 2008](#)).

In breast cancer, several recent meta-analyses of epidemiological studies have suggested increased risk among smokers with the *NAT2* slow acetylator genotype; such an association has been observed especially among long-term smokers and post-menopausal women ([Terry & Goodman, 2006](#); [Ambrosone et al., 2008](#); [Ochs-Balcom et al., 2007](#); [Baumgartner et al., 2009](#)).

In all, the role of the *NAT* gene polymorphisms in tobacco-related cancers, with the exceptions of increased risk of bladder cancer and possibly breast cancer in *NAT2* slow acetylators, remains largely open due to the incomplete understanding of phenotype-genotype relationships, and the interplay between these two genes and their polymorphisms ([Hein, 2002, 2006](#)).

#### (d) Others

Genes coding for *EPHX*, *UGT* and *SULT* enzymes, mainly but not exclusively involved in detoxification reactions, exhibit polymorphisms with numerous gene variants discovered

([Mackenzie et al., 1997](#); [London et al., 2000b](#); [Glatt et al., 2001](#); [Burchell, 2003](#)). Additional polymorphic genes studied for their significance in cancer susceptibility are the *NQO1* and *MPO* genes, with *NQO1* playing a dual role in the detoxification and activation of procarcinogens, and *MPO* converting lipophilic carcinogens into hydrophilic forms ([Nebert et al., 2002](#)). All these genes have been studied for their possible association with tobacco-related cancer risk to a varying extent and with variable outcomes ([London et al., 2000b](#); [Bamber et al., 2001](#); [Garte, 2001](#); [To-Figueras et al., 2001](#); [Tiemersma et al., 2002b](#); [Guillemette, 2003](#); [Wells et al., 2004](#); [Kiyohara et al., 2005](#); [Moreno et al., 2005](#); [Nagar & Remmel, 2006](#); [Gallagher et al., 2007](#)).

#### 4.2.3 Biomarkers of tobacco carcinogenesis and polymorphic genes of carcinogen metabolism

A myriad of studies have investigated association between various biomarkers of tobacco-related carcinogenesis and genetic variation of genes involved in carcinogen metabolism. For involvement in increased cancer susceptibility, a large variety of intermediate biomarkers have been studied, including PAH metabolites in urine, urinary mutagenicity, DNA and protein adducts, cytogenetic alterations, *HPRT* mutant lymphocytes, as well as somatic mutations of the tumour suppressor gene *TP53* and *KRAS* oncogene occurring in cancer tissue.

##### (a) PAH metabolites and mutagenicity in urine

##### (i) PAH metabolites in urine

Increased excretion of 1-hydroxypyrene in urine in association with the *GSTM1* null genotype has been reported in many studies on individuals with occupational or environmental exposure to PAHs ([Yang et al., 1999](#); [Alexandrie et al., 2000](#); [Lee et al., 2001](#); [Kuljukk-Rabb et al., 2002](#); [Kato et al., 2004](#)). The associations seen between *GSTT1* polymorphism and the

PAH metabolites are somewhat more variable. Similarly, the joint effect of *GSTM1* and *GSTT1* null genotypes, as well as the effects of some other genes of xenobiotic metabolism, such as *EPHX*, *CYP1A1*, *CYP1A2* and the aryl hydrocarbon receptor (*AhR*) gene have been either positive or negative ([Yang et al., 1999](#); [Alexandrie et al., 2000](#); [Lee et al., 2001](#); [Zhang et al., 2001](#); [Kuljukkka-Rabb et al., 2002](#); [Yang et al., 2003](#); [Chen et al., 2007](#); [Cocco et al., 2007](#); [Bin et al., 2008](#)).

Another PAH metabolite studied in this context is phenanthrene, the simplest PAHs with a bay region, a feature closely associated with carcinogenicity. A study quantified ratios of urinary products of metabolic activation (such as PhET) and detoxification (such as phenanthrols, HOPhe) of phenanthrene in 346 smokers, who were also genotyped for 11 polymorphisms in genes involved in PAHs metabolism, including the *CYP1A1* and *GSTM1* genes. A significant association between the presence of the *CYP1A1* Ile<sup>462</sup>Val polymorphism and high PhET/3-HOPhe ratios was found, particularly in combination with the *GSTM1* null polymorphism ([Hecht et al., 2006](#)).

Overall, the data on the influence of genetic variation in PAHs metabolism on the levels of the urinary metabolite biomarkers are variable, and currently inconclusive.

#### (ii) Urinary mutagenicity

One relatively early line of research investigated the relationship between urinary mutagenicity and genetic variation in activation or detoxification genes. These studies, however, have seldom been focused on smokers only but rather on other sources of exposure ([Pavanello & Clonfero, 2000](#)).

In some studies, *NAT2* slow acetylator genotype either alone or in combination with *GSTM1* null genotype has been associated with increased urinary mutagenicity in the *Salmonella* test in individuals with occupational, environmental or

medicinal PAH-related exposure, or in smokers ([Vineis & Malats, 1999](#); [Pavanello & Clonfero, 2000](#)). In another study, *CYP1A2* activity, but not *NAT2*, *GSTM1* or *GSTT1* genotypes influenced urinary mutagen excretion in smokers ([Pavanello et al., 2002](#)). A further study also suggested contribution of the *CYP1A2* gene variation to increased urinary mutagenicity in heavy smokers ([Pavanello et al., 2005](#)). Associations with variants of other xenobiotic-metabolising genes (such as *EPHX1*) have also been reported, with somewhat complex results ([Kuljukkka-Rabb et al., 2002](#)).

#### (b) DNA adducts

The relationship between the variants of polymorphic genes of carcinogen metabolism and tobacco smoke-related DNA adduct formation has been addressed in an abundant number of studies among smokers, occupationally exposed groups, and patients with smoking-related cancer. In addition, multiple *in vitro* studies on this relationship have been carried out ([Bartsch et al., 2000](#); [Pavanello & Clonfero, 2000](#); [Alexandrov et al., 2002](#); [Wiencke, 2002](#)).

The intensive efforts to study the relationship between *CYP1A1* and *GSTM1* gene polymorphism and the level of aromatic-hydrophobic/bulky PAH-DNA adducts in human lungs have so far provided little evidence for a role of a single metabolic genotype or their combinations on DNA adduct formation, with largely weak, non-significant or contradictory results. However, a trend of increasing adduct levels in subjects with the *CYP1A1*\*2-*GSTM1*\*0 genotype combination has been observed, which was reinforced when BPDE-DNA adducts were specifically assessed. These results suggest a gene-gene interaction, supported by biological data from other studies ([Bartsch et al., 2000](#); [Alexandrov et al., 2002](#); [Wiencke, 2002](#)). Such gene-gene interaction lends support to the increased risk for lung cancer found in carriers of these genotypes in Japanese, among whom the frequency of the

variant *CYP1A1* allele is much higher ([Bartsch et al., 2000](#); [Alexandrov et al., 2002](#)).

A wide selection of genes and genotypes included in the various studies have made it difficult to assess the overall role of the polymorphisms of *GSTM1* and other genes alone or in combination. Differences between the studies in the types of adducts determined, the various tissues, cell types and cancers studied, detection methods, variation in sources and types of exposure, sample size, gender differences, and sometimes poor knowledge regarding the alleles, genotypes and haplotypes under study also contribute to the large variability seen in these studies ([d'Errico et al., 1999](#); [Hemminki et al., 2001](#); [Alexandrov et al., 2002](#); [Wiencke, 2002](#)).

#### (c) Cytogenetic biomarkers of genotoxicity

##### (i) Chromosome aberrations and sister chromatid exchanges

Early studies investigating whether homozygosity for the *GSTM1* null allele affects prevalence of cytogenetic changes in lymphocytes of smokers reported positive results ([Seidegård et al., 1990](#); [van Poppel et al., 1992](#); [Cheng et al., 1995](#)). Since then, studies have investigated the association between genetic polymorphisms of xenobiotic-metabolising genes and cytogenetic biomarkers in smokers and in some occupational groups ([Rebbeck, 1997](#); [Autrup, 2000](#); [Pavanello & Clonfero, 2000](#); [Norppa, 2003, 2004](#)).

Collectively, the reported findings are in support of increased susceptibility of smokers to chromosomal effects in association with *GSTM1* and *GSTT1* null variants deficient in detoxification of tobacco smoke carcinogens. Exposure to genotoxicants generated from other environmental sources (e.g. polluted air, diet, endogenous sources such as reactive oxygen species) may contribute to the observed associations, and it is likely that other polymorphic metabolic genes such as *NAT2* may be involved ([Pavanello & Clonfero, 2000](#); [Norppa, 2001, 2003](#)).

##### (ii) Micronucleus induction

The relationship between formation of micronuclei and genetic polymorphisms of carcinogen metabolism has been addressed in a wide range of human population studies ([Norppa, 2003, 2004](#)). Induction of micronuclei in smokers may be little, if at all, affected by *GSTM1*, *GSTT1* or *NAT2* genotypes. In contrast, the *NAT1* rapid genotype appears to show an association with increased susceptibility to smoking-related micronuclei ([Norppa, 2004](#)).

A recent review evaluated more than seventy human studies on genetic polymorphisms and micronucleus frequency detected either in peripheral blood lymphocytes or exfoliated cells in populations exposed to various genotoxic agents. There were no significant genotype effects involved in micronucleus induction in smokers ([Iarmarcovai et al., 2008](#)). The relationship between genetic polymorphisms and micronucleus formation is complex, and is influenced to a variable extent by several genes of xenobiotic metabolism and DNA repair, as well as the variety of chromosomal alterations known to contribute to micronucleus formation ([Iarmarcovai et al., 2008](#)).

##### (iii) Chromosomal damage induced in vitro

The effects of genotypes or genotype combinations *in vitro* on the induction of various cytogenetic endpoints by tobacco-smoke carcinogens and their metabolites have been studied, initially focused on the *GSTM1* and *GSTT1* null genotypes ([Norppa, 2001, 2004](#)). In a study investigating NNK *in vitro*, lymphocytes from *GSTM1* null donors were more sensitive to induction of chromosomal aberrations and sister chromatid exchanges by NNK than lymphocytes from *GSTM1* positive donors ([Salama et al., 1999](#)).

(d) *Gene mutations*

(i) *HPRT mutant lymphocytes*

Associations between the frequencies of *HPRT* mutant T-lymphocytes in populations exposed to genotoxic agents, such as smokers, and the polymorphism of xenobiotic-metabolising genes have been studied. In the early studies, positive, weak, or negative associations were reported for *GSTM1* null genotype, and negative findings were published for *NAT2* slow acetylator genotype in occupationally exposed or non-exposed subjects (Rebbeck, 1997; Vineis & Malats, 1999). When healthy, non-smoking and occupationally non-exposed young adults were studied for *HPRT* mutant frequency and polymorphisms in *CYP1A1*, *GSTM1* and *NAT2* genes, none of these polymorphisms, analysed individually, were found to influence the *HPRT* mutant frequency (Davies *et al.*, 1999). A significant interaction between the *GSTM1* null genotype and *NAT2* slow acetylator was associated with higher mutant frequency, but no other genotype combinations (Davies *et al.*, 1999). Some later studies have reported variable associations between *HPRT* mutant frequency and polymorphisms for either individual genes (*GSTM1*, *GSTT1* or *EPHX1*) or some of the genotypes in combination among exposed (Viezzler *et al.*, 1999; Abdel-Rahman *et al.*, 2001, 2003).

(ii) *Mutations of the TP53 gene and other cancer-related genes*

Whether the frequency of somatic mutations detected in tumour tissue in cancer-related genes, primarily the *TP53* tumour suppressor gene and *KRAS* oncogene, may be modified by polymorphisms in carcinogen metabolizing genes was first investigated assessing the effects of the *GSTM1* genotype, alone or in combination with other genetic polymorphisms. Several, but not all, such studies showed significant association between *GSTM1* null genotype and either the frequency or type of *TP53* mutations in

smoking-induced lung cancer or other cancer type (Rebbeck, 1997; Vineis & Malats, 1999; Autrup, 2000). Fewer studies examined the association between *TP53* mutations and *GSTT1* polymorphism, and some results suggested the involvement of both null genotypes (Vineis & Malats, 1999; Autrup, 2000).

In smokers with non-small cell lung cancer, the risk of mutation was found to be the highest among the homozygous carriers of the *CYP1A1* rare allele *CYP1A1 MspI* (Ile<sup>462</sup>Val) who also exhibited the *GSTM1* null genotype (Kawajiri *et al.*, 1996). Similarly, positive associations between *K-RAS* mutations and homozygosity for the *CYP1A1* rare allele were observed; the risk of mutation was enhanced when the *CYP1A1* susceptible genotype was combined with *GSTM1* null genotype (Kawajiri *et al.*, 1996). In another study, also carried out in a Japanese study population, *K-RAS* mutations occurred with greater frequency in lung adenocarcinoma smoking patients and of the *GSTM1* null genotype as compared with the *GSTM1* positive genotype (Noda *et al.*, 2004).

Many of the studies that assessed *NAT2* acetylator genotypes have found non-significant associations with the frequency or type of *TP53* mutation in bladder, lung, or other cancers (Vineis & Malats, 1999; Autrup, 2000). A study on bladder cancer did not find an overall association between *TP53* mutation frequency and *GSTM1*, *GSTT1*, *GSTP1* or *NAT2* genotypes. However, among patients with *TP53* mutations, transversion mutations were more frequent in those with *GSTM1* null genotype as compared to those with *GSTM1* positive genotype; no significant associations were found for the *NAT2* gene (Ryk *et al.*, 2005).

In rectal cancer, overall negative results for an association between *TP53* or *KRAS* mutations and *GSTM1* and *NAT2* polymorphisms among smokers and non-smokers exposed to tobacco smoke were found (Curtin *et al.*, 2009). An interaction of second-hand tobacco smoke and *NAT2*



was found in *TP53* mutation positive tumours but not in smokers ([Curtin et al., 2009](#)). Earlier, an increased risk of *TP53* transversion mutations among *GSTM1* positive individuals who smoked cigarettes was found in colon cancer ([Slattery et al., 2002](#)).

A statistically significant association was observed between the *GSTT1* null genotype and *TP53* mutation status of breast tumour in one study ([Gudmundsdottir et al., 2001](#)), while in another larger study none of the genotypes for *CYP1B1*, *GSTM1*, *GSTT1* and *GSTP1* genes alone were associated with somatic *TP53* mutations ([Van Emburgh et al., 2008](#)).

In summary, data from various cancer types on the association between genetic polymorphisms of carcinogen-metabolizing genes and somatic mutations of the *TP53* and *K-RAS* genes vary widely and do not permit to conclude ([Rebbeck, 1997](#); [Vineis & Malats, 1999](#); [Autrup, 2000](#)).

### 4.3 Site-specific mechanisms of carcinogenicity of tobacco smoke

#### 4.3.1 Sites with sufficient evidence of carcinogenicity of tobacco smoking

##### (a) Lung

The conceptual model presented in Section 4.1 (Fig. 4.1) depicts the main mechanistic steps by which cigarette smoke causes cancer. Smokers inhale into their lungs carcinogens which, either directly or after metabolism, covalently bind to DNA, forming DNA adducts (see Section 4.1, Fig. 4.3). Tobacco smoke contains multiple strong lung carcinogens such as NNN, NNK, PAHs, 1,3-butadiene and cadmium. Levels of tobacco smoke-related DNA adducts, mainly <sup>32</sup>P-postlabelled aromatic-hydrophobic/PAH-related bulky DNA adducts, in the lung are higher in smokers than in non-smokers ([Phillips, 2002](#); [IARC, 2004a](#); [Hecht, 2008](#)). Higher levels

of DNA adducts have further been linked to increased risk for cancer in pooled and meta-analyses ([IARC, 2004a](#); [Veglia et al., 2008](#)).

Mutations in *TP53* and *K-RAS* genes, two central genes of human carcinogenesis, are more frequently mutated in smokers' lung cancer as compared to lung cancer from non-smokers ([DeMarini, 2004](#); [IARC, 2004a](#); [Lea et al., 2007](#); [Ding et al., 2008](#); see Section 4.1.3). In particular, *TP53* but also to some extent *K-RAS* mutations found in smoking-associated lung tumours exhibit mutational specificity that is consistent with the pattern produced by PAH diol epoxides in experimental studies and different from that observed in non-smokers' lung cancer ([Pfeifer et al., 2002](#); [DeMarini, 2004](#); [IARC, 2004a](#); [Le Calvez et al., 2005](#); Section 4.1.3). Keeping with such exposure-specific mutation profile, lung cancer in non-smokers exposed to second-hand tobacco smoke shows mutational similarity to smokers' lung cancer, although less data are available ([Husgafvel-Pursiainen, 2004](#); [IARC, 2004a](#); [Le Calvez et al., 2005](#); [Subramanian & Govindan, 2008](#)). The different pathways of lung carcinogenesis for smokers and non-smokers are likely to involve somatic mutations and other genetic alterations in a larger set of genes that are critical in controlling normal cellular growth via signal transduction ([Bode & Dong, 2005](#); [Lea et al., 2007](#); [Ding et al., 2008](#)).

Smoking-related lung carcinogenesis also involves a multitude of other alterations influencing the complex pathogenic pathways involved in lung cancer development, such as increased inflammation, aberrant apoptosis, increased angiogenesis, tumour progression and tumour metastasis ([Wolff et al., 1998](#); [Heeschen et al., 2001](#); [Schuller, 2002](#); [West et al., 2003](#); [Smith et al., 2006](#); [Lee et al., 2008b](#); Section 4.1.5). Continued exposure to toxicants, genotoxins, carcinogens, co-carcinogens and tumour promoters present in tobacco smoke has major effects on biological processes at all steps of multistep tumourigenesis of human lung ([Hecht,](#)

2003, 2008; Section 4.1). For example, nicotine in tobacco smoke is currently not described as a full carcinogen, but it exerts its biological effects via binding to nicotinic and other cellular receptors and likely enhances cell transformation and carcinogenicity through mechanisms not yet defined (Heeschen *et al.*, 2001; West *et al.*, 2003).

Numerous studies have provided evidence that the human genome may contain one or several loci that confer susceptibility to lung cancer. There are low-penetrance genes involved in the metabolism of tobacco smoke carcinogens, DNA repair and cell cycle control that may influence individual susceptibility to lung cancer (Spitz *et al.*, 2006). The role of the polymorphisms of these various classes of genes in lung carcinogenesis requires a systematic evaluation of the genetic evidence with stringent criteria (Ioannidis, 2008; Risch & Plass, 2008; Vineis *et al.*, 2009; Sections 4.1 and 4.2). Recently, genome-wide association studies have identified a susceptibility locus at chromosome 15q25.1 (Amos *et al.*, 2008; Hung *et al.*, 2008; Thorgeirsson *et al.*, 2008). The identity or function of the gene is not yet known, nor is the mechanism through which it may predispose to lung cancer. It is however likely that lung cancer susceptibility is related to the nicotine receptor gene residing at 15q25.1, and there is some evidence suggesting that it may be related to increased uptake of nicotine and NNK per cigarette (Le Marchand *et al.*, 2008).

In addition to genetic alterations, a growing body of evidence shows that epigenetic mechanisms, such as aberrant DNA methylation, histone modifications and RNA-mediated gene silencing are involved in cancer development (Jones & Baylin, 2007; Cortez & Jones, 2008). In lung carcinogenesis, gene promoter-associated (CpG island-specific) hypermethylation is an early and frequent event causing transcriptional inactivation of genes involved in regulation of cellular growth and differentiation (Belinsky, 2004). For example, several studies have indicated that the tumour suppressor gene *p16*

(*p16<sup>INK4a/CDKN2A</sup>*), a cell cycle regulator, is among the genes most frequently inactivated by aberrant methylation in lung cancer from smokers (Belinsky, 2004), with differences seen between smokers and never-smokers (Toyooka *et al.*, 2006). Significant associations have been established between smoking and promoter hypermethylation of tumour suppressor genes in lung tumours from smokers, and in plasma, serum or sputum DNA from cancer-free smokers (Belinsky, 2004; Belinsky *et al.*, 2005, 2006; Toyooka *et al.*, 2006).

#### (b) Oral cavity

PAHs can be carcinogenic at the site of application, which could include the human oral cavity. DMBA, a highly carcinogenic PAH not present in tobacco or tobacco smoke, is a standard model compound for induction of oral tumours in the hamster cheek pouch; less is known about the effects on the oral cavity of PAHs that do occur in tobacco products (Shklar, 1972; Rao, 1984; Vairaktaris *et al.*, 2008). A mixture of NNN and NNK induced oral tumours in rats when applied locally (Hecht *et al.*, 1986), and DNA adduct formation from NNN, NNK and NNAL has been observed in the rat oral cavity (Zhang *et al.*, 2009a, b). HPB-releasing DNA adducts from NNK and/or NNN have been reported in exfoliated oral cells from smokers and smokeless tobacco users (Heling *et al.*, 2008) and HPB-releasing haemoglobin adducts are elevated in smokeless tobacco users (IARC, 2007a). Unidentified DNA adduct levels are consistently elevated in oral cells and tissues from smokers compared to non-smokers (IARC, 2004a). Mutations in the *TP53* gene have been observed in oral tumours from smokers and smokeless tobacco users (IARC, 2006b, 2007a; Warnakulasuriya & Ralhan, 2007). Tobacco-associated genetic mutations including micronuclei, gene mutations, DNA polymorphisms, and chromosomal abnormalities have been reported in studies of buccal cells from smokers and smokeless tobacco users (Proia *et al.*, 2006). The use of lime by betel quid chewers is associated

with enhanced oxidative damage that could play a role in inflammation or tumour promotion ([IARC, 2004b](#)).

#### (c) *Larynx and nasopharynx*

Hamsters exposed to cigarette smoke by inhalation consistently developed benign and malignant tumours of the larynx; tumours were produced by inhalation of the particulate phase, but not the gas phase of cigarette smoke ([IARC, 1986](#)). In related studies in which hamsters were treated with DMBA by intratracheal instillation followed by exposure to cigarette smoke, a significantly higher incidence of laryngeal tumours was observed than in hamsters exposed only to cigarette smoke or to DMBA ([IARC, 1986](#)). Collectively, these results indicate an initiation-promotion mechanism for the production of laryngeal tumours, and are consistent with the results of experiments in which tobacco smoke condensate is applied to mouse skin ([IARC, 1986](#)). The combined data implicate PAHs and tumour promoters in tobacco smoke as potential etiological agents for cancer of the larynx in hamsters. Levels of DNA adducts measured by non-specific methods were higher in larynx tissue from smokers than from non-smokers ([IARC, 2004a](#)). Analyses of mutations in the *TP53* gene from tumours of the larynx in smokers show a pattern similar to that observed in lung tumours, and both are consistent with the pattern produced by PAH diol epoxides ([IARC, 2006b](#)). The available data are consistent with the conceptual framework illustrated in Fig. 4.1 ([Szyfter et al., 1999](#)).

Formaldehyde, a constituent of cigarette smoke, causes nasopharyngeal cancer in humans ([IARC, 2006a](#)). A recent study demonstrates a 10-fold higher level of the formaldehyde-DNA adduct N<sup>6</sup>-hydroxymethyldeoxyadenosine in leukocytes of smokers compared to non-smokers, suggesting its possible involvement in nasopharyngeal cancer in smokers ([Wang et al., 2009](#)). Acetaldehyde, another carcinogenic constituent of tobacco smoke, which also

forms genotoxic adducts (Section 4.1), may also contribute to the development of these forms of head and neck cancer.

#### (d) *Oesophagus*

Nitrosamines are probably the most effective oesophageal carcinogens known, with particularly strong activity in the rat ([Lijinsky, 1992](#)). NNN and NDEA are both present in cigarette smoke, and levels of NNN greatly exceed those of NDEA ([IARC, 2004a](#)). NNN is also present in considerable quantities in smokeless tobacco and betel quid containing tobacco ([IARC, 2004a, 2007a](#)). Thus, NNN is a likely candidate as a causative agent for esophageal cancer in smokers, smokeless tobacco users, and chewers of betel-quid with tobacco. While considerable mechanistic data are available from studies of NNN in laboratory animals ([Hecht, 1998](#); [Wong et al., 2005](#); [Lao et al., 2007](#); [Zhang et al., 2009a](#)), there are little comparable data in humans.

Increased acetaldehyde production derived both from tobacco smoke and from microbial alcohol oxidation may play a role in the synergistic carcinogenic action of alcohol and smoking on oesophagus, as well as on other upper aerodigestive locations ([Homann et al., 2000](#); [Salaspuro & Salaspuro, 2004](#); [Lee et al., 2007a](#)).

#### (e) *Stomach*

Hypermethylation of the E-cadherin 1 gene (*CDH1*) was observed preferentially in gastric tumours from smokers rather than non-smokers ([Poplawski et al., 2008](#)). *CDH1* can act as a tumour-suppressor gene, preventing cells from growing and dividing in an uncontrolled way to form a cancerous tumour. Because the protein encoded by this gene helps cells stick together, altered regulation may lead to metastasis.

[Boccia et al. \(2007\)](#) found an increased risk for stomach cancer among smokers who had the *SULT1A1* *His* genotype, and [Lee et al. \(2006\)](#) found an increased risk for those who had the *m2* allelic variant of *CYP1A1*. A nested case-control

study found that smokers had an increased risk of gastric cancer if they carried at least one variant allele A in Ex7+129 C > A (*Thr<sup>461</sup>Asn, m4*) of *CYP1A1* ([Agudo et al., 2006](#)). Stomach cancer tissue from smokers had higher levels of stable DNA adducts than did those from non-smokers; however, the number of non-smokers was quite small ([Dyke et al., 1992](#)).

#### (f) *Pancreas*

NNK and its metabolite NNAL are the only pancreatic carcinogens known to be present in tobacco and tobacco smoke. NNK was detected in the pancreatic juice of 15 of 18 samples from smokers, at levels significantly higher than in non-smokers; NNAL and NNN were also detected in some samples ([Prokopczyk et al., 2002](#)). DNA adducts of NNK and NNAL were present in pancreatic tissue of rats treated with these nitrosamines ([Zhang et al., 2009b](#)), but were not detected in most human pancreatic tissue samples ([Prokopczyk et al., 2005](#)).

#### (g) *Colorectum*

Tobacco smoke contains heterocyclic amines, such as 2-amino-1-methyl-6-phenylimidazo[4,5,6]pyridine (PhIP), which are intestinal carcinogens in rats and mutate the adenomatous polyposis coli (*Apc*) gene in mice ([Møllersen et al., 2004](#)). The *APC* gene is frequently mutated and has altered expression in human colon cancer ([Samowitz et al., 2007](#); [Samowitz, 2008](#)). A recent model of colon cancer by [Sweeney et al. \(2009\)](#) suggests that this disease can develop via at least three independent mechanistic pathways. One pathway is initiated by methylation of *MINT* (methylation in tumour) markers that proceeds down a pathway predisposing to microsatellite instability, followed by methylation of the mismatch repair gene mutL homologue 1 (*MHL1*) and the tumour-suppressor gene *TP16*, followed by mutation in *BRAF* (a homologue of a viral raf oncogen). A second independent pathway is initiated with a mutation in the *APC*

gene, followed by a mutation in the *TP53* gene. A third independent pathway involves only *KRAS2* mutations. One study found BPDE-DNA adducts at a higher frequency in colon DNA from smokers than from non-smokers ([Alexandrov et al., 1996](#)). Mutations or epigenetic changes in some or all of these genes have been found in smoking-associated colon or colorectal tumours.

Microsatellite instability, which is the expansion or contraction of short nucleotide repeats, occurs in approximately 10–15% of sporadic colorectal cancer, and is usually associated with smoking and hypermethylation of the promoter of the mismatch repair gene *MLH1* ([Samowitz, 2008](#)). Smoking-associated colorectal tumours also have high frequencies of methylation at CpG islands ([Samowitz, 2008](#)).

In a case-control study of colorectal cancer, [Kasahara et al. \(2008\)](#) found that the genetic polymorphism *APEX1/APE1* (apurinic/apyrimidinic endonuclease-1) Asp<sup>148</sup>Glu, which is a gene involved in DNA repair, was associated with risk for colorectal cancer among smokers but not non-smokers. Other studies have also found associations between polymorphisms in the DNA repair genes *XRCC1* and smoking and risk for colorectal cancer ([Stern et al., 2007](#); [Campbell et al., 2009](#)).

#### (h) *Liver*

Tobacco smoke contains liver carcinogens such as furan and certain nitrosamines. Liver tumours exhibit increased expression of *C-MYC*, *P16<sup>INK4A</sup>*, epidermal growth factor receptor (*EGFR*), telomerase, transforming growth factor- $\alpha$  (*TGF- $\alpha$* ), insulin-like growth factor-2 (*IGF-2*) and *RAF* oncogene ([Abou-Alfa, 2006](#)). Smokers show altered expression of some of these genes or of genes in the same or similar pathways ([Sen et al., 2007](#)). A genome-wide association study found that SNP rs1447295 in the 8q24 chromosome was positively associated with liver cancer among ever-smokers ([Park et al., 2008](#)). Thus, tobacco smoke appears to have epigenetic



effects on the liver that may contribute to hepatocellular carcinoma.

(i) *Urinary bladder*

Tobacco smoke contains aromatic amines such as 4-aminobiphenyl and 2-naphthylamine, which are human bladder carcinogens (see [IARC, 2012a](#)). In bladder tumours, smoking was associated with a more than twofold increase risk of methylation of the promoter region of the *PI6<sup>INK4A</sup>* gene and of the soluble Frizzled receptor protein (*SFRP*) gene ([Marsit et al., 2006](#)). In addition, [Tang et al. \(2009\)](#) suggested that epigenetic silencing of *Wnt* antagonists through hypermethylation may play a role in smoking-related invasive bladder cancer ([Tang et al., 2009](#)). SNP rs6983267 of the 8q24 chromosome was inversely associated with bladder cancer among ever-smokers ([Park et al., 2008](#)). Smokers generally have mutagenic urine and smoking is associated with specific cytogenetic changes and DNA breaks in bladder tumours ([DeMarini, 2004](#)). Smoking-associated stable DNA adducts have been found in bladder tissue or exfoliated urothelial cells, supporting a role for DNA damage in smoking-associated bladder cancer ([Phillips, 2002](#)).

(j) *Cervix*

The cervical mucus of smokers is more mutagenic than that of non-smokers, and cervical epithelia of smokers have higher frequencies of micronuclei than those of non-smokers ([DeMarini, 2004](#)). Several studies have found increased levels of DNA adducts in cervical tissue from smokers relative to non-smokers, suggesting a role for smoking-associated DNA damage in cervical cancer ([Phillips, 2002](#)).

(k) *Ovary*

It has been observed that the inverse associations reported for serous and endometrioid tumours with respect to parity and oral contraceptives did not hold for the mucinous tumours.

Based on these observations, [Risch et al. \(1996\)](#) suggested that mucinous ovarian tumours may be etiologically unrelated to the other types of epithelial tumours. Whereas mucinous elements such as gastric or intestinal type glands may be seen in mature teratomas, a form of germ cell neoplasia, overall mucinous tumours are classified as surface epithelial tumours because transitions among the subtypes may be observed. The major difference between mucinous and serous tumours is their biologic behaviour. Mucinous carcinomas of the ovary are slow growing tumours that appear to develop from their benign counterparts. The fact that the transitions between the benign, borderline, and malignant form of the disease can be seen in the same tumour suggests that over time, there is a progression from benign to malignant ([Riopel et al., 1999](#)). *K-ras* mutational analysis, for example, demonstrates a heterogeneous distribution of the mutation within different parts of the same neoplasm, suggesting that acquisition of the *K-ras* mutation occurs in malignant transformation ([Mandai et al., 1998](#)). Serous carcinomas seem to develop de novo rather than from a benign pre-existing lesion; alternatively, the rate of progression is rapid and the precursor lesion is obliterated before the detection of the tumour. In some data, current smoking is associated with a shorter interval to detection of mucinous than non-mucinous tumours. Because the mucinous tumour is slow growing, smoking could contribute to the malignant progression of the adenoma-carcinoma sequence, as the benign form of the tumour may have been present for some time.

(l) *Leukaemia*

Tobacco smoke contains known leukaemogens such as benzene, 1,3-butadiene and formaldehyde ([IARC, 2012a](#)). The mechanisms of leukaemogenesis are currently not well understood. Data indicate that leukaemogenic agents, such as benzene, cause toxicity to the

haematopoietic system, as well as genotoxicity at low levels, and that genetic polymorphisms may be involved in these processes ([Aksoy, 1989](#); [Lan et al., 2004](#); [Garte et al., 2008](#); [Hosgood et al., 2009](#); [Lau et al., 2009](#); [Rappaport et al., 2009](#)). Recent studies suggest the importance in carcinogen-related leukaemogenesis of damage to haematopoietic stem/progenitor cells circulating in the peripheral blood, or, alternatively, damage to primitive pluripotent progenitor cells present in other tissues ([Zhang et al., 2009c](#)). In these two models, damaged stem/progenitor cells would then travel to the bone marrow and become initiated leukaemic stem cells. Mechanisms considered central in these models are: disruption of bone marrow DNA, through e.g. formation of DNA adducts, DNA-protein crosslinks, the action of free radicals or active states of oxygen; intercalation of metals within the DNA structure; or inhibition of enzymes involved in cell division ([Zhang et al., 2007, 2009c](#)).

#### 4.3.2 Sites with limited evidence of carcinogenicity or evidence suggesting lack of carcinogenicity

##### (a) Breast

##### (i) Carcinogenic pathway

Carcinogens found in tobacco smoke pass through the alveolar membrane and into the blood stream, by means of which they can be transported to the breast via plasma lipoproteins ([Yamasaki & Ames, 1977](#); [Shu & Bymun, 1983](#); [Plant et al., 1985](#)). Tobacco smoke contains known rodent mammary carcinogens, including PAHs and aromatic amines ([IARC, 1986, 2004a](#); [el-Bayoumy, 1992](#); [Ambrosone & Shields, 1999](#); [Ambrosone, 2001](#); [Hoffmann et al., 2001](#)) which, due to their lipophilicity, can be stored in breast adipose tissue ([Obana et al., 1981](#); [Morris & Seifter, 1992](#)) and then metabolized and activated by human mammary epithelial cells ([MacNicoll et al., 1980](#)). Tobacco smoke constituents reach

the breast as demonstrated by the detection of cotinine in breast fluid ([Petrakis et al., 1978](#)). There is evidence suggesting the presence of mutagenic arylamines ([Thompson et al., 2002](#)) and PAHs ([Zanieri et al., 2007](#)) in human breast milk. Cigarette smoke condensate has been shown to transform normal human breast epithelial cells *in vitro* ([Narayan et al., 2004](#)), perhaps by blocking long-patch base excision repair ([Kundu et al., 2007](#)). Transformation and cytogenetic effects have been observed in human mammary epithelial cells after exposure to chemical carcinogens such as PAHs or arylamine ([Mane et al., 1990](#); [Eldridge et al., 1992](#); [Calaf & Russo, 1993](#)).

The formation of specific adducts from PAHs and aromatic amines has been observed in human breast epithelial cells *in vitro*, and unspecified-DNA adducts have been found in exfoliated ductal epithelial cells in human breast milk ([Gorlewska-Roberts et al., 2002](#); [Thompson et al., 2002](#)).

Mutations in the *TP53* tumour suppressor gene have been found in 15–30% of breast cancers ([Goldman & Shields, 1998](#); [Olivier & Hainaut, 2001](#)). An increased prevalence and altered spectrum of *TP53* mutations in breast tumours have been observed among current smokers compared with never smokers ([Conway et al., 2002](#)). The breast tumours with the most pronounced smoking-related mutational pattern (for example, a greater number of G:C→T:A transversions) were from women who had smoked for more than 20 years, although total *TP53* mutations were not associated with smoking duration ([Conway et al., 2002](#)). This increased frequency of G to T transversions in smokers versus non-smokers is also observed in the IARC *TP53* database ([IARC, 2006b](#); [Van Emburgh et al., 2008](#)).

Recent meta-analyses of epidemiological studies tend to show positive associations of breast cancer with long-term smoking among *NAT2* slow acetylators, especially among post-menopausal women (who are more likely than pre-menopausal women to be very long-term

smokers). [Firozi et al. \(2002\)](#) showed that breast tissue from NAT2 slow acetylators had significantly higher levels of the diagonal radioactive zone (smoking-related) DNA adduct pattern than that from fast acetylators.

High rates of breast cancer in women exposed to ionizing radiation during adolescence (aged 10–19 years at exposure) ([Tokunaga et al., 1987](#)) suggested that the adolescent breast may also be sensitive to the DNA-damaging effects of other exposures. This might also be true for the genotoxic compounds contained in tobacco smoke. Although some studies have supported such association, the results have been sparse and mixed. In addition, it is difficult to separate the effects of early life exposure to tobacco and smoking duration ([Terry & Rohan, 2002](#)).

Early age at first full-term pregnancy has been associated with reduced breast cancer risk ([Kelsey et al., 1993](#)), hypothetically due to terminal differentiation of the breast epithelium that occurs late in the first trimester. It has been suggested that in the early stages of pregnancy, when growth-promoting hormone levels are high, but before terminal differentiation ([Montelongo et al., 1992](#)), the breast may be particularly susceptible to the cancer-promoting chemicals in tobacco smoke. Several epidemiological studies compared measures of smoking before and after a first full-term pregnancy. Although suggestive, the data did not consistently show an increased risk for breast cancer among women who smoked before a first full-term pregnancy ([Adami et al., 1988](#); [Hunter et al., 1997](#); [Band et al., 2002](#); [Egan et al., 2003](#); [Gram et al., 2005](#); [Li et al., 2005](#); [Olson et al., 2005](#); [Cui et al., 2006](#)). Smoking was associated with a 50% increased risk among women with slow NAT2 acetylation genotype ([Egan et al., 2003](#)). Overall, studies of risk in association with the timing of smoking relative to a first pregnancy are inconclusive; nevertheless, the breast tissue appears to have a greater susceptibility to the carcinogenic chemicals in tobacco smoke

before compared to after terminal differentiation of breast epithelium.

## (ii) *Estrogenic pathway*

The “anti-estrogenic” mechanism through which tobacco smoking may inhibit breast cancer progression is unclear. Estrogen is a known risk factor for breast cancer and several hypotheses have been proposed: earlier age at menopause among smokers, a reduction in the gastrointestinal absorption or distribution of estrogen, enhanced metabolism of estradiol to inactive catechol estrogens, increased binding of estrogens by serum sex hormone-binding globulin, lowered levels of estrogen derived from adipose tissue ([Baron, 1984](#); [Baron et al., 1990](#); [Terry & Rohan, 2002](#)). Several studies of cigarette smoking and mammographically-defined breast density showed lower measures of breast density in current smokers than in non-smokers ([Sala et al., 2000](#); [Vachon et al., 2000](#); [Warwick et al., 2003](#); [Jeffreys et al., 2004](#); [Modugno et al., 2006](#); [Bremnes et al., 2007](#); [Butler et al., 2008](#)). Since exposure to estrogen has been associated positively with breast density, a strong risk factor for breast cancer ([McCormack & dos Santos Silva, 2006](#)), the results of these studies are consistent with an anti-estrogenic effect of cigarette smoking. Although smokers and non-smokers may have the same concentrations of estrogens overall, it may be the type rather than the absolute levels of circulating estrogens that is important. Smokers might have a lower concentration of more biologically active estrogens, primarily 16- $\alpha$ -hydroxyestrone (16 $\alpha$ -OHE1) ([Michnovicz et al., 1986, 1988](#); [Berta et al., 1992](#); [Berstein et al., 2000](#); [Terry et al., 2002b](#)). Estrogen can be metabolized along three pathways, to 16 $\alpha$ -OHE1 or to 2-OHE1 or to 4-OHE1. 16 $\alpha$ -OHE1 and 4-OHE1 have been observed to increase mammary epithelial cell proliferation rates in experimental studies ([Schütze et al., 1993, 1994](#); [IARC, 2007c](#)). In contrast, 2-OHE1 might decrease epithelial cell proliferation rates ([Bradlow et al., 1996](#);

[Muti et al., 2000](#)). If cigarette smoking increases estradiol 2-hydroxylation, as has been suggested ([Michnovicz et al., 1986](#)), thereby increasing the ratio of 2-OHE1:16- $\alpha$ -OHE1, an inverse association between smoking and breast cancer risk might be observed. However, only one study has directly examined 2-hydroxylation in relation to cigarette smoking ([Michnovicz et al., 1986](#)). Using injected radiolabelled estradiol, a 50% increased estradiol 2-hydroxylation was found in premenopausal women who smoked at least 15 cigarettes/day compared with non-smokers. Two studies of urinary estrogens found increased excretion of 2-OHE1 and decreased excretion of estriol among smokers ([Michnovicz et al., 1988](#); [Berstein et al., 2000](#)), which may also support the hypothesis that smoking decreases the formation of active estrogen metabolites along the 16 $\alpha$ -hydroxylation pathway. However, the ratio of urinary 2-OHE1:16 $\alpha$ -OHE1 was not related to breast cancer risk in the one case-control study that examined the association ([Ursin et al., 1999](#)). The 4-hydroxylation of estrogens is catalysed by CYP1B1, which is induced by tobacco smoke ([Nebert et al., 2004](#)). This has been postulated as an additional pathway that could lead to formation of DNA adducts via catechol estrogen-quinones ([Gaikwad et al., 2008](#)) and oxidative/DNA damage via redox-cycling ([Zhu & Conney, 1998](#)). The ratio of 2-OHE1:4-OHE1 has been studied in relation to breast cancer risk and smoking in one study ([Berstein et al., 2000](#)). Smokers carrying the CYP1B1 Val allele [associated with high hydroxylation activity] had a significantly higher risk for breast cancer compared to never smokers with the Leu/Leu [wildtype] genotype ([Saintot et al., 2003](#)).

#### (b) Endometrium

Exogenous estrogens unopposed by progesterone have been shown to increase the risk for endometrial cancer through increased mitotic activity of endometrial cells, increased number of DNA replication errors, and somatic mutations

resulting in the malignant phenotype ([IARC, 2007c, 2012c](#)). Hence, factors associated with estrogen absorption or metabolism may alter the risk of this malignancy. Several investigators have hypothesized that cigarette smoking might have anti-estrogenic effects, and through this mechanism reduce the risk of endometrial cancer ([Baron, 1984](#); [Baron et al., 1990](#); [Terry et al., 2002b, 2004a](#)).

Whether mediated through changes in the amount of adipose tissue, altered age at menopause, or anti-estrogenic effects, blood hormone concentrations might be an important link between smoking and the reduced risk of endometrial cancer observed in most epidemiological studies. The estrogens that have typically been studied in relation to cigarette smoking include estrone, sex hormone binding globulin (SHBG)-bound estradiol, and estriol. Blood concentrations of androgens, typically androstenedione and dehydroepiandrosterone sulfate (DHEAS), have also been studied, because these are biological precursors of estrone. Studies that have examined blood concentrations of SHBG are less common, and studies of unbound (free) estradiol are scarce.

Studies of cigarette smoking and blood hormone concentrations have been conducted mostly among post-menopausal women who were not taking HRT. Of these studies, nine examined serum ([Friedman et al., 1987](#); [Cauley et al., 1989](#); [Slemenda et al., 1989](#); [Schlemmer et al., 1990](#); [Cassidenti et al., 1992](#); [Austin et al., 1993](#); [Law et al., 1997](#)) or plasma ([Khaw et al., 1988](#); [Longcope & Johnston, 1988](#)) estrone, ten examined serum ([Friedman et al., 1987](#); [Cauley et al., 1989](#); [Slemenda et al., 1989](#); [Schlemmer et al., 1990](#); [Key et al., 1991](#); [Cassidenti et al., 1992](#); [Austin et al., 1993](#); [Law et al., 1997](#)) or plasma ([Khaw et al., 1988](#); [Longcope & Johnston, 1988](#)) estradiol, and two examined serum ([Cassidenti et al., 1992](#)) or plasma ([Longcope & Johnston, 1988](#)) free estradiol. These studies consistently showed little or no association between smoking



and blood estrogen concentrations among post-menopausal women who were not taking hormone replacement therapy. Among pre-menopausal women, three studies ([Longcope & Johnston, 1988](#); [Key et al., 1991](#); [Berta et al., 1992](#)) found no clear association between cigarette smoking and estrogen concentrations. Studies that adjusted hormone measurements for the effects of BMI (and other covariates) showed similar results to those that did not, suggesting that BMI is not a strong confounder of this association.

In two studies the association between cigarette smoking and blood estrogen concentrations after randomization of women to groups receiving either estradiol or placebo were examined ([Jensen & Christiansen, 1988](#); [Cassidenti et al., 1990](#)). In a small study of 25 post-menopausal women, unbound estradiol was significantly lower among smokers than non-smokers both at baseline and shortly after taking micronized estradiol orally ([Cassidenti et al., 1990](#)). No important differences were observed between smokers and non-smokers in serum concentrations of either estrone or bound estradiol. In contrast, in a study in which 110 post-menopausal women were randomized to take hormones (either orally or percutaneously) or a placebo ([Jensen & Christiansen, 1988](#)), smokers had lower concentrations of both estrone and bound estradiol than non-smokers after oral (but not percutaneous) hormone treatment for at least one year (concentrations of free estrogens were not examined). These results indicate that smoking might affect the absorption or metabolism of hormones used in replacement therapy.

Of the five studies that have examined the association between cigarette smoking and serum ([Lapidus et al., 1986](#); [Cassidenti et al., 1992](#); [Law et al., 1997](#)) or plasma ([Khaw et al., 1988](#); [Longcope & Johnston, 1988](#)) SHBG, none found any clear association. However, one of these studies ([Khaw et al., 1988](#)) found an inverse association between smoking and the ratio of bound estradiol to SHBG, a measure of estrogen

activity. In this context, [Cassidenti et al. \(1990\)](#) found unbound (but not SHBG-bound) estradiol was significantly lower among smokers than non-smokers both at baseline and after taking oral estradiol, suggesting an increased SHBG-binding capacity in the women who smoked.

In post-menopausal women, androgens are the major source of estrone, converted through aromatization in fat deposits. Thus, adiposity is positively correlated with estrogen concentrations in post-menopausal women. Of the nine studies in which blood concentrations of androstenedione were examined in smokers ([Friedman et al., 1987](#); [Khaw et al., 1988](#); [Longcope & Johnston, 1988](#); [Cauley et al., 1989](#); [Slemenda et al., 1989](#); [Schlemmer et al., 1990](#); [Cassidenti et al., 1992](#); [Austin et al., 1993](#); [Law et al., 1997](#)), higher circulating concentrations were found among current than among never or former smokers in all studies. However, there was no clear variation in blood estrone concentrations by smoking status, suggesting a reduced conversion of androstenedione to estrone among smokers. Of the five studies where cigarette smoking and DHEAS concentrations were examined, three ([Khaw et al., 1988](#); [Cassidenti et al., 1992](#); [Law et al., 1997](#)) found increased blood concentrations among current smokers, one ([Friedman et al., 1987](#)) found also an increase that was not statistically significant, whereas another ([Key et al., 1991](#)) found no clear differences according to smoking status.

Cigarette smoking and urinary estrogen concentrations have been examined in seven studies ([MacMahon et al., 1982](#); [Michnovicz et al., 1986](#); [Trichopoulos et al., 1987](#); [Michnovicz et al., 1988](#); [Berta et al., 1992](#); [Key et al., 1996](#); [Berstein et al., 2000](#)). Of these, three found no major differences according to smoking status ([Trichopoulos et al., 1987](#); [Michnovicz et al., 1988](#); [Berta et al., 1992](#)). The remaining four studies all showed lower urinary estriol concentrations among smokers than among non-smokers, but mixed results for urinary estrone and estradiol.

Two of these studies ([Michnovicz et al., 1988](#); [Berstein et al., 2000](#)) showed higher concentrations of 2-hydroxyestrone among smokers, than non-smokers but only after estrogen treatment in [Berstein et al. \(2000\)](#).

Age at natural menopause varies substantially under the influence of genetic and environmental factors ([McKinlay, 1996](#)). A relatively early age at menopause has been associated with reduced risk of endometrial cancer ([Kelsey et al., 1982](#); [Baron, 1984](#); [Baron et al., 1990](#); [Akhmedkhanov et al., 2001](#)). A one year decrease in age at menopause has been associated approximately with a 7% decrease in risk ([Kelsey et al., 1982](#)). It has been proposed that cigarette smoking decreases the age at natural menopause ([Baron et al., 1990](#)), more clearly with qualitative than quantitative smoking measures ([Parente et al., 2008](#)), and thus might reduce endometrial cancer risk through reduced exposure to endogenous estrogens. On average, smokers have menopause approximately 1 to 1.5 years earlier than non-smokers ([Terry et al., 2002b, 2004a](#)). Adjustment for obesity and other covariates did not alter the results ([Terry et al., 2002b](#)).

#### 4.4 Mechanistic considerations of the interaction of ethanol and tobacco carcinogens

The combined effects of alcoholic beverages and tobacco on the risk for cancer incidence and mortality have been widely studied in human populations. When tested for multiplicative and additive interactions, synergistic effects of alcoholic beverages and tobacco have been found, especially for oropharyngeal and oesophageal cancers ([Homann et al., 2000](#); [Castellsagué et al., 2004](#); [Salaspuro & Salaspuro, 2004](#); [Lee et al., 2005a](#); [Lee et al., 2007b](#)).

Data support at least four possible mechanisms for the modifying effects of alcoholic beverages on cancer risk due to tobacco.

1. Alcohol may have a local permeabilizing effect on penetration of the oral mucosa by tobacco carcinogens ([Du et al., 2000](#)), particularly important in the case of oropharyngeal and oesophageal cancer.
2. CYP2E1 and other CYPs may both activate and detoxify carcinogens present in tobacco smoke, including NDMA, NDEA, NNK, benzene and other tobacco-derived carcinogens in two ways: CYP induction increases metabolic activation of tobacco carcinogens leading to enhanced formation of proximate reactive chemical species at target sites; and alteration of phase II conjugation/detoxification enzymes by ethanol may also occur, changing the effective dose at the target site.
3. Competitive inhibition of CYP metabolism leads to reduced central hepatic and gastrointestinal clearance thus increasing dose delivery of carcinogens to peripheral target tissues (reviewed in [Meskar et al., 2001](#)).
4. Effects of acetaldehyde derived by microbial alcohol oxidation and from the tobacco smoke ([Homann et al., 2000](#); [Salaspuro & Salaspuro, 2004](#)).

Supportive evidence for ii) and iii) is briefly presented below.

##### 4.4.1 Effects of induction of CYPs by ethanol

###### (a) CYP2E1

Ethanol induces CYP2E1 in the human liver and in all species tested. Over 70 substrates of CYP2E1 have been compiled ([Raucy & Carpenter, 1993](#); [Guengerich et al., 1994](#); [Djordjević et al., 1998](#); [Klotz & Ammon, 1998](#); [Cederbaum, 2006](#)). Among those are tobacco carcinogens such as benzene, vinyl chloride, NDMA, NDEA and *N*-nitrosopyrrolidine, as well as many low-molecular-weight compounds. Induction of CYP2E1 by ethanol generated increased levels of toxic metabolites from the metabolism of many of these chemicals ([Novak & Woodcroft, 2000](#)). Pyridine, a constituent of tobacco smoke and

substrate of CYP2E1, generates DNA damaging products by redox-cycling ([Kim & Novak, 1990](#)).

In humans, in addition to the prominent CYP2E1 expression in the centrilobular regions of the liver, the enzyme is also detectable in the kidney cortex and, at lower levels, in organs such as the oropharynx, nasal mucosa, ovary, testis, small intestine, colon and pancreas ([Ingelman-Sundberg et al., 1994](#); [Lieber, 1999, 2004](#)).

In rats, ethanol induced CYP2E1 in epithelia of the cheek, tongue and oesophagus ([Shimizu et al., 1990](#)). As a result of CYP2E1 induction by ethanol in the upper respiratory tract and possibly of inhibition of carcinogen clearance, hamsters had a significant increase of nasal cavity and tracheal tumours after intraperitoneal injection of *N*-nitrosopyrrolidine ([McCoy et al., 1981](#)). Thus, induction of CYP2E1 by ethanol may participate in the genesis of cancers at several sites via metabolic activation of tobacco carcinogens into reactive species in target tissues.

#### (b) Other xenobiotic-activating CYPs

In addition to CYP2E1, several CYPs, including CYP3A4 and probably CYP1A2 in humans, and CYP1A1, 2B1 and 3A in rat liver, may be induced by ethanol. Of particular interest are members of the CYP3A family, which have wide substrate specificity and have been implicated in the activation of several known or suspected human carcinogens, including those derived from tobacco ([Wojnowski & Kamdem, 2006](#)). Both CYP3A4 and CYP1A2 metabolize NNK ([Jalas et al., 2005](#)). Based on the Michaelis constant (*K<sub>m</sub>*) data ([IARC, 2007a](#)), the relative efficiencies in NNK metabolism by human CYP are (from greatest catalyst to least): 2A13 > 2B6 > 2A6 > 1A2 ~1A1 > 2D6 ~2E1 ~3A4. As the amount of CYP enzymes with overlapping substrate specificity that participate in nitrosamine metabolism varies according to organ and species, it is difficult to determine their individual contribution at target sites.

#### 4.4.2 Effects of inhibition of CYPs by ethanol

Ethanol is a competitive inhibitor of CYP2E1 (reviewed in [Anderson, 1992](#)). It also inhibits the activities of CYP1A1, 2B6 and 2C19 but not those of CYP1A2.

Direct inhibition of CYPs by ethanol in target tissues may reduce metabolic activation of xenobiotics and hence local toxic and tumorigenic effects. Thus CYP inhibition in the liver could increase extrahepatic exposure to genotoxic metabolites from tobacco carcinogens that are substrates for these CYP enzymes. This mechanism is supported by several studies.

Ethanol caused a fivefold increase in oesophageal DNA adducts in rats induced by NDEA ([Swann, 1984](#)). In monkeys, *O*<sup>6</sup>-methylguanine-DNA adducts after an oral dose of NDMA with or without ethanol were increased by co-exposure to ethanol in all tissues except the liver ([Anderson et al., 1996](#)). Effects were seen in the oesophagus (17-fold increase), colonic mucosa (12-fold), pancreas (sixfold), urinary bladder (11-fold), ovary (ninefold), uterus (eightfold), brain (ninefold), spleen (13-fold) and nasal mucosa (fivefold). In these studies, ethanol treatment was acute, so that enzyme induction was improbable, and the oesophagus was not directly exposed to either ethanol or carcinogen. This indicates that a systemic interaction, most likely inhibition of hepatic carcinogen clearance, was responsible for the observed effects in the oesophagus and other extrahepatic tissues. The 17-fold increase in DNA adducts in the monkey oesophagus is similar to the 18-fold increased risk for human oesophageal cancers in tobacco smokers combined with heavy alcohol drinking ([Tuyens et al., 1977](#)).

The relevance of increased genotoxic effects in extrahepatic target sites by ethanol is confirmed by many rodent experiments. Oral dosing of mice with NDMA in ethanol resulted in nasal cavity tumours (olfactory neuroblastoma) that were not seen with NDMA or ethanol alone ([Griciute](#)

[et al., 1981](#)). Ethanol in the drinking-water led to a ninefold increase in oesophageal tumours in rats induced by NDEA ([Aze et al., 1993](#)). Ethanol given by gavage to nursing dams together with NDMA or NNK ([Chhabra et al., 2000](#)) increased O<sup>6</sup>-methylguanine-DNA adducts in maternal mammary glands, by 10-fold with NDMA and to a lesser extent with NNK. In the suckling infants, DNA adducts were detected in the lungs and kidneys after maternal exposure to NDMA and increased about fourfold after maternal co-treatment with ethanol. In mice, ethanol given with NDMA in the drinking-water resulted in a fourfold increase in lung tumours, but had no significant effect when NDMA was given intragastrically, intraperitoneally, subcutaneously or intravenously ([Anderson, 1992](#)). These negative findings support that direct inhibition of hepatic carcinogen clearance by ethanol is the main operative mechanism.

There is indirect evidence that ethanol can inhibit the *in vivo* clearance of the carcinogen NDMA in humans: individuals with chronic renal failure showed detectable blood and urine levels of NDMA, which were increased by consumption of ethanol ([Dunn et al., 1990](#)). Other studies that involved sources of NDMA from tobacco smoke, diet or pharmaceuticals are consistent with ethanol reducing its clearance rate in humans ([Anderson, 1992](#)).

Other possible modifying effects of ethanol in tobacco-related tumorigenesis are presented in Section 4 of the *Monograph on Consumption of Alcoholic Beverages* in this Volume.

## 4.5 Synthesis

### 4.5.1 Mechanisms of tobacco-related carcinogenesis

The pathways by which tobacco products cause cancer essentially recapitulate established mechanisms of carcinogenesis by individual compounds, which were elaborated by landmark

studies during the second half of the 20<sup>th</sup> century. These studies demonstrate that most carcinogens, either directly or after metabolism catalyzed by multiple cytochrome P450 enzymes, react with nucleophilic sites in DNA to form covalent binding products called adducts (a contraction for “addition products”). These DNA adducts, if left unrepaired by cellular DNA repair enzymes, persist and cause mistakes during DNA replication leading to incorporation of the wrong base in a DNA strand and consequent permanent mutations. If these permanent mutations occur in important regions of critical growth control genes such as the oncogene *KRAS* or the tumor suppressor gene *p53*, cellular growth processes can become severely unregulated and cancer can result. Multiple studies of mutations in *KRAS*, *p53*, and other growth control genes in lung tumours from smokers, some of which report thousands of mutations, are fully consistent with this overall concept.

It is the complexity of tobacco carcinogenesis which challenges investigators to identify specific mechanisms that fully explain the ways in which tobacco products cause each type of cancer. There are over 70 established carcinogens in cigarette smoke, and analyses of smokers’ urine and blood clearly demonstrate higher uptake of these compounds in smokers than in non-smokers. The urine of smokers is consistently mutagenic. Similar considerations apply to smokeless tobacco users, although there are fewer identified carcinogens. Multiple DNA adducts are present in the lungs and other tissues of smokers, and sister chromatid exchanges as well as other genetic effects are consistently observed. But much less is known about the specifics of the process. Only relatively few DNA adducts in smokers’ lungs have been structurally characterized and the relationship between specific adducts and the consequent mutations in critical genes is still somewhat unsettled.

There are other processes which contribute to cancer induction by tobacco products, based on



multiple studies in both laboratory animals and humans. These include inflammation, tumor promotion, oxidative damage, co-carcinogenesis, and direct activation of cellular growth pathways by constituents of smoke. Many studies demonstrate the involvement of these processes in tobacco carcinogenesis but the details by which they interact with the DNA damage pathways and their roles in specific cancers caused by tobacco products are still not fully understood.

#### 4.5.2 Genetic polymorphisms

Multiple studies have been carried out on the role of genetic polymorphisms of xenobiotic metabolism in smoking-related carcinogenesis in humans. These studies have covered various cancer types, with lung cancer representing one of the most intensively studied. The polymorphic genes, their variant forms, and the genotype combinations investigated in these studies have similarly been numerous. In addition to the associations with increased risk of cancer, much data have accumulated on relationships between the polymorphisms and the various biomarkers of tobacco carcinogenesis in non-cancer control populations, whether smokers or non-smokers, in subjects with work-related exposure or in patients with other cancers.

Despite the massive body of research, many observations remain ambiguous. Some associations between genetic polymorphism and increased risk for cancer, such as for the *GSTM1* null genotype, alone or in combination with *CYP1A1* polymorphism, in lung cancer, or the *NAT2* slow acetylator genotype in bladder cancer and breast cancer appear stronger and more consistent, but not without controversies. Similarly, the data on the various biomarkers of tobacco-related carcinogenesis exhibit inconsistencies.

The variability in the data is at least partially likely due to differences between the studies in the genes and gene variants included (many of

which are still of unknown functional or regulatory consequence), in the types of cancer studied, in levels and sources of exposure, in ethnic backgrounds, in sex, in histological types and in the features of the genome such as haplotype blocks and copy number variation resulting in linkage disequilibrium. In addition, gene-gene interactions and gene-environment interactions are likely to contribute to the discrepancies in current data. Mechanisms of tobacco-related carcinogenesis also involve genes from numerous other classes, such as those encoding for DNA repair proteins and many other regulators of cell cycle and growth. In addition; there are well described mechanistic pathways of carcinogenesis mediated via epigenetic alterations and genetic instability, to mention a few.

#### 4.5.3 Site-specific mechanisms

The Working Group reviewed the mechanistic evidence relative to specific target sites for which there is sufficient evidence of carcinogenicity in humans, i.e. lung, oral cavity, oesophagus, larynx and nasopharynx, pancreas, stomach, liver, urinary bladder, leukaemia, cervix and ovary. Genotoxic effects have been found in eight organ sites at which tobacco smoke causes cancer in humans ([DeMarini, 2004](#)).

Sites with limited evidence of carcinogenicity or evidence suggesting lack of carcinogenicity in humans include the breast and the endothelium and relevant mechanisms are presented below.

Breast — There are several plausible mechanisms by which smoking may increase breast cancer risk, and some data support such an effect, including the increased risk among long-term smokers with *NAT2* slow genotype. Despite the overall lack of clear association in epidemiological studies, and the potential anti-estrogenic effects of smoking, the possibility that smoking increases breast cancer risk is biologically plausible.

Endothelium — The mechanisms by which cigarette smoking reduces the risk for endometrial cancer among current smokers, mainly among postmenopausal, remain unclear.

#### 4.5.4 Interaction of ethanol and tobacco carcinogens

Data in rodents and non-human primates on the relationships between a) inhibition of hepatic clearance of nitrosamines by ethanol, b) the formation of promutagenic DNA adducts and c) tumours in extra-hepatic targets, likely also pertain in humans.

## 5. Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of tobacco smoking.

Tobacco smoking causes cancers of the lung, oral cavity, naso-, oro- and hypopharynx, nasal cavity and accessory sinuses, larynx, oesophagus, stomach, pancreas, colorectum, liver, kidney (body and pelvis), ureter, urinary bladder, uterine cervix and ovary (mucinous), and myeloid leukaemia. Also, a positive association has been observed between tobacco smoking and cancer of the female breast. For cancers of the endometrium (post-menopausal) and of the thyroid, there is *evidence suggesting lack of carcinogenicity*.

There is *sufficient evidence* in humans for the carcinogenicity of parental smoking. Parental smoking causes hepatoblastoma in children. Also, a positive association has been observed between parental smoking and childhood leukaemia (particularly acute lymphocytic leukaemia).

There is *sufficient evidence* in experimental animals for the carcinogenicity of tobacco smoke and of tobacco smoke condensates.

Tobacco smoking is *carcinogenic to humans* (Group 1).

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