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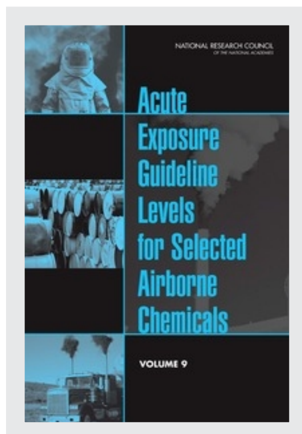

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Acute Exposure Guideline Levels for Selected Airborne Chemicals

VOLUME 9

Committee on Acute Exposure Guideline Levels

Committee on Toxicology

Board on Environmental Studies and Toxicology

Division on Earth and Life Studies

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This project was supported by Contract No. W81K04-06-D-0023 and EP-W-09-007 between the National Academy of Sciences and the U.S. Department of Defense and the U.S. Environmental Protection Agency. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the organizations or agencies that provided support for this project.

International Standard Book Number-13: 978-0-309-15944-9
International Standard Book Number-10: 0-309-15944-X

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Preface

Extremely hazardous substances (EHSs)[2] can be released accidentally as a result of chemical spills, industrial explosions, fires, or accidents involving railroad cars and trucks transporting EHSs. Workers and residents in communities surrounding industrial facilities where EHSs are manufactured, used, or stored and in communities along the nation's railways and highways are potentially at risk of being exposed to airborne EHSs during accidental releases or intentional releases by terrorists. Pursuant to the Superfund Amendments and Reauthorization Act of 1986, the U.S. Environmental Protection Agency (EPA) has identified approximately 400 EHSs on the basis of acute lethality data in rodents.

As part of its efforts to develop acute exposure guideline levels for EHSs, EPA and the Agency for Toxic Substances and Disease Registry (ATSDR) in 1991 requested that the National Research Council (NRC) develop guidelines for establishing such levels. In response to that request, the NRC published *Guidelines for Developing Community Emergency Exposure Levels for Hazardous Substances* in 1993. Subsequently, *Standard Operating Procedures for Developing Acute Exposure Guideline Levels for Hazardous Substances* was published in 2001, providing updated procedures, methodologies, and other guidelines used by the National Advisory Committee (NAC) on Acute Exposure Guideline Levels for Hazardous Substances and the Committee on Acute Exposure Guideline Levels (AEGLs) in developing the AEGL values.

Using the 1993 and 2001 NRC guidelines reports, the NAC—consisting of members from EPA, the Department of Defense (DOD), the Department of Energy (DOE), the Department of Transportation (DOT), other federal and state governments, the chemical industry, academia, and other organizations from the private sector—has developed AEGLs for approximately 200 EHSs.

In 1998, EPA and DOD requested that the NRC independently review the AEGLs developed by NAC. In response to that request, the NRC organized within its Committee on Toxicology (COT) the Committee on Acute Exposure Guideline Levels, which prepared this report. This report is the ninth volume in

[2]As defined pursuant to the Superfund Amendments and Reauthorization Act of 1986.

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the series *Acute Exposure Guideline Levels for Selected Airborne Chemicals*. It reviews the AEGLs for bromine, ethylene oxide, furan, hydrogen sulfide, propylene oxide, and xylenes for scientific accuracy, completeness, and consistency with the NRC guideline reports. It also includes a chapter addressing the use of physiologically based pharmacokinetic (PBPK) models to support the derivation of AEGLs.

The committee's review of the AEGL documents involved both oral and written presentations to the committee by the NAC authors of the documents. The committee examined the draft documents and provided comments and recommendations for how they could be improved in a series of interim reports. The authors revised the draft AEGL documents based on the advice in the interim reports and presented them for reexamination by the committee as many times as necessary until the committee was satisfied that the AEGLs were scientifically justified and consistent with the 1993 and 2001 NRC guideline reports. After these determinations have been made for an AEGL document, it is published as an appendix in a volume such as this one.

The nine interim reports of the committee that led to this report were reviewed in draft form by individuals selected for their diverse perspectives and technical expertise, in accordance with procedures approved by the NRC's Report Review Committee. The purpose of this independent review is to provide candid and critical comments that will assist the institution in making its published report as sound as possible and to ensure that the report meets institutional standards for objectivity, evidence, and responsiveness to the study charge. The review comments and draft manuscript remain confidential to protect the integrity of the deliberative process. We wish to thank the following individuals for their review of the nine committee interim reports, which summarize the committee's conclusions and recommendations for improving NAC's AEGL documents for bromine (twelfth and fifteenth interim reports, 2005 and 2008, respectively), ethylene oxide (tenth and fifteenth interim reports, 2004 and 2008, respectively), furan (sixth, eighth, and fifteenth interim reports, 2001, 2002, and 2008, respectively), hydrogen sulfide (third, sixth, seventh, eighth, and ninth interim reports, 2000, 2001, 2002, 2002, and 2003, respectively), propylene oxide (tenth interim report, 2004), xylenes (twelfth and fourteenth interim reports, 2005 and 2006, respectively), and the use of PBPK models to support the derivation of AEGLs (fifteenth interim report, 2008): Deepak Bhalla (Wayne State University), Harvey Clewell (The Hamner Institutes for Health Sciences), Rakesh Dixit (MedImmune/AstraZeneca Biologics, before he became a member of the committee), David Gaylor (Gaylor and Associates, LLC), Sidney Green (Howard University), A. Wallace Hayes (Harvard School of Public Health), Sam Kacew (University of Ottawa), Nancy Kerkvliet (Oregon State University), Florence K. Kinoshita (Hercules Incorporated [retired]), Kenneth Poirier (Toxicology Excellence for Risk Assessment),Charles R. Reinhardt (DuPont Haskell Laboratory [retired]), and Bernard M. Wagner (New York University Medical Center [retired]).

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Although the reviewers listed above have provided many constructive comments and suggestions, they were not asked to endorse the conclusions or recommendations, nor did they see the final draft of this volume before its release. The review of the third interim report, completed in 2000, was overseen by Mary Vore, University of Kentucky Medical Center. The reviews of the sixth interim report (2001), seventh interim report (2002), fourteenth interim report (2006), and fifteenth interim report (2008) were overseen by Robert Goyer, University of Western Ontario (retired). The reviews of the eighth interim report (2002) and tenth interim report (2004) were overseen by David H. Moore, Battelle Memorial Institute. The review of the ninth interim report (2003) was overseen by Judith A. Graham, American Chemistry Council (retired). The review of the twelfth interim report (2005) was overseen by David W. Gaylor, Gaylor and Associates, LLC. Appointed by the NRC, they were responsible for making certain that an independent examination of the interim reports was carried out in accordance with institutional procedures and that all review comments were carefully considered. Responsibility for the final content of this report rests entirely with the authoring committee and the institution.

The committee gratefully acknowledges the valuable assistance provided by the following persons: Ernest Falke, Marquea D. King, Iris A. Camacho, and Paul Tobin (all from EPA); and George Rusch (Honeywell, Inc.). The committee also acknowledges Raymond Wassel and Keegan Sawyer, the project directors for their work this project. Other staff members who contributed to this effort are James J. Reisa (director of the Board on Environmental Studies and Toxicology), Susan Martel (senior program officer for toxicology), Ruth Crossgrove (senior editor), Radiah Rose (manager of editorial projects), Mirsada Karalic-Loncarevic (manager of the Technical Information Center), Orin Luke (senior program assistant), and Tamara Dawson (program associate). Finally, I would like to thank all members of the committee for their expertise and dedicated effort throughout the development of this report.

Donald E. Gardner, *Chair*
Committee on Acute Exposure
Guideline Levels

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Acute Exposure Guideline Levels for Selected Airborne Chemicals

VOLUME 9

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National Research Council Committee Review of Acute Exposure Guideline Levels of Selected Airborne Chemicals

This report is the ninth volume in the series *Acute Exposure Guideline Levels for Selected Airborne Chemicals*.

In the Bhopal disaster of 1984, approximately 2,000 residents living near a chemical plant were killed and 20,000 more suffered irreversible damage to their eyes and lungs following accidental release of methyl isocyanate. The toll was particularly high because the community had little idea what chemicals were being used at the plant, how dangerous they might be, or what steps to take in an emergency. This tragedy served to focus international attention on the need for governments to identify hazardous substances and to assist local communities in planning how to deal with emergency exposures.

In the United States, the Superfund Amendments and Reauthorization Act (SARA) of 1986 required that the U.S. Environmental Protection Agency (EPA) identify extremely hazardous substances (EHSs) and, in cooperation with the Federal Emergency Management Agency and the U.S. Department of Transportation, assist local emergency planning committees (LEPCs) by providing guidance for conducting health hazard assessments for the development of emergency response plans for sites where EHSs are produced, stored, transported, or used. SARA also required that the Agency for Toxic Substances and Disease Registry (ATSDR) determine whether chemical substances identified at hazardous waste sites or in the environment present a public health concern.

As a first step in assisting the LEPCs, EPA identified approximately 400 EHSs largely on the basis of their immediately dangerous to life and health values, developed by the National Institute for Occupational Safety or Health. Although several public and private groups, such as the Occupational Safety and Health Administration and the American Conference of Governmental Industrial Hygienists, have established exposure limits for some substances and some exposures (e.g., workplace or ambient air quality), these limits are not easily or directly translated into emergency exposure limits for exposures at high levels

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but of short duration, usually less than 1 hour (h), and only once in a lifetime for the general population, which includes infants (from birth to 3 years of age), children, the elderly, and persons with diseases, such as asthma or heart disease.

The National Research Council (NRC) Committee on Toxicology (COT) has published many reports on emergency exposure guidance levels and spacecraft maximum allowable concentrations for chemicals used by the U.S. Department of Defense (DOD) and the National Aeronautics and Space Administration (NASA) (NRC 1968, 1972, 1984a,b,c,d, 1985a,b, 1986a, 1987, 1988, 1994, 1996a,b, 2000a, 2002a, 2007a, 2008a). COT has also published guidelines for developing emergency exposure guidance levels for military personnel and for astronauts (NRC 1986b, 1992, 2000b). Because of COT's experience in recommending emergency exposure levels for short-term exposures, in 1991 EPA and ATSDR requested that COT develop criteria and methods for developing emergency exposure levels for EHSs for the general population. In response to that request, the NRC assigned this project to the COT Subcommittee on Guidelines for Developing Community Emergency Exposure Levels for Hazardous Substances. The report of that subcommittee, *Guidelines for Developing Community Emergency Exposure Levels for Hazardous Substances* (NRC 1993), provides step-by-step guidance for setting emergency exposure levels for EHSs. Guidance is given on what data are needed, what data are available, how to evaluate the data, and how to present the results.

In November 1995, the National Advisory Committee (NAC)[1] for Acute Exposure Guideline Levels for Hazardous Substances was established to identify, review, and interpret relevant toxicologic and other scientific data and to develop acute exposure guideline levels (AEGLs) for high-priority, acutely toxic chemicals. The NRC's previous name for acute exposure levels—community emergency exposure levels (CEELs)—was replaced by the term AEGLs to reflect the broad application of these values to planning, response, and prevention in the community, the workplace, transportation, the military, and the remediation of Superfund sites.

AEGLs represent threshold exposure limits (exposure levels below which adverse health effects are not likely to occur) for the general public and are applicable to emergency exposures ranging from 10 minutes (min) to 8 h. Three levels—AEGL-1, AEGL-2, and AEGL-3—are developed for each of five exposure periods (10 min, 30 min, 1 h, 4 h, and 8 h) and are distinguished by varying degrees of severity of toxic effects. The three AEGLs are defined as follows:

AEGL-1 is the airborne concentration (expressed as ppm [parts per million] or mg/m^3 [milligrams per cubic meter]) 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

[1]NAC is composed of members from EPA, DOD, many other federal and state agencies, industry, academia, and other organizations. The NAC roster is shown on page 9.

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upon cessation of exposure.

AEGL-2 is the airborne concentration (expressed as ppm or mg/m^3) 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.

AEGL-3 is the airborne concentration (expressed as ppm or mg/m^3) of a substance above which it is predicted that the general population, including susceptible individuals, could experience life-threatening adverse health effects or death.

Airborne concentrations below AEGL-1 represent exposure levels that can produce mild and progressively increasing but transient and nondisabling odor, taste, and sensory irritation or certain asymptomatic nonsensory adverse effects. With increasing airborne concentrations above each AEGL, there is a progressive increase in the likelihood of occurrence and the severity of effects described for each corresponding AEGL. Although the AEGL values represent threshold levels for the general public, including susceptible subpopulations, such as infants, children, the elderly, persons with asthma, and those with other illnesses, it is recognized that individuals, subject to idiosyncratic responses, could experience the effects described at concentrations below the corresponding AEGL.

SUMMARY OF REPORT ON GUIDELINES FOR DEVELOPING AEGLS

As described in *Guidelines for Developing Community Emergency Exposure Levels for Hazardous Substances* (NRC 1993) and the NRC guidelines report *Standing Operating Procedures for Developing Acute Exposure Guideline Levels for Hazardous Chemicals* (NRC 2001a), the first step in establishing AEGLs for a chemical is to collect and review all relevant published and unpublished information. Various types of evidence are assessed in establishing AEGL values for a chemical. These include information from (1) chemical-physical characterizations, (2) structure-activity relationships, (3) in vitro toxicity studies, (4) animal toxicity studies, (5) controlled human studies, (6) observations of humans involved in chemical accidents, and (7) epidemiologic studies. Toxicity data from human studies are most applicable and are used when available in preference to data from animal studies and in vitro studies. Toxicity data from inhalation exposures are most useful for setting AEGLs for airborne chemicals because inhalation is the most likely route of exposure and because extrapolation of data from other routes would lead to additional uncertainty in the AEGL estimate.

For most chemicals, actual human toxicity data are not available or critical information on exposure is lacking, so toxicity data from studies conducted in laboratory animals are extrapolated to estimate the potential toxicity in humans. Such extrapolation requires experienced scientific judgment. The toxicity data

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for animal species most representative of humans in terms of pharmacodynamic and pharmacokinetic properties are used for determining AEGLs. If data are not available on the species that best represents humans, data from the most sensitive animal species are used. Uncertainty factors are commonly used when animal data are used to estimate risk levels for humans. The magnitude of uncertainty factors depends on the quality of the animal data used to determine the no-observed-adverse-effect level (NOAEL) and the mode of action of the substance in question. When available, pharmacokinetic data on tissue doses are considered for interspecies extrapolation.

For substances that affect several organ systems or have multiple effects, all end points (including reproductive [in both genders], developmental, neurotoxic, respiratory, and other organ-related effects) are evaluated, the most important or most sensitive effect receiving the greatest attention. For carcinogenic chemicals, excess carcinogenic risk is estimated, and the AEGLs corresponding to carcinogenic risks of 1 in 10,000 (1×10^{-4}), 1 in 100,000 (1×10^{-5}), and 1 in 1,000,000 (1×10^{-6}) exposed persons are estimated.

REVIEW OF AEGL REPORTS

As NAC began developing chemical-specific AEGL reports, EPA and DOD asked the NRC to review independently the NAC reports for their scientific validity, completeness, and consistency with the NRC guideline reports (NRC 1993, 2001a). The NRC assigned this project to the COT Committee on Acute Exposure Guideline Levels. The committee has expertise in toxicology, epidemiology, occupational health, pharmacology, medicine, pharmacokinetics, industrial hygiene, and risk assessment.

The AEGL draft reports are initially prepared by ad hoc AEGL development teams consisting of a chemical manager, two chemical reviewers, and a staff scientist of the NAC contractor—Oak Ridge National Laboratory. The draft documents are then reviewed by NAC and elevated from "draft" to "proposed" status. After the AEGL documents are approved by NAC, they are published in the *Federal Register* for public comment. The reports are then revised by NAC in response to the public comments, elevated from "proposed" to "interim" status, and sent to the NRC Committee on Acute Exposure Guideline Levels for final evaluation.

The NRC committee's review of the AEGL reports prepared by NAC and its contractors involves oral and written presentations to the committee by the authors of the reports. The NRC committee provides advice and recommendations for revisions to ensure scientific validity and consistency with the NRC guideline reports (NRC 1993, 2001a). The revised reports are presented at subsequent meetings until the subcommittee is satisfied with the reviews.

Because of the enormous amount of data presented in AEGL reports, the NRC committee cannot verify all of the data used by NAC. The NRC committee relies on NAC for the accuracy and completeness of the toxicity data cited in the

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AEGL reports. Thus far, the committee has prepared seven reports in the series *Acute Exposure Guideline Levels for Selected Airborne Chemicals* (NRC 2001b, 2002b, 2003, 2004, 2007b, 2008b, 2009, 2010). This report is the ninth volume in that series. AEGL documents for bromine, ethylene oxide, furan, hydrogen sulfide, propylene oxide, and xylenes are each published as an appendix in this report. This volume also contains a chapter on the use of physiologically based pharmacokinetic models to support the derivation of AEGLs. The committee concludes that the AEGLs developed in these appendixes are scientifically valid conclusions based on the data reviewed by NAC and are consistent with the NRC guideline reports. AEGL reports for additional chemicals will be presented in subsequent volumes.

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Appendixes

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1

Bromine[1]

Acute Exposure Guideline Levels

PREFACE

Under the authority of the Federal Advisory Committee Act (FACA) P.L. 92-463 of 1972, the National Advisory Committee for Acute Exposure Guideline Levels for Hazardous Substances (NAC/AEGL Committee) has been established to identify, review and interpret relevant toxicologic and other scientific data and develop AEGLs for high-priority, acutely toxic chemicals.

AEGLs represent threshold exposure limits for the general public and are applicable to emergency exposure periods ranging from 10 minutes (min) to 8 hours (h). Three levels—AEGL-1, AEGL-2, and AEGL—are developed for each of five exposure periods (10 and 30 min and 1, 4, and 8 h) and are distinguished by varying degrees of severity of toxic effects. The three AEGLs have been defined as follows:

AEGL-1 is the airborne concentration (expressed as parts per million [ppm] or milligrams per cubic meter [mg/m^3]) of a substance above which it is predicted that the general population, including susceptible individuals, could experience notable discomfort, irritation, or certain asymptomatic, nonsensory

[1]This document was prepared by the AEGL Development Team composed of Sylvia Talmage (Oak Ridge National Laboratory) and Chemical Manager Ernest Falke (National Advisory Committee [NAC] on Acute Exposure Guideline Levels for Hazardous Substances). The NAC reviewed and revised the document and AEGLs as deemed necessary. Both the document and the AEGL values were then reviewed by the National Research Council (NRC) Committee on Acute Exposure Guideline Levels. The NRC committee has concluded that the AEGLs developed in this document are scientifically valid conclusions based on the data reviewed by the NRC and are consistent with the NRC guideline reports (NRC 1993, 2001).

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effects. However, the effects are not disabling and are transient and reversible upon cessation of exposure.

AEGL-2 is the airborne concentration (expressed as ppm or mg/m^3) 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.

AEGL-3 is the airborne concentration (expressed as ppm or mg/m^3) of a substance above which it is predicted that the general population, including susceptible individuals, could experience life-threatening health effects or death.

Airborne concentrations below the AEGL-1 represent exposure levels that could produce mild and progressively increasing but transient and nondisabling odor, taste, and sensory irritation or certain asymptomatic, nonsensory effects. With increasing airborne concentrations above each AEGL, there is a progressive increase in the likelihood of occurrence and the severity of effects described for each corresponding AEGL. Although the AEGLs represent threshold levels for the general public, including susceptible subpopulations, such as infants, children, the elderly, persons with asthma, and those with other illnesses, it is recognized that individuals, subject to idiosyncratic responses, could experience the effects described at concentrations below the corresponding AEGL.

SUMMARY

The halogen bromine (Br_2) is a dark reddish-brown volatile liquid at room temperature. Its oxidizing potential lies between that of chlorine and iodine. Bromine is used as a water disinfectant, for bleaching fibers, and in the manufacture of medicinal bromine compounds, dyestuffs, flame retardants, agricultural chemicals, inorganic bromide drilling fluids, and gasoline additives.

Bromine is a skin, eye, and respiratory-tract irritant. Inhalation causes respiratory-tract irritation and pulmonary edema. Although accidental human exposures have occurred, concentrations were either not reported or were judged unreliable. The data on the inhalation toxicity of bromine are sparse and, at times, conflicting. Aside from old and anecdotal information, the database is limited to one study with human subjects and two lethality studies with the mouse as the test species. One of the lethality studies (Bitron and Aharonson 1978) provided data sufficient for derivation of the relationship between concentrations that result in lethality (LC_{50} values [concentration with 50% lethality]) and exposure duration: $C^{2.2} \times t = k$ (chemical concentration in air with a chemical-specific exponent applied to a specific end point × exposure time = response).

The AEGL-1 was based on exposures of 20 healthy human subjects to concentrations of 0.1 to 1.0 ppm for at least 30 min (Rupp and Henschler 1967). Eye irritation, but not nose or throat irritation, occurred during a 30-min exposure at 0.1 ppm. At concentrations of ≥0.5 ppm, there was a stinging and burning sensation of the conjunctiva. The 30-min exposure to 0.1 ppm, which caused

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mild irritation, was chosen as the basis for the AEGL-1. The 0.1-ppm concentration was divided by an intraspecies uncertainty factor of 3 to protect susceptible individuals. An intraspecies uncertainty factor of 3 was considered sufficient because workers have been occupationally exposed to 1 ppm with no symptoms other than "excess irritation" (Elkins 1959). The resulting 0.033-ppm concentration is 30-fold lower than the 1-ppm concentration that induced excess irritation in healthy workers. Effects at this low concentration appear to be limited to the eyes and upper respiratory tract; there is likely to be little penetration to the lower respiratory tract. Compared with the 0.5-ppm AEGL-1 concentration for chlorine, a chemical that more readily penetrates to the lower respiratory tract, the intraspecies uncertainty factor of 3 for bromine is considered adequate. An intraspecies uncertainty factor of 1 was applied to the 0.5-ppm test value for chlorine because this concentration failed to elicit an asthmatic response in atopic and asthmatic individuals. The resulting 30-min AEGL-1 value of 0.033 ppm was used for all AEGL-1 exposure durations, as adaptation to mild sensory irritation occurs.

The AEGL-2 was based on the exposure to approximately 1 ppm for 30 min, which the subjects in the above study (Rupp and Henschler 1967) found irritating (stinging and burning sensation of the conjunctiva and nose and throat irritation). The 30-min 1-ppm value was divided by an intraspecies uncertainty factor of 3 to protect susceptible individuals and time-scaled to the other AEGL-2 exposure durations by using the concentration-exposure duration relationship of $C^{2.2} \times t = k$ from the mouse lethality study. An intraspecies uncertainty factor of 3 was considered sufficient, as the symptoms may be below those defining the AEGL-2. Furthermore, compared with the 30-min AEGL-2 value of 2.8 ppm for chlorine, this value may be conservative. The 30-min value for the less well-scrubbed chlorine was based on transient changes in pulmonary parameters (without respiratory symptoms) in asthmatic and atopic individuals. No reliable studies with exposures to higher concentrations were located.

Both lethality studies with the mouse described the inhalation toxicity of both chlorine and bromine. However, both studies reported lower LC_{50} values for chlorine than those reported in more recent well-conducted studies. Nevertheless, the study that reported the lower lethal concentrations for chlorine was used for derivation of the AEGL-3 values for bromine (Schlagbauer and Henschler 1967). The data in this study showed a clear concentration-response relationship; the exposure duration was 30 min. Using probit analysis, a 30-min LC_{50} value of 204 ppm and a 30-min LC_{01} of 116 ppm were calculated. The 30-min LC_{01} of 116 ppm was used as the basis for calculation of AEGL-3 values. The 116-ppm LC_{01} was divided by a combined uncertainty factor of 10 (3 for interspecies differences [the mouse was the most sensitive species for lethal effects in tests with other halogens] and 3 for intraspecies differences [at high concentrations, bromine is corrosive to the mucous membranes of the respiratory system; effects are not expected to differ greatly among individuals]) and

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scaled across time using the relationship $C^{2.2} \times t = k$, derived from the Bitron and Aharonson (1978) study.

The calculated values are shown in Table 1-1.

1. INTRODUCTION

Bromine, a halogen, is a dark reddish-brown volatile liquid that vaporizes readily to a red vapor at room temperature. The diatomic state persists in the liquid, gas, and solid phases. Chemically, the electronegativity and oxidizing potential of halogens decrease as the atomic weight increases, thus making bromine intermediate in oxidizing potential between chlorine and iodine. All the halogens form an acid in water, and the reactivity of these acids shows the same relationship as the elemental halogens. The water solubility of bromine is greater than that of chlorine (O'Neil et al. 2001; Teitelbaum 2001). Additional chemical and physical properties are listed in Table 1-2.

The uses of bromine include water disinfection, bleaching fibers and silk, and the manufacture of medicinal bromine compounds and dyestuffs (O'Neil et al. 2001). The global market for bromine-containing compounds includes flame retardants, agricultural chemicals (principally methyl bromide), inorganic bromide drilling fluids such as calcium bromide, and gasoline additives (Glauser 2009). Production of ethylene dibromide, a gasoline antiknock agent for leaded fuels has decreased substantially over the past years. Likewise, the use of brominated fumigants and pesticides, such as ethylene dibromide and methyl bromide, has been restricted in the United States (Teitelbaum 2001). Commercially, bromine is recovered from soluble bromide salts in salt lakes, inland seas, brine wells and seawater. Seawater contains bromine at a concentration of 65 ppm (Teitelbaum 2001).

In 2005, world production was estimated at 587,000 metric tons, most of the bromine being used for brominated flame retardants ((Glauser 2009). Several production plants are located near natural brine sites in Arkansas (Jackisch 1992). Bromine is shipped in bulk quantities in 7,570-liter (L) and 15,140-L lead-lined pressure tank cars or 6,435- to 6,813-L nickel-clad pressure tank trailers filled to at least 92% capacity (Jackisch 1992). Bromine is also shipped in 600-, 1,200-, and 1,800-gallon tank trucks and 2,300- and 4,400-gallon tank cars (Great Lakes Chemical Corporation 1996).

Bromine is a skin, eye, and respiratory tract irritant (Teitelbaum 2001). All of the exposure data on humans and many of the experimental data on animals are extremely old, provide few experimental details, or conflict with more recent information. Therefore, many of the data are considered unreliable. Two inhalation studies with the mouse as the test species and using several concentrations and exposure durations were located. However, both of these studies report values for chlorine that are much lower than those of other researchers.

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TABLE 1-1 Summary of AEGL Values for Bromine

Classification	10 min	30 min	1 h	4 h	8 h	End Point (Reference)
AEGL-1 (Nondisabling)	0.033 ppm (0.22 mg/m^3)	0.033 ppm (0.22 mg/m^3)	0.033 ppm (0.22 mg/m^3)	0.033 ppm (0.22 mg/m^3)	0.033 ppm (0.22 mg/m^3)	Eye irritation in humans (Rupp and Henschler 1967)
AEGL-2 (Disabling)	0.55 ppm (3.6 mg/m^3)	0.33 ppm (2.2 mg/m^3)	0.24 ppm (1.6 mg/m^3)	0.13 ppm (0.85 mg/m^3)	0.095 ppm (0.62 mg/m^3)	Conjunctiva and nose and throat irritation in humans (Rupp and Henschler 1967)
AEGL-3 (Lethal)	19 ppm (124 mg/m^3)	12 ppm (78 mg/m^3)	8.5 ppm (55 mg/m^3)	4.5 ppm (29 mg/m^3)	3.3 ppm (21 mg/m^3)	30 min LC_{01} in mice (Schlagbauer and Henschler 1967)

TABLE 1-2 Chemical and Physical Data for Bromine

Parameter	Data	Reference
Synonyms	Dibromine	HSDB 2008
CAS registry number	7726-95-6	O'Neil et al. 2001
Chemical formula	Br_2	O'Neil et al. 2001
Structure	Br-Br	O'Neil et al. 2001
Molecular weight	159.9 (Br_2)	O'Neil et al. 2001
Physical state	Dark, reddish-brown fuming liquid, vaporizes rapidly at room temperature	O'Neil et al. 2001
Melting and boiling point	−7.25ºC/59.47ºC	O'Neil et al. 2001
Solubility	17 g/L in water at 20ºC	Teitelbaum 2001
Vapor pressure	175 mmHg at 20ºC	AIHA 2001
Vapor density (air = 1)	3.5	AIHA 2001
Liquid density (water = 1)	3.1	O'Neil et al. 2001
Flammability	Not flammable; may cause fire on contact with combustibles	DOT 1985
Conversion factors (Br_2)	1 ppm = 6.5 mg/m^3 1 mg/m^3 = 0.15 ppm	AIHA 2001

2. HUMAN TOXICITY DATA

2.1. Acute Lethality

Champeix et al. (1970) described the case of a worker exposed to an unknown concentration of vapor during an industrial accident. A postmortem examination revealed bromine burns to 20% of the body, extensive pulmonary and

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tracheal damage, and effects on the kidneys and liver. In another industrial accident, eight workers were exposed to an unknown concentration of bromine vapor (Suntych 1953). Three workers developed bronchopneumonia, one developed blepharospasm, and the remainder developed laryngitis. One worker died as the result of sudden circulatory failure associated with bronchopneumonia.

Carel et al. (1992) reported a transportation accident involving a semitrailer truck carrying liquid bromine on an isolated stretch of highway in the Negev Desert, Israel. The driver was pinned in the cabin of the truck and was unable to free himself or reach his protective equipment. He died of bromine intoxication 3 h after the accident occurred.

Using primarily the database on chlorine, the relationship between the toxicity of chlorine and bromine, and the relationship between concentration and time from animal lethality data, Withers and Lees (1986) calculated an LC_{50} for humans exposed to bromine. Their model incorporates the effects of physical activity, inhalation rate, the effectiveness of medical treatment, and the lethal toxic load function (the relationship between lethality, concentration, and time). Concentrations were based on the estimate that bromine is 1.5 times less toxic than chlorine. The estimated 30-min LC_{50} at a standard level of activity (inhalation rate of 12 L/min) for the regular, vulnerable, and average (regular + vulnerable) populations were 375, 150, and 315 ppm, respectively. Estimated LC_{10} values for a 10-min exposure were 325, 130, and 208 ppm, respectively.

2.2. Nonlethal Toxicity

2.2.1. Odor Threshold

The odor threshold for bromine has been variously reported at approximately 0.01 to 3.8 ppm (Rupp and Henschler 1967; Billings and Jonas 1981; Amoore and Hautala 1983; Ruth 1986). Ruth (1986) reported the threshold for irritation at 0.3 ppm, but the source of the data was not stated. Rupp and Henschler (1967) reported that healthy subjects had difficulty distinguishing between the odor of chlorine and the odor of bromine at concentrations up to 1 ppm, the highest concentration tested. The odor has been reported as suffocating by O'Neil et al. (2001) and bleachy and penetrating by Ruth (1986).

2.2.2. General Toxic Effects

The signs and symptoms associated with human exposure to low concentrations include upper airways irritation, inflammation of the eyelids, lacrimation, coughing, nosebleed, and a feeling of oppression, dizziness, and headache (Flury and Zernik 1931; Alexandrov 1983; Teitelbaum 2001). After several hours these symptoms may be followed by abdominal pain and diarrhea and a measles-like eruption on the trunk and extremities. Inhalation of "larger quantities" results in brown coloration of the eyes, tongue, and mucous membranes of

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the mouth, catarrh, salivation, coughing, feeling of suffocation, glottis cramps, hoarseness, bronchitis, and bronchial asthma (Flury and Zernik 1931). Bromine was reported to produce a stinging and burning sensation of the conjunctiva at exposures of ≥0.5 ppm (Rupp and Henschler 1967). Chronic exposure to bromine resulting in excessive tissue levels of bromide ions (bromism) may lead to slowing of cerebration, impaired memory, anorexia, skin rash, headache, slurring of speech, confusion, weakness, disturbed reflexes, drowsiness, and mild conjunctivitis (EPA 1988).

Irritant levels for bromine have been reported in several sources. Many of the data are extremely old and are compromised by inadequate descriptions of vapor-generation methods, analytic-measurement methods, and exposure durations. These data and reviews are cited here for completeness.

Henderson and Haggard (1943), relying on older data including Matt (1889) and Flury and Zernik (1931) who cite Lehmann (1887), stated that 40-60 ppm is dangerous for brief exposures, 4 ppm is the maximum concentration that can be tolerated for 0.5 to 1 h, and 0.1 to 0.15 ppm can be tolerated for prolonged periods of time. Flury and Zernik (1931) and Withers and Lees (1986) cited the data of Matt (1889) who exposed human volunteers to bromine vapor (bromine was poured into a room) for 16 min to 7.67 h. Under this exposure scenario, Matt (1889) stated that 1-2 ppm could be tolerated by workers indefinitely, 3.5 ppm is tolerable for 30 min to 1 h, and 4 ppm is intolerable for work conditions.

Workers regularly exposed to bromine concentrations at approximately 0.3 to 0.6 ppm for 1 year experienced headache; pain in the joints, stomach, and chest; irritability; and loss of appetite (Alexandrov 1983). Long-term exposure can lead to nervous system disorders, myocardial degeneration, and thyroid hyperplasia. The source of the Alexandrov (1983) data was not given. Elkins (1959), citing a personal communication, reported that 1 ppm in a Massachusetts plant handling liquid bromine was judged to be excessively irritating. Flury and Zernik (1931) cited the data of Lehmann (1887) who reported that exposure to 0.75 ppm in a workroom caused no symptoms in 6 h. OSHA (unpublished material, 1997) monitoring data taken from January 1, 1985, to January 1, 1997, and involving 22 samples from 10 area offices, showed that workers are currently exposed to concentrations between 0.00 and 0.18 ppm. “Total times” for the 0.18-ppm exposures ranged from 15 min to 7.5 h.

2.2.3. Clinical Study

In a clinical study, Rupp and Henschler (1967) determined the odor threshold and subjective irritation concentrations of both chlorine and bromine. These authors subjected 20 healthy students to “low concentrations” of bromine or chlorine in an 8 m^3 exposure chamber. Bromine gas was generated directly from a heated 2-L flask containing 50 mL of the liquid; dilutions were made

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with fresh air. Analytic determinations were made titrimetrically with thiosulfate solution (higher concentrations) or spectrophotometrically (concentrations below 0.1 ppm). Samples were collected in potassium iodide solution (higher concentrations) or by absorption by *o*-toluidine hydrochloride (lower concentrations). The odor threshold for bromine was tested at concentrations of 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, and 1.0 ppm over a 30-min duration. Odor intensity was evaluated with the descriptors: minimal, medium strong, strong, and very strong. Subjective eye, nose, and throat irritation was evaluated as concentrations increased from 0 to 0.9 ppm over a 1-h duration.

A total of 20 students were tested, with 3-4 entering the exposure chamber at one time. Upon chamber entry, odor of bromine was noted by all 20 individuals at a concentration of 0.01 ppm, with an intensity of minimal to medium strong. At 0.2 ppm, most subjects rated odor intensity between medium strong and very strong. In the second part of the study, the subjects recorded their irritation every 5 min over a 60-min period. Eye irritation was first noted at a bromine concentration of 0.1 ppm and occurred within the first 30 min of exposure. At concentrations of 0.2 ppm and higher, distinct nose, eye, and throat irritation occurred, with a rapidly increasing concentration response. Between 0.5 and 0.9 ppm, a 5-min exposure was perceived as uncomfortable (concentrations of 0.5 to 0.9 ppm were irritating to the conjunctiva, nose, and throat.); however, the intensity of effect did not increase above 0.5 ppm. Irritation appeared to be limited to the eyes, nose, and throat; a "compelling cough stimulus" was not attained at concentrations up to 0.9 ppm. At similar concentrations, bromine was found to be more irritating than chlorine. In evaluating their own experiment, the authors noted that the actual concentrations were approximately 40% (range 17-57%) less than the nominal concentrations reported above. Measurements were taken in the vicinity of a wall and not in the immediate area of the subjects. In evaluating the experimental results, Henschler considered the threshold for subjective discomfort to be 0.5 ppm (D. Henschler, Institut for Toxicology, Wurzburg, Germany, personal commun., Dec. 21, 1999).

In the same study, Rupp and Henschler (1967) reported sensory irritation for chlorine at concentrations that proved to be nonirritating in later well-conducted studies (reviewed in 54 Fed. Reg. 2455[1989]). For example, Rupp and Henschler (1967) reported conjunctival pain in several subjects after 15 min of exposure to chlorine at 0.5 ppm, whereas, in a study by Rotman et al. (1983), healthy subjects reported no serious subjective symptoms of irritation from chlorine at a concentration of 1 ppm for 8 h. The lack of controls in the Rupp and Henschler (1967) study as well some methodologic shortcomings in the chlorine part of the study are discussed by OSHA (54 Fed. Reg. 2455 [1989]). The more recent studies of odor thresholds reported higher concentrations than those reported in the Rupp and Henschler (1967) study. It should also be noted that concentration and exposure-duration data reported in the text and figures of the Rupp and Henschler study are conflicting.

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2.2.4. Accidents

On the morning of November 8, 1984, an accident at a chemical plant in Geneva, Switzerland, resulted in the release of 550 kg of liquid bromine (Morabia et al. 1988). Bromine in gaseous form was released via the ventilation system with sufficient force to form a dense brown cloud that drifted into the neighborhood. The cloud remained low over the ground and drifted through the center of the town before reaching Lake Geneva where it dissipated. The time elapsed from the release to disappearance of the cloud over the lake was approximately 5 h. An ozone analyzer located at the Ecotoxicological Centre of the Canton of Geneva (location not given) detected an oxidizing substance between 10 and 12 o'clock that morning. At an undefined time, the centre measured bromine concentrations (Draeger tubes equipped with chlorine reactive tubes) to define the outside limits of the potentially contaminated zone. These concentrations were between 0.2 and 0.5 ppm; concentrations were not measured initially or in the vicinity of the plant.

Ninety-one patients were admitted to the casualty, outpatient, and ophthalmology departments of the University Hospital at Geneva (Morabia et al. 1988). These patients reported signs and symptoms of eye irritation (90%), upper airways irritation (68%), cough (47%), expectoration (34%), and headache (46%). One patient, a worker at the plant, was treated for severe acute bronchitis; following hydrocortisone treatment, he rapidly recovered and was discharged the next day. In the remainder of the patients, symptoms were considered moderate and self-limiting. A 1-month follow-up of 62 of the patients indicated that there were no serious late complications.

Following the transportation accident described by Carel et al. (1990, 1992) in Section 2.1 above, several motorists were exposed to bromine vapor when they stopped to assist the driver of the truck. These exposures produced only mild respiratory symptoms and first and second degree burns to exposed areas of the skin. Four persons were treated with steroids because of shortness of breath; one, a heavy smoker, had diffuse lung wheezes. Six to eight weeks after the accident, four of the exposed motorists complained of cough, shortness of breath, chest tightness, eye irritation, headache, dizziness, fatigue, memory disturbances, and sleep and sexual disturbances. Clinical and laboratory examinations, however, revealed no abnormal findings.

2.3. Developmental and Reproductive Effects

No data concerning developmental and reproductive effects of bromine exposure in humans by the inhalation route were identified in the available literature. Chronic bromism has been associated with two cases of developmental problems (EPA 1988). The bromism was a result of ingestion of bromide salts.

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2.4. Genotoxicity

No data concerning the genotoxicity of bromine in humans were identified in the available literature.

2.5. Carcinogenicity

No data concerning the carcinogenicity of bromine in humans were identified in the available literature.

2.6. Summary

No inhalation studies on the developmental and reproductive toxicity, genotoxicity, or carcinogenicity of bromine in humans were located in the available literature. Human exposures may cause eye, skin, and mucous membrane irritation as well as headache, abdominal pain, and dyspnea (Teitelbaum 2001). Incidences of human exposures were found, but few clear concentration-exposure durations were reported. Some of these data indicate that concentrations of ≤1.0 ppm are irritating (Elkins 1959; Rupp and Henschler 1967; Alexandrov 1983; Ruth 1986). Other data are quoted from secondary and tertiary sources. A study using human subjects reported eye irritation at a concentration of 0.1 ppm and additional sensory irritation at concentrations of ≥0.2 ppm (Rupp and Henschler 1967). The results of parts of this study do not agree with data or statements of other, more recent investigators (Rotman et al. 1983; Ruth 1986; 54 Fed. Reg. 2455 [1989]).

3. ANIMAL TOXICITY DATA

3.1. Acute Lethality

Several recent sources cited the data of Flury and Zernik (1931), who cited the data of Lehmann (1887). These data are so old that they should be considered unreliable but are reported here for completeness. Lehmann (1887) reported that inhalation exposure of three animal species at 180 ppm (duration not reported) caused severe irritation and corneal clouding, the 7-h LC_{10} was 140 ppm for both the cat and guinea pig, and exposure at 300 ppm for 3 h caused deaths in rabbits and guinea pigs. Observations at the latter concentration-exposure time revealed pulmonary edema, deposits on the trachea and bronchi, and gastric hemorrhage.

Henderson and Haggard (1943) reported that a concentration of 1,000 ppm is rapidly fatal. Their source of data appears to be Hill (1915) who experimented with guinea pigs. The original data were not located.

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3.1.1. Rats

Ivanov et al. (1976) reported an LC_{50} of 415 ppm for the rat. Neither the exposure time nor the original citation were stated.

3.1.2. Mice

Ivanov et al. (1976) reported an LC_{50} of 4,46 ppm for the mouse. Neither the exposure time nor the original citation was stated.

Two other acute lethality studies, both using the mouse as the test species, provided details of the exposures. Bitron and Aharonson (1978) exposed 1-month-old male albino mice (28 to 126 mice/group) to concentrations of 240 or 750 ppm for four exposure times at each concentration and calculated 50% mortality as a function of exposure time (median lethal exposure time, or Lt_{50}). Bromine vapor was generated from the liquid, collected in an aqueous solution of potassium iodide, and determined by standard iodometry. Mice were restrained in cylindrical glass exposure chambers. Postexposure observations were made over a 30-day period. The data displayed a clear dose-response relationship and Lt_{50} values for the 240 and 750 ppm exposures were 100 and 9 min, respectively. Mortality at each concentration-exposure duration is listed in Table 1-3. Dose-response curves were presented graphically, and the values listed in Table 1-3 were estimated from the graph. The results of this work were unusual in that many of the deaths were delayed, occurring during the second week of the observation period, rather than during and immediately following exposure. The authors exposed similar groups to chlorine, and it was noted that chlorine is considerably more toxic to mice than bromine.

Using the method of Litchfield and Wilcoxon (1949), Bitron and Aharonson (1978) also computed 0 or 100% mortalities, which they presented graphically. For the 240-ppm concentration, no deaths were calculated to occur following an exposure for 20 min. For the 750-ppm concentration, no deaths were calculated to occur following approximately 5 min of exposure (same as the experimental value).

TABLE 1-3 Mortality in Mice Exposed to Bromine at 240 or 750 ppm

Concentration (ppm)	Exposure Duration (min)	Mortality (%)
240	24	7
	65	27
	120	50
	215	90
750	5	0
	7	44
	13	73
	24	95

Source: Bitron and Aharonson 1978. Reprinted with permission; copyright 1978, American Industrial Hygiene Association.

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Schlagbauer and Henschler (1967) exposed groups of 10 female NMRI mice (weight 18-23 g) to various concentrations of bromine for 30 min in order to calculate an LC_{50}. Generation and measurement methods were the same as in a companion study that used human subjects (Rupp and Henschler 1967). The data showed a clear dose-response relationship (Table 1-4). The authors calculated a 30-min LC_{50} of 174 ppm. The 30-min LC_{01} was 116 ppm.

In a second experiment (Schlagbauer and Henschler 1967), mice were exposed to bromine at concentrations of 22 or 40 ppm for 3 or 6 h, and mortalities were determined after 10 days. No deaths occurred at 22 ppm for 3 h; mortality was 70% for the 6-h exposure. At a concentration of 40 ppm, deaths occurred following the 3-h (3/10) and 6-h (8/10) exposures. Again, it was observed that chlorine was more toxic than bromine (the 30-min LC_{50} value of bromine was approximately 1.5 times that of chlorine). The present study did not use a control group. In reviewing the data on chlorine and bromine, Withers and Lees (1986) noted that the chlorine 30-min LC_{50} value of Schlagbauer and Henschler (1967) is lower than values of other researchers.

3.2. Nonlethal Toxicity

Ivanov et al. (1976) reported on exposures of groups of eight rats (a total of 800 rats) to concentrations of bromine ranging from 0.12 to 77 ppm. The exposure time was 4 h. The authors quoted an earlier source, and no details of the exposure methods or vapor generation or analytic techniques were given. A concentration of 1.5 ppm decreased respiratory frequency by 19% (134 respirations/min in the exposed group compared with 165 respirations/min in the controls). "Olfactory sharpness," the ability to react to or detect other compounds, appeared to be decreased, and the number of "free cells" in the upper respiratory pathways was increased. The authors considered this concentration the threshold for irritation, which they defined as "$Lim_{ir.}$" Respiratory, cardiac, vascular, neural ("SPP" and reflexes), and endocrine system (dynamics of ^{131}I accumulation and release by the thyroid) effects developed at 50 mg/m^3 (7.7 ppm), and spermatogenesis was affected at 100 mg/m^3 (15 ppm). No further details were reported.

Ivanov et al. (1976) also reported on subchronic (4 month) exposures of rats at three concentrations: 1.9, 0.2, and 0.02 ppm. A 4-month exposure to bromine at 1.9 ppm produced effects on the respiratory, olfactory, and endocrine systems. Exposure at 0.2 ppm for 4 months led to less pronounced changes, which were reversible after a 1-month recovery period. A concentration of 0.02 ppm had no effect.

In a 28-day feeding study with rats, liquid bromine (38%) administered at 20 mg/kg/day induced clinical signs of salivation and decreased activity; increased red-blood-cell count, hemoglobin and packed cell volume (all reversible in 14 days); increased serum glucose; and increased urinary volume with protein (EPA 2005).

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TABLE 1-4 Mortality in Mice Exposed to Bromine at 111 to 315 ppm for 30 Min

Concentration (ppm)	Morality
111	0/10
40	3/10
199	6/10
236	9/10
252	10/10
268	9/10
290	10/10
315	10/10

Source: Schlagbauer and Henschler 1967. Reprinted with permission; copyright 1967, *International Archives of Occupational and Environmental Health*.

3.3. Developmental and Reproductive Effects

No data concerning developmental effects of bromine in animals were found in the available literature. Ivanov et al. (1976) reported that a 4-h exposure to bromine at 15 ppm affected spermatogenesis in male mice; further details were not reported.

3.4. Genotoxicity

Liquid bromine, tested at a concentration of 38.0% and a volume of 10 µg/plate, was positive in the *Salmonella typhimurium* microsome reverse mutation assay with strains TA 1537 and TA 100 in the absence of S9 and with strain TA 1537 in the presence of S9 activation. Bromine was cytotoxic for all strains with and without metabolic activation at more than 3,333 µg/plate (EPA 2002).

3.5. Chronic Toxicity and Carcinogenicity

No data concerning the chronic toxicity or carcinogenicity of bromine in animals were located in the available literature. Potassium bromate ($KBrO_3$) has been shown to be a renal carcinogen in rats. The mechanism of action has been attributed to the generation of oxygen radicals by $KBrO_3$, a strong oxidizer. KBr is not a carcinogen (Kurokawa et al. 1990).

3.6. Summary

Data on the acute inhalation toxicity of bromine are sparse. Most of the older experimental data using animal species are of questionable reliability.

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Only two studies provide experimental methods and details adequate for consideration in derivation of AEGLs. Bitron and Aharonson (1978) determined Lt_{50} values for one species, the mouse. The Lt_{50} at a concentration of 750 ppm was 9 min, and the Lt_{50} at a concentration of 240 ppm was 100 min. No deaths occurred or were predicted to occur during a 5-min exposure at 750 ppm or during a 20-min exposure at 240 ppm. The study of Schlagbauer and Henschler (1967) reported much lower lethal values for bromine as well as for chlorine. Their 30-min LC_{50} value for the mouse was 174 ppm. A concentration of 15 ppm for 4 h affected spermatogenesis in male mice (Ivanov et al. 1976). Limited data were located concerning genotoxicity, and no data concerning developmental effects or carcinogenicity were located in the available literature.

4. SPECIAL CONSIDERATIONS

4.1. Metabolism and Disposition

No data on the absorption, distribution, metabolism, or excretion of bromine following inhalation exposures in humans or animals were located in the available literature. Alexandrov (1983) stated that bromine may enter the body following inhalation, ingestion, or skin application, but the source of the data was not provided. Bromine gas reacts at the site of contact and metabolic and kinetic considerations are not relevant regarding the determination of AEGL values.

4.2. Mechanism of Toxicity

Bromine, a strong oxidizing agent, is a respiratory irritant and can cause pulmonary edema in humans and animals (Teitelbaum 2001). Reaction with water results in the formation of hypobromous acid, HOBr, which slowly decomposes to hydrobromic acid and oxygen (Downs and Adams 1973).

4.3. Structure-Activity Relationships

The irritating potential of mucus membrane irritants may be related to their water solubility. At 20-25ºC, the water solubility of bromine is 0.214 mol/L, whereas that of chlorine is 0.092 mol/L (Teitelbaum 2001). Water solubility determines the scrubbing capacity or penetration of a gas into the respiratory tract. On the basis of water solubility, bromine would react more intensely in the upper respiratory tract and thus be better scrubbed than chlorine. As a result of being well-scrubbed in the upper nasal passages, bromine may produce a feeling of irritation at a lower concentration than chlorine. Chlorine, on the other hand, would more readily penetrate to the lower respiratory tract, resulting in lethality at concentrations that are lower than those for bromine. This differ-

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ence in lethality is substantiated by the study of Rupp and Henschler (1967). Bitron and Aharonson (1978) observed similar results for lethality in studies with the mouse. The 30-min LC_{50} values for chlorine and bromine are listed in Table 1-5. The 30-min Lt_{50} (LC_{50}) values for Bitron and Aharonson (1978) were determined using the relationship between concentration and exposure time as explained in Section 7.2; values for the two concentration-exposure durations were averaged. Because of the 30-day postexposure observation period in this study, the Lt_{50} can be defined as an LC_{50}. For comparison purposes, the 30-min LC_{50} value for fluorine in the mouse is 225 ppm (Keplinger and Suissa 1968).

Bromine is more water soluble than chlorine (Teitelbaum 2001) and would be scrubbed to a greater extent in the nasal passages than chlorine; relatively smaller amounts would reach the lungs. Because of its reactive potential and decomposition in the presence of water (Teitelbaum 2001), the solubility of fluorine has not been defined. In the respiratory tract of the rat, the relative inhalation toxicities of the hydrohalous acids formed is HF > HCl ≥ HBr (Kusewitt et al. 1989).

4.4. Other Relevant Information

4.4.1. Species Differences

No relatively recent data sufficient for comparing differences in species sensitivity for either irritation or lethality were located in the available literature. In the older data, no differences were found in lethality values in two separate exposures between the cat and guinea pig and guinea pig and rabbit (Lehmann 1887). For the halogens fluorine and chlorine, the mouse is the most sensitive tested species (NRC 2004, 2010).

4.4.2. Susceptible Populations

Individuals with asthma or other respiratory diseases may be more susceptible to the effects of respiratory irritants than healthy individuals. No data on bromine and the asthmatic population were located.

TABLE 1-5 Relative Toxicities of Chlorine and Bromine to the Mouse

Chemical	30-Min LC_{50}	Reference
Chlorine	203	Bitron and Aharonson 1978
	127	Schlagbauer and Henschler 1967
Bromine	424	Bitron and Aharonson 1978
	174	Schlagbauer and Henschler 1967

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4.4.3. Concentration-Exposure Duration Relationship

ten Berge et al. (1986) used the two Lt_{50} data points from the study by Bitron and Aharonson (1978) to determine the concentration and exposure duration relationship of $C^{2.2} \times t = k$, where C is concentration, t is time, and k is a constant.

5. DATA ANALYSIS FOR AEGL-1

5.1. Summary of Human Data Relevant to AEGL-1

Many of the data on bromine are old, unreferenced, anecdotal, or conflicting (Elkins 1959; Rupp and Henschler 1967; Alexandrov 1983; Ruth 1986; HSDB 2008). Rupp and Henschler (1967) reported that eye irritation occurred at 0.1 ppm. Current monitoring data from the Occupational Safety and Health Administration (OSHA) show that workers are exposed to concentrations up to 0.18 ppm, presumably without irritation. Ruth (1986) reported that the irritation threshold (undefined) was 0.3 ppm; workers exposed to 0.3 to 0.6 ppm for 1 year suffered various symptoms (Alexandrov 1983). Except for the study of Rupp and Henschler (1967), all of these values are either poorly documented or appear unusually low compared with more recent data for other halogens. The only clinical study, Rupp and Henschler (1967), reported eye irritation, but no nose or throat irritation, at a concentration of 0.1 ppm for 30 min. Concentrations of 0.5 to 0.9 ppm were irritating to the conjunctiva, nose, and throat. The intensity of the irritation was not well-described, and their values in related experiments (chlorine) are low in comparison to other researchers.

The more-robust database for chlorine rather than bromine can also be considered when addressing the irritation potential of halogens. In four clinical studies, some with atopic or asthmatic individuals, exposure to chlorine at 0.4 or 0.5 ppm for various periods of time did not produce airway hyper-reactivity or an asthmatic response (Anglen 1981; Rotman et al. 1983; D'Alessandro et al. 1996; Shusterman et al. 1998). Because sensitive individuals were tested, an intraspecies uncertainty factor of 1 was applied to derive an AEGL-1 value for chlorine (NRC 2004).

5.2. Summary of Animal Data Relevant to AEGL-1

No useful data were available. Ivanov et al. (1976) reported that the threshold of irritation for bromine for rats was 1.5 ppm during a 4-h exposure, but they quoted an earlier source and provided few experimental details.

5.3. Derivation of AEGL-1

The AEGL-1 was based on exposures of 20 healthy human subjects to

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concentrations of 0.1 to 0.9 ppm for up to 60 min (Rupp and Henschler 1967). Eye irritation, but no nose or throat irritation, occurred during a 30-min exposure at 0.1 ppm. At concentrations ≥0.5 ppm, there was a stinging and burning sensation of the conjunctiva. The 30-min 0.1 ppm concentration, which caused mild irritation, was divided by an intraspecies uncertainty factor of 3 to protect susceptible individuals. This adjustment was considered appropriate for acute exposure to chemicals in which the mechanism of action involves surface contact irritation of ocular or respiratory tract tissue or both rather than systemic activity following absorption and distribution of the parent chemical or a biotransformation product to a target tissue (NRC 2001). An intraspecies uncertainty factor of 3 was also considered sufficient because workers have been occupationally exposed to bromine at 1 ppm with no other symptoms than "excess irritation" (Elkins 1959). The resulting 0.033 ppm concentration is 30-fold lower than the 1 ppm concentration that induced excess irritation in healthy workers. Effects at this low concentration appear to be limited to the eyes and upper respiratory tract; there is likely to be little penetration to the lower respiratory tract. Compared with the AEGL-1 value of 0.5 ppm for chlorine, a chemical that more readily penetrates to the lower respiratory tract, the uncertainty factor of 3 is appropriate. The 0.5-ppm concentration of chlorine, with no uncertainty factor applied, was considered protective of the tested atopic and asthmatic individuals. The 30-min AEGL-1 value of 0.033 ppm for bromine was used across all exposure durations because adaptation occurs to mild sensory irritation (Table 1-6). Calculations for AEGL values are in Appendix A. Appendix B is a graph of the toxicity data in relationship to the AEGL values.

The proposed values are far below the 0.3 ppm threshold for irritation in humans reported by Ruth (1986). The values are 27- to 160-fold below the threshold for irritation in rats, as reported by Ivanov et al. (1976), which was 1.5 ppm for 4-h.

6. DATA ANALYSIS FOR AEGL-2

6.1. Summary of Human Data Relevant to AEGL-2

Elkins (1959) quoted 1 ppm as excessively irritating in workers, but these were chronic exposures. Alexandrov (1983) reported severe choking at 1.7-3.5 ppm, but his values were not documented. The healthy subjects in the study of Rupp and Henschler (1967) reported prickling or stinging of the eyes and nose and throat irritation at concentrations of 0.5 to 0.9 ppm (1.0 ppm for 30 min in the odor intensity study). These sensations were reported following exposures of greater than 30 min. The intensity of the symptoms was not well described.

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TABLE 1-6 AEGL-1 Values for Bromine

10 min	30 min	1 h	4 h	8 h
0.033 ppm ($0.22 mg/m^3$)	0.033 ppm ($0.22 mg/m^3$)	0.033 ppm ($0.22 mg/m^3$)	0.033 ppm ($0.22 mg/m^3$)	0.033 ppm ($0.22 mg/m^3$)

6.2. Summary of Animal Data Relevant to AEGL-2

Ivanov et al. (1976) reported that the threshold for acute effects for a 4-h exposure of rats to bromine was 7.7 ppm, but they quoted an earlier source and provided few details.

6.3. Derivation of AEGL-2

The AEGL-2 was based on the exposure to bromine of 1 ppm (rounded from 0.9 ppm in the odor intensity study) for 30 min, which the subjects in the Rupp and Henschler (1967) study found irritating (stinging and burning sensation of the conjunctiva and nose and throat irritation). The 30-min 1 ppm value was divided by an intraspecies uncertainty factor of 3 to protect susceptible individuals and time-scaled to the other AEGL-2 exposure durations using the concentration-exposure duration relationship of $C^{2.2} \times t = k$ from the mouse lethality study. An intraspecies uncertainty factor of 3 was considered sufficient, as the symptoms may be below those defining an AEGL-2. Furthermore, compared with the 30-min AEGL-2 value of 2.8 ppm for chlorine, this value may be conservative. The 30-min value for the less well-scrubbed chlorine was based on transient changes in pulmonary parameters (without respiratory symptoms) in asthmatic and atopic individuals (Rotman et al. 1983; D'Alessandro et al. 1996). No reliable studies with exposures to higher concentrations were located. The calculated values appear in Table 1-7 and calculations are contained in Appendix A. Appendix B is a category graph of the toxicity data in relation to AEGL values.

7. DATA ANALYSIS FOR AEGL-3

7.1. Summary of Human Data Relevant to AEGL-3

No reliable human data relevant to derivation of an AEGL-3 values were located in the available literature.

TABLE 1-7 AEGL-2 Values for Bromine

10 min	30 min	1 h	4 h	8 h
0.55 ppm ($3.6 mg/m^3$)	0.33 ppm ($2.2 mg/m^3$)	0.24 ppm ($1.6 mg/m^3$)	0.13 ppm ($0.85 mg/m^3$)	0.095 ppm ($0.62 mg/m^3$)

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7.2. Summary of Animal Data Relevant to AEGL-3

Two studies using the mouse provided data on lethality. Both studies provided lethality data on bromine and chlorine. Both studies reported lower LC_{50} values for chlorine than those reported for bromine in more recent well-conducted studies. Bitron and Aharonson (1978) calculated Lt_{50} values for bromine at a concentration of 750 ppm for 9 min in the mouse and at a concentration of 240 ppm for 100 min. The 240 and 750 ppm concentrations can be considered LC_{50} values for the tested times. Using the $C^{2.2} \times t = k$ relationship, these two values can be used to calculate 30-min LC_{50} values. The respective 30-min LC_{50} values are 415 and 434 ppm (average, 424 ppm). No deaths were calculated to occur at 750 ppm for 5 min or 240 ppm for 20 min.

The study by Schlagbauer and Henschler (1967) provided a lower LC_{50} for bromine for the mouse. The lethality data in their study showed a good concentration-response relationship and the exposure time was held constant at 30 min. Using probit analysis, a 30-min LC_{01} of 116 ppm was calculated.

7.3. Derivation of AEGL-3

Both lethality studies with the mouse described the inhalation toxicity of chlorine and bromine. However, both studies reported lower LC_{50} values for chlorine than those reported for bromine in more recent well-conducted studies. Nevertheless, the study that reported the lower lethal concentrations for chlorine was used for derivation of the AEGL-3 values for bromine (Schlagbauer and Henschler 1967). The data in this study showed a clear concentration-response relationship; furthermore, the exposure duration was longer than it was in the Bitron and Aharonson (1978) study. Using probit analysis, a 30-min LC_{50} value of 204 ppm and a 30-min LC_{01} of 116 ppm were calculated. The 30-min LC_{01} of 116 ppm was used as the basis for calculation of AEGL-3 values for bromine. The 116 ppm LC_{01} was divided by a combined uncertainty factor of 10 (3 for interspecies differences [the mouse was the most sensitive species for lethal effects in tests with other halogens] and 3 for intraspecies differences [at high concentrations, bromine is corrosive to the mucous membranes of the respiratory system; effects are not expected to differ greatly among individuals] [NRC 2001]) and scaled across time using the relationship of $C^{2.2} \times t = k$, which was derived from the Bitron and Aharonson (1978) study. Calculations are provided in Appendix A, and values appear in Table 1-8 below. Appendix B is a category graph of the toxicity data in relation to AEGL values.

The calculated AEGL-3 values for bromine are below those of chlorine (NRC 2004) and fluorine (NRC 2010) (Table 1-9). This result indicates that the values for bromine are protective, as chlorine is 1.5 times more toxic than bromine based on lethality in the mouse. As noted, the database for chlorine is extensive. In addition, the mice used by Bitron and Aharonson (1978) were young

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and were restrained in glass enclosures during the exposures. Both of these factors increase the sensitivity of the tested species to chemical toxicity.

8. SUMMARY OF AEGLs

8.1. AEGL Values and Toxicity End Points

The AEGLs for bromine were derived in the following manner. The AEGL-1 and AEGL-2 values were based on a study with 20 human subjects who were exposed to concentrations of 0.1 to 0.9-1.0 ppm (Rupp and Henschler 1967). Eye irritation noted within a 30- min exposure at 0.1 ppm was used as the basis for the AEGL-1. At 0.5 to 0.9 ppm (1.0 ppm), the nose and throat were also irritated. A 30-min exposure at 1 ppm was used as the basis for the AEGL-2 values. An intraspecies uncertainty factor of 3 was considered sufficient because irritation was confined to the eyes during the 0.1-ppm exposure (precluding an asthmatic response), and workers have been occupationally exposed at 1 ppm with no reported symptoms other than "excess irritation." The AEGL-1 values were not time-scaled, as adaptation to mild sensory irritation occurs. Time-scaling for the AEGL-2 was based on a lethality study with the mouse (Bitron and Aharonson 1978).

TABLE 1-8 AEGL-3 Values for Bromine

10 min	30 min	1 h	4 h	8 h
19 ppm ($124 mg/m^3$)	12 ppm ($78 mg/m^3$)	8.5 ppm ($55 mg/m^3$)	4.5 ppm ($29 mg/m^3$)	3.3 ppm ($21 mg/m^3$)

TABLE 1-9 Comparison of AEGL Values for Fluorine, Chlorine, and Bromine

Classification	Exposure Duration				
	10 min	30 min	1 h	4 h	8 h
AEGL-1					
Fluorine	1.7 ppm	1.7 ppm	1.7 ppm	1.7 ppm	1.7 ppm
Chlorine	0.5 ppm	0.5 ppm	0.5 ppm	0.5 ppm	0.5 ppm
Bromine	0.033 ppm	0.033 ppm	0.033 ppm	0.033 ppm	0.033 ppm
AEGL-2					
Fluorine	20 ppm	11 ppm	5.0 ppm	2.3 ppm	2.3 ppm
Chlorine	2.8 ppm	2.8 ppm	2.0 ppm	1.0 ppm	0.71 ppm
Bromine	0.55 ppm	0.33 ppm	0.24 ppm	0.13 ppm	0.095 ppm
AEGL-3					
Fluorine	36 ppm	19 ppm	13 ppm	5.7 ppm	5.7 ppm
Chlorine	50 ppm	28 ppm	20 ppm	10 ppm	7.1 ppm
Bromine	19 ppm	12 ppm	8.5 ppm	4.5 ppm	3.3 ppm

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The AEGL-3 values for bromine were based on a study using the mouse (Schlagbauer and Henschler 1967). Thirty-minute exposures to several concentrations were tested. The data showed a clear concentration-response relationship from which LC_{50} and LC_{01} values could be calculated. The 30-min LC_{01} of 116 ppm, derived by probit analysis, was divided by a total uncertainty factor of 10 (3 each for interspecies and intraspecies differences, as the mouse is the most sensitive species in studies with halogens, and at high concentrations, bromine is corrosive to the respiratory tissues, and effects are not expected to differ greatly among species or between individuals) and scaled to the other exposure times using $C^{2.2} \times t = k$. The resulting values are lower than those for chlorine, which is known to be more toxic than bromine.

The AEGL values for three levels and five exposure periods are summarized in Table 1-10. Data and derivations are summarized in Appendix C.

8.2. Comparisons with Other Standards and Guidelines

Standards and guidance levels for workplace and community exposures are listed in Table 1-11. The 1-h AEGL-1 and AEGL-2 values are below the respective emergency response planning guideline (ERPG) values. The ERPG values were based on an unobjectionable odor, 0.1 ppm (Rupp and Henschler 1967), and mild and transient health effects at slightly higher concentrations (Rupp and Henschler 1967; Morabia et al. 1986; D. Henschler, Institut for Toxicology, Wurzburg, Germany, personal commun., Dec. 21, 1999). The ERPG-2 was based on the same studies that reported that exposure to concentrations above 0.5 ppm caused coughing, dizziness, and intense irritation to the eyes, nose, and throat. The 1-h ERGP-3 was based on the mouse lethality studies of Bitron and Aharonson (1978) and Schlagbauer and Henschler (1967) and on the concentration- time relationship of Withers and Lees (1986). The 1-h AEGL-3 and ERPG-3 values are similar. The same references were used for derivation of all AEGL values.

TABLE 1-10 Summary of AEGL Values for Bromine

Classification	Exposure Duration				
	10 min	30 min	1 h	4 h	8 h
AEGL-1 (Nondisabling)	0.033 ppm (0.22 mg/m^3)	0.033 ppm (0.22 mg/m^3)	0.033 ppm (0.22 mg/m^3)	0.033 ppm (0.22 mg/m^3)	0.033 ppm (0.22 mg/m^3)
AEGL-2 (Disabling)	0.55 ppm (3.6 mg/m^3)	0.33 ppm (2.2 mg/m^3)	0.24 ppm (1.6 mg/m^3)	0.13 ppm (0.85 mg/m^3)	0.095 ppm (0.62 mg/m^3)
AEGL-3 (Lethal)	19 ppm (124 mg/m^3)	12 ppm (78 mg/m^3)	8.5 ppm (55 mg/m^3)	4.5 ppm (29 mg/m^3)	3.3 ppm (21 mg/m^3)

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The immediately dangerous to life and health (IDLH) value is based on several reviews and specifically cites the data of Flury and Zernik (1931) and Henderson and Haggard (1943). Full-day workplace standards are all 0.1 ppm, with short-term allowable exposures at 0.2 and 0.3 ppm. The 8-h AEGL-1 and AEGL-2 values are below workplace standards.

TABLE 1-11 Extant Standards and Guidelines for Bromine

Guideline	Exposure Duration				
	10 min	30 min	1 h	4 h	8 h
AEGL-1	0.033 ppm	0.033 ppm	0.033 ppm	0.033 ppm	0.033 ppm
AEGL-2	0.55 ppm	0.33 ppm	0.24 ppm	0.13 ppm	0.095 ppm
AEGL-3	19 ppm	12 ppm	8.5 ppm	4.5 ppm	3.3 ppm
ERPG-1 (AIHA)[a]			0.1 ppm		
ERPG-2 (AIHA)			0.5 ppm		
ERPG-3 (AIHA)			5 ppm		
IDLH (NIOSH)[b]		3 ppm			
REL-TWA (NIOSH)[c]					0.1 ppm
PEL-TWA (OSHA)[d]					0.1 ppm
TLV-TWA (ACGIH)[e]					0.1 ppm
REL-STEL (NIOSH)[f]					0.3 ppm
TLV-STEL (ACGIH)[g]					0.2 ppm
MAK (Germany)[h]					Withdrawn
MAC (The Netherlands)[i]					0.1 ppm

[a]ERPG (emergency response planning guidelines, American Industrial Hygiene Association (AIHA 2001): The ERPG-1 is the maximum airborne concentration below which it is believed nearly all individuals could be exposed for up to 1 h without experiencing other than mild, transient adverse health effects or without perceiving a clearly defined objectionable odor. The ERPG-2 is the maximum airborne concentration below which it is believed nearly all individuals could be exposed for up to 1 h without experiencing or developing irreversible or other serious health effects or symptoms that could impair an individual's ability to take protective action. The ERPG-3 is the maximum airborne concentration below which it is believed nearly all individuals could be exposed for up to 1 h without experiencing or developing life-threatening health effects.
[b]IDLH (immediately dangerous to life or health, National Institute for Occupational Safety and Health) (NIOSH 1996) represents the maximum concentration from which

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escape within 30 min would be possible without any escape-impairing symptoms or any irreversible health effects.
[c]REL-TWA (recommended exposure limits–time-weighted average, National Institute for Occupational Safety and Health) (NIOSH 2005) is analogous to the ACGIH TLV-TWA.
[d]PEL-TWA (permissible exposure limits–time-weighted average, Occupational Safety and Health Administration, OSHA) (29 CFR 1910.1000 [2003]) is analogous to the ACGIH TLV-TWA but is for exposures of no more than 10 h/d, 40 h/wk.
[e]TLV-TWA (Threshold Limit Value–time-weighted average, American Conference of Governmental Industrial Hygienists) (ACGIH 1996) is the time-weighted average concentration for a normal 8-h workday and a 40-h workweek to which nearly all workers may be repeatedly exposed, day after day, without adverse effect.
[f]REL-STEL (recommended exposure limits–short-term exposure limit, National Institute for Occupational Safety and Health) (NIOSH 2005) is analogous to the ACGIH TLV-TWA.
[g]TLV-STEL (Threshold Limit Value–short-term exposure limit, American Conference of Governmental Industrial Hygienists) (ACGIH 1996) is defined as a 15-min TWA exposure that should not be exceeded at any time during the workday even if the 8-h TWA is within the TLV-TWA. Exposures above the TLV-TWA up to the STEL should not be longer than 15 min and should not occur more than four times per day. There should be at least 60 min between successive exposures in this range.
[h]MAK (maximale argeitsplatzkonzentration [maximum workplace concentration], Deutsche Forschungsgemeinschaft [German Research Association]) (DFG 2007) is analogous to the ACGIH TLV-TWA. The MAK for bromine was withdrawn in 2007, and bromine was placed in category IIB, substances for which no MAK value can be established at present.
[i]MAC (maximaal aanvaarde concentratie [maximum accepted concentration], SDU Uitgevers [under the auspices of the Ministry of Social Affairs and Employment, The Hague, The Netherlands]) is analogous to the ACGIH TLV-TWA (MSZW 2004).

8.3. Data Adequacy and Research Needs

The data on the toxic effects of bromine are sparse. Because of the sparse-data, lower values were chosen for the AEGL-1 and AEGL-2 than might have been used in the presence of extensive data. No recent reliable human studies were available. Some of the studies that are quoted and requoted in toxicology books were performed as early as the 1880s; vapor generation and analytic techniques have improved since that time. The clinical study by Rupp and Henschler (1967) tested both bromine and chlorine and reported irritant values for chlorine that are lower than those in other studies. The values for bromine may be correspondingly low. Two lethality studies with the mouse as the test species were available for calculation of the AEGL-3 values. Both the key study, Schlagbauer and Henschler (1967), and the study by Bitron and Aharonson (1978) were noted to have lower lethality values for chlorine than those of many other investigators, and their values for bromine may be correspondingly low. Although bromine is less toxic than chlorine, the interim AEGL-3 values for bromine are less than those for chlorine (NRC 2004).

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In the absence of reliable studies that address the end point of irritation, a study to determine the exposure concentration producing a 50% decrease in the respiratory rate (RD_{50}) in the mouse would be of value, particularly as it would confirm the irritation potential of bromine relative to that of chlorine.

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APPENDIX A

DERIVATION OF AEGL VALUES FOR BROMINE

Derivation of AEGL-1

Key study: Rupp and Henschler 1967

Toxicity end point: Eye irritation in humans at 0.1 ppm for 30 min

Uncertainty factors: 3 for intraspecies variability

Time-scaling: Not applied; adaptation to mild sensory irritation

Modifying factor: None

Calculation: 0.1 ppm/3 = 0.033 ppm

Derivation of AEGL-2

Key study: Rupp and Henschler 1967

Toxicity end point: Eye, nose, and throat irritation in humans at 1.0 ppm for 30 min

Time-scaling: $C^{2.2} \times t = k$, based on mouse lethality study (Bitron and Aharonson 1978)

Uncertainty factors: 3 for intraspecies variability

Modifying factor: None

Calculations: $(\text{Concentration/uncertainty factors})^{2.2} \times t = k$
$(1 \text{ ppm}/3)^{2.2} \times 30 \text{ min} = k$
$2.676 \text{ ppm}^{2.2} \times \text{min} = k$

10-min AEGL-2: $(2.676 \text{ ppm}^{2.2} \times \text{min}/10 \text{ min})^{1/2.2} = 0.55$ ppm

30-min AEGL-2: 0.33 ppm

1-h AEGL-2: $(2.676 \text{ ppm}^{2.2} \times \text{min}/60 \text{ min})^{1/2.2} = 0.24$ ppm

4-h AEGL-2: $(2.676 \text{ ppm}^{2.2} \times \text{min}/240 \text{ min})^{1/2.2} = 0.13$ ppm

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8-h AEGL-2: $(2.676\ \text{ppm}^{2.2} \times \text{min}/480\ \text{min})^{1/2.2} = 0.095\ \text{ppm}$

Derivation of AEGL-3

Key study: Schlagbauer and Henschler 1967

Toxicity end point: 30-min LC_{01} of 116 ppm in the mouse, calculated by probit analysis

Time-scaling: $C^{2.2} \times t = k$, based on mouse lethality study (Bitron and Aharonson 1978)

Uncertainty factors: 3 for intraspecies variability
3 for interspecies variability

Modifying factor: None

Calculations: $(\text{Concentration/uncertainty factors})^{2.2} \times t = k$
$(116\ \text{ppm}/10)^{2.2} \times 30\ \text{min} = k$
$6{,}590.66\ \text{ppm}^{2.2} \times \text{min} = k$

10-min AEGL-3: $(6{,}590.66\ \text{ppm}^{2.2} \times \text{min}/10\ \text{min})^{1/2.2} = 19\ \text{ppm}$

30-min AEGL-3: 116/10 = 12 ppm

1-h AEGL-3: $(6{,}590.66\ \text{ppm}^{2.2} \times \text{min}/60\ \text{min})^{1/2.2} = 8.5\ \text{ppm}$

4-h AEGL-3: $(6{,}590.66\ \text{ppm}^{2.2} \times \text{min}/240\ \text{min})^{1/2.2} = 4.5\ \text{ppm}$

8-h AEGL-3: $(6{,}590.66\ \text{ppm}^{2.2} \times \text{min}/480\ \text{min})^{1/2.2} = 3.3\ \text{ppm}$

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APPENDIX B

CATEGORY GRAPH OF TOXICITY DATA AND AEGL VALUES

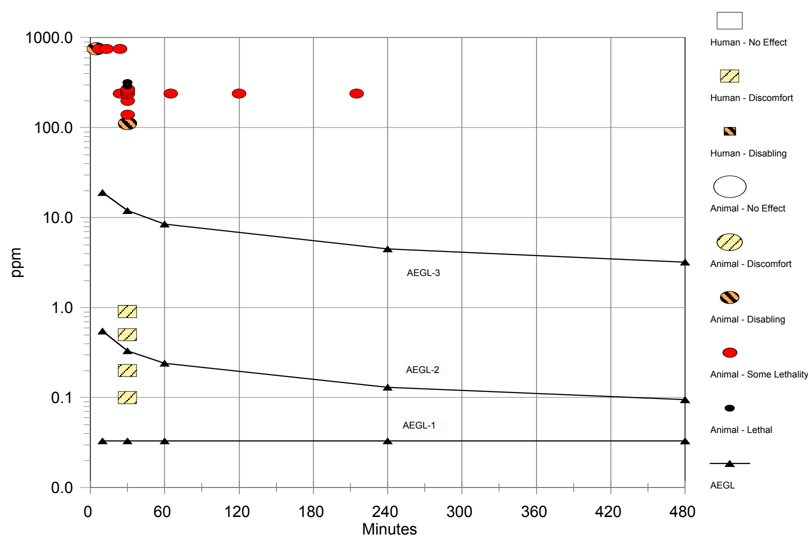

FIGURE B-1 Category graph for bromine.

TABLE B-1 Data Used in Category Graph

Source	Species	ppm	Min	Category[a]
AEGL-1		0.033	10	AEGL
AEGL-1		0.033	30	AEGL
AEGL-1		0.033	60	AEGL
AEGL-1		0.033	240	AEGL
AEGL-1		0.033	480	AEGL
AEGL-2		0.55	10	AEGL
AEGL-2		0.33	30	AEGL
AEGL-2		0.24	60	AEGL
AEGL-2		0.13	240	AEGL
AEGL-2		0.095	480	AEGL

(Continued)

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TABLE B-1 Continued

Source	Species	ppm	Min	Category[a]
AEGL-3		19	10	AEGL
AEGL-3		12	30	AEGL
AEGL-3		8.5	60	AEGL
AEGL-3		4.5	240	AEGL
AEGL-3		3.3	480	AEGL
Bitron and Aharonson 1978	Mouse	240	24	SL
	Mouse	240	65	SL
	Mouse	240	120	SL
	Mouse	240	215	SL
	Mouse	750	5	2
	Mouse	750	7	SL
	Mouse	750	13	SL
	Mouse	750	24	SL
Schlagbauer and Henschler 1967	Mouse	111	30	2
	Mouse	140	30	SL
	Mouse	199	30	SL
	Mouse	236	30	SL
	Mouse	252	30	SL
	Mouse	268	30	SL
	Mouse	290	30	3
	Mouse	315	30	3
Rupp and Henschler 1967	Human	0.1	30	1
	Human	0.2	30	1
	Human	0.5	30	1
	Human	0.9	30	1

[a]Category 0, no effect; 1, discomfort; 2, disabling, 3, lethal; SL, some lethality.

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APPENDIX C

ACUTE EXPOSURE GUIDELINE LEVELS FOR BROMINE

Derivation Summary for Bromine

AEGL-1 VALUES

10 min	30 min	1 h	4 h	8 h
0.033 ppm	0.033 ppm	0.033 ppm	0.033 ppm	0.033 ppm

Key Reference: Rupp, H., and D. Henschler. 1967. Effects of low concentrations of chlorine and bromine on man [in German]. Int. Arch. Arbeitsmed. 23(1):79-90.

Test Species/Strain/Number: 20 human subjects

Exposure Route/Concentrations/Durations: Inhalation, concentrations of 0.1 to 1.0 ppm for at least 30 min

Effects:
0.1 ppm: eye irritation
0.50 to 1.0 ppm: eye, nose, and throat irritation

End Point/Concentration/Rationale: Eye irritation but not nose or throat irritation at 0.1 ppm for 30 min; meets the AEGL-1 definition of notable discomfort.

Uncertainty Factors/Rationale:
Total uncertainty factor: 3
Interspecies: Not applied, human data used
Intraspecies: 3, Workers have been exposed to concentrations up to 1 ppm with irritation being the only reported symptom. Compared with the 0.5 ppm AEGL-1 for the less well-scrubbed chlorine, the value may be conservative. Chlorine at 0.5 ppm for 4 h failed to elicit an asthmatic response in sensitive subjects.

Modifying Factor: Not applied

Animal to Human Dosimetric Adjustment: Not applied.

Time-Scaling: Not applied, adaptation to mild sensory irritation.

Data Adequacy: Compared with the irritancy data on chlorine, these values may be conservative. Based on the small database for bromine, extra protectiveness was considered appropriate.

AEGL-2 VALUES

10 min	30 min	1 h	4 h	8 h
0.55 ppm	0.33 ppm	0.24 ppm	0.13 ppm	0.095 ppm

Key Reference: Rupp, H., and D. Henschler. 1967. Effects of low concentrations of chlorine and bromine on man [in German]. Int. Arch. Arbeitsmed. 23(1):79-90.

Test Species/Strain/Number: 20 human subjects

Exposure Route/Concentrations/Durations: Inhalation, concentrations of 0.1 to 1.0 ppm for at least 30 min

(Continued)

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AEGL-2 VALUES Continued

10 min	30 min	1 h	4 h	8 h
0.55 ppm	0.33 ppm	0.24 ppm	0.13 ppm	0.095 ppm

Effects:
0.1 ppm: eye irritation
0.5 to 1.0 ppm: eye, nose, and throat irritation

End Point/Concentration/Rationale: Throat irritation at the 1.0 ppm concentration.

Uncertainty Factors/Rationale:
Total uncertainty factor: 3
Interspecies: Not applied, human data used.
Intraspecies: 3. Symptoms are below those defining an AEGL-2, but no reliable studies with exposures to higher concentrations were located. Irritation appeared to be limited to the upper respiratory tract with likely little penetration to the lower respiratory tract. Compared with the 30-min AEGL-2 value of 2.8 ppm for chlorine (which was protective of sensitive subjects) the uncertainty factor of 3 is adequate.

Modifying Factor: Not applied.

Animal to Human Dosimetric Adjustment: Not applied.

Time-scaling: $C^{2.2} \times t = k$, based on a mouse lethality study.

Data Adequacy: Compared with the irritancy data on chlorine, these values may be conservative. But, based on the limited data base for bromine, extra protectiveness was considered appropriate.

AEGL-3 VALUES

10 min	30 min	1 h	4 h	8 h
19 ppm	12 ppm	8.5 ppm	4.5 ppm	3.3 ppm

Key Reference: Schlagbauer, M., and D. Henschler. 1967. Inhalation toxicity of chlorine and bromine with single and repeated exposures [in German]. Int. Arch. Arbetsmed. 23(1):91-98.

Test Species/Strain/Number: Mouse/NMRI/10 per exposure group

Exposure Route/Concentrations/Durations: Inhalation, 110.5 to 315 ppm for 30 min

Concentration:	Mortality:
110.5 ppm:	0/10
139.7 ppm:	3/10
198.9 ppm:	6/10
236.0 ppm:	9/10
252.1 ppm:	10/10
267.6 ppm:	9/10
290.3 ppm:	10/10
315.0 ppm	10/10

End Point/Concentration/Rationale: LC_{01} calculated by probit analysis

(Continued)

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AEGL-3 VALUES Continued

10 min	30 min	1 h	4 h	8 h
19 ppm	12 ppm	8.5 ppm	4.5 ppm	3.3 ppm
Uncertainty Factors/Rationale: Total uncertainty factor: 10 Interspecies: 3; the mouse was the most sensitive species in other studies with halogens Intraspecies: 3; at high concentrations, the corrosive action of irritants is not expected to differ greatly among individuals.				
Modifying Factor: Not applied.				
Animal to Human Dosimetric Adjustment: Not applied.				
Time-scaling: $C^{2.2} \times t = k$, based on a mouse lethality study.				
Data Adequacy: Compared with the lethality data on chlorine, these values may be conservative, but based on the small database for bromine, extra protectiveness was considered appropriate.				

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2

Ethylene Oxide[1]

Acute Exposure Guideline Levels

PREFACE

Under the authority of the Federal Advisory Committee Act (FACA) P.L. 92-463 of 1972, the National Advisory Committee for Acute Exposure Guideline Levels for Hazardous Substances (NAC/AEGL Committee) has been established to identify, review and interpret relevant toxicologic and other scientific data and develop AEGLs for high-priority, acutely toxic chemicals.

AEGLs represent threshold exposure limits for the general public and are applicable to emergency exposure periods ranging from 10 min to 8 h. AEGL-2 and AEGL-3, and AEGL-1 levels, as appropriate, will be developed for each of five exposure periods (10 and 30 min and 1, 4, and 8 h) and will be distinguished by varying degrees of severity of toxic effects. It is believed that the recommended exposure levels are applicable to the general population, including infants and children and other individuals who may be susceptible. The three AEGLs have been defined as follows:

AEGL-1 is the airborne concentration (expressed as parts per million [ppm] or milligrams per cubic meter [mg/m^3]) of a substance above which it is predicted that the general population, including susceptible individuals, could

[1]This document was prepared by the AEGL Development Team composed of Kowetha Davidson (Oak Ridge National Laboratory) and Chemical Managers Susan Ripple and Kyle Blackman (National Advisory Committee [NAC] on Acute Exposure Guideline Levels for Hazardous Substances). The NAC reviewed and revised the document and AEGLs as deemed necessary. Both the document and the AEGL values were then reviewed by the National Research Council (NRC) Committee on Acute Exposure Guideline Levels. The NRC committee has concluded that the AEGLs developed in this document are scientifically valid conclusions based on the data reviewed by the NRC and are consistent with the NRC guideline reports (NRC 1993, 2001).

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Acute Exposure Guideline Levels for Selected Airborne Chemicals: Volume 9

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 (expressed as ppm or mg/m^3) 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.

AEGL-3 is the airborne concentration (expressed as ppm or mg/m^3) of a substance above which it is predicted that the general population, including susceptible individuals, could experience life-threatening health effects or death.

Airborne concentrations below the AEGL-1 represent exposure levels that could produce mild and progressively increasing but transient and nondisabling odor, taste, and sensory irritation or certain asymptomatic, nonsensory effects. With increasing airborne concentrations above each AEGL, there is a progressive increase in the likelihood of occurrence and the severity of effects described for each corresponding AEGL. Although the AEGLs represent threshold levels for the general public, including susceptible subpopulations, such as infants, children, the elderly, persons with asthma, and those with other illnesses, it is recognized that individuals, subject to idiosyncratic responses, could experience the effects described at concentrations below the corresponding AEGL.

SUMMARY

Ethylene oxide is a highly flammable gas produced in very large quantities in the United States (5.3 to 6.3 billion pounds). It is very reactive with nucleophilic substances such as water, alcohols, halides, amines, and sulfhydryl compounds. Ethylene oxide is used as an intermediate in the production of ethylene glycol and nonionic surfactants; a small amount is used as a fumigant for sterilizing foods and heat-sensitive medical equipment.

The database on the toxicity of ethylene oxide vapor in humans and experimental animals is extensive, including data on all aspects of toxicity except lethality in humans. Pharmacokinetics data show that ethylene oxide is readily absorbed from the respiratory tract in humans and other animals. Ethylene oxide alkylates proteins and DNA, and it is metabolized primarily by nonenzymatic hydrolysis, enzymatic hydrolysis, and glutathione conjugation.

The odor detection threshold for ethylene oxide was reported to be 260 ppm by one investigator and 700 ppm by another. In humans, ethylene oxide vapors affect the eyes, respiratory tract, central and peripheral nervous systems, gastrointestinal tract (probably secondary effects to nervous system toxicity), hematopoietic system, and possibly the reproductive system and fetus. Acute exposure to ethylene oxide at the odor detection level (≥260 ppm) causes eye and upper respiratory tract irritation and signs and symptoms of effects on the central and peripheral nervous systems. Acute exposure to a calculated concen-

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tration of at least 500 ppm for 2 to 3 min caused hematologic effects and more severe effects on the central nervous system than those noted at the odor detection level. Most effects observed after acute exposure are reversible, including effects on the nervous system. Repeated exposures exacerbate peripheral nerve damage. Human studies have provided evidence suggestive of reproductive toxicity, some evidence of an association between exposure to ethylene oxide and genetic damage to somatic cells, and limited evidence of carcinogenicity.

Acute lethality studies in experimental animals showed that mice are the most sensitive species (4-h LC_{50} [concentration with 50% lethality] = 660 to 835 ppm), followed by the dog (4-h LC_{50} = 960 ppm) and rat (4-h LC_{50} = 1,537 to 1,972 ppm; 1-h LC_{50} = 4,439 to 5,748 ppm). Immediate deaths were likely due to respiratory failure and delayed deaths were due to secondary respiratory infections. Experimental animals exposed to lethal and nonlethal concentrations of ethylene oxide showed evidence of eye and respiratory tract irritation and effects on the central and peripheral nervous systems. Additional studies in animals exposed to ethylene oxide for up to 6 h/day provided evidence of reproductive toxicity (subchronic exposure), developmental toxicity, neurotoxicity, genetic toxicity in germ cells, and carcinogenicity.

Data were available for deriving AEGL-2 and -3 values. Values for AEGL-1 were not derived because concentrations causing mild sensory irritation are ≥260 ppm, which is above the AEGL-2 values and would not serve as a warning of potential exposure. Therefore, AEGL-1 values are not recommended. The absence of AEGL-1 values does not imply that exposure below the AEGL-2 is without adverse effects.

The AEGL-2 values were based on an acute neurotoxicity study in rats exposed to 0, 100, 300, or 500 ppm for 6 h (Mandella 1997a) and a developmental toxicity study with pregnant rats exposed to ethylene oxide at 10, 33, or 100 ppm for 6 h/day during organogenesis (Snellings et al. 1982a). The point of departure is 100 ppm, the no-observed-adverse-effect level (NOAEL) for neurotoxicity and developmental toxicity. The decrease in fetal body weight and the increase in litter incidence of delayed ossification of the vertebrae at 100 ppm were not toxicologically significant, and 100 ppm is the NOAEL for the collective neurotoxicity end points (droopy, half-closed eyelids; impaired locomotion; low arousal; and no response to approach). A total uncertainty factor of 10 was applied to the point of departure: 3 for interspecies sensitivity and 3 for intraspecies variability. An uncertainty factor of 3 was selected for interspecies sensitivity because similar neurotoxicity effects (distal axonal degeneration) have been observed in rats and humans. Direct alkylation of DNA, proteins, and other macromolecules—one potential mechanism of toxicity—is not expected to differ across species. Physiologically based pharmacokinetic (PBPK) models have shown that the area under the curve, peak blood levels, internal dose in milligram per kilogram of body weight (mg/kg), and hemoglobin adduct levels (measure of internal exposure) in humans are similar to or lower than the corresponding values for rats. In addition, the hemoglobin adduct level in rats and humans is proportional to exposure concentration. A factor of 3 was selected for

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intraspecies variability because glutathione-*S*-transferase polymorphism in humans modulates systemic exposure as measured by hemoglobin adducts. Ethylene oxide exposure measured by hemoglobin adduct levels is within a factor of 3 in individuals with the *GSTT1* genotype (conjugator) and the *GSTT1*-null genotype (nonconjugator). There is no evidence that individuals with respiratory diseases, including asthma, would respond differently to ethylene oxide concentrations far below odor detection or irritation levels. The time-scaling approach used ten Berge's equation in which $C^n \times t = k$ (chemical concentration in air with a chemical-specific exponent applied to a specific end point × exposure time = response), where n = 1.2, based on analysis of rat lethality data. The AEGL value for a 10-min exposure is the same as the 30-min value because of the uncertainty of extrapolating from a 6-h exposure to 10 min.

AEGL-3 values were derived from a lethality study with rats (Jacobson et al. 1956). An LC_{01} (concentration with 1% lethality) value (628 ppm), which is considered an approximation of the lethality threshold, was estimated from a 4-h acute inhalation study with rats. Uncertainty factors of 3 for interspecies sensitivity and 3 for intraspecies variability (total uncertainty factor of 10) were applied to the LC_{01}. The rationale for the interspecies uncertainty factor was the same as that described for AEGL-2 as a rat study was used to derive the AEGL values and the exposure concentration was within range for the PBPK model simulations showing linearity of systemic uptake. An intraspecies uncertainty factor of 3 was selected because glutathione-*S*-transferase polymorphism can modulate systemic exposure as measured by hemoglobin adduct levels, and individuals with asthma are not expected to be affected differently by ethylene oxide exposure. An interspecies or intraspecies uncertainty factor of 10 would lower the 10- and 30-min AEGL values below the odor detection or irritation thresholds and exposure concentrations associated with life-threatening events. Scaling to the different timeframes was based on ten Berge's equation ($C^n \times t = k$), where n = 1.2. The AEGL value for a 10-min exposures is the same as the 30-min value because of the uncertainty of extrapolating from a 4-h exposure to 10 min.

Assessment of carcinogenicity data (alveolar or bronchiolar adenomas or carcinomas in the lungs of female mice) showed that extrapolating the total cumulative exposure over 2 years to a single exposure and estimating a 10^{-4} risk resulted in AEGL-3 values of 1,300, 1,300, 640, 160, and 80 ppm for 10- and 30-min and 1-, 4-, and 8-h exposures, respectively. These values exceed those derived for AEGL-2 and AEGL-3.

AEGL values derived for ethylene oxide are summarized in Table 2-1.

1. INTRODUCTION

Ethylene oxide (a monoepoxide) is a gas at room temperature and normal atmospheric pressure; the vapor density is 1.49. The vapor is highly flammable at concentrations ranging from 3% to 100%, and it may undergo explosive de-

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composition (WHO 1985; Gardiner et al. 1993). Ethylene oxide is very reactive with nucleophiles such as water, alcohols, halides, amines, and sulfhydryl compounds (EPA 1985, WHO 1985). Physicochemical properties of ethylene oxide are presented in Table 2-2.

TABLE 2-1 Summary of AEGL Values for Ethylene Oxide

Classification	10 min	30 min	1 h	4 h	8 h	End Point (Reference)
AEGL-1[a] (Nondisabling)	Not Recommended					
AEGL-2 (Disabling)	80 ppm (144 mg/m^3)	80 ppm (144 mg/m^3)	45 ppm (81 mg/m^3)	14 ppm (25 mg/m^3)	7.9 ppm (14 mg/m^3)	NOAEL for neurotoxicity and developmental toxicity (Snellings et al. 1982; Mandella 1997a)
AEGL-3 (Lethal)	360 ppm (648 mg/m^3)	360 ppm (648 mg/m^3)	200 ppm (360 mg/m^3)	63 ppm (113 mg/m^3)	35 ppm (63 mg/m^3)	Lethality (Jacobson et al. 1956)

[a]The absence of AEGL-1 values does not imply that exposure below the AEGL-2 is without adverse effects.

TABLE 2-2 Physical and Chemical Data for Ethylene Oxide

Parameter	Value	Reference
Chemical name	Ethylene oxide	
Synonyms	1,2-epoxyethane, oxirane, dimethylene oxide, ethene oxide	
CAS registry no.	75-21-8	
Chemical formula	C_2H_4O	
Molecular weight	44.05	Budavari et al. 1996
Physical state	Colorless, flammable gas	Budavari et al. 1996
Boiling and freezing points	10.4°C and –112.5°C	Gardiner et al. 1993
Specific gravity	0.8966 at 0/4°C; 0.8711 at 20/20°C	Gardiner et al. 1993
Solubility	Soluble in water, acetone, acetone, benzene, ethanol, and diethyl ether	IARC 1994
Vapor pressure[a]	1.50 atm; 152 kPa, 1.52 bar at 21°C	Braker and Mossman 1980
Vapor density	1.49 at 40°C	Gardiner et al. 1993
Liquid density	0.8824 at 10/10°C	IARC 1994
Critical temperature	468.95 K, 195.8°C, 384.4°F	Braker and Mossman 1980
Autoignition temperature	702 K, 429°C, 804°F	Braker and Mossman 1980
Flammability limit	3.0-100%	Braker and Mossman 1980
Conversion factor	1 ppm = 1.8 mg/m^3 at 25°C, 1 atm	Gardiner et al. 1993

[a]atm, atmosphere; kPa, kilopascal.

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Ethylene oxide is produced in very large quantities in the United States and in other countries. Estimated U.S. production was 5.3 to 6.2 billion pounds in 1990 (Gardiner et al. 1993; IARC 1994) and 5.6 billion pounds in 1992 (IARC 1994). Worldwide production exceeded 12 billion pounds (IARC 1994) and may be as high as 16.5 billion pounds (Gardiner et al. 1993). Ethylene oxide is used as an intermediate in the production of ethylene glycol (antifreeze), which accounts for about 60% of its use; nonionic surfactants, which account for about 16%; ethanolamines, which account for about 8.5%; and glycol ethers, diethylene glycol, triethylene glycol, and other chemicals, which account for the remaining 16% (IARC 1994). A small amount of ethylene oxide is used as a fumigant for sterilizing heat-sensitive medical and dental equipment and foods, such as spices and nuts (Gardiner et al. 1993; IARC 1994).

Ethylene oxide is not persistent in the environment; the estimated degradation rate in the atmosphere is 37% in 5.8 days. The half-life is 12 to 14 days in fresh water and 4 days in salt water (EPA 1985; IARC 1994).

The database for ethylene oxide is very large; humans and experimental animal studies on acute toxicity, developmental and reproductive toxicity, genetic toxicity (somatic and germ cells), carcinogenicity, and pharmacokinetics and metabolism were available. These data were used to derive the AEGL values.

2. HUMAN TOXICITY DATA

2.1. Acute Lethality

No studies were available on lethality attributed to ethylene oxide exposure in humans. Marchand et al. (1957) reported the accidental death of three workers involved in the manufacture of ethylene oxide. They experienced vomiting, abdominal pain, diarrhea, headache, and severe nervous system effects that progressed to coma, circulatory collapse, and respiratory failure. Pulmonary edema and congestion of the meninges and brain were observed at the postmortem examination of one of them. The workers were exposed to glycol chlorohydrin, dichloroethane, and ethylene oxide; the deaths were attributed to glycol chlorohydrin and dichloroethane exposure and not to ethylene oxide.

2.2. Nonlethal Toxicity

2.2.1. Odor Threshold

Several human studies on ethylene oxide exposure were available in the literature. In one study, human volunteers sniffed ethylene oxide from an osmoscope (an apparatus attached to the nose) to determine the detection level and description of the odor (Jacobson et al. 1956). The ethylene oxide atmospheres were generated in a 0.7-m^3 chamber and drawn into the osmoscope. The concen-

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tration of ethylene oxide in the chamber was analyzed by collecting the chamber air into a solution of calcium chloride ($CaCl_2$) and hydrochloric acid (HCl) or a 50% solution of magnesium bromide ($MgBr_2$) containing 0.1 N sulfuric acid (H_2SO_4) and titrating with sodium hydroxide (NaOH). The subjects described the odor as pleasantly to sickeningly sweet, fruity, alcoholic, or acetone- or etherlike. The median detectable concentration was 700 ppm (1,260 mg/m^3) with a 95% confidence interval of 317 to 1,540 ppm (571 to 2,772 mg/m^3).

Hellman and Small (1974) conducted a study in which a trained panel of subjects ("trained odor panel") characterized the sensory odor properties of 101 petrochemicals, one of which was ethylene oxide. The properties were defined as (1) absolute odor threshold, the concentration at which 50% of the panel detected an odor; (2) 50% odor recognition threshold, the concentration at which 50% of the panel defined the odor as being representative of the odorant; (3) 100% odor recognition threshold, the concentration at which 100% of the panel defined the odor as being representative of the odorant; and (4) hedonic tone, the pleasure or displeasure associated with the odor quality as judged by the panel. They also derived an "odor index", which is the vapor pressure (ppm)/100% odor recognition threshold (ppm). The absolute odor threshold for ethylene oxide was 260 ppm (468 mg/m^3), and the 50% and 100% odor recognition thresholds were both 500 ppm (900 mg/m^3). The odor index was 2,000 ppm, which placed ethylene oxide in a category of low odor potential. The odor was considered to be sweet or olefinic and was judged as neutral with respect to odor pleasantness or unpleasantness. Hellman and Small (1974) did not report the number of subjects involved in this study or provide additional information on the "training" the subjects received. Cawse et al. (1980) reported that olfactory fatigue occurs upon repeated exposure to ethylene oxide, thus rendering ineffective the warning properties of odor.

The level of distinct odor awareness (LOA) for ethylene oxide calculated based on an odor threshold of 260 ppm and using the guidance provided by van Doorn et al. (2002) is 1,625 ppm. This value is similar to the 95% upper confidence limit on the median odor threshold reported by Jacobson et al. (1956). The derivation of the LOA is presented in Appendix C.

2.2.2. Case Reports and Anecdotal Data

The following case studies describe signs and symptoms of ethylene oxide intoxication and the concentrations and exposure durations at which they occurred.

Salinas et al. (1981) reported that a female nurse was exposed to ethylene oxide vapor while disposing of an ampule she accidentally dropped. Her exposure lasted 2 to 3 min and she showed immediate signs and symptoms of intoxication, including repeated episodes of nausea, stomach spasms, paleness, lightheadedness, short periods of unconsciousness, convulsive movements of her arms and legs, and periods of apnea (cessation of breathing). Muscle twitching,

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nausea, and malaise continued for 24 h after exposure; malaise and an inability to perform minor motor tasks continued for up to 1 week after exposure. Chest X-rays, laboratory studies, and arterial blood gases were normal. The patient was asymptomatic 2 months after exposure. The authors estimated maximum exposure as 500 ppm based on the release of 17 g of ethylene oxide into the sterilizer bag, resulting in a minimal peak concentration of 500 micrograms per milliliter (µg/mL) in the bag. Her exposure may have been considerably greater than the calculated concentration of 500 ppm.

Five hospital workers were exposed for 30 min to ethylene oxide vapors emitted from a leaky sterilizer at concentrations high enough to be detected by odor (≥260 ppm) (Deleixhe et al. 1986; Laurent 1988). The sterilizing gas consisted of a mixture of ethylene oxide and carbon dioxide (15/85, v/v). The equipment was operated under 6 atmospheres of pressure, and the concentration of ethylene oxide in the equipment was 1,200 mg/L. The investigators did not specify the method for monitoring the air concentrations after this accident, but a colorimetric method and flame-ionization detection had been used previously. The sterilizer workers experienced ethylene oxide concentrations at the odor threshold of 260 ppm, but it could have been higher. Measured concentrations were 15 to 50 ppm 2.5 h after the accident and about 5 ppm the next day. Two workers experienced only headache and diarrhea, which disappeared within 70 h after exposure; the other three workers experienced more serious signs of toxicity, which included irritation of the upper respiratory tract, dry mouth and thirst, conjunctival irritation, severe headache, and intense generalized pruritus, along with muscular weakness in one worker and dizziness in another. Muscular weakness may have been a sign of toxicity to the peripheral nervous system. Nausea, vomiting, and diarrhea started 20 h after exposure, lasted for 14 days, and cleared up by 21 days. Hemolysis was noted on days 9 to 11 and persisted until day 16.

Garry et al. (1979) described the symptoms experienced by 12 workers exposed to ethylene oxide in the instrument and materials sterilization area. Informed consent was obtained from this study population. Another group of 12 individuals represented an unexposed or incidentally exposed population. Freon gas was used as a carrier with the ethylene oxide to prevent an explosion. Ambient ethylene oxide concentrations were monitored over the entire sterilization cycle by infrared spectroscopy and gas chromatography. Gas chromatography identified the two constituents in the sterilizing gas but could not be used for measuring ethylene oxide because the humidified air resulted in poor absorption of ethylene oxide to the charcoal filter. The frequency of upper respiratory tract irritation indicated that exposure was intermittent, showing a bimonthly cycle over a 5-month period. During a 2-month period, 12 nurses experienced sore throat and dry mouth (most prominent symptoms), diarrhea, conjunctival irritation, headache, nausea, speech difficulty, recent memory loss, weakness, dizziness, and incoordination. The maximum ethylene oxide concentrations ranged from 36 ppm (64.8 mg/m^3) in the room about 15 feet from the sterilizer (probably representing the breathing zone) to 1,500 ppm (2,700 mg/m^3) in the open

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drain leading from the sterilizing unit. Garry et al. (1979) also reported that an investigator was exposed to 1,500 ppm for 5 min; symptoms of intoxication were not described. The signs and symptoms Garry et al. (1979) described cannot be attributed to a single exposure. However, the investigators noted that illnesses were periodic and alleviated by time away from the workplace.

Finelli et al. (1983) described the signs and symptoms experienced by three sterilizer operators accidentally exposed to ethylene oxide over 4-month to 12-year periods. Ambient ethylene oxide concentrations were not determined. Symptoms of intoxication included numbness, tingling, cramps, weakness, and incoordination in the lower extremities and cramps in the hands. In addition, frequent complaints reported by the sterilizer operators included eye irritation, headaches, smelling of fumes, sleeplessness, and nervousness. Neurologic examination showed distal abnormalities in the legs and feet (reflex, vibratory sensation, and flexion) but no abnormalities in cranial nerves. An abnormal gait was noted in one patient and bilateral footdrop was found in two patients. Nerve conduction studies showed abnormalities in motor and sensory conduction potential in the lower extremities in two patients and normal conduction potential in the third. Electromyograms showed abnormal potentials in the lower extremities. The resulting diagnosis was distal axonal neuropathy (peripheral neuropathy). Two patients were fully recovered within 7 months and one was almost fully recovered after 6 months.

The National Institute for Occupational Safety and Health conducted a survey to assess the effects of exposure to ethylene oxide on 10 hospital workers (Zey et al. 1994). The workers complained of headache, dizziness, mucous membrane irritation, nasal bleeding, vomiting, diarrhea, facial flushing and swelling, fatigue, nervousness, and a “sweet”-like odor. The 8-h time-weighted average (TWA) concentration in the breathing zone of the workers ranged from 0.23 to 0.56 ppm, with short-term excursions reaching 77 ppm in one area of the breathing zone and 11 ppm in another. The authors believed the concentrations were higher than those measured in the present investigation, because the employers noticed the ethylene oxide odor, which has a detection threshold higher than the measured concentration. The clinical signs also suggest exposure to higher concentrations.

Deschamps et al. (1992) described a case of persistent nonimmunologic asthma and slight peripheral neuropathy that developed in a worker exposed to ethylene oxide 4 h/day for 4 days. The worker was about 18 m from an ethylene oxide leak and he wore no protective equipment. The worker noticed an odor, suggesting that the concentration was ≥260 ppm. Signs and symptoms after the 4-day exposure included coughing, shortness of breath, and wheezing. Respiratory symptoms persisted and 1 year after the accident, pulmonary function tests showed bronchial obstruction and bronchial hyperreactivity. The forced vital capacity was 93% of the predicted value, forced expiratory volume in 1 s (FEV_1) was 74% of the predicted value, midexpiratory flow rate (forced expiratory flow 25% to 75%) was 44% of the predicted value, and the FEV_1 after 600 µg of acetylcholine showed a 20% decrease. The respiratory effects persisted for at least 3

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years after exposure. Immunologic tests showed no formation of immunoglobulin E antibodies to ethylene oxide. The investigators proposed that the time of onset of symptoms was too short to be explained by a sensitizing mechanism. They further suggested that the alkylating properties of ethylene oxide probably explained why the onset of symptoms occurred after the fourth day of exposure, because alkylating injuries take longer to appear than direct irritation or caustic injuries. A neurologic examination showed signs of proprioceptive axonal neuropathy. An additional five workers, including one with asthma, were exposed because of the leak; none of them experienced respiratory symptoms.

Gross et al. (1979) reported on three workers accidentally exposed for 2 weeks to 2 months to ethylene oxide vapor from a leaky sterilizer. Symptoms they experienced included irritation of the conjunctiva and mucous membranes, decreased sense of smell and taste, headaches, nausea, vomiting, and lethargy. One patient had recurrent major motor seizures, but there was no evidence of peripheral neuropathy. A second worker experienced muscle weakness and increased fatigue and showed evidence of peripheral neuropathy. A third worker had problems with memory and thinking, difficulty swallowing, cramps, numbness, and weakness in the arms and legs, along with clinical signs that included slurred speech, confusion, weakness of facial and distal muscles, and muscular incoordination. A neurologic test also showed evidence of peripheral neuropathy. The exposure concentrations for these workers were not monitored; however, intermittent odor detection of ethylene oxide suggested excursions greater than 260 ppm during work shifts.

2.2.3. Epidemiologic Studies

Bryant et al. (1989) surveyed sterilizer workers from 27 hospitals who were potentially exposed to ethylene oxide. Short-term symptoms were identified by means of a questionnaire sent to 241 workers; 182 responded, 165 of whom worked with ethylene oxide. The age of the cohort ranged from less than 20 years (1%) to greater than 60 years (9%). The sterilizers used in the hospitals included table top or portable sterilizers and built-in sterilizers with and without ventilation hoods. The portable sterilizers used cartridges containing 100% ethylene oxide, and the other sterilizers used a mixture containing ethylene oxide and an inert carrier gas. The investigators did not describe the analytic procedure for determining ethylene oxide concentrations. Ethylene oxide concentrations ranged from peaks of 11 to 23.5 ppm, decreasing to <1 ppm within 60 seconds (s) or from 8.5 ppm decreasing to 1 ppm within 160 s depending on the type of sterilizer used. The total exposure concentration per sterilizer cycle ranged from undetectable to 10.7 ppm with exposure durations per cycle ranging from 166 s (2.77 min) to 705 s (11.75 min). The mean concentration per cycle was 3.4 ppm. The detection of the ethylene oxide odor suggests that the concentrations exceeded 260 ppm, at least briefly. The most prevalent symptoms other than the odor of ethylene oxide included headaches, skin and eye irritation, dry mouth,

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and sore throat. Other symptoms included skin rash, runny nose, loss of sense of smell, shortness of breath, nausea, numbness in fingers, and drowsiness. A larger number of workers exposed to concentrations above the mean concentration reported more symptoms than workers exposed to concentrations below the mean, suggesting a concentration effect. Some symptoms may have been due to daily peak exposures and some were likely due to repeated exposures over a prolonged period.

2.3. Developmental and Reproductive Toxicity

Hemminki et al. (1982) conducted a cross-sectional study on the spontaneous abortion rate (number of spontaneous abortions per number of pregnancies) among the staff of 80 Finnish hospitals who used ethylene oxide to sterilize heat-sensitive equipment. Control groups exposed to ethylene oxide were identified by hospital nursing staff, who also distributed the questionnaires to the subjects. The return rate for the questionnaires was about 91% for both groups. Specific exposure data were not reported in this study, but the mean 8-h TWA ranged from 0.1 to 0.5 ppm, with the peak concentration reaching 250 ppm at Finnish hospitals. Data from about 24 hospitals showed that concentrations varied between 5 and 10 ppm for about 20 min when the sterilizer door was open (Hemminki et al. 1983). The data as summarized in Table 2-3 are presented as crude and adjusted rates (age, parity, decade of reported pregnancy, coffee and alcohol consumption, and smoking habits). Crude and adjusted spontaneous abortion rates were significantly elevated in female staff exposed to ethylene oxide compared with the unexposed control group. Data obtained from hospital discharge records produced similar results for the spontaneous abortion rates: 22.5% ($p < 0.05$, compared with controls) for the staff exposed to ethylene oxide and 9.2% for the controls. The abortion ratio (number of spontaneous abortions per number of births) based on hospital records was also higher in workers exposed to ethylene oxide (33.3% compared with 11.8% for controls, $p < 0.05$). The findings of this study are not conclusive; several weaknesses are evident. Both the exposed and control populations were identified by the nursing staff without corroborating exposure data. Hospital discharge records confirmed the results for only about one-third of the respondents. There are inherent recall biases when results are based on respondents' memories. The number of sterilizing staff exposed only to ethylene oxide during pregnancy was very small compared with the other groups.

Rowland et al. (1996) conducted a cross-sectional epidemiologic study on the reproductive outcome among California dental assistants potentially exposed to ethylene oxide and showed an increased risk of adverse reproductive outcome associated with exposure. The exposed population consisted of respondents who listed ethylene oxide as the method used to sterilize instruments at the last menstrual date of their last pregnancy. Adverse pregnancy outcomes included spontaneous abortion (<21 weeks), preterm delivery (21 to 36 weeks), and post-term

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delivery (≥42 weeks). Thirty-two women reported exposure to ethylene oxide; spontaneous abortion occurred in five, preterm birth occurred in three, and post-term birth occurred in five. Of the 1,288 unexposed women in the study; 88 reported a spontaneous abortion, 56 reported a preterm birth, and 141 reported a post-term birth. The adjusted relative risks of adverse outcomes are presented in Table 2-4. The small number of respondents exposed to ethylene oxide reduces the statistical power of the study and limits the analysis of confounding factors (unscavenged nitrous oxide, high use of amalgam, and cigarette smoking). However, the study authors conducted a sensitivity analysis and concluded that the missing nitrous oxide and smoking data did not bias their results on reproductive outcome. This study has a number of limitations and weaknesses. Exposure measures were not reported, but the authors noted that high concentrations were likely because of the type of sterilization system dental technicians use. The exposure status of the respondents was not confirmed, and the reproductive outcome of the respondents was not verified through hospital records. Although this study suggested that exposure to ethylene oxide can adversely affect the outcome of pregnancy and that the effect can occur at any stage of pregnancy, the results are not conclusive.

TABLE 2-3 Spontaneous Abortion Rates Among Hospital Sterilizing Staff and Controls

Group	Total Number of Pregnancies	Crude Rate (%)	Adjusted Rate[a] (%)
Sterilizing staff[b]	1,443	11.3	9.7
Exposed during pregnancy	545	16.7[c]	15.1[c]
Not exposed during pregnancy	605	6.0	4.6
Uncertain	293	123[c]	11.3[c]
Ethylene oxide alone			
Exposed during pregnancy	82	20.7[d]	16.1[c]
Not exposed during pregnancy	1,068	10.3	7.8
Control	1,179	10.6	10.5

[a]Adjusted for age, parity, decade of reported pregnancy, coffee and alcohol consumption, and cigarette smoking.
[b]Includes staff exposed to ethylene oxide, glutaraldehyde, and formaldehyde sterilants.
[c]$p < 0.05$ for exposed versus nonexposed pregnancies.
[d]$p < 0.01$ for exposed versus nonexposed pregnancies.
Source: Hemminki et al. 1982. Reprinted with permission; copyright 1982, *British Medical Journal*.

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TABLE 2-4 Adverse Pregnancy Outcomes Among Female Dental Assistants Exposed to Ethylene Oxide

Reproductive Outcome	Number Exposed	Relative Risk	95% Confidence Interval
Spontaneous abortion[a]	32	2.5	1.0-6.3
Preterm birth[a]	21	2.7	0.8-8.8
Post-term birth[a]	17	2.1	0.7-5.9
Spontaneous abortion, preterm birth[a]	32	2.6	1.3-5.4
Spontaneous abortion, preterm birth[b]	26	2.3	1.0-5.4
Spontaneous abortion, preterm or post-term birth[a]	25	2.7	1.2-6.1
Spontaneous abortion, preterm or post-term birth[b]	20	2.5	1.0-6.1

[a]Adjusted for age only.
[b]Adjusted for age, unscavenged nitrous oxide, and high use of amalgam.
Source: Rowland et al. 1996. Reprinted with permission; copyright 1996, *Epidemiology*.

2.4. Carcinogenicity

2.4.1. Epidemiologic Studies

Several epidemiologic studies have been conducted on the mortality experience of workers potentially exposed to ethylene oxide. Types of cancer that are of concern among workers exposed to ethylene oxide include lymphohematopoietic cancers (combined), leukemia, non-Hodgkin's lymphoma, and cancer of the brain, stomach, and pancreas.

Hogstedt et al. (1979a) reported three leukemia cases among 230 workers potentially exposed to ethylene oxide in a factory where hospital equipment was sterilized, whereas only 0.2 case was expected based on a rough estimate of the person-years of observation and sex- and age-specific rates in Sweden. Exposure concentrations ranged from 2 to 70 ppm with 8-h TWA concentrations of 20 ± 10 ppm in the breathing zone and 150 ppm at floor level. Hogstedt and coworkers followed these workers and two additional cohorts engaged in the production of ethylene oxide; one group produced ethylene oxide by the chlorohydrin method and another used direct oxidation of ethylene. The three cohorts composed a total of 709 Swedish workers with total followup extending from 1961 to 1985 for mortality and to 1983 for cancer (Hogstedt et al. 1979a,b; Hogstedt 1988). Ethylene oxide exposures varied over the years (Hogstedt et al. 1979b), ranging from a high of 1,300 mg/m^3 (~260 ppm, odor detection) to averages of <25 mg/m^3 (14 ppm) during the 1940s, 10 to 50 mg/m^3 (6 to 28 ppm) during the 1950s and early 1960s, and 1 to 10 mg/m^3 (0.6 to 6 ppm) during the 1970s. Con-

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founding exposures included ethylene chlorohydrin, ethylene dichloride, bis(2-chloroethyl)ether, other chlorinated chemicals, and ethylene glycol. The risk of all cancers combined, stomach cancer, and blood and lymphatic cancer—particularly the risk of stomach cancer and leukemia among male workers—was increased. The risk of cerebrovascular diseases among male workers exposed to ethylene oxide was also increased (Hogstedt 1988). Because of confounding exposures to other chemicals, the observed effects cannot be attributed to ethylene oxide alone.

Steenland et al. (1991) conducted a retrospective mortality study on 18,254 U.S. workers (55% female and 45% male) employed for at least 3 months at 14 facilities that produced sterilized medical supplies and spices. The average followup was 16 years. The average 8-h TWA concentration was 4.3 ppm (7.7 mg/m^3) for sterilizer operators and 2.0 ppm (3.6 mg/m^3) for other exposed workers. No statistically significant increases were observed for the number of deaths due to all causes, all cancers, all hematopoietic cancers, leukemia-aleukemia, non-Hodgkin's lymphoma, or stomach cancer compared with mortality rates for the general U.S. population. However, deaths due to hematopoietic cancers showed a significant positive trend ($p = 0.03$) with increasing time since first exposure (latency), and deaths due to kidney cancer were significantly increased ($p < 0.05$) when the latency was >20 years. Significant increases in the mortality rates for all hematopoietic cancers and lymphosarcoma and reticulosarcoma were noted for male workers. Steenland et al. (1991) noted that their study was limited by the small number of cases and short followup time.

Wong and Trent (1993) analyzed the data on the same cohort consisting of 18,728 workers. They also showed no statistically significant increases in mortality rates except for deaths due to non-Hodgkin's lymphoma among male workers; this increase did not show a trend associated with duration of employment or latency. However, the number of cases was very small. This study also was reported by UCCPC (1991).

Bisanti et al. (1993) conducted a study on 1,971 male Italian chemical workers: 637 were licensed for at least 1 year to handle only ethylene oxide and 1,334 were licensed for at least 1 year to handle ethylene oxide and other chemicals. The license was a qualitative indication of exposure. No quantitative exposure estimates were available. Followup of the entire cohort from 1940 to 1984 showed six deaths due to hematopoietic cancer and four due to lymphosarcoma or reticulosarcoma ($p < 0.05$). Five cases of hematopoietic cancers ($p < 0.05$) and three cases of lymphosarcomas and reticulosarcomas ($p < 0.001$) occurred in the subcohort licensed to handle only ethylene oxide.

Teta et al. (1993) followed the mortality of 1,896 chemical workers potentially exposed to ethylene oxide from 1940 to 1988. These investigators did not find a statistically significant increase in mortality due to all malignant neoplasms or lymphohematopoietic, stomach, brain, or pancreatic cancer.

Hagmar et al. (1995) analyzed the mortality experience of a cohort consisting of 2,170 workers (1,309 women and 861 men) employed for at least 1 year in facilities producing medical supplies sterilized with ethylene oxide. Eth-

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ylene oxide exposure was initially about 40 ppm at one facility and 75 ppm at the other; it decreased over the years so that only sterilizer operators were exposed to concentrations greater than 0.2 ppm in later years. These investigators failed to find statistically significant increases in the risks of malignant neoplasms, lymphohematopoietic neoplasms, and leukemia.

Shore et al. (1993) evaluated available epidemiologic studies and conducted a meta-analysis of 10 cohorts that included 29,800 workers with potential exposure to ethylene oxide. A total of 2,540 deaths were recorded. No association was found between ethylene oxide exposure and risk of leukemia, pancreatic cancer, brain and nervous system cancer, and total cancer. A suggested increased risk was observed for non-Hodgkin's lymphoma and stomach cancer; however, evaluations of intensity, frequency, and duration of exposure and latency did not support the conclusion. This study was also reported by UCCPC (1993).

2.4.2. Risk Assessment

In 1984, the Occupational Safety and Health Administration (OSHA) reported the results of a quantitative cancer risk assessment on occupational exposure to ethylene oxide. For a 45-year working lifetime exposure to 1 ppm, OSHA estimated 12 to 23 excess deaths due to cancer per 10,000 workers. OSHA (49 Fed. Reg. 25734[1984]) reported that Crump (no date provided) estimated 3.7 to 23 deaths per 10,000 workers, the Ethylene Oxide Industry Council estimated 18 to 79 deaths per 10,000 workers, and Sielken (no date provided) estimated 1 to 6 deaths per 10,000 workers.

The Environmental Protection Agency (EPA) reported a 95% upper bound on slope or q_1* of 1×10^{-4} µg/m^3 based on the total incidence of leukemia and brain gliomas in female Fischer 344 (F344) rats (from data reported by Snellings et al. 1981) (EPA 1985). Current estimates for single exposures to ethylene oxide are presented in Appendix B.

2.5. Genetic Toxicity

2.5.1. Epidemiologic and Case Studies

Various end points of genetic toxicity have been studied extensively in humans receiving accidental acute high-level exposures and long-term low-level exposures to ethylene oxide. The populations receiving the most attention are sterilizer operators and chemical manufacturing workers. The literature has been reviewed recently by Rhomberg et al. (1990), Dellarco et al. (1990), and IARC (1994). These reviews described both positive and negative associations between exposure to ethylene oxide and increased frequencies of sister chromatid

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exchanges (SCEs) and chromosome aberrations in peripheral lymphocytes. Because the literature is quite extensive, only a few studies are described in this report.

Although most studies involve long-term exposure to ethylene, two acute exposure studies with mixed results were located in the literature. Laurent (1988) reported increased SCE frequencies in peripheral lymphocytes of three sterilizer workers accidentally exposed for 30 min to ethylene oxide concentrations exceeding the odor detection level (260 ppm or 1,260 mg/m^3). Clinical symptoms are described in Section 2.2 of this document. SCE frequencies analyzed in the peripheral lymphocytes 5 days and 2 years after the accident were compared with a group of control or chronically exposed workers. Five days after the accident, the mean SCE frequency was significantly elevated, by 160% compared with the control group and by 144% compared with the chronically exposed group. The mean SCE frequency in the chronically exposed group was significantly elevated (112%) compared with the control group. A significant increase in the proportion of high-frequency cells (cells with more than 15 SCEs per cell) was observed in the exposed subjects 5 days after the accident; this increase accounted for the increased frequency of SCEs. By 2 years after the accident, SCE frequencies had returned to the preaccident level.

Tates et al. (1995) compared several end points of genetic damage in seven chemical workers incidentally exposed to ethylene oxide at concentrations ranging from 52 to 785 mg/m^3 (29 to 436 ppm, 8-h TWA concentrations) with a group of seven unexposed controls. Frequencies of SCEs, *hprt* mutants, and micronuclei were evaluated in peripheral lymphocytes harvested 89 to 180 days after exposure. Although the level of hemoglobin adducts also indicated very high exposures to ethylene oxide, the various genetic tests showed no positive results compared with the control group. These results differ from those obtained by Laurent (1988). However, Tates et al. (1995) did not conduct their genetic tests until 89 to 180 days (3 to 6 months) after exposure, and it is possible that any genetic lesions formed were repaired before that time or the lymphocytes were replaced by natural turnover of the cells. Tates et al. (1995) also did not see increases in the same genetic parameters in workers chronically exposed to ethylene oxide at average concentrations ranging from <0.006 ppm (0.01 mg/m^3) to <0.1 ppm (0.18 mg/m^3), which may have been too low to induce measurable genetic damage.

Garry et al. (1979) reported that four sterilizer operators exposed for 2 months to ethylene oxide at concentrations high enough to cause respiratory and neurologic symptoms had elevated SCE frequencies 3 and 8 weeks after the last exposure. A concentration of 36 ppm (64.8 mg/m^3) was measured 15 feet from the sterilizer and 1,500 ppm (2,700 mg/m^3) was found at floor level. The mean SCE frequency was 9.75 ± 0.75 per metaphase cell 3 weeks after exposure and 10.34 ± 2.55 per metaphase cell 8 weeks after exposure, compared with 5.98 ± 0.31 in the control group (eight subjects). In four asymptomatic workers inci-

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dentally exposed to ethylene oxide, including one subject exposed to 1,500 ppm (2,700 mg/m^3) for 5 min, the frequency of SCEs for the group was elevated (mean = 9.73 ± 0.98 SCEs per metaphase cell) 7 to 9 weeks after the last known exposure to ethylene oxide.

In a recent study, Major et al. (1996) compared genetic damage in two groups of nurses exposed to ethylene oxide with control groups. One group comprised 9 nurses exposed to ethylene oxide at 5 to 20 mg/m^3 (2.8 to 11 ppm) and 14 controls, and the other group comprised 10 nurses exposed to ethylene oxide at 5 to 100 mg/m^3 (2.8 to 55.6 ppm) and 27controls. A group of 48 "historic" controls was also used for comparison. Compared with their respective hospital controls, both groups of nurses showed increased frequency of SCEs, chromosome aberrations, or lectin-stimulated labeling index. Aberrations in exposed nurses included deletions, dicentrics, chromatid exchanges, and rings. The background rates in the two control populations varied, indicating differences in confounding factors (alcohol consumption, smoking, age). Overall, this study showed genetic damage in both exposed groups.

2.5.2. Risk Assessment

Rhomberg et al. (1990) calculated risk estimates for heritable translocations in offspring of fathers exposed to ethylene oxide; they used data reported by Generoso et al. (1990) for their estimates. For an exposure of 10 ppm for 8 h/day for 3 weeks or 15 days of exposure (1,200 ppm × hours), 16 translocation carriers are expected among 10,000 live offspring. Preston et al. (1995) reviewed the Rhomberg et al. (1990) assessment and concluded that the genetic risk for induction of reciprocal translocations would be negligible at low doses. They further noted that Rhomberg overestimated the risk by a factor of 10. Natarajan et al. (1995) used a parallelogram approach to assess genetic risk in humans based on dominant mutations. Their assessment considered genetic end points in germ cells and somatic cells in animals, including humans. They estimated a risk of 4×10^{-4} above background from occupational exposure to ethylene oxide at 1 ppm for 1 year.

2.6. Occupational Exposure

Workers have been exposed to ethylene oxide at concentrations ranging from undetectable to peaks at moderately high concentrations. Occasionally, very high concentrations have been experienced during accidental exposures but not in the routine work environment. Data on some occupational exposures to ethylene oxide are presented in Table 2-5. Additional information was presented by IARC (1994).

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TABLE 2-5 Occupational Exposure to Ethylene Oxide

Industry	Duration of Exposure	Concentration (ppm)	Signs and Symptoms of Exposure	Reference
Hospital sterilizer operation	5-min TWA	62.5 ± 46 (13-160)	Not reported	Sarto et al. 1984
	1 cycle	15.8 ± 9.8 (3.7-35.5)	Not reported	
	8-h TWA	10.7 ± 4.9 (3.7-20)	Not reported	
Hospital sterilizer operation	8-h TWA	0.1-0.5	Not reported	Hemminki et al.1982
Hospital sterilizer operation	Peak	Up to 250	Not reported	
	20 min	5-10	Not reported	Hemminki et al. 1983
Hospital sterilizer operation	Purge cycle	36-1500	Upper respiratory and neurologic symptoms	Garry et al. 1979
Hospital sterilizer operation	8-h TWA	ND to 6.3	Not reported	Elliott et al. 1988
	2-30 min	ND to 103	Not reported	
Hospital folding and packing	8-h TWA	ND to 6.7	Not reported	

Abbreviation: TWA, time-weighted average; ND, not detectable.

2.7. Summary

No adequate data are available on the lethality of ethylene oxide in humans. Nonlethal effects of ethylene oxide and the exposure concentrations at which the effects occur are summarized in Table 2-6. Primary targets for nonlethal effects include the eyes, respiratory tract, and central and peripheral nervous systems. Experimental studies, case reports, and epidemiologic studies have documented noncancer effects on the respiratory tract, eyes, central and peripheral nervous system, gastrointestinal tract (probably due to nervous system toxicity), hematopoietic system, and possibly the reproductive system and fetus. The absolute odor detection level for ethylene oxide is 260 ppm as reported by one author, and the median odor threshold is 700 ppm as reported by another. The odor recognition level is 500 ppm. As noted in Table 2-6, nonlethal effects occur after exposure to ethylene oxide concentrations approximating the odor threshold (≥260 ppm) for short periods (2 to 30 min) or repeatedly for a few days. Genetic damage to somatic cells occurs at concentrations below 260 ppm. Chronic exposure to low 8-h TWA concentrations is associated with the same effects as acute exposure, possibly due to daily high-level excursions.

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TABLE 2-6 Summary of Nonlethal Effects of Ethylene Oxide in Humans

Concentration		Exposure		
ppm	mg/m^3	Duration	Effects	Reference
1,3349	2,4028	10 s	Definitely irritating to nasal passages	Walker and Greeson 1932
2,670	4,806	Not reported	Slightly irritating to nasal passages, acetic acid-like odor	Walker and Greeson 1932
3,260	1,260	30 min	Odor, headache, gastrointestinal effects, eye and upper respiratory tract irritation, pruritus, muscle weakness, dizziness, hemolysis	Deleixhe et al. 1986; Laurent 1988
3,260	3,1260	4 h/d for 4 d	Coughing, shortness of breath, wheezing, slight peripheral neuropathy, nonimmunologic asthma	Deschamps et al. 1992
Excursions of 3,260	3,1260	2 wk to 2 mon	Eye and mucous membrane irritation, difficulty swallowing, headache, gastrointestinal effects, lethargy, fatigue, problems with memory and thinking, major motor seizures, peripheral neuropathy	Gross et al. 1979
3,500	900	2-3 min	Gastrointestinal effects, unconsciousness, apnea, muscle twitching, malaise, incoordination for up to 1 wk	Salinas et al. 1981
Not reported	Not reported	4 mon to 12 y	Eye irritation, headaches, smelling of fumes, distal axonal neuropathy	Finelli et al. 1983
36-1,500	65-2,700	Cyclic for 2-5 mon	Upper respiratory irritation, eye irritation, sore throat and dry mouth, gastrointestinal effects, headache, speech difficulty, recent memory loss, weakness, dizziness, and incoordination	Garry et al. 1979
0.23-0.56 (TWA)	0.4-1	Chronic	Sweetlike odor, headache, dizziness, irritation of mucous membranes, gastrointestinal effects, fatigue, and nervousness	Zey et al. 1994
Excursions of 11 or 77	19.8-139.6			

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Peak = 23.5	42.3	Up to 1 min	Odor, headache, skin and eye irritation, dry mouth, sore throat, runny nose, shortness of breath, nausea, and numbness in fingers	Bryant et al. 1989
Total up to 10.7	19.3	Up to 11.75 min		
Average 3.4	6.1	Not reported	Drowsiness	
0.1-0.5 (8-h TWA)	0.18-0.9 (8-h TWA)	During pregnancy	Increased risk of spontaneous abortion	Hemminki et al. 1982
Peak 250	450			
5-10 (20 min daily)	9-18			
Not reported	Not reported	Any duration during pregnancy	Increased risk of spontaneous abortion, preterm birth, or post-term birth	Rowland et al. 1996

Abbreviation: TWA, time-weighted average.

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Signs of toxicity occurring after short-term exposure to ethylene oxide include eye and upper respiratory tract irritation, nausea, vomiting, diarrhea, headache, dizziness, malaise, fatigue, muscle weakness, and signs and symptoms of peripheral neuropathy. Other effects noted in some studies include dry mouth, sore throat, runny nose, shortness of breath, apnea, memory loss, and seizures. Nonimmunologic asthma was reported in one study; this effect has not been confirmed and may not be due to ethylene oxide exposure. One worker with asthma exposed to ethylene oxide at the odor detection level experienced no symptoms suggestive of effects on the respiratory tract. Two epidemiologic studies presented evidence suggesting that exposure to ethylene oxide is associated with adverse reproductive outcomes: spontaneous abortions, preterm births, and post-term births. An increase in the rate of spontaneous abortions was reported for a cohort exposed to ethylene oxide at concentrations ranging from 0.1 ppm (8-h TWA), to 5 to 10 ppm for 20-min intervals, to peaks of 250 ppm.

Epidemiologic studies conducted to assess the effect of exposure to ethylene oxide on mortality due to malignant neoplasms in workers in chemical factories or sterilizer facilities have produced mixed results with regard to increased cancer risk. Some studies showed increased risks for lymphohematopoietic cancer in the entire cohort or in male subcohorts, whereas other studies showed no increased risk. IARC (1994) concluded that the evidence of carcinogenicity based on human studies is limited.

Human studies on exposure to ethylene oxide also showed that the frequency of SCEs is increased in peripheral lymphocytes of workers exposed to concentrations approximating the odor threshold (260 ppm) for 30 min, exposed to concentrations high enough to cause respiratory and neurologic symptoms for 2 months, incidentally exposed to concentrations of 36 to 1,500 ppm, or chronically exposed to concentrations of 2.8 to 55.6 ppm. The frequency of chromosome aberration was also increased by chronic exposure. Increased frequency of genetic damage was not associated with exposure to high incidental concentrations ranging from 29 to 436 ppm (8-h TWA) when cells were analyzed 89 to 180 days after exposure, suggesting that repair or cell turnover had likely occurred. No damage was observed after chronic exposures to concentrations less than 0.1 ppm.

3. ANIMAL TOXICITY DATA

3.1. Acute Lethality

3.1.1. Rats

Jacobson et al. (1956) exposed groups of 10 male white rats to ethylene oxide vapor at 2,298, 1,992, 1,843, 1,648, 1,343, or 882 ppm (4,140, 3,590, 3,320, 2,970, 2,420, or 1,590 mg/m^3) for 4 h and observed the animals for signs of toxicity and death for the next 14 days. Ethylene oxide in air was pumped into a 0.4-m^3 inhalation chamber operated under constant-flow conditions. The

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chamber atmosphere was analyzed by a colorimetric procedure in which ethylene oxide was collected in a solution of 60% $CaCl_2$ and 0.1 N HCl or a 50% solution of $MgBr_2$ and 0.1 N H_2SO_4 and titrated with NaOH. Both methods gave similar results. Clinical signs observed in the exposed groups included frequent movement and preening, clear nasal discharge, lacrimation, occasional salivation, diarrhea, gasping that increased in severity during exposure, and death. Mortality occurred in all groups. The mortality data are summarized in Table 2-7. The LC_{50} was 1,460 ppm (2,630 mg/m^3) (confidence interval [C.I.] = 620 to 2,550 ppm). Signs of upper respiratory tract irritation, tracheal congestion and petechial hemorrhages, and mild edema in the lungs and peribronchial region were seen upon gross examination. In addition, a secretion was noted around the eyes and nose, and the stomach was distended.

In another 4-h acute inhalation study, groups of five male and five female Sprague-Dawley rats were exposed to ethylene oxide (99.9%) vapor at 1,850, 1,443, or 1,021 ppm (3,330, 2,597, or 1,838 mg/m^3); groups of five males also were exposed to 2,182 or 2,026 ppm (3,928 or 3,647 mg/m^3) and five females were exposed to 1,637 ppm (2,947 mg/m^3) (Nachreiner 1991). The animals were exposed in a 1,300-L glass and stainless steel dynamic chamber. The chamber atmospheres were analyzed with a gas chromatograph equipped with a flame ionization detector. Surviving animals were observed for 14 days after exposure. This study is summarized in Tables 2-8 and 2-9. The LC_{50} was 1,972 ppm (C.I. = 1,887 to 2,061) for male rats, 1,537 ppm (C.I. = 1,391 to 1,698 ppm) for female rats, and 1,741 ppm (C.I. = 1,655 to 1,831 ppm) for the combined sexes. During exposure, signs of eye, nasal, and oral irritation (blepharospasm; wetness and encrustation around the eyes, nose, and mouth; swollen eye tissue), hypoactivity, and signs of respiratory distress (audible respiration, mouth breathing, increased or shallow respiration, and gasping) were noted (Table 2-8). Clinical signs immediately after exposure included tremors and an absence of tail and toe

TABLE 2-7 Mortality in Male White Rats Exposed to Ethylene Oxide Vapor for 4 Hours

Concentration		
ppm	mg/m^3	Mortality (%)
2,298	4,140	10/10 (100)
1,992	3,590	10/10 (100)
1,843	3,320	9/10 (90)
1,648	2,970	4/10 (40)
1,343	2,420	2/10 (20)
882	1,590	2/10 (20)

Source: Jacobson et al. 1956. Reprinted with permission; copyright 1956, *American Medical Association*.

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pinch reflex in some groups. Clinical signs indicative of eye and respiratory tract irritation and neurologic effects were observed during the first 3 or 4 days after exposure. No clinical signs were observed after the day of exposure in the 1,021-ppm group or after day 4 in the other exposure groups. Gross findings included brain hemorrhage, lung discoloration and hyperinflation, crusts and scabbing in the oral cavity and pharynx, and abnormal contents in the nose (see Table 2-9 for details). Microscopic findings consisted primarily of congestion and hemorrhage in males found dead in the 2,182-ppm group and females found dead in the 1,850-ppm group. Other lesions included alveolar histiocytosis, pulmonary edema, interstitial pneumonitis, and emphysema.

TABLE 2-8 Lethality and Clinical Signs in Male and Female Sprague-Dawley Rats Exposed to Ethylene Oxide Vapor for 4 Hours

	Concentration (ppm)								
	Males					Females			
Effects	2,182	2,026	1,850	1,443	1,021	1,850	1,637	1,443	1,021
Number of deaths	4/5	4/5	0/5	0/5	0/5	5/5	4/5	1/5	0/5
During Exposure									
Blepharospasm	+	+	+	+	+	+	+	+	+
Wetness around eyes and nose	+	+	+	+	+	+	+	+	+
Hyperactivity	+	+	+	+	+	+	+	+	+
Mouth breathing	+					+			
After Exposure									
Unkempt fur	+	+	+			+	+		
Wetness or encrustation around eyes, nose, and mouth	+	+	+	+	+	+	+	+	+
Swollen tissue around eyes					+				+
Mouth breathing	+	+	+	+	+	+	+	+	
Audible respiration	+	+	+			+	+	+	
Gasping	+	+	+			+			
Decreased, increased, or shallow respiration	+	+	+		+[a]	+	+		+[a]
Absence of tail and toe pinch reflex		+					+		
Hypoactivity	+	+	+	+		+	+	+	
Tremors		+		+				+	

[a]Increased respiration rate and shallow respiration only.
Source: Nachreiner 1991.

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TABLE 2-9 Gross Findings in Male and Female Sprague-Dawley Rats Exposed to Ethylene Oxide for 4 Hours

	Concentration (ppm)								
	Males					Females			
Effects	2,182	2,026	1,850	1,443	1,021	1,850	1,637	1,443	1,021
Brain: hemorrhage	3[a]	0	0	0	0				
Lungs: discoloration, diffuse or focal and multifocal	3	4	0	3	2	5	4	1	3
Lungs: hyperinflated	3	3	0	0	0	0	0	0	0
Nose: abnormal contents						3	0	0	0
Oral and pharyngeal: crust, scab, scale						3	0	0	0

[a]Number of animals with lesions; five animals per group were exposed.
Source: Nachreiner 1991.

In a 1-h acute inhalation study, groups of five male Sprague-Dawley rats were exposed to ethylene oxide at measured concentrations of 6,161, 5,546, or 4,827 ppm and groups of five females were exposed to concentrations of 4,287, 4,202, 4,064, 3,966, or 3,609 ppm (Nachreiner 1992). Ethylene oxide in air (4,000 to 7,000 ppm) was metered into a 120-L glass and stainless steel dynamic exposure chamber. The chamber atmospheres were analyzed with a gas chromatograph equipped with a flame ionization detector, and nominal concentrations were calculated based on the amount of ethylene oxide delivered to the chamber. All surviving animals were observed for 14 days. Mortality and clinical signs are summarized in Table 2-10 and gross findings are shown in Table 2-11. No deaths occurred in the male group exposed to 4,827 ppm or in the female group exposed to 3,609 ppm. The LC_{50} was 5,748 ppm (95% C.I. = 5,276 to 6,262 ppm,) for males, 4,439 ppm (C.I. = 4,034 to 4,884 ppm) for females, and 5,029 ppm (95% C.I. = 4,634 to 5,459 ppm) for the combined sexes. Because of extreme variations in the analytic concentrations (3,584 to 4,432 ppm), which probably explain the unusual mortality rate, the 4,064-ppm female group was not included in the calculation for the LC_{50}. Clinical signs of toxicity were observed in all groups during and after the 1-h exposure up to day 3 or 4 postexposure. Restlessness was observed in all groups during the first 10 min of exposure. In all groups of males and in the 4,827-ppm female group, only lacrimation was observed on the day of exposure; periocular wetness was observed in the

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remaining female groups. These findings suggest that ethylene oxide was irritating to the eyes and the respiratory tract and toxic to the nervous system. Gross examination showed effects in the nose, lungs, and kidneys (Table 2-11). Lung weights were elevated in animals that died before the study ended compared with the lungs of animals that survived until study termination, particularly in the male groups.

TABLE 2-10 Clinical Signs in Male and Female Sprague-Dawley Rats Exposed to Ethylene Oxide for 1 Hour

	Concentration (ppm)							
	Males			Females				
Effects	6,161	5,546	4,827	4,827	4,202	4,064	3,966	3,609
Mortality (%)	4/5 (80)	1/5 (20)	0/5 (0)	5/5 (100)	1/5 (20)	5/5 (100)	2/5 (40)	0/5 (0)
During Exposure								
Restlessness	+	+	+	+	+	+	+	+
Wetness around eyes	+	+	+	+	+	+	+	+
Lacrimation	+	+	+	+				
Mouth breathing	+							
Hypoactivity	+	+	+	+	+	+	+	+
No acoustic startle reflex	+	+	+	+				
After Exposure								
Unkempt fur	+	+		+	+	+	+	+
Encrustation or wetness: eyes, mouth, nose			+	+	+			+
Decreased respiration	+	+		+	+	+	+	
Hypoactivity	+	+		+		+	+	+
Ataxia	+				+	+	+	+
Tremors	+	+			+	+	+	

Source: Nachreiner 1992.

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TABLE 2-11 Gross Findings in Male and Female Sprague-Dawley Rats Exposed to Ethylene Oxide for 1 Hour

	Concentration (ppm)							
	Male			Female				
Effects	6,161	5,546	4,827	4,827	4,202	4,064	3,966	3,609
Encrustation in the nose	2[a]	1	0	2	1	3	2	B
Lungs: discoloration, diffuse or focal and multifocal	4	1	0	5	4	5	3	B
Lungs: hyperinflated	1	0	0	0	1	1	0	B
Kidneys: diffuse color change				0	0	3	0	B

[a]Number of animals with lesions; five animals per group were exposed.
Source: Nachreiner 1992.

Hollingsworth et al. (1956) conducted several experiments in which rats and other species were exposed to ethylene oxide (97.0% to 98.6%) vapor for various durations in a 450-L metal chamber. Ethylene oxide concentration in the chamber was determined by a colorimetric procedure using H_2SO_4 and $MgBr_2$ titrated with NaOH. Controls were included but not described. The investigators reported that all 10 male and 10 female rats died after exposure to ethylene oxide at a concentration of 841 ppm (1,510 mg/m^3) for 7 h/day, 5 days/week for eight exposures. Gross and microscopic effects were assessed on rats after two or three exposures to ethylene oxide and killed 1 or 3 days after the last exposure. Microscopic effects occurred in the lungs (interstitial edema, congestion, alveolar hemorrhage), liver (fatty degeneration), kidneys (congestion and cloudy swelling of the convoluted tubules), and adrenal glands (fat vacuoles). Renal effects were more severe 3 days after exposure than on the first day after exposure. Exposure to 357 ppm (640 mg/m^3) for 7 h/day, 5 days/week for seven exposures resulted in the death of 2/20 rats (10 males and 10 females exposed). Severe lung irritation and secondary pulmonary effects were observed in these animals. In another experiment, 10 male and 10 female rats were exposed to ethylene oxide vapor at 357 ppm for 33 to 59 exposures for 48 to 85 days. Growth was retarded, and by the 38th exposure 18 rats (90%) had died because of secondary respiratory effects. Near the end of the exposure period, neuromuscular impairment at the lumbar and sacral region manifested as paralysis and muscular atrophy of the hindlimbs was observed. The two surviving rats (males) were allowed to recover after 42 exposures.

Jacobson et al. (1956) exposed 20 male white rats to ethylene oxide vapor at 440 ppm (720 mg/m^3) for 6 h/day, 5 days/week for 6 weeks; they included an

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equal number of unexposed animals as controls. Chamber description and analytic procedure were the same as described for a single exposure to rats and mice except the chamber size was 0.7 m^3. Thirteen deaths (65%) occurred among the 20 exposed rats. Clinical signs observed in the exposed rats included a reddish discharge from the nose, diarrhea, labored breathing, hindlimb weakness followed by hindlimb paralysis during the last 2 weeks of exposure, and progressive weight loss. No significant pathologic effects were noted except for marked hemosiderosis in the spleen of a few animals. The weight loss and paralysis were reversible in five rats observed for several months after terminating exposure.

3.1.2. Mice

Jacobson et al. (1956) exposed groups of 10 female white mice to ethylene oxide at 1,365, 1,343, 960, 882, 860, or 533 ppm (2,460, 2,420, 1,730, 1,590, 1,550, or 960) for 4 h and observed them for 14 days or until death. Ethylene oxide in air was pumped into a 0.4-m^3 chamber operated under constant flow conditions. The chamber atmosphere was monitored by collecting chamber air into a solution of 60% $CaCl_2$ and 0.1 N HCl or a 50% solution of $MgBr_2$ and 0.1 N H_2SO_4 and titrating the mixture with NaOH. Similar results were obtained by both methods. The mice showed clinical signs similar to those in the rat, which included frequent movement and preening, clear nasal discharge, lacrimation, occasional salivation, gasping followed by severe dyspnea, and death. Mortality data are summarized in Table 2-12. The LC_{50} for mice was 835 ppm (1,504 mg/m^3) (C.I. = 623 to 1,040 ppm). The only gross finding reported for mice was distension of the stomach.

In a National Toxicology Program (NTP) (1987) inhalation study, groups of five male and five female B6C3F_1 mice were exposed to ethylene oxide (>99%) vapor at concentrations of 0, 100, 200, 400, 800, or 1,600 ppm (180, 360, 720, 1,440, or 2,880 mg/m^3) for 4 h and observed for 14 days. Analytic concentrations were determined with a photoionization detector or gas chromatograph equipped with a flame ionization detector. Analytic concentrations were within 5% of target concentrations. Mortality data are summarized in Table 2-13. No animals of either sex died after exposure to 100 to 400 ppm. All males exposed to 800 ppm died 2 to 6 days after exposure and four females exposed to 800 ppm died 1 to 3 days after exposure. All male and female mice exposed to 1,600 ppm died within 4 h after exposure. Lacrimation and dyspnea were observed at 800 ppm; severe dyspnea, incoordination, semiconsciousness, and diarrhea were observed in animals exposed to 1,600 ppm. No clinical signs were described for the 100- and 400-ppm groups. An LC_{50} value of 660 ppm (95% C.I. = 509 to 856 ppm) was calculated for female mice; an LC_{50} value was not calculated for male mice. Postmortem examinations were not conducted on these animals.

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TABLE 2-12 Mortality in Female White Mice Exposed to Ethylene Oxide Vapor for 4 Hours

Concentration		Mortality (%)
ppm	mg/m^3	
1,365	2,460	10/10 (100)
1,343	2,420	10/10 (100)
960	1,730	7/10 (70)
882	1,590	3/10 (30)
860	1,550	6/10 (60)
533	960	1/10 (10)

Source: Jacobson et al. 1956. Source: Jacobson et al. 1956. Reprinted with permission; copyright 1956, *American Medical Association*.

TABLE 2-13 Mortality in Male and Female B6C3F$_1$ Mice Exposed to Ethylene Oxide Vapor for 4 Hours

Concentration		Mortality (%)	
ppm	mg/m^3	Male	Female
100	180	0/5	0/5
200	360	0/5	0/5
400	720	0/5	0/5
800	1,440	5/5 (100%)	4/5 (80%)
1,600	2,880	5/5 (100%)	5/5 (100%)

Source: NTP 1987.

NTP (1987) also conducted a 14-day study in which male and female B6C3F$_1$ mice were exposed to ethylene oxide at concentrations of 0, 50, 100, 200, 400, or 800 ppm (90, 180, 360, 720, or 1,440 mg/m^3), 6 h/day, 5 days/week. Analytic concentrations were determined with a photoionization detector or gas chromatograph equipped with a flame ionization detector. All five male and four of five female mice exposed to 800 ppm died within 1 day of exposure; one female died within 2 days of exposure, thus confirming the lethality of 800 ppm in the single-exposure study. Clinical signs at 800 ppm included hunched posture and listlessness. All animals exposed to 50 to 400 ppm survived except for two females exposed to 200 ppm; their deaths were not related to exposure.

Hollingsworth et al. (1956) reported that all five female mice died after exposure to ethylene oxide at a concentration of 841 ppm (1,510 mg/m^3), 7 h/day, 5 days/week for eight exposures. Four of 10 female mice died after seven exposures to ethylene oxide at 357 ppm (640 mg/m^3). Moderate loss of body weight and severe lung injury indicative of irritation and secondary pulmonary effects were observed in these animals. Another 10 female mice similarly exposed to 357 ppm for 33 exposures over 48 days showed growth retardation and

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all died due to secondary respiratory infection. The concentration of ethylene oxide in the chamber atmosphere was determined by a colorimetric procedure using H_2SO_4 and $MgBr_2$ titrated with NaOH.

In a 6-week inhalation study, Jacobson et al. (1956) exposed 30 female white mice to ethylene oxide vapor at 400 ppm (720 mg/m^3) for 6 h/day, 5 days/week for 6 weeks. An equal number of unexposed animals were included as controls. Chamber description and analytic procedure were the same as described for the single exposure of rats and mice except the chamber size was 0.7 m^3. A slight weight loss was observed in exposed animals compared with controls. Twenty-four (80%) mice exposed to ethylene oxide died during the study compared with three (10%) controls. No significant pathologic changes were reported.

3.1.3. Guinea Pig

Waite et al. (1930) exposed guinea pigs to ethylene oxide (99.5%) vapor at concentrations of 8.5%, 6.3% to 6.4%, 5.1%, 4%, 1.4% to 2.5%, 0.7%, 0.3%, 0.13%, 0.05%, and 0.025% for various durations ranging from 1 to 480 min. The concentrations correspond to ethylene oxide vapor in air at 85,000, 63,000 to 64,000, 51,000, 40,000, 14,000 to 25,000, 7,000, 3,000, 1,300, 500, and 250 ppm. The concentration of ethylene oxide was determined by drawing the vapor into 2 N HCl and titrating the solution with barium hydroxide in the presence of a colorimetric indicator. One to four guinea pigs were exposed to each concentration. Twenty-four guinea pigs from the same colony were used as controls. Deaths occurred during exposure to 8.5% ethylene oxide for 33 min. Deaths also occurred within 24 h after exposure to concentrations of 6.3% to 6.4% for 10 or 20 min, 2.5% for 60 min, 1.4% for 60 or 107 min, 0.7% for 150 min, and 0.3% for 330 min. Deaths occurred between 1 and 8 days in groups exposed to 5.1% for 6 min, 4% for 20 min, 1.4% for 20 min, 0.7% for 60 min, 0.3% for 190 min, and 0.13% for 480 min. No deaths occurred in the groups exposed to 1.4% for 10 min, 0.7% for 20 min, 0.3% for 70 min, 0.13% for up to 290 min, and 0.025% or 0.05% for 480 min. Clinical signs of toxicity were observed at all concentrations. Changes in rate, depth, and amplitude of respiration occurred at all concentrations. Nasal irritation, profuse lacrimation, blinking, and squinting (signs of eye irritation) were observed at all concentrations except 0.025%. The eyes showed reddening of the conjunctiva and prominent vessels in the sclera immediately after exposure but not after 24 h. Bloody and frothy nasal exudate were observed at concentrations ≥ 0.3%; an unsteady gait, staggering, and falling on their sides were noted at concentrations ≥ 0.7%; and dyspnea progressing to gasping occurred at concentrations ≥ 0.3%. The onset and duration of these signs varied depending on the concentration of ethylene oxide. Gross pathologic examination showed lung congestion and edema, frothy serous exudate from the trachea and bronchi, and hyperemia of the liver and kidneys of animals dying during exposure or within 24 h after exposure. Animals killed

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immediately after exposure showed evidence of lung congestion, whereas those surviving for 2 to 4 days also showed changes in their kidneys. Animals killed after 4 days showed slight lung congestion and slight changes in their kidneys or no signs of serious injury; no pathology was observed 8 days after exposure.

Hollingsworth et al. (1956) reported that all eight male and eight female guinea pigs died after exposure to ethylene oxide at 841 ppm (1,510 mg/m^3) for 7 h/day, 5 days/week for eight exposures. Gross and microscopic effects were assessed in guinea pigs exposed two or three times and killed 1 or 3 days after exposure. Toxic effects occurred in the lungs (interstitial edema, congestion, alveolar hemorrhage), liver (fatty degeneration), kidneys (congestion and cloudy swelling of the convoluted tubules), and adrenal glands (fat vacuoles). The renal effects were more severe after 3 days than after 1 day. A control group was included but not described. The ethylene oxide concentration in the chamber was determined by a colorimetric procedure using H_2SO_4 and $MgBr_2$ titrated with NaOH and a colorimetric indicator.

3.1.4. Dogs

Three male beagle dogs per group were exposed to ethylene oxide vapor at concentrations of 2,830, 1,393, 710, or 327 ppm (5,100, 2,510, 1,282, or 590 mg/m^3) for 4 h followed by a 14-day observation period (Jacobson et al. 1956). Chamber description and analytic procedure were the same as described for a single exposure of rats and mice except the chamber size was 0.7 m^3. Death occurred only in the groups exposed to 2,830 and 1,393 ppm, and all deaths occurred within 24 h after exposure. The LC_{50} for dogs was 960 ppm (1,730 mg/m^3). Mortality data are summarized in Table 2-14. Clinical signs observed at 2,830 ppm included lacrimation; clear nasal discharge; frothy, colorless, mucous vomitus; diarrhea; convulsions; dyspnea; and death. Dogs exposed to 1,393 ppm showed similar signs except for diarrhea, convulsion, and dyspnea. No clinical signs were observed at 710 or 327 ppm. Pathologic changes included moderate lung congestion, dilation of perivascular lymphatic spaces, perivascular edema, and distension of the stomach.

TABLE 2-14 Mortality in Male Beagle Dogs Exposed to Ethylene Oxide Vapor for 4 Hours

Concentration		
ppm	mg/m^3	Mortality (%)
2,830	5,100	3/3 (100)
1,393	2,510	3/3 (100)
710	1,280	0/3 (0)
327	590	0/3 (0)

Source: Jacobson et al. 1956. Source: Jacobson et al. 1956. Reprinted with permission; copyright 1956, *American Medical Association*.

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3.1.5. Other Species

One female rabbit, one male rabbit, and one female monkey died after exposure to ethylene oxide at 841 ppm (1,510 mg/m^3), 7 h/day, 5 days/week for eight exposures (Hollingsworth et al. 1956). One rabbit of each sex and one female monkey were similarly exposed to ethylene oxide for 33 to 59 exposures in 48 to 85 days. Ethylene oxide concentration in the chamber was determined by a colorimetric procedure using H_2SO_4 and $MgBr_2$ titrated with NaOH and a colorimetric indicator. The male rabbit died after 48 exposures. In rabbits and monkeys, neuromuscular impairment of the lumbar and sacral region manifested as paralysis, and muscular atrophy of the hindlimbs occurred during the latter part of the exposure. Complete recovery was attained 100 to 132 days after exposure was terminated.

3.2. Nonlethal Toxicity

Section 3.1 contains data on effects occurring at concentrations not causing death of the animals under study. These data may be used to further assess nonlethal toxicity in laboratory animals.

3.2.1. Rats

Embree et al. (1977) reported that 15 Long-Evans male rats exposed by inhalation to ethylene oxide at 1,000 ppm (1,800 mg/m^3) for 4 h showed signs of toxicity including "central depression," diarrhea, and eye and respiratory tract irritation. These animals were used in a dominant-lethal study and were not further investigated for general toxicity.

3.2.2. Mice

Snellings et al. (1984a) reported the results of a subchronic inhalation study using B6C3F$_1$ mice exposed to ethylene oxide (99.9%) vapor. Groups of 30 male and 30 female mice were exposed to ethylene oxide vapor in a 4,350-L stainless steel and glass chamber at target concentrations of 0, 10, 50, 100, or 250 ppm, 6 h/day, 5 days/week for 10 weeks (males) or 11 weeks (females). Analytic concentrations were determined with a gas chromatograph equipped with a flame ionization detector; concentrations were within 6% of the targets. No treatment-related clinical signs of toxicity or body weight changes were observed in animals exposed to ethylene oxide. Erythrocyte parameters were depressed, suggesting slight anemia in males and females at 250 ppm. Spleen weights were depressed in both sexes and testes weights were depressed in males; no corresponding histopathologic effects were observed, suggesting that the organ weight changes were not toxicologically significant. Neuromuscular

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screening tests performed on five females at 6 weeks and on five mice of each sex at study termination showed treatment-related effects for five parameters (toe and tail pinch reflex, righting reflex, gait, and locomotor activity) at 250 ppm and for two parameters (gait and locomotor activity) at 50 and 100 ppm.

Groups of 10 male and 10 female $B6C3F_1$ mice were exposed to ethylene oxide at concentrations of 0, 50, 100, 200, 400, or 600 ppm (90, 180, 360, 720, or 1,080 mg/m^3), 6 h/day, 5 days/week for 14 weeks (NTP 1987). Analytic concentrations were determined with a photoionization detector or gas chromatograph equipped with a flame ionization detector. All mice exposed to 400 or 600 ppm died within the first 4 weeks of the study. Clinical signs observed in mice exposed to ethylene oxide at 600 ppm included anorexia, dyspnea, decreased activity, bloatedness, and listlessness. One male in each of the remaining groups died before the end of the experiment. Treatment-related histopathologic effects were observed in the kidneys of males (≥100 ppm) and females ((≥200 ppm), thymus of males and females ((≥200 ppm), nasal cavity of both sexes ((≥200 ppm), and spleen of both sexes (600 ppm). No treatment-related effects occurred at 50 ppm.

3.2.3. Dogs

Two of three male beagle dogs exposed to ethylene oxide vapor at 290 ppm (523 mg/m^3) for 6 h/day, 5 days/week for 6 weeks showed clinical signs of toxicity, including vomiting, occasional tremors, and transient weakness in the hindlimbs (Jacobson et al. 1956). The chamber and the analytic procedure were the same as described for a single exposure of rats and mice except the chamber size was 0.7 m^3. A mild anemia developed in all three dogs. Pathologic effects included lung congestion and moderate alveolar collapse, which was probably due to irritant effects of ethylene oxide, and muscular atrophy (fat replaced muscle fibers), which caused weakness in the hindlimbs. No deaths occurred among the exposed or control animals.

3.3. Neurotoxicity

In an acute neurotoxicity study, groups of 10 male and 10 female Sprague-Dawley rats were exposed by whole body inhalation to ethylene oxide at 0, 100, 300, or 500 ppm for 6 h and observed for 14 days after exposure (Mandella 1997a). The rats were exposed under dynamic conditions with monitoring of chamber atmosphere by gas chromatography four times during the exposure cycle. Nominal concentrations were determined based on the amount of test material consumed during the exposure cycle. The mean analytic concentrations were within 2% of the target concentrations. Neurobehavioral assessments that included the standard functional observational battery (FOB) and motor activity

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tests were performed on all animals on day 1 and on days 8 and 15 after exposure. At study termination, five rats of each sex per group were killed and perfused in situ for neuropathologic examination and the brain and peripheral nerves of the control and 500-ppm groups were examined microscopically. The results of the FOB assessment showing exposure-related effects are presented in Table 2-15. The FOB assessment showed increased incidences of the following findings among a total of 20 males and females (combined) exposed to 0, 100, 300, and 500 ppm and assessed on day 1: drooping, half-closed eyelids; slightly impaired locomotion; low level of arousal; and no reaction to approach. The incidences of low arousal and no response to approach were significantly increased in male rats and both sexes combined at 300 and 500 ppm, and the incidence of droopy, half-closed eyelids was significantly increased in both sexes at 500 ppm. The increased incidence of slightly impaired locomotion was not significant but showed a clear exposure-related trend and was considered related to exposure to ethylene oxide. The incidence of low arousal was increased at 100 ppm, but not significantly, compared with controls. According to the investigator, these end points were indicative of a decrease in alertness and reflex response. The low arousal state, which was characterized by a decrease in the normal exploratory activity of the animals, and the slightly droopy or half-closed eyelids were indicative of decreased alertness. The lack of response to approach was indicative of a decreased reflex response. Motor activity also was decreased in both sexes at 500 ppm and in males at 300 ppm and was correlated with the decrease in normal exploratory activity. Overall, this study showed exposure-related acute neurotoxicity in male and female rats on day 1 after a single exposure to ethylene oxide at 300 or 500 ppm. The effects were reversible because no clear exposure-related effects were observed on day 8 or 15. The NOAEL for this study was 100 ppm.

Ohnishi et al. (1985) studied the effect of inhaled ethylene oxide vapor on neuropathy in rats. Five male Wistar rats were exposed to ethylene oxide at a concentration of 500 ppm, 6 h/day, 3 days/week for 13 weeks. Five pair-fed animals exposed to ambient air served as controls. Clinical signs in the exposed rats included an awkward gait at weeks 5 to 8 and slight to moderate hindlimb ataxia starting at week 9 or 10. Light and electron microscopic examination of peripheral nerves showed axonal degeneration of myelinated fibers in the fasciculus gracilis and hindlimb nerves. The degenerative changes accounted for the ataxia observed in these animals.

In a 4-week range-finding study, groups of five male and five female Sprague-Dawley rats were exposed by whole-body inhalation to ethylene oxide vapor at concentrations of 0, 100, 300, 400, or 500 ppm (Mandella 1997b). Chamber atmospheres were within 2% of the target concentrations. No exposure-related effects were observed at 100 ppm. One female rat in the 500-ppm group was found dead on day 18. Clinical signs observed at 500 ppm included irregular gait, decreased fecal volume, lethargy, prostration, emaciation, yellow anogenital staining, moist rales, labored breathing, paleness, and black and

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TABLE 2-15 Summary of the Acute Neurotoxicity Study in Rats

Observation	0 ppm	100 ppm	300 ppm	500 ppm
Males (n = 10)				
Drooping, half-closed eyelids	1 (10%)	1 (10%)	0	5 (50%)
Slightly impaired locomotion	0	0	2 (20%)	1 (10%)
Low arousal	0	2 (20%)	5* (50%)	9** (90%)
Approach response—no reaction	1 (10%)	2 (20%)	6* (60%)	6* (60%)
Females (n = 10)				
Drooping, half-closed eyelids	1 (10%)	1 (10%)	5 (25%)	3 (30%)
Slightly impaired locomotion	0	0	0	2 (20%)
Low arousal	0	1 (10%)	1 (10%)	6* (60%)
Approach response—no reaction	1 (10%)	1 (10%)	1 (10%)	4 (20%)
Males + Females (n = 20)				
Drooping, half-closed eyelids	2 (10%)	2 (10%)	5 (25%)	8** (40%)
Slightly impaired locomotion	0	0	2 (10%)	3 (15%)
Low arousal	0	3 (15%)	6** (30%)	15** (75%)
Approach response—no reaction	2 (10%)	3 (15%)	7* (35%)	10** (50%)

*$p < 0.05$, **$p < 0.01$ compared with controls.
Source: Mandella 1997a.

brown stains on the snout. Body weights of males and females exposed to 300, 400, or 500 ppm decreased by 12% to 42% at study termination and food consumption decreased by 15% and 18% in females and males, respectively, during the first week. The neurologic assessment at weeks 3 and 4 showed that hindlimb grip strength decreased 22% to 36% in both sexes at 300, 400, and 500 ppm; this effect was more severe at 400 and 500 ppm. Landing foot splay decreased 29% to 42% in both sexes at week 3 or 4 at 400 and 500 ppm; this effect was more severe at 500 ppm. The postmortem examination showed decreased absolute brain weight in males with 500-ppm exposure. No exposure-related gross lesions were observed, and only minimal to slight vacuolation of the white matter of the thalamus and medulla oblongata was observed in both sexes at 500 ppm. The NOAEL for the 4-week inhalation study was 100 ppm.

In a subchronic neurotoxicity study, groups of 15 male and 15 female Sprague-Dawley rats were exposed by whole body inhalation to ethylene oxide vapor at concentrations of 0, 25, 50, 100, or 200 ppm for 14 weeks (Mandella 1997c). Chamber atmospheres were analyzed by gas chromatography; mean analytic concentrations were within 1% of target concentration. Neurobehavioral assessments (functional observational battery) were conducted on 10 rats of each sex after exposure for 5, 9, and 14 weeks and after a 13-week recovery period.

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Five rats of each sex were assessed for gross and microscopic lesions after exposure for 14 weeks and after the 13-week recovery period. No exposure-related effects were observed at 100 ppm and no exposure-related effects were observed for clinical signs, mortality, or cholinesterase activity at any concentration. Body weight gain decreased 16% to 17% during exposure to 200 ppm with a concomitant decrease in food consumption. The neurobehavioral assessment showed no exposure-related effect except for a 25% decrease in hindlimb grip strength in females exposed to 200 ppm. The level of motor activity did not differ between exposed and control rats. Postmortem examination showed no exposure-related gross or microscopic lesions in nervous system tissue. The NOAEL for this study was 100 ppm.

Groups of 12 cynomolgus monkeys were exposed whole body to ethylene oxide (>99% pure) in 3.5-m^3 stainless-steel and glass chambers at concentrations of 0, 50, or 100 ppm for 7 h/day, 5 days/week for 24 months (Setzer et al. 1996). The monkeys obtained from the wild were of unknown age; the mean body weights were 5.26, 5.39, and 5.21 kg for the control, 50-, and 100-ppm groups, respectively. The controls were exposed to room air in the chambers. Chamber atmospheres were analyzed by gas chromatography; the analytic concentrations were within 10% of target and ethylene oxide was absent from the control atmosphere. Body weight was measured weekly followed by monthly measurements after 2 months. Maximum nerve conduction velocity for the sciatic-tibial nerves and electroencephalographic (EEG) measurements were conducted five times during exposure. Two animals from each group were sacrificed at the end of the exposure period for neuropathologic examination. The remaining animals were maintained for an additional 7 years without ethylene oxide exposure, at which time two additional animals per group were subjected to neuropathologic examination.

Mean body weight of monkeys exposed to 50 ppm was similar to that of controls, but the 100-ppm group weighed significantly less than controls from week 25 to the termination of exposure. The maximum nerve conduction velocity of the monkeys exposed to ethylene oxide did not differ significantly from that of controls at any time during exposure, but it was consistently lower in the 100-ppm group than in controls from 12 months to the termination of exposure. The investigators noted that maximum nerve conduction velocity of two animals in the 100-ppm group showed a large decline between 12 months and the termination of exposure. The maximum nerve conduction velocity was not significantly affected in animals exposed to ethylene oxide at the end of the 7-year recovery period. No significant effect was observed on EEG measurements. Neuropathologic examination of two monkeys per group after exposure for 2 years showed lesions indicating axonal dystrophy in the medulla oblongata, restricted to the nucleus gracilis at 50 and 100 ppm. The lesions were negative/trace or negative in the two controls, slight or severe in the two 50-ppm monkeys, and negative or slight in the two 100-ppm monkeys. Demyelination in the extreme distal portion of the fasciculus gracilis was seen in one monkey in each group; the lesion was severe at 100 ppm. Neuropathologic examination of

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two monkeys per group maintained for the additional 7 years showed slight or moderate axonal dystrophy in the two monkeys in each group, including controls. This study is inconclusive because it is difficult to draw conclusions about the neuropathologic lesions based on examination of only two animals from each group. The investigators noted that one animal in the 100-ppm group showing the decline in maximum nerve conduction velocity also showed severe demyelination of the fasciculus gracilis.

3.4. Reproductive and Developmental Toxicity

3.4.1. Rats

Groups of 17 to 22 pregnant F344 rats were exposed to ethylene oxide (99.9) vapor at concentrations of 0 (two control groups), 10, 33, or 100 ppm (18, 59, or 180 mg/m^3) for 6 h/day on gestation day (GD) 6 to 15 inclusive (Dow Chemical Co. 1982; Snellings et al. 1982a). The rats were exposed in a 4,400-L stainless steel and glass chamber, and chamber atmosphere was monitored by a gas chromatograph equipped with a flame ionization detector. The dams were killed on GD 20 for evaluation of maternal, reproductive, and developmental parameters. No effects were noted on maternal body weight gain, preimplantation loss, resorptions, or fetal deaths. The weights of male (3.1 g versus 3.3 or 3.4 g for controls) and female (2.9 g versus 3.0 or 3.1 for controls) fetuses were significantly ($p < 0.05$) reduced at the 100-ppm exposure; crown-to-rump length was not affected. There were no gross external or visceral malformations. Delays in vertebrae ossification occurred in a higher percentage of litters in the 100-ppm group than in either control group; the increase was not statistically significant. Variations in ossification occurred in the distal thoracic vertebral centra. Although the incidence of delayed ossification at 100 ppm did not achieve statistical significance ($p = 0.10$), the effect is considered treatment related, because delayed ossification is a definite effect at 125 ppm (see BRRC 1993 below). These effects suggest a mild growth retardation with no corresponding effect on maternal body weight gain. No effects occurred at 10 or 33 ppm.

In another developmental toxicity study, groups of 25 pregnant CD rats (Sprague-Dawley stock) were exposed to ethylene oxide (99.8%) vapor at concentrations of 0 (control), 50, 125, or 225 ppm (90, 225, or 405 mg/m^3), 6 h/day on GD 6 to 15 inclusive (BRRC 1993). The dams were exposed in 900-L stainless steel and glass chambers, and the chamber atmosphere was analyzed with a gas chromatograph equipped with a flame ionization detector. The analytic concentrations were within 3% of target concentrations. The dams were killed on GD 21 for evaluation of maternal and developmental parameters. No treatment-related clinical signs of toxicity or maternal mortality occurred. Absolute maternal body weight and body weight gain were significantly decreased at 125 and 225 ppm. Food consumption during the exposure period also was significantly reduced at 225 ppm. Mean fetal weights were significantly reduced in

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male (96%, 95%, and 90% of control weights) and female (97%, 95%, and 90% of control weights) fetuses at 50, 125, and 225 ppm, respectively. The incidences of litters with skeletal variations (primarily unossified or poorly ossified areas) in the head region, phalanges, forelimbs and hindlimbs, and sternum were increased at 125 and 225 ppm. Twelve types of variations were observed in the 225-ppm group, and three were observed at 125 ppm. No increase in the incidence of delayed ossification occurred at 50 ppm. Therefore, the minimal effect on fetal weight at 50 ppm and the lack of a statistical effect on the incidence of delayed ossification suggest that the small reduction in fetal weight approximated the threshold for growth retardation.

Saillenfait et al. (1996) conducted a developmental toxicity study with pregnant Sprague-Dawley rats exposed to ethylene oxide on GD 6 to 15 inclusive. Exposure conditions were 0, 400, 800, or 1,200 ppm for 0.5 h once a day and 0, 200, or 400 ppm or 0, 800, or 1,200 ppm for 0.5 h three times a day. The animals were exposed in a 200-L chamber, and the chamber atmosphere was monitored by gas liquid chromatography. Analytic concentrations were within 17% of target concentrations. The animals were observed daily, and body weights were recorded on GD 6, 11, 16, and 21. All surviving dams were killed on GD 21 for evaluation of maternal and developmental parameters. No treatment-related clinical signs of toxicity were observed in any group exposed to any concentration once daily; however, one dam exposed to 1 × 800 ppm died due to causes unrelated to exposure. Maternal weight gain was not significantly affected in groups exposed to any concentration once a day. Maternal body weight gain in the 3 × 1,200-ppm group was 26% ($p < 0.01$) of the control value from GD 6 to 11, 32% ($p < 0.01$) of the control value from GD 11 to 16, and 30% of the control value from GD 6 to 15. Absolute maternal weight gain (less gravid uterine weight) in the 3 × 1,200-ppm group declined to 41% ($p < 0.01$) of control weight, and it declined to 82% ($p > 0.05$) of control weight in the 3 × 800-ppm group. Body weights of male and female fetuses in the 3 × 1,200-ppm and the 3 × 800-ppm groups significantly ($p < 0.01$) decreased to 90% and 93% to 94% of the control value, respectively, compared with control weights. Mean fetal weight at 3 × 200 ppm, but not at 3 × 400 ppm, also decreased significantly compared with its control because of the large size of the control fetuses. This study used three control groups, and the mean live litter size of the control group for the 3 × 200-ppm and 3 × 400-ppm exposure groups was small, resulting in larger fetuses compared with the other control groups. This study either showed no fetal weight changes or did not show a clear concentration-response relationship except for the 3 × 800- and the 3 × 1,200-ppm groups.

The Saillenfait et al. (1996) study also showed a significant increase in the incidences of litters with dilated renal pelvis (13/18 versus 4/18 for controls, $p < 0.01$) and dilated ureter (14/18 versus 7/18 for controls, $p < 0.01$) in the group exposed to 1 × 1,200 ppm. Considering the wide variations that can occur in renal development (Woo and Hoar 1972), it is doubtful that the variations in the renal pelvis and ureter are of biological significance.

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Hackett et al. (1982) (also reported by Hardin et al. 1983) reported on a study in female Sprague-Dawley CD rats exposed to filtered air (control) or 99.7% ethylene oxide vapor at 150 ppm (270 mg/m^3) for 7 h/day, 5 days/week for 3 weeks before mating. The rats were exposed in a 2,350-L stainless steel chamber; ethylene oxide concentration in the exposure chamber was monitored by gas chromatography. After the 3-week premating period, rats exposed to filtered air were subdivided: group 1 was exposed to filtered air throughout gestation, group 2 was exposed to filtered air from GD 1 to 6 and to ethylene oxide from GD 7 to 16, and group 3 was exposed to ethylene oxide from GD 1 to 16. This study is summarized in Table 2-16. The rats exposed to ethylene oxide for 3 weeks continued on the same treatment throughout gestation (group 4). There were 41 pregnant rats per group except for group 4, which had 39 rats. The mean body weight of female rats exposed to ethylene oxide vapor before mating and throughout gestation (group 4) was significantly lower (4% to 6%) than that of controls (group 1) during the latter part of the premating period and throughout gestation. The investigators reported no other treatment-related or biologically significant maternal effects. No statistical differences occurred in the pregnancy rate or in the number of live fetuses per litter compared with the control. Fetal parameters showing treatment-related effects included decreases in male and female fetal weights and increases in the incidence of litters with reduced ossification of the skull and sternebrae in all groups exposed for any duration during gestation compared with controls. No treatment-related malformations were observed. These results showed that ethylene oxide exposure produced fetal effects whether administered over a prolonged period or during organogenesis, and the effects were indicative of growth retardation.

In a one-generation reproduction study, groups of 30 male and 30 female F344 rats were exposed to ethylene oxide (99.9%) vapor at concentrations of 0 (control), 10, 33, or 100 ppm (18, 59,or 180 mg/m^3) for 6 h/day for 12 weeks before mating (5 days/week), during mating (7 days/week), and during GD 0 to 19 and day 5 to 21 postpartum (7 days/week) (Snellings et al. 1982b). Two control groups were included in the study. The animals were exposed in a 4,400-L stainless steel and glass chamber and ethylene oxide concentrations were monitored with a gas chromatograph equipped with a flame ionization detector. The survival rates and fertility indices of male and female rats were not affected by exposure to ethylene oxide. The length of gestation increased in 7/14 dams at 100 ppm compared with controls. In addition, the median number of pups born (4 versus 10 for controls), median number of implantation sites (6 versus 10 for controls), and median number of fetuses born per implantation site (0.57 versus 0.92 to 1.0 for controls) were significantly lower in dams at 100 ppm. No effect was observed on pup survival. At 100 ppm, mean F_1 pup weight on day 4 postpartum was significantly greater than that of controls, and F_1 male pup weight in the 33-ppm group was significantly less than that of the control groups on day 21 postpartum. The mixed results suggest no treatment-related effect on pup weight in this study.

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TABLE 2-16 Maternal, Reproductive, and Developmental Effects of Exposure to Ethylene Oxide Vapor by Inhalation in Rats

Parameter	Exposure Group[a]			
	Group 1	Group 2	Group 3	Group 4
Maternal Body Weight (g)				
Day 21, premating	278[b]	277	280	267*
GD 6	298	298	293	279*
GD 11	315	314	308	295*
GD 16	339	335	328	317*
GD 21	382	381	378	360*
Reproductive				
No. live litters/no. pregnant	41/41	41/41	41/41	38/39
No. implantation sites/dam	14.7	14.0	14.8	14.3
No. resorptions/litter	0.75	0.71	0.92	1.60*
No. live fetuses/litter	13.9	13.5	13.8	12.7
Fetal Parameters				
Weight of female (g)	3.56	3.35*	3.23*	3.12*
Weight of male (g)	3.73	3.53*	3.47*	3.34*
Crown-to-rump length (mm), female	36.1	35.3*	34.7*	34.8*
Crown-to-rump length (mm), male	36.5	36.1*	35.8*	35.6*
Morphologic alterations[c]				
Reduced ossification, skull	3/2 (4.9)	16/9 (22.0)*	10/9 (22.0)*	14/10 (26.3)*
Reduced ossification, sternebrae	69/23 (56.1)	145/36 (87.8)*	159/36 (87.8)*	155/33 (85.8)*

[a]Group 1, unexposed during gestation; group 2, exposed GD 7-16; group 3, exposed GD 1-16; group 4, exposed from premating through GD 1-16.
[b]Mean values except when presented as incidence.
[c]Number of fetuses per number of litters; numbers in parentheses are percentage of affected litters relative to controls.
*$p \leq 0.05$, compared with controls.
Source: Hackett et al. 1982.

Mori et al. (1991) studied the effects of inhalation exposure to ethylene oxide on spermatogenesis in Wistar rats. Groups of six male rats were exposed to ethylene oxide vapor at concentrations of 50, 100, or 250 ppm (90, 180, or 450 mg/m^3) for 6 h/day, 5 days/week for 13 weeks and killed 40 h after the last exposure. The control group consisted of 12 males rats exposed to clean air. The animals were exposed in a 0.2-m^3 inhalation chamber; the chamber atmosphere was monitored with a gas chromatograph equipped with a flame ionization detector (Mori et al. 1989). No treatment-related effects were observed on mean body weight, testicular weight, testicular lactate dehydrogenase activity, or epididymal sperm count. The following treatment-related effects were observed at 250 ppm: degenerative changes in the seminiferous tubules, decrease in epididymal weight (80% or control weight), marked decrease in sperm count in the

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epididymis body plus tail, and marked increase in incidence of sperm head abnormalities (immature and teratic types combined and immature types separately). When analyzed separately, the incidence of teratic types was significantly elevated in all exposure groups. No other changes were observed in groups exposed to ethylene oxide at 50 or 100 ppm.

Mori et al. (1989) conducted another study in which groups of six to eight male Wistar rats were exposed to clean air or ethylene vapor at 500 ppm (900 mg/m^3) for 6 h/day, 3 days/week for 2, 4, 6, or 13 weeks. Chamber description and the monitoring procedure were as described above. End points evaluated included body weights (controls were pair-fed), weight and histopathology of the testes and epididymides, and testicular enzyme activities. No significant effect was observed on body weight at any time during the study. The testes of exposed rats were atrophic after 13 weeks of treatment and the relative testicular weights showed corresponding decreases (82%, 59%, and 46% of control weight) after 4, 6, and 13 weeks, respectively. Relative epididymal weight decreased (86%, 71%, and 59% of control weight) at the same time points. Histopathologic examination of the testes showed degenerative changes in the seminiferous tubules manifested as mild degeneration of germ cells at 2 weeks, conspicuous degeneration at 4 weeks, exfoliation of germ cells at 6 weeks, and a marked reduction in germ cells in about 50% of seminiferous tubules, which contained only Sertoli cells, at 13 weeks. Plasma testosterone levels were not affected. Testicular glutathione reductase activity was reduced at all time points, glutathione peroxidase activity was decreased at 2 weeks and increased at 6 and 13 weeks, and glutathione-*S*-transferase activity was increased at 4 to 13 weeks.

3.4.2. Mice

Generoso et al. (1987) and Rutledge and Generoso (1989) showed that exposure to ethylene oxide before mating or within 24 h of mating can have pronounced effects on mouse fetal development.

In the study to assess the effect of inhaled ethylene oxide on preovulatory oocytes, Generoso et al. (1987) exposed female mice to ethylene oxide at 0 or 1,200 ppm (2160 mg/m^3) for 1.5 h/day for 4 consecutive days before mating or to 300 ppm (540 mg/m^3) for 6 h/day for 10 exposures over a 14-day premating period. Chamber and analytic procedures were not described. The dams were killed on GD 17 to assess the effect on resorptions, midgestational deaths, and late fetal deaths. The number of implants per female was significantly reduced at 300 ppm but not at 1,200 ppm. However, the percentage of resorptions in both groups of females exposed before mating was significantly elevated by 10.8% (3.0% in controls) and 41.1% (6.4% in controls) at 1,200 and 300 ppm, respectively. Midgestational deaths and late fetal deaths were slightly elevated but not significantly; the induced loss of conceptuses was 15.7% at 1,200 ppm and 58.2% at 300 ppm, showing that exposure to the lower concentration for a longer time was more effective than the high concentration for a short time.

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Rutledge and Generoso (1989) exposed groups of female (C3H × C57BL)F_1 and (SEC × C57BL)F_1 mice to ethylene oxide vapor at 0 (control) or 1,200 ppm (2,160 mg/m^3) for 1.5 h beginning 1, 6, 9, or 25 h after a 30-min mating period; the mice were killed on GD 17. The following exposure times correspond to different developmental stages of the zygote: 1 h, sperm entry; 6 h, early pronuclear stage before DNA synthesis; 9 h, pronuclear DNA synthesis stage; and 25 h, early two-cell stage. Additional experimental groups were included to study the effects of ethylene oxide exposure on the preovulatory oocytes. Two additional groups of female mice were exposed similarly to 0 or 1,800 ppm (3,240 mg/m^3) 6 h after mating and were killed serially on GD 11 to 15 to determine the effect on midgestational development. A marked reduction was observed in the number of live fetuses from female mice exposed to ethylene oxide vapor 1 h after mating (6 fetuses per dam versus 9.72 for controls) and 6 h after mating (1.81 fetuses per dam versus 10.11 for controls). In addition, the incidence of abnormal fetuses markedly increased when females were exposed 1 h (14.7% versus 0.2% for controls) and 6 h (39.2% versus 1.7% for controls) after mating. The predominant types of abnormalities were hydrops (different degrees of edema ranging from thick neck to a "balloon-like fetus") and eye defects. Defects in the limbs and tail occurred in females exposed 6 h after mating. Other abnormalities included abdominal wall defect, cleft palate, exencephaly, and small size. Generoso et al. (1987) reported that the percentage of resorptions was significantly elevated at all times, but the greatest effect occurred in females exposed 6 h (52.9%) after mating; the induced loss of conceptus was 82.25%. Significant, but less severe, effects occurred when females were exposed at 9 and 25 h after mating. Analysis of the uterine content of females exposed to 1,800 ppm and killed on GD 11 to 15 showed significant increases in fetal deaths, particularly on GD 15 (late deaths). The number of defective living fetuses per dam significantly increased, whereas the number of living fetuses per dam decreased. Most dead fetuses were hydropic (Rutledge and Generoso 1989).

Ribeiro et al. (1987) evaluated the effect of inhaling ethylene vapor at 0, 200, or 400 ppm on sperm morphology in mice. Male Swiss Webster mice were exposed 6 h/day for 5 days, and killed 1, 3, and 5 weeks after exposure. The results showed that ethylene oxide induced concentration-related increases in the incidences of abnormal spermatozoa, spermatids, and preleptotene spermatogonial cells compared with the incidences in controls. The increases were statistically significant at both doses.

Weller et al. (1999) conducted a developmental toxicity study in mice to ascertain whether Haber's rule (concentration × time [C × T]) was valid for ethylene oxide toxicity. One to three female C57BL/6J mice were mated with one male of the same strain, and the day a vaginal plug was found was designated as GD 0. The female mice were assigned randomly to groups based on weight gain between GD 0 and GD 4 or 5. On GD 7, groups of female mice were exposed by whole-body inhalation to ethylene oxide vapor at 0, 2,100, or 2,700 ppm-h in a dynamic chamber (45 L/min) equipped for continuous monitoring of ethylene

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oxide. Individual concentrations ranged from 350 to 1,800 ppm and exposure times ranged from 1.5 to 6 h (see Table 2-17 for individual exposure conditions). The analytic concentrations were within 5% of the target concentrations. Maternal toxicity was assessed based on survival, clinical signs, and body weight gain. The dams were killed on GD 18, the uteri were removed, and developmental toxicity was assessed based on number of resorptions, viability of fetuses, fetal weight, crown-to-rump length, external and internal abnormalities, and skeletal malformations.

The results are summarized in Table 2-17. Maternal deaths attributed to ethylene oxide exposure occurred at 2,700 ppm-h at concentrations of 1,350 ppm and above and at 2,100 ppm-h at a concentration of 1,400 ppm; maternal weight loss occurred at 2,100 and 2,700 ppm-h, with the higher concentrations being more effective than the lower concentrations. The clinical signs assessed included fur appearance, movement, arousal, eyes (crusty or normal), and breathing (labored or normal). One or more of the clinical signs were observed 30 min after exposure to 2,100 and 2,700 ppm-h, with the incidence showing an increasing trend with increasing exposure concentration at 2,100 ppm-h, and the incidence was 95% or greater at 2,700 ppm-h. The overall incidence of clinical signs at 24 h was lower than that observed 30 min after exposure and showed an increasing trend with exposure concentrations at 2,100 and 2,700 ppm-h. Developmental toxicity was exhibited by increased resorptions, decreased fetal body weight, decreased crown-to-rump length, and increased litter incidences of eye defects after exposure to ethylene oxide. The percentage resorptions at 2,100 ppm-h and reduction in fetal weight and crown-to-rump length at 2,100 and 2,700 ppm-h showed increasing severity with exposure concentration. The number of litters at 1,350 ppm and above was too small to assess an exposure-related trend for eye defects. Nevertheless, the data showed that the litter incidence was greater in groups exposed to 700 and 900 ppm for 3 h than in groups exposed to 350 and 450 ppm for 6 h, which was greater than the total incidence in controls. No treatment-related skeletal defects were observed in fetuses from dams exposed to ethylene oxide. This study showed developmental effects at the lowest concentration tested (Weller et al. 1999).

3.4.3. Rabbits

Groups of 30 female New Zealand White rabbits were artificially inseminated and exposed to ethylene oxide vapor (99.7% purity) at 150 ppm for 7 h/day from GD 1 to 19 or GD 7 to 19 (Hackett et al. 1982). The rabbits were exposed in a 2,350-L stainless steel chamber; ethylene oxide concentration in the exposure chamber was monitored by gas chromatography. Another group of 30 inseminated rabbits was exposed to filtered air throughout the study. All rabbits were killed on GD 30. No statistically significant effects were observed on mean food consumption, mean body weight, organ weights, histopathologic lesions, or maternal, reproductive, or developmental parameters.

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TABLE 2-17 Developmental Toxicity in C57BL6 Mice Exposed Whole Body to Ethylene Oxide on GD 7

		Maternal Effects					Developmental Effects							
Concentration (ppm) × Time (h)	Exposed (sperm +)	Number Deaths (%)	Weight Lost (%)	% with Clinical Signs 30 min	% with Clinical Signs 24 h	Number with Fetuses (%)	Number Implants	Number Resorptions (%)	Number Dead Fetuses (%)	Fetal Weight (g)	C-R Length (mm)	Number Offspring (litters)	Eye Defects (Offspring/ Litters)[a]
0 × 1.5	50	0	1.2	2.3	0	28	203	28 (13.8)	0	0.92	19.22	175 (28)	13 (6)
0 × 1.75	8	0	0.7	12.5	12.5	6	50	3 (6.0)	0	0.97	20.03	47 (6)	5 (3)
0 × 2	28	1 (3.6)	0.3	0	0	14	95	11 (11.6)	1 (1.1)	0.99	20.70	83 (14)	4 (3)
0 × 3	38	0	3.4	2.6	0	19	141	15 (10.6)	1 (0.7)	0.93	19.71	125 (19)	5 (4)
0 × 6	30	1 (3.3)	3.8	6.7	0	19	150	14 (9.3)	0	0.99	19.52	136 (19)	12 (6)
Total	154	2 (1.3)	1.9	4.8	2.5	86 (55.8%)	639	71 (11.1)	2 (2.1)	0.96	19.84	566 (86)	39 (22)
C × t = 2,100 ppm-h													
1,400 × 1.5	39	3 (7.7)	7.2	100.0	20.7	8 (22.2)	62	24 (38.7)	17 (27.4)	0.72 (75)	16.89 (85)	21 (8)	7 (3)
700 × 3	41	0	6.6	81.6	5.3	22 (53.7)	168	27 (16.0)	3 (1.8)	0.88 (92)	19.24 (97)	139 (22)	53 (15)
350 × 6	33	0	4.7	53.1	3.1	19 (57.6)	152	13 (8.6)	1 (0.7)	0.97 (101)	19.90 (100)	138 (19)	20 (8)
C × t = 2,700 ppm-h													
1,800 × 1.5	73	41 (56.2)	13.0	100.0	66.2	3 (9.4)	22	14 (63.6)	0	0.70 (73)	16.66 (84)	8 (3)	7 (1)
1,543 × 1,.75	23	15 (65.2)	13.5	95.7	72.2	1 (12.5)	7	1 (14.3)	0	0.76 (79)	17.83 (90)	6 (1)	6 (1)
1,350 × 2	76	27 (35.5)	11.4	100.0	39.7	7 (14.3)	20	9 (45.0)	1 (5.0)	0.86 (90)	18.74 (94)	10 (7)	3 (2)
900 × 3	50	1 (2.0)	8.8	98.0	24.0	11 (22.5)	86	22 (25.6)	5 (5.8)	0.82 (85)	18.42 (93)	59 (11)	34 (9)
450 × 6	41	0 (0)	6.2	95.1	2.4	20 (40.1)		28 (18.9)	0	0.97 (101)	19.32 (97)	120 (20)	13 (10)

[a]Includes anophthalmia and microthalmia.
Abbreviation: C-R, crown-to-rump.
Source: Weller et al. 1999. Reprinted with permission; copyright 1999, American Industrial Hygiene Association.

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3.5. Carcinogenicity

Inhalation carcinogenicity studies have been conducted in mice and rats. Adkins et al. (1986) conducted a 6-month lung tumor bioassay in female A/J mice exposed to ethylene oxide at 0, 70, or 200 ppm (126 or 360 mg/m^3) and showed an increased incidence of lung adenomas (Adkins et al. 1986). The number of lung tumors per tumor-bearing mouse statistically significantly increased (1.62, 1.53, and 2.47, for 0, 70, and 200 ppm, respectively) in the 200-ppm group.

A study reported by the NTP (1987) with B6C3F$_1$ mice exposed to ethylene oxide vapor at 50 or 100 ppm for 102 weeks showed statistically significant increases in the incidences of alveolar and bronchiolar adenomas or carcinomas and Harderian gland tumors in male and female mice and increases in uterine tumors and malignant lymphomas in female mice. Snellings et al. (1984b) and Garman et al. (1985) reported on a study using groups of 120 male and 120 female F344 rats exposed to ethylene oxide concentrations of 0, 10, 33, or 100 ppm for 2 years. Ten rats per group were killed at 6 and 12 months; 20 rats per group were killed at 18 months and all survivors were killed when the study terminated. The results showed statistically increased incidences in mononuclear cell leukemia in males and females and in subcutis fibromas in males. Garman et al. (1985) specifically analyzed the brain tumors and reported increased incidences at 33 and 100 ppm and a dose-related trend for both male and female rats. The incidence of gliomas showed a statistically significant increase at 100 ppm in male rats. These data are summarized in Table 2-18. Another study with male F344 rats exposed to ethylene oxide at 50 or 100 ppm showed increased incidences of mixed cell gliomas of the brain at 50 ppm and peritoneal mesotheliomas at 100 ppm (Lynch et al. 1984a).

3.6. Genetic Toxicity

Ethylene oxide readily alkylates DNA and other macromolecules; it has been studied extensively in numerous genetic toxicity systems using both prokaryotic and eukaryotic cells in vitro and in vivo. The results of these tests showed that ethylene oxide is genotoxic in bacteria, yeast and other fungi, *Drosophila melanogaster*, and rodent and human cells, causing gene mutations, gene conversions, sex-linked lethal mutations, and heritable translocations in the nonmammalian systems and unscheduled DNA synthesis, gene mutations, SCEs, chromosomal aberrations, and micronuclei in cultured mammalian cells (Golberg 1986, Dellarco et al. 1990; IARC 1994). The formation of DNA adducts with ethylene oxide in several mammalian systems shows that ethylene oxide alkylates genetic material. There is also a wealth of information showing that inhalation exposure to ethylene oxide causes genetic damage in somatic and germ cells in rodents, monkeys, and rabbits. A few of the studies are presented in this report; reviews by Golberg (1986), Dellarco et al. (1990), and IARC (1994) are sources for additional information.

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TABLE 2-18 Inhalation Exposure to Ethylene Oxide: Summary of Carcinogenicity Studies

Animal Description				Response		
Species/ Strain	Sex	Number in Group	Exposure Protocol	Tissue and Tumor Type	Incidence[a]	Reference
Mouse/A/J	F	30	0, 70, or 200 ppm, 6 h/d, 5 d/wk, for 6 mon	Lung adenoma	28%, 56%, and 87%; 0.46, 0.86,* 2.14* tumors/mouse	Adkins et al. 1986
Mouse/ $B6C3F_1$	M	50	0, 50, or 100 ppm, 6 h/d, 5 d/wk, for 102 wk	Alveolar or bronchiolar adenoma or carcinoma	11/50, 19/50, and 26/50*	NTP 1987
	M	50	0, 50, or 100 ppm, 6 h/d, 5 d/wk, for 102 wk	Harderian gland papillary cystadenoma	1/43, 9/44,* and 8/42*	NTP 1987
	F	50	0, 50, or 100 ppm, 6 h/d, 5 d/wk, for 102 wk	Alveolar or bronchiolar adenoma or carcinoma	2/49, 5/48, and 22/49*	NTP 1987
	F	50	0, 50, or 100 ppm, 6 h/d, 5 d/wk, for 102 wk	Harderian gland papillary cystadenoma	1/46, 6/46, and 8/47*	NTP 1987
	F	50	0, 50, or 100 ppm, 6 h/d, 5 d/wk, for 102 wk	Uterus, adenoma or adenocarcinoma	0/49, 4/47, and 5/49*	NTP 1987
	F	50	0, 50, or 100 ppm, 6 h/d, 5 d/wk, for 102 wk	Malignant lymphoma	9/49, 6/48, and 22/49*	NTP 1987
Rat, F344	M	50	0, 0, 10, 33, or 100 ppm, 6 h/d, 5 d/wk, 2 y	Spleen, mononuclear cell leukemia[c]	5/48, 8/49, 9/51, 12/39,*[b] and 9/30*[b]	Snellings et al. 1984b
	M	100	0, 0, 10, 33, or 100 ppm, 6 h/d, 5 d/wk, 2 y	Brain, gliomas[d]	1/181 (both control), 0/92, 3/85, 6/87*	Garman et al. 1985
	M	50	0, 0, 10, 33, or 100 ppm, 6 h/d, 5 d/wk, 2 y	Skin, subcutis fibroma[c]	2/49, 1/48, 9/51,* 1/39, and 11/30*	Snellings et al. 1984b
	F	50	0, 0, 10, 33, or 100 ppm, 6 h/d, 5 d/wk, 2 y	Spleen, mononuclear cell leukemia[c]	6/56, 5/60, 11/54, 14/48,* and 15/26*	Snellings et al. 1984b
	F	100	0, 0, 10, 33, or 100 ppm, 6 h/d, 5 d/wk, 2 y	Brain, gliomas[d]	0/187, 1/94, 2/90, and 2/78	Garman et al. 1985
Rat, F344	M	80	0, 50, or 100 ppm 7 h/d, 5 d/wk, 104 wk	Brain, mixed cell glioma	0/76, 2/77, and 5/79*	Lynch et al. 1984a

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	M	80	0, 50, or 100 ppm 7 h/d, 5 d/wk, 104 wk	Body cavity, peritoneal mesothelioma	3/78, 7/79, and 21/79*	Lynch et al. 1984a
	M	80	0, 50, or 100 ppm 7 h/d, 5 d/wk, 104 wk	Spleen, mononuclear cell leukemia	24/77, 38/79,* and 30/76	Lynch et al. 1984a

[a]Tumor incidence presented in order of exposure groups as shown in "Exposure Protocol" column.
[b]Statistically significant when compared with the combined control groups.
[c]Incidences based on number of rats killed at 24 months.
[d]Incidences based on number of animals at risk.
*$p < 0.05$, test group compared with control.

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3.6.1. Germ Cells

Table 2-19 summarizes the data on genetic toxicity in germ cells in rats and mice.

Sega et al. (1988) exposed male (C3H × B/10)F_1 mice to ethylene oxide at concentrations of 450 ppm (810 mg/m^3) for 4 h, 900 ppm (1620 mg/m^3) for 2 h, or 1,800 ppm (3240 mg/m^3) for 1 h and showed increased DNA strand breaks and unscheduled DNA synthesis as measured by incorporation of [^{3}H]thymidine into DNA. An exposure-rate effect was observed; 1,800 ppm for 1 h was more effective than 900 ppm for 2 h, which was more effective than 450 ppm for 4 h.

Sega et al. (1991) also examined the effect of exposure rate on DNA alkylation of reproductive targets (sperm and testes) and hemoglobin. Male (C3HRl × B/10Rl)F_1 mice were exposed to [^{3}H]ethylene oxide at 75 ppm for 4 h, 150 ppm for 2 h, or 300 ppm for 1 h (300 ppm × hours); alkylation of DNA was measured 90 min and 1, 3, and 6 days after terminating exposure. This study showed that epididymal and vas sperm were alkylated by ethylene oxide; the amount of alkylation was greater in epididymal sperm than in vas sperm, suggesting a greater susceptibility in developing sperm. Alkylation of both epididymal and vas sperm increased with exposure rate. There was no suggestion of repair, as the binding level did not decrease as a function of time after exposure. Alkylation of hemoglobin also showed an exposure rate effect, with no decrease with time after exposure.

The dominant-lethal assay is one test used to screen for mutagenicity in germ cells. Embree et al. (1977) showed that dominant lethality is induced in male Long-Evans rats exposed to ethylene oxide at 1,000 ppm (1,800 mg/m^3) for 4 h. The exposed males were mated with female rats each week for 10 consecutive weeks. Significant increases in postimplantation deaths were observed during the first 5 weeks of mating for ethylene oxide–exposed rats compared with controls. Postimplantation deaths are indicated by an increased number of dead implants per female (weeks 2, 3, and 5) and number of dead implants per total implants (mutagenic index) (weeks 1, 2, 3, and 5). There was a significant decrease in the fertility index (number of pregnant females per number of females mated) during weeks 3 and 4 and in the total number of implants per total number of pregnancies during week 2. Preimplantation losses were not affected. The increase in postimplantation deaths during the first 5 weeks suggests that ethylene oxide affected germ cells after meiosis.

Generoso et al. (1983) repeatedly exposed (101 × C3H)F_1 male mice to 255 ppm (459 mg/m^3) for 6 h/day, 5 days/week for 2 or 11 weeks and evaluated dominant lethality after mating the exposed males with (C3H ×C57BL)F_1 females for 3.5 days after the last exposure. Both treatments produced marked increases in the number of dead implants (average = 37% and 50% after 2 and 11 weeks, respectively) and dominant lethality (average = 39% and 55% after 2 and 11 weeks, respectively). The effect after 11 weeks of treatment was slightly greater than that after 2 weeks.

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TABLE 2-19 Genotoxic Effects of Inhaled Ethylene Oxide on Germ Cells in Male Rodents

Species and Strain	Assay	Experimental Protocol	C × t	Results	Reference
Rat, Long-Evans	Dominant lethality[a]	1,000 ppm for 4 h; mated with females weekly for 10 wk	4,000 ppm-h	Positive: increase in dead implants per pregnancy (wk 2, 3, 5) and dead implants per total implants (wk 1, 2, 3, 5)	Embree et al. 1977
Mouse, (C3H × B110)F_1	DNA strand breaks and UDS	450 ppm for 4 h, 900 ppm for 2 h, or 1,800 ppm for 1 h	1,800 ppm-h	Positive: DNA strand breaks and UDS; exposure-rate effect: 1,800 ppm >900 ppm >450 ppm	Sega et al. 1988
Mouse, (C3H × B110)F_1	DNA alkylation of sperm and hemoglobin	75 ppm for 4 h, 150 ppm for 2 h, or 300 ppm for 1 h	300 ppm-h	DNA alkylation of epididymal and vas sperm and hemoglobin	Sega et al. 1991
Mouse, (101 × $C3HF_1$)	Dominant lethality[b]	255 ppm, 6 h/d, 5 d/wk for 2 or 11 wk	15,300 ppm-h or 84, 150 ppm-h	Positive: dominant lethals produced after 2 (39%) and 11 (55%) wk	Generoso et al. 1983
Mouse, (C3H × 101)F_1	Dominant lethality	Control, 300, 400, or 500 ppm, 6 h/d for 4 d	7,200 ppm-h 9,600 ppm-h, 12,000 ppm-h	Positive: exposure-related increase; 4%, 27%, and 62% dominant lethality	Generoso et al. 1986
Mouse, (C3H × 101)F_1	Dominant lethality	Control, 300 ppm for 6 h/d, 600 ppm for 3 h/d, or 1,200 ppm for 1.5 h/d for 4 d	1,800 ppm-h	Positive: exposure-rate increase; 11%, 32%, and 64% dominant lethality	Generoso et al. 1986
Mouse, (C3H × 101)F_1	Dominant lethality	Control, 165, 204, 250, or 300 ppm 6 h/d, 5 d/wk for 6 wk, then 7 d/wk for 2.5 wk	47,025-85,500 ppm-h	Positive: dose-related increase; 6%-8%, 13%-14%, 23%-24%, and 45%-60% dominant lethality	Generoso et al. 1990
Mouse, (C3H ×101)F_1	Heritable translocation	Control, 165, 204, 250, or 300 ppm 6 h/d, 5 d/wk for 6 wk, then 7 d/wk for 2.5 wk	47,025-85,500 ppm-h	Positive: dose-related increase; 0.05%, 2.80%, 5.09%, 10.84%, and 25.53% translocation carriers in combined female strains	Generoso et al. 1990

[a]Defined as number of dead implants per total implants.
[b]Defined as average number of living embryos in experimental group per average number for controls.
Abbreviation: UDS, unscheduled DNA synthesis.

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Generoso et al. (1986) also conducted dominant lethality tests with (C3H × 101)F_1 male mice exposed to ethylene oxide at 300, 400, or 500 ppm (540, 720, or 900 mg/m^3) for 6 h/day for 4 days; the total concentrations were 7,200, 9,600, or 12,000 ppm-h. Each exposure group was accompanied by a control. The treated animals were mated each day with a different female ([SEC × C57BL]F_1) starting on the day after exposure ended and continuing for 12 days (500 ppm) or 8 days (300 and 400 ppm). The results showed that the maximum effects occurred during mating days 4.5 to 7.5 at 500 ppm; a marked decrease was observed for the number of living embryos and marked increases were observed in the number of dead implants and the number of females with one or more dead implants. Clear, but less pronounced, effects were seen at 400 ppm and only marginal effects were seen at 300 ppm. The overall dominant lethality showed a clear concentration-response relationship.

In another study, Generoso et al. (1986) examined the effect of exposure rate on dominant lethality. Male mice of the same hybrid strain were exposed to ethylene oxide at 300 ppm for 6 h/day, 600 ppm for 3 h/day, or 1,200 ppm for 1.5 h/day for 4 days (total concentration = 1,800 ppm-h/day). The exposed males were mated with (SEC ×C57BL)F_1 females starting 5 days after the last exposure. Each exposure group was accompanied by a control. A clear exposure-related increase in the frequency of dominant lethality was observed.

In 1990, Generoso et al. evaluated the effect of inhaled ethylene oxide on dominant lethality and heritable translocations. Groups of (C3H × 101)F_1 male mice were exposed to ethylene oxide at 165, 204, 250, or 300 ppm (297, 367, 450, or 540 mg/m^3) for 6 h/day, 5 days/week, for 6 weeks followed by exposure for 7 days/week for 2.5 weeks. During the last 10 days of exposure and 1 day after the last exposure, the male mice were mated with T-stock or (SEC × C57BL)F_1 females. No significant dominant lethality was seen at 165 ppm in either strain as assessed by the number of live embryos. At 204 ppm, there was a significant decrease in the number of live embryos in one strain and a significant increase in the number of females with one or more dead implants in both strains; this dose showed an overall marginal effect on dominant lethality. At 250 and 300 ppm, clear effects on dominant lethality were indicated in both strains by decreases in the number of live embryos, increases in the number of dead implants, and increases in the number of females with one or more dead implants. The frequency of dominant lethals showed a concentration-related increase that was not linear.

In the experiment on heritable translocations, Generoso et al. (1990) evaluated the frequency of semisterile and sterile male offspring and analyzed the carriers for translocations. Each female strain had a concentration-related increase in the frequency of translocation carriers; the increases achieved statistical significance ($p < 0.01$ compared with controls) at all concentrations. The response curves were not linear.

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3.6.2. Somatic Cells

Other genetic toxicity tests including SCE and chromosome aberration have been performed on peripheral lymphocytes and bone marrow cells in laboratory animals exposed to ethylene oxide by inhalation. Kligerman et al. (1983) compared the frequencies of SCEs in peripheral lymphocytes taken from male F344 rats exposed to ethylene oxide at target concentrations of 0, 50, 150, or 450 ppm for 6 h/day for 1 or 3 days. The frequency of SCEs per metaphase significantly increased only at 450 ppm (10.4 versus 7.8 in controls) after a single exposure, whereas the frequencies significantly increased at all concentrations (7.5, 9.1, 10.3, and 13.6 for 0, 50, 150, and 450 ppm, respectively) after three exposures. The frequency of SCEs was similar for a single exposure to 450 ppm (2,700 ppm-h) and repeated exposures to 150 ppm (2,700 ppm-h). In addition, only repeated exposures caused increases in the number of high-frequency cells with ≥20 SCEs per metaphase.

A study conducted by Ong et al. (1993) showed that SCEs are induced in spleen and bone marrow cells of male F344 rats exposed to ethylene oxide at 100 ppm for 6 h/day, 300 ppm for 2 h/day, or 600 ppm for 1 h/day, 5 days/week, for 3, 6, or 9 months. The frequency of SCEs in spleen cells did not show a clear concentration-response relationship at any time point, but a cumulative response was seen as duration of exposure increased. The frequency of SCEs in bone marrow cells was highest at the lowest concentration and there was no clear increase with duration of exposure.

The frequency of SCEs also increased in lymphocytes of New Zealand White rabbits exposed to ethylene oxide at 200 or 400 ppm, 6 h/day or 1,500 ppm for 15 min two times a day, 5 days/week up to a cumulative concentration of about 48,000 ppm-h (Yager 1987). A clear exposure-rate effect was not observed.

Vergnes and Pritts (1994) reported that male F344 rats and male $B6C3F_1$ mice exposed to ethylene oxide at 200 ppm for 6 h/day, 5 days/week, for 4 weeks had significantly elevated frequencies of micronuclei in polychromatic erythrocytes in the bone marrow. The mean percentage of micronucleated cells was 0.79% for rats (0.30% for controls) and 0.72% for mice (0.22% for controls).

Lynch et al. (1984b) reported that the frequency of SCEs and chromosome aberrations significantly increased at both concentrations in lymphocytes of adult male cynomolgus monkeys exposed to ethylene oxide at 50 or 100 ppm for 7 h/day, 5 days/week, for 2 years. Mitotic activity of the lymphocytes was also reduced.

3.6.3. DNA Alkylation

Ethylene oxide is a reactive epoxide that readily alkylates DNA and proteins without metabolic activation (Golberg 1986). Ehrenberg et al. (1974) de-

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tected radioactive binding to the nucleic acid fraction of tissues from mice exposed to radioactive ethylene oxide at 29 ppm for 82 min and analyzed 73 min after exposure. The relative binding activity in the tissues was as follows: kidney > spleen > lung > liver > testes > brain. Ehrenberg et al. (1974) further identified 7-(2-hydroxyethyl)guanine (7-HEG) as one of the DNA adducts formed after exposure to ethylene oxide. Potter et al. (1989) exposed male F344 rats by nose-only inhalation to [^{14}C]ethylene oxide at 1, 10, or 33 ppm for 6 h and isolated DNA from brain, lung, liver, spleen, kidney, and testes. A linear relationship was observed for the formation of 7-HEG and concentration of ethylene oxide in air. Alkylation frequencies ranged from 0.0786 to 0.118, 0.777 to 0.964, and 3.03 to 3.66 nanomoles of 7-HEG per g of DNA at concentrations of 1, 10, and 33 ppm, respectively, for all tissues except testis, which was 60% lower (0.065, 0.466, and 2.00, respectively). Bolt and Leutbecher (1993) exposed male Sprague-Dawley rats to [^{14}C]ethylene oxide in a closed system until the ethylene oxide disappeared from the atmosphere; a linear increase was again observed for the formation of 7-HEG adducts in liver and spleen (exposure concentration in ppm was not provided). The animals were sacrificed immediately after exposure.

In a time-course study, Walker et al. (1990) exposed male F344 rats to ethylene oxide at 300 or 500 ppm (6 h/day, 5 days/weeks) for 1 or 3 days or 1, 2, or 4 (300 ppm only) weeks and measured DNA adducts in brain, kidneys, liver, lungs, spleen, and testes 1 h after cessation of exposure. DNA adducts were detected in all tissues after the first exposure, with adduct levels increasing with repeated exposure time particularly during the second week after exposure to 500 ppm and after the first exposure at 300 ppm. The highest concentrations were found in the lungs and the lowest concentrations were in testes. The disappearance of adducts after a 4-week exposure to 300 ppm was gradual and linear, with some adducts persisting 10 days after cessation of exposure. The half-life of disappearance of adducts was approximately 7 days.

Walker et al. (1992a) conducted a similar experiment in male B6C3F$_1$ mice exposed to ethylene oxide at 100 ppm for 1 or 3 days or 1, 2, or 4 weeks (6 h/day, 5 days/week) and compared the results with those of male F344 rats exposed to 300 ppm for 4 weeks. DNA adducts were measured in lungs, kidneys, liver, spleen, testes, and brain. In control mice, 2 to 6 picomoles (pmol) of 7-HEG per mg of DNA was detected. At the early time points (not further described), formation of 7-HEG adducts was similar to that of controls, but as exposure duration increased adduct formation increased, attaining a steady-state concentration only in lungs by 4 weeks. After exposure for 4 weeks, 7-HEG adducts showed a greater persistence in rat tissues than in the mouse, except for the kidney. The half-life of adduct disappearance was 6.9 days in mouse kidney and 1 to 2.3 days in other tissues in the mouse. In this study, the half-life of disappearance of DNA adducts in rats ranged from about 2.9 to 5.8 days in all tissues. Two minor adducts (O^6-(2-hydroxyethyl)guanine [O-HEG] and 3-(2-hydroxyethyl)guanine [3-HEG)]) were also detected in the rats exposed to 300 ppm for 1 to 4 weeks. Steady-state concentrations of 1.0-1.2 pmol of O-HEG per

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mg of DNA was achieved by 2 weeks in brain, kidney, lung, and spleen, whereas steady state was achieved after a few days for 3-HEG adducts (1 pmol of adduct per mg of DNA). After a 4-week exposure the concentration of these adducts was 250- to 300-fold lower than the concentration of 7-HEG.

Walker et al. (1992a) conducted another study with male mice and rats exposed to ethylene oxide at 0, 3, 10, 33, or 100 ppm for 6 h/day, 5 days/week, for 4 weeks. Another group of rats was exposed similarly to 300 ppm. After a 4-week exposure to 100 ppm, formation of 7-HEG adducts was similar in all mouse tissues (21 to 38 pmol of 7-HEG per micromole [µmol] of guanine), with testes containing the lowest level. In rats exposed to 100 ppm for 4 weeks, 7-HEG formation was lower in liver, kidney, and testes (44 to 55 pmol of 7-HEG per µmol of guanine) than in other tissues (81 to 105 pmol of 7-HEG per µmol of guanine); the lowest amount was found in testes. The dose-response relationship for 7-HEG formation was nonlinear for rats and mice (lung, brain, and spleen).

3.7. Summary

Acute lethality data are summarized in Table 2-20. Mice are the most sensitive species, followed by dogs and rats. The LC_{50} values for 4-h exposures ranged from 660 ppm for female mice to 1,972 ppm for male rats, and 1-h LC_{50} values ranged from 4,439 ppm for female rats to 5,748 ppm for male rats; a 1-h study was not available for the mouse. The lowest concentration causing death in a 4-h exposure study was 533 ppm (20% mortality) for female mice. A slightly higher concentration of 800 ppm causes 100% mortality in male mice. The lowest concentration causing death in a study of 1-h exposure of female rats was 3,966 ppm (40% mortality). Lethal concentrations of ethylene oxide vapor are irritating to the eyes and upper and lower respiratory tract. Death in all cases did not appear to involve severe respiratory tract irritation but was due to respiratory failure, probably involving the central nervous system (Golberg 1986). Lethal concentrations of ethylene oxide also cause neurologic effects manifested by absence of tail, toe pinch, and startle reflexes; ataxia; semiconsciousness; and convulsions. In addition, vomiting occurs in dogs and diarrhea occurs in rats and dogs, which also may be due to a neurologic mechanism and not to a direct effect on the gastrointestinal tract. Pathologic lesions develop in the liver, kidney, and respiratory tract of animals exposed to lethal concentrations.

Clinical signs observed in animals that survived a single exposure to ethylene oxide vapor were similar to those observed in animals that died. Eye and respiratory tract irritation and neurologic signs were the primary effects observed in animals surviving exposure to ethylene oxide. The effects were usually reversible within a few days after exposure, depending on the concentration of ethylene oxide vapor. Several studies on repeated exposures (6 h/day, 5 days/week) to ethylene oxide vapor were available; durations ranged from

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TABLE 2-20 Summary of Lethality Data for Experimental Animals

Species and Sex	LC_{50}[a]		Exposure	Comments	Reference
	ppm	mg/m³	Time (min)		
Rat, male	1,460	2,630	240	Lowest experimental concentration causing death was 882 ppm (20%)	Jacobson et al. 1956
Rat, male	1,972	3,550	240	Lowest experimental concentration causing death was 2,026 ppm (80%); no deaths at 1,850 ppm	Nachreiner 1991
Rat, female	1,537	2,767	240	Lowest experimental concentration causing death was 1,443 ppm (20%); no deaths at 1,021 ppm	Nachreiner 1991
Rat, male and female	1,741	3,134	240	No comments	Nachreiner 1991
Rat, male	5,748	10,346	60	Lowest experimental concentration causing death was 5,546 ppm (20%); no deaths at 4,827 ppm	Nachreiner 1992
Rat, female	4,439	7,990	60	Lowest experimental concentration causing death was 3,966 ppm (40%); no deaths at 3,609 ppm	Nachreiner 1992
Rat, male and female	5,029	9,052	60	No comments	Nachreiner 1992
Mouse, female	835	1,504	240	Lowest experimental concentration causing death was 533 ppm (20%); lowest concentration tested	Jacobson et al. 1956
Mouse, male	ND	ND	240	LC_{50} was not calculated; 100% mortality at 800 ppm; no deaths at 400 ppm	NTP 1987
Mouse, female	660	1,188	240	Lowest experimental concentration causing death was 800 ppm (80%); no deaths at 400 ppm	NTP 1987
Dog, male	960	1,730	240	No deaths occurred at 710 pm	Jacobson et al. 1956
Guinea pig	ND	ND	480	1,300 ppm caused death	Waite et al. 1930
Guinea pig	ND	ND	330	3,000 ppm caused death	Waite et al. 1930
Guinea pig	ND	ND	190	3,000 ppm caused death	Waite et al. 1930
Guinea pig	ND	ND	150	7,000 ppm caused death	Waite et al. 1930
Guinea pig	ND	ND	60	25,000 ppm caused death	Waite et al. 1930
Guinea pig	ND	ND	10	63,000 ppm caused death	Waite et al. 1930

[a] LC_{50} (concentration with 50% lethality) or percentage mortality at the lowest experimental concentration causing death.
Abbreviation: ND, not determined.

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6 weeks with dogs, to 10 to 14 weeks with rats and mice, to 24 months with monkeys. Clinical signs observed after repeated exposures were similar to those observed after a single exposure. However, respiratory tract irritation progressed to secondary effects; neurologic effects progressed to hindlimb weakness, muscle atrophy, and paralysis, depending on the concentration. Growth retardation, mild anemia, and pathologic lesions in adrenal gland, thymus, nasal cavity, kidney, and spleen occurred after repeated exposures to ethylene oxide vapor. Neurologic effects, including hindlimb paralysis, were reversible and resolved several months after exposure was terminated. Neurotoxicity studies showed effects related to locomotion, arousal, approach response, and closed eyes after a single exposure and grip strength after repeated exposure for 4 or 14 weeks. Neuropathologic effects included axonal degeneration of myelinated fibers in the fasciculus gracilis and hindlimb nerves in rats after exposure for 13 weeks and axonal dystrophy in the medulla oblongata and demyelination of the fasciculus gracilis in monkeys after exposure for 24 months.

Developmental and reproductive toxicity studies using rats, mice, and rabbits exposed to ethylene oxide are summarized in Table 2-21. There are some inconsistencies in the developmental toxicity studies. The studies by Snellings et al. (1982a) and BRRC (1993) showed developmental effects at ≥50 ppm in rats exposed for 6 h/day (300 ppm-h), whereas the study by Saillenfait et al. (1996) showed developmental effects only at ≥800 ppm in rats exposed for 0.5 h three times per day (1,200 ppm-h). The difference is not due to strain sensitivity, because the BRRC (1993) and Saillenfait et al. (1996) studies used Sprague-Dawley rats. No developmental effects were observed in rabbits exposed to 150 ppm. Reproductive toxicity studies showed effects at ≥50 ppm in rats and ≥200 ppm in mice after repeated exposures. The study by Weller et al. (1999) in mice, which is summarized in Table 2-17, showed developmental effects manifested as resorptions, growth retardation, and eye defects after a single inhalation exposure to ethylene oxide at ≥350 ppm.

Ethylene oxide is a direct alkylating agent that is genotoxic in numerous in vitro and in vivo test systems. Ethylene oxide vapor is genotoxic in mammalian germ cells as evidenced by induction of dominant lethality, heritable translocations, DNA strand breaks, and unscheduled DNA synthesis (see Table 2-19). It is genotoxic in somatic cells as indicated by induction of SCEs, chromosome aberrations, or micronuclei in peripheral lymphocytes, spleen cells, or bone marrow cells. In addition to its genotoxic activity in somatic cells, ethylene oxide is carcinogenic in mice and rats. Positive results have been obtained with the mouse lung tumor bioassay (70 ppm) and the standard 2-year bioassays in mice and rats at ≥100 ppm. The carcinogenicity results are summarized in Table 2-18. IARC (1994) concluded that the animal data provided sufficient evidence of carcinogenicity of ethylene oxide.

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TABLE 2-21 Developmental and Reproductive Effects of Ethylene Oxide Vapor

Species	Exposure	Effect	Reference
Rat	0, 10, 33, 100 ppm, 6 h/d, GD 6-15	33 ppm, NOEL 100 ppm, mild growth retardation of fetus	Snellings et al. 1982a
Rat	0, 50, 125, 250 ppm, 6 h/d, GD 6-15	50 ppm, slight fetal growth retardation 125 ppm, maternal effects and fetal growth retardation 250 ppm, more severe maternal effects and fetal growth retardation	BRRC 1993
Rat	0, 150 ppm, 7 h/d, 5 d/wk, premating, GD 7-16 or 1-16	Growth retardation of fetus regardless of stage of exposure	Hackett et al. 1982
Rat	0, 400, 800, 1,200 ppm, 0.5 h/d, GD 6-15	No effects on fetus at any concentration	Saillenfait et al. 1996
Rat	0, 200, 400, 800, 1,200 ppm, 0.5 h, 3 times per day, GD 6-15	800 ppm, fetal growth retardation 1,200 ppm, maternal effects and fetal growth retardation	Saillenfait et al. 1996
Mouse	0, 1,200 ppm, 12 h, GD 1	Fetal deaths, hydrops, and other malformations	Rutledge and Generoso 1989
Mouse	0, 200, 400 ppm, 6 h/d, 5, 15, or 25 exposures	200 ppm, abnormal spermatozoa 400 ppm, abnormal spermatozoa	Ribeiro et al. 1987
Rat	0, 10, 33, 100 ppm, 6 h/d, one-generation reproduction	33 ppm, NOEL 100 ppm, reproductive and fetal effects	Snellings et al. 1982b
Rat, males	0, 50, 100, 250 ppm, 6 h/d, subchronic	50 ppm, abnormal sperm, teratic type 100 ppm, abnormal sperm, teratic type 250 ppm, abnormal sperm, testicular degeneration	Mori et al. 1991
Rabbits	0, 150 ppm, 7 h/d, GD 7-19 or 1-19	No developmental effects	Hackett et al. 1982

Abbreviation: NOEL, no-observed-effect level; GD, gestation day.

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4. SPECIAL CONSIDERATIONS

4.1. Metabolism, Disposition, and Kinetics

Ethylene oxide is metabolized or biotransformed primarily by two pathways: hydrolysis and glutathione conjugation. According to Golberg (1986), ethylene oxide is not a substrate for epoxide hydrolase but forms ethylene glycol via nonenzymatic hydrolysis and oxalic acid after several additional steps. Martis et al. (1982) showed that ethylene oxide, administered to dogs by intravenous (i.v.) injection, is rapidly hydrolyzed to ethylene glycol. Tardif et al. (1987) identified the following metabolites in the urine of mice administered ethylene oxide by i.v. injection (20 or 60 mg/kg) or after inhalation exposure (200 ppm for 6 h): 2-hydroxyethylmercapturic acid (HMA), *S*-(2-hydroxyethyl)-L-cysteine, *S*-carboxymethyl-L-cysteine, and ethylene glycol. Only HMA and ethylene glycol were identified in rat urine and ethylene glycol was found in rabbit urine. The metabolites were qualitatively similar for both routes of exposure for each species but were quantitatively different. The proportion of the total dose excreted was small, with the rat excreting the larger portion followed by the mouse and rabbit, which excreted only 2% to 3% of the i.v. or inhalation dose. Tardif et al. (1987) examined the urine for specific metabolites and may not have identified all metabolites, particularly those of ethylene glycol.

Brown et al. (1996) found no difference in the in vitro production of ethylene glycol by heat-inactivated and active mouse liver cytosol, suggesting that production of ethylene glycol was not due to cytosolic epoxide hydrolase activity. Brown et al. (1996) also noted that the high rate of nonenzymatic hydrolysis relative to enzymatic hydrolysis in rats made it impossible to accurately determine the Michaelis constant for enzymatic hydrolysis. Brown et al. (1996) also compared microsomal and cytosolic ethylene oxide metabolizing activity in liver and kidney in rats and mice and found most of the activity in the cytosolic fraction. Therefore, microsomal metabolism, including microsomal epoxide hydrolase activity, would contribute little to ethylene oxide metabolism.

Ethylene oxide reacts with chloride ions to form 2-chloroethanol followed by glutathione conjugation (glutathione *S*-transferase) and the formation of *S*-(2-hydroxyethyl)-L-cysteine (Golberg 1986; Fennell 1996). Metabolism of ethylene oxide via the glutathione-*S*-transferase pathway is saturable because of the depletion of glutathione. McKelvy and Zemaitis (1986) demonstrated that glutathione in various tissues in the rat and mouse was depleted after 4-h exposures to 1,200 and 900 ppm, respectively. Glutathione was depleted by as much as 82% to 85% in the liver of both species. Glutathione levels returned to control levels within 24 h after exposure in all tissues except bone marrow and testes in the rat and bone marrow, testes, and lungs in the mouse. Physiologically-based pharmacokinetic (PBPK) model simulation of rats exposed to ethylene oxide at 100, 600, and 1,200 ppm for 4 h estimated glutathione depletion to be 10% to 15% in the liver, 60% to 70% in the lungs, and 60% to 80% in the testes (Krishnan et al. 1992). PBPK modeling of mice exposed to ethylene oxide for 4 h es-

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timated glutathione depletion to be about 27%, 51%, 79%, and 83% in the liver and 45%, 62%, 78%, and 79% in the lungs at 100, 200, 300, and 400 ppm, respectively. Glutathione was depleted by 41% and 63% in kidney and by 19% and 15% in testes at 300 and 400 ppm, respectively (Brown et al. 1998). In 1996, Fennell reported that PBPK models indicated that glutathione conjugation accounted for about 10% of ethylene oxide metabolism in humans, 50% in rats, and 75% in mice, with most of the remaining ethylene oxide undergoing nonenzymatic hydrolysis. Fennell and Brown (2001) estimated the relative contributions of conjugation, hydrolysis, and exhalation of unchanged ethylene oxide at concentrations up to 600 ppm and showed that at ≤200 ppm about 80% of ethylene oxide is metabolized by glutathione conjugation and <60% is metabolized at concentrations up to 600 ppm in mice. In rats, about 60% of ethylene oxide at concentrations ≤ 400 ppm is metabolized by glutathione conjugation and a slightly lower percentage is metabolized at 600 ppm. In humans, only about 20% of ethylene oxide is metabolized by glutathione conjugation at concentrations ≤ 600 ppm. The remaining ethylene oxide undergoes hydrolysis or is exhaled unchanged. Hydrolysis accounted for about 10% and 20% of ethylene oxide biotransformation in mice exposed to concentrations up to 200 and 600 ppm, respectively, 30% to 35% in rats, and 60% in humans.

Brugnone et al. (1986) studied workers exposed to ethylene oxide at 0.2 to 22.5 mg/m^3 (0.11 to 12.3 ppm) and reported that, at steady state, 75% to 80% of inhaled ethylene oxide is absorbed into the body. The concentration of ethylene oxide in alveolar air ranged from 0.05 to 7 mg/m^3 (0.03 to 3.8 ppm). The venous blood:alveolar air coefficients ranged from 12 to 17 and the venous blood:environmental air coefficients ranged from 2.5 to 3.3. Brugnone et al. (1986) calculated a mean absorption of 7.2 to 7.7 mg of ethylene oxide for an 8-h exposure to 2 mg/m^3 (1.11 ppm) at an alveolar ventilation rate of 10 L/min. Filser et al. (1992) used the data presented by Brugnone et al. (1986) to calculate a mean half-life of 42 min for ethylene oxide in humans.

Maples and Dahl (1993) reported that blood uptake gradually increased during the first 15 min and reached a plateau at about 60 ng per g of blood in male F344 rats exposed to ethylene oxide vapor at 5 ppm for 60 min. Blood concentrations in mice after inhalation exposure to 50, 100, 200, 300, and 400 ppm for 4 h were relatively constant during exposure to ≤200 ppm but continued to rise during exposure to concentrations exceeding 200 ppm (Brown et al. 1998). The terminal blood concentrations were linear up to 200 ppm but showed a definite deviation from linearity above 200 ppm. The data on glutathione depletion explain the nonlinear increase in ethylene oxide concentration in the blood of mice exposed to >200 ppm. On the basis of PBPK modeling, Fennell and Brown (2001) estimated that ethylene oxide concentrations in the blood of mice, rats, and humans would be similar up to 100 ppm, followed by a steep increase in mice at concentrations above 200 ppm and a continued linear increase in humans and rats. Ehrenberg et al. (1974) calculated a biological half-life (t_2) of 9 min for male CBA mice exposed to [1,2-^{3}H]ethylene oxide at concentrations ranging from 1.15 to 33 ppm (average concentrations) for 60 to 107

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min. These investigators inferred that 2.5 µmol/kg (approximately equal to 2.5 µmol/L) is absorbed after an exposure of 1 ppm-h. Ehrenberg et al. (1974) concluded that the degree of protein alkylation could be used to monitor the tissue dose of alkylating agent. Martis et al. (1982) reported a mean half-life of about 33 min for a dog given ethylene oxide by i.v. injection. They also noted that elimination kinetics was not dose dependent in dogs.

Brown et al. (1996) reported t_2 for ethylene oxide clearance from the blood as 13.8 ± 3.0 min for male rats and 10.8 ± 2.4 min for female rats exposed to 100 ppm; similar values were obtained for animals exposed to 330 ppm. For mice, the t_2 was 3.12 ± 0.2 and 5.4 ± 0.5 min for male mice and 2.4 ± 0.2 and 5.6 ± 0.2 min for female mice exposed to 100 and 330 ppm, respectively. The authors noted that the increase in t_2 in mice exposed to 330 ppm was due to saturation of metabolism in mice probably because of glutathione depletion. They measured ethylene oxide concentrations in blood, muscle, brain, and testes 2 to 10 min after a 4-h exposure. Peak tissue concentrations were similar for all tissues except testes, which were 50% and 20% lower in the mouse and rat, respectively, than in other tissues. Ethylene oxide concentrations were slightly higher in the tissues of the rat (except for testes) exposed to 100 ppm than in mice, whereas at 330 ppm, the concentrations were slightly higher in mice than in rats. The authors also noted that clearance of ethylene oxide from the tissues was similar to that from blood.

In the PBPK model developed by Csanady et al. (2000), systemic uptake of ethylene oxide in blood was predicted to be 58% in rats and 79% in humans, with half-lives of about 19 min and 1 h, respectively. This model also predicted that 92% of ethylene oxide in humans is metabolized and about 8% is exhaled unchanged.

Simulation of tissue distribution showed that uptake into and elimination from tissues (peak ethylene oxide concentration) and blood, except for testes, is similar within each species of mice and rats exposed to 100 or 330 ppm for 4 h (Brown et al. 1996). This study demonstrated that blood concentrations are representative of the concentration of ethylene oxide in other tissues. Fennell and Brown (2001) simulated blood concentration, area under the curve (AUC), and dose in mg/kg by PBPK modeling. The model used pulmonary uptake in mouse obtained from nose-only exposures (40%) and in rats obtained from nose-only (43%) and unrestrained exposures (60%). Pulmonary uptake in humans was set at 78%. The model output underestimated the parameters for restrained and unrestrained rats; the results presented below are for unrestrained rats compared with humans and restrained rats compared with mice. After 6-h exposures to 100 ppm, the model shows that the peak blood concentration was 10% less, the AUC was 14% less, and the dose in mg/kg was 70% less in humans than in rats. The model showed that the peak blood concentration and AUC were almost identical, but the dose in mg/kg is about 70% less in restrained rats than in restrained mice. The AUC and peak blood concentration in restrained rats were below the levels in unrestrained rats. Of the three parameters modeled, dose in mg/kg ap-

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peared to best represent the species differences for mice, rats, and humans. The ratio of the dose in mg/kg/day was 8:2:1 for mice:rats:humans. Therefore, the PBPK model showed that (based on pharmacokinetics) humans are not likely to be more sensitive than rodents at concentrations up to 100 ppm and that humans are pharmacokinetically more like rats than mice. Fennell and Brown (2001) did not model concentrations above 100 ppm to compare concentrations that saturate the glutathione-*S*-transferase pathway in mice. Under saturating concentrations, the difference between rats and humans compared with mice is expected to be greater.

Osterman-Golkar et al. (1976) demonstrated that alkylation of hemoglobin in mice could be used as a measure of exposure to ethylene oxide. Potter et al. (1989) reported that hemoglobin adduct formation showed a linear trend and no evidence of saturation in male F344 rats exposed to 0, 3, 10, or 33 ppm for 6 h. Adduct formation measured as nmol of N^1-(2-*N*-hydroxyethyl)histidine per g of globin was 0.136, 1.03, and 4.64 for 3, 10, and 33 ppm, respectively. Walker et al. (1992b) also showed that hemoglobin adduct formation exhibited a linear trend in mice and rats exposed to ethylene oxide at 3 to 33 ppm (6 h/day, 5 days/week, for 4 weeks) but was nonlinear over the exposure ranges 3 to 100 ppm for mice and 3 to 300 ppm for rats.

Mayer et al. (1991) reported that ethylene oxide concentration in the workplace was significantly associated with *N*-hydroxyethylvaline (HEV) hemoglobin adducts after adjusting for smoking. In a review of the literature, Kolman et al. (2002) noted that two investigators reported similar levels of HEV hemoglobin adduct formation in individuals when occupational exposure to ethylene oxide was standardized to 1 ppm. Hemoglobin adduct formation also correlated with the smoking status of individuals in the population exposed to ethylene oxide via conversion of ethylene found in cigarette smoke (Kolman et al. 2002). Because ethylene oxide is metabolized by glutathione conjugation, the level of HEV adducts also was correlated with the polymorphic genotype, with higher levels found in homozygous nonconjugators compared with heterozygous or homozygous conjugators. Fennell et al. (2000) studied the level of HEV adducts in smokers and nonsmokers and noted that the *GSTT1* genotype but not the *GSTM1* genotype had an impact on the HEV adduct levels. Fennell et al. (2000) concluded that HEV adducts (a measure in internal dose levels) were increased by 50% to 70% in smokers who had the *GSTT1*-null genotype (nonconjugators). Yong et al. (2001) reported that workers with the *GSTT1*-null genotype (nonconjugators) exposed to ethylene oxide had significantly higher (2-fold) HEV levels than conjugators. Ehrenberg and Tornqvist (1995) concluded that the blood dose in humans would be about the same as that in the rodents. They noted uncertainty in their estimate because of the uncertainty in determining time-weighted occupational exposures for humans. Their estimates of HEV adduct formation per 1 ppm-h were12, 16, and 12 pmol/g/ppm-h for humans, rats, and mice, respectively.

Tavares et al. (1994) studied HEV hemoglobin adducts in blood samples taken from smoking and nonsmoking mothers and their newborn infants (<48 h

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old). Cigarette smoke contains ethylene and ethylene oxide, with ethylene occurring in much higher levels than ethylene oxide. The investigators found that the level of hemoglobin adducts in newborns of smokers was significantly higher than in those of nonsmokers, and a linear relationship was found for maternal and newborn HEV levels. The newborn adduct levels, however, were lower than the maternal levels, with the ratio of maternal:newborn ranging from 1.1 to 3.1 (mean = 1.8) for nonsmokers and 1.4 to 4.1 (mean 2.7) for smokers.

Farmer et al. (1996) studied HEV hemoglobin adducts in maternal blood from smokers and nonsmokers along with the adduct levels in umbilical cord (fetal) blood. They found a linear relation between the maternal and fetal HEV hemoglobin adduct levels, with higher levels found in maternal blood at a ratio of 2.7 to 2.8. This study also showed a linear correlation between maternal and newborn HEV adduct levels. The *GSTM1* genotype has no statistically significant influence on the adduct levels in smokers or nonsmokers. Both studies showed a linear correlation between maternal and fetal and newborn HEV hemoglobin adducts. The differences between maternal and newborn levels may have been due to differences in the makeup of the polypeptide chains of the maternal and fetal and newborn hemoglobin.

Mayer et al. (1991) noted that the hemoglobin adduct level correlated with the frequency of SCEs in peripheral lymphocytes. Yong et al. (2001) found a significant association between ethylene oxide exposure and frequency of SCEs after adjusting for smoking and other confounders. The frequency of SCEs was unexpectedly lower in nonconjugators (*GSTM1*-null genotype) than in conjugators (*GSTM1* genotype) (Yong et al. 2001). The authors noted that the decreased SCEs may have been due to nonchemical specificity of the end point and the lack of expression of the isozyme in lymphocytes.

In summary, evaluation of the uptake, metabolism, and excretion of ethylene oxide showed qualitative similarities among species (humans, rats, and mice). Uptake of ethylene oxide after inhalation is proportionally similar among species. The database showed that humans are more like rats than mice with regard to metabolism (detoxification) of ethylene oxide. Therefore, the rat is a better surrogate than the mouse for AEGL development. HEV adducts in hemoglobin can be used as a measure of exposure and the level of adduct formation correlates with glutathione-*S*-transferase polymorphism; nonconjugators have higher HEV adduct levels than conjugators. These results showed that genotypic diversity of glutathione *S*-transferase can modulate the detoxification of ethylene oxide and should be considered in AEGL development.

4.2. Mechanism of Toxicity

Ethylene oxide is a direct-acting alkylating agent; it alkylates DNA and proteins. Ethylene oxide is also a mild primary irritant and a central nervous system depressant.

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Finelli et al. (1983) noted that distal axonal neuropathy is characterized by primary axonal degeneration with secondary demyelination affecting distal segments of long tract fibers without involving the neuronal bodies. They postulated that ethylene oxide affects the peripheral and central nervous systems by interfering with metabolism of neuronal perikaryon or axonal transport, thus inhibiting delivery of essential metabolites to nerve terminals. Deschamps et al. (1992) noted that workers exposed to ethylene oxide for 4 h/day for 4 days showed signs of proprioceptive axonal neuropathy. Ohnishi et al. (1985) noted axonal degeneration of myelinated nerve fibers in rats after subchronic inhalation exposure to ethylene oxide. Because distal axonal neuropathy (peripheral neuropathy) has been diagnosed in humans exposed to ethylene oxide (Finelli et al. 1983), the mechanism in humans and rats may be similar.

Deschamps et al. (1992) proposed that the alkylating properties of ethylene oxide played a role in respiratory tract irritation. The time of onset of symptoms indicated that direct irritation or caustic injury was not the mechanism causing respiratory tract irritation.

The mechanisms by which ethylene oxide induces developmental and testicular toxicity (not including genetic damage to germ cells) are not known. Protein alkylation may be involved—that is, alkylation of enzymes in testicular toxicity (Mori et al. 1989). It is likely that protein and DNA alkylation also are involved in inducing developmental toxicity.

Genetic toxicity of ethylene oxide is probably mediated by alkylation of DNA or proteins, which can alter the structure and functional activities of genes, chromosomes, and protein. Genetic toxicity indicated by increased frequencies of SCEs, chromosome aberrations, and micronuclei is probably caused by DNA alkylation. Carcinogenicity is probably mediated by genetic toxicity resulting from DNA alkylation.

Generoso et al. (1986) suggested that dominant lethality involves alkylation of chromosomes. In another study, heritable translocations were confirmed by cytogenetic analysis of the offspring, thus showing structural alterations in chromosomes that could be due to DNA alkylation. Although the mechanism by which ethylene oxide induced effects in the zygote leading to fetal deaths, resorptions, and structural fetal defects is not known, Generoso et al. (1987) and Katoh et al. (1989) noted that genetic damage is a likely candidate. However, cytogenetic analysis of zygotes and midgestational fetuses ruled out numerical and structural alterations in chromosomes (Katoh et al. 1989). Russell et al. (1984) also ruled out gene mutations and specific locus mutations. Katoh et al. (1989) proposed that the effects may be mediated by a nonmutagenic process involving changes in gene expression.

4.3. Structure-Activity Relationships

Ethylene oxide is structurally similar to but more toxic than propylene oxide.

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4.4. Other Relevant Information

4.4.1. Species Variability

Acute lethality studies showed that mice are the most sensitive species, followed by dogs and rats. The LC_{50} value was about three times greater for male rats than for female mice exposed for 4 h. It is difficult to compare the toxicity in humans with that of laboratory animals because of the lack of quantitative exposure data, and most human studies involved repeated exposures under occupational conditions.

4.4.2. Susceptible Populations

Developmental toxicity studies showed that the developing embryo and fetus are more sensitive than the adult to toxic effects of ethylene oxide. No studies have been conducted on preweanling or weanling pups to determine whether the young animals are as sensitive to ethylene oxide exposure as the developing embryo and fetus. AEGL-2 values were derived from a developmental toxicity study, thereby incorporating data for the susceptible population.

4.4.3. Concentration-Exposure Duration Relationship

The LC_{50} data for 1- and 4-h inhalation exposures of rats can be used to determine the relationship between exposure concentration and exposure duration for ethylene oxide. The 1-h LC_{50} value is 5,029 ppm (Nachreiner 1992) and the 4-h LC_{50} values are 1,460 (Jacobson et al. 1956) and 1,741 (Nachreiner 1991) ppm. The regression line using the three data points representing only two exposure durations is presented in Figure 2-1. Although only two exposure durations were available for calculating the value of n, this method is better than using the default values of n to derive AEGL values for ethylene oxide. The calculated value of n is 1.2.

4.4.4. Concurrent Exposure Issues

There are no known concurrent exposure issues related to ethylene oxide.

5. DATA ANALYSIS FOR AEGL-1

5.1. Human Data Relevant to AEGL-1

There are no human data directly related to AEGL-1 derivation. Humans have been exposed to ethylene oxide at a wide range of concentrations. These studies did not correlate effects with exposure concentrations.

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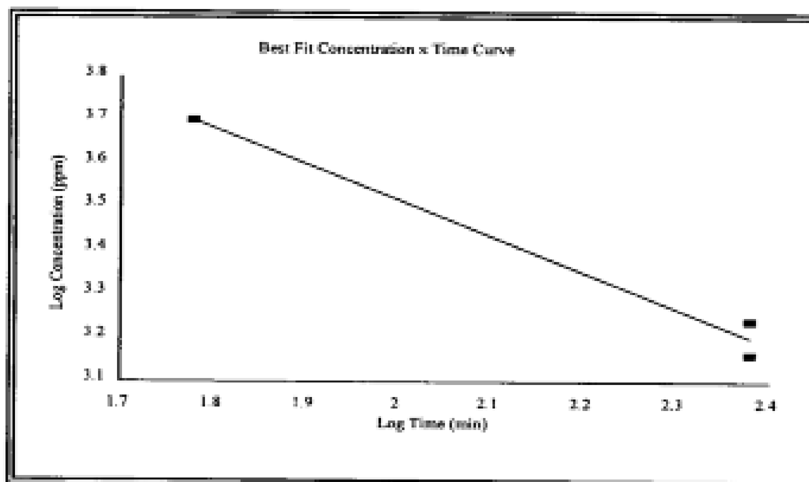

FIGURE 2-1 Rat data: Concentration-time curve for LC_{50} values for ethylene oxide.

5.2. Animal Data Relevant to AEGL-1

The same data sets described for AEGL-2 were considered for deriving AEGL-1 values.

5.3. Derivation of AEGL-1

No AEGL-1 values are recommended (see Table 2-22). The odor threshold and sensory irritation occur at ethylene oxide concentrations higher than those causing systemic effects. In addition, all AEGL-2 values are below the odor threshold. The absence of AEGL-1 values does not imply that exposure below the AEGL-2 level is without adverse effects.

6. DATA ANALYSIS FOR AEGL-2

6.1. Human Data Relevant to AEGL-2

Nonlethal effects of ethylene oxide in humans are summarized in Table 2-6. The epidemiologic studies provided evidence suggesting adverse reproductive outcomes (Hemminki et al. 1982; Rowland et al. 1996); however, these studies had a number of limitations and lacked exposure data for quantitative evaluation of AEGL values. Other nonlethal effects were shown to be reversible upon termination of exposure; however, some effects, particularly those occurring in the

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TABLE 2-22 AEGL-1 Values for Ethylene Oxide

10 min	30 min	1 h	4 h	8 h
Not recommended				

nervous system, are reasons for concern. Several human studies described effects due to ethylene oxide, but only three involved single or very short-term exposures. The study by Deschamps et al. (1992) showed respiratory tract irritation, nonimmunologic asthma, and peripheral neuropathy in one subject accidentally exposed to concentrations at the odor threshold (≥260 ppm) for 4 h/day for 4 days. Respiratory tract irritation could be attributed to each daily exposure, whereas peripheral neuropathy could have been caused by a single exposure and exacerbated upon repeated exposures. Other studies have shown that peripheral neuropathy may be exacerbated by repeated exposures to ethylene oxide (Finelli et al. 1983). The study by Laurent (1988) showed respiratory tract irritation, nervous system effects, and hemolysis in five workers exposed to ethylene oxide at the odor threshold for 30 min. Salinas et al. (1981) described nervous system effects leading to unconsciousness, apnea, and muscle twitching in an individual exposed to a calculated concentration of 500 ppm for 2 to 3 min. There is considerable uncertainty about the exposure concentration reported by Salinas et al. (1981); therefore, this study should not be used to derive AEGL values. In the remaining studies showing nonlethal effects, duration of exposure was not reported or subjects were exposed repeatedly for durations ranging from 2 weeks to more than a year. Genetic lesions in somatic cells were observed in individuals after a single exposure. These genetic lesions are not relevant end points for evaluating AEGL-2 levels, because the only disease state associated with genetic lesions in somatic cells is carcinogenicity.

6.2. Animal Data Relevant to AEGL-2

The primary animal studies that can be used to derive AEGL-2 values are an acute neurotoxicity study in rats (Mandella 1997a), developmental toxicity studies in rats (Snellings et al. 1982a; BRRC 1993; Saillenfait et al. 1996), a developmental toxicity study in mice (Weller et al., 1999), and a reproductive toxicity study in mice (Ribeiro et al. 1987). Acute neurotoxicity in rats included drooping, closed eyelids; impaired locomotion; low arousal; and no approach response after exposure to concentrations ≥ 300 ppm for 6 h (Mandella 1997a). Decreased hindlimb grip strength was observed in rats exposed to 300 ppm for 4 weeks (Mandella 1997b) or to 200 ppm for 14 weeks (Mandella 1997c). Ohnishi et al. (1985) showed that axonal degeneration of myelinated fibers in the fasciculus gracilis and hindlimb nerves appeared to be related to hindlimb ataxia in rats exposed to 500 ppm for at least 9 weeks. A developmental toxicity study in mice showed effects after exposure to 350 or 450 ppm for 6 h or 700 or 900 ppm for 3 h (Weller et al. 1999). Exposure to ethylene oxide > 900 ppm caused ma-

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ternal deaths in mice. The BRRC (1993) and Snellings et al. (1982a) studies showed developmental effects in rats exposed 6 h to 100 ppm and higher during organogenesis. The Saillenfait et al. (1996) study showed developmental toxicity in rats exposed to 800 or 1,200 ppm for 3 h and maternal effects at 1,200 ppm but not at the lower concentrations. The Saillenfait et al. (1996) study had inconsistencies and the results differed considerably from those of other developmental toxicity studies. Although developmental toxicity was observed in the rat after repeated exposures during organogenesis, developmental toxicity was also observed after a single exposure of mice and the critical time for inducing developmental toxicity on GD 7. The critical end point (growth retardation) in the developmental toxicity studies in rats exposed repeatedly also was observed in mice after a single exposure. Therefore, a cumulative effect due to exposure is not necessary to explain growth retardation in rat fetuses. Ribeiro et al. (1987) reported abnormal spermatozoa in mice exposed to 200 or 400 ppm for 6 h/day for only 5 days. The remaining studies on nonlethal toxicity involved repeated exposures of monkeys, rats, mice, and dogs and effects after the first or second exposure were not described.

6.3. Derivation of AEGL-2

AEGL-2 values were based on two rat studies, a neurotoxicity study by Mandella (1997a) and a developmental toxicity study by Snellings et al. (1982a). Rat studies are more appropriate for deriving AEGL-2 values because the exposure concentrations are below saturating levels, and developmental toxicity and neurotoxicity in the rat are the two most sensitive effects caused by exposure to ethylene oxide. The NOAEL for both studies was 100 ppm for a 6-h exposure. In the developmental toxicity study, pregnant female rats were exposed to 0, 10, 33, or 100 ppm for 6 h/day from GD 6 to 15 (Snellings et al. 1982a). Fetal body weight was slightly, but significantly, decreased and the incidence of delayed ossification was increased but not significantly; therefore, the effect at 100 ppm is not considered adverse. In another study, the increased litter incidence of poorly or unossified skeletal areas was statistically significant at 125 ppm and, taken together with decreased fetal body weight, the effects were considered adverse (BRRC 1993). In the acute neurotoxicity study, rats were exposed to 0, 100, 300, or 500 ppm for 6 h before assessment for neurotoxicity. The incidences of two FOB end points (low arousal and no reaction to approach) were significantly increased and the incidences of two additional FOB end points (droopy or half-closed eyelids and impaired locomotion) were increased but not significantly at 300 ppm, the next concentration above the NOAEL. The FOB was assessed after exposure was terminated, and this slight delay may have reduced the magnitude of the response assessed in the FOB evaluation. Three of the FOB end points (low arousal, no reaction to approach response, and impaired locomotion) suggested that ethylene oxide affected mobility of the animals, which was demonstrated explicitly as decreased mobility at 500 ppm. A

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decrease in mobility may affect the ability to escape. More serious neurotoxicity was observed during repeated exposure to ethylene oxide (Ohnishi et al. 1985; Mandella 1997b,c).

The NOAEL of 100 ppm for developmental toxicity and neurotoxicity in the rat is selected as the point of departure (POD) for AEGL-2 development. The benchmark dose approach was not used for AEGL-2 derivation because no single FOB end point was considered more toxicologically significant than another. Each end point showed a clear increasing trend when analyzed separately but not when the data were combined. Therefore, the NOAEL for all the neurotoxic effects was considered appropriate for deriving AEGL-2 values. The rat is considered the most appropriate species for AEGL derivation because pharmacokinetic data and the PBPK models (particularly for glutathione conjugation) show that the rat is more like humans than the mouse. A total uncertainty factor of 10 (3 for interspecies sensitivity and 3 for intraspecies variability) was applied to the POD. An uncertainty factor of 3 was selected for interspecies sensitivity. Very limited information is available on the mechanism of ethylene oxide–induced neurotoxicity in rats or humans; however, similar effects (distal axonal degeneration and neuropathy) have been observed in both species. Ethylene oxide directly alkylates DNA and other macromolecules; this mechanism of toxicity is not expected to differ across species. PBPK models have shown that the AUC, peak blood levels, dose in mg/kg of body weight, and hemoglobin adduct levels (measure of internal exposure) in humans are similar to or lower than those of rats (Fennell and Brown 2001). HEV adduct formation in hemoglobin is proportional to air concentrations across species and adduct formation per ppm-h exposure is similar for rats, mice, and humans (Ehrenberg and Tornqvist 1995). Therefore, the dosimetry is not expected to differ considerably in rats and humans. An uncertainty factor of 3 was selected for intraspecies variability. Glutathione-*S*-transferase polymorphism accounts for some variation within the population. The level of HEV hemoglobin adducts used as a measure of systemic exposure in humans is affected by glutathione-*S*-transferase polymorphism expressed by conjugator (*GSTM1* or *GSTT1*) or nonconjugator (*GSTM1*-null or *GSTT1*-null) genotypes. Muller et al. (1998) examined the HEV hemoglobin adducts in smoking and nonsmoking individuals with conjugator or nonconjugator genotypes and found that the level of HEV hemoglobin adducts in nonsmoking individuals with the *GSTT1*-null genotype was at least twice that in the nonsmoking individuals with the *GSTT1* genotype (Muller et al. 1998). Fennell et al. (2000) also reported that the level of HEV hemoglobin adducts was significantly higher in smoking individuals with the *GSTT1*-null genotype than in individuals with the *GSTT1* genotype. These results indicate that ethylene oxide levels in individuals with the *GSTT1*-null genotype would be higher than in those with the *GSTT1* genotype after exposure to ethylene oxide. However, the variation in exposure as measured by hemoglobin adducts appears to be within a factor of 3. There is no evidence that individuals with respiratory diseases, including asthma, respond differently to ethylene oxide exposure. One

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study showed no effect on an individual with asthma exposed to ethylene oxide at odor detection levels (≥260 ppm) (Deschamps et al. 1992).

Timeframe extrapolation was performed according to ten Berge's equation (ten Berg et al. 1986), where n = 1.2 was derived from the rat lethality data (described in Section 4.4.3). The AEGL-2 value for a 10-min exposure is the same as that derived for a 30-min exposure because of the uncertainty of extrapolating from a 6-h exposure to a 10-min exposure. The resulting AEGL-2 values are summarized in Table 2-23.

AEGL-2 values were derived from acute neurotoxicity and developmental toxicity studies. The NOAEL for developmental toxicity is supported by a study showing growth retardation at 125 ppm (BRRC 1993). The NOAEL of 100 ppm for neurotoxicity is supported by 4- and 13-week neurotoxicity studies (Mandella 1997b,c) that had effects at 200 and 300 ppm and NOAELs at 100 ppm.

7. DATA ANALYSIS FOR AEGL-3

7.1. Human Data Relevant to AEGL-3

No human lethality data are available for deriving AEGL-3 values. Epidemiologic data provided limited evidence that exposure to ethylene oxide is associated with an increased risk of lymphatic and hematopoietic cancer (IARC 1994). Quantitative assessments of human cancer data will not be attempted for data sets providing only limited evidence of carcinogenicity.

7.2. Animal Data Relevant to AEGL-3

Several lethality studies are available for deriving AEGL-3 values. One-hour inhalation studies have been conducted in male and female rats (Nachreiner 1992), and 4-h inhalation studies have been conducted in male and female rats (Jacobson et al. 1956; Nachreiner 1991), male and female mice (Jacobson et al. 1956; NTP 1987), and male dogs (Jacobson et al. 1956). The LC_{50} values varied for the three species studied: mice were slightly more sensitive than dogs, which were more sensitive than rats. All studies were well-conducted; the Nachreiner (1991, 1992) studies were more comprehensive with regard to the end points evaluated. There were some intraspecies variations in the rat and mouse studies. The mice and rats used by Jacobson et al. (1956) were of unspecified strain, the Nachreiner study (1991, 1992) used Sprague-Dawley rats, and the NTP (1987) study used $B6C3F_1$ mice. Therefore, strain differences could have accounted for some of the variations in LC_{50} values. Probit analysis of the dog data consisted of either 100% mortality or 100% survival, thereby producing a highly uncertain LC_{50} value that should be interpreted with caution.

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TABLE 2-23 AEGL-2 Values for Ethylene Oxide

10 min	30 min	1 h	4 h	8 h
80 ppm ($144 mg/m^3$)	80 ppm ($144 mg/m^3$)	45 ppm ($81 mg/m^3$)	14 ppm ($25 mg/m^3$)	7.9 ppm ($14 mg/m^3$)

Long-term exposure studies have shown that inhaled ethylene oxide is carcinogenic to the mouse (Adkins et al. 1986; NTP 1987) and rat (Lynch et al. 1984a; Snellings et al. 1984b; Garman et al. 1985). Concentrations associated with significant increases in tumor incidences are 100 ppm for 6 h/day repeatedly for 2 years or 200 ppm for 6 months.

A study in mice exposed to 1,200 ppm for 1.5 h on the day of mating (Rutledge and Generoso 1989) should be considered when evaluating data pertinent to deriving AEGL-3 values. Rutledge and Generoso (1989) observed a high incidence of late fetal deaths and hydrops. Although the exposure time was critical (the first 24 h after mating) for inducing these effects, any value derived for AEGL-3 should be protective of the zygote.

7.3. Derivation of AEGL-3

Lethality thresholds (LC_{01}) were derived from the mouse, rat, and dog data (Table 2-24) The LC_{01} for dogs exposed for 4 h is much lower than the values obtained for the other species; however, the results from the dog data have a higher degree of uncertainty than the results for other species. The LC_{01} values derived from the mouse and rat data range from 264 to 922 ppm for a 4-h exposure, with the mouse values being lower than those for the rat.

The rat is not the species most sensitive to acute inhalation exposure to ethylene oxide, but it is the more appropriate species for deriving AEGL-3 values, because pharmacokinetic data indicate that the rat is more like humans than the mouse. The rat study by Jacobson et al. (1956) was selected to derive the AEGL-3 values because it presented a better dose-response relationship and a more conservative POD than the Nachreiner (1991, 1992) studies. The LC_{01} value of 628 ppm is the POD derived from the Jacobson et al. (1956) rat data. The LC_{01} is an estimate of the lethality threshold and is below the lowest concentration causing death. The uncertainty factors are 3 for interspecies sensitivity and 3 for intraspecies variability (total = 10). Death after ethylene oxide exposure is attributed to respiratory failure, probably involving the central nervous system (Golberg 1986). Although the exact mechanism of these effects is not known, alkylation of macromolecules may be involved and this reaction is not expected to differ considerably among species. In addition, the exposure concentrations were within range for the PBPK model simulations showing linearity of systemic uptake (Fennell and Brown 2001). PBPK models have shown that the AUC, peak blood levels, dose in mg/kg, and hemoglobin adduct levels (measure

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TABLE 2-24 Estimates of the Threshold for Lethality (LC_{01}) of Ethylene Oxide

Species	Exposure Duration (h)	LC_{50} (ppm)	LC_{01} (ppm)	Reference
Dog	4	960	120	Jacobson et al. 1956
Mouse	4	623[a]	264	NTP 1987
	4	835	406	Jacobson et al. 1956
Rat	4	1460	628	Jacobson et al. 1956
	4	1,741[a]	922	Nachreiner 1991
Rat	1	5,029[a]	2494	Nachreiner 1992

[a]Combined data sets for males and females.
Abbreviations: LC_{50}, concentration with 50% lethality; LC_{01}, concentration with 1% lethality.

of internal exposure) for humans are similar to or lower than the corresponding values for rats (Fennell and Brown 2001). An intraspecies uncertainty factor of 3 was selected because a glutathione-*S*-transferase polymorphism expressed by conjugator (*GSTM1* or *GSTT1*) or nonconjugator (*GSTM1*-null or *GSTT1*-null) genotypes can modulate systemic exposure as measured by hemoglobin adduct levels (Muller et al. 1998). The variation in exposure as measured by hemoglobin adducts in conjugators and nonconjugators appears to be within a factor of 3. There is no evidence that individuals with respiratory diseases, including asthma, respond differently to ethylene oxide exposure. In addition, an interspecies or intraspecies uncertainty factor of 10 would produce10- and 30-min AEGL-3 values far below concentrations expected to be associated with life-threatening events.

The ten Berge equation ($C^n \times t = k$, where n = 1.2) was used to extrapolate to other time points. The value of n was derived empirically from the 1 and 4-h LC_{50} values for rats. The AEGL-3 values for 10 and 30 min and for 1, 4, and 8 h are 360, 360, 200, 63, and 35 ppm. The AEGL-3 value for a 10-min exposure is the same as that derived for a 30-min exposure, because the AEGL values were derived from a 4-h exposure, which increases the uncertainty for deriving the 10-min value. The AEGL-3 values are summarized in Table 2-25.

Ethylene oxide is carcinogenic in laboratory animals. Dose-response data were used to estimate the excess lifetime risk associated with a single exposure to ethylene oxide. Calculations for AEGL-3 estimates are presented in Appendix B of this document. EPA’s linearized multistage model (GLOBAL86) (Howe et al. 1986) was used to derive a unit risk for ethylene oxide from incidence data on alveolar and bronchiolar adenomas and carcinomas in female mice exposed to ethylene oxide vapor for 2 years. This site showed the highest incidence and was therefore considered to be the most sensitive target. AEGL-3 values based on carcinogenicity data are 1,300, 1,300, 640, 160, and 80 ppm for a 10^{-4} risk

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TABLE 2-25 AEGL-3 Values for Ethylene Oxide

10 min	30 min	1 h	4 h	8 h
360 ppm ($648 mg/m^3$)	360 ppm ($648 mg/m^3$)	200 ppm ($360 mg/m^3$)	63 ppm ($113 mg/m^3$)	35 ppm ($63 mg/m^3$)

level at exposure durations of 10 and 30 min and 1, 4, and 8 h, respectively. These values are higher than those based on a lethality threshold.

The proposed AEGL-3 value for a 30-min exposure is higher than the odor threshold of 260 ppm as reported by Hellman and Small (1974). Deschamps et al. (1992) reported toxic effects, but no lethality, for 4-h exposures at the odor detection level. Laurent (1988) also reported toxic effects, but no lethality, for 30-min exposures at the odor detection level. The odor detection level has been reported to range from 260 to as high as 1,540 ppm (upper 95% C.I.). Salinas reported very serious neurologic effects after a 2- to 3-min exposure to a reported concentration of 500 ppm (calculated concentration); this concentration is probably too low for the initial exposure.

8. SUMMARY OF PROPOSED AEGLs

8.1. Proposed AEGLs

The AEGL values are presented in Table 2-26. No lethality data for humans were available for deriving the AEGL-3 values; the limitations in the epidemiologic studies precluded an unequivocal conclusion on carcinogenicity based on human data. Data from long-term animal studies were used to estimate AEGL-3 values based on carcinogenicity. The calculations showed that the AEGL-3 values based on the excess lifetime cancer risk from a single exposure to ethylene oxide is greater than the concentration associated with the lethality threshold. Therefore, the AEGL-3 values were derived from an estimate of the lethality threshold (LC_{01}) for rats.

AEGL-2 values were derived from rat developmental and neurotoxicity end points; the NOAEL was 100 ppm for a 6-h exposure in both studies.

AEGL-1 values were not derived because the odor threshold and sensory irritation occur at concentrations above those causing systemic toxicity and would not serve as a warning of exposure. In addition, the proposed AEGL-2 and -3 values are below the odor threshold with the exception of AEGL-3 values for 10 and 30 min.

8.2. Comparison of AEGLs with Other Standards and Criteria

Table 2-27 summarizes standards and guidelines established by various agencies and organizations. The American Conference of Governmental Indus-

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trial Hygienists Threshold Limit Value, National Institute for Occupational Safety and Health (NIOSH) recommended exposure limits, NIOSH short-term exposure limit, and Occupational Safety and Health Administration permissible exposure limits are based on cancer risk associated with lifetime occupational exposures and should not be compared with the acute values derived for emergency standards. The NIOSH standard for immediately dangerous to life or health is based on an LC_{50} value of 800 ppm for a 4-h exposure. This value was adopted for a 30-min exposure. The emergency response planning guideline (ERPG)-3 value of 500 ppm is considerably higher than the AEGL-3 value of 200 ppm for 1 h, and the ERPG-2 value of 50 ppm is similar to the AEGL-2 value of 45 ppm for 1 h (AIHA 2005). The ERPG-3 level for 1 h was based primarily on the acute inhalation study of Jacobson et al. (1956) and the observation that short-term exposure of humans to high concentrations has not been associated with mortality. The ERPG-2 value was based on reproductive and developmental toxicity studies. The NRC (1986b) recommended an emergency exposure guidance level of 20 ppm for a 1-h exposure and 1 ppm for a 24-h exposure.

8.3. Data Quality and Research Needs

A number of uncertainties are associated with deriving AEGL values for ethylene oxide. The lack of definitive exposure data precluded using human data to derive AEGL-1 and AEGL-2 values. Estimates of exposure were based on the odor detection threshold of 260 ppm. Human studies with ethylene oxide to fill data gaps are precluded because of the potential carcinogenicity of the substance.

TABLE 2-26 AEGL Values for Ethylene Oxide

Classification	10 min	30 min	1 h	4 h	8 h	End Point (Reference)
AEGL-1 (Nondisabling)	Not recommended[a]					
AEGL-2 (Disabling)	80 ppm (144 mg/m^3)	80 ppm (144 mg/m^3)	45 ppm (81 mg/m^3)	14 ppm (25 mg/m^3)	7.9 ppm (14 mg/m^3)	NOAEL for neurotoxicity and developmental toxicity (Snellings et al. 1982a; Mandella 1997a)
AEGL-3 (Lethal)	360 ppm (648 mg/m^3)	360 ppm (648 mg/m^3)	200 ppm (360 mg/m^3)	63 ppm (113 mg/m^3)	35 ppm (63 mg/m^3)	Lethality (Jacobson et al. 1956)

[a]The absence of AEGL-1 values does not imply that exposure below the AEGL-2 value is without adverse effects.

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TABLE 2-27 Extant Standards and Guidelines for Ethylene Oxide

Guideline	Exposure Duration					
	10 min	15 min	30 min	1 h	4 h	8 h
AEGL-1	Not recommended					
AEGL-2	80 ppm		80 ppm	45 ppm	14 ppm	7.9 ppm
AEGL-3	360 ppm		360 ppm	200 ppm	63 ppm	35 ppm
ERPG-1 (AIHA)[a]						
ERPG-2 (AIHA)[a]				50 ppm		
ERPG-3 (AIHA)[a]				500		
EEGL (NRC)[b]				20 ppm		1 ppm (24 h)
IDLH (NIOSH)[c]			800 ppm			
REL-TWA (NIOSH)[d]						<0.1 ppm
PEL-TWA (OSHA)[e]	1 ppm					
TLV-TWA (ACGIH)[f]						1 ppm (A2)
EL (OSHA)[g]	5 ppm (15-min excursion limit)					
REL-STEL (NIOSH)[h]	5 ppm (ceiling)					
MAC (The Netherlands)[i]						0.5 ppm
MAK (Germany)[j]	No value (carcinogenicity category 2, germ cell mutagen category 2)					

[a]ERPG (emergency response planning guidelines of the American Industrial Hygiene Association) (AIHA 2001): The ERPG-1 is the maximum airborne concentration below which it is believed nearly all individuals could be exposed for up to 1 h without experiencing other than mild, transient adverse health effects or without perceiving a clearly defined objectionable odor. The ERPG-2 is the maximum airborne concentration below which it is believed nearly all individuals could be exposed for up to 1 h without experiencing or developing irreversible or other serious health effects or symptoms that could impair an individual's ability to take protective action. The ERPG-3 is the maximum airborne concentration below which it is believed nearly all individuals could be exposed for up to 1 h without experiencing or developing life-threatening health effects.
[b]EEGL (emergency exposure guidance levels of the National Research Council) (NRC 1986b) is the concentration of contaminants that can cause discomfort or other evidence of irritation or intoxication in or around the workplace but avoids death, other severe acute effects, and long-term or chronic injury.
[c]IDLH (immediately dangerous to life or health standards of the National Institute for Occupational Safety and Health) (NIOSH 1996) represents the maximum concentration from which escape within 30 min would be possible without any escape-impairing symptoms or irreversible health effects.
[d]REL-TWA (recommended exposure limits–time-weighted average of the National Institute for Occupational Safety and Health) (NIOSH 2005) is analogous to the American

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Conference of Governmental Industrial Hygienists Threshold Limit Value–time-weighted average (ACGIH TLV-TWA).
[e]PEL-TWA (permissible exposure limits–time-weighted average of the Occupational Safety and Health Administration (OSHA) (29 CFR 1910.1000 [2003]) is analogous to the ACGIH TLV-TWA but is for exposures of no more than 10 h/d, 40 h/wk.
[f]TLV-TWA (Threshold Limit Value–time-weighted average of the American Conference of Governmental Industrial Hygienists) (ACGIH 1996) is the time-weighted average concentration for a normal 8-h workday and a 40-h work week to which nearly all workers may be repeatedly exposed, day after day, without adverse effect. A2 indicates that ethylene oxide is a suspected human carcinogen.
[g]EL (excursion limit of the Occupational Health and Safety Administration) (OSHA) (29 CFR 1910.1047 [2007]) is analogous to the ACGIH Threshold Limit Value–short-term exposure limit (TLV-STEL).
[h]REL-STEL (recommended exposure limits–short-term exposure limit of the National Institute for Occupational Safety and Health (NIOSH 2005) is analogous to the ACGIH TLV-TWA.
[i]MAC (maximaal aanvaarde concentratie [maximum accepted concentration], SDU Uitgevers [under the auspices of the Ministry of Social Affairs and Employment, The Hague, The Netherlands]) is analogous to the ACGIH TLV-TWA (MSZW 2004).
[j]MAK (maximale argeitsplatzkonzentration [maximum workplace concentration], Deutsche Forschungsgemeinschaft [German Research Association]) (DFG 2007) is analogous to the ACGIH TLV-TWA.

The database of animal studies is robust, containing acute inhalation studies in several species, an acute neurotoxicity, developmental and reproductive toxicity studies in several species, in vivo germ cell studies, and carcinogenicity studies in two species. However, a single-day developmental toxicity study would clear up some of the concerns about fetal growth retardation as a single-exposure or repeat-exposure event. In addition, pharmacokinetics and metabolism studies were available. Therefore, the toxicity of ethylene oxide is well characterized in animals.

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APPENDIX A

DERIVATION OF AEGL VALUES FOR ETHYLENE OXIDE

Derivation of AEGL-2

Key studies:	Snellings et al. 1982a; Mandella 1997a
Toxicity end point:	NOAEL for neurotoxicity and developmental toxicity in rats, 100 ppm
Time-scaling:	ten Berge's equation: $C^n \times t = k$, where n = 1.2 derived from rat data
Uncertainty factors:	Total = 10 3 for interspecies sensitivity 3 for intraspecies variability
Calculations:	
6-h exposure (experimental)	C = 100 ppm/10 (uncertainty factor) = 10 ppm $C^n \times t = k$; n = 1.2 C = 10 ppm, t = 6 h, k = 95.09 ppm-h
10-min AEGL-2	80 ppm, same as the 0.5-h value
30-min AEGL-2	$C = (k/t)^{1/1.2} = (95.09\ \text{ppm-h}\ /0.5\ \text{h})^{1/1.2} = 80\ \text{ppm}$ C = 80 ppm
1-h AEGL-2	$C = (k/t)^{1/1.2} = (95.09\ \text{ppm-h}\ /1\ \text{h})^{1/1.2} = 45\ \text{ppm}$ C = 45 ppm
4-h, AEGL-2	$C = (k/t)^{1/1.2} = (95.09\ \text{ppm-h}\ /4\ \text{h})^{1/1.2} = 14\ \text{ppm}$ C = 14 ppm
8-h AEGL-2	$C = (k/t)^{1/1.2} = (95.09\ \text{ppm-h}\ /8\ \text{h})^{1/1.2} = 7.9\ \text{ppm}$ C = 7.9 ppm

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Derivation of AEGL-3

Key study: Jacobson et al. 1956

Toxicity end point: Lethality; the LC_{50} for white male rats was 1,460 ppm for a 4-h exposure. The data were extrapolated to a LC_{01} (628 ppm) to approximate the lethality threshold.

Time-scaling: ten Berge's equation: $C^n \times t = k$, where n = 1.2 derived from rat data

Uncertainty factors: Total = 10
3 for interspecies sensitivity
3 for intraspecies variability

Calculations: C = 628 ppm/10 (uncertainty factor) = 62.8 ppm
$C^n \times t = k$; c = 62.8 ppm, n= 1.2, t = 4 h,
k = 574.93 ppm-h

10-min AEGL-3: 360 ppm (same as the 0.5-h value)

30-min AEGL-3: $C = (k/t)^{1/1.2} = (574.93 \text{ ppm-h} / 0.5 \text{ h})^{1/1.2} =$ 355 ppm
C = 360

1-h AEGL-3: $C = (k/t)^{1/1.2} = (574.93 \text{ ppm-h} / 1 \text{ h})^{1/1.2} = 199$ ppm
C = 200 ppm

4-h AEGL-3: $C = (k/t)^{1/2} = (574.93 \text{ ppm-h} / 4 \text{ h})^{1/1.2} = 62.8$ ppm
C = 63 ppm

8-h AEGL-3: $C = (k/t)^{1/1.2} = (574.93 \text{ ppm-h} / 8 \text{ h})^{1/1.2} = 35$ ppm
C = 35 ppm

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APPENDIX B

PRELIMINARY CANCER ASSESSMENT OF ETHYLENE OXIDE

In 1985, EPA reported a unit risk or q_1* for inhalation exposure to ethylene of 1 $\times 10^{-4}$ µg/m^3 based on the combined incidences of leukemia and brain gliomas in F344 rats as reported by Snellings et al. (1981) (EPA 1985). A study by NTP (1987) was completed after EPA conducted its risk assessment of ethylene oxide. This study was summarized in Table 2-15 of the text and the data for lung tumors in female mice will be used to calculate another unit risk (q_1*). The calculations of the unit risk and the AEGL values for carcinogenicity are presented below.

Data summary (NTP 1987): Groups of 50 male and 50 female B6C3F$_1$ mice were exposed to 0, 50, or 100 ppm for 6 h/day, 5 days/week, for 102 weeks. The incidence of lung adenomas and carcinomas in females was 2/49, 5.48, or 22/49 for 0, 50, or 100 ppm, respectively.

Derivation of the unit risk for ethylene oxide:
Convert exposure concentrations for 6 h/day and 5 days/week
to continuous exposure:

$$50 \text{ ppm} \times 6/24 \times 5/7 = 8.93 \text{ ppm} \times 1.8 = 16.1 \text{ mg/m}^3$$
$$100 \text{ ppm} \times 6 \text{ h}/24 \text{ h} \times 5 \text{ days}/7\text{days} = 17.86 \text{ ppm} \times 1.8 = 32.2 \text{ mg/m}^3$$

The unit risk (q_1*) derived from the linearized multistage model is 8.82×10^{-3} (mg/m^3)$^{-1}$.

The calculations for AEGL values following the method presented by NRC (1986a) are presented below.

To calculate a “virtually safe dose” of d at a cancer risk of 10^{-4}:

$$d = 10^{-4}/(8.82 \times 10^{-3} \text{ (mg/m}^3))^{-1} = 1.13 \times 10^2 \text{ mg/m}^3$$

To calculate the total cumulative dose for a total lifetime exposure of 70 years, which is equivalent to 25,600 days:

$$\text{total d} = \text{d} \times 25{,}600 = 1.13 \times 10^{-2} \text{ mg/m}^3 \times 25{,}600 = 2.90 \times 10^2 \text{ mg/m}^3$$

In the adjustment to allow for uncertainties in assessing potential cancer risks under short-term exposures under the multistage model (Crump and Howe 1984), the total dose is divided by a factor of 6:

$$2.90 \times 10^2 \text{ mg/m}^3/6 = 4.8 \times 10^1 \text{ mg/m}^3 = 48 \text{ mg/m}^3 = 26.7 \text{ ppm.}$$

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Therefore, a 24-h exposure concentration associated with a 10^{-4} risk is 26.7 ppm. The 10^{-4} cancer risk associated with exposures for 10, 30, 60, 240, and 480 min can be calculated from the following equation:

$$C \times t = k, \text{ where c = concentration, t = time, and k is a constant.}$$

The AEGL values associated with risks of 10^{-4}, 10^{-5}, and 10^{-6} are presented in Table B-1.

TABLE B-1 AEGL Values Associated with Different Risks

Exposure Time	10^{-4}	10^{-5}	10^{-6}
10 min	1,300 ppm	130 ppm	13 ppm
30 min	1,300 ppm	130 ppm	13 ppm
1 h	640 ppm	64 ppm	6.4 ppm
4 h	160 ppm	16 ppm	1.6 ppm
8 h	80 ppm	8 ppm	0.8 ppm

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APPENDIX C

DERIVATION OF THE LEVEL OF DISTINCT ODOR AWARENESS (LOA) FOR ETHYLENE OXIDE

The level of distinct odor awareness (LOA) represents the concentration above which it is predicted that more than one-half of the exposed population will experience at least a distinct odor intensity and about 10% of the population will experience a strong odor intensity. The LOA should help chemical emergency responders in assessing the public awareness of the exposure due to odor perception. The LOA derivation follows the guidance given by van Doorn et al. (2002).

The odor detection threshold (OT_{50}) for ethylene oxide is calculated from the odor threshold of 260 ppm reported by Hellman and Small (1974) and adjusted by Van Doorn et al. (2002):

$$260 \text{ ppm} \times 40 \text{ ppm}/100 \text{ ppm} = 104 \text{ ppm}$$

The concentration (C) leading to an odor intensity (I) of distinct odor detection (I = 3) is derived by using the Fechner function:

$$I = k_w \times \log(C/OT_{50}) + 0.5.$$

For the Fechner coefficient, the default $k_w = 2.33$ is used because of the lack of chemical-specific data:

$$3 = 2.33 \times \log(C/104) + 0.5, \text{ which can be rearranged to}$$
$$\log(C/104) = (3 - 0.5)/2.33 = 1.07, \text{ and results in}$$
$$C = (10^{1.07}) \times 104 = 1{,}222 \text{ ppm}.$$

The resulting concentration is multiplied by an empirical field correction factor. It takes into account that in everyday life, factors, such as sex, age, sleep, smoking, upper airway infections, and allergy, as well as distraction, increase the odor detection threshold by up to a factor of 4. In addition, it takes into account that odor perception is very fast (about 5 s), which leads to the perception of concentration peaks. Based on the current knowledge, a factor of 1/3 is applied to adjust for peak exposure. Adjustments for distraction and peak exposure lead to a correction factor of 4/3 = 1.33.

$$LOA = C \times 1.33 = 1{,}222 \text{ ppm} \times 1.33 = 1625 \text{ ppm (van Doorn et al. 2002)}$$

Therefore, the LOA for ethylene oxide is 1,625 ppm.

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APPENDIX D

ACUTE EXPOSURE GUIDELINE LEVELS FOR ETHYLENE OXIDE

Derivation Summary for Ethylene Oxide

AEGL-1 VALUES

10 min	30 min	1 h	4 h	8 h
Not recommended				

Key reference: Not applicable

Test species, strain, number: Not applicable

Exposure route, concentration, durations: Not applicable

Effects: Not applicable

End point, concentration, rationale: Not applicable

Uncertainty factors/rationale:
Total uncertainty factor: Not applicable
Interspecies: Not applicable
Intraspecies: Not applicable

Modifying Factor: Not applicable

Animal to human dosimetric adjustment: Not applicable

Time-scaling: Not applicable

AEGL-2 VALUES

10 min	30 min	1 h	4 h	8 h
80 ppm	80 ppm	45 ppm	14 ppm	7.9 ppm

Key references:
Snellings, W.M., R.R. Maronpot, J.P. Zelenak, and C.P. Laffoon. 1982a. Teratology study in Fischer 344 rats exposed to ethylene oxide by inhalation. Toxicol. Appl. Pharmacol. 64(3):476-481.
Mandella, R.C. 1997a. An Acute Inhalation Neurotoxicity Study of Ethylene Oxide (498-95-A) in the Rat Via Whole-Body Exposure. Final Report. Study No. 95-6097. Prepared by Huntingdon Life Sciences, East Millstone, NJ , for Allied Signal, Inc, Morristown, NJ, and ARC Chemical Division, Balchem Corporation, Slate Hill, NY.

Test species, strain, number: Sprague-Dawley rats, 10/sex/group

Exposure route, concentration, durations: Inhalation; 0, 100, 300, or 500 ppm for 6 h

Effects:
Developmental toxicity
10 ppm: no effect
33 ppm: no effect

(Continued)

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AEGL-2 VALUES Continued

10 min	30 min	1 h	4 h	8 h
80 ppm	80 ppm	45 ppm	14 ppm	7.9 ppm

Effects:
100 ppm: statistically significant decrease in fetal body weight and non-statistically significant increase in the litter incidence of delayed ossification (not considered toxicologically significant)

Neurotoxicity:
0 ppm: droopy, half-closed eyelids (10%) and no response to approach (10%); other end points (0%)
100 ppm: droopy, half-closed eyelids (10%); low arousal (15%); and no response to approach (15%)
300 and 500 ppm: droopy, half-closed eyelids (25%, 40%**); impaired locomotion (10%, 15%); low arousal (30%,** 75%**); and no response to approach (35%,* 50%**)
*$p < 0.05$; **$p < 0.01$

End point, concentration, rationale: NOAEL for neurotoxicity at 100 ppm; low arousal was observed at 100 ppm, but the incidence did not reach statistical significance ($p = 0.12$, Fisher's exact test); the next higher concentration of 300 ppm caused significant increases in the incidences of low arousal and no reaction to approach response.

Uncertainty factors and rationale:
Total uncertainty factor:10
Interspecies: 3, one potential mechanism of toxicity, direct alkylation of DNA and proteins, is not expected to differ across species. Neurotoxicity similar in rats and humans (distal axonal degeneration, neuropathy); PBPK models have shown that the AUC, peak blood levels, internal dose in mg/kg of body weight, and hemoglobin adduct level (measure of internal exposure) for humans are similar to or lower than the corresponding values for rats.
Intraspecies: 3, An uncertainty factor of 3 was selected for intraspecies variability because glutathione-*S*-transferase polymorphism can modulate systemic exposure as measured by hemoglobin adducts but appears to be within a factor of 3 within the population. Individuals with asthma are not expected to respond differently to ethylene oxide concentrations below the odor detection and irritation thresholds.

Modifying factor: 1

Animal to human dosimetric adjustment: 1

Time-scaling: $C^n \times t = k$, where $n = 1.2$ as determined from empirical LC_{50} data for the rat for 1 and 4 h.

Data quality and support for the AEGL values: Human studies to evaluate adverse effects of ethylene oxide on reproduction and development have not been conclusive. However, multiple animal studies showed that ethylene oxide is a developmental toxicant in rats and mice. Humans and rats show similar manifestations of peripheral neurotoxicity; legs and hindlimbs are primary targets, with distal axonal degeneration and peripheral neuropathy developing in humans and rats. The AEGL-2 values are below the concentrations that cause respiratory tract irritation and are below the odor detection threshold.

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AEGL-3 VALUES

10 min	30 min	1 h	4 h	8 h
360 ppm	360 ppm	200 ppm	63 ppm	35 ppm

Key reference: Jacobson, K.H., E.B. Hackley, and l. Feinsliver. 1956. The toxicity of inhaled ethylene oxide and propylene oxide vapors. AMA Arch. Ind. Health 13(3): 237-244.

Test species, strain, number: White male rats, 10 per group

Exposure route, concentration, durations: Inhalation, 882, 1,343, 1,648, 1,843, 1,992, 2,298 ppm for 4 h.

Effects:
Clinical signs: Frequent movement and preening, clear nasal discharge, lacrimation, salivation, diarrhea, gasping, and death.
Gross observations: Signs of upper respiratory tract irritation, tracheal congestion, and petechial hemorrhages and mild edema in the lungs and peribronchial region.
Mortality: 882 ppm (2/10), 1,343 ppm (2/10), 1,648 ppm (4/10), 1,843 ppm (9/10), 1,992 ppm (10/10), and 2,298 ppm (10/10); LC_{50} = 1460 ppm.

End point, concentration, rationale: Lethality; LC_{01} = 628 ppm for 4 h, the estimated threshold for lethality derived by probit analysis of the data.

Uncertainty factors and rationale:
Total uncertainty factor: 10
Interspecies: 3, one potential mechanism of toxicity, direct alkylation of DNA and proteins, is not expected to differ across species. PBPK models have shown that the AUC, peak blood levels, internal dose in mg/kg of body weight, and hemoglobin adduct level (measure of internal exposure) for humans are similar to or lower than the corresponding values for rats.
Intraspecies: 3, An uncertainty factor of 3 was selected for intraspecies variability because glutathione-*S*-transferase polymorphism can modulate systemic exposure as measured by hemoglobin adducts but appears to be within a factor of 3 within the population. Individuals with asthma are not expected be respond differently to ethylene oxide concentrations below or slightly above the odor detection and irritation thresholds.
An interspecies or an intraspecies uncertainty factor of 10 would place AEGL-3 values below concentrations likely to be associated with life-threatening events.

Modifying factor: 1

Animal to human dosimetric adjustment: 1

Time-scaling: $C^n \times t = k$, where n = 1.2 as determined from empirical LC_{50} data for the rat for 1 and 4 h.

Data quality and support of AEGL values: AEGL-3 values for ethylene oxide were derived from one of several well-conducted studies. AEGL-3 values are below the estimated 10^{-4} risk associated with the lifetime risk of developing cancer after a single exposure. The 10- and 30-min values exceed the lower limit on the odor detection threshold. Respiratory tract irritation may occur at these concentrations and reversible neurologic effects may occur at the AEGL-3 concentrations, but life-threatening events are unlikely to occur.

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APPENDIX E

CATEGORY PLOT FOR ETHYLENE OXIDE

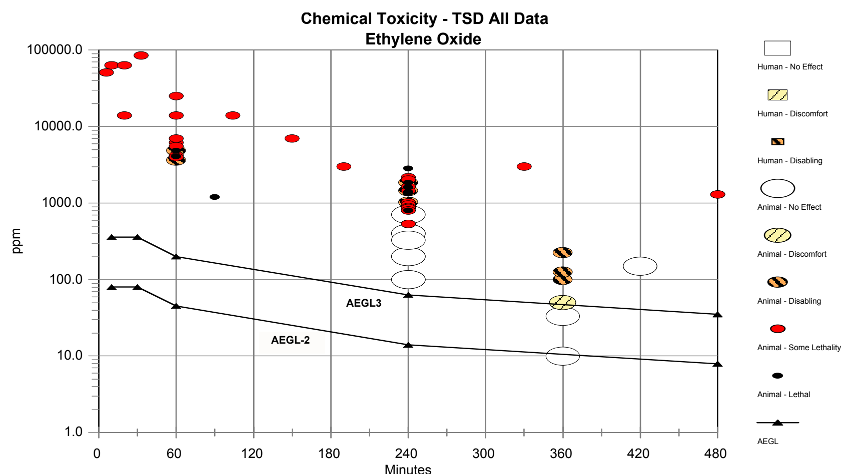

FIGURE D-1 Category plot for ethylene oxide.

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3

Furan[1]

Acute Exposure Guideline Levels

PREFACE

Under the authority of the Federal Advisory Committee Act (FACA) P.L. 92-463 of 1972, the National Advisory Committee for Acute Exposure Guideline Levels for Hazardous Substances (NAC/AEGL Committee) has been established to identify, review, and interpret relevant toxicologic and other scientific data and develop AEGLs for high-priority, acutely toxic chemicals.

AEGLs represent threshold exposure limits for the general public and are applicable to emergency exposure periods ranging from 10 minutes (min) to 8 hours (h). Three levels—AEGL-1, AEGL-2, and AEGL-3—are developed for each of five exposure periods (10 and 30 min and 1, 4, and 8 h) and are distinguished by varying degrees of severity of toxic effects. The three AEGLs have been defined as follows:

AEGL-1 is the airborne concentration (expressed as parts per million [ppm] or milligrams per cubic meter [mg/m^3]) of a substance above which it is predicted that the general population, including susceptible individuals, could experience notable discomfort, irritation, or certain asymptomatic, nonsensory

[1]This document was prepared by the AEGL Development Team composed of Claudia Troxel (Oak Ridge National Laboratory) and Chemical Manager George Rodgers (National Advisory Committee [NAC] on Acute Exposure Guideline Levels for Hazardous Substances). The NAC reviewed and revised the document and AEGLs as deemed necessary. Both the document and the AEGL values were then reviewed by the National Research Council (NRC) Committee on Acute Exposure Guideline Levels. The NRC committee has concluded that the AEGLs developed in this document are scientifically valid conclusions based on the data reviewed by the NRC and are consistent with the NRC guideline reports (NRC 1993, 2001).

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effects. However, the effects are not disabling and are transient and reversible upon cessation of exposure.

AEGL-2 is the airborne concentration (expressed as ppm or mg/m^3) 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.

AEGL-3 is the airborne concentration (expressed as ppm or mg/m^3) of a substance above which it is predicted that the general population, including susceptible individuals, could experience life-threatening health effects or death.

Airborne concentrations below the AEGL-1 represent exposure levels that could produce mild and progressively increasing but transient and nondisabling odor, taste, and sensory irritation or certain asymptomatic, nonsensory effects. With increasing airborne concentrations above each AEGL, there is a progressive increase in the likelihood of occurrence and the severity of effects described for each corresponding AEGL. Although the AEGLs represent threshold levels for the general public, including susceptible subpopulations, such as infants, children, the elderly, persons with asthma, and those with other illnesses, it is recognized that individuals, subject to idiosyncratic responses, could experience the effects described at concentrations below the corresponding AEGL.

SUMMARY

Furan is a colorless, highly flammable liquid with a strong, ethereal odor. It is used primarily as an industrial intermediate. Occupational exposure to furan is limited because it is handled in closed containers and is used in a closed system in industrial processes. The general public is typically exposed to furan on a daily basis. The chemical has been detected in cooked foods, the gas-phase component of cigarette smoke, wood smoke, exhaust gas from diesel and gasoline engines, and oils obtained by distilling rosin-containing pine wood. If furan is released, it is predicted to exist almost entirely in the vapor phase in the atmosphere because of its relatively high vapor pressure.

Quantitative toxicology data on effects after inhalation exposure to furan were limited to one study in rats. Oral administration of furan resulted in hepatocarcinogenicity and toxicity, and a number of studies determined that a reactive metabolite was responsible for most of the hepatic effects furan induced. In particular, metabolism studies indicate that furan is bioactivated in the liver to a reactive metabolite, *cis*-2-butene-1,4-dial, by cytochrome P-450 2E1. On the basis of a chronic oral carcinogenicity study in which clear evidence of carcinogenicity was noted in male and female rats and mice, the National Toxicology Program (NTP) classifies furan as “reasonably anticipated to be a human carcinogen” and the International Agency for Research on Cancer (IARC) lists furan as a Group 2B carcinogen (possibly carcinogenic to humans). The U.S. En-

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vironmental Protection Agency (EPA) has not classified furan according to its carcinogenicity.

AEGL-1 values were not derived for furan. No human or animal data relevant to the derivation of any AEGL-1 for furan were located.

The Terrill et al. (1989) study was used as the basis for the AEGL-2 and -3 derivations. Groups of five male and five female Sprague-Dawley rats were exposed to furan for 1 h at analytic concentrations of 1,014, 2,851, or 4,049 ppm in a dynamic inhalation chamber. The rats were observed for 14 days, at which time a gross necropsy was conducted on the surviving animals. Signs of furan intoxication during exposure included respiratory distress, increased secretory response, and death. The degrees of respiratory distress and increased secretory response at each concentration (or chemical) were not provided. Body weight (b.w.) declined in the mid- and high-concentration groups (actual b.w. not provided). No treatment-related lesions were observed in surviving animals. Mortality was not observed at the low or middle concentrations, but all males and four of five females died at the high concentration. A general statement was made that "in many instances, deaths were delayed until the end of the first week and the beginning of the second week."

The AEGL-2 derivation is based on the threshold for adverse effects in male and female rats at a concentration of 1,014 ppm for 1 h (Terrill et al. 1989). Although the severity of the clinical signs (respiratory distress, increased secretory response) was not reported, this lowest exposure group did not exhibit a decrease in b.w. as did the rats exposed to 2,851 or 4,049 ppm.

The AEGL-3 derivation is based on the highest nonlethal concentration in male and female rats of 2,851 ppm for 1 h (Terrill et al. 1989). Rats exposed to 1,014, 2,851, or 4049 ppm exhibited clinical signs, including respiratory distress and increased secretory response: however, the degree of the symptoms at each concentration was not provided. Death occurred in the highest exposure group.

For the AEGL-2 and -3 derivations, an uncertainty factor of 10 was applied for species-to-species extrapolation because there are inadequate data to properly assess interspecies variability. Terrill et al. (1989) was the only published furan toxicity study that investigated the toxicity of inhaled furan, and it evaluated only one species (rat). Therefore, insufficient empirical data were available to examine species differences in response to inhaled furan. A physiologically based pharmacokinetic (PBPK) simulation of inhalation exposure to furan predicted that the absorbed dose of furan in mice and rats would be ~10- and 3.5-fold higher, respectively, than that in humans, whereas the integrated liver exposure to furan metabolites would be ~6- and 3-fold higher, respectively, than that in humans. However, oral toxicity data indicate that rats are more sensitive than mice despite PBPK modeling predictions that mice would have a 3-fold higher absorbed dose and 2-fold higher integrated liver exposure to furan metabolites than rats. Therefore, there are too many uncertainties about the response to furan of the rat, mouse, and human liver to base an uncertainty factor on PBPK modeling predictions.

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An intraspecies uncertainty factor of 3 was applied for the following reasons:

1. Assuming death was the result of a progression of the toxicity present at the lower concentrations, the steep dose-response curve for lethality indicates there is not much variability in the response (0/10 rats died at 1,014 and 2,851 ppm, whereas 9/10 rats died at 4,049 ppm). The delayed deaths in the high-concentration group suggest that hepatotoxicity was the cause of death.
2. If no hepatotoxicity is present, the clinical signs are likely due to a direct contact effect, which is not expected to vary much among individuals.
3. If hepatotoxicity is present, it is due to the reactive metabolite produced in the liver. PBPK modeling data indicate that production of the metabolite is blood flow limited. Therefore, variations in cytochrome P-450 2E1 levels are not likely to be a significant factor (Kedderis and Held 1996). Using hepatocytes as the basis, PBPK modeling indicates that when adults and children (ages 6, 10, and 14 years) are exposed to the same furan concentrations, the blood concentration of furan is likely to be greater in children than in adults by a factor of only 1.5 (at steady state), and the maximum factor of adult-child differences in liver concentration of furan metabolite is about 1.25 (Price et al. 2003).

A modifying factor of 5 was applied to account for a limited data set (only one data set addressing furan toxicity after inhalation exposure was available; this study was not repeated, and there was no information on furan toxicity in other species). Therefore, a total uncertainty factor and modifying factor of 150 was applied to the AEGL-2 and -3 values.

The experimentally derived exposure values were scaled to AEGL time-frames using the concentration-time relationship given by the equation $C^n \times t = k$, where C = concentration, t = time, k is a constant, and n generally ranges from 0.8 to 3.5 (ten Berge et al. 1986). The value of n was not empirically derived because of insufficient data; therefore, the default value of n = 1 was used for extrapolating from shorter to longer exposure periods and a value of n = 3 was used to extrapolate from longer to shorter exposure periods.

The derived AEGL values are listed in Table 3-1.

1. INTRODUCTION

Furan is a colorless, highly flammable liquid with a strong, ethereal odor. An odor threshold value for furan could not be located in the available literature. Furan is miscible with most organic solvents but is only slightly soluble in water. It has low boiling and flash points and is often stabilized with butylated hydroxytoluene to inhibit the formation of explosion-prone peroxides upon exposure to air (EPA 1987). Furan is produced by decarbonylation of furfural (Kottke 1991). The industrial uses of furan are predominantly as an intermediate

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TABLE 3-1 Summary of AEGL Values for Furan

Classification	10 min	30 min	1 h	4 h	8 h	End Point (Reference)
AEGL-1 (Nondisabling)	NR[a]	NR	NR	NR	NR	Not applicable
AEGL-2 (Disabling)	12 ppm (33 mg/m^3)	8.5 ppm (24 mg/m^3)	6.8 ppm (19 mg/m^3)	1.7 ppm (4.7 mg/m^3)	0.85 ppm (2.4 mg/m^3)	1,014 ppm for 1 h: threshold for adverse effects in rats; clinical signs: although the severity of respiratory distress and increased secretory response not reported, no decrease in body weight occurred (Terrill et al. 1989)
AEGL-3 (Lethality)	35 ppm (97 mg/m^3)	24 ppm (67 mg/m^3)	19 ppm (53 mg/m^3)	4.8 ppm (13 mg/m^3)	2.4 ppm (6.7 mg/m^3)	2,851 ppm for 1 h: threshold for lethality in rats (Terrill et al. 1989)

[a]NR: not recommended. Numeric values for AEGL-1 are not recommended because of the lack of available data. Absence of an AEGL-1 does not imply that exposure below the AEGL-2 is without adverse effects.

in the production of tetrahydrofuran, pyrrole, and thiophene; in the formation of lacquers and solvents for resins; in the production of pharmaceuticals; in agricultural chemicals; and in stabilizers (IARC 1995). Furan is an EPA high-production-volume chemical (revised Sept. 6, 2001), with production exceeding 1 million pounds annually. Occupational exposure to furan is predicted to be minimal as it is handled in closed containers because of its volatility, and industrial processes that use furan are conducted in closed systems (NTP 1993).

The general public is typically exposed to furan on a daily basis. The chemical has been detected in the gas-phase component of cigarette smoke, wood smoke, exhaust gas from diesel and gasoline engines, and oils obtained by distilling rosin-containing pine wood (Budavari et al. 1989; IARC 1995). Furan is also present in cooked foods: analysis of approximately 300 food samples found furan levels ranging from nondetectable (below the limits of detection of the method) to 175 parts per billion (FDA 2009). Food and Drug Administration (FDA) calculations found that mean daily furan exposure ranged from 0.26 µg/kg of b.w. per day for adults to 0.41 µg/kg/day for infants consuming baby food and 0.9 µg/kg/day for those consuming infant formula (FDA 2007). Common sources of exposure in adults include coffee, juices, snack foods, nutritional drinks, and gravies; common sources in infants (up to 1 year old) are jarred baby foods and canned infant formulas (Becalski et al. 2005; FDA 2007; Zoller et al. 2007). FDA has posted these furan data on the agency's Web site at http://www.cfsan.fda.gov/~lrd/pestadd.html#furan. It is postulated that the primary source of furans in food is from thermal degradation and rearrangement of organic compounds, especially carbohydrates (69 CFR 25911[2004]).

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If it is released, furan is predicted to exist almost entirely in the vapor phase in the atmosphere because of its relatively high vapor pressure. The primary removal mechanism during daylight is predicted to be the reaction with photochemically generated hydroxyl radicals, with an estimated half-life of 2 to 6 h (EPA 1987) or 9.5 h (Atkinson 1989). Reaction with nitrate radicals is predicted to be the primary removal mechanism during night hours (approximate half-life of 2 h) (EPA 1987).

Although furan is present in cigarette smoke (Newsome et al. 1965), no human data were available regarding acute nonlethal toxicity of this compound. The NTP report (1993) summarized possible human exposure data. While it was reported that approximately 35 employees were potentially exposed to furan at 14 plants, no further details, such as health effects, were provided.

The physicochemical data on furan are presented in Table 3-2.

TABLE 3-2 Chemical and Physical Data for Furan

Parameter	Value	Reference
Synonyms	Furfuran, oxole, tetrole, divinylene oxide, 1,4-epoxy-1,3-butadiene, oxacyclopentadiene	Budavari et al. 1989
CAS registry number	110-00-9	
Chemical formula	C_4H_4O	Budavari et al. 1989
Molecular weight	68.07	Budavari et al. 1989
Physical state	Liquid	Budavari et al. 1989
Color	Colorless, turns brown upon standing	Garcia and James 2000
Melting point	−86ºC	Garcia and James 2000
Boiling point	31.36ºC at 760 mmHg 32ºC at 758 mmHg	Budavari et al. 1989
Liquid density (water = 1)	0.9371 at 19.4/4ºC	Budavari et al. 1989
Vapor density (air = 1)	2.36	Kottke 1991
Solubility	Freely soluble in alcohol and ether; solubility in water: 1% at 25ºC	Budavari et al. 1989 Kottke 1991
Vapor pressure	658 mmHg at 20ºC 600 mmHg at 20ºC	Kottke 1991 HSDB 2003
Conversion factors	1 ppm = 2.78 mg/m^3 1 mg/m^3 = 0.359 ppm Calculated: ppm × molecular weight = mg/m^3 at 24.45 ºC	Garcia and James 2000

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2. HUMAN TOXICITY DATA

2.1. Acute Lethality

No data were available regarding the acute lethality of furan in humans.

2.2. Nonlethal Toxicity

No human inhalation toxicity data for furan were available.

2.3. Developmental and Reproductive Effects

No human developmental and reproductive toxicity data concerning furan were found in the available literature.

2.4. Genotoxicity

No human genotoxicity data on furan were found in the available literature.

2.5. Carcinogenicity

No human data were found in the available literature regarding the carcinogenic potential of inhaled furan.

2.6. Summary

No data were found in the available literature regarding lethal and nonlethal toxicity, developmental and reproductive toxicity, genotoxicity, and carcinogenicity of inhaled furan in humans. Although it was reported that approximately 35 employees were potentially exposed to furan at 14 plants, no further details, such as health effects, were provided.

3. ANIMAL TOXICITY DATA

3.1. Acute Lethality

3.1.1. Dogs

A 10-kg dog (sex, age, and strain unspecified) was anesthetized with ether and then injected with 0.2 cubic centimeters (cm^3) of furan (Koch and Cahan 1925). Blood pressure immediately decreased, followed by an increase in the amplitude of the heartbeat and a rapid recovery of blood pressure to slightly higher than the initial reading. Three more injections gave similar results. It was

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concluded that furan stimulated the vagus mechanism because intravenous administration of furan after injection of 3 cm^3 of 1% atropine resulted in a decrease in blood pressure but no increase in the amplitude of the heartbeat. Furan was then substituted for ether (concentration of furan and protocol for furan inhalation were not provided). Blood pressure rapidly decreased, followed by an increased amplitude of the heartbeat. Respiration then ceased, followed by cardiac arrest. Necropsy revealed marked dilation of the blood vessels in the viscera; blood that was a bright, cherry red; and hyperemic lungs. The authors concluded that the immediate cause of death was asphyxia resulting from paralysis of the medulla.

3.1.2. Rats

Groups of five male and five female Sprague-Dawley rats were exposed for 1 h to furan vapor at analytic concentrations of 1,014, 2,851, and 4,049 ppm (Terrill et al. 1989). The vapor was generated with a bubbler, and exposures were conducted in a modified, 1-m^3 Hinner's-type, glass and stainless steel chamber (a Hinner's-type chamber is a vertical-flow chamber with cubic exposure sections, tangential inlets, and pyramid-shaped upper and lower sections) (McClellan and Henderson 1995). An infrared analyzer was used to monitor the exposure concentrations beginning at 15 min of exposure and continuing every 5 to 15 min thereafter. Animals were observed for 14 days, at which time a gross necropsy was conducted on the surviving animals. Signs of furan intoxication during exposure included respiratory distress, increased secretory response, and death. The degrees of respiratory distress and secretory response at each concentration were not provided. Body weight decreased in the middle- and high-concentration groups (actual b.w. not provided). No exposure-related lesions were observed in surviving animals. The mortality at each concentration was recorded and is presented in Table 3-3. Mortalities were not observed at the low and middle concentrations, but all males and four of five females died at the high concentration. The 1-h LC_{50} (concentration with 50% lethality) values and 95% confidence intervals were 3,398 ppm (2,683 to 4,303 ppm) for males, 3,550 ppm (2,726 to 4,623 ppm) for females, and 3,464 ppm (2,905 to 4,131 ppm) for both sexes combined.

TABLE 3-3 Mortality in Sprague-Dawley Rats Exposed to Furan

	Mortality[a]	
Concentration (ppm)	Male	Female
1,014 ± 36.6	0/5	0/5
2,851 ± 246.7	0/5	0/5
4,049 ± 227.8	5/5	4/5

[a]Number dead/number exposed.
Source: Terrill et al. 1989. Reprinted with permission; copyright 1989, American Industrial Hygiene Association.

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A rat was exposed to furan by inhalation via saturated cotton held over the nose (Koch and Cahan 1925). After a short struggle, the rat collapsed. There was an increase in the rate of respiration and the rat exhibited complete analgesia and relaxation lasting 2 to 3 min. The authors then stated that this experiment was repeated, but it is unclear if they meant both the exposure and the clinical signs or just the exposure. Although the rat appeared normal when replaced in its cage after treatment, it was dead the next morning.

3.1.3. Mice

Groups of three or four Swiss mice weighing 18 to 21 grams (g) were exposed to furan vapor ranging in concentration from 10.5 to 350 ppm for 1 h (Egle and Gochberg 1979). Information about the sex of the animals, individual vapor concentrations, method of vapor analysis, and period of observation after exposure was not provided. The vapor was generated by passing air through pure furan at room temperature and then transferring it with a 100-cm^3 syringe into a 5.2-liter (L) sealed glass desiccator. The 1-h LC_{50} was calculated to be 42 ppm. Gross necropsy revealed pulmonary inflammation and fluid accumulation, although it was not stated if these findings were limited to decedents or were also seen in survivors. Clinical signs of toxicity in mice that died during the 1-h exposure included hyperactivity for 5 to 15 min, followed by labored breathing and death soon after. As addressed by Garcia and James (2000), it is likely that hypoxia contributed to the toxicity observed in this study. According to their calculations, four mice placed in a closed system for 1 h would breathe 9.6 L of air (4 mice × 40 milliters [mL]/min × 60 min). The desiccator in which the exposure occurred was only a 5.2-L desiccator. The closed system in which the mice were exposed did not provide enough oxygen for the number of mice tested. Therefore, the mortality observed in the mice was most likely confounded by the hypoxic conditions, and the study is considered unacceptable.

3.1.4. Rabbits

A rabbit was exposed to furan through saturated cotton held over the nose (Koch and Cahan 1925). The rabbit struggled and collapsed. As the animal became sedated, respiration ceased but the heart continued to beat. After artificial respiration, breathing returned. Furan was administered a second time, but this time, respiration could not be restored after cessation. Necropsy revealed marked dilation of the blood vessels in the viscera; blood that was a bright, cherry red; and hyperemic lungs. The authors concluded that the immediate cause of death was asphyxia resulting from paralysis of the medulla.

3.2. Nonlethal Toxicity

Limited acute nonlethal exposure data were available on rats from furan

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kinetic studies (Kedderis et al. 1993). Using a closed recirculating chamber, gas uptake studies were conducted with three male Fischer 344 (F344) rats per group, with initial furan concentrations of 100, 500, 1,050, and 3,850 ppm. From the graph provided, it appears that rats were kept in the chamber up to 6 h. In a later study, 12 male rats per group were exposed for 4 h to furan at 52, 107, or 208 ppm. The liver and blood were sampled after exposure to determine furan concentrations. These studies were designed to develop and validate a PBPK model. Therefore, no data on possible toxicity resulting from the furan exposures were provided. It appears that all rats survived the exposures as there was no mention of mortality. The authors stated that 4-h exposures to concentrations higher than 300 ppm were not simulated by the PBPK model because the exposures would probably be lethal (Kedderis and Held 1996).

3.3. Developmental and Reproductive Effects

Data addressing the developmental and reproductive effects of furan in animals were not available.

3.4. Genotoxicity

Furan (up to 10,000 µg per plate) tested negative for genotoxicity in *Salmonella typhimurium* strains TA100, TA1535, TA1537, and TA98 in the presence and absence of exogenous metabolic activation (Mortelmans et al. 1986; NTP 1993) and in the induction of sex-linked recessive lethal mutations in germ cells from male *Drosophila melanogaster* when administered by feeding (10,000 ppm) or injection (25,000 ppm) (NTP 1993). Furan tested positive for genotoxicity in a number of in vitro and in vivo mammalian systems: furan induced trifluorothymidine resistance in mouse L5178Y lymphoma cells in the absence of metabolic activation (concentrations of 1,139 to 3,800 µg per plate, equivalent to ~16.5 to 45 micromolars [µ*M*]) (McGregor et al. 1988; NTP 1993); induced chromosome aberrations in Chinese hamster ovary (CHO) cells with metabolic activation at concentrations of 100 to 200 millimolars (m*M*) (Stich et al. 1981), while another study reported induction of chromosome aberrations and sister chromatid exchanges in CHO cells with and without metabolic activation (NTP 1993); and induced chromosomal aberrations (intraperitoneal [i.p.] concentration of 250 mg/kg) but not sister chromatid exchange (i.p. concentration up to 350 mg/kg) in bone marrow cells after i.p. injections to male $B6C3F_1$ mice (NTP 1993). Kong et al. (1988) reported positive findings in the micronucleus test (species and route of administration not provided) but negative findings in the SOS chromotest and the *umu* test at furan concentrations of 400 mg/kg.

Furan tested negative for genotoxicity when evaluated in the in vivo hepatocyte DNA repair assay (Wilson et al. 1992). For this assay, unscheduled DNA repair was measured in hepatocytes that were isolated from male F344 rats after

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a single gavage administration of furan at 5, 30 or 100 mg/kg or from male B6C3F$_1$ mice after administration of 10, 50, 100, or 200 mg/kg.

3.5. Chronic Toxicity and Carcinogenicity

No data were available assessing the potential carcinogenicity of inhaled furan. Therefore, the data addressing carcinogenicity after oral exposure are included below.

Furan was administered at doses of 0, 2, 4, or 8 mg/kg in corn oil by gavage to groups of 50 male or 50 female F344/N rats for 5 days/week for 2 years (NTP 1993). All groups of dosed rats exhibited an increased incidence of cholangiocarcinomas (males: 0/50, 43/50, 48/50, and 49/50; females: 0/50, 49/50, 50/50, and 48/50 for the 0, 2, 4, and 8-mg/kg groups, respectively). Cholangiocarcinomas were also present in animals examined at the 9- and 15-month interim evaluation. Male rats had an increased combined incidence of hepatocellular adenomas or carcinomas (1/50, 5/50, 22/50, and 35/50), while female rats had an increased incidence of hepatocellular adenomas (0/50, 2/50, 4/50, and 7/50). Nonneoplastic liver lesions that occurred in both male and female treated rats included biliary tract fibrosis, hyperplasia, chronic inflammation, proliferation and hepatocyte cytomegaly, cytoplasmic vacuolization, degeneration, nodular hyperplasia, and necrosis. An increased incidence of mononuclear cell leukemia was observed in rats treated with furan at 4 or 8 mg/kg (males: 8/50, 11/50, 17/50, and 25/50; females: 8/50, 9/50, 17/50, and 21/50). Nephropathy was observed in all dosed animals; severity increased with the dose. The nephropathy was accompanied by an associated increased incidence of parathyroid hyperplasia (renal secondary hyperparathyroidism). Treated male and female rats exhibited forestomach hyperplasia (males: 1/50, 4/49, 7/50, and 6/50; females: 0/50, 2/50, 5/50, and 5/50), and female rats had an increased incidence of subacute inflammation of the forestomach (0/50, 1/50, 5/50, and 6/50). The NTP concluded that there was clear evidence of carcinogenic activity of furan in male and female F344/N rats based on increased incidences of cholangiocarcinoma and hepatocellular neoplasms of the liver and on increased incidences of mononuclear cell leukemia.

Fifty male F344/N rats were administered furan at 30 mg/kg of b.w. in corn oil by gavage for 13 weeks and then maintained for 2 years without additional furan dosing (NTP 1993). Cholangiocarcinoma was present in all dosed animals, and hepatocellular carcinoma occurred with an overall incidence of 15%.

Groups of 50 male and 50 female B6C3F$_1$ mice were administered furan at 0, 8, or 15 mg/kg in corn oil by gavage for 5 days/week for 2 years (NTP 1993). Treated mice had an increased incidence of hepatocellular adenomas (males: 20/50, 33/50, and 42/50; females: 5/50, 31/50, and 48/50) and carcinomas (males: 7/50, 32/50, and 34/50; females: 2/50, 7/50, and 27/50). A significant number of nonneoplastic hepatocellular lesions were also observed, including

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hepatocyte cytomegaly, degeneration, necrosis, multifocal hyperplasia, cytoplasmic vacuolization and biliary tract dilation, fibrosis, hyperplasia, and inflammation. Benign pheochromocytoma and focal hyperplasia of the adrenal medulla were increased in dosed animals (benign pheochromocytoma, males: 1/49, 6/50, and 10/50; females: 2/50, 1/50, and 6/50). Male mice also exhibited an increased incidence of forestomach squamous papilloma (0/49, 1/50, and 3/50), focal inflammation of the forestomach (9/49, 13/50, and 21/50), and papillary hyperplasia of the forestomach (7/49, 14/50, and 22/50). The NTP concluded that there was clear evidence of carcinogenic activity of furan in male and female B6C3F_1 mice based on increased incidences of hepatocellular neoplasms of the liver and benign pheochromocytomas of the adrenal gland. On the basis of evidence of cancer in experimental animals, NTP classifies furan as "reasonably anticipated to be a human carcinogen" (NTP 2005).

A group of 100 female B6C3F_1 mice was dosed with furan at 0.5 mg/kg/day; a group of 75 mice was dosed with 1.0 mg/kg/day; and groups of 50 mice were dosed with 0, 2, 4, or 8 mg/kg/day (Moser et al. 2009). No significant, dose-related differences were observed in mortality rate or b.w. At gross necropsy, the 4- and 8-mg/kg/day dose groups had increased absolute and relative liver weight (values not given) and an increased incidence of grossly observed liver nodules (60% and 100% affected, respectively, compared with 8% for controls and 17% to 20% in the remaining dose groups). The largest nodules were observed in high-dose mice. Histopathologic examination of liver sections revealed a dose-related increase in hepatic cytotoxicity as assessed by the incidence and severity of hepatic subcapsular inflammation. Statistically significant increases were seen in the incidence of mild hepatic cytotoxicity at doses of 1.0 mg/kg/day and higher, of moderate hepatic cytotoxicity at 4 mg/kg/day and higher, and of marked hepatic cytotoxicity at 8 mg/kg/day (data provided in graph; exact values not provided). A dose-related increase was observed in the incidence of foci of altered hepatocytes, adenomas, carcinomas, and adenomas or carcinomas; the incidences generally attained statistical significance at 4 and 8 mg/kg/day. Two of the carcinomas in the high-dose group metastasized to the lung. In general, there was a dose-related decrease in the latency period or time to first tumor.

IARC (1995) concluded that there is inadequate evidence in humans of the carcinogenicity of furan and sufficient evidence in experimental animals of the carcinogenicity of furan. Therefore, IARC states that furan is possibly carcinogenic to humans (Group 2B). EPA has not classified furan as to carcinogenicity (EPA 2003).

3.6. Summary

Quantitative inhalation toxicity data were limited to the Terrill et al. (1989) study in rats. A 1-h exposure to 1,014 or 2,851 ppm was not lethal, whereas exposure to 4,049 ppm killed 9/10 animals. Clinical signs during expo-

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sure included respiratory distress and increased secretory response; however, the degree of the signs at each concentration was not provided. The study by Egle and Gochberg (1979) in which a 1-h LC_{50} of 42 ppm was determined in Swiss mice was unacceptable. The closed system in which the mice were exposed did not provide enough oxygen for the number of mice tested. Therefore, the mortality observed in the mice was most likely confounded by the hypoxic conditions. In a qualitative study, signs of inhalation exposure to furan included decreased blood pressure, increased amplitude of the heartbeat, and respiratory and cardiac arrest in a dog; increased respiration rate, complete analgesia, and relaxation with eventual death in a rat; and respiratory arrest in a rabbit (Koch and Cahan 1925). Necropsy of the dog and rabbit revealed visceral hemorrhage; vessel dilation; and bright, cherry red blood. The authors concluded that the animals died due to asphyxia resulting from paralysis of the medulla.

The NTP (1993) concluded that there was clear evidence of carcinogenic activity of furan in male and female F344/N rats after oral exposure based on increased incidences of cholangiocarcinoma and hepatocellular neoplasms of the liver and increased incidences of mononuclear cell leukemia. The NTP also concluded that there was clear evidence of carcinogenic activity of furan in male and female $B6C3F_1$ mice after oral exposure based on increased incidences of hepatocellular neoplasms of the liver and benign pheochromocytomas of the adrenal gland. Therefore, NTP classifies furan as “reasonably anticipated to be a human carcinogen” (NTP 2005). IARC has listed furan as a Group 2B carcinogen (possibly carcinogenic to humans), while the EPA has not classified furan as to carcinogenicity (EPA 2003).

4. SPECIAL CONSIDERATIONS

4.1. Metabolism and Disposition

On the basis of indirect evidence, it was proposed that the reactive intermediate formed during oxidation of furan was a reactive aldehyde (Burka et al. 1991; Parmar and Burka 1993). Chen et al. (1995) provided evidence that furan is metabolized to the reactive aldehyde *cis*-2-butene-1,4-dial, which is a major hepatic, microsomal metabolite of furan. It is likely that the formation of such an intermediate is responsible for the protein binding and cytochrome P-450 inhibition that occurs after treatment with furan (Burka et al. 1991; Parmar and Burka 1993). Using rat liver microsomes from untreated and acetone-pretreated rats and human cytochrome P-450 2E1 supersomes, Peterson et al. (2005) estimated the kinetics of furan oxidation to *cis*-2-butene-1,4-dial; the Michaelis constants (K_m) were 37.6, 18.5, and 65.1 μM, respectively; the maximum velocities (V_{max}) were 2.5, 5.9, and 15.4 mmoles/min/mg of protein, respectively; and the V_{max}/K_m values were 0.066, 0.32, and 0.324, respectively.

Burka et al. (1991) investigated the distribution and metabolism of furan after gavage with [2,5-^{14}C]furan at 8 mg/kg in male F344 rats. Twenty-four

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hours after a single dose of [^{14}C]furan, 40% of the radioactivity was recovered in expired air, 20% in the urine, 22% in the feces, and 19% remained in the tissues, primarily in the liver (13%). Of the 40% recovered in the expired air, 16% of the original dose was expired unchanged (11% in the first hour) and 26% was expired as CO_2, indicating a ring opening with later oxidation to CO_2. Most of the radioactivity remaining in the liver (80%) was associated with protein; none was associated with DNA. High-performance liquid chromatography analysis of urine collected over 24 h after a single dose revealed at least 10 unidentified metabolites.

Egle and Gochberg (1979) investigated the effects of respiratory rate and chemical concentration on respiratory tract retention of furan in dogs. Retention was determined in the total respiratory tract, lower respiratory tract, and upper respiratory tract (above the tracheal bifurcation) in groups of at least five mongrel dogs (mix of males and females weighing between 9 and 23 kg). The dogs were anesthetized with pentobarbital sodium and allowed to breathe spontaneously from a respirometer for the total and lower tract experiments, or they were artificially ventilated for the upper tract experiments. Furan vapor was generated by passing air through pure furan at room temperature, transferring the air to a recording respirometer, and diluting it to the desired concentration. The percentage of furan retained by the respiratory tract was estimated as the difference in the amount inhaled or in contact with the tissue and the amount recovered. Total respiratory tract retention of furan in the concentration range of 140 to 210 ppm varied from 91% to 95%, while lower tract retention varied from 87% to 93%. One-way (air moving to the distal end of the trachea) and two-way (air moving toward the distal end of the trachea and then back through the nose) upper respiratory tract retention values were reported to be identical, ranging between 85% and 90% (the authors did not speculate on the reason for the identical retention values). An inverse relationship between ventilation rate (between 6 and 26 inhalations per min) and retention was observed in all experiments, although the relationship did not always achieve statistical significance. The effect of concentration on total respiratory retention was also investigated, and it was found that retention increased with increasing concentrations (ranging from 80% retention for an average furan concentration of 110 ppm to 93% retention for an exposure concentration of 180 ppm). The authors did not provide a theory for the observation of increasing retention with increasing concentration over this narrow concentration range. Differences in tidal volume did not affect the total respiratory tract retention of furan.

Using freshly isolated hepatocytes from male F344 rats, Carfagna et al. (1993) demonstrated that furan induced time- and concentration-dependent cytolethality (as measured by lactate dehydrogenase leakage) and glutathione depletion at furan suspension concentrations of 4, 8, and 12 m*M*. When cells were exposed to the cytochrome P-450 inhibitor 1-phenylimidazole before exposure to 12 m*M* furan, glutathione depletion was delayed for 6 h, but cytolethality was not affected. To more closely mimic the typical in vivo exposure concentrations as predicted by a PBPK model, hepatocytes were then exposed for 1 to 4 h to 2

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to 100 µ*M* furan in suspension. Furan exposure produced time- and concentration-dependent cytolethality. Unlike exposure to higher furan concentrations, pretreatment with 1-phenylimidazole prevented both cytolethality and glutathione depletion. Conversely, hepatocytes from rats pretreated with acetone, a cytochrome P-450 2E1 inducer, showed enhanced cytolethality and glutathione depletion after exposure to furan. These results indicate that cytochrome P-450-mediated bioactivation of furan is required for cytolethality and glutathione depletion.

In vivo and in vitro furan kinetics were investigated in male F344 rats (Kedderis et al. 1993). Gas uptake studies were conducted using three rats per group, with initial furan concentrations of 100, 500, 1,050, and 3,850 ppm. Some rats were pretreated (by i.p. injection) with pyrazole, a cytochrome P-450 inhibitor, 30 min before exposure. The results showed a single saturable process following Michaelis-Menten kinetics. A V_{max} of 27.0 µmoles/h and K_m of 2.0 µ*M* were estimated for a 250-g rat using a PBPK model to fit the kinetic data (the PBPK model assumed that all furan biotransformation took place in the liver and followed Michaelis-Menten kinetics). On the basis of these data, it was stated that furan biotransformation is a high-affinity process. Pretreatment with pyrazole completely inhibited furan biotransformation. To validate the PBPK model, the concentration of furan was measured in the blood and liver of rats after a 4-h inhalation exposure to furan at 52, 107, or 208 ppm (12 rats per group). The predicted levels were similar to the actual levels, with furan uptake from the 4-h inhalation exposure having a $V_{max}C$ of 69.2 µmoles/h/kg and a K_m of 1.7 µ*M*.

Furan uptake into freshly isolated rat liver hepatocytes was then determined to compare in vitro and in vivo biotransformation (Kedderis et al. 1993). Rat hepatocytes were exposed to an initial furan headspace concentration of 0.8 to 10.8 µ*M*. Gas uptake by rat hepatocytes was also described by a single saturable process, with a K_m of 0.4 µ*M* and a V_{max} of 0.018 µmoles/h/10^6 cells. When the kinetic parameters from the in vitro experiment were used in the PBPK model, they accurately predicted the in vivo gas uptake data. The effect of various cytochrome P-450 inhibitors on furan biotransformation was also investigated by adding cytochrome P-450 inhibitors dissolved in media to the hepatocyte suspensions immediately before the addition of furan vapor. Furan biotransformation was inhibited by many of these inhibitors, including 1-phenylimidazole, ethanol, isopropanol, dimethyl sulfoxide, *N*-methylpyrazole, and aminobenzotriazole, but it was not inhibited by metyrapone or SKF 525A. Furan oxidation was not influenced by pretreating rats with phenobarbital, a cytochrome P-450 2B inducer. However, pretreatment with acetone resulted in a 5-fold increase in oxidation, indicating that cytochrome P-450 2E1 is a major catalyst in the oxidation of furan.

As discussed above, Kedderis et al. (1993) demonstrated that in vitro determination of furan biotransformation kinetics in freshly isolated rat hepatocytes accurately predicted in vivo furan pharmacokinetics. Therefore, hepatocytes from male $B6C3F_1$ mice and from three humans were exposed to various

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concentrations of furan vapor to develop species-specific pharmacokinetic models for furan biotransformation (Kedderis and Held 1996). The predicted bioactivation and hepatic dosimetry for each of the three species were then compared. As in the previous rat study, furan metabolism in human hepatocytes and mice hepatocytes was described by a single saturable process following Michaelis-Menten kinetics. The hepatocytes from all three species rapidly metabolized furan with a high affinity, with mice metabolizing it the most rapidly, followed by humans and then rats. Simulations of dosimetry models were then used to predict various parameters after inhalation exposure to 10 ppm for 4 h. The predicted absorbed dose of furan (mg/kg) was greatest in mice (10-fold higher) followed by rats (3.5-fold higher) when compared with humans. This difference in absorbed dose was accounted for by the fact that humans are larger and physiologically slower than mice and rats. The liver exposure to the toxic metabolite of furan followed the same pattern. Steady-state blood concentrations were predicted to be reached approximately 1 h after inhalation exposure to furan at 10 ppm. Humans had a slightly lower predicted steady-state blood concentration than rodents. A comparison of the projected rate of furan liver perfusion with furan oxidation revealed that furan oxidation was much greater than furan delivery to the liver via blood flow in all species (13-, 24-, and 37-fold greater in rats, mice, and humans, respectively). Therefore, hepatic blood flow will be the limiting factor in the biotransformation of furan. The authors state that the initial rate of furan bioactivation is so rapid relative to hepatic blood flow that hepatic cytochrome P-450 2E1 concentrations would have to decrease almost 40-fold before the bioactivation rate would decrease below the blood-flow limitation. This finding implies that interindividual variation in human cytochrome P-450 2E1 levels will not be a factor in the bioactivation of furan.

4.2. Mechanism of Toxicity

Furan is metabolized to the reactive aldehyde *cis*-2-butene-1,4-dial, a major hepatic, microsomal metabolite (Burka et al. 1991; Parmar and Burka 1993; Chen et al. 1995). In vitro work with rat hepatocytes demonstrated that furan produces time- and concentration-dependent cytolethality (Carfanga et al. 1993). Through the use of cytochrome P-450 inhibitors and inducers, it was determined that cytochrome P-450-mediated (particularly cytochrome P-450 2E1) oxidation of furan to a reactive intermediate is required for cytolethality (Carfanga et al. 1993; Kedderis et al. 1993).

The liver is the major target organ for furan-induced toxicity after oral exposure, although the kidney and other systems developed toxicity with repeated exposure at concentrations that produce significant hepatotoxicity. Hepatotoxicity was characterized by degenerative and regenerative lesions of hepatocytes and the biliary tract (NTP 1993). Degenerative lesions included cytoplasmic vacuolization, degeneration and necrosis of hepatocytes, and multifocal atrophy of the liver parenchyma (which was secondary to the necrosis of hepatocytes),

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while regenerative changes included cytomegaly and multifocal hyperplasia. Biliary tract changes were noted in most of the portal areas, and included hyperplasia of the bile ducts, which was usually accompanied by fibrosis encompassing many of the hyperplastic ducts, and chronic inflammation. Rats developed changes in the biliary tract at much lower concentrations than mice. Other lesions that have been noted in rats at concentrations producing significant hepatotoxicity include nephropathy, parathyroid hyperplasia and cardiomyopathy (secondary to the increased severity of nephropathy), bone marrow hyperplasia and congestion and proliferation of hematopoietic cells in the spleen (considered secondary to the inflammatory liver lesions), and dilation of the medullary sinuses (from an altered flow of lymph fluid secondary to the extensive hepatic damage). In mice, other lesions include focal hyperplasia of the adrenal medulla, focal inflammation and papillary hyperplasia of the forestomach in male mice, and hematopoietic cell proliferation in the spleen (considered secondary to the inflammatory liver lesions).

Although there is no direct evidence that inhaled furan causes hepatotoxicity, available data make it reasonable to expect that the liver would be a target organ. To compare human furan pharmacokinetics with those of rodents, a PBPK model for inhaled furan was developed and validated in rats to predict the absorbed liver dose after inhalation exposure to defined concentrations and durations (Kedderis et al. 1993). After demonstrating that furan biotransformation kinetics determined with freshly isolated rat hepatocytes in vitro accurately predicted furan pharmacokinetics in vivo, freshly isolated mouse or human hepatocytes were used to develop a mouse- or human-specific PBPK model (Kedderis and Held 1996). To compare interspecies differences, a simulation of a 4-h inhalation exposure to furan at 10 ppm was done for mice, rats, and humans. The absorbed dose of furan (inhaled furan minus exhaled furan divided by kg b.w.) in mice and rats would be ~10- and 3.5-fold higher, respectively, than that in humans (4.1, 1.4, and 0.4 mg/kg for mice, rats, and humans, respectively), while the integrated liver exposure to furan metabolites would be ~6- and 3-fold higher, respectively, than that in humans (1,075, 480, and 168 μM for mice, rats, and humans, respectively).

Much discussion has centered on the likelihood that the hepatic carcinogenic potential of furan is not due to genotoxicity but results from cell proliferation secondary to cytotoxicity. NTP (1993) and Garcia and James (2000) are excellent references. As summarized from these references, evidence that furan affects DNA indirectly through a mechanism involving cytotoxicity and not direct interaction includes the following: furan was negative in genotoxicity assays using *Salmonella*; a single oral bolus administration of radiolabeled furan in rats did not produce metabolites that bound covalently to DNA; single gavage administration of furan did not result in unscheduled DNA synthesis in hepatocytes isolated from rats and mice; administration of a single dose of furan at a concentration that was carcinogenic in a 2-year bioassay resulted in cytotoxicity as measured by clinical chemistry parameters; loss of ATP from uncoupling of oxidative phosphorylation was observed in hepatocytes treated in vitro and in

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mitochondria isolated from furan-exposed rats, which results in depletion of energy needed to maintain cellular membrane calcium pumps and leads to activation of cytotoxic enzymes; and administration of furan 6 or 8 h after a partial hepatectomy in rats did not result in initiation of hepatocarcinogenesis when followed by 52 weeks of promotion with oral administration of phenobarbital. The most current evidence is provided by a 2-year oral gavage study in mice by Moser et al. (2009). An association was seen among the furan-induced dose-response curve for hepatotoxicity, cell death, compensatory cell replication, and formation of liver tumors at doses of 4 mg/kg/day and higher; no tumors were produced at 0.5 or 1 mg/kg/day, concentrations that were not significantly hepatotoxic.

In contrast, evidence supporting the idea that furan could act as a direct hepatic carcinogen (genotoxic mechanism) is limited to the different patterns of mutation leading to oncogene activation that were seen in liver tumor cells from furan-exposed rodents compared with unexposed rodents and the positive results seen in some of the in vivo chromosomal aberration tests and eukaryotic cell mutation tests (such as the cultured mouse lymphoma cells, CHO cells, and $B6C3F_1$ mice) (NTP 1993; Garcia and James 2000).

No information was found concerning a potential mechanism for the development of mononuclear cell leukemia seen in male and female rats exposed to furan.

4.3. Structure-Activity Relationships

Although there are furan derivatives, they vary widely in toxicity, with many having different target organs. Data are insufficient to use structure-activity relationships to derive AEGL values.

4.4. Concentration-Exposure Duration Relationship

The relationship between concentration and duration of exposure as related to lethality was examined by ten Berge et al. (1986) for approximately 20 irritant or systemically acting vapors and gases. The authors subjected the individual animal data sets to probit analysis with exposure duration and exposure concentration as independent variables. An exponential function $C^n \times t = k$, where the value of n ranged from 0.8 to 3.5 for different chemicals was found to be an accurate quantitative descriptor for the chemicals evaluated. Approximately 90% of the values of n range from 1 to 3. Consequently, these values were selected as the reasonable lower and upper bounds of n. A value of n = 1 is used when extrapolating from shorter to longer time periods because the extrapolated values represent the most conservative approach in the absence of other data. Conversely, a value of n = 3 is used when extrapolating from longer to shorter time periods because the extrapolated values are more conservative in the absence of other data.

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4.5. Other Relevant Information

4.5.1. Oral Toxicity Data

The LD_{50} (dose with 50% lethality) of furan in solution after i.p. injection was 6.94 mg/kg for Swiss mice (mix of males and females) and 5.20 mg/kg for male Sprague-Dawley rats (Egle and Gochberg 1979). An oral furan dose of 0.25 cm^3 (234 mg/kg) in dogs and rabbits resulted in a copious flow of bloody saliva, vomiting (dogs), hemorrhage, and death (Koch and Cahan 1925). Intravenous furan administration of 1.5 cm^3 (1.41 mg/kg) in dogs resulted in convulsions, respiratory and cardiac arrest, visceral hemorrhage, and death (Koch and Cahan 1925). A 200-g rat injected with 0.1 cm^3 (94 or 470 mg/kg) of furan had respiratory arrest within 0.5 min. Noted gross effects included hyperemic condition of the liver and intestines; bright, cherry-red blood; and dilation of the blood vessels (Koch and Cahan 1925).

Acute, subchronic, and chronic oral toxicity data were available on rats and mice. The liver was the primary target organ, although the kidney and other systems developed toxicity with chronic exposure at significantly hepatotoxic concentrations. Acute oral exposure generally resulted in transient hepatotoxicity (Wilson et al. 1992). No mortality was seen at doses up to 30 mg/kg/day in rats and 50 mg/kg/day in mice, which were the highest doses tested (Wilson et al. 1992). Subacute exposure resulted in mortality in mice at doses of 40 mg/kg/day and higher and in rats at 80 mg/kg day and higher (NTP 1993). Subchronic exposure produced substantial hepatotoxicity in rats, with cholangiofibrosis and hyperplasia of the biliary tract occurring at the lowest dose tested (4 mg/kg/day). Hepatotoxicity became more severe with concentration, with liver changes including cholangiofibrosis and bile duct hyperplasia, pigmented Kupffer cells, and hepatocyte changes (degeneration, cytomegaly, necrosis, and nodular hyperplasia). Kidney lesions (tubule dilation, necrosis of tubule epithelium), thymic atrophy, and testicular or ovarian atrophy also occurred at the top doses. Similar hepatotoxic lesions were observed in dosed mice, but the lesions generally did not become significant until doses of 30 mg/kg/day and higher. Subchronic exposure to 30 mg/kg/day produced neoplasia in rats. One study reported that rats exposed for 6, 9, 12, or 13 weeks and then followed for an additional 16 months developed hepatic adenocarcinomas (Elmore and Sirica 1993), while another study found that a 13-week exposure produced cholangiocarcinoma in all dosed rats and hepatocellular carcinomas in a few rats by 2 years (NTP 1993).

Chronic oral dosing with furan resulted in neoplasia in rats and mice. At 2 years, almost all dosed rats had developed cholangiocarcinomas (at 2 mg/kg/day and higher), and male rats had an increased combined incidence of hepatocellular adenomas and carcinomas, while female rats had an increased incidence of hepatocellular adenomas (4 mg/kg/day and higher) (NTP 1993). An increased incidence of mononuclear cell leukemia was also observed in rats at 4 mg/kg/day. Nonneoplastic liver lesions included biliary tract fibrosis, hyperpla-

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sia, chronic inflammation, and proliferation; hepatocyte cytomegaly, cytoplasmic vacuolization, degeneration, nodular hyperplasia, and necrosis; and pigmentation of Kupffer cells. Nephropathy was observed in all dosed animals; the severity increased with dose. Other findings were generally considered to be a secondary effect of liver or kidney damage. Mice had an increased incidence of hepatocellular adenomas and carcinomas and benign pheochromocytoma at or above 8 mg/kg/day. Nonneoplastic liver lesions included cytoplasmic vacuolization, focal hyperplasia, and mixed cell cellular infiltration; dilation of the bile duct; biliary tract chronic inflammation, fibrosis, and hyperplasia; hepatocyte cytomegaly, degeneration, and necrosis; Kupffer cell pigmentation; and focal atrophy of the liver parenchyma. The incidence of focal hyperplasia of the adrenal medulla was also increased. A dose-related increase in the incidence of hematopoietic cell proliferation in the spleen was considered secondary to the inflammatory liver lesions. In another study, no increases in neoplasia were noted in mice dosed with furan at 0.5 or 1 mg/kg/day for 2 years; a dose-related increase in the incidence of foci of altered hepatocytes, adenomas, carcinomas, and adenomas or carcinomas occurred at 2.0 mg/kg/day and higher (Moser et al. 2009). In general, there was a dose-related decrease in the latency period or time to first tumor.

4.5.2. Sensitive Populations

Furan-induced hepatotoxicity is caused by the reactive metabolite produced in the liver. PBPK modeling data indicate that production of the metabolite is blood flow limited. Therefore, variations in cytochrome P-450 2E1 levels are not likely to be a significant factor (Kedderis and Held 1996). With hepatocytes used as the basis, PBPK modeling indicates that when adults and children (ages 6, 10, and 14 years) are exposed to the same furan concentrations, the blood concentration of furan is likely to be greater in children than in adults by a factor of only 1.5 (at steady state), and the maximum factor of adult-child differences in liver concentration of furan metabolite is about 1.25 (Price et al. 2003).

4.5.3. Species Variability

Limited data are available to evaluate interspecies variability. Empirical inhalation toxicity data are limited to only one study, which investigated only one species (rats) (Terrill et al. 1989). A PBPK simulation of a 4-h inhalation exposure to furan at 10 ppm was done for mice, rats, and humans to compare interspecies differences in internal dose (Kedderis and Held 1996). The absorbed dose of furan in mice and rats would be ~10- and 3.5-fold higher, respectively, than that in humans, while the integrated liver exposure to furan metabolites would be ~6- and 3-fold higher, respectively, than that in humans. From these data, it would appear that mice are more sensitive to furan toxicity than rats. However, oral toxicity data indicate that the rat is more sensitive, develop-

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ing cholangiofibrosis at the lowest chronic oral gavage dose of furan at 2 mg/kg/day (NTP 1993). In contrast, mice did not develop significant hepatotoxicity until approximately 4.0 mg/kg/day; hepatic effects at 2.0 mg/kg/day did not attain statistical significance (Moser et al. 2009).

5. DATA ANALYSIS FOR AEGL-1

5.1. Summary of Human Data Relevant to AEGL-1

No human data relevant to an AEGL-1 derivation were available.

5.2. Summary of Animal Data Relevant to AEGL-1

No animal data relevant to an AEGL-1 derivation were available in the searched literature.

5.3. Derivation of AEGL-1

AEGL-1 values were not derived for furan. Although a 40-mL puff of cigarette smoke was reported to contain 8.4 µg of furan (0.21 mg/L; 75 ppm), this value should not be the basis for an AEGL derivation because exposure to the puff of cigarette smoke is for too short a duration to extrapolate to the longer time periods used for AEGLs, and furan is one of many components of cigarette smoke that have the potential to cause adverse health effects. The health effects resulting from exposure to the mix of chemicals in cigarette smoke compared with the effects of exposure to furan alone are not known at this time. Therefore, AEGL-1 values were not recommended because of insufficient data (Table 3-4).

6. DATA ANALYSIS FOR AEGL-2

6.1. Summary of Human Data Relevant to AEGL-2

No human data relevant to the derivation of the AEGL-2 values were found in the available literature.

6.2. Summary of Animal Data Relevant to AEGL-2

Groups of Sprague-Dawley male or female rats exposed for 1 h to furan at 1,014, 2,851, or 4,049 ppm exhibited signs of toxicity, including respiratory distress and increased secretory response; mortality was observed at the highest concentration (Terrill et al. 1989). The severity of the signs at each concentration was not provided. It was stated that b.w. decreased in the middle- and high-concentration groups, but b.w. data were not provided.

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TABLE 3-4 AEGL-1 Values for Furan

10 min	30 min	1 h	4 h	8 h
NR[a]	NR	NR	NR	NR

[a]NR: Not recommended.

The exposure data on rats used to develop a PBPK model are not appropriate for use in AEGL derivations (Kedderis et al. 1993). Data from the gas-uptake studies are not appropriate because furan was introduced into the exposure chamber only at the beginning of the exposure; therefore, the exposure concentrations were not held constant over time. The other exposure data (12 rats per group exposed to furan at up to 208 ppm) are not appropriate for use in an AEGL derivation because the design of the study was not to evaluate toxicity but to validate a PBPK model; therefore, no details were provided about possible toxicity resulting from the exposures.

6.3. Derivation of AEGL-2

The AEGL-2 derivation is based on an exposure of 1,014 ppm for 1 h in rats (Terrill et al. 1989). Although the severity of the reported clinical signs (respiratory distress, increased secretory response) was not reported, this lowest exposure concentration group did not exhibit a decrease in b.w. as did the rats exposed to 2,851 and 4,049 ppm. An uncertainty factor of 10 was applied for species-to-species extrapolation because there are inadequate data to properly assess interspecies variability. Of the published furan toxicity studies, Terrill et al. (1989) was the only study that investigated the toxicity of inhaled furan, and it evaluated only one species (rat). Therefore, insufficient empirical data were available to examine species differences in response to inhaled furan. A PBPK simulation of inhalation exposure to furan predicted that the absorbed dose of furan in mice and rats would be ~10- and 3.5-fold higher, respectively, than that in humans, while the integrated liver exposure to furan metabolites would be ~6- and 3-fold higher, respectively, than that in humans. However, oral toxicity data indicate that the rat is more sensitive than the mouse despite PBPK modeling predictions that the mouse would have a 3-fold higher absorbed dose and 2-fold higher integrated liver exposure to furan metabolites than the rat. Therefore, there are too many uncertainties about the response of the rat, mouse, and human liver to furan to base an uncertainty factor on the PBPK modeling predictions.

An intraspecies uncertainty factor of 3 was applied for the following reasons:

1. Assuming death was the result of a progression of the toxicity present at the lower concentrations, the steep dose-response curve for lethality indicates there is not much variability in the response (0/10 rats died at 1,014 or 2,851

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ppm, while 9/10 rats died at 4,049 ppm). The delayed deaths in the high-concentration group suggest that hepatotoxicity was the cause of death.

2. If no hepatotoxicity is present, the clinical signs are likely due to a direct contact effect, which is not expected to vary much among individuals.

3. If the effect is hepatotoxicity, it is due to the reactive metabolite produced in the liver. PBPK modeling data indicate that production of the metabolite is blood flow limited. Therefore, variations in cytochrome P-450 2E1 levels are not likely to be a significant factor (Kedderis and Held 1996). Using hepatocytes as the basis, PBPK modeling indicates that when adults and children (ages 6, 10, and 14 years) are exposed to the same furan concentrations, the blood concentration of furan is likely to be greater in children than in adults by a factor of only 1.5 (at steady state), and the maximum factor of adult-child differences in the liver concentration of furan metabolites is about 1.25 (Price et al. 2003).

A modifying factor of 5 was applied to account for a limited data set (only one data set addressing furan toxicity after inhalation exposure was available); this study was not repeated, and there was no information on furan inhalation toxicity in other species. Therefore, a total uncertainty factor and modifying factor of 150 was applied to the AEGL-2 value.

The experimentally derived exposure values were then scaled to AEGL timeframes by using the concentration-time relationship given by the equation $C^n \times t = k$, where C = concentration, t = time, k is a constant, and n generally ranges from 0.8 to 3.5 (ten Berge et al. 1986). The value of n was not empirically derived because of insufficient data; therefore, a default value of n = 1 was used for extrapolating from shorter to longer exposure periods and a value of n = 3 was used to extrapolate from longer to shorter exposure periods.

AEGL-2 values are presented in Table 3-5.

7. DATA ANALYSIS FOR AEGL-3

7.1. Summary of Human Data Relevant to AEGL-3

No human data were available for derivation of AEGL-3 values.

7.2. Summary of Animal Data Relevant to AEGL-3

Terrill et al. (1989) determined 1-h LC_{50} values from Sprague-Dawley rats exposed to furan at 1,014, 2,851, and 4,049 ppm. The 1-h LC_{50} for male rats was 3,398 ppm, for female rats was 3,550 ppm, and for males and females combined was 3,464 ppm. The dose-response curve, however, was such that no deaths occurred at 1,014 or 2,851 ppm, but 9/10 died at 4,049 ppm. Exposed rats exhibited clinical signs, including respiratory distress and increased secretory response as well as decreased b.w.; however, the degree of the signs and actual

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TABLE 3-5 AEGL-2 Values for Furan

10 min	30 min	1 h	4 h	8 h
12 ppm ($33 mg/m^3$)	8.5 ppm ($24 mg/m^3$)	6.8 ppm ($19 mg/m^3$)	1.7 ppm ($4.7 mg/m^3$)	0.85 ppm ($2.4 mg/m^3$)

b.w. values at each concentration were not provided. The study, which determined a 1-h LC_{50} value of 42 ppm in Swiss mice, was inappropriate for use in deriving an AEGL because the exposure methods were unacceptable (see Section 3.1.3) (Egle and Gochberg 1979).

The rat exposure data used to develop a PBPK model are not appropriate for use in AEGL derivations (Kedderis et al. 1993). Data from the gas uptake studies are not appropriate because furan was introduced into the exposure chamber only at the beginning of the exposure; therefore, the exposure concentrations were not held constant over time. The other exposure data (12 rats per group exposed to furan at up to 208 ppm) are not appropriate for use in an AEGL derivation because the design of the study was not to evaluate toxicity but rather to validate a PBPK model; therefore, no details were provided about possible toxicity resulting from the exposures.

7.3. Derivation of AEGL-3

The AEGL-3 derivation is based on the highest nonlethal concentration in male and female rats of 2,851 ppm for 1 h (Terrill et al. 1989). An uncertainty factor of 10 was applied for species-to-species extrapolation because there are inadequate data to properly assess interspecies variability. Of the published furan toxicity studies, Terrill et al. (1989) was the only one that investigated the toxicity of inhaled furan, and it evaluated only one species (rat). Therefore, insufficient empirical data were available to examine species differences in response to inhaled furan. A PBPK simulation of inhalation exposure to furan predicted that the absorbed dose of furan in mice and rats would be ~10- and 3.5-fold higher, respectively, than that in humans, while the integrated liver exposure to furan metabolites would be ~6- and 3-fold higher, respectively, than that in humans. However, oral toxicity data indicate that the rat is more sensitive than the mouse despite PBPK modeling predictions that the mouse would have a 3-fold higher absorbed dose and 2-fold higher integrated liver exposure to furan metabolites than the rat. Therefore, there are too many uncertainties about the response of the rat, mouse, and human liver to furan to base an uncertainty factor on the PBPK modeling predictions.

An intraspecies uncertainty factor of 3 was applied for the following reasons:

1. Assuming death was the result of a progression of the toxicity present at the lower concentrations, the steep dose-response curve for lethality indicates there is not much variability in the response (0/10 rats died at 1,014 and 2,851

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ppm, while 9/10 rats died at 4,049 ppm). The delayed deaths in the high-concentration group suggest that hepatotoxicity was the cause of death.

2. If no hepatotoxicity is present, the clinical signs are likely due to a direct contact effect and are not expected to vary much among individuals.

3. If the effect is hepatotoxicity, it is due to the reactive metabolite produced in the liver. PBPK modeling data indicate that production of the metabolite is blood flow limited. Therefore, variations in cytochrome P-450 2E1 levels are not likely to be a significant factor (Kedderis and Held 1996). With hepatocytes used as the basis, PBPK modeling indicates that when adults and children (ages 6, 10, and 14 years) are exposed to the same furan concentrations, the blood concentration of furan is likely to be greater in children than in adults by a factor of only 1.5 (at steady state), and the maximum factor of adult-child differences in liver concentration of furan metabolite is about 1.25 (Price et al. 2003).

A modifying factor of 5 was applied to account for a limited data set (only one data set addressing furan toxicity after inhalation exposure was available); this study was not repeated, and there was no information on furan toxicity in other species. Therefore, a total uncertainty factor and modifying factor of 150 was applied to the AEGL-3 value.

The experimentally derived exposure values were then scaled to AEGL timeframes using the concentration-time relationship given by the equation $C^n \times t = k$, where C = concentration, t = time, k is a constant, and n generally ranges from 0.8 to 3.5 (ten Berge et al. 1986). The value of n was not empirically derived because of insufficient data; therefore, a default value of n = 1 was used for extrapolating from shorter to longer exposure periods and a value of n = 3 was used to extrapolate from longer to shorter exposure periods. AEGL-3 values are presented in Table 3-6.

A quantitative carcinogenicity risk assessment for a single exposure to furan was not considered appropriate. Data indicate that furan is a threshold carcinogen dependent on cell proliferation secondary to cytotoxicity at the target site. Therefore, a one-time exposure to furan would not be expected to result in tumor development. This probability is supported by a study in rats and mice administered a single oral gavage dose of 30 or 50 mg/kg/day, respectively, and sacrificed at 12 h or 1, 2, 4, or 8 days postexposure to assess hepatotoxicity (Wilson et al. 1992). Hepatotoxicity (as assessed by increases in liver enzyme activity, hepatocellular proliferation, and histopathology changes) peaked between 1 and 2 days postexposure. By 8 days postexposure, the liver had returned to near normal in the mice and rats, except that rats had some small foci of inflammation and some scarring.

TABLE 3-6 AEGL-3 Values for Furan

10 min	30 min	1 h	4 h	8 h
35 ppm ($97 mg/m^3$)	24 ppm ($67 mg/m^3$)	19 ppm ($53 mg/m^3$)	4.8 ppm ($13 mg/m^3$)	2.4 ppm ($6.7 mg/m^3$)

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8. SUMMARY OF AEGLs

8.1. AEGL Values and Toxicity End Points

A summary of the AEGL values for furan is presented in Table 3-7. AEGL-3 values are derived from the highest nonlethal concentration in rats, AEGL-2 values are based on the threshold for adverse effects in rats, and insufficient data were available to derive AEGL-1 values.

8.2. Comparisons with Other Standards

No occupational standards currently exist for furan. NTP (1993) postulates that it is because occupational exposure to furan should be minimal as it is used and handled in closed systems and containers (NTP 1993). A workplace environmental exposure level (WEEL) is not recommended by the American Industrial Hygiene Association (AIHA 1993) because of the highly toxic and carcinogenic potencies of furan and the lack of a no-observable-effect level for these effects (AIHA 1993). Instead, the recommended WEEL guide states that "worker exposure by all routes should be minimized to the fullest extent possible". The 1-h AEGL-2 value of 6.8 ppm is slightly higher than the 1-h spacecraft maximum allowable concentration (SMAC) of 4 ppm, which is based on an estimated no-observed-adverse-effect level (NOAEL) for hepatoxicity in rats (Garcia and James 2000). Calculation of the SMAC value is as follows: the 1-h LC_{50} of 3,500 ppm (9,700 mg/m^3) calculated by Terrill et al. (1989) was used as the basis for deriving an acceptable concentration for hepatoxicity. This value was extrapolated to a nonhepatotoxic concentration by adjusting with the minute volume for rats and the respiratory retention estimated from dogs and comparing with the oral NOAEL. This value was then adjusted by a total factor of 900 (30 for extrapolation from an LC_{50} to a NOAEL, 3 for interspecies extrapolation, and 10 to account for inadequate database).

TABLE 3-7 Summary of AEGL Values for Furan

Classification	10 min	30 min	1 h	4 h	8 h
AEGL-1 (Nondisabling)	NR[a]	NR	NR	NR	NR
AEGL-2 (Disabling)	12 ppm (33 mg/m^3)	8.5 ppm (24 mg/m^3)	6.8 ppm (19 mg/m^3)	1.7 ppm (4.7 mg/m^3)	0.85 ppm (2.4 mg/m^3)
AEGL-3 (Lethal)	35 ppm (97 mg/m^3)	24 ppm (67 mg/m^3)	19 ppm (53 mg/m^3)	4.8 ppm (13 mg/m^3)	2.4 ppm (6.7 mg/m^3)

[a]NR, not recommended. Numeric values for AEGL-1 are not recommended because of the lack of available data. Absence of an AEGL-1 does not imply that exposure below the AEGL-2 is without adverse effects.

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8.3. Data Adequacy and Research Needs

Quantitative inhalation toxicology data in animals were limited to one study in rats. Much of the literature on furan focused on metabolism and disposition. Research needs are many and include acute inhalation toxicity studies including gross and microscopic examination of exposed animals, inhalation data on multiple species, inhalation developmental and reproductive toxicity studies, and carcinogenicity evaluations after inhalation of furan. Detailed inhalation studies would elucidate whether the target organ is primarily the liver (as it is after oral or i.p. administration) or if other organs are also affected. The studies would also determine whether inhalation exposure to furan produces a direct contact effect, such as irritation.

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APPENDIX A

DERIVATION OF AEGL VALUES FOR FURAN

Derivation of AEGL-1

10-min AEGL-1:	Not recommended based on insufficient data
30-min AEGL-1:	Not recommended based on insufficient data
1-h AEGL-1:	Not recommended based on insufficient data
4-h AEGL-1:	Not recommended based on insufficient data
8-h AEGL-1:	Not recommended based on insufficient data

Derivation of AEGL-2

Key study: Terrill et al. 1989

Toxicity end point: Exposure concentration of 1,014 ppm for 1 h in rats (Terrill et al. 1989). Although the severity of the reported clinical signs (respiratory distress, increased secretory response) was not reported, this lowest exposure concentration group did not exhibit a decrease in b.w. like the rats exposed to 2,851 or 4,049 ppm.

Time-scaling: $C^n \times t = k$ (this document; default of n = 1 for shorter to longer exposure periods and n = 3 for longer to shorter exposure periods)

Uncertainty factors: 10 for interspecies variability
3 for intraspecies variability

Modifying factor: 5 for limited data set

Total uncertainty factors and modifying factor: 150

Calculations: $\text{C/uncertainty factors})^n \times t = k$
$(1{,}014 \text{ ppm}/150)^1 \times 1 \text{ h} = 6.76 \text{ ppm-h}$
$(1{,}014 \text{ ppm}/150)^3 \times 1 \text{ h} = 308.9 \text{ ppm-h}$

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10-min AEGL-2: $C^3 \times 0.167\ h = 308.9$ ppm-h
$C^3 = 1{,}849.7$ ppm
$C = 12.3$ ppm $= 12$ ppm

30-min AEGL-2: $C^3 \times 0.5\ h = 308.9$ ppm-h
$C^3 = 617.8$ ppm
$C = 8.5$ ppm

1-h AEGL-2: $C \times 1\ h = 6.76$ ppm-h
$C = 6.76$ ppm
$C = 6.8$ ppm

4-h AEGL-2: $C^1 \times 4\ h = 6.76$ ppm-h
$C^1 = 1.69$ ppm
$C = 1.7$ ppm

8-h AEGL-2: $C^1 \times 8\ h = 6.76$ ppm-h
$C^1 = 0.845$ ppm
$C = 0.85$ ppm

Derivation of AEGL-3

Key study: Terrill et al. 1989

Toxicity end point: Highest nonlethal exposure concentration in rats of 2,851 ppm for 1 h

Time-scaling: $C^n \times t = k$ (this document; default of n = 1 for shorter to longer exposure periods and n = 3 for longer to shorter exposure periods)

Uncertainty factors: 10 for interspecies variability
3 for intraspecies variability

Modifying factor: 5 for limited data set

Combined uncertainty factors and modifying factor: 150

Calculations: $(C/\text{uncertainty factors})^n \times t = k$
$[(2{,}851\ \text{ppm})/150]^1 \times 1\ h = 19.01$ ppm-h
$[(2{,}851\ \text{ppm})/150]^3 \times 1\ h = 6866.2$ ppm-h

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10-min AEGL-3: $C^3 \times 0.167\ h = 6866.2$ ppm-h
$C^3 = 41{,}114.97$ ppm
C = 34.5 ppm = 35 ppm

30-min AEGL-3: $C^3 \times 0.5\ h = 6866.2$ ppm-h
$C^3 = 13{,}732.4$ ppm
C = 23.9 ppm = 24 ppm

1-h AEGL-3: $C \times 1\ h = 19.01$ ppm-h
C = 19.01 ppm
C = 19 ppm

4-h AEGL-3: $C^1 \times 4\ h = 19.01$ ppm-h
$C^1 = 4.75$ ppm
C = 4.8 ppm
$C^1 \times 8\ h = 19.01$ ppm-h
$C^1 = 2.376$ ppm
C = 2.4 ppm

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APPENDIX B

ACUTE EXPOSURE GUIDELINE LEVELS FOR FURAN

Derivation Summary for Furan

AEGL-1 VALUES

10 min	30 min	1 h	4 h	8 h
Not recommended	Not recommended	Not recommended	Not recommended	Not recommended
Reference: Not applicable				
Test Species/Strain/Number: Not applicable				
Exposure Route/Concentrations/Durations: Not applicable				
Effects: Not applicable				
End Point/Concentration/Rationale: Not applicable				
Uncertainty Factors/Rationale: Not applicable				
Modifying Factor: Not applicable				
Animal to Human Dosimetric Adjustment: Not applicable				
Time-scaling: Not applicable				
Data Adequacy: Insufficient data to propose AEGL values				

AEGL-2 VALUES

10 min	30 min	1 h	4 h	8 h
12 ppm	8.5 ppm	6.8 ppm	1.7 ppm	0.85 ppm
Reference: Terrill, J.B., W.E. Van Horn, D. Robinson, and D.L. Thomas. 1989. Acute inhalation toxicity of furan, 2 methylfuran, furfuryl alcohol, and furfural in the rat. Am. Ind. Hyg. Assoc. J. 50(5):A359-A361.				
Test Species/Strain/Number: Sprague-Dawley rats, 5/sex/exposure group				
Exposure Route/Concentrations/Durations: 1,014, 2,851, and 4,049 ppm for 1 h				
Effects: Signs of toxicity during exposure included respiratory distress, increased secretory response (severity at each concentration not provided). 1,014 ppm: 0/10 died, no changes in b.w. 2,851 ppm: 0/10 died, decreased b.w. 4,049 ppm: 9/10 died, decreased b.w.				
End Point/Concentration/Rationale: Exposure concentration of 1,014 ppm for 1 h in rats. Although the severity of the reported clinical signs (respiratory distress, increased secretory response) was not reported, this lowest exposure concentration group did not exhibit a decrease in b.w. like the rats exposed to 2,851 and 4,049 ppm.				
Total Uncertainty Factor and Modifying Factor = 150				

(Continued)

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AEGL-2 VALUES Continued

10 min	30 min	1 h	4 h	8 h
12 ppm	8.5 ppm	6.8 ppm	1.7 ppm	0.85 ppm

Uncertainty Factors and Rationale:

Interspecies: 10; applied because there are inadequate data to properly assess interspecies variability. Terrill et al. (1989) was the only published furan toxicity study that investigated the toxicity of inhaled furan, and it evaluated only one species (rat). Therefore, insufficient empirical data were available to examine species differences in response to inhaled furan. A PBPK simulation of inhalation exposure to furan predicted that the absorbed dose of furan in mice and rats would be ~10- and 3.5-fold higher, respectively, than that in humans, while the integrated liver exposure to furan metabolites would be ~6- and 3-fold higher, respectively, than that in humans. However, oral toxicity data indicate that the rat is more sensitive than the mouse despite PBPK modeling predictions that the mouse would have a 3-fold higher absorbed dose and 2-fold higher integrated liver exposure to furan metabolites than the rat. Therefore, there are too many uncertainties about the response of the rat, mouse, and human liver to furan to base an uncertainty factor on the PBPK modeling predictions.

Intraspecies: 3; applied for the following reasons:

1. Assuming death was the result of a progression of the toxicity present at the lower concentrations, the steep dose-response curve for lethality indicates there is not much variability in the response (0 of 10 rats died at 1,014 or 2,851 ppm, while 9 of 10 rats died at 4,049 ppm). The delayed deaths in the high-concentration group suggest that hepatotoxicity was the cause of death.
2. If no hepatotoxicity is present, the clinical signs are likely due to a direct contact effect, which is not expected to vary much among individuals.
3. Higher, respectively, than that in humans. If the effect is hepatotoxicity, it is due to the reactive metabolite produced in the liver. PBPK modeling data indicate that production of the metabolite is blood flow limited. Therefore, variations in cytochrome P-450 2E1 levels are not likely to be a significant factor (Kedderis and Held 1996). Additionally, when using hepatocytes as the basis, PBPK modeling indicates that when adults and children (ages 6, 10, and 14 years) are exposed to the same furan concentrations, the blood concentration of furan is likely to be greater in children than in adults by a factor of only 1.5 (at steady state), and the maximum factor of adult-child differences in liver concentration of furan metabolite is about 1.25 (Price et al. 2003).

Modifying Factor: 5; applied to account for a limited data set (only one data set addressing furan toxicity after inhalation exposure was available). This study was not repeated, and there was no information on furan toxicity in other species.

Animal to Human Dosimetric Adjustment: Not applicable

Time-scaling: $C^n \times t = k$, where the default value of n = 1 was used for extrapolating from shorter to longer exposure periods and a value of n = 3 was used to extrapolate from longer to shorter exposure periods.

Data Adequacy: Only limited data were available to assess the inhalation toxicity of furan.

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AEGL-3 VALUES

10 min	30 min	1 h	4 h	8 h
35 ppm	24 ppm	19 ppm	4.8 ppm	2.4 ppm

Reference: Terrill, J.B., W.E. Van Horn, D. Robinson, and D.L. Thomas. 1989. Acute inhalation toxicity of furan, 2 methylfuran, furfuryl alcohol, and furfural in the rat. Am. Ind. Hyg. Assoc. J. 50(5):A359-A361.

Test Species/Strain/Number: Sprague-Dawley rats, 5/sex/exposure group

Exposure Route/Concentrations/Durations: 1,014, 2,851, and 4,049 ppm for 1 h

Effects: Signs of toxicity during exposure included respiratory distress, increased secretory response (severity at each concentration not provided).
1,014 ppm: 0/10 died
2,851 ppm: 0/10 died
4,049 ppm: 9/10 died

End Point/Concentration/Rationale: Highest nonlethal concentration in rats

Total Uncertainty Factor and Modifying Factor = 150

Uncertainty Factors and Rationale:
Interspecies: 10; applied because there are inadequate data to properly assess interspecies variability. Terrill et al. (1989) was the only published furan toxicity study that investigated the toxicity of inhaled furan, and it evaluated only one species (rat). Therefore, insufficient empirical data were available to examine species differences in response to inhaled furan. A PBPK simulation of inhalation exposure to furan predicted that the absorbed dose of furan in mice and rats would be ~10- and 3.5-fold higher, respectively, than that in humans, while the integrated liver exposure to furan metabolites would be ~6- and 3-fold higher, respectively, than that in humans. However, oral toxicity data indicate that the rat is more sensitive than the mouse despite PBPK modeling predictions that the mouse would have a 3-fold higher absorbed dose and 2-fold higher integrated liver exposure to furan metabolites than the rat. Therefore, there are too many uncertainties about the response of the rat, mouse, and human liver to furan to base an uncertainty factor on the PBPK modeling predictions.
Intraspecies: 3; applied for the following reasons:

1. Assuming death was the result of a progression of the toxicity present at the lower concentrations, the steep dose-response curve for lethality indicates there is not much variability in the response (0/10 rats died at 1,014 or 2,851 ppm, while 9/10 rats died at 4,049 ppm). The delayed deaths in the high-concentration group suggest that hepatotoxicity was the cause of death.
2. If no hepatotoxicity is present, the clinical signs are likely due to a direct contact effect, which is not expected to vary much among individuals.
3. If the effect is hepatotoxicity, it is due to the reactive metabolite produced in the liver. PBPK modeling data indicate that production of the metabolite is blood flow limited. Therefore, variations in cytochrome P-450 2E1 levels are not likely to be a significant factor (Kedderis and Held 1996). Additionally, when using heptatocytes as the basis, PBPK modeling indicates that when adults and children (ages 6, 10, and 14 years) are exposed to the same furan concentrations, the blood concentration of furan is likely to be greater in children than in adults by a factor of only 1.5 (at steady state), and the maximum factor of adult-child differences in liver concentration of furan metabolite is about 1.25 (Price et al. 2003).

(Continued)

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AEGL-3 VALUES Continued

10 min	30 min	1 h	4 h	8 h
35 ppm	24 ppm	19 ppm	4.8 ppm	2.4 ppm

Modifying Factor: 5; applied to account for a limited data set (only one data set addressing furan toxicity after inhalation exposure was available; this study was not repeated; there was no information on furan toxicity in other species.

Animal to Human Dosimetric Adjustment: Not applicable

Time-scaling: $C^n \times t = k$, where the default value of n = 1 was used for extrapolating from shorter to longer exposure periods and a value of n = 3 was used to extrapolate from longer to shorter exposure periods.

Data Adequacy: Only limited data were available to assess the inhalation toxicity of furan.

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APPENDIX C

Category Plot for Furan

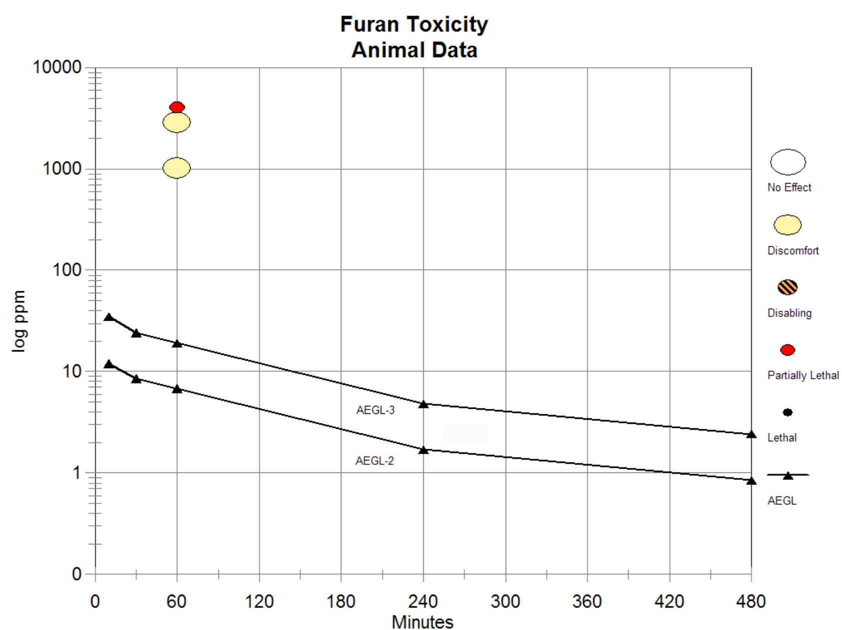

FIGURE 3-1 Category plot of animal toxicity data for furan compared with AEGL values.

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4

Hydrogen Sulfide[1]

Acute Exposure Guideline Levels

PREFACE

Under the authority of the Federal Advisory Committee Act (FACA) P.L. 92-463 of 1972, the National Advisory Committee for Acute Exposure Guideline Levels for Hazardous Substances (NAC/AEGL Committee) has been established to identify, review, and interpret relevant toxicologic and other scientific data and develop AEGLs for high-priority, acutely toxic chemicals.

AEGLs represent threshold exposure limits for the general public and are applicable to emergency exposure periods ranging from 10 minutes (min) to 8 hours (h). Three levels—AEGL-1, AEGL-2, and AEGL—are developed for each of five exposure periods (10 and 30 min and 1, 4, and 8 h) and are distinguished by varying degrees of severity of toxic effects. The three AEGLs have been defined as follows:

AEGL-1 is the airborne concentration (expressed as parts per million [ppm] or milligrams per cubic meter [mg/m^3]) of a substance above which it is predicted that the general population, including susceptible individuals, could experience notable discomfort, irritation, or certain asymptomatic, nonsensory

[1]This document was prepared by the AEGL Development Team composed of Cheryl Bast (Oak Ridge National Laboratory) and Chemical Manager Steve Barbee (National Advisory Committee [NAC] on Acute Exposure Guideline Levels for Hazardous Substances). The NAC reviewed and revised the document and AEGLs as deemed necessary. Both the document and the AEGL values were then reviewed by the National Research Council (NRC) Committee on Acute Exposure Guideline Levels. The NRC committee has concluded that the AEGLs developed in this document are scientifically valid conclusions based on the data reviewed by the NRC and are consistent with the NRC guideline reports (NRC 1993, 2001).

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effects. However, the effects are not disabling and are transient and reversible upon cessation of exposure.

AEGL-2 is the airborne concentration (expressed as ppm or mg/m^3) 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.

AEGL-3 is the airborne concentration (expressed as ppm or mg/m^3) of a substance above which it is predicted that the general population, including susceptible individuals, could experience life-threatening health effects or death.

Airborne concentrations below the AEGL-1 represent exposure levels that could produce mild and progressively increasing but transient and nondisabling odor, taste, and sensory irritation or certain asymptomatic, nonsensory effects. With increasing airborne concentrations above each AEGL, there is a progressive increase in the likelihood of occurrence and the severity of effects described for each corresponding AEGL. Although the AEGLs represent threshold levels for the general public, including susceptible subpopulations, such as infants, children, the elderly, persons with asthma, and those with other illnesses, it is recognized that individuals, subject to idiosyncratic responses, could experience the effects described at concentrations below the corresponding AEGL.

SUMMARY

Hydrogen sulfide (H_2S) is a colorless, flammable gas at ambient temperature and pressure. It has an odor similar to that of rotten eggs and is both an irritant and an asphyxiant. The air odor threshold ranges between 0.008 and 0.13 ppm, and olfactory fatigue may occur at 100 ppm. Paralysis of the olfactory nerve has been reported at 150 ppm (Beauchamp et al. 1984). Mean ambient air concentrations for H_2S range between 0.00071 and 0.066 ppm.

Controlled human data were used to derive AEGL-1 values. Three of 10 volunteers with asthma exposed to H_2S at 2 ppm for 30 min complained of headache and 8 of 10 experienced nonsignificant increased airway resistance (Jappinen et al. 1990). As there were no clinical symptoms of respiratory difficulty and there were no significant changes in forced vital capacity (FVC) or forced expiratory volume in 1 second (FEV_1), the AEGL-1 was based exclusively on increased complaints of headache in the three volunteers (Jappinen et al. 1990). A modifying factor of 3 was applied to account for the wide variability in complaints associated with the foul odor of H_2S and the shallow concentration response at the relatively low concentrations that are consistent with definition of the AEGL-1. The 30-min experimental value was scaled to the 10-min and 1-, 4-, and 8-h time points by using the concentration-exposure duration relationship, $C^{4.4} \times t = k$, where C is concentration, t is time, and k is a constant. The exponent 4.4 was derived from rat lethality data ranging from 10-min to 6-h exposures.

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The level of distinct odor awareness (LOA) for H_2S is 0.01 ppm (see Appendix C for LOA derivation). The LOA represents the concentration above which it is predicted that more than half the exposed population will experience at least a distinct odor intensity, and about 10% of the population will experience a strong odor intensity. The LOA should help chemical emergency responders in assessing public awareness of the exposure due to odor perception. Thus, the derived AEGL-1 values are considered to have warning properties.

The AEGL-2 was based on the induction of perivascular edema in rats exposed to H_2S at 200 ppm for 4 h (Green et al. 1991; Khan et al. 1991). An uncertainty factor of 3 was applied as rat and mouse data suggest little interspecies variability. An intraspecies uncertainty factor of 3 was applied to account for sensitive individuals. The intraspecies uncertainty factor of 3 is considered sufficient because applying the default uncertainty factor of 10 would result in a total uncertainty factor of 30, which would yield AEGL-2 values inconsistent with the total database for H_2S. AEGL-2 values derived with larger uncertainty factors are essentially identical to or below the 10-ppm concentration causing no adverse health effects in humans exercising to exhaustion for up to 30 min (Bhambhani and Singh 1991; Bhambhani et al. 1994, 1996a,b, 1997). Therefore, the total uncertainty factor applied in the derivation of AEGL-2 is 10. The 4-h experimental value was then scaled to the 10- and 30-min and 1- and 8-h time points, using $C^{4.4} \times t = k$. The exponent 4.4 was derived from empirical rat lethality data ranging from 10-min to 6-h exposures.

The AEGL-3 was based on the highest concentration causing no mortality in the rat after a 1-h exposure (504 ppm) (MacEwen and Vernot 1972). An uncertainty factor of 3 was used to extrapolate from animals to humans as rat and mouse data suggest little interspecies variability. An uncertainty factor of 3 was applied to account for sensitive individuals. The intraspecies uncertainty factor of 3 is considered sufficient because applying the default uncertainty factor results in AEGL-3 values inconsistent with the data. AEGL-3 values derived with larger uncertainty factors were equal to or less than twice the concentration that failed to produce adverse health effects in humans exercising to exhaustion for up to 30 min (Bhambhani and Singh 1991; Bhambhani et al. 1994, 1996a,b, 1997). Increased mortality or irreversible medical conditions consistent with the definition of AEGL-3 are unlikely at such concentrations. Therefore, the total uncertainty factor is 10. The value was then scaled to the 10- and 30 min and 1-, 4-, and 8-h time points, using $C^{4.4} \times t = k$. The exponent 4.4 was derived from rat lethality data ranging from 10-min to 6-h exposures.

The AEGL values are listed in Table 4-1.

1. INTRODUCTION

Hydrogen sulfide is a colorless, flammable gas at ambient temperature and pressure (NIOSH 1977). The National Fire Protection Association (NFPA 1974) placed H_2S in the highest flammability classification. Precautions against fire

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and explosion must be exercised to maintain airborne H_2S below 0.43%. It has an odor similar to that of rotten eggs and it is both an irritant and an asphyxiant. The odor threshold is between 0.008 and 0.13 ppm, and olfactory fatigue, resulting in a lack of detection of odor, may occur at 100 ppm. Paralysis of the olfactory nerve has been reported at 150 ppm (Beauchamp et al. 1984). Mean ambient air concentrations in the United States range between 0.00071 and 0.066 ppm (NRC 1977; Graedel et al. 1986; Warneck 1988).

Approximately 90% of the H_2S in the atmosphere occurs from natural sources. Hydrogen sulfide arises through bacterial reduction of sulfates and organic sulfur-containing compounds. It is emitted from crude oil, stagnant or polluted water, sewers, and manure or coal pits with low oxygen content. A small amount of H_2S is emitted from volcanoes, vents, mudpots, and similar geologic formations (ATSDR 2006).

Hydrogen sulfide is synthesized commercially for use in rayon manufacturing, as an agricultural disinfectant, and as an additive in lubricants. It is also used as an intermediate in sulfuric acid and inorganic sulfide manufacturing and it is a by-product of pulp and paper manufacturing (Jaakkola et al. 1990) and geothermal operations (Kage et al. 1998); it is present in "sour" crude petroleum (NIOSH 1977; Guidotti 1994), roofing tar (Hoidal et al. 1986), natural gas, and shale oil (Ahlborg 1951; Kilburn 1993). Hydrogen sulfide has been manufactured in ton quantities for use in production of heavy water and as a moderator in nuclear reactors (NRC 1977). In 1997, it was manufactured in the United States by three companies at five sites (ATSDR 1999). Most H_2S is made and used captively or transported by pipeline. As of 2007, total domestic commercial production in the United States exceeded 1.1×10^6 tons/year (Kroshwitz and Seidel 2007).

The physicochemical properties of H_2S are presented in Table 4-2.

TABLE 4-1 Summary of AEGL Values for Hydrogen Sulfide

Classification	10 min	30 min	1 h	4 h	8 h	End Point (Reference)
AEGL-1 (Nondisabling)	0.75 ppm (1.05 mg/m^3)	0.60 ppm (0.84 mg/m^3)	0.51 ppm (0.71 mg/m^3)	0.36 ppm (0.50 mg/m^3)	0.33 ppm (0.46 mg/m^3)	Headache in humans with asthma (Jappinen et al. 1990)
AEGL-2 (Disabling)	41 ppm (59 mg/m^3)	32 ppm (45 mg/m^3)	27 ppm (39 mg/m^3)	20 ppm (28 mg/m^3)	17 ppm (24 mg/m^3)	Perivascular edema in rats (Green et al. 1991; Khan et al. 1991)
AEGL-3 (Lethality)	76 ppm (106 mg/m^3)	59 ppm (85 mg/m^3)	50 ppm (71 mg/m^3)	37 ppm (52 mg/m^3)	31 ppm (44 mg/m^3)	Highest concentration causing no mortality in the rat after a 1-h exposure (MacEwen and Vernot 1972)

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TABLE 4-2 Chemical and Physical Data for Hydrogen Sulfide

Parameter	Data	Reference
Common name	Hydrogen sulfide	ATSDR 2006
Synonyms	Hydrosulfuric acid, stink damp, sulfur hydride, sulfurated hydrogen, dihydrogen monosulfide, sewer gas, swamp gas, rotten-egg gas	ATSDR 2006
CAS registry number	7783-06-4	ATSDR 2006
Chemical formula	H_2S	ATSDR 2006
Molecular weight	34.08	ATSDR 2006
Physical state	Colorless gas	ATSDR 2006
Melting, boiling, and flash points	−85.49°C, −60.33°C, and 26°C	ATSDR 2006
Density	1.5392 grams/liter at 0°C	ATSDR 2006
Density in air	1.192	ATSDR 2006
Solubility	1 gram in 242 milliliters of water at 20°C; soluble in alcohol, ether, glycerol, gasoline, kerosene, crude oil, carbon disulfide	ATSDR 2006
Vapor pressure	15,600 mmHg at 25°C	ATSDR 2006
Conversion factors in air	1 ppm = 1.4 mg/m^3 1 mg/m^3 = 0.7 ppm	AIHA 2000

2. HUMAN TOXICITY DATA

2.1. Acute Lethality

According to U.S. Occupational Safety and Health Administration records, there were 80 fatalities in 57 H_2S incidents from 1984 to 1994 (Fuller and Suruda 2000). Nineteen deaths and 36 H_2S-induced injuries occurred among people attempting to rescue victims overcome by the gas.

The clinical toxicology of H_2S has been reviewed (Smith and Gosselin 1979; Gosselin et al. 1984; Reiffenstein et al. 1992). Literature accounts of human fatalities after inhalation of H_2S are abundant; however, exposure concentrations and durations in these accidents are generally not rigorously defined. Vapor concentrations on the order of 500 to 1,000 ppm or more are usually fatal within minutes (API 1948; Ahlborg 1951; Reiffenstein et al. 1992). Most fatalities occur in confined spaces (sewers, animal processing plants, manure tanks) and result from respiratory failure, initially presenting with respiratory insufficiency, noncardiogenic pulmonary edema, coma, or cyanosis. In many cases,

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people lose consciousness after only one or two breaths, termed "slaughterhouse sledgehammer" (ATSDR 2006). Osbern and Crapo (1981) reported a typical, unfortunate accident involving an underground liquid manure storage pit. A farmer drained the liquid manure to a depth of 45 cm and entered the pit to retrieve a lid that a cow had kicked into the tank. The farmer was overcome within a few minutes, as were three men who attempted to rescue him. Three of the men lapsed into unconsciousness and died before reaching the hospital. Autopsy showed massive liquid manure pulmonary aspiration in two individuals and fulminant pulmonary edema without manure aspiration in the third. "Increased heart-blood sulfide levels" indicated significant H_2S exposure. The clinical course of the surviving patient was complicated by hemodynamic instability, respiratory distress syndrome, and pulmonary infection. Air samples taken a week after the accident detected H_2S at 76 ppm; however, pit air concentrations were likely higher at the time of exposure because of temperature and manure concentration.

Two workers collapsed and died within 45 min after entering a sewer manhole (NIOSH 1991). A concentration of 200 ppm was measured in sewer air 6 days after the accident. In another accident, a worker at a poultry-processing plant died after exposure to an estimated H_2S concentration of 2,000 to 4,000 ppm for an estimated 15 to 20 min (Breysse 1961). Pulmonary, intracranial, and cerebral edema and cyanosis were observed at autopsy.

Hsu et al. (1987) reported 10 cases of accidental H_2S poisoning. The H_2S concentration was 429 ppm 4 h after the accident. Five victims died at the site of exposure. Four lost consciousness within 2 to 20 min of the accident and fell into a deep coma for approximately 48 h, regaining consciousness only after extensive hyperbaric oxygen therapy. Electrocardiograms indicated T-wave changes in all five survivors and changes in the P-wave in the patient remaining in the coma for 2 days. By day 9 after the accident, the electroencephalograms (EEGs) were essentially normal in four victims, while the P-wave returned to normal on day 21 and the T-wave returned to normal on day 36 in the most severely poisoned patient. On day 3, blood urea nitrogen increased to 39.2 milligrams per deciliter and remained above normal through day 13, while serum glutamic pyruvic transaminase activity remained increased through day 8. No pulmonary edema or long-term neurologic abnormalities were identified.

Autopsy of H_2S victims often reveals pulmonary edema (Adelson and Sunshine 1966; Winek et al. 1968) and petechial hemorrhage into the lungs and brain along with gray-green cyanosis or a purple-to-green cast to the cerebral cortex, viscera, and blood (Freireich 1946; Breysse 1961; Adelson and Sunshine 1966). Postmortem formation of sulfhemoglobin contributes to these discolorations. Pulmonary edema is not always associated with death in H_2S-induced central respiratory arrest inasmuch as very high (1,000 ppm) exposures induce prompt unconsciousness, apnea, and anoxic convulsions with risus sardonicus and opisthotonos (Hurwitz and Taylor 1954). If victims are promptly evacuated, recovery can be rapid. It is at lower "but nevertheless fatal" exposure concentra-

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tions where the development of pulmonary and other systemic signs of H_2S intoxication are permitted (Gosselin et al. 1984).

2.2. Nonlethal Toxicity

2.2.1. Case Reports

Albuminuria and hematuria (Osbern and Crapo 1981), brain stem and cortical damage (Hurwitz and Taylor 1954), neurasthenia, amnesia, and other psychic disorders and difficulties with equilibrium to frank tremor can afflict survivors of acute H_2S intoxication. People acutely exposed to lower but nonfatal concentrations commonly experience lacrimation, photophobia, corneal opacity, tachypnea, dyspnea, tracheobronchitis (with elevated risk for bronchopneumonia), gastrointestinal distress (nausea, vomiting, diarrhea), arrhythmias, and palpitations, but these changes generally resolve promptly upon evacuation to fresh air. Patients can be left with a residual cough, hyposmia, dysosmia, or phantosmia (Kilburn and Warshaw 1995; Hirsch and Zavala 1999). At much higher concentrations, recovery from coma can be relatively rapid, and the clinical course is usually complete but slow in those patients who do not die. Artificial respiration is appropriate in victims with depressed or absent breathing, as are supportive steps to combat development of pulmonary edema (Gosselin et al. 1984). Oxygen is indicated in those patients with acute respiratory distress syndrome (Smith 1996).

Case reports concerning nonlethal H_2S effects in humans are abundant; however, exposure parameters, concentration, and duration are often either unreported or only estimated. Symptoms of acute H_2S exposure include ocular and respiratory tract irritation, nausea, headaches, loss of equilibrium, memory loss, olfactory paralysis, loss of consciousness, tremors, and convulsions (ATSDR 2006). Among tunnel, rayon, and sewer workers exposed for several hours to days, keratoconjunctivitis (“gas eye”) is commonplace (Vanhoorne et al. 1995). This condition is characterized by tearing, burning, and scratchy irritation of the cornea and conjunctivae, and the symptoms generally resolve without intervention or sequelae after cessation of exposure (Grant 1974). The threshold for ocular irritation by H_2S alone has been reported as 10 to 20 ppm (WHO 1981), but accounts of eye pain, burning, and photophobia in the presence of related sulfides put the threshold at no more than 6 ppm (Vanhoorne et al. 1995).

Parra et al. (1991) reported cases of 14 workers likely poisoned with H_2S from toilet facilities. The toilets were connected to a manure pit without a siphon. Workers complained of eye, nose, and throat irritation; nausea; dizziness; vomiting; and dyspnea. (One worker died a few hours after hospital admission. Hemorrhagic bronchitis and asphyxia were identified at autopsy.) Most workers recovered uneventfully; however, after a symptom-free period of 3 weeks, one worker reported dyspnea, chest tightness, and hemoptysis. A mild, bilateral, interstitial fibrosis was found on a chest X-ray and pulmonary function tests

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showed mild restrictive pulmonary disease. Five months after the accident, the patient was asymptomatic except for residual exertion dyspnea.

Six patients were examined 5 to 10 years after accidental exposures to unknown concentrations of H_2S (Tvedt et al. 1991a,b). They had been unconscious for 5 to 20 min in the H_2S atmospheres. Persistent neurologic symptoms included impaired vision, memory loss, decreased motor function, tremors, ataxia, abnormal learning and retention, and slight cerebral atrophy. One patient was severely demented.

In another report, 37 workers (ages 24 to 50 years) were accidentally exposed to an undetermined concentration of H_2S while drilling a pit to lay the foundation for a municipal sewage pumping station (Snyder et al. 1995). Symptoms included headache, dizziness, breathlessness, cough, burning discomfort in the chest, throat and eye irritation, nausea, and vomiting. Most workers recovered uneventfully; however, one worker died and another remained in a coma for 5 days. The comatose patient was aggressively treated with hyperbaric oxygen. He was discharged from the hospital on day 15 with slow speech, impaired attention span, easy distractibility, isolated retrograde amnesia, decreased ability to communicate, impaired visual memory, and poor retention of new information. His condition was unchanged at 12 and 18 months after exposure. Numerous other reports of permanent or persistent neurologic effects after exposure to H_2S have been published (Wasch et al. 1989; Kilburn 1993; Kilburn and Warshaw 1995; Kilburn 1997). As with the other case studies, these reports lack definitive exposure parameters.

In May and June 1964, a H_2S emission from an industrial landfill in Terre Haute, Indiana, resulted in nearby residents complaining about odor and nausea, loss of sleep, shortness of breath, and headache (HEW 1964). Samples collected from five sites around the city indicated H_2S concentrations ranging from <2 to >300 parts per billion (ppb); however, the observations are confounded by concurrent exposure to other malodorous pollutants such as smoke from burning garbage and sulfurous coal tar. Data summarized and experiments carried out by the State of California Department of Health Services showed that the geometric mean of the threshold odor concentration for H_2S was approximately 0.008 ppm (Amoore 1985). It was also stated that, as a provisional rule, it appears that when an unpleasant odor reaches approximately 5 times its odor threshold concentration, the mean concentration for complaints of odor annoyance is attained. Factors responsible for odor annoyance were categorized as the unpleasant odor sensation itself, effects on social life, and instigation of headache or nausea (Amoore 1985).

In another report, Ruth (1986) reported an odor threshold range of 0.0005 to 0.01 ppm and listed an irritating H_2S concentration of 10 ppm.

Members of the Mobile Monitoring Team, Source Sampling Team, Technical Support Team, and Systems Planning and Implementation Team of the Texas Natural Resource Conservation Commission (TNRCC) conducted a mobile laboratory sampling trip to the Corpus Christi, Texas, area from January 31 to February 6, 1998 (TNRCC 1998). The mean H_2S concentration downwind

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from an oil refinery was 0.09 ppm (30-min downwind average). The six staff members complained of persistent objectionable odors, eye and throat irritation, headache, and nausea. In most cases, the symptoms subsided within a few hours after leaving the sampling site; throat irritation persisted in two staff members through the following day. The exposure duration was about 5 h. Sulfur dioxide, benzene, methyl *t*-butyl ether, and toluene were also detected, and it is possible that these chemicals contributed to the complaints.

Some authors hypothesize that odors, such as the rotten egg smell associated with H_2S, may trigger asthma attacks; however, quantitative data supporting this premise are limited, and it is uncertain whether a toxicologic mechanism or stress-induced anxiety is involved. It is clear that objectionable odor can affect behavior; individuals detecting odors may stay indoors, temporarily leave the neighborhood or area, complain to officials, or consider a change of residence (Shusterman 1992). Many objectionable odor sources have been implicated in asthma attacks, annoyance, and behavioral modifications and include municipal odors (landfills, sewage treatment plants), agricultural odors (composting, feed lots), industrial odors (pulp mills, refineries, hazardous waste sites) (Shusterman 1992), and household (perfumes, flowers, cleaning products, food/cooking odors) and bodily odors (Stein and Ottenberg 1958; Herbert et al. 1967). Bruvold et al. (1983) used a survey questionnaire to determine whether people living downwind of two sewage treatment plants in California (Pacifica and Novato) detected H_2Sodor and experienced odor annoyance more frequently than people living in two control communities. Hydrogen sulfide concentrations in the test communities ranged from 1 to 6 ppb. Odor was reported by 49 of 54 respondents in Pacifica compared with 4 of 54 respondents in the Pacifica control community and 19 of 50 respondents in Novato compared with 1 of 48 respondents from the Novato control community. When respondents were asked to rate odor annoyance on a scale of 0 (no annoyance) to 10 (extreme annoyance), the following median annoyance scores were obtained: Pacifica affected = 7.9, Pacifica control = 5.5, Novato affected = 4.3, Novato control = 1.0. One in nine respondents in the “exposed” neighborhoods also reported that they or a family member had been made sick by the odors; however, only 1% of this group sought medical attention and no odor-induced asthma was reported in these communities.

Rossi et al. (1993) examined the association between emergency room visits for asthma attacks and weather (temperature, humidity, barometric pressure, rainfall), levels of air pollutants (nitrogen dioxide, sulfur dioxide, H_2S, total suspended particles), and pollen counts. No association was found between pollen counts and weather conditions, except for an inverse correlation with temperature. The most significant correlation was found for nitrogen dioxide, with lesser correlations for sulfur dioxide, H_2S, and total suspended particles. The daily mean H_2S concentration was 0.0022 ppm (range 0 to 0.02 ppm) and the daily maximum was 0.01 ppm (range 0 to 0.12 ppm). Only the relationship between nitrogen dioxide and asthma attacks held after controlling for temperature.

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Stein and Ottenberg (1958) interviewed 25 hospitalized patients with asthma to determine whether odors precipitated asthma attacks. Initially, the patients were asked what would precipitate an asthma attack. If odors were not mentioned, they were then specifically asked about odors. The responses were then analyzed for the "character of the odorous substance." Twenty-two of the 25 subjects stated that odors precipitated attacks; of these 22, about half initially specified odor as a precipitating factor, whereas the others included odor only when prompted by the interviewer. The most common (74%) "precipitating odors" were categorized as "cleanliness/uncleanliness and included urine, sweat, feces, disinfectant, bleach, camphor, dirty/musty, smoke, sulfur, chemicals, paint, horses, and barn; followed by "romantic odors" (21%) including perfume, spring, and flowers; and foods (5%) such as bacon, onion, and garlic. In another report, Herbert et al. (1967) administered questionnaires to two groups of patients with asthma, one from a psychiatric hospital and one from a general hospital, to determine whether odors precipitated asthma attacks. Approximately 80% of patients reported that odor triggered asthma attacks, with the most common triggers being paint, tobacco fumes, wood smoke, household odors, and paraffin. The authors concluded that emotional distress could precipitate an asthmatic attack. There was no clear differentiation between the two groups of patients. In both the Stein and Ottenberg (1958) and Herbert et al. (1967) reports, the results are compromised by possible undefined concurrent exposures, no measure of repeatability, and the fact that neither the patient nor the interviewer was blinded to the inquiry.

2.2.2. Epidemiologic Studies

Jappinen et al. (1990) studied a cohort of 26 male pulp mill workers (mean age 40.3 years, range 22 to 60 years) to assess the possible effects of H_2S on respiratory function. The workers experienced daily exposure to H_2S "usually below the maximum permitted concentration of 10 ppm." Bronchial responsiveness, FVC, and FEV_1 were measured after at least 1 day off work and at the end of a workday. No significant changes in respiratory function or bronchial responsiveness were observed at the end of the workday compared with control values.

Studies of communities located near pulp mills have reported increased incidences of respiratory system symptoms (irritation and cough) and central nervous system symptoms (headaches, migraine) (Partti-Pellinin et al. 1996). Although H_2S concentrations have been reported in these studies, the populations were also exposed to relatively high concentrations of other malodorous sulfur compounds such as sulfur dioxide and mercaptans. Thus, it is difficult to define a concentration-response relationship for H_2S from these reports.

Hessel et al. (1997) studied a group of 175 oil and gas workers (mean age 35 years) in Alberta, Canada. Hydrogen sulfide exposure concentrations were not available; therefore, exposure groups were determined by questioning the

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workers about "exposures strong enough to cause symptoms," "exposures that resulted in loss of consciousness (knockdown)," or no exposure. Exposures strong enough to cause symptoms were reported by 51 workers, "knockdown" was reported by 14 workers, and 110 workers reported no exposure. Exposures strong enough to cause symptoms were not associated with lower spirometric values. Knockdowns were not associated with lower spirometric values but were associated with shortness of breath while hurrying on the level or up a slight hill, wheezing with chest tightness, and wheezing attacks.

In another study, 21 swine confinement facility owner-operators were tested by spirometry immediately before and after a 4-h work period (Donham et al. 1984). The confinement workers had statistically significant ($p < 0.05$) reductions in pulmonary flow rates ranging from 3.3% to 11.9% mean forced expiratory flow (FEF) after the 4-h work period. The report states that the work environment was sampled for particulates and gases during the exposure period and that evidence suggested a concentration-response relationship between carbon dioxide and H_2S exposure and lung function decrements. However, these monitoring data were not presented in the study report.

2.2.3. Experimental Studies

Jappinen et al. (1990) exposed a group of 10 people with asthma (3 men age 33 to 50 years and 7 women age 31 to 61 years) to H_2S at 2 ppm for 30 min. The subjects had been diagnosed with bronchial asthma for 1 to 13 years and were under medical supervision. Severe asthma patients were excluded from the protocol. Two volunteers were exposed simultaneously in a 10-m^3 sealed tile-walled exposure chamber with an oxygen flow of 2 liters (L)/min. Hydrogen sulfide concentration was monitored continuously with a sulfur dioxide analyzer connected to a converter that transformed H_2S into sulfur dioxide at 840°C. The H_2S was supplied to the chamber from laminated plastic bags through plastic tubing. All asthma subjects complained of an unpleasant odor and nasal and pharyngeal dryness at the initiation of exposure. Three of the 10 complained of headache after exposure. There were no significant effects on FVC, FEV_1, or FEF values after exposure to H_2S. Airway resistance (R_{aw}) value was slightly decreased in two and increased in eight subjects. The range of R_{aw} differences was –5.95% to +137.78%, with an average increase of 26.3%; no accompanying clinical symptoms were observed. The range of specific airway conductance (SG_{aw}) differences was –57.7% to +30%, with an average decrease of 8.4%. These effects were not statistically significant; however, in two subjects, changes were greater than 30% in both R_{aw} and SG_{aw}.

Bhambhani and Singh (1991) exposed 16 healthy male volunteers (age 25.2 ± 5.5 years) to H_2S at 0, 0.5, 2.0, or 5.0 ppm during graded cycle exercise performed to exhaustion (up to 16 min). Filtered air from a pressurized cylinder was passed through a stainless steel humidifying chamber at a flow rate of 50 L/min. After humidification, the air was mixed with H_2S at 1,000 ppm in nitro-

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gen regulated from a pressurized cylinder to obtain the desired concentration of H_2S in the test atmosphere. The H_2S was then collected in three polyethylene bags connected in a series and suspended from the ceiling. The flow of gas into and out of each bag was controlled with a two-way stopcock and the H_2S concentration was continuously monitored with a sulfur dioxide analyzer connected to a converter that transformed H_2S into sulfur dioxide. Each subject completed a preliminary graded exercise test (H_2S at 0 ppm) designed to determine maximum oxygen uptake (VO_{2max}) and to identify the anaerobic and respiratory compensation thresholds by use of respiratory gas-exchange criteria. The subject began pedaling an electronically braked cycle ergonometer at zero load at a speed of 60 revolutions/min for 4 min. The power output was then increased by 200 kilopond-meters (kpm)/min every 2 min. Until volitional fatigue was achieved, there was no further increase in oxygen uptake with increasing power output (when VO_{2max} was achieved). After the preliminary test, each subject completed four additional tests, one every 2 weeks, while breathing H_2S at 0, 0.5, 2.0, or 5.0 ppm in a random order. After a 4-min warmup period at zero load, the power output was increased so that it was midway between zero load and ventilatory threshold 1 (VT_1) from the preliminary test. The subject pedaled at this power output for 3 min, after which the power output was increased midway between VT_1 and VT_2 from the preliminary test. Pedaling was continued for an additional 3 min and then raised to the VT_2 level. After 3 min of pedaling, power output was increased 200 kpm/min every minute until the VO_{2max} was achieved. There was no effect on heart rate or expired ventilation as a result of H_2S exposure during submaximal or maximal exercise. There was a tendency for oxygen uptake to increase and for carbon dioxide output to decrease with increasing H_2Sconcentration; however, these effects were significant ($p < 0.05$) only at 5.0 ppm. Blood lactate levels also increased significantly ($p < 0.05$) as a result of exposure to H_2S at 5.0 ppm. Maximal power output was not affected, however, thus questioning the biologic and toxicologic significance of the measured effects.

In a followup study, Bhambhani et al. (1994, 1996a) exposed 25 healthy volunteers (13 men, age 24.7 ± 4.6 years and 12 women, age 22 ± 2.1 years) to H_2S at 0 or 5 ppm for 30 min while exercising on a cycle ergonometer at 50% of their VO_{2max}. The exposure protocol is essentially identical to that described by Bhambhani and Singh (1991). There were no treatment-related effects on oxygen uptake, carbon dioxide production, respiratory exchange ratio, heart rate, blood pressure, arterial blood oxygen and carbon dioxide tensions or pH, or perceived exertion ratings (Bhambhani et al. 1994). In men, the muscle citrate synthetase decreased 19% ($p < 0.05$) after H_2Sexposure compared with controls. Muscle lactate and lactic acid dehydrogenase increased 24% (not significant [n.s.]) and 6% (n.s.), respectively, and cytochrome oxidase decreased 9% (n.s.) after H_2Sexposure compared with the control condition. In women, the muscle citrate synthetase decreased 19% (n.s.) after H_2S exposure compared with controls. Muscle lactate and lactic acid dehydrogenase were not affected in women and cytochrome oxidase increased 23% (n.s.) after H_2S exposure compared with

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the control condition (Bambhani et al. 1996a). None of the subjects reported adverse health effects after H_2S exposure.

In another followup study, Bhambhani et al. (1996b) exposed 19 healthy volunteers (9 men, age 27.4 ± 6.4 years and 10 women, age 21.8 ± 3.0 years) to H_2S at 0 or 10 ppm for 15 min while exercising on a cycle ergonometer at 50% of their VO_{2max}. The exposure protocol is essentially identical to that described by Bhambhani and Singh (1991). There were no treatment-related effects on FVC, FEV_1, peak expiratory flow rate, FEF rate, or maximal ventilation volume in either sex.

In another study, Bambhani et al. (1996b), exposed 28 healthy volunteers (15 men, age 23.4 ± 5.2 years and 13 women, age 21.8 ± 3.0 years) to H_2S at 0 or 10 ppm for 30 min while exercising on a cycle ergonometer at 50% of their VO_{2max}. The exposure protocol is essentially identical to that described by Bhambhani and Singh (1991). There were no treatment-related effects on oxygen uptake, carbon dioxide production, respiratory exchange ratio, heart rate, blood pressure, arterial blood oxygen, and carbon dioxide tensions. Muscle lactate increased 33% (n.s.) in men and 16% (n.s.) in women after exposure to H_2S. Muscle cytochrome oxidase decreased by 16% in men, whereas it increased by 11% in women after exposure to H_2S. None of the subjects reported adverse health effects after H_2S exposure.

2.3. Developmental and Reproductive Toxicity

Xu et al. (1998) conducted a retrospective epidemiologic study of female workers in a large petrochemical facility in Beijing, China. The study was designed to investigate the association between petrochemical exposure and spontaneous abortion. The facility consisted of 17 major production plants, which are divided into separate workshops, allowing for the assessment of exposure to specific chemicals. Married women (n = 2,853) between the ages of 20 and 44 years who had never smoked and did not consume ethanol participated in the study. These women reported at least one pregnancy during their employment at the plant. The association between occupational exposures and spontaneous abortion were adjusted for age, education level, plant shift work, standing and kneeling hours at work, noise level, dust concentration, passive smoke at home and work, and diet. There were 106 women (3.7% of the study population) exposed to only H_2S and the data showed a significant association between occupational H_2S exposure and increased frequency of spontaneous abortion. The spontaneous abortion rate was 12.3% (odds ratio 2.3, 95% confidence interval 1.2 to 4.4) in workers exposed to H_2S compared with 2.9% in the control group. No H_2S exposure concentrations were reported.

2.4. Genotoxicity

Genotoxic studies on acute human exposure to H_2S were not available.

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2.5. Carcinogenicity

Carcinogenicity studies on human exposure to H_2S were not available. However, no increase in cancer incidence was found in a cohort living downwind from natural gas refineries in Alberta, Canada, from 1970 to 1984 (ATSDR 2006).

2.6. Summary

Hydrogen sulfide is both an irritant and an asphyxiant. At relatively low concentrations (<10 ppm), minor ocular and respiratory irritation occur; at higher concentrations (hundreds to thousands of ppm), the central nervous system is affected and paralysis of the respiratory center may lead to rapid death. Ocular effects described after inhalation of H_2S include acute conjunctivitis (gas eye) at concentrations of 50 to 100 ppm and are believed to be result from direct contact of H_2S with the eye. The threshold for eye irritation is reportedly 6 to 20 ppm (WHO 1981; Vanhoorne et al. 1995; ACGIH 1996). There are numerous reports of accidental poisonings with H_2S; however, neither reliable concentrations nor duration parameters are described for lethal or nonlethal end points. Several reports suggest that neurologic effects may persist in survivors of severe H_2S poisonings. Some reports hypothesize that odors, such as the rotten egg smell associated with H_2S, may trigger asthma attacks and other health effects; however, it is uncertain whether a toxicologic mechanism or a nontoxicologic odor-related mechanism is involved. No adverse effects were observed in male or female volunteers exposed to H_2S at 5 or 10 ppm for up to 30 min while exercising to exhaustion (Bambhani and Singh 1991; Bambhani et al. 1994, 1996a,b). Headache in 30% and clinically insignificant increased airway resistance were observed in asthma patients exposed to H_2S at 2 ppm for 30 min (Jappinen et al. 1990). An association between spontaneous abortion and occupational exposure to H_2S was identified in a cohort of female petroleum plant workers; however, no H_2S concentrations were provided (Xu et al. 1998). No information on potential human genotoxicity or carcinogenicity was located.

3. ANIMAL TOXICITY DATA

3.1. Acute Lethality

3.1.1. Rats

Groups of five male and five female Wistar rats were exposed to different concentrations of H_2S for 10, 30, or 60 min (Arts et al. 1989; Zwart et al. 1990). The test atmospheres were monitored at the inlet and outlet of the glass exposure chambers (0.90 m long and 0.15 m diameter; flow rate 25 to 40 L/min). The 10-min LC_{50} (concentration with 50% lethality) value was 835 ppm, whereas the 30- and 50-min LC_{50} values were 726 and 683 ppm, respectively.

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MacEwen and Vernot (1972) exposed groups of 10 male Sprague-Dawley rats to H_2S at 400, 504, 635, or 800 ppm for 1 h. The exposures were conducted in a 30-L glass bell jar at an air flow rate of 30 L/min and chamber concentrations were continuously monitored with an ion-specific sulfide electrode. Gasping was observed during exposure. Rats surviving the 14-day observation period exhibited normal weight gains; however, congestion and mottling of the kidney and liver accompanied by moderate to severe fatty changes in the liver were observed at necropsy. A 1-h LC_{50} value of 712 ppm was calculated. Mortality data are summarized in Table 4-3.

Tansy et al. (1981) exposed groups of five male and five female Sprague-Dawley rats to H_2S at 0 to 600 ppm for 4 h, followed by a 14-day observation period. Animals were exposed simultaneously in a 75-L glass chamber. A 4-h LC_{50} of 444 ppm was calculated.

Prior et al. (1988) exposed groups of male and female Long Evans, Sprague-Dawley, and Fischer 344 (F344) rats to different concentrations of H_2S for 2, 4, or 6 h, followed by a 14-day observation. Exposure chambers were clear, acrylic horizontal flow chambers with two removable stainless steel cones with a total volume of 69 L. Hydrogen sulfide concentrations were monitored by gas chromatography four times per hour. LC_{50} values of 587, 501, and 335 ppm were calculated for the 2-, 4-, and 6-h time points, respectively. No strain differences were observed.

In another study, Khan et al. (1990) exposed groups of 12 male F344 rats to H_2S at 0, 10, 50, 200, 400, or 500 to 700 ppm for 4 h. Animals exposed to H_2S at 500 to 700 ppm died from the exposure. This study, including nonlethal effects observed at lower concentrations, is described in Section 3.2.1.

Groups of five male Sprague-Dawley rats were exposed to H_2S at 0 or 1,655.4 ± 390.9 ppm (Lopez et al. 1989). The H_2S was passed sequentially through a regulator and flowmeter and then mixed with a supply of conditioned air. The H_2S flow was adjusted to maintain the target concentration and the test atmosphere was analyzed by gas chromatography. All test rats died within 3 min, but none of the control rats died from exposure. Rats exposed to H_2S exhibited dyspnea characterized by exaggerated abnormal, audible respiration and had frothy fluid from the nose and mouth during exposure. Pulmonary edema was evident in exposed rats at necropsy. No abnormalities were observed in control animals.

TABLE 4-3 Mortality of Rats Exposed to Hydrogen Sulfide for 1 Hour

Concentration (ppm)	Mortality
400	0/10
504	0/10
635	1/10
800	9/10

Source: MacEwen and Vernot 1972.

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3.1.2. Mice

Groups of five male and five female Swiss mice were exposed to different concentrations of H_2S for 10, 30, or 50 min (Arts et al. 1989; Zwart et al. 1990). The test atmospheres were monitored at the inlet and outlet of the glass exposure chambers (0.90 m long and 0.15-m diameter; flow rate 25 to 40 L/min). The 10-min LC_{50} value was 1,160 ppm, and the 30- and 50-min LC_{50} values were 800 and 676 ppm, respectively.

MacEwen and Vernot (1972) exposed groups of 10 male CF1 mice to H_2S at 400, 504, 635, or 800 ppm for 1 h. The exposures were conducted in a 30-L glass bell jar at an air flow rate of 30 L/min and chamber concentrations were continuously monitored with an ion-specific sulfide electrode. Gasping and convulsions were observed during exposure. Mice surviving the 14-day observation exhibited normal weight gains; however, one mouse each from the 635- and 800-ppm groups had a blocked urethral opening due to encrustation of the external orifice, and consequently the bladders were distended upon examination at 14 days. A 1-h LC_{50} value of 634 ppm was calculated. Mortality data from this study are summarized in Table 4-4, and rat and mouse LC_{50} values are summarized in Table 4-5.

TABLE 4-4 Mortality of Mice Exposed to Hydrogen Sulfide for 1 Hour

Concentration (ppm)	Mortality
400	2/10
504	0/10
635	5/10
800	8/10

Source: MacEwen and Vernot 1972.

TABLE 4-5 Summary of LC_{50} Values of Rats and Mice Exposed to Hydrogen Sulfide

	LC_{50} (ppm)		
Exposure Duration	Rat	Mouse	Reference
10 min	835	1,160	Zwart et al. 1990
30 min	726	800	Zwart et al. 1990
60 min[a]	683	676	Zwart et al. 1990
1 h	712	634	MacEwen and Vernot 1972
2 h	587	ND	Prior et al. 1988
4 h	501	ND	Prior et al. 1988
4 h	444	ND	Tansy et al. 1981
6 h	335	ND	Prior et al. 1988

[a]Zwart et al. (1990) reported a 50-min LC_{50} value. However, because the exposure was 60 min, it is assumed that this value is a 60-min LC_{50} value.
Abbreviation: LC_{50}, concentration with 50% lethality; ND, no data.

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3.2. Nonlethal Toxicity

3.2.1. Rats

Groups of 12 male F344 rats were exposed to H_2S at 0, 10, 200, or 400 ppm for 4 h (Lopez et al. 1987, 1988). Exposure chambers were clear, acrylic horizontal flow chambers with two removable stainless steel cones with a total volume of 69 L. Hydrogen sulfide was passed sequentially through a regulator and flowmeter and was then mixed with a supply of filtered air. The flow of H_2S and air was adjusted to maintain the target concentration in the exposure chambers. Gas flow volume was 17 L/min. Hydrogen sulfide concentrations were monitored by gas chromatography three times per hour. Actual concentrations were within 4% of nominal. Four rats from each group were sacrificed at 1, 20, and 44 h postexposure. No animals died during exposure. No effects were noted at 10 ppm. At the end of the 4-h exposure, rats in the 400-ppm group were lethargic but rapidly recovered. Lactate dehydrogenase (LDH) and protein in nasal passages were initially increased in rats administered 400 ppm but had returned to baseline levels 20 h after exposure (Lopez et al. 1987). Bronchoalveolar cell counts were decreased only in the 400-ppm group. Alkaline phosphatase (400 ppm) and LDH (200 and 400 ppm) activities in lung lavage fluid were increased up to 90%. Lung protein concentrations were increased 3,000% and remained elevated through 44 h postexposure at 400 ppm. Treatment-related nasal cavity lesions were observed only at 400 ppm (Lopez et al. 1988). Necrosis and exfoliation of respiratory and olfactory mucosal cells were observed; however, squamous epithelial cells were not affected. Lesions were distributed midway along the nasal passages involving nasal and maxillary conchae but not ethmoidal conchae. Injured respiratory mucosa was undergoing repair at 44 h; however, olfactory mucosa continued to exfoliate after 44 h.

Green et al. (1991) exposed groups of six male F344 rats to H_2S at 0, 200, or 300 ppm for 4 h utilizing the exposure system described by Lopez et al. (1987, 1988). Animals were sacrificed 1 h after exposure. Actual exposure concentrations were 0, 194.1 ± 3.7, and 290 ± 3 ppm. No adverse clinical signs were observed in animals exposed to 200 ppm; however, there was a significant ($p < 0.001$) increase in protein and LDH in lavage fluid from these animals compared with controls. Focal areas of perivascular edema and occasional collections of proteinaceous material in the alveoli were observed in animals from the 200-ppm group. Rats exposed to H_2S at 300 ppm were visibly stressed during exposure and their lungs showed focal areas of red atelectasis and patchy alveolar edema with perivascular and peribronchial interstitial edema. Significantly ($p < 0.001$) increased protein concentration and LDH activity were found in lung lavage fluid from animals exposed to 300 ppm. Exposure to H_2S at 300 ppm produced significant ($p < 0.01$) abnormalities of surfactant activity parameters.

Khan et al. (1990) exposed groups of 12 male F344 rats to H_2S at 0, 10, 50, 200, 400, or 500 to 700 ppm for 4 h utilizing the exposure system described by Lopez et al. (1987, 1988). Four rats from each group were sacrificed at 1, 24,

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or 48 h postexposure. No effects were observed at 10 ppm. Cytochrome *c* oxidase activity in lung mitochondria was significantly ($p < 0.05$) decreased at 50 ppm (15%), 200 ppm (43%), and 400 ppm (68%) at 1 h postexposure compared with controls. Cytochrome *c* oxidase activity returned to normal 24 h postexposure in the 50-ppm animals, was about 89% of normal at 24 and 48 h postexposure at 200 ppm, and remained at 70% of normal 24 and 48 h postexposure in the 400-ppm group. Animals inhaling H_2S at 500 to 700 ppm died and had >90% inhibition of cytochrome *c* oxidase activity. Succinate oxidase was significantly ($p < 0.001$) decreased at 200 ppm (40%) and 400 ppm (63%) at the 1-h time point. Succinate-cytochrome *c* reductase and NADH-cytochrome *c* reductase were unaffected by exposure to H_2S.

Kahn et al. (1991) studied groups of six male F344 rats inhaling H_2S at 0, 50, 200, or 400 ppm for 4 h using an exposure system identical to that described by Lopez et al. (1987). All rats were sacrificed immediately after cessation of exposure, and lungs were lavaged. Greater than 90% of the cells lavaged were pulmonary alveolar macrophages. Exposure to H_2S at 50 and 200 ppm did not affect the number of cells recovered; however, cell viability was significantly ($p < 0.05$) reduced in animals exposed to 400 ppm. This experiment also showed complete inhibition of zymosan-induced stimulation of respiratory rates of the macrophages from animals exposed to H_2S at 200 and 400 ppm.

In a third study, Kahn et al. (1998) exposed groups of male F344 rats to H_2S at 0, 1, 10, or 100 ppm, 8 h/day, 6 days/week, for 5 weeks. Lung, brain, and liver mitochondria were analyzed for cytochrome oxidase activities, and erythrocytes were analyzed for superoxide dismutase and glutathione peroxidase activities. No effects were noted at 1 ppm. Rats exposed to H_2S at 10 and 100 ppm exhibited concentration-related reductions in cytochrome *c* oxidase activity in lung ($p < 0.05$) mitochondria. Cytochrome *c* oxidase activity in liver mitochondria was not affected by exposure. A small (8%) decrease in superoxide dismutase activity was noted in 100-ppm rats compared with controls.

Male F344 rats (number per group not stated) were exposed to H_2S at 0 or 400 ppm for 4 h or 1,500 ppm for 4 min (Lefebvre et al. 1991). The exposure system was similar to that described by Lopez et al. (1987, 1988). Immediately after exposure, the eyes were washed with 0.4 milliliter of saline and the lavage fluid was collected for exfoliative cytology. The number of exfoliated cells was increased in animals exposed to H_2S (44 cells/microliter [μL] at 400 ppm; 35 cells/μL at 1,500 ppm) compared with control (19 cells/μL). Exposure to H_2S also increased the proportion of conjunctival to corneal epithelial cells recovered compared with controls.

Kohno et al. (1991) exposed groups of male Wistar rats to H_2S at 75 ppm for 20 to 60 min. Heart rates were decreased 10% to 27% during exposure and up to 1 h postexposure compared with controls, which was thought to be a possible compensatory response. Necropsy showed slight lung congestion in exposed animals.

Dorman et al. (2002) exposed groups of male CD rats to H_2S at 10, 30, 80, 200, or 400 ppm for 3 h and examined hindbrain, lung, liver, and nasal cyto-

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chrome oxidase activity and sulfide concentrations immediately after exposure. Lung sulfide and sulfide metabolite concentrations were also analyzed at 0, 1.5, 3, 3.25, 3.5, 4, 5, and 7 h postexposure to 400 ppm. Lung sulfide concentrations increased during exposure and returned to baseline levels within 15 min after exposure to 400 ppm, and lung sulfide metabolite concentrations transiently increased immediately after the end of exposure. Decreased cytochrome oxidase activities were noted in the olfactory epithelium at 30, 80, 200, and 400 ppm. Increased olfactory sulfide concentrations were noted after exposure to 400 ppm. No effects were noted for hindbrain and nasal respiratory sulfide concentrations. Nasal respiratory epithelial cytochrome oxidase activity decreased in animals exposed to H_2S at 30, 80, 200, and 400 ppm. Liver sulfide concentrations were increased at 200 and 400 ppm, and liver cytochrome oxidase activity was increased in all treatment groups.

Brenneman et al. (2000) exposed groups of male Sprague-Dawley rats to H_2S at 0, 10, 30, or 800 ppm for 6 h/day, 7 days/week, for 10 weeks. Multifocal, bilaterally symmetrical olfactory neuron loss and basal cell hyperplasia, limited to the olfactory mucosa, were observed in rats exposed to 30 or 80 ppm. Lesions were observed in the dorsal medial meatus and dorsal and medial areas of the ethmoid recess. No treatment-related effects were noted at 10 ppm.

Brenneman et al. (2002) then exposed groups of male Sprague-Dawley rats to H_2S at 0, 30, 80, 200, or 400 ppm for 3 h/day for 1 or 5 consecutive days. Nasal passages were examined histologically 24 h postexposure and lesion recovery was assessed 2 and 6 weeks after the 5-day exposure. Regeneration of the respiratory mucosa and full thickness necrosis of the olfactory mucosa localized to the ventral and dorsal medial meatus were noted after the single 3-h exposure to H_2S at 80, 200, or 400 ppm. A single exposure to 400 ppm resulted in severe mitochondrial swelling in sustentacular cells and olfactory neurons, progressing to olfactory epithelial necrosis and sloughing. Repeated exposure to 80, 200, or 400 ppm resulted in necrosis of the olfactory mucosa with early mucosal regeneration extending from the dorsomedial meatus to the caudal regions of the ethmoid recess.

In a repeated exposure study, groups of five male Sprague-Dawley rats were exposed to H_2S at 0 (nitrogen air mixture), 25, 50, 75, or 100 ppm for 3 h/day for 5 days (Skrajny et al. 1996). The rats were implanted with hippocampal electrodes in the dentate gyrus or CA1 region to determine the effects of H_2S on EEG activity in the hippocampus and neocortex. Exposures occurred in a 33-L acrylic chamber designed to allow observation of the animal during recording of the EEG during exposure. Filtered room air was drawn through the chamber with a vacuum pump and mixed with H_2S in nitrogen. The mixture was passed through a diffuser in the top of the chamber at an airflow of 11 L/min. The H_2S concentration was continuously monitored by gas chromatography. Exposure to H_2S at 100 ppm resulted in increased hippocampal theta activity but did not change the basic behavior-EEG correlation. Total hippocampal theta activity increased in a cumulative manner in both the dentate gyrus and CA1 regions during exposure to H_2S at 25, 50, 75, and 100 ppm. The increase was significant

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($p < 0.05$) after exposure on days 3, 4, and 5 and did not return to control levels during the 24-h period between exposures. Complete recovery of the 100-ppm animals required about 2 weeks.

In a subchronic study, groups of 15 male and 15 female Sprague-Dawley rats were exposed to H_2S at 0, 10.1, 30.5, or 80.0 ppm for 6 h/day, 5 days/week, for 90 days (CIIT 1983a). No animals died during the study. Clinical signs included crustiness around the ear tags; crusty noses, eyes, and muzzles; red-stained fur; swollen red ears; rales; lacrimation; and swollen muzzles and eyes. Decreased body weight gain and decreased brain weights were observed in high-concentration animals of both sexes. No treatment-related effects were observed with regard to food consumption, ophthalmology, neurologic function, clinical pathology, gross pathology, histopathology, and neuropathology.

3.2.2. Mice

In a subchronic study, groups of 10 male and 12 female $B6C3F_1$ mice were exposed to H_2S at 0, 10.1, 30.5, or 80.0 ppm for 6 h/day, 5 days/week, for 90 days (CIIT 1983b). Two control mice were found dead, and one 30.5-ppm mouse was accidentally killed during the study. Two 80-ppm mice exhibiting prostration or hypoactivity were sacrificed in extremis. Clinical observations at 80 ppm included alopecia, missing anterior appendage, and loss of use of an anterior appendage. Decreased body weight gain was observed in high-concentration animals. Neurologic function studies revealed two 80-ppm animals not responding to an artificial light stimulus and two others with an irregular gait due to missing appendages. At sacrifice, 89% of high-concentration males and 78% of high-concentration females displayed inflammation of the anterior nasal mucosa in the anterior segments of the nose. No effects were noted in controls or in the low- or midconcentration animals. No treatment-related effects were observed with regard to ophthalmology, hematology, serum chemistry, and urinalyses.

3.2.3. Rabbits

Mixed-breed rabbits exposed to H_2S at 72 ppm for 1.5 h developed ventricular repolarization (Kosmider et al. 1966). Rabbits exposed to 72 ppm for 0.5 h/day for 5 days exhibited cardiac arrhythmia. Histochemical staining of myocardial cells from exposed rabbits showed a decrease in ATP phosphohydrolase and $NADPH_2$ oxidoreductase.

3.2.4. Monkeys

Rhesus monkeys exposed to H_2S at 500 ppm for 22 min developed ataxia, anorexia, and parenchymal necrosis in the brain, while exposure to 500 ppm for

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35 min resulted in conjunctival irritation, sudden loss of consciousness, and respiratory and cardiac arrest (Lund and Wieland 1966). Monkeys exposed to H_2S at 500 ppm for 25 min, followed by a 17-min exposure to 500 ppm 3 days later had extensive changes in gray matter and moderate liver hyperemia upon necropsy (Lund and Wieland 1966). No other details on tissue pathology results were provided. No other experimental details were presented.

3.3. Developmental and Reproductive Toxicity

Dorman et al. (2000) exposed groups of 12 male and 12 female Sprague-Dawley rats to H_2S at 0, 10, 30, or 80 ppm for 6 h/day, 7 days/week, for 2 weeks before breeding. Exposures continued during a 2-week mating period and then from gestation days 0 to 19. Exposure of the dams and pups (eight rats per litter after culling) resumed between postnatal day 5 and 18. Adult male rats were exposed for 70 consecutive days. Offspring were evaluated for motor activity, passive avoidance, functional observational battery, acoustic startle response, and neuropathology. A significant ($p < 0.05$) decrease in food consumption was observed in F_0 males only in the 80-ppm group during the first week of exposure. There were no deaths and no treatment-related adverse clinical signs in F_0 males or females. There were no significant effects on reproductive performance of the F_0 rats as assessed by the number of females with live pups, average gestation length, and average number of implants per pregnant female. No treatment-related effects in pups were noted with regard to growth, development, and behavioral tests. No other effects were noted at any concentration.

Saillenfait et al. (1989) exposed groups of eight pregnant Sprague-Dawley rats to H_2S at 0, 50, 100, or 150 ppm for 6 h/day on days 6 to 20 of gestation. Hydrogen sulfide (10% in nitrogen) was delivered from a compressed gas cylinder by a pressure-regulating valve into a 200-L stainless steel dynamic flow inhalation chamber. Prenatal exposure to H_2S at 100 and 150 ppm resulted in a slight (−4%) decrease ($p < 0.01$) in fetal body weight. However, the biologic significance of this finding is questionable as the dams also lost weight and there were a greater numbers of live fetuses per litter at these concentrations. No maternal or fetal effects were noted at 50 ppm.

Groups of 17 to 24 pregnant Sprague-Dawley rats (and offspring) were exposed to H_2S at 0, 20, 50, or 75 ppm for 7 h/day from gestation day 1 through delivery and up to postnatal day 21 (Hayden et al. 1990a). Each group was culled to litters with 12 pups each at parturition. Animals were exposed in a 90-L acrylic dome-shaped chamber. Air supply to the chamber was room air drawn through a HEPA (high-efficiency particulate air) filter with a vacuum blower and mixed with H_2S in nitrogen. The mixture was passed through an orifice plate through a diffuser at the top of the chamber. Actual H_2S concentrations were within 5% of nominal concentrations. Maternal blood glucose increased about 50% ($p < 0.05$) on day 21 postpartum in all exposure groups. This increase was accompanied by a possible decrease in serum triglyceride in dams and pups

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on day 21. No treatment-related effects were noted for serum alkaline phosphatase, LDH, or serum glutamic-oxaloacetic transaminase.

In a similar study, groups of five or six pregnant Sprague-Dawley rats were exposed to H_2S at 0, 20, 50, or 75 ppm for 7 h/day from gestation day 6 through postpartum day 21 (Hayden et al. 1990b). There was a concentration-dependent increase in parturition time (measured in minutes) of about 10%, 20%, and 40% over matched controls at 20, 50, and 75 ppm, respectively. The biologic significance of this effect is questionable because parturition time was quite variable among control groups. Maternal liver cholesterol increased ($p < 0.05$) in the 75-ppm group. No other treatment-related effects were noted in the dams or their offspring.

Hannah and Roth (1991) exposed Sprague-Dawley rats to H_2S at 0, 20, or 50 ppm for 7 h/day from gestation day 5 through postpartum day 21. The mean Purkinje cell terminal path length significantly increased in the 20- and 50-ppm groups compared with controls; however, the biological significance of this finding is unclear as no concentration response was observed. The study neither mentioned whether there were any adverse effects on maternal health nor provided neurobehavioral parameters in followup studies of H_2S-exposed offspring.

Skrajny et al. (1992) exposed groups of 20 pregnant Sprague-Dawley rats to H_2S at 0, 20, or 75 ppm for 7 h/day from day 5 postcoitus through postpartum day 21. Animals were exposed in a 90-L chamber designed to allow continuous observation. Air flow was 30 L/min and H_2S concentrations were verified by gas chromatography. Increased ($p < 0.05$) serotonin levels were observed in the frontal cortex on day 21 postpartum of pups exposed to H_2S at 20 ppm, and increased ($p < 0.01$) serotonin levels were observed in the cerebellum and frontal cortex on postpartum days 14 and 21 in pups exposed to H_2S at 75 ppm. Norepinephrine concentrations increased ($p < 0.05$) at 75 ppm in the cerebellum at postpartum days 7, 14, and 21 but significantly increased in the frontal cortex only at postpartum day 21. At 20 ppm, frontal cortex norepinephrine levels decreased compared with controls on days 14 and 21. No neurobehavioral correlates were provided in preliminary or followup investigations to assist in determining the biologic significance of those findings.

3.4. Genotoxicity

Studies on the genotoxic potential of H_2S are limited. Hydrogen sulfide was negative in an Ames reverse mutation assay in *Salmonella typhimurium* strains TA97, TA98, and TA100 with and without hepatic S9 from male Sprague-Dawley rat and Syrian hamster liver (ATSDR 2006). No other genotoxicity studies were located.

3.5. Carcinogenicity

No information on the carcinogenic potential of H_2S was located in the available literature.

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3.6. Summary

Animal lethality studies from various laboratories have produced similar results. All F344 rats died when exposed to H_2S at 500 to 700 ppm for 4 h (Khan et al. 1990), whereas no F344 rats died when similarly exposed to H_2S at up to 400 ppm for 4 h (Lopez et al. 1987, 1988; Khan et al. 1990, 1991; Green et al. 1991; Lefebvre et al. 1991). Rat LC_{50} values range from 683 to 712 ppm for periods of 10 min to 1 h (MacEwen and Vernot 1972; Zwart et al. 1990) and from 335 to 587 ppm for periods of 2 to 6 h in Sprague-Dawley, Long Evans, and F344 rats (Tansy et al. 1981; Prior et al. 1988). Mouse LC_{50} values range from 634 to 1,160 ppm for periods of 10 min to 1 h (MacEwen and Vernot 1972; Zwart et al. 1990).

Toxicity studies identified the respiratory and nervous systems as the primary targets for H_2S, with some cardiac involvement. Nasal pathology and decreased bronchiolar macrophage counts have been observed in rats exposed to 400 ppm for 4 h (Lopez et al. 1988; Kahn et al. 1991), and enzyme activity changes (LDH, cytochrome *c* oxidase, succinate oxidase) have been observed in rats exposed to H_2S at 50 to 400 ppm for 4 h (Lopez et al. 1987, 1988; Khan et al. 1990; Green et al. 1991). Pulmonary edema and atelectasis developed in rats exposed to H_2S at 300 ppm for 4 h (Green et al. 1991). Changes in EEG activity were identified in rats repeatedly exposed to H_2S at 25 to 100 ppm (Skrajny et al. 1996), and biochemical brain changes were identified in mice exposed to H_2S at 100 ppm for 2 h.

Data suggesting that H_2S exposure can impair prenatal neurologic development in rats are equivocal; inconsistencies in some developmental studies make the data difficult to interpret. Genotoxicity data are extremely limited and no carcinogenicity data were located.

4. SPECIAL CONSIDERATIONS

4.1. Metabolism and Disposition

Hydrogen sulfide is present as an endogenous substance in normal mammalian (Warenycia et al. 1990; Kage et al. 1992; Mitchell et al. 1993) tissues. Normal tissues contain relatively high (μg/g) concentrations of endogenous sulfide ion (HS^-) (Dorman et al. 2002). Endogenous sulfide also arises from bacterial activity in the lower bowel (NRC 1977) and there is some evidence that H_2S participates in normal nerve transmission (Kimura 2000).

The major metabolic and excretory pathway of H_2S involves oxidation of the sulfide to sulfate. The exact mechanism of the oxidation is unknown; however, both enzymatic (sulfide oxidase) and nonenzymatic catalytic systems have been proposed. Glutathione stimulates mitochondrial oxidation of thiosulfate to sulfite in vitro, and it is possible that a sulfite intermediate may be converted to

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sulfate by sulfite oxidase. After oxidation, H_2S is excreted either as free sulfate or as conjugated sulfate in the urine (Beauchamp et al. 1984).

Two other metabolic pathways for H_2S have been identified as follows: methylation of H_2S to produce methanethiol and dimethylsulfide and reaction of hydrosulfide with metallo- or disulfide-containing enzymes (Dorman et al. 2002). Data suggest that thiol *S*-methyltransferase catalyzes the methylation of H_2S to yield less toxic methanethiol and dimethylsulfide (Beauchamp et al. 1984). The reaction of H_2S with metalloenzymes, such as cytochrome oxidase, is a mechanism of toxicity and is described in Section 4.2.

Hydrogen sulfide may also reduce disulfide bridges in proteins and this reaction is likely responsible for H_2S-induced inhibition of succinic dehydrogenase. Oxidized (but not reduced) glutathione protects against H_2S poisoning. This protective mechanism is likely due to the scavenging of hydrosulfide by the oxidized glutathione disulfide linkage, thus preventing the reaction of the sulfide with other enzymatic sites (Beauchamp et al. 1984).

4.2. Mechanism of Toxicity

Hydrogen sulfide acts similarly to cyanide by interrupting the electron transport chain through inhibition of cytochrome oxidase. Tissues with high oxygen demand (e.g., cardiac muscle, brain) are particularly sensitive to sulfide inhibition of electron transport (Ammann 1986). At physiologic pH, the dissociated hydrosulfide anion and undissociated H_2S ($K_1 = 1.0 \times 10^{-7}$) (Williams and Williams 1967) are in equilibrium. At high, nonphysiologic concentrations, H_2S (as HS^-) acts similarly to cyanide (as CN^-) by direct, reversible inhibition of mitochondrial cytochrome *c* oxidase (ferrocytochrome *c*-oxygen oxidoreductase) (Nicholls 1975; Smith et al. 1977; Kahn et al. 1990), the principal terminal oxidase of aerobic metabolism. The sulfide anion forms a reversible complex with the heme A prosthetic groups of cytochromes *a* and a_3 (electrons received from cytochrome *c* by cytochrome *a* heme are normally transferred to the cytochrome a_3 heme). Spectral data demonstrate that sulfide and cyanide anions bind to the trivalent cytochrome a_3 iron (Nicholls 1975); interestingly, the affinities of cyanide and hydrosulfide for cytochrome oxidase binding sites are of the same order of magnitude (Wever et al. 1975). As a result of the electron transfer blockage, oxidative phosphorylation and aerobic metabolism are compromised, peripheral tissue partial pressure of oxygen increases, and the unloading gradient for oxyhemoglobin decreases. High concentrations of oxyhemoglobin are thus found in the venous return, resulting in flushed skin and mucous membranes. Lactic acidemia occurs as a result of the increased demand placed on glycolysis. Although the signs of H_2S poisoning are essentially identical to those of cyanide poisoning, H_2S has a greater tendency to produce conjunctivitis and pulmonary edema (Smith 1996).

Hydrogen sulfide can also directly stimulate the chemoreceptors of the carotid and aortic bodies to produce hyperpnea and cardiac irregularity. However,

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death is due to respiratory arrest that may occur within minutes at high H_2S concentrations (Smith 1996).

4.3. Concurrent Exposure Issues

Data suggest that ethanol consumption may potentiate the toxic effects of H_2S. Poda (1966) reported that "it took very little" H_2S to overcome six persons who had been drinking heavily during a 16- to 24-h period before reporting to work. Rats pretreated with ethanol (0.33 or 0.66 gram per kilogram of body weight, intraperitoneally) 0.5 h before exposure to H_2S at 790 to 800 ppm for a maximum of 20 min lost consciousness in 35% less time than rats exposed only to H_2S (Beck et al 1979). However, the results of the rodent study are equivocal because all rats receiving only the 20-min H_2S exposure died but some rats pretreated with ethanol survived.

Increased chronic and recurrent headaches were observed in Finnish pulp workers exposed simultaneously to H_2S (peak up to 20 ppm), methyl mercaptans, and sulfur dioxide (ATSDR 2006). In another study, no increase in respiratory symptoms or pulmonary function abnormalities were noted in Canadian pulp and paper mill workers exposed to H_2S at 0.05 ppm, sulfur dioxide at 0.3 ppm, carbon monoxide at 8.3 ppm, total particulates at 0.8 ppm, and chlorine at <0.05 ppm (ATSDR 2006). No other details were reported.

4.4. Structure-Activity Relationships

As previously stated, H_2S and cyanide are potent inhibitors of the cytochrome oxidase system and produce similar effects. Both compounds selectively react with cytochrome aa_3. Also, similar to cyanide, H_2S may inhibit other metalloproteins containing alkali metals such as horseradish peroxidase, potato polyphenol oxidase, and catalase. Inhibition of the latter enzymes occurs only at concentrations much greater than those required to inhibit cytochrome oxidase. The toxicologic significance of these enzyme inhibitions is unclear as the critical position of cytochrome *c* oxidase in oxidative metabolism makes its inhibition felt earliest and strongest (Smith 1996).

The hydrosulfide ion complexes with methemoglobin to form sulfmethemoglobin, which is analogous to cyanmethemoglobin. The dissociation constant for cyanmethemoglobin is 2×10^{-8} mole (mol)/L, whereas the dissociation constant for sulfmethemoglobin is about 6×10^{-6} mol/L. In both cases, nitrite-induced methemoglobinemia provides protection and had antidotal effects against H_2S poisoning (Smith 1996).

4.5. Temporal Extrapolation

The concentration-exposure time relationship for many irritant and systemically acting vapors and gases can be described by the relationship $C^n \times t =$

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k, where the exponent n ranges from 0.8 to 3.5 (ten Berge et al. 1986). However, mortality data suggest that the value of n for H_2S does not fall within this range (see Figure 4-1). For rat lethality data, the derived values of n approach 8 for times up to 1 h. As longer time points are included, the value of n decreases, suggesting that at short time points (and relatively low concentrations) a rather broad change in exposure duration (not concentration) is associated with a relatively small change in response. It is likely that at some point (between 1 and 6 h) there is a steep threshold (providing for an all-or-none response); however, given the available data, it is not possible to quantitate this point on the concentration-response curve. Because rat data were used to derive AEGL-2 and AEGL-3 values and because AEGL values are derived for periods of 10 min to 8 h, it is most appropriate to select an n value from this species for the period of time closest to the time periods encompassed by the AEGL values. Furthermore, use of default time-scaling parameters yields AEGL values less consistent with the overall data set. Thus, the value n = of 4.4, derived from rat lethality data from 10 min to 6 h (r^2 = 0.92), was selected for extrapolation of H_2S response across time.

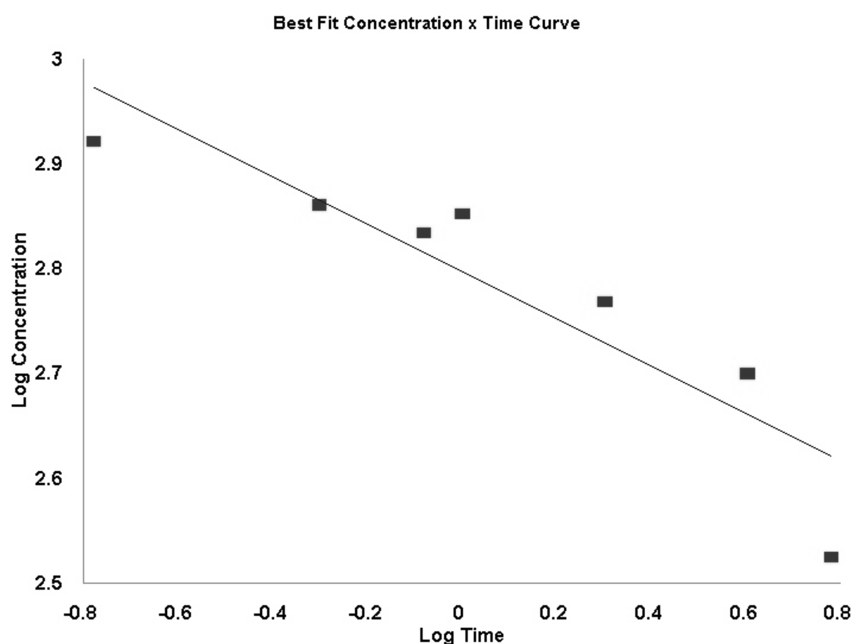

FIGURE 4-1 Log concentration vs. log time of rat LC_{50} values.

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5. RATIONALE AND PROPOSED AEGL-1

5.1. Human Data Relevant to AEGL-1

Complaints of headache were recorded in 3 of 10 adult volunteers with asthma exposed by controlled inhalation of H_2S at 2 ppm for 30 min (Jappinen et al. 1990). Bhambhani et al. (1991; 1994; 1996a,b) exposed 88 exercising healthy male and female volunteers (ranging in age from 18 to 61 years) to H_2S at 0.5 to 10 ppm for up to 30 min. No treatment-related respiratory or cardiac effects or complaints of headache were observed.

5.2. Animal Data Relevant to AEGL-1

Effects observed from inhalation exposure of experimental animals to H_2S are generally no-effect levels or more severe than those defined by AEGL-1.

5.3. Derivation of AEGL-1

Controlled human data were used to derive AEGL-1 values. Three of 10 volunteers with asthma exposed to H_2S at 2 ppm for 30 min complained of headache and 8 of 10 experienced nonsignificant increased airway resistance (Jappinen et al. 1990). As there were no clinical symptoms of respiratory difficulty and no significant changes in FVC or FEV_1, the AEGL-1 was based exclusively on increased complaints of headache in the three volunteers (Jappinen et al. 1990). A modifying factor of 3 was applied to account for the wide variability in complaints associated with the foul odor of H_2S and the shallow concentration response at the relatively low concentrations that are consistent with definition of the AEGL-1. The concentration-time relationship for many irritant and systemically acting vapors and gases may be described by $C^n \times t = k$ (ten Berge et al. 1986). For scaling the AEGL values for H_2S across time, the empirically derived chemical-specific value of 4.4 (derived from rat lethality data ranging from 10-min to 6-h exposure durations; Figure 4-1) was used as the exponent n. The AEGL-1 values for H_2S are presented in Table 4-6, and the calculations for these AEGL-1 values are presented in Appendix A.

The level of distinct odor awareness (LOA) for H_2S is 0.01 ppm (see Appendix C for LOA derivation). The LOA represents the concentration above which it is predicted that more than half of the exposed population will experience at least a distinct odor intensity, and about 10% of the population will experience a strong odor intensity. The LOA should help chemical emergency responders in assessing public awareness of the exposure due to odor perception. Thus, the derived AEGL-1 values are considered to have warning properties.

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TABLE 4-6 AEGL-1 Values for Hydrogen Sulfide

Classification	10 min	30 min	1 h	4 h	8 h
AEGL-1	0.75 ppm (1.05 mg/m^3)	0.60 ppm (0.84 mg/m^3)	0.51 ppm (0.71 mg/m^3)	0.36 ppm (0.50 mg/m^3)	0.33 ppm (0.46 mg/m^3)

6. RATIONALE AND PROPOSED AEGL-2

6.1. Human Data Relevant to AEGL-2

Case reports describing human poisonings with H_2S leading to effects consistent with the definition of AEGL-2 are abundant. However, because of a lack of reliable concentration and duration parameters, these data are not appropriate for derivation of AEGL-2 values.

6.2. Animal Data Relevant to AEGL-2

Nasal passage necrosis and exfoliation and increased lung lavage fluid bronchiolar cell counts and enzyme activities were observed in rats exposed to H_2S at 400 ppm for 4 h (Lopez et al. 1988). Decreased cytochrome *c* oxidase activity (Khan et al. 1990) and decreased pulmonary alveolar cell macrophage activity (Kahn et al. 1991) were also observed in rats exposed to H_2S at 400 ppm for 4 h. Focal areas of red atelectasis and patchy alveolar edema with perivascular and peribronchial interstitial edema, increased protein concentration and LDH activity in lung lavage fluid, and abnormal surfactant activity were observed in rats exposed to H_2S at 300 ppm for 4 h (Green et al. 1991). Rats exposed to H_2S at 200 ppm for 4 h exhibited minor perivascular edema and a significant increase in protein and LDH in lung lavage fluid (Green et al. 1991). Khan et al. (1991) observed no change in pulmonary alveolar macrophage counts in rats exposed to 200 ppm for 4 h.

6.3. Derivation of AEGL-2

The focal areas of perivascular edema in rats exposed to H_2S at 200 ppm for 4 h (Green et al. 1991; Khan et al. 1991) are used as the basis for AEGL-2 values. An uncertainty factor of 3 is used to extrapolate from animals to humans as rat and mouse data suggest little interspecies variability. An uncertainty factor of 3 will also be applied to account for sensitive individuals. The intraspecies uncertainty factor of 3 is sufficient because application of 10 yields AEGL-2 values inconsistent with the total database. AEGL-2 values derived with a total default uncertainty factor were less than the10-ppm concentration causing no effects in humans exercising to exhaustion (Bhambhani and Singh 1991; Bhambhani et al. 1994, 1996a,b, 1997). Thus, the total uncertainty factor is 10.

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The concentration-time relationship for many irritant and systemically acting vapors and gases may be described by $C^n \times t = k$ (ten Berge et al. 1986). For scaling the AEGL values for H_2S across time, the empirically derived chemical-specific value of 4.4 (derived from rat lethality data ranging from 10 min to 6 h exposure duration, Figure 4-1) was used as the exponent n. The AEGL-2 values for H_2S are presented in Table 4-7, and the calculations for these AEGL-2 values are presented in Appendix A.

7. RATIONALE AND PROPOSED AEGL-3

7.1. Human Data Relevant to AEGL-3

Human lethality data were anecdotal and lacked reliable concentration and time parameters. Thus, those reports were not appropriate for establishing AEGL-3 values.

7.2. Animal Data Relevant to AEGL-3

Well-conducted LC_{50} studies are available for rats (MacEwen and Vernot 1972; Tansy et al. 1981; Prior et al. 1988; Zwart et al. 1990) and mice (MacEwen and Vernot 1972; Zwart et al. 1990).

7.3. Derivation of AEGL-3

The highest H_2S concentration causing no mortality in the rat after a 1-h exposure is 504 ppm (MacEwen and Vernot 1972). This value is used as the basis for deriving AEGL-3 values. An uncertainty factor of 3 is used to extrapolate from animals to humans as rat and mouse data suggest little interspecies variability. An uncertainty factor of 3 will also be applied to account for sensitive individuals. The intraspecies uncertainty factor of 3 is sufficient because application of the default 10 yields AEGL-3 values inconsistent with the total database. AEGL-3 values derived with a total default uncertainty factor were less than twice the concentration causing no effects in humans exercising to exhaustion (Bhambhani and Singh 1991; Bhambhani et al. 1994, 1996a,b, 1997). Effects consistent with the definition of AEGL-3 are unlikely at such concentrations. Therefore, the total uncertainty factor is 10. The concentration-time relationship for many irritant and systemically acting vapors and gases may be described by $C^n \times t = k$ (ten Berge et al. 1986). For scaling the AEGL values for H_2S across time, the empirically derived chemical-specific value of 4.4 (derived from rat lethality data ranging from 10 min to 6 h exposure duration; Figure 4-1) was used as the exponent n. The AEGL-3 values for H_2S are presented in Table 4-8, and the calculations for these AEGL-3 values are presented in Appendix A.

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These values are supported by the fact that no deaths were observed in rats exposed to H_2S at 80 ppm for 6 h/day, 5 days/week, for 90 days (CIIT 1983a).

8. SUMMARY OF PROPOSED AEGLS

8.1. AEGL Values and Toxicity End Points

The derived AEGL values for various levels of effects and durations of exposure are summarized in Table 4-9. Headache in humans with asthma was used as the basis for AEGL-1, and focal areas of perivascular edema in rats were used as the basis for AEGL-2. The highest concentration causing no mortality in rats after a 1-h exposure was used to calculate the AEGL-3.

The odor threshold ranges between 0.008 and 0.13 ppm, olfactory fatigue may occur at 100 ppm, and paralysis of the olfactory nerve has been reported at 150 ppm (Beauchamp et al. 1984). Furthermore, the LOA for H_2S is 0.01 ppm (see Appendix C for LOA derivation). The LOA represents the concentration above which it is predicted that more than half of the exposed population will experience at least a distinct odor intensity, and about 10% of the population will experience a strong odor intensity. The LOA should help chemical emergency responders in assessing public awareness of the exposure due to odor perception. Thus, the derived AEGL-1 values are considered to have warning properties, and the characteristic odor will also be present at AEGL-2 and AEGL-3 concentrations.

TABLE 4-7 AEGL-2 Values for Hydrogen Sulfide

Classification	10 min	30 min	1 h	4 h	8 h
AEGL-2	41 ppm ($59\ mg/m^3$)	32 ppm ($45\ mg/m^3$)	27 ppm ($39\ mg/m^3$)	20 ppm ($28\ mg/m^3$)	17 ppm ($24\ mg/m^3$)

TABLE 4-8 AEGL-3 Values for Hydrogen Sulfide

Classification	10 min	30 min	1 h	4 h	8 h
AEGL-3	76 ppm ($106\ mg/m^3$)	59 ppm ($85\ mg/m^3$)	50 ppm ($71\ mg/m^3$)	37 ppm ($52\ mg/m^3$)	31 ppm ($44\ mg/m^3$)

TABLE 4-9 Summary of AEGL Values for Hydrogen Sulfide

Classification	10 min	30 min	1 h	4 h	8 h
AEGL-1 (Nondisabling)	0.75 ppm ($1.05\ mg/m^3$)	0.60 ppm ($0.84\ mg/m^3$)	0.51 ppm ($0.71\ mg/m^3$)	0.36 ppm ($0.50\ mg/m^3$)	0.33 ppm ($0.46\ mg/m^3$)
AEGL-2 (Disabling)	41 ppm ($59\ mg/m^3$)	32 ppm ($45\ mg/m^3$)	27 ppm ($39\ mg/m^3$)	20 ppm ($28\ mg/m^3$)	17 ppm ($24\ mg/m^3$)
AEGL-3 (Lethality)	76 ppm ($106\ mg/m^3$)	59 ppm ($85\ mg/m^3$)	50 ppm ($71\ mg/m^3$)	37 ppm ($52\ mg/m^3$)	31 ppm ($44\ mg/m^3$)

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8.2. Other Exposure Criteria

The criteria for H_2S exposure have been established and are shown in Table 4-10.

TABLE 4-10 Extant Standards and Guidelines for Hydrogen Sulfide

Guideline	Exposure Duration				
	10 min	30 min	1 h	4 h	8 h
AEGL-1	0.75 ppm	0.60 ppm	0.51 ppm	0.36 ppm	0.33 ppm
AEGL-2	41 ppm	32 ppm	27 ppm	20 ppm	17 ppm
AEGL-3	76 ppm	59 ppm	50 ppm	37 ppm	31 ppm
ERPG-1[a]	0.1 ppm				
ERPG-2[a]	30 ppm				
ERPG-3[a]	100 ppm				
Acute MRL (ATSDR)[b]	0.07 ppm				
EEGL (NRC)[c]	50 ppm (10 min)				10 ppm (24 h)
SEAL 1[h], SEAL 2[h] (NRC)[d]					15 ppm (10 d) 30 ppm (24 h)
IDLH (NIOSH)[e]	100 ppm				
TLV-TWA (ACGIH)[f]					10 ppm
PEL-TWA (OSHA)[g]					20 ppm
PEL-acceptable maximum peak, 10 min, once only per 8-hr workshift (OSHA)[g]	50 ppm				
TLV-STEL (ACGIH)[h]	15 ppm				
REL-STEL (NIOSH)[i]	10 ppm				
MAK (Germany)[j]					10 ppm
MAC (The Netherlands)[k]					10 ppm
OELV- LLV (Sweden)[l]					10 ppm
OELV-CLV (Sweden)[l]	15 ppm (15min)				

[a]ERPG (emergency response planning guidelines, American Industrial Hygiene Association) (AIHA 2001): The ERPG-1 is the maximum airborne concentration below which it is believed nearly all individuals could be exposed for up to 1 h without experiencing other than mild, transient adverse health effects or without perceiving a clearly defined objectionable odor. The ERPG-2 is the maximum airborne concentration below which it is believed nearly all individuals could be exposed for up to 1 h without experiencing or developing irreversible or other serious health effects or symptoms that could impair an individual's ability to take protective action. The ERPG-3 is the maximum airborne con-

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centration below which it is believed nearly all individuals could be exposed for up to 1 h without experiencing or developing life-threatening health effects.
[b]MRL (minimal risk level, Agency for Toxic Substances and Disease Registry) (ATSDR 2009) is an estimate of daily human exposure that is likely to be without appreciable risk of adverse noncancer health effects over a specified duration.
[c]EEGL (emergency exposure guidance levels, National Research Council) (NRC 1985) is the concentration of contaminants that can cause discomfort or other evidence of irritation or intoxication in or around the workplace but avoids death, other severe acute effects, and long-term or chronic injury. The EEGL for H_2S is based on keratoconjunctivitis.
[d]SEAL (submarine escape action levels, National Research Council) (NRC 2002): SEAL1 is the maximum concentration of a gas in a disabled submarine below which healthy submariners can be exposed for up to 10 days without experiencing irreversible health effects. SEAL2 is the maximum concentration of a gas in a disabled submarine below which healthy submariners can be exposed for up to 24 h without experiencing irreversible health effects. The SEALs for H_2S are based on ocular toxicity.
[e]IDLH (immediately dangerous to life or health, National Institute for Occupational Safety and Health) (NIOSH 1996) represents the maximum concentration from which one could escape within 30 min without any escape-impairing symptoms or any irreversible health effects. The IDLH for H_2S is based on acute inhalation toxicity data in humans and animals.
[f]TLV-TWA (Threshold Limit Value–time-weighted average, American Conference of Governmental Industrial Hygienists) (ACGIH 1996) is the time-weighted average concentration for a normal 8-h workday and a 40-h workweek to which nearly all workers may be repeatedly exposed, day after day, without adverse effect. ACGIH (2000) lists a "notice of intended changes" for 2001; proposed value: 5 ppm.
[g]PEL-TWA (permissible exposure limits–time-weighted average, Occupational Safety and Health Administration, OSHA) (29 CFR 1910.1000 [2003]) is analogous to the ACGIH TLV-TWA but is for exposures of no more than 10 h/d, 40 h/wk.
[h]TLV-STEL (Threshold Limit Value–short-term exposure limit, American Conference of Governmental Industrial Hygienists) (ACGIH 1996) is the short-term exposure limit for a 15-min exposure.
[i]REL-STEL (recommended exposure limits–short-term exposure limit, National Institute for Occupational Safety and Health) (NIOSH 2005) is analogous to the ACGIH TLV-STEL.
[j]MAK (maximale argeitsplatzkonzentration [maximum workplace concentration], Deutsche Forschungsgemeinschaft [German Research Association]) (DFG 2000) is analogous to the ACGIH TLV-TWA.
[k]MAC (maximaal aanvaarde concentratie [maximum accepted concentration], SDU Uitgevers [under the auspices of the Ministry of Social Affairs and Employment, The Hague, The Netherlands]) is analogous to the ACGIH TLV-TWA (MSZW 2004).
[l]OELV-LLV (occupational exposure limit value-level limit value) and OELV-CLV (occupational exposure limit value-ceiling limit value) (Swedish Work Environment Authority 2005). OELV-LLV is the maximum acceptable average concentration (time-weighted average) of an air contaminant in respiratory air. An OELV is either a level limit value (one working day) or a ceiling limit value (15 min or some other reference time period) and short time value (a recommended value consisting of a time-weighted average for exposure during a reference period of 15 min).

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8.3. Data Adequacy and Research Needs

The database for human exposure for effects defined by AEGL-1 is relatively good as controlled chamber studies with asthma patients and otherwise healthy volunteers are available. These studies, when considered together, provide good concentration-response information and are appropriate for derivation of AEGL-1 values. Case reports of accidental human exposure to H_2S leading to effects consistent with the definitions of AEGL-2 and AEGL-3 did not include concentration or duration parameters adequate for derivation of AEGL-2 or AEGL-3 values. However, numerous animal studies were available for derivation of AEGL-2 and AEGL-3 values. The animal studies were well-conducted in a number of species over a broad range of exposure durations and were adequate for derivation of AEGL-2 and AEGL-3 values for H_2S.

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APPENDIX A

TIME-SCALING CALCULATIONS FOR HYDROGEN SULFIDE

Derivation of AEGL-1

Key study: Jappinen et al. 1990

Toxicity end point: Headache in human asthma subjects

Scaling: $C^{4.4} \times t = k$
$(2\ ppm)^{4.4} \times 0.5\ h = 10.27$ ppm-h

Modifying factor: 3, wide variability in complaints associated with the foul odor of H_2S and the shallow concentration response at the relatively low concentrations that are consistent with definition of the AEGL-1

Calculations:

10-min AEGL-1:
$C^{4.4} \times 0.167\ h = 10.27$ ppm-h
$C^{4.4} = 61.5$ ppm
C = 2.6 ppm
10-min AEGL-1 = 2.6/3 = 0.75 ppm

30-min AEGL-1:
$C^{4.4} \times 0.5\ h = 10.27$ ppm-h
$C^{4.4} = 20.54$ ppm
C = 2.00 ppm
30-min AEGL-1 = 2.0/3 = 0.60 ppm

1-h AEGL-1:
$C^{4.4} \times 1\ h = 10.27$ ppm-h
$C^{4.4} = 10.27$ ppm
C = 1.71 ppm
1-h AEGL-1 = 1.7/3 = 0.51 ppm

4-h AEGL-1:
$C^{4.4} \times 4\ h = 10.27$ ppm-h
$C^{4.4} = 2.57$ ppm
C = 1.28 ppm
4-h AEGL-1 =1.28/3 = 0.36 ppm

8-h AEGL-1:
$C^{4.4} \times 8\ h = 10.27$ ppm-h
$C^{4.4} = 1.28$ ppm
C = 1.06 ppm
8-h AEGL-1 = 1.06/3 = 0.33 ppm

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Derivation of AEGL-2

Key studies: Green et al. 1991; Khan et al. 1991

Toxicity end points: Minor perivascular edema present and significant increase in protein and LDH in lung lavage fluid.

Scaling: $C^{4.4} \times t = k$
$(200 \text{ ppm})^{4.4} \times 4 \text{ h} = 4.31 \times 10^{10}$ ppm-h

Uncertainty factors: 3 for interspecies variability
3 for intraspecies variability

Calculations:

10-min AEGL-2: $C^{4.4} \times 0.167 \text{ h} = 4.31 \times 10^{10}$ ppm-h
$C^{4.4} = 2.58 \times 10^{11}$ ppm
C = 414.4 ppm
10-min AEGL-2 = 414.4/10 = 41.4 ppm

30-min AEGL-2: $C^{4.4} \times 0.5 \text{ h} = 4.31 \times 10^{10}$ ppm-h
$C^{4.4} = 8.62 \times 10^{10}$ ppm
C = 322.2 ppm
30-min AEGL-2 = 322.2/10 = 32.2 ppm

1-h AEGL-2: $C^{4.4} \times 1 \text{ hr} = 4.31 \times 10^{10}$ ppm-h
$C^{4.4} = 4.31 \times 10^{10}$ ppm
C = 274 ppm
1-h AEGL-2 =274/10 = 27.4 ppm

4-h AEGL-2: $C^{4.4} \times 4 \text{ hr} = 4.31 \times 10^{10}$ ppm-h
$C^{4.4} = 1.08 \times 10^{10}$ ppm
C = 199.9 ppm
4-h AEGL-2 =199.9/10 = 19.9 ppm

8-h AEGL-2: $C^{4.4} \times 8 \text{ h} = 4.31 \times 10^{10}$ ppm-h
$C^{4.4} = 5.39 \times 10^{9}$ ppm
C = 170.6 ppm
8-h AEGL-2 =170.6/10 = 17.1 ppm

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Derivation of AEGL-3

Key study: MacEwen and Vernot 1972

Toxicity end point: Highest concentration causing no death after a 1-h exposure in rats

Scaling: $C^{4.4} \times t = k$
$(504\ \text{ppm})^{4.4} \times 1\ \text{h} = 6.06 \times 10^{11}$ ppm-h

Uncertainty factors: 3 for interspecies variability
3 for intraspecies variability

Calculations:

10-min AEGL-3: $C^{4.4} \times 0.167\ \text{h} = 6.06 \times 10^{11}$ ppm-h
$C^{4.4} = 3.63 \times 10^{12}$ ppm
C = 759.8 ppm
10-min AEGL-3 = 759.8/10 = 75.9 ppm

30-min AEGL-3: $C^{4.4} \times 0.5\ \text{h} = 6.06 \times 10^{11}$ ppm-h
$C^{4.4} = 1.21 \times 10^{12}$ ppm
C = 590.8 ppm
30-min AEGL-3 = 590.8/10 = 59.1 ppm

1-h AEGL-3: $C^{4.4} \times 1\ \text{h} = 6.06 \times 10^{11}$ ppm-h
$C^{4.4} = 6.06 \times 10^{11}$ ppm
C = 503.9 ppm
1-h AEGL-3 =503.9/10 = 50.4 ppm

4-h AEGL-3: $C^{4.4} \times 4\ \text{h} = 6.06 \times 10^{11}$ ppm-h
$C^{4.4} = 1.52 \times 10^{11}$ ppm
C = 366.7 ppm
4-h AEGL-3 =366.7/10 = 36.7 ppm

8-h AEGL-3: $C^{4.4} \times 8\ \text{hr} = 6.06 \times 10^{11}$ ppm-h
$C^{4.4} = 7.58 \times 10^{10}$ ppm
C = 312.8 ppm
8-h AEGL-3 = 312.8/10 = 31.3 ppm

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APPENDIX B

ACUTE EXPOSURE GUIDELINES FOR HYDROGEN SULFIDE

Derivation Summary for Hydrogen Sulfide

AEGL-1 VALUES

10 min	30 min	1 h	4 h	8 h
0.75 ppm	0.60 ppm	0.51 ppm	0.36 ppm	0.33 ppm

Key Reference: Jappinen, P., V. Vilkka, O. Marttila, P. Jappinen, and T. Haahtela. 1990. Exposure to hydrogen sulfide and respiratory function. Br. J. Ind. Med. 47(12):824-828.

Test Species/Strain/Number: Human/10 asthma patients

Exposure Route/Concentrations/Durations: Inhalation/2 ppm /30 min

Effects: Odor and pharyngeal dryness at the beginning of exposure; headache (3/10); increased R_{aw} (significant in 2/10) with no accompanying clinical signs or lung function effects

End Point/Concentration/Rationale: headache/2 ppm

Uncertainty Factors/Rationale:
Interspecies 1: subjects were human

Modifying Factor: 3 to account for the wide variability in complaints associated with the foul odor of H_2S and the shallow concentration response at the relatively low concentrations that are consistent with definition of the AEGL-1

Animal to Human Dosimetric Adjustment: NA

Time-Scaling: $C^n \times t = k$, where n = 4.4; value derived from rat lethality data ranging from 10 min to 6 h. Data point used for AEGL-1 derivation was 30 min. Other time points were based on extrapolation.

Data Quality and Research Needs: These values are supported by the fact that no adverse effects were observed in healthy humans exposed to H_2S at 5 ppm for 30 min or 10 ppm for 15 min while exercising to exhaustion (Bhambhani and Singh 1991; Bhambhani et al. 1994, 1996a,b). Using these concentrations and applying an uncertainty factor of 10 for sensitive human subpopulations, the following AEGL-1 values would be obtained: 0.64, 0.50, 0.43, 0.31, and 0.26 ppm for the 10- and 30-min and 1-, 4-, and 8-h time points, respectively, for the 5-ppm exposure for 30 min; and 1.1, 0.85, 0.73, 0.53, and 0.45 ppm for the 10- and 30-min and 1-, 4-, and 8-h time points, respectively, for the 10-ppm exposure for 15 min. These values suggest that the proposed AEGL-1 values are protective.

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AEGL-2 VALUES

10 min	30 min	1 h	4 h	8 h
41 ppm	32 ppm	27 ppm	20 ppm	17 ppm

Key References: Green, F.H., S. Schurch, G.T. DeSanctis, J.A. Wallace, S. Cheng, and M. Prior. 1991. Effects of hydrogen sulfide exposure on surface properties of lung surfactant. J. Appl. Physiol. 70(5):1943-1949. Khan, A.A., S. Yong, M.G. Prior, and L.E. Lillie. 1991. Cytotoxic effects of hydrogen sulfide on pulmonary alveolar macrophages in rats. J. Toxicol. Environ. Health 33(1):57-64.

Test Species/Strain/Number: (1) Rat/F344/6 males; (2) Rat/F344/6 males

Exposure Route/Concentrations/Durations:
Inhalation/0, 200, or 300 ppm/4 h;
Inhalation/0, 50, 200, or 400 ppm/4 h

Effects:
200 ppm: No adverse clinical signs or gross lung pathology, increased protein and LDH in lavage fluid
300 ppm: Clinical signs during exposure, increased protein and LDH in lavage fluid, lung atelectasis, and edema
50 and 200 ppm: No effect on viability of pulmonary alveolar macrophages
300 ppm: Decreased viability of pulmonary alveolar macrophages (200 ppm for 4 h was determinant for AEGL-2)

End Point/Concentration/Rationale: (1) No-effect level for gross lung pathology, minor perivascular edema, increased protein and LDH in lung lavage fluid. (2) No-effect level for pulmonary alveolar macrophage viability/200 ppm

Uncertainty Factors/Rationale:
Interspecies 3: rat and mouse data suggest little interspecies variability
Intraspecies 3: The intraspecies uncertainty factor of 3 is considered sufficient because application of the default uncertainty factor of 10 would result in a total uncertainty factor of 30, which would yield AEGL-2 values inconsistent with the total database. AEGL-2 values derived with a total uncertainty factor of 30 would be 14 ppm for 10 min, 11 ppm for 30 min, 9.0 ppm for 1 h, 6.7 ppm for 4 h, and 5.7 ppm for 8 h, values essentially identical to or below the 10-ppm concentration causing no effects in humans exercising to exhaustion (Bhambhani and Singh 1991; Bhambhani et al. 1994, 1996a,b, 1997).
Total Uncertainty Factor: 10. The total adjustment is 10 because each of the factors of 3 represents a logarithmic mean (3.16) of 10; therefore, $3.16 \times 3.16 = 10$.

Modifying Factor: NA

Animal to Human Dosimetric Adjustment: NA

Time-Scaling: $C^n \times t = k$, where n = 4.4; value derived from rat lethality data ranging from 10 min to 6 h. Data point used for AEGL-2 derivation was 4 h. Other time points were based on extrapolation.

Data quality and research needs: Two well-conducted studies in rats support one another.

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AEGL-3 VALUES

10 min	30 min	1 h	4 h	8 h
76 ppm	59 ppm	50 ppm	37 ppm	31 ppm

Key Reference: MacEwen, J.D., and E.H. Vernot. 1972. Acute toxicity of hydrogen sulfide. Pp. 66-70 in Toxic Hazards Research Unit Annual Report: 1972. Report No. ARML-TR-72-62. Aerospace Medical Research Laboratory, Air Force Systems Command, Wright-Patterson Air Force Base, OH.

Test Species/Strain/Sex/Number: Sprague-Dawley rats/10 males/concentration

Exposure Route/Concentrations/Durations: Rats/inhalation: 400, 504, 635, or 800 ppm/1 h
(Highest concentration causing no death in rats after a 1-h exposure (504 ppm) was determinant for AEGL-3.)

End Point/Concentration/Rationale: Highest concentration causing no death in rats after a 1 h-exposure/504 ppm/threshold for death for 1-h exposure in rats.

Effects:
Concentration Mortality
400 ppm 0/10
504 ppm 0/10
635 ppm 1/10
800 ppm 9/10

Uncertainty Factors/Rationale:
Total uncertainty factor: 10
Interspecies: 3 for rat and mouse data suggest little interspecies variability
Intraspecies: 3 is considered sufficient because application of the default uncertainty factor of 10 would result in a total uncertainty factor of 30, which would yield AEGL-3 values inconsistent with the total database. AEGL-3 values derived with a total uncertainty factor of 30 would be 25 ppm for 10 min, 20 ppm for 30 min, 17 ppm for 1 h, 12 ppm for 4 h, and 10 ppm for 8 h, values equal to or less than 2-fold the concentration causing no effects in humans exercising to exhaustion (Bhambhani and Singh 1991; Bhambhani et al. 1994, 1996a,b, 1997). Effects consistent with the definition of AEGL-3 would be unlikely to occur at such concentrations.
Total Uncertainty Factor: 10. The total adjustment is 10 because each of the factors of 3 represents a logarithmic mean (3.16) of 10; therefore, $3.16 \times 3.16 = 10$.

Modifying Factor: NA

Animal to Human Dosimetric Adjustment: Insufficient data

Time-Scaling: $C^n \times t = k$, where $n = 4.4$, value derived from rat lethality data ranging from 10 min to 6 h. Data point used for AEGL-3 derivation was 1 h. Other time points were based on extrapolation.

Data Quality and Research Needs: Well-conducted study with appropriate end point for AEGL-3.

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APPENDIX C

Derivation of the Level of Distinct Odor Awareness for Hydrogen Sulfide

The level of distinct odor awareness (LOA) represents the concentration above which it is predicted that more than half of the exposed population will experience at least a distinct odor intensity, and about 10% of the population will experience a strong odor intensity. The LOA should help chemical emergency responders in assessing public awareness of the exposure due to odor perception. The LOA derivation follows the guidance given by Ruijten et al. (2009).

The odor detection threshold (concentration at which 50% of people detect an odor; OT_{50}) for H_2S was calculated to be 0.0006 ppm (Ruijten et al. 2009).

The concentration (C) leading to an odor intensity (I) of distinct odor detection (I = 3) is derived with the Fechner function:

$$I = k_w \times \log(C/OT_{50}) + 0.5.$$

For the Fechner coefficient, the default of $k_w = 2.33$ is used because of a lack of chemical-specific data:

$$3 = 2.33 \times \log(C/0.0006) + 0.5, \text{ which can be rearranged to}$$
$$\log(C/0.0006) = (3 - 0.5)/2.33 = 1.07 \text{ and results in}$$
$$C = (10^{1.07}) \times 0.0006 = 0.0071 \text{ ppm}.$$

The resulting concentration is multiplied by an empirical field correction factor. It takes into account that in everyday life factors such as sex, age, sleep, smoking, upper airway infections, and allergy as well as distraction increase the OT_{50} by a factor of 4. In addition, it takes into account that odor perception is very fast (about 5 seconds) which leads to the perception of concentration peaks. On the basis of current knowledge, a factor of 1/3 is applied to adjust for peak exposure. Adjustment for distraction and peak exposure lead to a correction factor of 4/3 = 1.33.

$$LOA = C \times 1.33 = 0.0071 \text{ ppm} \times 1.33 = 0.01 \text{ ppm}.$$

The LOA for H_2S is 0.01 ppm.

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5

Propylene Oxide[1]

Acute Exposure Guideline Levels

PREFACE

Under the authority of the Federal Advisory Committee Act (FACA) P.L. 92-463 of 1972, the National Advisory Committee for Acute Exposure Guideline Levels for Hazardous Substances (NAC/AEGL Committee) has been established to identify, review, and interpret relevant toxicologic and other scientific data and develop AEGLs for high-priority, acutely toxic chemicals.AEGLs represent threshold exposure limits for the general public and are applicable to emergency exposure periods ranging from 10 minutes (min) to 8 hours (h). Three levels—AEGL-1, AEGL-2, and AEGL-3—are developed for each of five exposure periods (10 and 30 min and 1, 4, and 8 h) and are distinguished by varying degrees of severity of toxic effects. The three AEGLs have been defined as follows:

AEGL-1 is the airborne concentration (expressed as parts per million [ppm] or milligrams per cubic meter [mg/m^3]) 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.

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[1]This document was prepared by the AEGL Development Team composed of Claudia Troxel (Oak Ridge National Laboratory) and Chemical Manager Jim Holler (National Advisory Committee [NAC] on Acute Exposure Guideline Levels for Hazardous Substances). The NAC reviewed and revised the document and AEGLs as deemed necessary. Both the document and the AEGL values were then reviewed by the National Research Council (NRC) Committee on Acute Exposure Guideline Levels. The NRC committee has concluded that the AEGLs developed in this document are scientifically valid conclusions based on the data reviewed by the NRC and are consistent with the NRC guideline reports (NRC 1993, 2001).

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AEGL-2 is the airborne concentration (expressed as ppm or mg/m^3) 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.

AEGL-3 is the airborne concentration (expressed as ppm or mg/m^3) of a substance above which it is predicted that the general population, including susceptible individuals, could experience life-threatening health effects or death.

Airborne concentrations below the AEGL-1 represent exposure levels that could produce mild and progressively increasing but transient and nondisabling odor, taste, and sensory irritation or certain asymptomatic, nonsensory effects. With increasing airborne concentrations above each AEGL, there is a progressive increase in the likelihood of occurrence and the severity of effects described for each corresponding AEGL. Although the AEGLs represent threshold levels for the general public, including susceptible subpopulations, such as infants, children, the elderly, persons with asthma, and those with other illnesses, it is recognized that individuals, subject to idiosyncratic responses, could experience the effects described at concentrations below the corresponding AEGL.

SUMMARY

Propylene oxide is an extremely flammable, highly volatile, colorless liquid. Its odor has been described as sweet and alcoholic, and it has reported odor thresholds ranging from 10 to 200 ppm (Jacobson et al. 1956; Hellman and Small 1974; Amoore and Hautala 1983). The primary industrial uses of propylene oxide include in the production of polyurethane foams and resins, propylene glycol, functional fluids (such as hydraulic fluids, heat transfer fluids, and lubricants), and propylene oxide–based surfactants. It is also used as a food fumigant, soil sterilizer, and acid scavenger.

Data addressing inhalation toxicity of propylene oxide in humans were limited to one case report, general environmental work surveys, and molecular biomonitoring studies. Studies addressing lethal and nonlethal inhalation toxicity of propylene oxide in animals were available in monkeys, dogs, rats, mice, and guinea pigs. General signs of toxicity after acute exposure to propylene oxide vapor included nasal discharge, lacrimation, salivation, gasping, lethargy and hypoactivity, weakness, and incoordination. Repeated exposures resulted in similar but generally reversible signs of toxicity. Much of the toxicologic evidence suggests that propylene oxide reacts at the site of entry. Therefore, inhalation of propylene oxide results in respiratory tract irritation, eventually leading to death. Possible neurotoxic effects have also been observed in rodents and dogs after inhalation exposure to higher concentrations of propylene oxide.

Propylene oxide is a direct alkylating agent that covalently binds to DNA and proteins. Consequently, it has tested positive in a number of in vitro tests but has produced equivocal results in in vivo test systems. Data addressing the po-

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tential carcinogenicity of propylene oxide in animals is considered adequate for establishing propylene oxide as a carcinogen in experimental animals.

The AEGL-1 is based on a workplace survey that measured exposure concentrations of 380 ppm for 177 min, 525 ppm for 121 min, 392 ppm for 135 min, and 460 ppm for 116 min in the breathing zone of three workers during drumming operations (CMA 1998). Strong odor and irritation were noted in the survey. The nature of the irritation was not provided, but occasional eye irritation was noted as the reason for the monitoring program. Because the effects were considered mild irritation, the AEGL values would be set equal across time. Therefore, the four exposure concentrations can be averaged, resulting in a point of departure of 440 ppm. A total uncertainty factor and modifying factor of 6 is applied. An interspecies uncertainty factor was not needed, because the data were from human exposures. An intraspecies uncertainty factor of 3 was applied, because irritation is a point-of-contact effect and is not expected to vary greatly among individuals. A modifying factor of 2 is applied, because the defined effects are above an AEGL-1 (undefined irritation) but below an AEGL-2 end point.

No human data were available for deriving an AEGL-2. When considering animal data for deriving an AEGL-2, dyspnea in mice was the most sensitive end point consistent with the AEGL-2 definition, and mice were the most sensitive to the toxic effects of propylene oxide vapor. Therefore, the AEGL-2 values are based on data from the NTP (1985) study in which mice exposed to 387 ppm for 4 h exhibited dyspnea. Although a no-effect level was not established for dyspnea at this concentration, no other adverse effects were noted. In addition, compared with other studies investigating propylene oxide toxicity in mice, the NTP study reported toxic effects occurring at much lower concentrations than were observed in other studies. An interspecies uncertainty factor of 1 was applied, because mice are the most sensitive laboratory species in terms of the lethal effects of propylene oxide as well as clinical signs of toxicity, and available data indicate that mice are equally or slightly more sensitive than humans in the manifestation of clinical signs. The NTP (1985) study reported toxic effects at much lower concentrations than those observed in other studies. An intraspecies uncertainty factor of 3 was applied because the mechanism of toxicity—irritation—is a point-of-contact effect and is not expected to vary greatly among individuals. Therefore, a total uncertainty factor of 3 was applied.

Although the mechanism of action appears to be a direct irritant effect, it is not appropriate to set the values equally across time, because the irritation is no longer considered mild but is part of the continuum of respiratory tract irritation leading to death. The experimentally derived exposure value was therefore scaled to AEGL timeframes using the concentration-time relationship given by the equation $C^n \times t = k$, where C is concentration, t is time, k is a constant, and n is 1.7 as calculated with the rat lethality data reported by Rowe et al. (1956) (ten Berge et al. 1986). The 10-min value was set equal to the 30-min value because of the uncertainty in extrapolating from the exposure duration of 4 h to 10 min.

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The AEGL-3 derivation is based on the calculated 4-h $BMCL_{05}$ (benchmark concentration, 95% lower confidence limit at the 5% response rate) of 1,161 ppm, the lowest $BMCL_{05}$ value in rats (NTP 1985). Lethality data in the dog, a nonobligate nose breather, support use of the $BMCL_{05}$ value in the rat, but the dog values should not be used as the basis for the AEGL-3 derivation because two of three animals in the high-dose group died before they were removed from the exposure chamber. Mouse data were not used because the mouse is overly sensitive to propylene oxide compared with the other species tested. The $BMCL_{05}$ values in mice are 282 and 673 ppm (Jacobson et al. 1956; NTP 1985), compared with 1,161 to 3,328 ppm in rats (Jacobson et al. 1956; Shell Oil Co. 1977; NTP 1985) and 1,117 ppm in dogs (Jacobson et al. 1956). Other data demonstrating that the mouse $BMCL_{05}$ values are unreasonably low include the studies in which only minimal effects were noted in monkeys exposed to 300 ppm for 6 h/day, 5 days/week, for 2 years (Sprinz et al. 1982; Lynch et al. 1983; Setzer et al. 1997), or to 457 ppm for 7 h/day for 154 days (Rowe et al. 1956), and the highest documented human exposure of 1,520 ppm for 171 min, which caused irritation that was not severe enough for the worker to cease working (CMA 1998). These data support the 4-h $BMCL_{05}$ of 1,161 ppm in rats as a reasonable point of departure. An intraspecies uncertainty factor of 3 was applied, because the mechanism of toxicity—irritation—is a point-of-contact effect and is not expected to vary greatly among individuals. An interspecies uncertainty factor of 1 was applied because of the supporting data in dogs (similar 4-h $BMCL_{05}$) and monkeys (2-year studies, which produced minimal effects). The 4-h AEGL-3 value using a total uncertainty factor of 3 is 387 ppm, which is conservative compared with the 300- or 457-ppm chronic exposure in monkeys producing minimal effects. Therefore, a total uncertainty factor of 3 was considered reasonable.

As for the AEGL-2 derivation, the point of departure for the AEGL-3 derivation was scaled to AEGL timeframes using the concentration-time relationship given by the equation $C^n \times t = k$, where C is concentration, t is time, k is a constant, and n is 1.7 as calculated with the rat lethality data reported by Rowe et al. (1956) (ten Berge et al. 1986). The value was extrapolated across time, because the irritation is no longer considered mild; rather, the concentration represents the threshold for lethality. The 10-min value was set equal to the 30-min value because of the uncertainty in extrapolating from the exposure duration of 4 h to 10 min.

A level of distinct odor awareness (LOA) for propylene oxide of 21 ppm was derived on the basis of the odor detection threshold from the study of Hellman and Small (1974). The LOA represents the concentration above which it is predicted that more than half the exposed population will experience at least a distinct odor intensity; about 10% of the population will experience a strong odor intensity. The LOA should help chemical emergency responders in assessing public awareness of the exposure due to odor perception.A quantitative carcinogenicity assessment for a single exposure to propylene oxide is not considered appropriate. Data indicate that propylene oxide is a threshold carcinogen

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that depends on increased cell proliferation and hyperplasia at the target site and would require repeated exposure to produce tumorigenesis. Therefore, a one-time exposure even to high concentrations of propylene oxide is not expected to result in tumor development. This conclusion is supported by the Sellakumar et al. (1987) study in which no tumors were observed when 12-week-old male Sprague-Dawley rats were exposed to propylene oxide at 433 or 864 ppm for 30 days or to 1,724 ppm for 8 days (exposures were for 6 h/day, 5 days/week) and allowed to die naturally.

The derived AEGL values are listed in Table 5-1.

1. INTRODUCTION

Propylene oxide is an extremely flammable, highly volatile, colorless liquid, with a boiling point of 35°C (Meylan et al. 1986; Budavari et al. 1996). The chemical has a high vapor pressure and limited solubility in water but is miscible with a number of organic solvents (ARCO 1983; Budavari et al. 1996). The odor of propylene oxide has been described as sweet and alcoholic, and it has reported odor thresholds ranging from 10 to 200 ppm (Jacobson et al. 1956; Hellman and Small 1974). The physicochemical data on propylene oxide are presented in Table 5-2.

TABLE 5-1 Summary of AEGL Values for Propylene Oxide

Classification	10 min	30 min	1 h	4 h	8 h	End Point (Reference)
AEGL-1 (Nondisabling)	73 ppm (170 mg/m^3)	73 ppm (170 mg/m^3)	73 ppm (170 mg/m^3)	73 ppm (170 mg/m^3)	73 ppm (170 mg/m^3)	Humans: strong odor and irritation noted in monitoring study; average of four exposure concentrations and durations:380 ppm for 177 min, 525 ppm for 121 min, 392 ppm for 135 min, 460 ppm for 116 min (CMA 1998)
AEGL-2 (Disabling)	440 ppm (1,000 mg/m^3)	440 ppm (1,000 mg/m^3)	290 ppm (690 mg/m^3)	130 ppm (310 mg/m^3)	86 ppm (200 mg/m^3)	Dyspnea in mice at 387 ppm for 4 h (NTP 1985)
AEGL-3 (Lethality)	1,300 ppm (3,100 mg/m^3)	1,300 ppm (3,100 mg/m^3)	870 ppm (2,100 mg/m^3)	390 ppm (930 mg/m^3)	260 ppm (620 mg/m^3)	Calculated 4-h $BMCL_{05}$ of 1,161 ppm in rats (NTP 1985)

Abbreviation: $BMCL_{05}$, benchmark concentration, 95% lower confidence limit with 5% response.

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Propylene oxide is produced primarily by one of two processes: from direct oxidation of propylene with air or oxygen or via the intermediate propylene chlorohydrin (Gardiner et al. 1993). The largest use of propylene oxide is in production of polyurethane foams and resins, followed by its use in production of propylene glycol resulting from its hydrolysis. Other common applications include its use in manufacturing functional fluids (such as hydraulic fluids, heat transfer fluids, and lubricants) and propylene oxide–based surfactants, and its use as a food fumigant and acid scavenger (ARCO 1983). The *Chemical Economics Handbook* (SRI International 1995) has estimated that 3,575 to 3,650 million pounds of propylene oxide were produced in the United States in 1998. Worldwide annual capacity for propylene oxide production was estimated at 8.8 billion pounds on Jan. 1, 1994 (SRI International 1995).

Data addressing the toxicity of propylene oxide in humans were limited to one case report, general environmental health surveys, and molecular biomonitoring studies. Studies addressing lethal and nonlethal toxicity of propylene oxide in several species of experimental animals were available.

TABLE 5-2 Chemical and Physical Data for Propylene Oxide

Parameter	Value	Reference
Chemical name	Propylene oxide	
Synonyms	1,2-Epoxypropane, methyloxidrane, propene oxide, 1,2-propylene oxide	ACGIH 1996
CAS registry number	75-56-9	
Molecular formula	C_3H_6O	
Molecular weight	58.08	Budavari et al. 1996
Physical state	Liquid	Budavari et al. 1996
Color	Colorless	Budavari et al. 1996
Melting and boiling points	–112.13ºC and 34.23ºC	Budavari et al. 1996
Solubility	40.5% by wt in water at 20ºC; 59% by wt in water at 25ºC	Budavari et al. 1996; Gardiner et al. 1993
Specific gravity	0.8304 at 20ºC; 0.826 at 25ºC	Gardiner et al. 1993
Density (water = 1)	2.0	Gardiner et al. 1993
Vapor pressure	445 torr at 20ºC	ACGIH 1996
Conversion factors	1 ppm = 2.376 mg/m^3 at 25ºC 1 mg/m^3 = 0.421 ppm	Gardiner et al. 1993

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2. HUMAN TOXICITY DATA

2.1. Acute Lethality

No data were found in the literature regarding lethality in humans after acute exposure to propylene oxide.

2.2. Nonlethal Toxicity

2.2.1. Odor Threshold

Reported ranges of odor threshold vary widely. In one study, 14 subjects found the odor of propylene oxide to be "sweet, alcoholic, and like natural gas, ether, or benzene" (Jacobson et al. 1956). By using an osmoscope, median detectable concentrations were computed by the time-percent effect method of Litchfield, and the median detectable odor concentration was calculated to be 200 ppm (95% confidence interval [C.I.]: 114 to 353 ppm). Amoore and Hautala (1983) reported an odor threshold of 44 ppm; Hellman and Small (1974) reported a threshold of 10 ppm for odor detection and 35 ppm for odor recognition. Subjects classified the odor as neutral to pleasant. The American Industrial Hygiene Association (AIHA 1989) and the Environmental Protection Agency (EPA) (1992) have critiqued studies with odor threshold data, and both have classified the studies by Jacobson et al. (200 ppm) and Hellman and Small (10 and 35 ppm) as acceptable.

2.2.2. Case Reports

One case report of acute exposure was reported in the Russian literature (Beliaev et al. 1971). A 43-year-old male worker was accidentally exposed to propylene oxide vapor for 10 to 15 min while cleaning up a spill. The exposure concentration was exceedingly high, 1,400 to 1,500 milligrams per liter (mg/L) (590,000 ppm), evoking doubt about the accuracy of the measurement. Shortly after exposure, he developed eye and lung irritation, burning behind the sternum, and restlessness. Headache, general weakness, and diarrhea followed 1.5 h later, and within 2 h he was cyanotic and had collapsed. He was given oxygen and antihistamines and was treated for shock. He regained consciousness but remained weak, had diarrhea, and vomited periodically. His pulse and blood pressure returned to normal 2 h later, and he was discharged from the hospital in satisfactory condition after 10 days.

2.2.3. Workplace Exposures

An environmental health survey in 1949 measured propylene oxide levels over drums being filled with polypropylene glycol (1% to 8% free propylene

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oxide content) (CMA 1998). Two 30-min samples contained propylene oxide at 348 and 913 ppm (vol/vol). Another sample collected for 12 min over the opening to a polypropylene glycol mixing tank during purging contained 28 ppm. Workers complained of eye irritation after 2 weeks of steady operation. No fatalities in the 23 potentially exposed workers occurred within 5 months of the sampling.

In 1968, air sampling was conducted in the breathing zone of three workers during typical drumming operations of propylene oxide (CMA 1998). The sampling was conducted to evaluate the effectiveness of local exhaust ventilation in response to worker complaints of occasional eye irritation. Samples were taken starting 5 min after overhead heater fans were turned on (providing additional ventilation) or starting 5 min after the overhead heater fans were off (when worker complaints were typically noted). Air samples were collected in airtight Saran® bags and analyzed by vapor-phase chromatography. Results of the sampling are presented in Table 5-3.

TABLE 5-3 Summary Results of Personal Exposure Monitoring for Propylene Oxide During Typical Drumming Operations

Sample Number	Description of Samples (taken in breathing zone of operators during drumming of propylene oxide)	Personnel Monitored	Sampling Duration (min)	TWA for Monitoring Period (ppm)
1	Sampling initiated 5 min after overhead heater fan turned on, heater fan on for duration of monitoring	Drumming operator 1	177	380
2	Sampling initiated 5 min after overhead heater fan turned off, heater fan off for duration of monitoring	Drumming operator 1	171	1520
3	Sampling initiated 5 min after overhead heater fan turned off, heater fan off for duration of monitoring	Drumming operator 2	124	1310
4	Sampling initiated 5 min after overhead heater fan turned off, heater fan off for duration of monitoring	Drumming operator 2	121	525
5	Sampling initiated 5 min after overhead heater fan turned on, heater fan on for duration of monitoring	Drumming operator 3	135	392
6	Sampling initiated 5 min after overhead heater fan turned on, heater fan on for duration of monitoring	Drumming operator 3	116	460

Abbreviation: TWA, time-weighted average.
Source: CMA 1998.

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Exposures were 1,520 ppm (vol/vol) for 171 min, 1,310 ppm for 124 min, and 525 ppm for 121 min with the overhead heater fan turned off and 380 ppm for 177 min, 392 ppm for 135 min, and 460 ppm for 116 min with the overhead heater fan turned on (CMA 1998). The industrial hygienist was in the drumming booth during the monitoring periods and stated that "the odor was quite strong during the sampling; however, the irritation was not intolerable." Other observations noted by the hygienist included the following: "odor was quite obvious but not objectionable"; "pronounced odor, nonobjectionable"; and "general area in drumming room, about 10 feet from drumming station, odor was detectable but faint." No fatalities in the 30 potentially exposed workers (including the hygienist) occurred within 5 months of sampling, indicating that the measured exposures to propylene oxide were not fatal.

Background propylene oxide concentrations were measured over three 8-h shifts in a plant in 1975 to perform baseline routine annual monitoring (CMA 1998). The concentration of the samples in ambient air ranged from none detected (<0.1 ppm) to 31.8 ppm (vol/vol). Propylene oxide concentrations were also measured in the breathing zones of workers using Sipin personal sampler pumps over the 8-h work periods. Measured concentrations ranged from 13.2 to 31.8 ppm as 8-h time-weighted averages (TWAs) measured over the 3-day sampling period (see Table 5-4). No worker complaints were noted in the report.

2.3. Developmental and Reproductive Toxicity

No human developmental and reproductive toxicity data on propylene oxide were found in the literature.

2.4. Genotoxicity

Unscheduled DNA synthesis after in vitro challenge with the carcinogen *N*-acetoxy-2-acetylaminofluorene was measured in lymphocytes of 23 process workers exposed to propylene oxide (Pero et al. 1982). The control population consisted of workers in a nearby mechanical industry factory. Five of the most exposed workers had an estimated TWA of 0.6 to 12 ppm during 5 working days, and some workers had short exposures to concentrations as high as 1,000 ppm. Exposed workers showed a decreased capacity for unscheduled DNA synthesis, a step in the enzymatic repair of DNA lesions. Osterman-Golkar et al. (1984) reported a good correlation between the estimated exposures of eight workers to propylene oxide vapor and hemoglobin adduction at the *N*-(2-hydroxypropyl)histidine residues. Workers exposed to the highest estimated concentration of approximately 10 ppm for 25% to 75% of their work time had adduct levels in the range of 4.5 to 13 nanomoles (nmol) per gram (g) of hemoglobin. Pero et al. (1985) also found a significant increase in the alkylation of histidine residues of hemoglobin in relation to propylene oxide exposure and a significant decrease in proficiency of DNA repair as measured by unscheduled

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DNA synthesis. Linear regression of the hemoglobin adducts versus DNA repair proficiency revealed a significant correlation ($r = -0.64$; $p < 0.03$).

Cytogenetic monitoring studies measuring chromosomal aberrations were carried out on groups of employees of the Shell petrochemical company (de Jong et al. 1988). From 1976 to 1981, these employees were potentially exposed to a number of genotoxic chemicals, including propylene oxide, with exposures occurring well below the occupational exposure limits. One group under investigation was limited in its exposure to propylene oxide alone, and the average air concentration of propylene oxide in the plant where the group worked was 0.042 ppm (geometric mean; range <0.042 to 2.74 ppm). The authors concluded that no correlation could be made between increased chromosomal aberrations and work exposure to low levels of propylene oxide or to any of the other genotoxic chemicals under investigation.

Högstedt et al. (1990) measured cytogenic end points in the blood of 20 male individuals exposed to propylene oxide for 1 to 20 years in a plant that produced alkylated starch. Average concentrations of propylene oxide measured in the breathing zones during 2- to 4-h measuring periods ranged from 0.33 to 11.4 ppm, with a peak concentration of 56 ppm measured during a shorter 20-min sampling period. Micronulei and chromosomal aberrations were measured; however, there was no control group with which to compare the results. A correlation was observed between measured propylene oxide air concentrations and the presence of the hemoglobin adduct hydroxypropylvaline in the exposed workers.

TABLE 5-4 Summary Results of Personal Exposure Monitoring

			Propylene Oxide		
				Mean Job Class Concentration[a] (ppm)	
Job Classification	Number of Persons Monitored	Number of Samples	Concentration Range (ppm)	Mean	95% UCL
Maintenance personnel	5	8	14.9-18.9	17.4	18.30
Laboratory personnel	2	2	30.2-31.8	31.0	36.05
Engineer	1	1	30.2	30.2	N/A
Foreman	2	4	16.1-23.8	20.58	24.49
Operator	6	11	13.2-23.3	18.69	20.31

[a]Calculated arithmetic mean and 95% upper confidence level (UCL) for the associated job class. Job classes were identified and monitored by homogenous exposure groups rather than job titles.
Abbreviation: UCL, 95% upper confidence level; N/A, not applicable.
Source: CMA 1998.

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2.5. Carcinogenicity

Data on the potential carcinogenicity of propylene oxide in humans are limited and no definitive conclusions can be drawn. A retrospective cohort study of alkylene oxide–exposed workers conducted by Thiess et al. (1982) and a mortality study by Egedahl et al. (1989) did not find increased mortality from cancer or any other cause, but the studies were confounded by exposure to multiple alkene oxides as well as other chemicals. A nested case-control study investigating cancer incidences in workers ever exposed to propylene oxide versus never exposed did not result in significant associations of specific cancers with exposure (Ott et al. 1989).

2.6. Summary

Available data on human exposure to propylene oxide were limited. Odor detection threshold values for propylene oxide ranged from 10 to 200 ppm. The only case report of an occupational exposure was of a male worker accidentally exposed to a high concentration of propylene oxide for 10 to 15 min. Symptoms of exposure included eye and lung irritation, burning sensation in the chest, restlessness, headache, general weakness, diarrhea, and vomiting. The worker reportedly recovered. Other workplace exposure information was reported in environmental health surveys. Measured exposure concentrations of propylene oxide were as high as 1,520 ppm for 171 min with no reports of fatality. A strong odor and undefined irritation were noted at this concentration. In another report, 8-h TWAs measured over a 3-day sampling period indicated propylene oxide exposures ranging from 13.2 to 31.8 ppm. Ambient air concentrations of propylene oxide ranged from none detected to 41.8 ppm. The report noted no worker complaints.Molecular biomonitoring studies of workers exposed to low concentrations of propylene oxide have revealed a good correlation between hemoglobin adduction, decreased proficiency for DNA repair, and estimated exposure to propylene oxide. Cytogenetic studies have not found a significant correlation between in vivo propylene oxide exposure and micronuclei or chromosomal aberrations. Data on the potential carcinogenicity of propylene oxide in humans are limited and no definitive conclusions can be drawn.

3. ANIMAL TOXICITY DATA

3.1. Acute Lethality

3.1.1. Dogs

Jacobson et al. (1956) exposed groups of three male beagle dogs to measured propylene oxide vapor concentrations of 1,363, 2,005, 2,030, or 2,481 ppm for 4 h. Animals were exposed in constant-flow gassing chambers with a capac-

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ity of 0.4 m^3. The propylene oxide vapor was generated by passing a stream of nitrogen through the liquid. Chamber air was sampled by drawing chamber air through a series of bubblers (the first containing a solution of calcium chloride with water [$CaCl_2 \cdot 2H_2O$] in 0.1 N hydrochloric acid [HCl], and the second containing water to trap any acid). Chamber concentrations were calculated from the results of titration of the HCl that had not reacted with propylene oxide in the bubblers with a standard sodium hydroxide solution. The dogs were observed for signs of toxicity and mortality for 14 days. Signs of toxicity in the animals included lacrimation, salivation and nasal discharge, vomiting, and death. At least one dog in each group exposed to 2,005 ppm or higher exhibited motor weakness after exposure. Congestion of the tracheal mucosa and lungs, spotty alveolar edema, marked perivascular and peribronchial edema, and focal areas of subepithelial edema and necrobiosis of bronchiolar epithelium were noted during postmortem examination in the dogs exposed to 2,030 and 2,481 ppm. Some exposed animals also had subpleural hemorrhage. Incidences of subendocardial ecchymoses were considered a secondary effect of the anoxia. Mortality occurred in all groups exposed to 2,005 ppm or higher (see Table 5-5). Death occurred within 24 h of exposure. The authors did not calculate an LC_{50} (concentration with 50% lethality) value for dogs because of inadequate data (two of three dogs in the 2,481-ppm group were dead when they were removed from the chamber; the third dog probably would have died soon and was therefore killed immediately). If one conducts a probit analysis of the data, a 4-h LC_{50} value of 1,941 ppm is obtained.

3.1.2. Rats

Jacobson et al. (1956) exposed groups of 10, white male rats (strain not given) to measured conentrations of propylene oxide vapor at 945, 1,329, 2,684, 3,448, 4,490, or 5,254 ppm for 4 h. Animals were exposed in constant-flow gassing chambers of 0.4-m^3 capacity. The propylene oxide vapor was generated by passing a stream of nitrogen through the liquid. Chamber air was sampled by drawing chamber air through a series of bubblers (the first containing a solution of $CaCl_2 \cdot 2H_2O$ in 0.1 N HCl, and the second containing water to trap any acid). Chamber concentrations were calculated from the results of titrating the HCl that had not reacted with propylene oxide in the bubblers with a standard sodium hydroxide solution. The rats were observed for 14 days for signs of toxicity and mortality. Signs of toxicity in exposed rats included frequent movement and preening, clear nasal discharge, lacrimation, salivation, gasping that increased in intensity during exposure, and death. The only pathologic change revealed during postmortem examination was a distended stomach in exposed rats. Mortality occurred in rats exposed to 3,448 ppm or higher (see Table 5-6). Death occurred by day 6. The calculated 4-h LC_{50} was 4,000 ppm (9,486 mg/m^3; Bliss-Finney method) (95% C.I. 3,550 to 4,470 ppm).

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TABLE 5-5 Mortality of Male Dogs Exposed to Propylene Oxide Vapor for 4 Hours

Concentration		
ppm	mg/m^3	Mortality (%)
1,363	3,230	0/3 (0)
2,005	4,750	1/3 (33)
2,030	4,810	2/3 (67)
2,481	5,880	3/3 (100)

Source: Jacobson et al. 1956. Reprinted with permission; copyright 1956, American Medical Association.

TABLE 5-6 Mortality of Male Rats Exposed to Propylene Oxide Vapor for 4 Hours

Concentration		
ppm	mg/m^3	Mortality (%) [day of death]
945	2,240	0/10 (0)
1,329	3,150	0/10 (0)
2,684	6,360	0/10 (0)
3,448	8,170	3/10 (30) [1,[a] 1, 3]
4,490	10,640	7/10 (70) [1, 1, 1, 2, 2, 2, 5]
5,254	12,450	9/10 (90) [1,[a] 1,[a] 1,[a] 1,[a] 1, 1, 1, 5, 6]

[a]Death within the first hour postexposure.

Source: Jacobson et al. 1956. Reprinted with permission; copyright 1956, American Medical Association.

Groups of female albino rats were exposed to nominal air concentrations of propylene oxide vapor at 2,000, 4,000, 8,000, or 16,000 ppm for various times (see Table 5-7) and were then observed for 14 days for signs of toxicity and mortality (Rowe et al. 1956; methods reported by Spencer et al. 1951). Measured concentrations ranged from 64% to 110% of nominal, averaging 87% of nominal concentrations (actual measured concentrations of the individual exposure concentrations not provided). During the exposure, animals exhibited eye and nasal irritation, difficulty breathing, drowsiness, weakness, and occasionally some incoordination. The severity of the signs increased with concentration and duration of exposure. Some rats continued to experience wheezing after the exposure, and three of those with respiratory difficulty developed pneumonia. The surviving animals generally exhibited weight loss, but they recovered within 14 days. Mortality occurred in most groups exposed to 4,000 ppm and higher, and death typically occurred during the exposure or within 24 h after exposure. Mortality data are presented in Table 5-7.

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TABLE 5-7 Mortality of Female Albino Rats Exposed to Propylene Oxide Vapor

Concentration (ppm)	Duration (h)	Mortality (%)
2,000	7.0	0/10 (0)
4,000	7.0	10/10 (100)
	4.0	4/10 (40)
	2.0	4/10 (40)
	1.0	0/5 (0)
8,000	2.0	10/10 (100)
	1.0	5/10 (50)
	0.5	2/10 (20)
	0.25	0/10 (0)
16,000	0.5	10/10 (100)
	0.25	0/15 (0)

Source: Rowe et al. 1956. Reprinted with permission; copyright 1956, American Medical Association.

Exposure to propylene oxide vapor for 4 h at a concentration of 4,000 ppm killed 6/6 Sherman rats and 4/6 albino rats (time to death not reported) (Smyth et al. 1948; Weil et al. 1963; also reported by Smyth et al. 1969; methods described by Smyth et al. 1962). These studies do not mention controls, and the authors stated that the concentrations were not precise but rather were estimates. In another experiment, liquid propylene oxide was placed in a shallow tray in a sealed container for at least 24 h. Six albino rats were then introduced into the chamber for 5 min. This exposure killed all six rats.

In an acute inhalation exposure study by NTP (1985), groups of five Fischer 344 (F344)/N rats of each sex were exposed to air containing measured concentrations of propylene oxide vapor at 0, 1,277, 2,970, 3,794, or 3,900 ppm for 4 h. Propylene oxide vapor was generated by vaporizing propylene oxide at room temperature followed by dilution with air. Propylene oxide chamber air concentrations were measured 8 to 12 times per exposure period with a gas chromatograph. After exposure, animals were observed for 14 days for signs of toxicity and mortality. Mortality and toxic effects (red nasal discharge and dyspnea) occurred in all groups exposed to 2,970 ppm or higher (Table 5-8). There were no gross pathologic changes in any of the treated animals. NTP did not report an LC_{50} value. A probit analysis of the data predicts a 4-h LC_{50} of 3,205 ppm (both sexes combined).

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TABLE 5-8 Mortality of F344/N Rats Exposed to Propylene Oxide for 4 Hours

	Mortality (%) [day of death]		
Concentration (ppm)	Males	Females	Other Effects
1,277	0/5 (0)	0/5 (0)	None observed
2,970	1/5 (20) [3]	2/5 (40) [1, 2]	Dyspnea, red nasal discharge
3,794	4/5 (80) [1, 3, 4, 5]	4/5 (80) [1, 1, 3,5]	Dyspnea, red nasal discharge
3,900	3/5 (60) [1, 2, 2]	3/5 (60) [1, 1, 2]	Dyspnea, red nasal discharge

Source: NTP 1985.

Groups of four male and four female Wistar rats were exposed to measured concentrations of propylene oxide vapor at 3,000, 3,450, 4,050, 4,280, 4,500, 5,260, or 5,970 ppm for 4 h (Shell Oil Company 1977). Animals were exposed in dynamic, cylindrical glass exposure chambers. Test chamber air was drawn from the mixing chamber exit through an infrared gas analyzer for concentration verification and then returned to the exposure chamber inlet manifold. After exposure, animals were observed for toxicity signs and mortality for 14 days. During the exposure, all animals exhibited signs of toxicity, including excessive lacrimation and eye irritation, sedation, piloerection, mucous discharge (frequently bloodstained) from the nose and mouth, and respiratory difficulty that continued for several hours after exposure. The time to onset of these signs was concentration related. Rats surviving the 14-day observation period appeared normal; however, necropsy was not performed on any of the animals. Mortality generally occurred during or within the first 24 h after exposure (mortality data are presented in Table 5-9). Propylene oxide exhibited a steep concentration-response curve for mortality: no deaths were observed at 3,450 ppm but all animals died at 5,260 ppm (slope factor of 32.2). The calculated 4-h LC_{50} value (both sexes combined) was 4,197 ppm (95% C.I.: 3,902 to 4,394 ppm).

In a repeated-exposure study, groups of three male and three female Wistar rats were exposed 6 h/day to air containing measured propylene oxide vapor concentrations of 0 or 997 ppm for 10 days or 1,940 ppm for 9 days (Shell Oil Company 1977). Signs of toxicity in exposed animals were similar to but less severe than those observed in an acute exposure (described above) and included excessive lacrimation and eye irritation, sedation, piloerection, mucous discharge (frequently bloodstained) from the nose and mouth, and respiratory difficulty that continued for several hours after exposure. The toxicity signs in the repeated-exposure study, however, disappeared after day 3 of exposure, except for lethargy, which developed and progressed in the 1,940-ppm group. None of the rats in the 1,940-ppm group would have survived to day 10 of exposure, and they were therefore necropsied after exposure day 9. Macroscopic examination revealed subcutaneous edema of the face, anal region, and feet and distended urinary bladders in females; however, no histologic changes in the tissues were noted. No macroscopic changes were noted in the 997-ppm group, and male rats had decreased kidney and heart weights that were not accompanied by histopa-

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thologic changes. Microscopic examination of the lungs of animals in the 997- and 1,940-ppm groups revealed concentration-related necrosis and inflammation of the respiratory epithelium from the nasal cavity to the major bronchi, accompanied by epithelial proliferation and focal hyperplasia and metaplasia.

Rowe et al. (1956) exposed male and female albino rats to various concentrations of propylene oxide for 7 h/day, 5 days/week. Ten male rats exposed to 457 ppm 79 times and 10 female rats exposed to 457 ppm 138 times experienced eye and respiratory irritation, slight alveolar hemorrhage, and pulmonary edema. Although the authors stated that there was an increase in mortality due to pneumonia, they did not reveal the incidence.

3.1.3. Mice

Jacobson et al. (1956) exposed groups of 10, white female mice (strain not given) to measured concentrations of propylene oxide vapor at 945, 1,329, 1,755, 2,684, 3,448, 4,490, or 5,254 ppm for 4 h. The propylene oxide vapor was generated by passing a stream of nitrogen through the liquid. Chamber air was sampled by drawing it through a series of bubblers (the first containing a solution of $CaCl_2{\cdot}2H_2O$ in 0.1 N HCl and the second containing water to trap any acid). Chamber concentrations were calculated from the results of titrating the HCl that had not reacted with propylene oxide in the bubblers with a standard sodium hydroxide solution. Animals were observed for signs of toxicity and mortality for the next 14 days. Signs of toxicity in exposed mice were similar to those observed in exposed rats (see Section 3.1.2) and included frequent movement and preening, clear nasal discharge, lacrimation, salivation, gasping that increased in intensity during exposure, and death. As in rats, the only pathologic change in mice was a distended stomach. Mortality occurred in all exposed groups (see Table 5-10). The calculated 4-h LC_{50} was 1,740 ppm (4,126 mg/m^3; Bliss-Finney method) (95% C.I. 1,380 to 2,120 ppm).

TABLE 5-9 Mortality of Wistar Rats Exposed to Propylene Oxide for 4 Hours

Concentration (ppm)	Mortality (%) [day of death]		
	Males	Females	Total
3,000	0/4	0/4	0/8
3,450	0/4	0/4	0/8
4,050	0/4	3/4 (75) [1, 2, 2]	3/8 (38)
4,280	2/4 (50) [2, 6]	2/4 (50) [1, 2]	4/8 (50)
4,500	3/4 (75) [1, 1, 7]	4/4 (100) [1, 1, 1, 1]	7/8 (88)
5,260	4/4 (100) [1, 1, 1, 2]	4/4 (100) [1, 1, 1, 1]	8/8 (100)
5,970	4/4 (100) [1, 1, 2, 3]	4/4 (100) [1, 1, 1, 1]	8/8 (100)

Source: Shell Oil Company 1977.

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TABLE 5-10 Mortality of Female Mice Exposed to Propylene Oxide Vapor for 4 Hours

Concentration		
ppm	mg/m^3	Mortality (%) [Day of death]
945	2,240	2/11 (18) [1, 1[a]]
1,329	3,150	1/10 (10) [1[a]]
1,755	4,160	5/10 (50) [1,1,1,1,3]
2,684	6,360	9/10 (90) [1, 1, 1, 1, 1, 1, 1, 6, 13]
3,448	8,170	10/10 (100) [1, 1, 1, 1, 1, 1, 1, 1, 1, 1]
4,490	10,640	10/10 (100) [1, 1, 1, 1, 1, 1, 1, 1, 1, 2]
5,254	12,450	9/10 (90) [1, 1, 1, 1, 1, 1, 1, 1, 1]

[a]Death after first hour postexposure; other deaths on day 1 occurred within 1 h postexposure.

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In an acute inhalation exposure study by NTP (1985), groups of five B6C3F$_1$ mice of each sex were exposed to air containing measured concentrations of propylene oxide vapor—generated by vaporizing propylene oxide at room temperature followed by dilution with air—at 0, 387, 859, 1,102, 1,277, or 2,970 ppm for 4 h. Propylene oxide chamber air concentrations were measured 8 to 12 times per exposure period with a gas chromatograph. After exposure, the animals were observed for 14 days for signs of toxicity and mortality. All exposed mice exhibited dyspnea, mice exposed to 1,227 and 2,970 ppm exhibited sedation, and the highest concentration group also exhibited lacrimation. No pathologic changes were observed at necropsy. Mortality data are presented in Table 5-11. Although one female mouse died in the 387-ppm group, the mortality did not appear to be treatment related. No females died at the next higher concentration (859 ppm), and almost all other mice in the study that died from propylene oxide exposure died on the first day (one mouse died on day 2), whereas the 387-ppm mouse died on test day 6. NTP (1985) also conducted a 2- and 13-week study in B6C3F$_1$ mice; therefore, the data from the 4-h exposure study could be further evaluated by placing the results in context with the other studies. No mortalities occurred in mice (five animals per sex per concentration) exposed to propylene oxide at 0, 20, 47, 99, 196, or 487 ppm for 6 h/day, 5 days/week, for 2 weeks. Mice in the 196- and 487-ppm groups exhibited dyspnea, and the highest exposure groups were hypoactive. No mortalities occurred in mice (10 animals per sex per concentration) exposed to propylene oxide at 0, 31, 63, 125, 250, or 500 ppm for 6 h/day, 5 days/week, for 13 weeks except for one male mouse in the 125-ppm group on day 14. In this repeat-exposure study, the 500-ppm groups had lower body weights than controls, but gross or microscopic pathologic evaluation did not reveal any compound-related effects. Signs

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TABLE 5-11 Mortality of $B6C3F_1$ Mice Exposed to Propylene Oxide for 4 Hours

Concentration (ppm)	Mortality (%) [day of death]		Other Effects
	Males	Females	
387	0/5 (0)	1/5 (20) [6]	Dyspnea
859	0/5 (0)	0/5 (0)	Dyspnea
1,102	2/5 (40) [1, 1]	4/5 (80) [1, 1, 1, 2]	Dyspnea
1,277	2/5 (40) [1, 1]	5/5 (100) [1, 1, 1, 1, 1]	Dyspnea, sedation
2,970	5/5 (100) [1, 1, 1, 1, 1,]	5/5 (100) [1, 1, 1, 1, 1]	Dyspnea, sedation, lacrimation

Source: NTP 1985.

of toxicity were not stated, so it is unclear whether any were noted or simply were not reported. On the basis of the overall experimental results discussed above, the one death in the female group exposed to 387 ppm for 4 h did not appear to be related to exposure. The calculated 4-h LC_{50} for both sexes combined was 1,160 ppm (using probit analysis of the acute exposure data, excluding the death in the 387-ppm group).

3.1.4 Guinea Pigs

Rowe et al. (1956) exposed groups of female guinea pigs to nominal air concentrations of propylene oxide vapor at 2,000, 4,000, 8,000, or 16,000 ppm for various times (see Table 5-12). Measured concentrations ranged from 64% to 110% of nominal, averaging 87% of nominal concentrations (actual measured concentrations of the individual exposure concentrations not provided). The animals were observed for signs of toxicity and mortality for 14 days after exposure. During the exposure, animals exhibited eye and nasal irritation, difficulty breathing, drowsiness, weakness, and occasionally some incoordination. The severity of the signs increased with concentration and duration of exposure. The surviving animals generally exhibited weight loss but recovered within 14 days. Death typically occurred during or within 24 h after exposure. Mortality data are presented in Table 5-12.

3.2. Nonlethal Toxicity

3.2.1. Nonhuman Primates

To investigate the potential neuropathologic effects of propylene oxide, male monkeys (two per group) were exposed to propylene oxide at 0, 100, or 300 ppm for 6 h/day, 5 days/week, for 24 months and were sacrificed at the termination of exposure (Sprinz et al. 1982). The only observable differences noted

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between treated and control monkeys were signs of axonal dystrophy in the medulla oblongata of the brain. Setzer et al. (1997) found no neurophysiologic or neuropathologic changes in groups of 12 cynomolgus monkeys exposed to propylene oxide vapor at 0, 100, or 300 ppm for 7 h/day, 5 days/week, for 24 months.

One female rhesus monkey exposed 154 times to 457 ppm, two female rhesus monkeys exposed 154 times to 195 ppm, and two female rhesus monkeys exposed 154 times to 102 ppm for 7 h/day, 5 days/week, showed no adverse effects (Rowe et al. 1956).

3.2.2. Dogs

As discussed in Section 3.1.1., no deaths were observed in three male beagle dogs exposed to propylene oxide vapor at 1,363 ppm for 4 h (Jacobson et al. 1956). Signs of toxicity in the animals included lacrimation, salivation, and nasal discharge.

3.2.3. Rats

No mortalities occurred in groups of 10 white male rats exposed to measured concentrations of propylene oxide vapor at 945, 1,329, or 2,684 ppm for 4 h (see Section 3.1.2; Table 5-5; Jacobson et al. 1956). Signs of toxicity in exposed rats included frequent movement and preening, clear nasal discharge, lacrimation, salivation, and gasping that increased in intensity during exposure. It was stated that the severity of toxic signs increased with increasing concentration.

TABLE 5-12 Mortality of Female Guinea Pigs Exposed to Propylene Oxide Vapor

Concentration (ppm)	Duration (h)	Mortality (%)
2,000	7.0	0/5 (0)
4,000	7.0	2/5 (40)
	4.0	1/5 (20)
	2.0	0/5 (0)
8,000	4.0	10/10 (100)
	2.0	1/5 (20)
	1.0	0/10 (0)
16,000	1.0	5/5 (100)
	0.5	0/5 (0)

Source: Rowe et al. 1956. Reprinted with permission; copyright 1956, American Medical Association.

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Groups of female albino rats survived exposure to propylene oxide vapor at nominal concentrations of 16,000 ppm for 15 min, 8,000 ppm for 15 min, 4,000 ppm for 1 h, or 2,000 ppm for 7 h (see Section 3.1.2; Table 5-6; Rowe et al. 1956). During the exposures, animals exhibited eye and nasal irritation, difficulty breathing, drowsiness, weakness, and occasionally some incoordination. The severity of the signs increased with concentration and duration of exposure (see Table 5-6 for complete exposure data). Rowe et al. (1956) also exposed groups of five female albino rats to propylene oxide at nominal concentrations of 4,000 ppm for 30 min, 2,000 ppm for 2 h, or 1,000 ppm for 7 h. Twenty-four hours after exposure, animals were killed, organs were weighed, and gross and microscopic evaluation of the tissues was performed. No treatment-related effects were observed.

NTP (1985) conducted a 4-h acute inhalation study in groups of five male and five female F344/N rats (see Section 3.1.2; Table 5-7). No deaths and no treatment-related effects occurred at the lowest propylene oxide exposure concentration of 1,277 ppm.

Groups of four male and four female Wistar rats were exposed to measured concentrations of propylene oxide vapor at 3,000, 3,450, 4,050, 4,280, 4,500, 5,260, or 5,970 ppm for 4 h and then observed for toxicity signs and mortality for 14 days (see Section 3.1.2; Table 5-8; Shell Oil Company 1977). During the exposure, all animals exhibited signs of toxicity, including excessive lacrimation and eye irritation, sedation, piloerection, mucous discharge (frequently bloodstained) from the nose and mouth, and respiratory difficulty that continued for several hours after exposure. The time to onset of these signs was concentration related. No mortalities occurred in male rats exposed to 3,000, 3,450, or 4,050 ppm or in female rats exposed to 3,000 or 3,450 ppm. Rats surviving the 14-day observation period appeared normal; however, necropsy was not performed on any of the animals.

Groups of six white male rats were exposed for 4 h to measured concentrations of propylene oxide vapor at 4.6 or 8.4 ppm (11.0 or 20.0 mg/m^3) to determine the threshold for acute effects (Pugaeva et al. 1970). Signs of acute toxicity included impairment of the central nervous system, liver, and hemodynamic functions, with a threshold of 8.4 ppm (20 mg/m^3). No further details were provided. The concentrations reported in this study are extremely low compared with other published data. The reason for this discrepancy is not clear.

In a repeat-exposure study, groups of three male and three female Wistar rats survived exposure to propylene oxide vapor at 0 or 997 ppm for 6 h/day for 10 days (see Section 3.1.2; Shell Oil Company 1977). Signs of toxicity in exposed animals included excessive lacrimation and eye irritation, sedation, piloerection, mucous discharge (frequently bloodstained) from the nose and mouth, and respiratory difficulty that continued for several hours after exposure. The toxicity signs disappeared after day 3 of exposure. No macroscopic changes were noted in the 997-ppm group, and male rats had decreased kidney and heart weights that were not accompanied by histopathologic changes. Microscopic

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examination of the lungs of animals in the 997-ppm group revealed necrosis and inflammation of the respiratory epithelium from the nasal cavity to the major bronchi, accompanied by epithelial proliferation and focal hyperplasia and metaplasia.

In a repeat-exposure study by NTP (1985), five male and five female F344/N rats were exposed to propylene oxide at 0, 47, 99, 196, 487, or 1,433 ppm for 6 h/day, 5 days/week, for 2 weeks. No deaths occurred in males exposed to 487 ppm or less or in females exposed to 1,433 ppm or less. Toxic signs observed at the highest concentration included dyspnea, hypoactivity, gasping, ataxia, diarrhea, and reduced body weight compared with controls.

No deaths occurred when groups of 10 male and 10 female F344/N rats were exposed to 0, 31, 63, 125, 250, or 500 ppm for 6 h/day, 5 days/week, for 13 weeks (NTP 1985). Rats exposed to propylene oxide at 500 ppm had slightly decreased weight gain. Gross and microscopic pathologic examination did not reveal any compound-related effects, but it was noted that all rats were infected with chronic murine pneumonia.

Rowe et al. (1956) exposed male and female albino rats for 7 h/day, 5 days/week to various measured concentrations of propylene oxide vapor. No signs of toxicity and no exposure-related mortality occurred in rats exposed to 457 ppm for 11 to 13 exposures, 195 ppm for 11 to 14 exposures, or 195 or 102 ppm for 138 exposures. Five rats of each sex exposed 25 to 27 times to 457 ppm developed alveolar hemorrhage and edema and interstitial edema and congestion. Ten male rats exposed to 457 ppm 79 times and 10 female rats exposed to 457 ppm 138 times developed eye and respiratory irritation, slight alveolar hemorrhage, and pulmonary edema.

Groups of 12 male Wistar rats were exposed to propylene oxide at 1,500 ppm or filtered air for 6 h/day, 5 days/week, for 7 weeks (Ohnishi et al. 1988). Rats were sacrificed at the end of the 5- to 7-week exposure or after a recovery period of 7 to 8 weeks after the completion of exposure. Exposed rats had significantly decreased body weight compared with controls and developed ataxia in the hindlimbs, without foot drop or muscular atrophy. Pathologic examination of the exposed rats revealed changes compatible with central-peripheral distal axonopathy.

Five groups of 10 male and 10 female specific pathogen-free–reared rats were exposed to measured concentrations of propylene oxide vapor at 0, 76, 149, 298, or 600 ppm for 6 h/day, 5 days/week, for 13 weeks (Dow Chemical Company 1981). All animals survived to scheduled sacrifice. No changes were noted in the 76-ppm group. Transient restless behavior was noted in the 149-, 298-, and 600-ppm exposure groups during the first 3 days of exposure, and piloerection and salivation occasionally occurred in the 600-ppm group. Males in the 298-ppm group had a slight transitory decrease in body weight gain. Animals exposed to propylene oxide at 600 ppm also exhibited a decreased body weight gain that was more pronounced in males. In addition, histopathologic examination revealed degenerative and hyperplastic changes in the nose, includ-

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ing edema in the submucosa and focal atrophy and squamous metaplasia of the olfactory epithelium in animals from the 600-ppm group.

No evidence of neurotoxicity was found in groups of 30 male F344 rats exposed to propylene oxide vapor at 0, 30, 100, or 300 ppm for 6 h/day for 24 weeks (exposures were 5 days/week for the first 14 weeks and 7 days/week thereafter) (Young et al. 1985). End points examined included clinical toxicity signs, hindlimb grip strength, open-field activity test, and histopathologic examination of the central and peripheral nervous systems from 10 control and 10 high-concentration-group rats.

Groups of 40 white rats were exposed to measured propylene oxide concentrations of 4.1, 10.5, or 15.6 ppm (9.7, 25.0, or 37.0 mg/m^3) in an exposure chamber for 6 months (Pugaeva et al. 1970). Chronic exposure resulted in changes in brain bioelectric activity, adrenocorticotropic activity in blood, and arterial blood pressure. The threshold for chronic effects was listed as close to 4.1 ppm (9.7 mg/m^3). The concentrations reported by this study are extremely low compared with other published data. The reason for the discrepancy is not clear.

Groups of 50 male and 50 female F344/N rats were exposed to propylene oxide at 0, 200, or 400 ppm for 6 h/day, 5 days/week, for 2 years (NTP 1985). Mean body weight of high-dose rats was lower than that of controls. Pathologic evaluation of the nasal cavity revealed dose-related suppurative inflammation of the respiratory epithelium, and increased incidences of epithelial hyperplasia and squamous metaplasia in the high-dose rats.

To investigate the effects of propylene oxide vapor on nasal epithelial cell proliferation, groups of 10 F344 rats were exposed to propylene oxide vapor at 0, 10, 20, 50, 150, or 525 ppm for up to 4 weeks for 6 h/day, 5 days/week (Eldridge et al. 1995). Animals were killed and examined after 1 or 4 weeks of exposure or 1 or 4 weeks postexposure. At 1 week of exposure, cell proliferation as measured by bromodeoxyuridine (BrdU) incorporation was statistically increased in the respiratory epithelium in rats from the 525-ppm exposure group and in the olfactory epithelium in rats from the 50-, 150-, and 525-ppm exposure groups. Half the rats in the 525-ppm exposure group also showed hyperplasia as measured by histologic evaluation in the respiratory epithelium. At 4 weeks of exposure, increased cell proliferation was still observed in the olfactory epithelium in rats from the 150- and 525-ppm exposure groups and in the respiratory epithelium in rats from the 525-ppm exposure group. Olfactory degeneration was also present in 525-ppm group rats (8/9), and hyperplasia of the respiratory epithelium was observed in the 150- and 525-ppm groups. By 1 week postexposure, only rats from the 525-ppm exposure group still had olfactory epithelium degeneration and increased cell proliferation of the respiratory epithelium, both of which regressed to control levels by 4 weeks postexposure.

A later study investigated the effects of propylene oxide vapor on nasal respiratory epithelial and hepatic cell proliferation in groups of six F344 rats exposed to propylene oxide vapor at 0, 5, 25, 50, 300, or 500 ppm for 6 h/day, 5 days/week, for 3 or 20 days (Ríos-Blanco et al. 2003b). Three days before ne-

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cropsy, osmotic pumps containing BrdU were implanted in the animals. Animals were killed after cessation of the last exposure and histopathologic examination of the nasal cavity and liver was performed. Cell proliferation was assessed by evaluating BrdU incorporation in the respective tissues. Of six assigned anatomic locations in the nasal passages, only level 1 (immediately posterior to the upper incisor teeth through the naso- and maxilloturbinates; included nasal stratified squamous and respiratory epithelium) and level 5 (posterior to the middle of the first upper molar teeth through the ethmoturbinates; included olfactory epithelium and nasal respiratory epithelium lining the nasopharyngeal meatus) were evaluated. Within level 1, two regions were evaluated for cell proliferation. Region 1 comprised mucociliary epithelium lining the medial septum, dorsal medial meatus, and medial surface of the nasoturbinate; region 2 contained the transitional epithelium lining the lateral surface of the nasoturbinate. The authors noted that level 1 contains the specific region where nasal tumors appeared during the cancer bioassays in F344 rats. Histopathologic lesions were confined to hyperplastic lesions in the nasal respiratory epithelium lining the septum, medial, and dorsal surfaces at level 1 (region 1). The hyperplastic response was associated with mucous secretory cells arranged in mucous cell nests, and the average number of mucous cell nests was statistically increased only in rats exposed to ≥300 ppm for 20 days. No histopathologic abnormalities were noted in rats exposed to ≤50 ppm for 20 days or in any group exposed to propylene oxide for 3 days. Cell proliferation as measured by BrdU incorporation was increased ($p < 0.01$) in region 1 after exposure to 300 and 500 ppm for 3 or 20 days and in region 2 and in the epithelial lining of the nasopharyngeal meatus after 3 days of exposure to 500 ppm and after 20 days of exposure to 300 and 500 ppm.

3.2.4. Mice

Five male and five female B6C3F$_1$ mice exposed to measured propylene oxide concentrations of 387 or 859 ppm for 4 h did not show compound-related mortality (see Section 3.1.2; Table 5-10; NTP 1985). The death of one female mouse in the 387-ppm group did not appear to be treatment related. As discussed in Section 3.1.3., no females died at the next higher concentration (859 ppm), whereas 4/5 died at 1,102 ppm. Additionally, almost all other mice that died after propylene oxide exposure died on day 1(one mouse died on day 2), but the 387-ppm mouse died on test day 6. Dyspnea was observed in mice at all doses, but no compound-related effects were observed at gross necropsy.

Groups of five male and five female B6C3F$_1$ mice were exposed to propylene oxide at 0, 20, 47, 99, 196 or 487 ppm for 6 h/day, 5 days/week, for 2 weeks (NTP 1985). No mice died. Mice in the 196- and 487-ppm groups exhibited dyspnea, and those in the highest concentration groups were hypoactive.

Groups of 10 male and 10 female B6C3F$_1$ mice were exposed to propylene oxide at 0, 31, 63, 125, 250, or 500 ppm for 6 h/day, 5 days/week, for 13

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weeks (NTP 1985). No mortalities occurred except for one male mouse in the 125-ppm group on day 14. The high-dose groups had lower body weight than controls. Gross or microscopic pathologic evaluation did not reveal any compound-related effects. Signs of toxicity were not stated, so it is unclear whether any were noted or were simply not reported.

In a chronic toxicity and carcinogenicity study, groups of 50 male and 50 female $B6C3F_1$ mice were exposed to propylene oxide at 0, 200, or 400 ppm for 6 h/day, 5 days/week, for 2 years (NTP 1985). High-concentration-group males and females had lower mean body weight (21% and 10% below controls, respectively) and a significantly lower rate of survival compared with controls (58% and 20%, respectively). No treatment-related clinical signs were observed. Exposed mice had an increased incidence of inflammation of the nasal cavity.

Aranyi et al. (1986) exposed female CD1 mice to propylene oxide vapor at 0 or 20 ppm for a single 3-h exposure (135 mice per group) or for five, 3-h exposures (162 mice per group, 3 h/day, 5 days/week). The treated and control mice were then challenged with an aerosol of *Streptococcus zooepidemicus* pneumonia five or six times to determine whether exposure to propylene oxide altered the mice's susceptibility to this infection, and the bactericidal activity of the lungs was assessed by measuring pulmonary bactericidal activity to *Klebsiella pneumoniae*. Exposure to propylene oxide vapor at 20 ppm did not increase the mice's susceptibility to respiratory infection, nor did it reduce the pulmonary bactericidal activity.

3.2.5. Guinea Pigs

Groups of female guinea pigs survived exposure to propylene oxide vapor at nominal air concentrations of 16,000 ppm for 30 min, 8,000 ppm for 1 h, 4,000 ppm for 2 h, or 2,000 ppm for 7 h (see Section 3.1.4; Table 5-12; Rowe et al. 1956). Measured concentrations ranged from 64% to 110% of nominal, averaging 87% of nominal concentrations. The animals were observed for signs of toxicity and mortality for 14 days after exposure. During the exposure, animals exhibited eye and nasal irritation, difficulty breathing, drowsiness, weakness, and occasionally some incoordination.

Rowe et al. (1956) conducted several repeated-exposure experiments for 7 h/day, 5 days/week to various measured concentrations of propylene oxide vapor in male and female guinea pigs. No adverse effects were observed in male or female guinea pigs exposed 11 to 13 times to 457 ppm, 11 to 14 or 128 times to 195 ppm, or 128 times to 102 ppm. Guinea pigs (five of each sex) exposed to 457 ppm for 25 to 27 exposures exhibited a moderate depression in growth, and males had moderate alveolar hemorrhage and edema and interstitial edema and congestion. Females had no histopathologic changes. Guinea pigs (eight of each sex) exposed 110 times to 457 ppm had eye and respiratory passage irritation, slightly depressed growth, and increased average lung weight. Females also had

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slight alveolar hemorrhage and edema, and males had slight fatty liver degeneration.

3.2.6. Rabbits

Albino rabbits (one or two of each sex) exposed to propylene oxide vapor 25 to 27 or 154 times at 457 ppm, 154 times at 195 ppm, or 154 times at 102 ppm for 7 h/day, 5 days/week exhibited no adverse effects (Rowe et al. 1956).

3.3. Developmental and Reproductive Effects

Spermatogenic functions were evaluated in male cynomolgus monkeys after exposure to propylene at 0, 100, or 300 ppm for 7 h/day, 5 days/week, for 24 months (Lynch et al. 1983, abstract). Exposed monkeys had statistically significant decreases in sperm count and sperm motility and an increase in drive range. However, no increases were noted in sperm head abnormalities.

Groups of 25 mated female F344 rats were exposed to propylene oxide vapor at 0, 100, 300, or 500 ppm for 6 h/day during gestation days (GD) 6 to 15 (Harris et al. 1989). Animals were killed on GD 20, and fetuses were removed by cesarean section. Pregnant rats in the 500-ppm group had a significant decrease in body weight accompanied by a significant decrease in food consumption during the exposure period (GD 6 to 15) and during GD 0 to 20. Rats exposed to 300 ppm or less exhibited no signs of maternal toxicity. The only developmental effect was an increased incidence of a seventh cervical rib in fetuses from the 500-ppm group. This fetal variation was believed to be linked to the maternal toxicity observed at that dose.

The potential developmental effects of propylene oxide in rats were investigated in a study by Hackett et al. (1982; also reported by Hardin et al. 1983a). Young adult female Sprague-Dawley rats were exposed to propylene oxide at 500 ppm for either 3 weeks before gestation to GD 16 (43 rats) or on GD 1 to 16 (41 rats) or GD 7 to 16 (44 rats). Control animals (46 rats) were exposed to filtered air. All exposures were conducted for 7 h/day, 5 days/week. Animals received no exposures from GD 17 to 20 and were killed on GD 21. Treated pregnant rats had significantly decreased body weight compared with controls. The minor differences in organ weight in treated groups (decreased absolute liver and spleen weights and increased relative kidney weights) were ascribed to the lower body weight observed in these groups. Although exposure to propylene oxide did not affect the mating performance of the rats, animals exposed during the pregestation period had significantly decreased numbers of corpora lutea per dam, implantations per dam, and live fetuses per litter. Signs of fetal toxicity observed in all exposed groups included a significant decrease in fetal growth (decreased fetal body weight and crown-to-rump length) and an increased incidence of rib dysmorphology (wavy ribs). No major malformations related to exposure were observed.

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Hackett at al. (1982; reported by Hardin et al. 1983a) also investigated the potential developmental effects in New Zealand White rabbits after exposure to propylene oxide vapor at 500 ppm. Groups of artificially inseminated female rabbits were assigned to one of three groups. Group 1 (controls, 17 rabbits) was exposed to filtered air during GD 1 to 19, group 2 (11 rabbits) was exposed to filtered air during GD 1 to 6 and to propylene oxide during GD 7 to 19, and group 3 (19 rabbits) was exposed to propylene oxide during GD 1 to 19. All exposures were for 7 h/day, 5 days/week. Animals were not exposed from GD 20 to 29, and they were killed on GD 30. Effects of propylene oxide exposure in rabbits from GD 1 to 19 and 7 to 19 were limited to decreased food consumption on GD 11 to 15 and 16 to 20 and decreased body weight on GD 15. There were no differences in the number of corpora lutea or implantation sites per dam or in the number of resorptions or dead fetuses per litter of treated rabbits compared with controls. There was also no evidence of developmental toxicity.

To assess reproductive toxicity of propylene oxide, groups of 30 male and 30 female F344 rats were exposed to propylene oxide vapor at 0, 30, 100, or 300 ppm for 6 h/day, 5 days/week, for 14 weeks, and the animals were then mated to generate F_1 litters (Hayes et al. 1988). Next, 30 male and 30 female weanling pups from each group were exposed to propylene oxide vapor for 17 weeks and then mated to produce F_2 litters (sibling mating was avoided). The mean body weights of both F_0 and F_1 adult male and female rats were significantly decreased in the 300-ppm treatment groups, with males having a more pronounced decrease. Decreased body weight was also noted in the 100-ppm group F_0 males and in the 30- and 100-ppm group F_1 males. Despite the toxicity observed in the adults, exposure to propylene oxide vapor at up to 300 ppm did not produce any exposure-related changes in reproductive parameters such as mating, conception, survival indices for litters, litter size, and mean pup weight. In addition, gross and histopathologic examination of pups and adults revealed no changes that could be attributed to exposure.

Nasal, respiratory, and developmental toxicities of propylene oxide were examined by exposing groups of 10 male and 10 female Sprague-Dawley rats to propylene oxide vapor by inhalation at a concentration of 0, 125, 500, or 1,000 ppm for 6 h/day, 7 days/week, during a 5- to 6-week period, including premating (2 weeks), mating (2 weeks), and postmating (males; 2 weeks) or gestation (females; GD 0 to 19) (Okuda et al. 2006). The females were allowed to deliver naturally. Dams and pups were killed on postnatal day 4, and males were killed after the postmating period. Effects in males and females exposed to propylene oxide at 1,000 ppm included reduced survival (three males and four females died or were sacrificed moribund; death was from respiratory failure), ataxic gait, reduced body weight starting on day 7 of the premating period that continued throughout the study, inflammation and alveolar macrophage aggregates in the lung, and lesions in the upper and lower respiratory tract—particularly in the respiratory and olfactory mucosa of the nasal cavity (respiratory epithelium showed regeneration with replacement squamous epithelium or that migrated to the cuboidal epithelium; olfactory epithelium exhibited necrosis, atrophy, and

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regeneration). Effects in the 500-ppm group included reduced body weight in males and similar nasal cavity and pulmonary histopathologic lesions in males and females, although the incidences and severities of the lesions were reduced compared with those observed at 1,000 ppm. The only effect observed in rats exposed to propylene oxide at 250 ppm was slight atrophy of the olfactory epithelium in the nasal cavity in 5/10 male rats. Reproductive toxicity was also observed at exposure to propylene oxide at 1,000 ppm. Females in the high-concentration group had a decreased fertility index (78% versus 100% for controls): one female did not copulate and two were not pregnant. Although the pregnant females in the 1,000-ppm group had a comparable number of corpora lutea and only a slightly decreased number of implantations (12.1 versus 14.1 for controls), no pup was born to any of the high-concentration group dams. Whether it was the result of early or late intrauterine death was not stated. Males exposed to propylene oxide at 1,000 ppm for 6 weeks had significantly increased incidences of germ cell necrosis in the seminiferous tubule, decreased sperm and debris of spermatic elements in the epididymis, and decreased serum testosterone with a concomitant increase in luteinizing hormone and follicle-stimulating hormone. Reproductive toxicity was not noted in males or females in any of the other exposure groups.

Okuda et al. (2006) conducted an additional inhalation experiment to further examine developmental toxicity. Groups of five pregnant Sprague-Dawley rats were exposed to propylene oxide vapor at 0, 125, 250, 500, 750, or 1,000 ppm for 6 h/day during GD 6 to 19. Dams were sacrificed on GD 20 and pups were delivered by cesarean section. The numbers of live and dead fetuses were recorded, and live fetuses were weighed, sexed, and examined for external, skeletal, and visceral abnormalities. Exposure to 750 and 1,000 ppm resulted in a concentration-related decrease in mean maternal body weight on GD 13 (–9% and –11% of controls, respectively) and GD 20 (–18% and –20%, respectively). Fetal effects observed at 750 and 1,000 ppm included a concentration-related decrease in fetal body weight (~–22% and –26% of controls, respectively) and a reduced number of ossified sacral-caudal vertebrae (6.1 and 5.6, respectively, versus 7.6 for controls). No maternal or developmental effects were observed at propylene oxide exposure concentrations up to 500 ppm.

3.4. Genotoxicity

3.4.1. In Vitro

Mutagenicity tests have revealed that propylene oxide is a direct-acting mutagen that causes base-pair substitutions. Short-term in vitro mutagenicity assays of propylene oxide have revealed positive results in *Salmonella typhimurium* strains TA1535 and TA100 with and without metabolic activation and to *Bacillus subtilis* and *Escherichia coli* WP2 *uvrA*. Propylene oxide also induced forward mutations in Chinese hamster ovary cells. Negative results were

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found in *S. typhimurium* strains TA98, TA1537, and TA1538 and in T2 bacteriophage. Propylene oxide tested positive in a number of cytogenic assays, including inducing chromosomal aberrations in cultured dividing human lymphocytes, a rat epithelial cell line, and cultured rat liver cells; increasing in a dose-dependent manner the amount of chromatid damage and number of chromatid gaps in flask cultured cells; and inducing chromosomal aberrations and sister chromatid exchanges in Chinese hamster ovary cells with and without metabolic activation (reviewed by Giri 1992; IARC 1994; Meylan et al. 1986).

3.4.2. In Vivo

In vivo genotoxicity studies have given equivocal results. Chromosomal aberrations and sister chromatid exchanges were not found in lymphocytes from male cynomolgus monkeys exposed to air containing propylene oxide at 100 or 300 ppm for 7 h/day, 5 days/week, for 2 years (Lynch et al. 1984b). Male mice gavaged twice with propylene oxide at 100 to mg/kg (mg/kg) of body weight did not show an increased incidence of micronucleated, polychromatic erythrocytes in bone marrow; although two intraperitoneal injections of 300 mg/kg did produce an increase, lower doses did not produce that effect (Bootman et al. 1979). Sex-linked recessive lethal mutations in *Drosophila* were induced after a 24-h vapor exposure to propylene oxide at 645 ppm. Rat dominant-lethal and mouse sperm-head morphology assays, in which male rats and mice were exposed to propylene oxide vapor at 300 ppm for 7 h/day on 5 consecutive days, did not produce positive results (Hardin et al. 1983b).

3.4.3. DNA and Hemoglobin Alkylation

Propylene oxide is a direct alkylating agent that covalently binds to DNA and proteins by introducing a 2-hydroxypropyl group. When propylene oxide underwent reaction in vitro with calf thymus DNA at 37°C for 4 h at pH 7.4 at a concentration of 0.2 millimole (mmol) of propylene oxide per mg of DNA, the order of deoxynucleoside activity was found to be deoxyguanosine (46%) > deoxyadenosine (38%) > deoxycytosine (24%) > deoxythymidine (15%) (Djuric et al. 1986). Solomon et al. (1988) characterized and quantified the 2-hydroxypropyl DNA adducts formed after in vitro incubation of propylene oxide with calf thymus DNA at 37°C for 10 h at pH 7.0 to 7.5 (100 mmol of propylene oxide per 150 mg of DNA). The adducts were N^7-guanine (133 nmol per mg of DNA), N^3-adenine (14 nmol per mg of DNA), N^3-uridine (13 nmol per mg of DNA), and N^6-uridine (1 nmol per mg of DNA).

In an experiment assessing in vivo propylene oxide binding, male CBA mice were injected intraperitoneally with [^{14}C]propylene oxide (Svensson et al. 1991). Three and 10 h after injection, the N^7-(2-hydroxypropyl)guanine (7-HPG) adduct was found in DNA from a number of organs, primarily in the liver and spleen (liver > spleen > kidney > testis > lung). DNA adduction in the respiratory

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mucosa was assessed in groups of three male F344 rats exposed by nose only to [^{3}H]propylene oxide vapor at 6, 12, 18, 28 , or 46 ppm for ~2 h (until the rats had inhaled ~20 liters of air) (Snyder and Solomon 1993). At 48 ppm, DNA binding was greatest in the nasal mucosa (17 × 10^6 adducts per base), followed by the trachea (5.8 × 10^6 adducts per base) and lung (3.3 × 10^6 adducts per base). The persistence of the adducts after exposure to 19.5 ppm for 2 h was measured and revealed little clearance of the radiolabeled DNA from lungs and trachea over 10 days. The nasal mucosa showed biexponential clearance; the clearance half-lives of the DNA adducts during the rapid and slow phases were 8 h and 5.3 days, respectively. The authors surmised that the rapid phase may be due to depurination of the major adducts (7-HPG and N^3-(2-hydroxypropyl)adenine), while the slower phase may represent cell turnover in the rat nose.

Ríos-Blanco et al. (1997) measured guanine adducts in the nasal and hepatic tissue of male F344 rats sampled 7 h or 3 days after exposure to propylene oxide vapor at 0 or 500 ppm for 6 h/day, 5 days/week, for 4 weeks. Guanine adducts were measured by ^{32}P-postlabeling and by gas chromatography–high-resolution mass spectrometry. Distribution of the 7-HPG adduct was nasal respiratory tissue > nasal olfactory tissue > hepatic tissue in both of the exposed groups, with the number of nasal tissue adducts being 21 to 24 times greater than the number of adducts in hepatic tissue. Both respiratory and hepatic tissue exhibited similar disappearance rates for adducts when measured 3 days after exposure ceased. In a later study, Ríos-Blanco et al. (2000) also measured DNA adduction levels (7-HPG adducts) in additional tissues from male F344 rats exposed to propylene oxide at 500 ppm for 4 weeks (6 h/day, 5 days/week). Tissues were again sampled 7 h or 3 days after the end of the last exposure, and 7-HPG was quantified by gas chromatography–high-resolution mass spectrometry. The distribution of 7-HPG adducts was nasal respiratory tissue > nasal olfactory tissue > lung > spleen > liver > testis. The actual amounts of 7-HPG (picomoles per micromole [pmol/µmol] of guanine) at 7 h postexposure were 606.2, 297.5, 69.8, 43.0, 27.5, and 14.2, respectively; at 3 days postexposure they were 393.3, 222.7, 51.5, 26.7, 18.0, and 10.4, respectively. The lungs formed only ~12% of the number of adducts measured in the nasal respiratory epithelium. Similar disappearance rates of the adduct were again noted in all tissues. It appears a similar study was also conducted by Segerbäck et al. (1998) using the identical exposure protocol but quantifying 7-HPG adducts by using the ^{32}P-postlabeling assay. The adduct levels (adducts per 10^6 nucleotides) at 7 h postexposure were respiratory nasal epithelium (98.1) > olfactory nasal epithelium (58.5) > lung (16.3) > lymphocytes (9.92) ≥ spleen (9.26) > liver (4.64) > testis (2.95). By 3 days postexposure, the adduct levels had generally decreased to approximately one-quarter of the 7-h postexposure values. Segerbäck et al. (1998) concluded that the degree of loss corresponds to the spontaneous rate of depurination for this adduct and suggests a low efficiency of repair for 7-HPG in the rat.

To assess concentration-dependent accumulation of 7-HPG in the nasal respiratory epithelium, lungs, and liver, groups of five male F344 rats were exposed to propylene oxide by inhalation of 0, 5, 25, 50, 300 or 500 ppm for 6

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h/day, 5 days/week, for 3 or 20 days (Ríos-Blanco et al. 2003a). 7-HPG was quantified by gas chromatography–high-resolution mass spectrometry. A linear increase in the accumulation of 7-HPG in DNA was observed for all three tissues after exposure to propylene oxide for 3 days and for the nasal respiratory epithelium and lung after 20 days of propylene oxide exposure, while the liver exhibited a sublinear accumulation of 7-HPG after 20 days of propylene oxide exposure. Increased binding was present in nasal respiratory epithelium at the lowest concentration of 5 ppm. Overall, the nasal respiratory epithelium contained the highest concentrations of 7-HPG, with concentrations 6- and 13-fold greater than in lung and liver, respectively.

Propylene oxide reacts with hemoglobin to form histidine, cysteine, and N-terminal valine adducts. In an experiment assessing in vivo propylene oxide binding, male CBA mice were intraperitoneally injected with 0.065, 0.10, or 0.19 mmol of [^{14}C] propylene oxide per kg of body weight (Svensson et al. 1991). Propylene oxide formed hemoglobin adducts at the terminal valine and histidine nitrogens. The N-terminal valine adducts followed a dose-related linear increase. Farmer et al. (1982) reported that the hydroxypropylhistidine adduct of hemoglobin showed a linear concentration response over an inhaled propylene oxide vapor concentration range of 0 to 2,000 ppm in female LAC Porton-derived Wistar rats. Segerbäck et al. (1992, 1994) also found a good correlation between the inhaled and intraperitoneally administered dose of propylene oxide and adduction of hemoglobin at the N-terminal valine in $B6C3F_1$ mice, F344 rats, and beagle dogs. The dose of propylene oxide in blood (as measured by hemoglobin adduction) did not appear to depend on the route of administration, as blood levels were similar whether propylene oxide was administered by injection or by inhalation, and did not vary greatly among species. Alkylation efficiency increased slightly with increasing body weight (the levels of hemoglobin adduction in the dog were 2.9-fold greater than in the mouse); the difference was not large enough to be accounted for by surface area-based extrapolation. To assess concentration-dependent accumulation of *N*-(2-hydroxypropyl)valine in hemoglobin, groups of male F344 rats were exposed to propylene oxide by inhalation of 0, 5, 25, 50, 300, or 500 ppm for 6 h/day, 5 days/week, for 3 or 20 days (Ríos-Blanco et al. 2000). Animals were killed 5 h after exposure. Accumulation of the *N*-(2-hydroxypropyl)valine adduct exhibited a linear concentration response in rats exposed to propylene oxide for 3 days, but the response was slightly sublinear in rats exposed to propylene oxide for 20 days.

3.5. Carcinogenicity

Propylene oxide is classified as a Group 2B carcinogen (possibly carcinogenic to humans) by IARC (1994) and as B2 (probable human carcinogen) by the EPA (1994). These classifications are based on inadequate evidence in humans and sufficient evidence in animals. Propylene oxide appears to cause cancer in animals at the site of contact. Intragastric administration of propylene ox-

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ide to Sprague-Dawley rats resulted in tumors of the forestomach, and subcutaneous injections in rats generated sarcomas at the injection site (Walpole 1958; Dunkelberg 1982). Inhalation studies with propylene oxide have demonstrated carcinogenic activity in mice and rats based on nasal cavity tumors. The inhalation carcinogenicity studies are summarized in Table 5-13.

No tumors were observed when 12-week-old male Sprague-Dawley rats were exposed to propylene oxide at 433 or 864 ppm for 30 days or to 1,724 ppm for 8 days (exposures were for 6 h/day, 5 days/week) and allowed to die naturally (Sellakumar et al. 1987).

The NTP (1985; also reported by Renne et al. 1986) reported clear evidence of carcinogenicity of propylene oxide in C57CL/6 × C3H mice exposed to 400 ppm for 6 h/day, 5 days/week, for 103 weeks based on statistically significant increases in the incidence of nasal submucosa hemangiomas and hemangiosarcomas in male and female mice (see Table 5-13). No evidence of carcinogenicity was found at 200 ppm. Nonneoplastic effects of propylene oxide on the nasal turbinates of mice included acute and chronic inflammation (incidence at 0, 200, and 400 ppm; 50 animals per group examined; males: 1, 14, and 38, respectively; females: 0, 14, and 18, respectively), suppurative inflammation (males: 0, 8, and 4, respectively; females: 0, 16, and 23, respectively), and serous inflammation (males: 0, 13, and 2, respectively; females: 2, 6, and 2, respectively). F344/N rats exposed to propylene oxide at 400 ppm had an increased incidence of nasal epithelial papillary adenomas (statistical significance not achieved; see Table 5-19), indicating some evidence of carcinogenicity. No evidence of carcinogenicity was found at 200 ppm. Nonneoplastic effects of propylene oxide on the nasal turbinates of rats included suppurative inflammation (incidence at 0, 200, and 400 ppm; 50 animals per group examined; males: 9, 21, and 38, respectively; females: 3, 5, and 23, respectively), epithelial hyperplasia (males: 0, 1, and 11, respectively; females: 1, 0, and 5, respectively), and squamous metaplasia (males: 1, 3, and 21, respectively; females: 1, 2, and 11, respectively).

Groups of 80 male F344/N rats exposed to propylene oxide vapor at 100 or 300 ppm for 104 weeks had a statistically significant increase in the incidence of adrenal pheochromocytomas and a concentration-dependent increase in nasal epithelial hyperplasia, although the latter was probably influenced by a *Mycoplasma pulmonis* infection that occurred toward the beginning of the study (Lynch et al. 1984a). Kuper et al. (1988) exposed four groups of 100 Wistar rats of each sex to propylene oxide vapor at 0, 30, 100, or 300 ppm for 28 months. Ten rats per sex per group were killed at 12, 18, and 24 months to provide interim data; the survivors were killed at study termination. Male and female rats from all exposed groups showed degenerative and hyperplastic changes in the nasal mucosa. Although increased incidences of adenocarcinomas and fibroadenomas of the mammary glands were found in females exposed to 300 ppm, mammary tumors have not been observed in any other animals treated with propylene oxide in other studies.

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TABLE 5-13 Inhalation Exposure to Propylene Oxide: Summary of Carcinogenicity Studies

Animal Description				Tissue and Tumor Type		
Species and Strain	Sex	Number per Group	Exposure Protocol	Response	Incidence[a]	Reference
Mouse: C57BL/6 × C3H	M	50	0, 200, 400 ppm 6 h/d, 5 d/wk, 103 wk	Nasal cavity: Hemangioma Hemangiosarcoma Hemangioma and hemangiosarcoma	0/50, 0/50, 5/50* 0/50, 0/50, 5/50* 0/50, 0/50, 10/50*	NTP 1985
				Nasal cavity: Squamous cell carcinoma, papilloma	0/50, 0/50, 2/50	
	F	50	0, 200, 400 ppm 6 h/d, 5 d/wk, 103 wk	Nasal cavity: Hemangioma Hemangiosarcoma Hemangioma and hemangiosarcoma	0/50, 0/50, 3/50 0/50, 0/50, 2/50 0/50, 0/50, 5/50*	
				Nasal cavity: Adenocarcinoma	0/50, 0/50, 2/50	
Rat: F344/N	M	50	0, 200, 400 ppm 6 h/d, 5 d/wk, 103 wk	Nasal cavity: Papillary adenoma	0/50, 0/50, 2/50	NTP 1985
	F	50	0, 200, 400 ppm 6 h/d, 5 d/wk, 103 wk	Nasal cavity: Papillary adenoma	0/50, 0/50, 3/50**	
Rat: F344/N	M	80	0, 100, 300 ppm 7 h/d, 5 d/wk, 104 wk	Adrenal glands: Pheochromocytoma	8/78, 25/78,* 22/80*	Lynch et al. 1984a
Rat: Wistar	F	70	0, 30, 100, 300 ppm 6 h/d, 5 d/wk, 124 wk	Mammary glands: Adenocarcinoma	3/69, 6/71, 5/69, 8/70*	Kuper et al. 1988
				Mammary glands: Fibroadenoma	32/69, 30/71, 39/69, 47/70*	
Rat: Sprague–Dawley	M	50	0, 435 ppm 6 h/d, 5 d/wk, 30 d;	No tumors	N/A	Sellakumar et al. 1987
			1,740 ppm 6 h/d, 5 d/wk, 8 d	No tumors	N/A	

[a]Incidence presented in order of exposure groups as shown in "Exposure Protocol" column.
*$p < 0.05$ compared with controls.
**$p < 0.05$ by trend test.
Abbreviations: M, male; F, female; N/A, not applicable.

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3.6. Summary

Summaries of acute lethality data in laboratory animals are presented in Tables 5-14 and 5-15. Mice were the most likely to die after propylene oxide exposure, followed by dogs and rats. Four-hour inhalation LC_{50} values for propylene oxide vapor were 1,160 to 1,740 ppm for mice, 1,941 ppm for dogs, and 3,205 to 4,197 ppm for rats. General signs of toxicity after acute exposure to propylene oxide vapor in dogs, rats, mice, and guinea pigs included nasal discharge (clear or bloody), lacrimation, salivation, gasping, lethargy and hypoactivity, weakness, and incoordination (see Table 5-16). Many of these signs increased in severity with increasing concentration and duration of exposure. Necropsy of exposed animals revealed limited findings: pulmonary congestion and edema were found in dogs, but in mice and rats only distended stomachs or no gross pathologic changes were observed. Repeated exposure in rats resulted in similar but generally reversible signs of toxicity. Necropsy revealed pathologic changes in the nasal cavity, including degenerative and hyperplastic effects, concentration-related suppurative inflammation of respiratory epithelium, and metaplasia. Studies assessing the potential neurotoxicity of propylene oxide after chronic exposure reported effects at higher doses in rats but not at lower doses in rats or monkeys. Some of the clinical signs observed in acute and repeated-exposure studies—such as lethargy, hypoactivity, weakness, incoordination, and hindlimb ataxia—are suggestive of nervous system effects.

Propylene oxide vapor exposure in male monkeys resulted in decreased sperm counts and motility, and in male rats it resulted in increased incidences of germ cell necrosis in the seminiferous tubule, decreased sperm and debris of spermatic elements in the epididymis, and decreased serum testosterone levels with a concomitant increase in luteinizing hormone and follicle-stimulating hormone. No reproductive effects were observed in rabbits. Developmental effects were limited to decreased fetal growth and increased incidence of rib dysmorphology or a reduced number of ossified sacral-caudal vertebrae in rats.

Propylene oxide is a direct alkylating agent that covalently binds to DNA and proteins. Consequently, it has generally tested positive for mutagenicity in in vitro tests. Equivocal mutagenicity results have been obtained in in vivo test systems. Data on potential carcinogenicity in animals is considered adequate for establishing propylene oxide as a carcinogen. Exposure to propylene oxide vapor has induced nasal cavity tumors in mice and rats.

4. SPECIAL CONSIDERATIONS

4. 1. Metabolism and Disposition

Much of the in vitro metabolism of propylene oxide has been summarized by IARC (1994). The two primary pathways of metabolism appear to be hydrolysis to propylene glycol via epoxide hydrolase (EH) and glutathione conju-

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gation by glutathione *S*-transferase (GST); glutathione conjugation can also occur nonenzymatically. Propylene glycol may be excreted or further metabolized to lactic and pyruvic acids. These metabolic pathways represent detoxification pathways, as propylene oxide is a direct alkylating agent.

Faller et al. (2001) investigated the kinetics of GST and EH in liver and lung cytosolic and microsomal fractions at 37°C in male B6C3F$_1$ mice, F344 rats, and humans (Table 5-17). The measurement maximum velocity/Michaelis constant indicated microsomal EH in human liver and lung had a greater capacity for propylene oxide metabolism compared with mice and rats, while human lung GST activity was much lower than that of the mouse but greater than that of the rat.

To assess the pharmacokinetics of the chemical, two male Sprague-Dawley rats were exposed to unspecified concentrations of propylene oxide for an unspecified time (Golka et al. 1989; discussed by IARC 1994). Rats exposed to air containing propylene oxide at up to 3,000 ppm did not exhibit saturation kinetics, with 96% of the inhaled propylene oxide being metabolized and only 3% being exhaled unchanged. The transfer rate of propylene oxide from the chamber to the body of one rat (uptake clearance) was calculated as 75 milliliters (mL)/min, representing 64% of alveolar ventilation. Inhaled propylene oxide was rapidly eliminated from the rat. Animals exposed to concentrations greater than 3,000 ppm experienced acute toxic effects (effects not described).

TABLE 5-14 Summary of Propylene Oxide 4-Hour Inhalation LC$_{50}$ Data in Laboratory Animals

Species	Concentration (ppm)	LC$_{50}$ Method of Calculation	Reference
Dog	1,941	Probit analysis (with caution: 2 of 3 dogs in high-dose group were dead before removal from chamber; calculated for the purpose of this technical document)	Jacobson et al. 1956
Rat	4,000	Bliss-Finney method	Jacobson et al. 1956
Rat	3,205	Probit analysis (calculated for the purpose of this technical document)	NTP 1985
Rat	4,197	Not given	Shell Oil Co. 1977
Mouse	1,740	Bliss-Finney method	Jacobson et al. 1956
Mouse	1,160	Probit analysis (calculated for the purpose of this technical document)	NTP 1985

Abbreviation: LC$_{50}$, concentration with 50% lethality.

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TABLE 5-15 Summary of Propylene Oxide Acute Lethal Inhalation Data in Laboratory Animals

Species	Concentration (ppm)	Duration (h)	Mortality and Other Effects	Reference
Dog	2,005 1,363	4	Lowest concentration causing death (1/3) No mortality	Jacobson et al. 1956
Rat	4,000	4	Killed 6/6	Smyth and Carpenter 1948
Rat	4,000	4	Killed 4/6	Weil et al. 1963
Rat	3,448 2,684	4	Lowest concentration causing death (3/10) No mortality	Jacobson et al. 1956
Rat	16,000	0.50.25	Death (10/10)No mortality (0/10)	Rowe et al. 1956
Rat	8,000	0.5	Longest duration causing smallest number of deaths (2/10); no mortality at 0.25 h of exposure.	Rowe et al. 1956
Rat	4,000	2.0	Longest duration causing smallest number of deaths (4/10); no mortality at 1 h exposure	Rowe et al. 1956
Rat	2,970 1,277	4	Lowest concentration causing death (M, 1/5; F, 2/5) No mortality at 1,277 ppm	NTP 1985
Rat (M)	4,280 4,050	4	Lowest concentration causing death (2/4) No mortality	Shell Oil Co. 1977
Rat (F)	4,050 3,450	4	Lowest concentration causing death (3/4) No mortality	Shell Oil Co. 1977
Mouse	945	4	Lowest concentration causing death (lowest concentration tested)	Jacobson et al. 1956

(Continued)

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TABLE 5-15 Continued

Species	Concentration (ppm)	Duration (h)	Mortality and Other Effects	Reference
Mouse (M)	1,102 387	4	Lowest concentration resulting in death (2/5) No mortality	NTP 1985
Mouse (F)	387 859 1,102	4	Death not related to exposure in 1/5 No death: 0/5 Death: 4/5	NTP 1985
Guinea pig	16,000	1 0.5	Death: 5/5 No death: 0/5	Rowe et al. 1956
Guinea pig	8,000	2	Longest duration at this concentration resulting in smallest number of deaths (1/5); no mortality at 1 h of exposure.	Rowe et al. 1956
Guinea pig	4,000	4	Longest duration at this concentration resulting in smallest number of deaths (1/5); no mortality at 2 h of exposure.	Rowe et al. 1956

Abbreviations: M, male; F, female.

TABLE 5-16 Summary of Propylene Oxide Nonlethal Inhalation Data in Laboratory Animals

Species	Concentration (ppm)	Duration	Effects	References
Monkey	100 300	6 h/d, 5 d/wk for 2 y	Investigated potential neuropathologic effect; only observable effect signs of axonal dystrophy in medulla oblongata	Sprinz et al. 1982
Monkey	100 300	6 h/d, 5 d/wk for 2 y	No neurophysiologic or neuropathologic changes noted	Setzer et al. 1997
Monkey	100 300	7 h/d, 5 d/wk for 2 y	In vivo genotoxicity: no chromosomal aberrations or sister chromatid exchanges found in lymphocytesSpermatogenic functions: 9 sperm count and sperm motility, increase in drive range; no sperm head abnormalities	Lynch et al. 1983, 1984b

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Monkey	102 195 457	154 times for 7 h/d, 5 d/wk	No adverse effects	Rowe et al. 1956
Dog	1,363	4 h	Highest concentration causing no mortality Lacrimation, salivation, nasal discharge	Jacobson et al. 1956
Rat	2,684	4 h	Highest concentration causing no mortality Frequent movement and preening, nasal discharge, lacrimation, salivation, gasping	Jacobson et al. 1956
Rat	1,277	4 h	No mortality, no clinical signs or gross pathology changes	NTP 1985
Rat (M)	4,050	4 h	Highest concentration causing no mortality Lacrimation, eye irritation, sedation, piloerection, mucous discharge from nose and mouth, respiratory difficulty	Shell Oil Co. 1977
Rat (F)	3,450	4 h	Highest concentration causing no mortality Lacrimation, eye irritation, sedation, piloerection, mucous discharge from nose and mouth, respiratory difficulty	Shell Oil Co. 1977
Rat	600	6 h/d, 5 d/wk	Transient restless behavior observed only during first 3 days of exposure; occasional salivation and piloerection noted	Dow Chemical Company 1981
Mouse (M)	859	4 h	Highest concentration causing no mortality; dyspnea; no compound-related effects at gross necropsy	NTP 1985
Mouse (F)	387	4 h	1/5 died (not treatment related); dyspnea; no compound-related effects at gross necropsy	NTP 1985
	859	4 h	No mortality; dyspnea; no compound-related effects at gross necropsy	
Guinea pig	16,000 8,000 4,000 2,000	30 min 1 h 2 h 7 h	Highest concentrations and longest durations causing no mortality Signs of toxicity in all groups: eye and nasal irritation, breathing difficulty, drowsiness, weakness	Rowe et al. 1956

Abbreviations: M, male; F, female.

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TABLE 5-17 In Vitro Metabolism of Propylene Oxide by Subcellular Fractions of Mouse, Rat, and Human Lung and Liver Tissue

		Cytosolic Glutathione *S*-Transferase			Microsomal Epoxide Hydrolase		
Tissue	Species	V_{max}	K_m	V_{max}/K_m	V_{max}	K_m	V_{max}/K_m
Liver	Mouse	N/A	N/A	33 ± 1.7	16 ± 3.0	3.7 ± 0.77	4.4 ± 1.2
	Rat	N/A	N/A	13 ± 0.8	13 ± 1.3	1.3 ± 0.16	9.9 ± 1.6
	Human A	24 ± 1.7	1.8 ± 0.16	13 ± 1.5	80 ± 3.2	2.1 ±0.11	38 ±2.5
	Human B	21 ± 3.5	1.8 ± 0.37	12 ± 3.1	46 ± 5.6	2.5 ± 0.39	19 ± 3.8
Lung	Mouse	395 ± 33	3.7 ± 0.45	106 ± 16	7.1 ± 1.6	0.84 ± 0.22	8.5 ± 2.9
	Rat	N/A	N/A	14 ± 1.6	N/A	N/A	5.5 ±1.4
	Human C	N/A	N/A	51 ± 11	35 ± 4.2	1.1 ± 0.16	31 ±5.8
	Human D	62	3.0	21 ± 14	38 ± 9.0	0.83 ± 0.23	46 ± 17

Abbreviations: V_{max}, maximum velocity; K_m, Michaelis constant; N/A, not applicable.
Source: Faller et al. 2001. Reprinted with permission; copyright 2001, *Toxicology and Applied Pharmacology*.

Male F344/N rats exposed by nose only to propylene oxide vapor at 14 ppm for 60 min showed increasing blood propylene oxide concentrations during the first 10 min of exposure, with concentrations leveling off at 3 nanograms per g of blood for the remainder of the exposure (Maples and Dahl 1993). Blood propylene oxide measurements were not made after exposure ceased.

Male Wistar/Lewis rats were exposed to propylene oxide vapor at 80, 143, 217, 283, 625, or 904 ppm for 6 h (Nolan et al. 1980). Low propylene oxide blood concentrations were found in all exposed animals, suggesting that propylene oxide is rapidly absorbed and metabolized by the rat. A disproportionate increase in propylene oxide blood levels after 143 ppm indicated nonlinear kinetics. A concentration-dependent depression of hepatic nonprotein sulfhydryls (NPSH) was observed in exposed animals, indicating that glutathione conjugation was a major detoxification reaction. Levels were maximally depressed in rats exposed to 625 or 904 ppm, while no depression was seen in the 80-ppm group. NPSH measured in the lungs, kidneys, and blood from the 625-ppm group revealed depressed levels in the lungs and kidneys but not in the blood. Ríos-Blanco et al. (1997) reported that rats exposed to propylene oxide exhibited a 60% decrease in NPSH in nasal tissue (further details not provided).

Uptake efficiency of inhaled propylene oxide and the resultant depletion of NPSH in the upper respiratory tract (URT) were investigated in groups of male F344 rats with surgically isolated URTs (Morris et al. 2004). Respiratory

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and olfactory nasal NPSH contents were measured in the rats immediately after exposure in a nose-only inhalation chamber for 1 h to propylene oxide at 0, 50, 100, 300, or 500 ppm. Respiratory nasal mucosal NPSH content decreased ($p < 0.05$) to 70%, 45%, 35%, and 15% of control values, respectively, while decreases in olfactory mucosal NPSH content did not follow a clear concentration response. Uptake efficiency in the URT was assessed during exposure to propylene oxide for 1 h at 0, 25, 50, 100, or 300 ppm at a flow rate of 50 mL/min (about one-half of the predicted minute volume) or 200 mL/min (about twice the predicted minute volume). Uptake efficiency remained stable during exposure and was similar at all concentrations at the same flow rate. Greater uptake efficiency was noted at the lower flow rate of 50 mL/min (~25%) compared with the flow rate of 200 mL/min (~11%), but the delivered dose at each concentration at the flow rate of 200 mL/min was greater than that at a flow rate of 50 mL/min. NPSH content in respiratory nasal mucosa was similar to that already measured. Olfactory mucosal NPSH content again did not differ significantly from the control value. With the results of this investigation, the authors concluded that highly efficient uptake by the rat nose is unlikely, and uptake by the human nose is even less likely because of the simpler structure of the human nose compared with that of the rat. While NPSH depletion was fairly extensive with up to an 85% loss compared with controls, propylene oxide conjugation with GSH represents only a small fraction of the total amount of propylene oxide absorbed. A total of 3,000 to 5,400 nmol was absorbed over the 1-h exposure to propylene oxide, while the NPSH content in the respiratory mucosa is estimated to be ~80 nmol. The steady-state uptake efficiency of propylene oxide in the nose is probably maintained by clearance of propylene oxide by the circulation.

Uptake efficiency of inhaled propylene oxide and the resultant depletion of NPSH in the URT were next investigated in groups of male $B6C3F_1$ mice using the same protocol as that used by Morris et al. (2004) in rats (Morris and Pottenger 2006). The mice with surgically isolated URTs were exposed to propylene oxide in a nose-only inhalation chamber for 1 h at 0, 25, 50, 100, 300, or 500 ppm at a flow rate of 12 mL/min (about one-half of the predicted minute volume) or 50 mL/min (about twice the predicted minute volume). In contrast with the rats, flow rate had no effect on respiratory or nasal olfactory mucosal NPSH levels at each concentration in mice. NPSH levels were significantly depleted at 300 and 500 ppm in respiratory mucosa (51% and 31% of controls, respectively) and olfactory mucosa (63% and 48% of controls, respectively) but were not statistically different at 25, 50, or 100 ppm compared with controls. URT uptake efficiency differed with exposure concentration (the efficiency at 300 ppm and a flow rate of 12 mL/min being 26% of the inspired concentration versus 30% for controls and the efficiency at 300 and 500 ppm and a flow rate of 50 mL/min being 11% and 12%, respectively, versus 16% for controls), suggesting saturation of an uptake pathway.

Groups of male F344N rats were exposed to propylene oxide by three different exposure protocols to investigate the resultant propylene oxide blood con-

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centrations and NPSH levels in respiratory nasal mucosa (RNM), lung, blood, and liver (Lee et al. 2005). In one protocol, rats had a single 6-h exposure to propylene oxide at 0, 10, 25, 50, 100, 150, 300, 500, 625, or 750 ppm. In another protocol, they were exposed to propylene oxide at 0, 50 or 100 ppm for 6 h/day for 1 to 5 days. Third, rats were exposed to propylene oxide at 0, 5, 25, 50, 300, or 500 ppm for 6 h/day for 3 days or for 6 h/day, 5 days/week, for 4 weeks. After a single 6-h exposure, blood propylene oxide concentrations increased linearly with the exposure concentration. Daily 6-h exposures for 3 days or 4 weeks (5 days/week) resulted in a linear increase in propylene oxide blood concentration only up to 300 ppm, with a steeper slope observed between exposure concentrations of 300 and 500 ppm. The repeated exposures to propylene oxide at ≤300 ppm resulted in lower propylene oxide blood levels than after a single exposure, suggesting possible induction of metabolic elimination of propylene oxide. Repeated exposure to 500 ppm for 3 days resulted in a similar blood concentration produced by a single exposure, while exposure to 500 ppm for 4 weeks resulted in propylene oxide blood concentrations ~31% higher. The increase in propylene oxide blood levels at 500 ppm is likely the result of glutathione (GSH) depletion in the liver. The time course of blood accumulation of propylene oxide followed first-order kinetics with a $t_{1/2}$ of 59 min. A similar half-life was observed when propylene oxide was added directly to fresh blood samples ($t_{1/2}$ of 54 min). NPSH levels in RNM after a single 6-h exposure decreased sharply with increasing propylene oxide exposure concentrations: exposure to propylene oxide at 50 ppm and ≥300 ppm resulted in NPSH levels that were ~43% and ~16% of control values, respectively. The decrease in NPSH levels followed a nonlinear curve similar to a hyperbola. The decreases in lung NPSH levels were more moderate in lung tissue, with the concentration response being almost linear. NPSH levels were ~20% of control values after exposure to propylene oxide at 750 ppm. The liver NPSH levels followed a nonlinear decrease. The loss of NPSH in the liver at lower propylene oxide exposure concentrations was less pronounced than that produced in the RNM but was comparable at higher propylene oxide concentrations (liver NPSH levels with propylene oxide at 300 ppm were ~16% of control values). Repeated exposure to propylene oxide generally did not affect the extent of NPSH depletion in the RNM, lung, or liver. Overall, the RNM tissue exhibited the most severe and most distinct concentration-dependent depletion of NPSH. The authors proposed that the extensive depletion is likely due to the high propylene oxide burden of the nasal mucosa being in direct contact with propylene oxide in the inhaled air and the high GST activity in this tissue.

4.2. Mechanism of Toxicity

Propylene oxide is a direct alkylating agent that has been shown to alkylate proteins and DNA. In addition, it has irritant properties, such as inducing lacrimation and mucous discharge. Much of the toxicologic evidence suggests

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that propylene oxide reacts at the site of entry. Because rodents are obligate nose breathers, upper respiratory tract damage was produced after inhalation exposure to propylene oxide. Acute inhalation exposure in laboratory rodents resulted in dyspnea, gasping, and mucous discharge from the nose and mouth (Jacobson et al. 1956; Shell Oil Company 1977; NTP 1985). Postmortem examinations of these animals either did not reveal any remarkable findings or revealed only a distended stomach, correlating with a gasping attempt to breathe by obligate nose breathers. Findings from repeat inhalation exposure studies in rodents revealed upper respiratory tract lesions, such as rhinitis and squamous metaplasia, hyperplasia, necrosis, and suppurative inflammation of the upper respiratory tract epithelium (Shell Oil Company 1977; Dow Chemical Company 1981; NTP 1985; Kuper et al. 1988; Eldridge et al. 1995). Only one study reported postmortem findings after repeated inhalation exposure to propylene oxide indicative of lower respiratory tract damage (Rowe et al. 1956). Some of the rats and guinea pigs repeatedly exposed to propylene oxide had alveolar hemorrhage and edema and interstitial edema and congestion of the lungs. As would be predicted, respiratory tract damage in dogs (nonobligate nose breathers) after inhalation exposure occurred on more distal parts of the respiratory system. Gross necropsy of dogs exposed to propylene oxide concentrations up to 2,481 ppm for 4 h revealed congestion of the tracheal mucosa and lungs, spotty alveolar edema, marked perivascular and peribronchial edema, and focal areas of subepithelial edema and necrobiosis of bronchiolar epithelium.Toxicity does not appear to be limited to the site of entry, however. Possible neurotoxic effects have been observed in rodents and dogs after inhalation exposure to higher concentrations of propylene oxide. In dogs, motor weakness and vomiting were observed in some animals exposed to 2,005 ppm and greater. Some of the signs noted in rodents exposed to propylene oxide included drowsiness, sedation, weakness, incoordination, hypoactivity, ataxia, diarrhea, and transient restless behavior (Jacobson et al. 1956; Rowe et al. 1956; Shell Oil Company 1977; Dow Chemical Company 1981; NTP 1985; Ohnishi et al. 1988). Rats exposed to propylene oxide at 1,500 ppm for 7 weeks exhibited hindlimb ataxia, and pathologic examination revealed changes compatible with central-peripheral distal axonopathy (Ohnishi et al. 1988). Repeated exposures to lower concentrations of propylene oxide (up to 300 ppm), however, resulted in no or minimal evidence of neurotoxicity in rats or monkeys (Sprinz et al. 1982; Young et al. 1985; Setzer et al. 1997). Possible neurotoxic signs, including restlessness, headache, general weakness, diarrhea, and vomiting, were also reported in the case report of a Russian worker exposed to high concentrations of propylene oxide (Beliaev et al. 1971).Propylene oxide appears to cause cancer in animals at the site of contact. Intragastric administration of propylene oxide to Sprague-Dawley rats resulted in tumors of the forestomach, and subcutaneous injections in rats generated sarcomas at the injection site (Walpole 1958; Dunkelberg 1982). Inhalation studies with propylene oxide have demonstrated carcinogenic activity in mice and rats manifested as nasal cavity tumors (NTP 1985). Specifically, inhalation of propylene oxide at 400 ppm for 2 years produced tumors (submucosa hemangiomas

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and hemangiocarcinomas) in the nasal cavity of mice, and an increased incidence of nasal epithelial papillary adenomas indicated some evidence of carcinogenicity in rats (NTP 1985). No evidence of carcinogenicity was found at 200 ppm. Because propylene oxide covalently binds to DNA by introducing a 2-hydroxypropyl group, the mode of action of nasal tumorigenesis in rodents was hypothesized to be the result of direct DNA damage. The primary DNA adduct formed in rats after exposure to propylene oxide is 7-HPG; the highest level was found in nasal tissue—specifically, nasal respiratory tissue (Ríos-Blanco et al. 1997, 2000, 2003a). The accumulation of 7-HPG in nasal respiratory tissue increased linearly with propylene oxide exposure concentrations ranging from 5 to 500 ppm (Ríos-Blanco et al. 2003a). The investigators concluded that adduct accumulation in the nasal respiratory tissue was not sufficient to induce tumor formation as it had a linear concentration response, while nasal tumor formation had a nonlinear concentration response. In contrast, cell proliferation in the nasal respiratory epithelium was nonlinear and correlated better with tumor formation (Eldridge et al. 1995; Ríos-Blanco et al. 2003b). Hyperplastic lesions were present in the same region where nasal tumors developed in the NTP (1985) cancer bioassay in rats. The cell proliferation may be a result of depletion of NPSH (includes GSH) in the respiratory nasal mucosa of rats and mice, the levels of which were depleted to significant levels after exposure to propylene oxide at 300 and 500 ppm (Morris et al. 2004; Lee et al. 2005; Morris and Pottenger 2006). Lee et al. (2005) proposed that depletion of GSH as a cosubstrate for the conjugation reaction with propylene oxide (a detoxification pathway) results in continuous and severe perturbation of GSH in the respiratory nasal mucosa of rodents repeatedly exposed to high concentrations of propylene oxide, which leads to inflammatory lesions and cell proliferation. In conclusion, data indicate that propylene oxide is a threshold carcinogen dependent on increased cell proliferation and hyperplasia at the target site and therefore would require repeated exposure to produce tumorigenesis. This conclusion is supported by the Sellakumar et al. (1987) study in which no tumors were observed when 12-week-old male Sprague-Dawley rats were exposed to propylene oxide at 433 or 864 ppm for 30 days or to 1,724 ppm for 8 days (exposures were for 6 h/day, 5 days/week) and allowed to die naturally.

4.3. Structure-Activity Relationships,

Propylene oxide is not as toxic as ethylene oxide, a structurally related chemical that also is a direct alkylating agent and undergoes similar biotransformation. According to a comparison of the 4-h LC_{50} values for the two chemicals, propylene oxide is 2 to 3 times less toxic than ethylene oxide (data are presented in Table 5-18). Ethylene oxide is mutagenic to germ cells as well as somatic cells in species such as rodents, monkeys, and rabbits, and it has been found to be 5 to 10 times more effective than propylene oxide in gene conversion, reverse mutations, and sister chromatid conversion in yeast (Agurell et al.

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1991; Gardiner et al. 1993). The two chemicals have about the same potency for inducing in vitro point mutations in bacteria and sister chromatid exchanges in human lymphocytes (Agurell et al. 1991). In vivo, ethylene oxide is more effective than propylene oxide at inducing chromosomal aberrations in humans and sister chromatid exchanges and chromosomal aberrations in monkeys (Lynch et al. 1984b; Högstedt et al. 1990). The number of hemoglobin adducts formed in mice after exposure to propylene oxide has been estimated to be 4 times lower than the number formed by exposure to ethylene oxide (Farooqi et al. 1993). After intraperitoneal injection of each chemical, propylene oxide binding in mouse liver DNA was 1/20th that of ethylene oxide (IARC 1994).

4.4. Other Relevant Information

4.4.1. Species Differences

Available data indicate that differences in sensitivity among species generally differ by a factor of 3. The 4-h LC_{50} values for the different species tested differ at most by a factor of 3.5, with mice being the most sensitive, followed by dogs and then rats. Measured hemoglobin adduct levels after inhalation exposure in rats, mice, and dogs varied at most by a factor of 2.9 (Segerbäck et al. 1992, 1994). Examination of clinical signs revealed that dyspnea was the most sensitive end point and occurred at lower concentrations in mice when compared with rats or when compared with other clinical signs reported in other species including dogs and humans. Data addressing the in vitro metabolism of propylene oxide in human, rat, and mouse lung and liver microsomes indicate that human microsomal EH has a greater capacity for propylene oxide metabolism than the rat and mouse EH. The human lung cytosolic GST activity level appeared to be between that of the mouse and the rat (Faller et al. 2001).

TABLE 5-18 Summary of Calculated 4-Hour LC_{50} and LC_{01} Values for Ethylene Oxide and Propylene Oxide

	Ethylene Oxide (ppm)			Propylene Oxide (ppm)		
Species	LC_{50}	Estimated LC_{01}	Reference	LC_{50}	Estimated LC_{01}	Reference
Dog	960	120	Jacobson et al. 1956	1,941	930	Jacobson et al. 1956
Rat	1,460 1,741	628 922	Jacobson et al. 1956 Nachreiner 1991	4,000 3,205	2,280 1,037	Jacobson et al. 1956 NTP 1985
Mouse	835 623	406 264	Jacobson et al. 1956 NTP 1987	1,740 1,160[a]	624 451[a]	Jacobson et al. 1956 NTP 1985

[a]Probit analysis excluding the one female mouse death at 387 ppm, based on the assumption that the death was not treatment related.
Abbreviations: LC_{50}, concentration with 50% lethality; LC_{01}, concentration with 1% lethality.

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4.4.2. Concentration-Exposure Duration Relationship

For mild irritation, AEGL values are set equal across time because mild irritation generally does not vary greatly over time. Evidence indicates that death after propylene oxide inhalation is the result of respiratory tract irritation (see Section. 4.2.). Although the mechanism of action appears to be a direct irritant effect, it is not appropriate to set the values equal across time because the irritation is no longer considered mild but is part of the continuum of respiratory tract irritation leading to lethality. The experimentally derived exposure values are therefore scaled to AEGL timeframes using the concentration-time relationship given by the equation $C^n \times t = k$, where C is concentration, t is time, k is a constant, and n is 1.7 as calculated with the rat lethality data reported by Rowe et al. (1956) (ten Berge et al. 1986) (see Appendix B).

5. RATIONALE FOR AEGL-1

5.1. Human Data Relevant to AEGL-1

The reported odor thresholds for propylene oxide in humans range from 10 to 35 ppm (Hellman and Small 1974) to 200 ppm (Jacobson et al. 1956). In an environmental health survey, 8-h TWAs measured over a 3-day sampling period indicated propylene oxide exposures ranging from 13.2 to 31.8 ppm. No worker complaints were noted in the report (CMA 1998). In another workplace survey, measured propylene oxide exposure concentrations in the breathing zone of three workers during drumming operations were 1,520 ppm for 171 min, 1,310 ppm for 124 min, and 525 ppm for 121 min with the local heater fan turned off and 380 ppm for 177 min, 392 ppm for 135 min, and 460 ppm for 116 min with the heater fan turned on (CMA 1998). A strong odor was noted during sampling, but the irritation was "not intolerable" (occasional eye irritation was noted in the report as the reason for the monitoring program). Another survey of propylene oxide concentrations during drumming operations found concentrations of 348 and 913 ppm for 30 min and 28 ppm during purging for 12 min. Eye irritation was reported to occur after 2 weeks of steady work in the drumming operation.

5.2. Animal Data Relevant to AEGL-1

No animal toxicity data relevant to an AEGL-1 derivation were available.

5.3. Derivation of AEGL-1

The AEGL-1 derivation is based on the workplace survey that measured exposure concentrations of 380 ppm for 177 min, 525 ppm for 121 min, 392

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ppm for 135 min, and 460 ppm for 116 min in the breathing zone of three workers during drumming operations (CMA 1998). Strong odor and irritation were noted in the workplace survey (exact nature of the irritation, other than the strong odor, was not provided, but occasional eye irritation was noted in the report as the reason for the monitoring program). Because the irritant effects are mild, the values would be set equal across time. Therefore, the four exposure concentrations can be averaged together, resulting in a point of departure of 440 ppm. A total uncertainty factor and modifying factor of 6 is applied. An interspecies uncertainty factor was not needed as the data were from human exposures; an intraspecies uncertainty factor of 3 was applied because irritation is a point-of-contact effect and is not expected to vary greatly among individuals; and a modifying factor of 2 is applied because the defined effects are above an AEGL-1 (undefined irritation) but below an AEGL-2 end point. The resultant value of 73 ppm was set equal across time because mild irritation is not expected to vary greatly.

AEGL-1 values are presented in Table 5-19.

6. RATIONALE FOR AEGL-2

6.1. Human Data Relevant to AEGL-2

No human data were relevant for derivation of an AEGL-2 value. Workplace exposure data from environmental health surveys reported health effects of irritation that are below the defined AEGL-2 end point (CMA 1998).

6.2. Animal Data Relevant to AEGL-2

Data relevant to AEGL-2 were available for dogs, rats, mice, and guinea pigs. Dogs exposed to propylene oxide vapor at 1,363 ppm for 4 h exhibited lacrimation, salivation, and nasal discharge (Jacobson et al. 1956). Much of the rat data were from lethality studies in which signs of toxicity were discussed. Generally, the severity of toxic signs increased with increasing concentration and duration, but the severity of each toxic sign at the respective concentrations was not clear. In addition, lethal concentrations were included in the general discussion of the observed toxic signs, which was the case in the following studies by Rowe et al. (1956) and Shell Oil Company (1977). Exposure to 16,000 or 8,000 ppm for 15 min, 4,000 ppm for 1 h, or 2,000 ppm for 7 h resulted in nasal irritation, difficulty breathing, drowsiness, weakness, and occasionally some

TABLE 5-19 AEGL-1 Values for Propylene Oxide

Classification	10 min	30 min	1 h	4 h	8 h
AEGL-1	73 ppm (170 mg/m^3)	73 ppm (170 mg/m^3)	73 ppm (170 mg/m^3)	73 ppm (170 mg/m^3)	73 ppm (170 mg/m^3)

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incoordination (Rowe et al. 1956). Wistar rats exposed to 3,000 or 3,450 ppm for 4 h exhibited excessive lacrimation and eye irritation, sedation, piloerection, mucous discharge (frequently bloodstained) from the nose and mouth, and respiratory difficulty (Shell Oil Co. 1977). Jacobson et al. (1956) noted frequent moving and preening, clear nasal discharge, lacrimation, salivation, and gasping in rats during a 4-h exposure to 945, 1,329, or 2,684 ppm. However, the severity of each toxic sign at the respective concentrations was not clear. Rats exposed to 149, 298, or 600 ppm for 6 h/day, 5 days/week, for 13 weeks exhibited transient restless behavior only during the first 3 days of exposure (Dow Chemical Company 1981). The 600-ppm group also exhibited occasional salivation and piloerection. No treatment-related effects were noted in rats exposed to 1,277 ppm for 4 h (NTP 1985); 4,000 ppm for 30 min, 2,000 ppm for 2 h, or 1,000 ppm for 7 h (Rowe et al. 1956); 47, 99, 196, or 487 ppm for 6 h/day, 5 days/week, for 2 weeks (NTP 1985); or 31, 63, 125, or 250 ppm for 6 h/day, 5 days/week, for 13 weeks (NTP 1985). Studies in mice were more limited. Dyspnea was observed in mice exposed for 4 h to propylene oxide vapor at 387 ppm, the lowest concentration tested in the acute study (NTP 1985). No exposure-related effects were observed in this group at necropsy. In a repeat-exposure study in which mice were exposed to propylene oxide vapor at 0, 20, 47, 99, 196, or 487 ppm for 6 h/day, 5 days/week, for 2 weeks, dyspnea was not observed in mice exposed to 98.5 ppm or less (NTP 1985). Mice in the 196- and 487-ppm groups exhibited dyspnea, and the highest exposure groups were also hypoactive. No effects were noted in mice exposed to 31, 63, 125, or 250 ppm for 6 h/day, 5 days/week, for 13 weeks (NTP 1985).

Guinea pigs exposed to 16,000 ppm for 30 min, 8,000 ppm for 1 h, 4,000 ppm for 2 h, or 2,000 ppm for 7 h exhibited eye and nasal irritation, difficulty breathing, drowsiness, weakness, and occasional incoordination (Rowe et al. 1956). As in the rat studies previously discussed, the severity of toxic signs increased with increasing concentration and duration, but the severity of each toxic sign at the respective concentrations was not clear. In addition, lethal concentrations were included in the general discussion of the observed toxic signs.

6.3. Derivation of AEGL-2

No human data were available for derivation of an AEGL-2. When considering animal data for derivation of an AEGL-2, dyspnea in mice was the most sensitive end point consistent with the AEGL-2 definition, and mice were the most susceptible to the toxic effects of propylene oxide vapor. Therefore, the AEGL-2 values are based on the data from the NTP (1985) study in which mice exposed to 387 ppm for 4 h exhibited dyspnea. Although a no-effect level was not established for dyspnea at this concentration, no other adverse effects were noted. In addition, compared with other studies investigating propylene oxide toxicity in mice, the NTP study reported toxic effects at much lower concentrations than those observed in other studies. An interspecies uncertainty factor of 1

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was applied because mice are the most sensitive laboratory species tested both for the lethal effects of propylene oxide and for clinical signs of toxicity, and available data indicate that mice are equally or slightly more sensitive than humans in manifesting clinical signs (see Section 4.4.1.). This NTP (1985) study reported toxic effects at much lower concentrations than those observed in other studies. An intraspecies uncertainty factor of 3 was applied because the mechanism of toxicity, irritation, is a point-of-contact effect and is not expected to vary greatly among individuals. Therefore, a total uncertainty factor of 3 was applied. Although the mechanism of action appears to be a direct irritant effect, it is not appropriate to set the values equal across time because the irritation is no longer considered mild but is part of the continuum of respiratory tract irritation leading to lethality. The experimentally derived exposure value was therefore scaled to AEGL timeframes using the concentration-time relationship given by the equation $C^n \times t = k$, where n was 1.7 as calculated with the rat lethality data reported by Rowe et al. (1956) (ten Berge et al. 1986). The 10-min value was set equal to the 30-min value because of the uncertainty in extrapolating from the exposure duration of 4 h to 10 min.

AEGL-2 values are presented in Table 5-20.

7. RATIONALE FOR AEGL-3

7.1. Human Data Relevant to AEGL-3

No reports of human mortality after exposure to propylene oxide were located. In a human workplace report, exposure to propylene oxide at 1,520 ppm for 171 min did not result in mortality in an exposed worker. This exposure concentration represents the highest documented exposure concentration in humans. It was noted during sampling that Athe odor was quite strong during the sampling; however, the irritation was not intolerable (CMA 1998).

7.2. Animal Data Relevant to AEGL-3

Lethality studies appropriate for an AEGL-3 derivation were available for several species. Using the individual mortality data, the 4-h benchmark concentration with 1% response (BMC_{01}), and $BMCL_{05}$ values were calculated by a log-probit analysis with EPA Benchmark Dose Software Version 1.4.1b (see Appendix C). When not calculated by the study author, the LC_{50} was calculated

TABLE 5-20 AEGL-2 Values for Propylene Oxide

Classification	10 min	30 min	1 h	4 h	8 h
AEGL-2	440 ppm (1,000 mg/m^3)	440 ppm (1,000 mg/m^3)	290 ppm (690 mg/m^3)	130 ppm (310 mg/m^3)	86 ppm (200 mg/m^3)

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by probit analysis. Table 5-21 summarizes the LC_{50}, BMC_{01}, and $BMCL_{05}$ values calculated by using lethality data in dogs, rats, and mice. Mice were the most sensitive to propylene oxide exposure, having 4-h LC_{50} values ranging from 1,160 to 1,740 ppm (Jacobson et al. 1956; NTP 1985). Mice were followed by dogs (4-h LC_{50} of 1,941 ppm) and then rats (4-h LC_{50} values ranging from 3,205 to 4,197 ppm) (Jacobson et al. 1956; Shell Oil Company 1977; NTP 1985). The value for dogs should be interpreted with caution because two of three animals in the high-dose group died before they were removed from the exposure chamber.

7.3. Derivation of AEGL-3

The AEGL-3 derivation is based on the calculated 4-h $BMCL_{05}$ value of 1,161 ppm, the lowest $BMCL_{05}$ value in rats (NTP 1985). Lethality data on the dog, a nonobligate nose breather, support the use of the $BMCL_{05}$ value in the rat, but the dog values should not be used as the basis for the AEGL-3 derivation because two of three animals in the high-dose group died before they were removed from the exposure chamber. Mouse data were not used because the mouse is overly sensitive to propylene oxide compared with the other species tested. The $BMCL_{05}$ values in mice are 282 and 673 ppm (Jacobson et al. 1956; NTP 1985), compared with 1,161 to 3,328 ppm in rats (Jacobson et al. 1956; and Shell Oil Co. 1977; NTP 1985) and 1,117 ppm in dogs (Jacobson et al. 1956). Other data demonstrating that the mouse $BMCL_{05}$ values are unreasonably low include the studies in which only minimal effects were noted in monkeys exposed to 300 ppm for 6 h/day, 5 days/week, for 2 years (Sprinz et al. 1982; Lynch et al. 1983; Setzer et al. 1997) or to 457 ppm for 7 h/day for 154 days (Rowe et al. 1956) and the highest documented human exposure of 1,520 ppm

TABLE 5-21 Summary of 4-Hour LC_{50}, BMC_{01}, and $BMCL_{05}$ Values for Propylene Oxide

Species	LC_{50} (ppm)	Calculated BMC_{01} (ppm)	Calculated $BMCL_{05}$ (ppm)	Reference
Dog	1,941	1,773	1,117	Jacobson et al. 1956
Rat	3,205	1,845	1,161	NTP 1985
	4,000	2,482	2,254	Jacobson et al. 1956
	4,197	3,556	3,328	Shell Oil Co. 1977
Mouse	1,740	113	282	Jacobson et al. 1956
	1,160[a]	783[a]	673[a]	NTP 1985

[a]Data exclude the death of the one animal in the 387-ppm group.
Abbreviations: LC_{50}, concentration with 50% lethality; BMC_{01}, benchmark concentration with 1% response; and $BMCL_{05}$, benchmark concentration, 95% lower confidence limit with 5% response.

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for 171 min, which caused irritation that was not severe enough to cause the worker to cease working (CMA 1998). These data support the 4-h $BMCL_{05}$ of 1,161 ppm in rats as a reasonable point of departure. An intraspecies uncertainty factor of 3 was applied because the mechanism of toxicity, irritation, is a point-of-contact effect and is not expected to vary greatly among individuals. An interspecies uncertainty factor of 1 was applied because of the supporting data in dogs (similar 4-h $BMCL_{05}$) and monkeys (2-year studies that produced minimal effects). The 4-h AEGL-3 value using a total uncertainty factor of 3 is 387 ppm, which is conservative compared with the 300- or 457-ppm chronic exposure in monkeys producing minimal effects. Therefore, a total uncertainty factor of 3 was considered reasonable. As for the AEGL-2 derivation, the experimentally derived exposure value for the AEGL-3 derivation was scaled to AEGL time-frames by using the concentration-time relationship given by the equation $C^n \times t = k$, where n was 1.7 as calculated with the rat lethality data reported by Rowe et al. (1956) (ten Berge et al. 1986). The value was extrapolated across time because the irritation is no longer considered mild, and the concentration represents the threshold for lethality. The 10-min value was set equal to the 30-min value because of the uncertainty in extrapolating from the exposure duration of 4 h to 10 min. AEGL-3 values are presented in Table 5-22.

A carcinogenicity assessment was not appropriate for an acute exposure scenario on the basis that the proposed mechanism of carcinogenicity suggests a nonlinear mode of action requiring continued exposure (see Appendix D). Therefore, a one-time exposure even to high concentrations of propylene oxide would not be expected to result in tumor development. This conclusion is supported by the Sellakumar et al. (1987) study in which no tumors were observed when 12-week-old male Sprague-Dawley rats were exposed to propylene oxide at 433 or 864 ppm for 30 days or 1,724 ppm for 8 days (exposures were for 6 h/day, 5 days/week) and allowed to die naturally.

8. SUMMARY OF AEGLs

8.1. AEGL Values and Toxicity End Points

The AEGL-1 values are based on the average of four propylene oxide exposure concentrations measured in the breathing zone of three workers (380 ppm for 177 min, 525 ppm for 121 min, 392 ppm for 135 min, or 460 ppm for 116 min) (CMA 1998). A strong odor was noted, but irritation was not intolerable.

TABLE 5-22 AEGL-3 Values for Propylene Oxide

Classification	10 min	30 min	1 h	4 h	8 h
AEGL-3	1,300 ppm (3,100 mg/m^3)	1,300 ppm (3,100 mg/m^3)	870 ppm (2,100 mg/m^3)	390 ppm (930 mg/m^3)	260 ppm (620 mg/m^3)

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The AEGL-2 values are based on dyspnea in mice at 387 ppm for 4 h (NTP 1985). Dyspnea is the most sensitive end point, and mice are the most susceptible to it. Although a no-effect level was not established at this concentration, no other effects were noted. This NTP (1985) study reported toxic effects at much lower concentrations than those observed in other studies. The AEGL-3 values are based on the calculated 4-h $BMCL_{05}$ of 1,161 ppm in rats (NTP 1985). The AEGL values for propylene oxide are summarized in Table 5-23.

A useful way to evaluate the AEGL values in the context of existing empirical data is presented in Figure 5-1. For this plot, toxic responses were placed in severity categories. The severity categories fit into definitions of the AEGL health effects: 0 = no effects; 1= discomfort; 2 = disabling; 3 = lethal, and SL = partially lethal (an experimental concentration at which some animals died and some did not). The effects that place an experimental result in a particular category vary according to the spectrum of data available on a specific chemical and the effects from exposure to that chemical. The concentrations often span a number of orders of magnitude, especially when human data exist. Therefore, the concentration is placed on a logarithmic scale. The graph in Figure 5-1 plots the propylene oxide AEGL values along with the existing acute human and animal toxicity data for propylene oxide in terms of the categories assigned to them. From this plot, one sees that the AEGL-1 values are below any exposure concentration resulting in any effects; the AEGL-2 values are below concentrations that produce discomfort; and the AEGL-3 values are in the range of concentrations that produce discomfort but below concentrations that produce disabling or lethal effects. Therefore, the AEGL values should be protective of human health.

TABLE 5-23 Summary of AEGL Values

Classification	Exposure Duration				
	10 min	30 min	1 h	4 h	8 h
AEGL-1 (Nondisabling)	73 ppm (170 mg/m^3)	73 ppm (170 mg/m^3)	73 ppm (170 mg/m^3)	73 ppm (170 mg/m^3)	73 ppm (170 mg/m^3)
AEGL-2 (Disabling)	440 ppm (1,000 mg/m^3)	440 ppm (1,000 mg/m^3)	290 ppm (690 mg/m^3)	130 ppm (310 mg/m^3)	86 ppm (200 mg/m^3)
AEGL-3 (Lethal)	1,300 ppm (3,100 mg/m^3)	1,300 ppm (3,100 mg/m^3)	870 ppm (2,100 mg/m^3)	390 ppm (930 mg/m^3)	260 ppm (620 mg/m^3)

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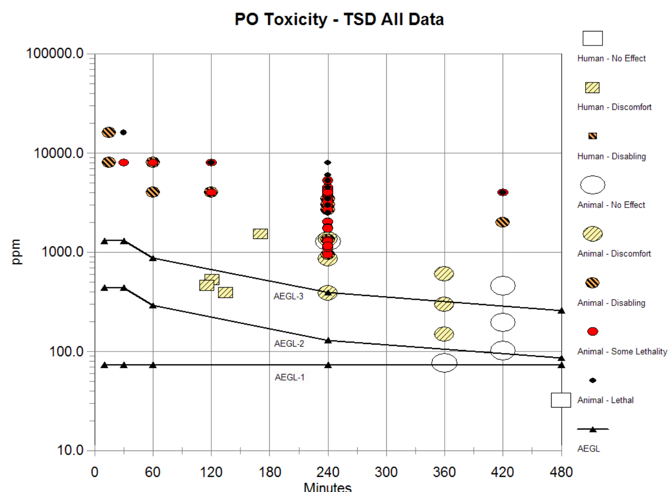

FIGURE 5-1 Category plot of animal toxicity data compared with AEGL values.

8.2. Comparisons with Other Standards

Standards and guidance levels for workplace and community exposures are listed in Table 5-24. The 30-min AEGL-3 value of 1,300 ppm is greater than the 30-min immediately dangerous to life or health value of 400 ppm. The 8-h AEGL-2 of 86 ppm is below the Occupational Safety and Health Administration time-weighted average of 100 ppm and above the American Conference of Governmental Industrial Hygienists Threshold Limit Value–TWA of 20 ppm. Compared with the emergency response planning guideline (ERPG) values, the 1-h AEGL-1, AEGL-2, and AEGL-3 values are similar to the ERPG-1, ERPG-2, and ERPG-3 values, respectively.

8.3. Data Adequacy and Research Needs

Limited data consistent with a defined AEGL-2 end point were available. Animal studies reporting clinical signs often did not report the severity of the signs at each respective exposure concentration but rather gave only a general statement. Additional data consistent with a defined AEGL-2 end point in multiple species would be helpful in further defining the AEGL-2 levels. The AEGL-1 derivation would be improved if additional data on the degree of human irritation after exposure to propylene oxide were available. Animal studies reporting the severity of clinical signs at each respective exposure in multiple species would also be beneficial.

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TABLE 5-24 Extant Standards and Guidelines for Propylene Oxide

Guideline	Exposure Duration				
	10 min	30 min	1 h	4 h	8 h
AEGL-1	73 ppm	73 ppm	73 ppm	73 ppm	73 ppm
AEGL-2	440 ppm	440 ppm	290 ppm	130 ppm	86 ppm
AEGL-3	1,300 ppm	1,300 ppm	870 ppm	390 ppm	260 ppm
ERPG-1 (AIHA)[a]			50 ppm		
ERPG-2 (AIHA)			250 ppm		
ERPG-3 (AIHA)			750 ppm		
IDLH (NIOSH)[b]	400 ppm				
REL-TWA (NIOSH)[c]					Cancer; lowest feasible concentration
PEL-TWA (OSHA)[d]					100 ppm [240 mg/m^3]
TLV-TWA (ACGIH)[e]					2 ppm
MAK (Germany)[f]					Not established: considered carcinogenic to humans
MAC (The Netherlands)[g]					2.5 ppm [6 mg/m^3]

[a]ERPG (emergency response planning guidelines of the American Industrial Hygiene Association) (AIHA 1996): The ERPG-1 is the maximum airborne concentration below which it is believed nearly all individuals could be exposed for up to 1 h without experiencing other than mild, transient adverse health effects or without perceiving a clearly defined objectionable odor. The ERPG-1 for propylene oxide is based on possibility of mild, transient adverse effects without perceiving objectionable odor—repeated 7-h exposures at concentrations as high as 200 ppm were well tolerated by rats, rabbits, guinea pigs, and one monkey (Rowe et al. 1956).The ERPG-2 is the maximum airborne concentration below which it is believed nearly all individuals could be exposed for up to 1 h without experiencing or developing irreversible or other serious health effects or symptoms that could impair an individual's ability to take protective action. The ERPG-2 for propylene oxide is based on following data: at 250 ppm, the odor should be easily detected, and some irritation - probably minor- might occur; rats repeatedly exposed to 870 ppm did not show increases in adverse health effects or severe irritation (Sellakumar et al. 1987).The ERPG-3 is the maximum airborne concentration below which it is believed nearly all individuals could be exposed for up to 1 h without experiencing or developing life-threatening health effects. The ERPG-3 for propylene oxide is based on the following data: 1/10th the concentration not causing evident systemic toxicity in female guinea pigs exposed for 1 h; 1/3rd the concentration not producing systemic effects in female rats exposed for 2 h; about twice the concentration not causing effects in dogs, other than

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some motor weakness in one of three dogs exposed for 4 h (Rowe et al. 1956; Jacobson et al. 1956).
[b]IDLH (immediately dangerous to life or health, National Institute for Occupational Safety and Health) (NIOSH 1996, 2005) represents the maximum concentration from which one could escape within 30 min without any escape-impairing symptoms or any irreversible health effects. The IDLH for propylene oxide is based on acute inhalation toxicity data in animals (Jacobson et al. 1956).
[c]REL-TWA (recommended exposure limits–TWA, National Institute for Occupational Safety and Health) (NIOSH 2005) is defined analogous to the American Conference of Governmental Industrial Hygienists Threshold Limit Value–TWA (ACGIH TLV-TWA).
[d]PEL-TWA (permissible exposure limits–TWA, Occupational Health and Safety Administration) (29 CFR 1910.1000[1996]) is defined analogous to the ACGIH-TLV-TWA but is for exposures of no more than 10 h/day, 40 h/week.
[e]TLV-TWA (Threshold Limit Value–TWA, American Conference of Governmental Industrial Hygienists) (ACGIH 2001) is the TWA concentration for a normal 8-h workday and a 40-h workweek, to which nearly all workers may be repeatedly exposed, day after day, without adverse effect.
[f]MAK (maximale argeitsplatzkonzentration [maximum workplace concentration], Deutsche Forschungsgemeinschaft [German Research Association]) (DFG 1999) is analogous to the ACGIH-TLV-TWA.
[g]MAC (maximaal aanvaaarde concentratie [maximal accepted concentration], SDU Uitgevers [under the auspices of the Ministry of Social Affairs and Employment, The Hague, The Netherlands]) is analogous to the ACGIH-TLV-TWA (MSZW 2004).

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APPENDIX A

DERIVATION OF AEGL VALUES FOR PROPYLENE OXIDE

Derivation of AEGL-1

Key study:	CMA 1998
Toxicity end point:	Average of four propylene oxide exposure concentrations (380 ppm for 177 min, 525 ppm for 121 min, 392 ppm for 135 min, and 460 ppm for 116 min) measured in the breathing zone of three workers (average = 440 ppm)
Scaling:	Values were set equal across time because end point is mild irritation
Uncertainty factors:	1 for interspecies variability 3 for intraspecies variability
Modifying factor:	2
Combined uncertainty factors and modifying factor:	6
Calculations:	C/(uncertainty factor) = 440 ppm/3 = 73 ppm
10-min, 30-min, 1-h, 4-h, 8-h AEGL-1 values set equal across time:	73 ppm

Derivation of AEGL-2

Key study:	NTP 1985
Toxicity end point:	Dyspnea in mice exposed to 387 ppm for 4 h
Scaling:	$C^{1.7} \times t = k$ where n of 1.7 was derived from rat lethality data reported by Rowe et al. (1956) using the method of ten Berge et al. (1986)
Uncertainty factors:	1 for interspecies variability

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	3 for intraspecies variability Combined uncertainty factor of 3
Modifying factor:	Not applicable
Calculations:	$[(C/\text{uncertainty factor})]^n \times t = k$ $[(387\ \text{ppm})/3]^{1.7} \times 4\ \text{h} = 15{,}490.4$ ppm-h
10-min AEGL-2	The 10-min value was set equal to the 30-min value of 440 ppm because of the uncertainty in extrapolating from the exposure duration of 4 h to 10 min
30-min AEGL-2	$C^n \times 0.5\ \text{h} = 15{,}490.4$ ppm-h $C^{1.7} = 30{,}980.8$ ppm C = 438.4 ppm = 440 ppm
1-h AEGL-2	$C^n \times 1\ \text{h} = 15{,}490.4$ ppm-h $C^{1.7} = 15{,}490.4$ ppm C = 291.6 ppm = 290 ppm
4-h AEGL-2	$C^n \times 4\ \text{h} = 15{,}490.4$ ppm-h $C^{1.7} = 3{,}872.6$ ppm C = 129.00 ppm = 130 ppm
8-h AEGL-2	$C^n \times 8\ \text{hr} = 15{,}490.4$ ppm-h $C^{1.7} = 1{,}936.3$ ppm C = 85.8 ppm = 86 ppm

Derivation of AEGL-3

Key study:	NTP 1985
Toxicity end point:	Calculated 4-h $BMCL_{05}$ of 1,161 ppm using rat lethality data
Scaling:	$C^{1.7} \times t = k$ where n of 1.7 was derived from rat lethality data reported by Rowe et al. (1956) using the method of ten Berge et al. (1986)
Uncertainty factors:	1 for interspecies variability 3 for intraspecies variability
Modifying factor:	None

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Calculations:	$(C/[\text{uncertainty factors}])^n \times t = k$ $(1{,}161\ \text{ppm})/3^{1.7} \times 4\ \text{h} = 100{,}269.4$ ppm-h
10-min AEGL-3	The 10-min value was set equal to the 30-min value of 1,300 ppm because of the uncertainty in extrapolating from the exposure duration of 4 h to 10 min
30-min AEGL-3	$C^n \times 0.5\ \text{h} = 100{,}269.4$ ppm-h $C^{1.7} = 200{,}538.8$ ppm C = 1,315.0 ppm = 1,300 ppm
1-h AEGL-3	$C^n \times 1\ \text{h} = 100{,}269.4$ ppm-h $C^{1.7} = 100{,}269.4$ ppm C = 874.7 ppm = 870 ppm
4-h AEGL-3	$C^n \times 4\ \text{h} = 100{,}269.4$ ppm-h $C^{1.7} = 25{,}067.4$ ppm C = 387.0 ppm = 390 ppm
8-h AEGL-3	$C^n \times 8\ \text{h} = 100{,}269.4$ ppm-h $C^{1.7} = 12{,}533.7$ ppm C = 257.4 ppm = 260 ppm

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APPENDIX B

TIME-SCALING CALCULATIONS

Filename:
PO for Log Probit Model
Date: 05 November 2007 Time: 09:30:11

Sequence	Conentration			
Number	ppm	Minutes	Exposed	Responded
1	2,000	420	10	0
2	4,000	420	10	10
3	4,000	240	10	4
4	4,000	120	10	4
5	4,000	60	5	0
6	8,000	120	10	10
7	8,000	60	10	5
8	8,000	30	10	2
9	8,000	15	10	0
10	16,000	30	10	10
11	16,000	15	15	0

Observations 1 through 11 considered

Sequence	Concentration			
Number	(ppm)	Minutes	Exposed	Responded
1	2,000	420	10	0
2	4,000	420	10	10
3	4,000	240	10	4
4	4,000	120	10	4
5	4,000	60	5	0
6	8,000	120	10	10
7	8,000	60	10	5
8	8,000	30	10	2
9	8,000	15	10	0
10	16,000	30	10	10
11	16,000	15	15	0

Used probit equation: $Y = B0 + B1 \times X1 + B2 \times X2$
X1 = concentration ppm, ln-transformed
X2 = minutes, ln-transformed

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Chi square = 26.64
Degrees of freedom = 8
Probability model = 8.17E-04

Ln(likelihood) = –20.39

B 0 = –3.9364E+01	Student t test = –2.9553
B 1 = 3.8770E+00	Student t test = 3.2821
B 2 = 2.3053E+00	Student t test = 3.3066
Variance B 0 0 =	1.7742E+02
Covariance B 0 1 =	-1.5682E+01
Covariance B 0 2 =	-8.9312E+00
Variance B 1 1 =	1.3954E+00
Covariance B 1 2 =	7.7204E-01
Variance B 2 2 =	4.8607E-01

Estimation ratio between regression coefficients of ln(concentration) and ln (minutes)
Point estimate = 1.682
Lower limit (95% confidence limit) = 1.265
Upper limit (95% confidence limit) = 2.099

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APPENDIX C

BENCHMARK CALCULATIONS

Benchmark Calculations

The benchmark calculations are based on the study by NTP (1985) using a range of four concentrations in rats. For derivation of 10- and 30-min and 1-, 4-, and 8-h AEGL-3 values, a $BMCL_{05}$ of 1,161 ppm, derived with the log-probit model, was used.

$BMCL_{05}$ = 1,161 ppm
BMC_{01} = 1,845 ppm

Probit Model. (Version: 2.8; Date: 02/20/2007)
Input Data File: C:\BMDS\PO\RATNTP.(d)
Gnuplot Plotting File: C:\BMDS\PO\RATNTP.plt
Fri Nov 02 17:38:56 2007

BMDS model run:

The form of the probability function is
P[response] = background + (1 – background) ×
CumNorm(intercept + slope × log(dose)),
where CumNorm(.) is the cumulative normal distribution function.

Dependent variable = mortality
Independent variable = concentration
Slope parameter is not restricted
Total number of observations = 5
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative function convergence has been set to 1e-008
Parameter convergence has been set to 1e-008

User has chosen the log-transformed model.

Default initial (and specified) parameter values
Background = 0
Intercept = –15.8272
Slope = 1.96437.

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Asymptotic correlation matrix of parameter estimates:

	Intercept	Slope
Intercept	1	–1
Slope	–1	1

(The model parameters backgrounds have been estimated at a boundary point or have been specified by the user and do not appear in the correlation matrix.)

Parameter estimates:

			95.0% Wald Confidence Interval	
Variable	Estimate	Standard Error	Lower Confidence Limit	Upper Confidence Limit
Background	0	NA[a]		
Intercept	-31.3034	15.5575	-61.7955	-0.811365
Slope	3.85333	1.90441	0.12075	7.58591

[a]NA indicates that this parameter has hit a bound implied by some inequality constraint and thus has no standard error.

Analysis of deviance table:

Model	Log(likelihood)	No. of Parameters	Deviance	Test d.f.[a]	p Value
Full model	–17.8428	5			
Fitted model	–18.5238	2	1.36197 3	3	0.7145
Reduced model	–32.0518	1	28.418	4	<0.000

[a]d.f., degrees of freedom.

Akaike information criterion: 41.0475

Goodness of Fit					
Dose	Estimated Probability	Expected	Observed	Scaled Size	Residual
0.0000	0.0000	0.0000	0	10	0.0000
1,277.0000	0.0001	0.0001	0	10	–0.030
2,970.0000	0.3117	3.117	3	10	–0.080
3,794.0000	0.6746	6.746	8	10	0.847
3,900.0000	0.7118	7.118	6	10	–0.781

Chi square = 1.33, degrees of freedom = 3, p = 0.7211.

Benchmark dose computation:

Specified effect = 0.05
Risk type = extra risk
Confidence level = 0.95
BMD = 2201.43
BMDL = 1,160.91

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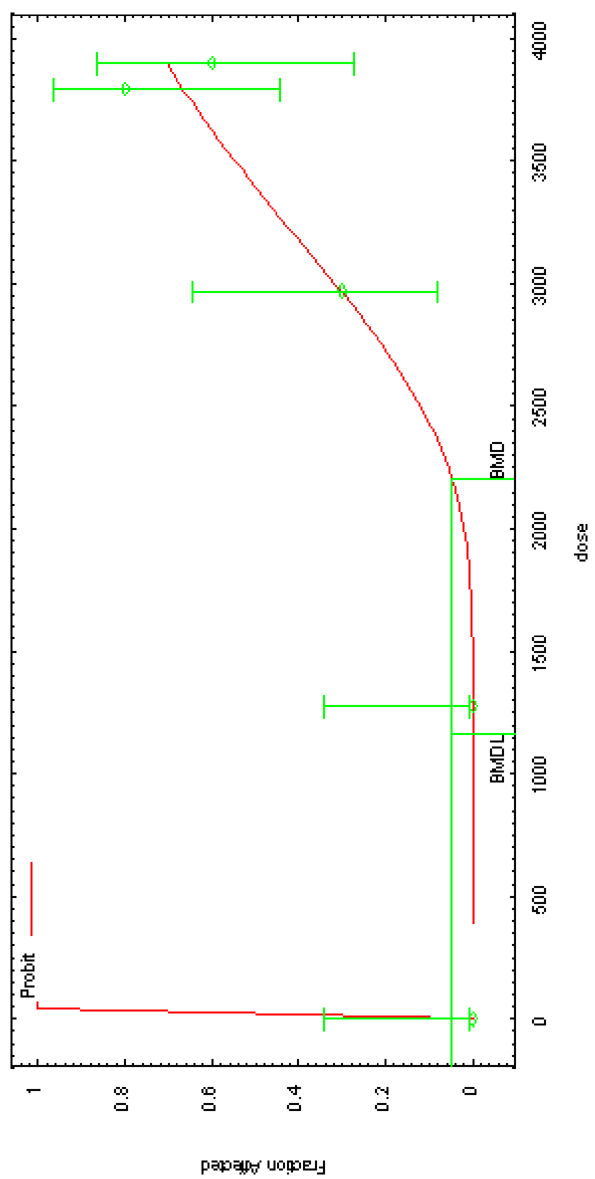

FIGURE C-1 Probit model with 95% confidence level.

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APPENDIX D

CARCINOGENICITY ASSESSMENT

Discussion of Cancer Assessment of Propylene Oxide

Propylene oxide appears to cause cancer in animals at the site of contact. Intragastric administration of propylene oxide to Sprague-Dawley rats resulted in tumors of the forestomach, subcutaneous injections in rats generated sarcomas at the injection site, and inhalation exposure caused nasal cavity tumors in mice and rats (Walpole 1958; Dunkelberg 1982; NTP 1985). The nasal cavity tumors (nasal submucosa hemangiomas and hemangiosarcomas) in male and female C57CL/6 × C3H mice resulted from whole-body inhalation exposure to propylene oxide at 400 ppm for 6 h/day, 5 days/week, for 103 weeks (NTP 1985; also reported by Renne et al. 1986). No evidence of carcinogenicity was found at 200 ppm. Nonneoplastic effects of propylene oxide on the nasal turbinates of mice included acute and chronic inflammation, suppurative inflammation, and serous inflammation. F344/N rats exposed to propylene oxide at 400 ppm had an increased incidence of nasal epithelial papillary adenomas, although statistical significance was not achieved (NTP 1985). The tumor incidence indicates some evidence of carcinogenicity at 400 ppm but no evidence of carcinogenicity was found at 200 ppm. Nonneoplastic effects of propylene oxide on the nasal turbinates of rats included suppurative inflammation, epithelial hyperplasia, and squamous metaplasia.

Studies investigating the mode of action of propylene-oxide-induced nasal cavity tumors support the hypothesis that propylene oxide is a threshold carcinogen dependent on increased cell proliferation and hyperplasia at the target site. Propylene oxide covalently binds to DNA by introducing a 2-hydroxypropyl group, and the primary DNA adduct formed in rats after inhalation exposure to propylene oxide is the N^7-(2-hydroxypropyl)guanine (7-HPG), particularly in nasal tissue (Ríos-Blanco et al. 1997, 2000, 2003a). The accumulation of 7-HPG in nasal respiratory tissue increased linearly with propylene oxide exposure concentrations ranging from 5 up to 500 ppm (Ríos-Blanco et al. 2003a). The investigators concluded that adduct accumulation in the nasal respiratory tissue was not sufficient to induce tumor formation as it had a linear concentration response, while nasal tumor formation had a nonlinear concentration response. In contrast, cell proliferation in the nasal respiratory epithelium was nonlinear and correlated better with tumor formation (Eldridge et al. 1995; Ríos-Blanco et al. 2003b). Hyperplastic lesions were present in the same region where nasal tumors developed in the NTP (1985) cancer bioassay in rats. The cell proliferation may be a result of the depletion of NPSH (includes GSH) in the respiratory nasal mucosa of rats and mice, the levels of which were depleted significantly after exposure to propylene oxide at 300 and 500 ppm (Morris et al. 2004; Lee et al. 2005; Morris and Pottenger 2006). Lee et al. (2005) proposed that de-

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pletion of GSH as a cosubstrate for the conjugation reaction with propylene oxide (a detoxification pathway) results in continuous and severe perturbation of GSH in the respiratory nasal mucosa of rodents repeatedly exposed to high concentrations of propylene oxide, which leads to inflammatory lesions and cell proliferation.

On the basis of these data, propylene oxide is a threshold carcinogen, and repeated exposure would be required to produce tumorigenesis. Therefore, it is inappropriate to conduct a carcinogen assessment for a single exposure to propylene oxide, because a one-time exposure even to a high concentration of propylene oxide is not be expected to result in tumor development. This conclusion is supported by the Sellakumar et al. (1987) study in which no tumors were observed when 12-week-old male Sprague-Dawley rats were exposed to propylene oxide 433 or 864 ppm for 30 days or at 1,724 ppm for 8 days (exposures were for 6 h/day, 5 days/week) and allowed to die naturally.

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APPENDIX E

CALCULATION OF LEVEL OF DISTINCT ODOR AWARENESS FOR PROPYLENE OXIDE

Derivation of the Level of Distinct Odor Awareness (LOA)

The level of distinct odor awareness (LOA) represents the concentration above which it is predicted that more than half the exposed population will experience at least a distinct odor intensity, and about 10% of the population will experience a strong odor intensity. The LOA should help chemical emergency responders in assessing the public awareness of the exposure due to odor perception. The LOA derivation follows the guidance given by van Doorn et al. (2002). For derivation of the odor detection threshold (OT_{50}), a study is available in which the odor threshold for the reference chemical *n*-butanol (odor detection threshold 0.04 ppm) has also been determined:Hellman and Small (1974):Odor detection threshold for propylene oxide: 9.9 ppmOdor detection threshold for *n*-butanol: 0.3 ppmCorrected odor detection threshold (OT_{50}) for propylene oxide: 9.9 ppm × 0.04 ppm/0.3 ppm = 1.32 ppmThe concentration (C) leading to an odor intensity (I) of distinct odor detection (I = 3) is derived by using the Fechner function:$I = k_w \times \log(C/OT_{50}) + 0.5$For the Fechner coefficient, the default of $k_w = 2.33$ is used because of the lack of chemical-specific data:$3 = 2.33 \times \log(C/1.32) + 0.5$which can be rearranged to$\log(C/1.32) = (3 - 0.5)/2.33 = 1.07$ and results in$C = (10^{1.07}) \times 1.32 = 11.8 \times 1.32 = 15.576$ ppmThe resulting concentration is multiplied by an empirical field correction factor. It takes into account that everyday life factors—such as sex, age, sleep, smoking, upper airway infections, and allergy as well as distraction—increase the odor detection threshold by a factor of 4. In addition, it takes into account that odor perception is very fast (about 5 s), which leads to the perception of concentration peaks. On the basis of current knowledge, a factor of 1/3 is applied to adjust for peak exposure. Adjustment for distraction and peak exposure lead to a correction factor of 4/3 = 1.33.LOA = C ×1.33 = 15.576 ppm × 1.33 = 20.7 ppm = 21 ppm. The LOA for propylene oxide is 21 ppm.

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APPENDIX F

ACUTE EXPOSURE GUIDELINE LEVELS FORPROPYLENE OXIDE

Derivation Summary for Propylene Oxide

AEGL-1 VALUES

10 min	30 min	1 h	4 h	8 h
73 ppm	73 ppm	73 ppm	73 ppm	73 ppm

Reference: Chemical Manufacturers Association (CMA 1998). Human Experience with Propylene Oxide. Prepared by Chemical Manufacturers Association for National Advisory Committee, (NAC)/AEGLs, October 16, 1998.

Test Species/Strain/Number: 3 male workers

Exposure Route/Concentrations/Durations: Inhalation: four propylene oxide exposure concentrations measured in the breathing zone of three workers: 380 ppm for 177 min, 525 ppm for 121 min, 392 ppm for 135 min, and 460 ppm for 116 min

Effects: A notation was made by the hygienist that a strong odor was present during sampling; however, the irritation was not intolerable. The nature of the irritation, other than the strong odor, was not provided, but occasional eye irritation was noted in the report as the reason for the monitoring program.

End Point/Concentration/Rationale: The AEGL-1 values are based on the average of four propylene oxide exposure concentrations measured in the breathing zone of the three workers, 440 ppm.

Uncertainty Factors/Rationale:
Total uncertainty factor: 3
  Interspecies: Not applicable
  Intraspecies: 3, the mechanism of toxicity, irritation, is not expected to differ greatly among individuals

Modifying Factor: 2, because the defined effects are above an AEGL-1 tier (undefined irritation), but below an AEGL-2 end point

Animal to Human Dosimetric Adjustment: Not applicable

Time-Scaling: Mild irritant effects are set equal across time

Data Adequacy: The AEGL-1 derivation would be improved if additional data on the degree of human irritation after exposure to propylene oxide were available. Animal studies reporting the severity of clinical signs at each respective exposure in multiple species would also be beneficial.

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Acute Exposure Guideline Levels for Selected Airborne Chemicals: Volume 9

AEGL-2 VALUES

10 min	30 min	1 h	4 h	8 h
440 ppm	440 ppm	290 ppm	130 ppm	86 ppm

Reference: National Toxicology Program (NTP 1985). Toxicology and Carcinogenesis Studies of Propylene Oxide (CAS No. 75-56-9) in F344/N Rats and $B6C3F_1$ Mice (Inhalation Studies). NTP TR 267, NIH 85-2527, U.S. Department of Health and Human Services, Public Health Service, National Institute of Health, National Toxicology Program, Research Triangle Park, NC.

Test Species/Strain/Sex/Number: five $B6C3F_1$ mice/sex/group

Exposure Route/Concentrations/Durations: Inhaled 0, 387, 859, 1,102, 1,277, or 2,970 ppm for 4 h

Effects:

Conc. (ppm)	Mortality Males	Females	Other Effects
387	0/5	1/5	Dyspnea
859	0/5	0/5	Dyspnea1
102	2/5	4/5	Dyspnea1
277	2/5	5/5	Dyspnea, sedation
2,970	5/5	5/5	Dyspnea, sedation, lacrimation

End Point/Concentration/Rationale: 387 ppm for 4 h based on dyspnea; dyspnea in mice is the most sensitive end point, and mice are the most susceptible species. Although a no-effect level was not established at this concentration, no other adverse effects were noted. This NTP (1985) study reported toxic effects at much lower concentrations than those observed in other studies. The death of a mouse at 387 ppm did not appear to be exposure related.

Uncertainty Factors/Rationale:
Total uncertainty factor: 3
Interspecies: 1, mice are the most sensitive species tested in terms of lethality and clinical signs of toxicity, and available data indicate that mice are equally or slightly more sensitive than humans in manifesting clinical signs. The clinical sign of dyspnea was by far the most sensitive end point. This NTP (1985) study reported toxic effects at much lower concentrations than those observed in other studies.
Intraspecies: 3, The mechanism of toxicity, irritation, is a point-of-contact effect and is not expected to differ greatly among individuals.

Modifying Factor: Not applicable

Animal to Human Dosimetric Adjustment: Not applicable

Time-Scaling: Although the mechanism of action appears to be a direct irritant effect, it is not appropriate to set the values equal across time because the irritation is part of the continuum of respiratory tract irritation leading to death. The experimentally derived exposure value was therefore scaled to AEGL timeframes by using the concentration-time relationship given by the equation $C^n \times t = k$, where C is concentration, t is time, k is a constant, and n is 1.7 as calculated by using the rat lethality data reported by Rowe et al. (1956) (ten Berge et al. 1986). The 10-min value was set equal to the 30-min value because of the uncertainty in extrapolating from the exposure duration of 4 h to 10 min.

(Continued)

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AEGL-2 VALUES Continued

10 min	30 min	1 h	4 h	8 h
440 ppm	440 ppm	290 ppm	130 ppm	86 ppm

Data Adequacy: Limited data consistent with a defined AEGL-2 end point were available. Animal studies reporting clinical signs often did not report the severity of the signs at each exposure concentration but rather gave only a general statement. Additional data consistent with a defined AEGL-2 end point in multiple species would be helpful in further defining the AEGL-2 levels.

AEGL-3 VALUES

10 min	30 min	1 h	4 h	8 h
1,300 ppm	1,300 ppm	870 ppm	390 ppm	260 ppm

Reference: NTP 1985. Toxicology and Carcinogenesis Studies of Propylene Oxide (CAS No. 75-56-9) in F344/N Rats and B6C3F_1 Mice (Inhalation Studies). NTP TR 267, NIH 85-2527, U.S. Department of Health and Human Services, Public Health Service, National Institute of Health, National Toxicology Program, Research Triangle Park, NC.

Test Species/Strain/Sex/Number: five F344/N rats/sex/group

Exposure Route/Concentrations/Durations: inhaled 0, 1,277, 2,970, 3,794, or 3,900 ppm for 4 h

Effects:

Conc. (ppm)	Mortality Males	Mortality Females	Other Effects
1,277	0/5	0/5	None observed
2,970	1/5	2/5	Dyspnea, red nasal discharge
3,794	4/5	4/5	Dyspnea, red nasal discharge
3,900	3/5	3/5	Dyspnea, red nasal discharge

Calculated $BMCL_{05}$: 1,161 pm

End Point/Concentration/Rationale: The 4-h $BMCL_{05}$ of 1,161 ppm was used as the point of departure.

Uncertainty Factors/Rationale:
Total uncertainty factor: 3
Interspecies: 1, based on supporting data in dogs (similar 4-h $BMCL_{05}$ of 1,116 ppm) and a 2-year study in primates that demonstrated no mortality at 300 ppm for 6 h/day, 5 days/week
Intraspecies: 3, the mechanism of toxicity, irritation, is a point of contact effect and is not expected to differ greatly among individuals.

Modifying Factor: NA

Animal to Human Dosimetric Adjustment: Not applicable

Time-Scaling: As for the AEGL-2 derivation, the experimentally derived exposure value for the AEGL-3 derivation was scaled to AEGL timeframes by using the concentration-time relationship given by the equation $C^n \times t = k$, where C is concentration, t is time, k is a constant, and n is 1.7 as calculated by using the rat lethality data reported by Rowe et al. (1956) (ten Berge et al. 1986). The value was extrapolated across time because the

(Continued)

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AEGL-3 VALUES Continued

10 min	30 min	1 h	4 h	8 h
1,300 ppm	1,300 ppm	870 ppm	390 ppm	260 ppm
irritation is no longer considered mild; the concentration represents the threshold for lethality. The 10-min value was set equal to the 30-min value because of the uncertainty in extrapolating from the exposure duration of 4 h to 10 min.				
Data Adequacy: Data were adequate for derivation of an AEGL-3. The resulting values were supported by dog data (similar no-effect level of mortality in a nonobligate nose breather; Jacobson et al. 1956); monkey data, 300 ppm 6 h/day for 2 years not lethal (Sprinz et al. 1982; Lynch et al. 1983; Setzer et al. 1997); 457 ppm for 7 h/day for 154 days not lethal (Rowe et al. 1956); and human data (exposure to 1,520 ppm for 171 min not lethal) (CMA 1998).				

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6

Xylenes[1]

Acute Exposure Guideline Levels

PREFACE

Under the authority of the Federal Advisory Committee Act (FACA) P.L. 92-463 of 1972, the National Advisory Committee for Acute Exposure Guideline Levels for Hazardous Substances (NAC/AEGL Committee) has been established to identify, review, and interpret relevant toxicologic and other scientific data and develop AEGLs for high-priority, acutely toxic chemicals. AEGLs represent threshold exposure limits for the general public and are applicable to emergency exposure periods ranging from 10 minutes (min) to 8 hours (h). Three levels—AEGL-1, AEGL-2, and AEGL-3—are developed for each of five exposure periods (10 and 30 min and 1, 4, and 8 h) and are distinguished by varying degrees of severity of toxic effects. The three AEGLs have been defined as follows:

AEGL-1 is the airborne concentration (expressed as parts per million [ppm] or milligrams per cubic meter [mg/m^3]) of a substance above which it is predicted that the general population, including susceptible individuals, could experience notable discomfort, irritation, or certain asymptomatic, nonsensory

[1]This document was prepared by the AEGL Development Team composed of Claudia Troxel (Oak Ridge National Laboratory) and Chemical Manager Loren Koller (National Advisory Committee [NAC] on Acute Exposure Guideline Levels for Hazardous Substances). The NAC reviewed and revised the document and AEGLs as deemed necessary. Both the document and the AEGL values were then reviewed by the National Research Council (NRC) Committee on Acute Exposure Guideline Levels. The NRC committee has concluded that the AEGLs developed in this document are scientifically valid conclusions based on the data reviewed by the NRC and are consistent with the NRC guideline reports (NRC 1993, 2001).

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effects. However, the effects are not disabling and are transient and reversible upon cessation of exposure.

AEGL-2 is the airborne concentration (expressed as ppm or mg/m^3) 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.

AEGL-3 is the airborne concentration (expressed as ppm or mg/m^3) of a substance above which it is predicted that the general population, including susceptible individuals, could experience life-threatening health effects or death.

Airborne concentrations below the AEGL-1 represent exposure levels that could produce mild and progressively increasing but transient and nondisabling odor, taste, and sensory irritation or certain asymptomatic, nonsensory effects. With increasing airborne concentrations above each AEGL, there is a progressive increase in the likelihood of occurrence and the severity of effects described for each corresponding AEGL. Although the AEGLs represent threshold levels for the general public, including susceptible subpopulations, such as infants, children, the elderly, persons with asthma, and those with other illnesses, it is recognized that individuals, subject to idiosyncratic responses, could experience the effects described at concentrations below the corresponding AEGL.

SUMMARY

Xylene is found in a number of consumer products, including solvents, paints and coatings, and as a blend in gasoline. Mixed xylenes are composed of three isomers: *m*-xylene, *o*-xylene, and *p*-xylene, with the *m*-isomer predominating. Ethylbenzene is also present in the technical product formulation. Absorbed xylene is rapidly metabolized and is excreted almost exclusively in the urine as methylhippuric acid isomers in humans and as methylhippuric acid isomers and toluic acid glucuronides in animals. Xylene causes mucus irritation and affects the central nervous system (CNS) in humans and animals after acute inhalation exposure. Hepatic effects have been noted in humans after acute inhalation exposure to high concentrations and in rats after subchronic oral or inhalation exposure. No consistent developmental or reproductive effects were observed in the studies found in the available literature. Commercial xylene and all three isomers have generally tested negative for genotoxicity. Xylenes are currently not classifiable as to carcinogenicity by the International Agency for Research on Cancer (IARC) or the Environmental Protection Agency (EPA).

The AEGL-1 is based on the no-effect level for notable discomfort in human subjects. Only mild eye irritation was noted during a 30-min exposure to mixed xylenes at 400 ppm (Hastings et al. 1984). An interspecies uncertainty factor was not applied because the key study used human data. An intraspecies uncertainty factor of 3 was applied because slight eye irritation is caused by a

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direct effect of the chemical and the response is not expected to vary greatly among individuals. Because irritation is considered a threshold effect, which should not vary over time, the AEGL-1 value was not scaled across time, but rather the same value is applied at all times. The resulting value of 130 ppm is supported by several other studies, including a 150-ppm *p*-xylene exposure resulting in eye irritation in a contact lens wearer (Hake et al. 1981); a 15-min exposure to mixed xylenes at 230 ppm resulting in mild eye irritation and dizziness with no loss of coordination in one individual (Carpenter et al. 1975b); and a 3-h exposure to *m*- or *p*-xylene at 200 ppm (Ogata et al. 1970), a 4-h exposure to *m*-xylene at 200 ppm (Savolainen et al. 1981), and a 5.5-h exposure to *m*-xylene at 200 ppm (Laine et al. 1993), all representing no-effect levels for notable discomfort.

The AEGL-2 is based on the no-effect level for impaired ability to escape. During a 4-h exposure to mixed xylenes at 1,300 ppm, rats developed poor coordination (slight coordination loss) after 2 h of exposure, returning to normal coordination postexposure (Carpenter et al. 1975b). The point of departure of 1,300 ppm for 2 h therefore represents the threshold for reversible equilibrium disturbances and the no-effect level for impaired ability to escape. This concentration and end point are consistent with the preponderance of available data for 4-h exposures in rats: the median effective concentration (EC_{50}) for decreased rotarod performance was 1,982 ppm (Korsak et al. 1993); the minimum narcotic concentration for *m*-, *o*-, and *p*-xylene ranged from 1,940 to 2,180 ppm (Molnár et al. 1986); and exposure to *p*-xylene at 1,600 ppm resulted in hyperactivity, fine tremor, and unsteadiness (Bushnell 1989) and caused changes in the flash evoked potential suggestive of increased arousal (Dyer et al. 1988). It is assumed that the CNS response observed after xylene exposure is directly related to the concentration of parent material reaching the brain, and that venous blood concentrations (CV) correlate with brain concentrations. Therefore, the CV of xylene after a 2-h exposure to xylene at 1,300 ppm is expected to provide an internal dose measurement correlating with the clinical sign of poor coordination. With a physiologically based pharmacokinetic (PBPK) model (see Appendix C), the internal dose (CV) producing impaired coordination in rats was determined. Then, the human PBPK model was run for each defined AEGL time period to determine the equivalent exposure concentration producing the target CV.

The AEGL-3 derivation is based on reversible prostration in rats and a no-observed-effect level (NOEL) for death in rats exposed to 2,800 ppm for 4 h (Carpenter et al. 1975b). Although coordination initially remained poor, it returned to normal the next day. This concentration represents a threshold for marked CNS depression, which could lead to death. As for the AEGL-2, it is assumed that the CNS effects observed after xylene exposure are directly related to the concentration of parent material reaching the brain. Therefore, PBPK modeling (see Appendix C) was again used to calculate the internal dose (CV) correlating with an exposure of rats to 2,800 ppm for 4 h that produced prostra-

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tion. The human PBPK model was then run for each defined AEGL time period to determine the equivalent exposure concentration producing the target CV.

A total uncertainty factor of 3 was applied to the AEGL-2 and -3 dose metrics. An intraspecies uncertainty factor of 3 was applied for the pharmacokinetic and pharmacodynamic uncertainty because the minimum alveolar concentration (MAC) for volatile anesthetics should not vary by more than 2- to 3-fold among humans (NRC 2002). An interspecies uncertainty factor of 3 would usually be applied. PBPK modeling reduced the toxicokinetic component of the uncertainty factor to 1, but the pharmacodynamic component would normally be retained and assigned a 3 (although it appears that similar CNS effects occur in humans and animals, it is not known if they occur at the same tissue dose). A total uncertainty factor of 10, however, drives the 8-h AEGL-2 value to 180 ppm and the 4-h AEGL-3 value to 447 ppm. These amounts are exposure concentrations that humans are known to tolerate with minimal or no adverse effects. With regard to the AEGL-2, humans exposed to *p*-xylene at 150 ppm for 7.5 h exhibited no effects on performance tests and noted only mild eye irritation (Hake et al. 1981). With regard to the AEGL-3, numerous human studies investigated the effects of exposure to *m*-xylene at 130 to 200 ppm for 4 to 6 h, with 20-min peaks of 400 ppm with or without exercise, and found no effect or reported minimal CNS effects (Savolainen and Linnavuo 1979; Savolainen et al. 1984, 1985a,b; Seppalainen et al. 1989, 1991; Laine et al. 1993). Therefore, the interspecies uncertainty factor is reduced to 1, and a total uncertainty factor of 3 is applied to the AEGL-2 and AEGL-3 values (NRC 2002).

The proposed xylene AEGL values apply to all three xylene isomers or a mixture of xylene isomers. No significant differences in the potency of the isomers after oral or inhalation exposure were identified, metabolism of each isomer proceeds via the same pathways, and PBPK model predictions indicate that the internal dose (CV) after exposure does not vary significantly among the individual isomers.

The AEGL values are listed in Table 6-1. AEGL-2 and AEGL-3 values are greater than 10% of the lower explosive limit.

1. INTRODUCTION

Commercial or mixed xylene is composed of three isomers: *meta*-xylene (*m*-xylene), *ortho*-xylene (*o*-xylene), and *para*-xylene (*p*-xylene), of which the *m*-isomer usually predominates (40% to 70% of the mixture) (Fishbein 1988; ATSDR 2007). The exact composition of the isomers depends on the xylene formulation. Ethylbenzene is often present in mixed xylenes; in fact, the technical product contains approximately 40% *m*-xylene and approximately 20% each *o*- and *p*-xylene and ethylbenzene (Fishbein 1988). Other minor contaminants of xylene include toluene and C_9 aromatic fractions. Mixed xylenes are used in production of the individual isomers or ethylbenzene, as a solvent, in paints and coatings, and as a blend in gasoline (Fishbein 1988; ATSDR 2007). The annual

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production capacity of mixed xylenes has been estimated at 13.1 billion pounds, with 1990 and 1991 production estimates of about 6 billion pounds (ATSDR 2007). The individual isomers are used primarily as chemical intermediates (OECD 2003). Almost all *o*-xylene produced in the United States is consumed in the manufacture of phthalic anhydride. Other minor uses include the use of *o*-xylene as a feedstock in the production of bactericides, soybean herbicides, and dyes. Most *m*-xylene is used as a chemical intermediate in the production of isophthalic acid. Small amounts of *m*-xylene are also consumed in the production of *m*-tolic acid, isophthalonitrile, and other compounds. Almost all U.S. production of *p*-xylene is consumed in the manufacture of dimethyl terephthalate and terephthalic acid, which are used in the production of polyester fiber and plastics.

The physical and chemical properties of xylenes are presented in Table 6-2. The odor of xylenes is described as an aromatic hydrocarbon odor. The odor threshold ranges between 0.8 and 40 ppm, and the irritating concentration is 100 ppm (Ruth 1986).

TABLE 6-1 Summary of Proposed AEGL Values for Xylenes

Classification	10 min	30 min	1 h	4 h	8 h	End Point (Reference)
AEGL-1 (Nondisabling)	130 ppm (560 mg/m^3)	130 ppm (560 mg/m^3)	130 ppm (560 mg/m^3)	130 ppm (560 mg/m^3)	130 ppm (560 mg/m^3)	Eye irritation in human volunteers exposed to mixed xylenes at 400 ppm for 30 min (Hastings et al. 1984)
AEGL-2 (Disabling)	2,500 ppm[a] (11,000 mg/m^3)	1,300 ppm[a] (5,600 mg/m^3)	920 ppm[a] (4,000 mg/m^3)	500 ppm (2,200 mg/m^3)	400 ppm (1,700 mg/m^3)	Rats exposed to mixed xylenes at 1,300 ppm exhibited poor coordination 2 h into a 4-h exposure (Carpenter et al. 1975b)
AEGL-3 (Lethal)	—[b]	3,600 ppm[a] (16,000 mg/m^3)	2,500 ppm[a] (11,000 mg/m^3)	1,300 ppm[a] (5,600 mg/m^3)	1,000 ppm[a] (4,300 mg/m^3)	Rats exposed to mixed xylenes at 2,800 ppm for 4 h exhibited prostration followed by a full recovery (Carpenter et al. 1975b)

[a]Concentrations are at or higher than 1/10th of the lower explosive limit (LEL) for all forms of xylene (*o*-xylene LEL, 9,000 ppm; *m*- and *p*-xylene LEL, 11,000 ppm). Therefore, safety considerations against the hazard of explosion must be taken into account.
[b]10-min AEGL-3 = 7,200 ppm is ≥50% of LEL. Therefore, extreme safety considerations against the hazards of explosions must be taken into account.

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TABLE 6-2 Physical and Chemical Data for Xylenes

Parameter	Value	Reference
Synonyms	Dimethylbenzene (1,2-; 1,3-; or 1,4-); xylol, *m*-xylene (*m*-isomer); *o*-xylene (*o*-isomer); *p*-xylene (*p*-isomer); methyltoluene	ACGIH 1991; Budavari et al. 1996
CAS registry no.	1330-20-7 108-38-3 (*m*-isomer) 95-47-6 (*o*-isomer) 106-42-3 (*p*-isomer)	
Chemical formula	C_8H_{10}	Budavari et al. 1996
Molecular weight	106.17	Budavari et al. 1996
Physical state	Liquid	Budavari et al. 1996
Color	Colorless	Budavari et al. 1996
Melting point	No data for mixture −47.4°C (*m*-isomer) −25°C (*o*-isomer) 13-14°C (*p*-isomer)	Budavari et al. 1996
Boiling point	137-140°C	Budavari et al. 1996
Solubility	Practically insoluble in water; 106 mg/L at 25°C	ATSDR 2007
Vapor pressure at 21°C	6.72 mmHg	ATSDR 2007
Density	0.864 g/cm^3	ATSDR 2007
Log K_{OW}	3.12-3.20	ATSDR 2007
Conversion factors in air	1 ppm = 4.34 mg/m^3 1 mg/m^3 = 0.23 ppm	NRC 1984

2. HUMAN TOXICITY DATA

2.1. Acute Lethality

Three men were employed to paint a double-bottomed tank in the engine room of a ship (Morley et al. 1970). Solvent composed 34% of the total weight of the paint, with xylene composing in excess of 90% of the solvents, and only a trace amount of toluene was present. The men started work at 10:30 am, and after being reported missing later that evening were found unconscious at 5:00 am the next day. The first patient was dead upon admission to the hospital. Autopsy revealed severe pulmonary congestion with focal alveolar hemorrhage and acute pulmonary edema, hepatic congestion with swelling and vacuolization of many cells in the centrilobular areas, and microscopic petechial hemorrhages in

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both the gray and white matter of the brain. In addition, evidence of axonal neuronal damage was indicated by swelling and loss of Nissl substance. The second patient was admitted to the hospital unconscious, exhibiting only a slight response to painful stimuli. He was also hypothermic, had a flushed face, and had peripheral cyanosis. Medium-grade moist sounds were present in his lungs, and a chest x-ray revealed patchy diffuse opacity in both lungs. Five hours after treatment with tracheal aspiration and oxygen, the patient regained consciousness, but was amnesic for 2 to 3 days. Evidence of renal damage was indicated by an increase in blood urea of 59 mg/100 milliliters (mL) to 204 mg/100 mL 3 days after admission. Endogenous creatinine clearance was also reduced at this time. Slight hepatic impairment was indicated by a rise in serum transaminase activity to 100 international units (IU) over 48 h, followed by a return to normal levels. Patient 3 recovered consciousness after admission and was confused and amnesic, had slurred speech, and was ataxic upon walking. Within 24 h of admission, he was fully conscious and alert, and the ataxia disappeared over 48 h. There was no evidence of renal impairment, and slight hepatic impairment was indicated by a slight rise in serum transaminase activity (52 IU) over 48 h, followed by a return to normal levels.

The circumstances of the accident were re-created by the study authors. On the basis of the quantity of paint applied, the volume of the space, and the assumption of still air conditions (based on the limited ventilation present), the probable xylene concentration was estimated to have been 10,000 ppm. Although two cans of cleaning fluid composed primarily of toluene were also found at the scene, neither of the survivors remembered using the cleaning fluid. Therefore, it was assumed by the study authors that exposure was mainly to xylene.

2.2. Nonlethal Toxicity

2.2.1. Case Reports

Two case reports of seizures after exposure to xylene-based products have been reported in the literature. Goldie (1960) reported a case in which eight painters were exposed to paint containing 80% xylene and 20% methylglycolacetate. When they were painting the inside of a gun tower, adequate ventilation was not present because the ventilation system created too great a draft for painting. The workers complained of headache, vertigo, gastric discomfort, dryness of the throat, and slight drunkenness after 30 min of exposure; therefore, the painters worked in the unventilated area for 30 min at a time followed by 10-min breaks to breathe fresh air. After working for about 2 months, an 18-year-old male exhibited behavior indicative of a convulsive seizure one day after leaving work. Signs included weakness, dizziness, inability to speak, unconsciousness, eyes and head rotated to one side, chewing but no foaming, and short, sharp interrupted jerks of the upper and lower limbs. The patient recov-

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ered consciousness 20 min later. Although the patient experienced another shorter seizure after admission to the hospital, hospital tests were unable to confirm the diagnosis. In another case, Arthur and Curnock (1982) reported that an adolescent male developed major and minor seizures after using a xylene-based glue used for building model airplanes. Neither case report provides an exposure concentration, and exposures were not limited to xylene.

Klaucke et al. (1982) reported that during work one day, 15 male and female employees of a small community hospital reported at least two of the following symptoms lasting from 2 to 48 h: headache, nausea, vomiting, dizziness or vertigo, eye irritation, and nose or throat irritation. The frequency of the symptoms was as follows: headache, 12/15; nausea, 10/15; eye irritation, 8/15; nose or throat irritation, 7/15; dizziness or vertigo, 7/15; and vomiting, 6/15. Fourteen of the 15 employees noted an unusual odor 15 to 30 min before the onset of symptoms. After investigation, it was determined that the "illness" was caused when 1 L of liquid xylene was poured down a drain in a pathology laboratory, and the vapors were then drawn into the room, which contained a ventilation fan that distributed the vapors throughout the affected area of the hospital. It was estimated that workers were exposed to levels as high as 700 ppm.

2.2.2. Controlled Exposures

Twenty-three male volunteers (mean age 23 years) were divided in groups of four or five and exposed to air containing measured concentrations of *m*-xylene, *p*-xylene, or toluene at 100 or 200 ppm for 3 h or for 7 h with a 1-h lunch break (Ogata et al. 1970). Vapor concentrations were analyzed every half-hour by gas chromatography. Systolic and diastolic blood pressure, pulse rate, flicker value, and reaction time were assessed in all volunteers at the beginning and end of exposures. Exposure to *m*- or *p*-xylene did not significantly influence any of these parameters.

Six or seven7 volunteers (21 to 60 years of age; sex not provided) were exposed to air containing measured concentrations of mixed xylenes (*p*-xylene, 7.84%; *m*-xylene, 65.01%; *o*-xylene, 7.63%; ethylbenzene, 19.27%) for 15 min in the following order: 230, 110, 460, or 690 ppm, with exposures limited to one per day (Carpenter et al. 1975a,b). Volunteers provided written responses at 1-min intervals throughout the 15-min exposure. Xylene concentration was analyzed by gas chromatography. Results of the exposure are summarized in Table 6-3. Complaints at 110 ppm were limited to mild throat discomfort in one volunteer during the first and seventh minute of exposure; this individual did not experience discomfort during exposure to 230 ppm. Exposure to 230 ppm resulted in one volunteer complaining of eye irritation during the 4th, 5th, and 15th minute of exposure; another noting sleepiness at the 13th minute followed by eye wetness (but no tears formed) at the 14th and 15th minute of exposure; one volunteer reporting possible mild nasal irritation; and another volunteer reporting

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TABLE 6-3 Xylene Irritation Thresholds (Subjects Exposed for 15 Min)

Condition of Subjects	Concentrations (ppm)			
	110	230	460	690
Volunteers	6	7	6	6
Throat irritation	1	0	1	2
Eye irritation	0	1	4	4
Tears	0	1	1	2
Dizziness and light-headedness	0	1	1	4

Source: Carpenter et al. 1975b. Reprinted with permission; copyright 1975, *Toxicology and Applied Pharmacology*.

intermittent dizziness and light-headedness (with no loss of coordination) during the last 2 min of exposure. At 460 ppm, four volunteers reported intermittent or continuous mild eye irritation, with one additionally reporting eye wetness when leaving the chamber; one volunteer noted mild dizziness at the sixth minute of exposure that persisted throughout the 15-min exposure (the same individual who noted dizziness at 230 ppm); and one volunteer reported possible mild nasal and throat irritation (the same individual who reported nasal irritation at 230 ppm). Exposure to 690 ppm resulted in dizziness and light-headedness in four volunteers. Three of the volunteers reported the dizziness to be mild and not associated with a loss of balance, while the other volunteer reported a slight loss of balance. Eye, nose, and throat irritation was noted during exposure but ceased within 10 min postexposure. Carpenter and associates (1975b) concluded that exposure to xylene at 100 ppm would not be objectionable to most people, while none of the volunteers thought that 690 ppm could be tolerated over an 8-h work day.

Volunteer male college students 18 to 30 years old were exposed to air containing mixed xylenes at 0, 100, 200, or 400 ppm (0, 0.43, 0.86, and 1.72 mg/L) for 30 min (*p*-xylene, 7.84%; *m*-xylene, 65.01%; *o*-xylene, 7.63%; ethylbenzene, 19.27%) (Hastings et al. 1984). The students were exposed using an olfactometer delivery hood made of transparent Lucite, which allowed adequate air flow. Solvent or distilled water (for control exposures) was delivered with a motorized syringe, and heating tapes vaporized the solvent or water before introduction into the hood. Samples of air taken from the breathing zone in the hood were analyzed by gas chromatography for the actual exposure concentrations and were acceptable. A contact electrode was taped near the skin of the outer canthus of one eye on each subject to measure eye blinks, and an indifferent electrode was clipped to the ipsilateral ear. Respiratory measurements were recorded with the aid of a thermistor placed near one naris. Behavioral tests, two measuring psychomotor performance (consisting of the Michigan Eye-Hand Coordination Test and a visuomotor-skill TV game) and one measuring cognitive performance (choice reaction time), were administered before, during, and

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after exposure (10 min after placement in the hood when exposed to control air, during the last 5 min of the 30-min exposure to xylene, and 10 min after the subjects were again exposed to control air for 10 min). The reader is referred to the study for additional details about the behavioral testing. The subjects were asked every 5 min during the experiment if they detected any odor or experienced eye, nose, or throat irritation.

The only clear concentration-related effect of exposure in the Hastings et al. (1984) study was mild eye irritation reported by 56%, 60%, 70%, and 90% of the subjects in the 0-, 100-, 200-, and 400-ppm groups, respectively (not statistically significant). No concentration-related increase in the percentage of exposed subjects experiencing nose or throat irritation was observed and the number of eye blinks per minute and respiration rate (breaths per minute) were not statistically increased in any of the exposure groups compared with the controls, confirming that the reported irritation was mild. No statistically significant differences in the performance of the behavioral tasks by the exposed subjects were observed compared with controls.

Gamberale et al. (1978) conducted two series of experiments assessing the effects of xylene exposure in healthy male volunteers age 21 to 33 years. In the first investigation, groups of five males were exposed to xylene at 0, 100, or 300 ppm for 70 min on day 1, 2, or 3, with the sequence of the exposure balanced among the three groups (on day 1, groups 1, 2, and 3 were exposed to 0, 300, and 100 ppm, respectively). In the second investigation, a group of eight volunteers (who had also participated in the first series) were exposed to xylene at 300 ppm for 70 min; they exercised four times per day on a bicycle equipped with an ergometer at 100 watts [W]) for the first 30 min of exposure and sat in a chair the remaining 40 min of exposure. In both experiments, a breathing valve with low resistance was used to supply the air or xylene, and menthol crystals were placed in the tube of the mouthpiece to mask the odor of solvent. A total hydrocarbon analyzer was used to continuously measure the inspired xylene concentration during exposure, and a gas chromatographic technique was used to measure the alveolar air concentration of xylene (further details were not provided). Heart rate was checked regularly. Five performance tests were administered to volunteers during the exposures: one administered at the beginning of the exposure period and all five during the last 35 min of exposure. The performance tests included critical flicker fusion, reaction time addition, simple reaction time, short-term memory, and choice reaction time. All the tests utilized visual stimulation with electronic recording of responses. After each exposure trial, subjects were asked to fill out a questionnaire addressing subjective symptoms experienced during exposures.

The concentration of xylene in the alveolar air at 30 and 70 min of exposure corresponded to the nominal concentration: the alveolar air concentration in the 300-ppm group was three times that of the 100-ppm group (Gamberale et al. 1978). After subjects exercised during exposure to 300 ppm, the alveolar xylene concentration increased 3.7- and 2.2-fold at 30 and 70 min, respectively, compared with exposure to 300 ppm at rest. No exposure-related changes in heart

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rate were observed. Although a slight increase in the frequency of headache and feeling of "sickness" were noted, the number of subjects with these complaints was not provided. However, the authors stated that most subjects reported no or negligible symptoms. Xylene exposure at rest did not significantly affect the results of the performance tests of subjects exposed to xylene at 100 or 300 ppm. When xylene exposure was combined with 100 W of work, impaired performance was observed on all tests, significantly so ($p < 0.05$) in the reaction time addition test and the short-term memory test (further details not provided).

Exposure of groups of four male volunteers to *p*-xylene at 70 ppm, toluene at 80 ppm, or a combination of toluene at 50 ppm and *p*-xylene at 20 ppm for 4 h did not affect the results of choice reaction time, simple reaction time, or short-term memory performance tests as assessed by microcomputers immediately upon entry into the exposure chamber, after 2 h of exposure, or after 4 h of exposure compared with control air exposure (Olson et al. 1985). Solvent exposure did not affect heart rate or the reporting of subjective symptoms recorded by questionnaire at the end of the exposures.

Groups of two healthy male volunteers aged 22 to 35 years were exposed in random sequence to air containing toluene at 100 ppm, xylene at 100 ppm, a mixture of toluene at 50 ppm and xylene at 50 ppm, or control air for 4-h sessions, with each exposure session separated by 7-day intervals (Dudek et al. 1990). No information about the purity or composition of xylene was provided. Exposures occurred in a chamber, with the test solvent concentrations controlled by monitoring with gas chromatography and infrared spectrophotometry. Terpon vapors were used to mask the odor of the test solvents. A battery of nine psychological tests was used to evaluate the effects of the solvents on the subjects during exposure. The tests evaluated memory (Sperling's test), interference of cognitive processes (Stroop's test), cognitive processes (Sternberg's test), motor-visual coordination (Flanagan's test), speed and precision of hand movements (aiming), psychomotor efficiency (simple reaction time, choice reaction time, and Santa Ana), and mood (profile of mood state). The volunteers completed a training session on these performance tests 1 week before the exposure. On the day of the exposure, the performance tests were administered 1 h before exposure, at the commencement of exposure, and 3 h into the exposure; only the results with xylene are reported here. Xylene exposure for 3 h resulted in significant reductions in performance of the simple reaction time test (prolongation of simple reaction time; $p < 0.001$) and the choice reaction time test ($p < 0.001$). No statistically significant effects were observed in any of the other psychological tests.

An average of 10 subjects (mix of males and females) were exposed to xylene in a 1,200-cubic-foot gas chamber for 3 to 5 min, and the level of irritation experienced by the subjects was recorded upon exit from the chamber (Nelson et al. 1943). Further experimental details were not provided. The study authors reported that exposure to xylene at 200 ppm resulted in eye, nose, and throat irritation in most subjects and it was classified as objectionable.

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In 1981, Hake et al. published the results of a study that established the relationship between exposure concentration and *p*-xylene body burden as measured by urine, blood, breath, and saliva, and they evaluated the effects of repeated *p*-xylene vapor exposure in humans. Nine adult Caucasian males and seven adult Caucasian females were exposed at rest to *p*-xylene vapors. The subjects were subdivided into three daily groups for 7.5, 3, or 1 h of daily exposures. Males and females were exposed the first week (five consecutive days) to *p*-xylene at 100 ppm. Males were also exposed to 20 ppm the second week, 150 ppm the third week, and fluctuating concentrations of 50 to 150 ppm (for a time-weighted average [TWA] of 100 ppm) the fourth week. Subjects were exposed to 0 ppm Thursday and Friday of the week preceding xylene exposure, and Monday and Tuesday of the week following the last week of xylene exposure to provide control data. Exposures were conducted in a controlled environment chamber (20 × 20 × 8 feet). The *p*-xylene vapor was introduced into the chamber's circulating air by a stream of air-sweeping vapor from a warm flask. The *p*-xylene chamber vapor concentrations were continuously monitored and an infrared spectrometer with a gas chromatograph served as a back-up monitor. End points selected to evaluate *p*-xylene included neurologic testing (modified Romberg, heel-to-toe test, electroencephalogram [EEG], and visual evoked potentials [VEPs]), cardiopulmonary function tests, cognitive testing (Flanagan coordination test, time estimation tests, arithmetic test, inspection test), and subjective responses (noted by these volunteers during the exposure or during the first 3 h of exposure).

Irritation was the only subjective response noted that was related to xylene exposure (Hake et al. 1981). In males, eye irritation was noted seven times and eight times during the week-long exposures to 100 and 150 ppm, respectively, compared with three mentions during the four control days. Of these notations, one individual in the 7.5-h exposure group wearing contact lenses noted eye irritation almost every day, while another subject complained twice at 100 ppm and three times at 150 ppm. No irritation was noted by males during any 3-h exposure, but one subject complained of eye irritation during a 1-h exposure to 150 ppm. No visible reddening of the eyes or conjunctiva was observed. Irritation was also noted by females, but it was confined to nose and throat irritation. During the 5-day exposure to 100 ppm, irritation was noted 17 times, compared with 5 times during two control days. No significant neurologic, cardiopulmonary, or cognitive abnormalities were definitively correlated with exposure to any concentration of *p*-xylene. Although a decrement in the performance of the Flanagen coordination test was noted in males exposed to *p*-xylene at 150 ppm for 7.5 h/day, the decrement was almost entirely due to the performance of one subject who had previously been ill. The changes in EEG activity (increase in delta activity) observed in males exposed for 7.5 h to *p*-xylene at 100 or 150 ppm could not definitely be ascribed to exposure because the changes were not evident during every exposure and were not correlated with exposure concentration.

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Nine male student volunteers (aged 20 to 25 years) were exposed at rest to *m*-xylene at 200 ppm, trichloroethane at 200 or 400 ppm, or a combination of xylene at 200 ppm and trichloroethane at 400 ppm for 4 h/day, once a week, with a 6-day interval between succeeding exposures over six consecutive weeks (Savolainen et al. 1981; Seppalainen et al. 1983). The exposures were single blind, with each subject acting as his own control. The end points examined by each author were body sway, reaction times, flicker fusion, and subjective symptoms (Savolainen et al. 1981) and pattern VEP (Seppalainen et al. 1983). Body sway along the anteroposterior and lateral axes was recorded with a strain gauge platform with the eyes open and closed and was measured 1 h before exposure and after 20 min and 3.75 h of exposure. Reaction time (manual response to stimuli) and tapping of the dominant hand was measured before exposure, and after 1 and 3 h of exposure. Flicker fusion was assessed before exposure and at 1.5 and 3.5 h of exposure. Pattern VEP was measured before exposure and 5 to 30 min after exposure ended. Exposure to xylene alone did not result in any marked adverse effects. A slight improvement in performance was observed as a slight decrease in body sway and slightly shortened reaction time (Savolainen et al. 1981). No effects on tapping speed were observed, and a slight increase in the critical fusion thresholds were noted in the afternoon sessions (Savolainen et al. 1981). No statistically significant effects were observed in the pattern VEP after exposure to *m*-xylene (Seppalainen et al. 1983).

Nine male student volunteers (mean age 21 years) in three groups of three, were exposed for 3 h in the morning and 40 min in the afternoon, with a 40-min lunch break, to air containing a fixed concentration of *m*-xylene at 200 ppm, a basal concentration of *m*-xylene at 135 ppm with 20-min peak concentrations of 400 ppm at the beginning of the morning and afternoon sessions, or to control air (Seppalainen et al. 1989, 1991; Laine et al. 1993). The subjects were exposed sedentary or with 10 min of exercise (100 W) at the beginning of each exposure session. The exposures occurred on six separate days, with a minimum of a 5-day interval separating the xylene exposures. The subjects were exposed in a dynamic chamber, and the concentration of atmospheric xylene was continuously monitored (further details not provided). Peppermint oil was used to mask control exposure and the solvent odor, with the experiment being a single-blind experiment with crossover design, with each subject acting as his own control. The end points examined by each author were VEPs and brainstem auditory evoked potentials (BAEPs) (Seppalainen et al. 1989), EEG recordings (Seppalainen et al. 1991), and body sway and reaction times (Laine et al. 1993).

VEPs were recorded in the morning before the subjects entered the exposure chamber and at the end of the morning and afternoon exposure session (Seppalainen et al. 1989). For pattern VEPs (pattern reverse stimulus), latencies of P50, N70, P100, N135, P170, and the peak-to-peak amplitude of N70 to P100 were measured. For flash VEPs (light flash), latencies of P50, N70, P100, N150, P200, and the peak-to-peak amplitude of P100 to N150 were measured. BAEPs were also recorded. The results from the study demonstrated that xylene exposure at rest did not result in any consistent effects on VEPs, while xylene expo-

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sure combined with exercise resulted in minor but statistically significant decreases in the latency of N135 in the pattern VEP and of P200 in the flash VEP at fluctuating concentrations of 400 ppm. No exposure-related changes were noted in BAEPs. The study authors suggested that "the most intensive exposure situations" may result in an aroused state but that the changes did not indicate hazards to healthy workers.

EEG recordings were made during the first 18 min of the morning and afternoon exposure sessions and included 10 min of exercise and 3 to 4 min after exercise ceased on the days subjects were exercising (Seppalainen et al. 1991). Five-minute recordings were also made 1 and 2 h after the subject entered the chamber in the morning and 45 min after the afternoon exposure ceased. Exposure to *m*-xylene resulted in minor changes that suggested a stimulating, excitatory effect (slight alpha activation).

Body sway and reaction times were measured before exposure in the morning, 20 and 120 min after the beginning of the morning exposure, 20 min after the beginning of the afternoon exposure, and 50 min after termination of the afternoon exposure (Laine et al. 1993). Body sway along the anteroposterior and lateral axes was recorded with a strain gauge platform with the eyes open and closed. Simple reaction time of the dominant hand after visual stimuli and choice reaction time after auditory and visual stimuli were used to assess reaction times. The authors also measured gaze deviation nystagmus. Exposure to peak concentrations of *m*-xylene (400 ppm) resulted in decreased body sway in sedentary and exercising subjects. No definitive conclusions on the effects of *m*-xylene exposure on reaction times could be drawn because reaction times did not consistently change with the intensity of exposure. In the afternoon, longer simple reaction times were noted after exposure to peaks of *m*-xylene at rest, while prolonged audiomotor choice reaction times were prolonged after exposure to peaks of *m*-xylene while exercising. No nystagmus was noted.

Laine et al. (1993) also exposed 12 healthy male volunteers (four groups of three) at rest to stable concentrations of *m*-xylene at 200 ppm for 5 h and 30 min on 2 days, with 1 week separating the exposures. Body sway and reaction time (auditory, visual, and associative signals) were measured in the morning before exposure and after cessation of the exposure. Body movement while the subjects slept was recorded the night of the exposure at each subject's home using a static charge sensitive bed. No effect on body sway was observed, and no statistical difference in reaction times was noted. The only statistically significant effect observed when the subjects were sleeping was a slightly decreased number of body movements. No effect on active and quiet sleep was observed. No differences in body sway or reaction time were observed the morning after the exposure.

Nine male student volunteers (mean age 21 years) in three groups of three were exposed for 3 h in the morning and 1 h in the afternoon, with a 40-min lunch break, to air containing a fixed concentration of *m*-xylene at 200 ppm, a basal concentration of *m*-xylene at 135 ppm with 20-min peak concentrations of 400 ppm at the beginning of the morning and afternoon sessions, or to control

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air (Savolainen et al. 1984, 1985a,b). The subjects were exposed sedentary or with 10 min of exercise (100 W) at the beginning of each exposure session. The exposure occurred at 6-day intervals during six succeeding weeks. The subjects were exposed in a dynamic chamber, and the concentration of atmospheric xylene was continuously monitored with an infrared monitor. Peppermint oil was used to mask control exposure and the solvent odor, with the experiment being a single-blind experiment with crossover design, with each subject acting as his own control. The end points examined by each author were body sway (Savolainen et al. 1985a) and body sway and reaction time (Savolainen et al. 1984, 1985b). Body sway along the anteroposterior and lateral axes was recorded with a strain gauge platform with the eyes open and closed and was measured before exposure in the morning, at the time of peak exposure and exercise (about 15 to 20 min into exposure), and after exposure. Simple reaction time of the dominant hand following visual stimuli and choice reaction time following auditory stimuli or auditory and visual stimuli were used to assess reaction times (Savolainen et al. 1984, 1985b).

Savolainen et al. (1984) found that body sway along the anteroposterior axis was impaired by exposure to peak xylene concentrations at rest but improved with peak xylene concentrations with exercise. Opposite results were observed when body sway was measured along the lateral axis: only fluctuating concentration with exercise impaired balance, while body sway improved (decreased) with exposure at rest. Savolainen et al. (1985b) found that body sway was negatively correlated with xylene concentration; that is, xylene exposure improved (decreased) body sway, while no correlation was evident between blood xylene concentration and body sway. In contrast, Savolainen et al. (1985a) reported that changes in body sway were positively correlated with blood xylene concentration from exposure to stable and fluctuating *m*-xylene concentrations.

No consistent, significant effects on reaction time after exposure to *m*-xylene were found by Savolainen et al. (1985b). Savolainen et al. (1984) reported that choice reaction times as assessed by using auditory stimuli were statistically impaired in subjects exposed to peak *m*-xylene concentrations with exercise during the afternoon session. In the afternoon sessions, simple reaction times were impaired in sedentary subjects after peak exposure to *m*-xylene but improved in subjects exposed to peak concentration with exercise.

A total of 22 male student volunteers (mean age 24 years) were exposed to laboratory grade *m*-xylene (Riihimaki and Savolainen 1980; Savolainen and Riihimaki 1981). Six sedentary subjects were exposed for 6 h/day (with a 1-h lunch break) for five consecutive days and for 1 to 3 days after a 2-day weekend. Exposures on Monday to Friday were to stable concentrations of 100 ppm for the morning and afternoon sessions except on Friday afternoon when the concentration was doubled to 200 ppm. Exposures on Monday to Wednesday of the following week were to fluctuating concentrations of *m*-xylene: subjects were exposed to an approximate baseline concentration of 70 ppm with peaks of 200 ppm lasting 10 min (TWA of 100 ppm). On Wednesday afternoon, exposure concentrations were approximately doubled (baseline concentration of 130 ppm

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with peaks of 400 ppm; TWA of 200 ppm). The remaining 16 subjects were divided into two groups of eight. Both groups exercised on a bicycle ergometer at 100 W 4×/day for 10 min, exercising at 1 and 2 h of exposure in the morning and afternoon sessions. One group was exposed to a stable concentration of *m*-xylene at 100 ppm, with concentrations doubled to 200 ppm Friday afternoon, and the other group was exposed to fluctuating concentrations (baseline concentration of 70 ppm with hourly peaks of 200 ppm over 10 min; mean concentration of 100 ppm), with concentrations doubled on the last day (baseline concentration of 130 ppm with peaks of 400 ppm; mean concentration of 200 ppm). The subjects were exposed in a dynamic chamber, with peppermint oil used to mask control exposure and the solvent odor. Control days preceded and succeeded exposure days, so that each subject acted as his own control. The end points examined by each author included body sway (Riihimaki and Savolainen 1980; Savolainen and Riihimaki 1981), subjective symptoms, choice and simple reaction times, critical flicker fusion, Santa Ana manual dexterity test, nystagmus, and EEG recordings on a limited number of subjects (Riihimaki and Savolainen 1980).

Body balance was affected when a rapid increase in blood xylene concentration occurred (after peak exposures to fluctuating concentrations, particularly to 400 ppm), with tolerance developing upon continuing exposure (Riihimaki and Savolainen 1980; Savolainen and Riihimaki 1981). Changes in EEG recordings suggestive of a slight decrease in vigilance (increased number of slow occipital transients) were observed in 4/4 subjects after exposure to peak concentrations of xylene combined with exercise (Riihimaki and Savolainen 1980). One volunteer also exhibited bilateral spike and wave complexes. Although Riihimaki and Savolainen (1980) reported impairment of simple and choice reaction times after exposure to xylene with the development of tolerance upon continuing exposure, further details were not provided. Xylene exposure did not affect nystagmus, Santa Ana manual dexterity test, or critical flicker fusion (Riihimaki and Savolainen 1980). Symptoms were limited to mild nose and throat irritation reported by 1/6 sedentary subjects during the 400-ppm peaks (Riihimaki and Savolainen 1980). The results of testing the 6 sedentary subjects and the 16 volunteers divided into 2 groups of 8 were also reported by Savolainen et al. (1979b) and Savolainen et al. (1980), respectively.

Savolainen and Linnavuo (1979) assessed body balance in 17 healthy male volunteers (mean age 24 years) by means of a strain gauge transducer. Then, 6 of the 17 volunteers were exposed to *m*-xylene in the morning for 3 h to a TWA of 100 ppm with hourly peaks of 200 ppm and in the afternoon for 3 h to a TWA of 200 ppm with hourly peaks of 400 ppm, with a 1-h lunch break separating the morning and afternoon exposures. Body balance was assessed 1 h before the morning exposure and at the end of the morning and afternoon sessions. Control days preceded and succeeded exposure days. Although no differences in body balance were observed after xylene exposure in the morning session, impaired body balance was noted in the subjects during the afternoon session, particularly with the eyes closed.

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With six xylene concentrations (composition not specified) and 18 subjects familiar with the smell of xylene, the odor threshold for xylene was reported as 0.1 to 0.4 ppm (reported as 0.6 to 1.9 mg/m^3) for the minimum perceptible concentration and 0.09 to 0.3 ppm (0.4 to 1.4 mg/m^3) for the maximum imperceptible concentration (Gusev 1965). EEG recordings of four subjects exposed to xylene for 6 min indicated reductions in the electrical activity of the cerebral cortex at 0.07 ppm (0.32 mg/m^3) but not at 0.05 ppm (0.21 mg/m^3). The reason for this effect at this relatively low exposure concentration is unknown.

2.3. Developmental and Reproductive Effects

A limited number of studies suggest an association between xylene exposure and an increased risk of spontaneous abortion (Windham et al. 1991; Taskinen et al. 1994) or developmental toxicity (Kucera 1968; Holmberg and Nurminen 1980; Taskinen et al. 1989). A number of limitations preclude the usefulness of these studies, including small sample sizes, no quantified exposure concentrations, and concurrent exposures to other solvents.

2.4. Genotoxicity

No increase in the frequency of sister chromatid exchanges was observed in peripheral lymphocytes from individuals exposed to xylene in occupational (Haglund et al. 1980; Pap and Varga 1987) or experimental settings (Richer et al. 1993).

2.5. Carcinogenicity

Occupational exposure to xylene has been associated with an increased risk of leukemia (Arp et al. 1983; Wilcosky et al. 1984; Anttila et al. 1995); non-Hodgkin's lymphoma (Wilcosky et al. 1984; Anttila et al. 1995); or cancer of the rectum, colon (Siemiatycki 1991; Gérin et al. 1998), or nervous system (Spirtas et al. 1991; Anttila et al. 1995). Despite these associations, however, a number of limitations preclude the usefulness of these data, including small sample sizes, no quantified exposure concentrations, and concurrent exposures to other solvents including benzene.

2.6. Summary

A summary of the effects of xylene exposure on humans is provided in Table 6-4. CNS disturbances after acute and chronic inhalation exposure to xylene include headache, vertigo, nausea, fatigue, irritability, dizziness, impaired concentration, and confusion. Case reports of xylene inhalation have included signs and symptoms such as seizures, unconsciousness and coma, acute pulmonary edema, and transient renal and hepatic impairment. Death occurred due to pulmonary failure after inhalation of mixed xylenes at about 10,000 ppm.

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TABLE 6-4 Summary of Controlled Human Exposures to Xylene[a]

Concentration (ppm)	Duration	Isomer	Effect	Reference
110	15 min	Mixed	Intermittent throat irritation (1/6)	Carpenter et al. 1975b
230			Eye irritation (1/7 affected); dizziness (1/7)	
460			Eye irritation (4/6 affected); dizziness (1/6), mild nose/ throat irritation	
690			Eye irritation (4/6 affected); dizziness (4/6, with one having loss of balance), eye/nose/throat irritation	
0, 100, 200, 400	30 min	Mixed	Eye irritation reported, but incidence not statistically significant: (56%, 60%, 70%, 90%, respectively) No nose or throat irritation No change in behavioral tests (performance or cognitive) or respiratory measurements	Hastings et al. 1984
0, 100, 300	70 min	Unknown	No effect on 5 performance tests, heart rate, subjective symptoms Note: exposure via a breathing valve; used menthol to mask odor	Gamberale et al. 1978
300 w/exercise	70 min	Unknown	Significantly decreased performance on short-term memory and reaction time (2/5 tests) Note: exposure via a breathing valve; used menthol to mask odor	Gamberale et al. 1978
100	3 h	Unknown	Significantly affected performance of simple and choice reaction time tests Note: used terpon to mask odor	Dudek et al. 1990
100, 200	3 h or 7h w/ 1 hr break	*m*-, *p*-	No effect on blood pressure, pulse rate, flicker value, or reaction time (total of 23 volunteers)	Ogata et al. 1970
70	4 h	*p*-	No effect on choice reaction time, simple reaction time, short-term memory, heart rate, or subjective symptoms	Olson et al. 1985

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200	4 h	*m*-	No adverse effect on VEP, tapping speed, body sway, reaction time, critical flicker fusion	Savolainen et al. 1981; Seppalainen et al. 1983
200	5.5 h	*m*-	No effect on body sway, reaction times, active or quiet sleep; only effect was sleep movements slightly decreased during night after exposure	Laine et al. 1993
100 or 150	7.5 h	*p*-	Mild eye irritation, primarily in person wearing contacts No effect on performance tests	Hake et al. 1981

[a]Exposures separated by a lunch break are not included in this table.

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3. ANIMAL TOXICITY DATA

3.1. Acute Lethality

3.1.1. Cats

Four male cats of mixed breed were exposed to air containing a mean measured concentration of mixed xylenes at 9,500 ppm (*p*-xylene, 7.84%; *m*-xylene, 65.01%; *o*-xylene, 7.63%; ethylbenzene, 19.27%) (Carpenter et al. 1975b). Clinical signs during the exposure included salivation, ataxia, tonic and clonic spasms, and anesthesia. All cats were dead within 2 h of exposure. Necropsy failed to reveal any exposure-related histologic lesions.

3.1.2. Rats

Groups of 10 female, Sprague-Dawley rats were exposed to a mixture of xylenes (*o*-, *p*-, and *m*-xylene; percentage of each not provided) by inhalation in a 60-L chamber for 4 h (Lundberg et al. 1986). Xylene concentrations were not provided, but it was stated that they were administered in a geometric series. Solvent concentrations in the chamber were monitored by infrared analysis of a stream of chamber air continuously drawn through an infrared analyzer, and exposure levels were adjusted accordingly. Animals were observed for mortality for 24 h after the start of the exposure. A 4-h 50% lethal concentration (LC_{50}) value of 11,000 ppm (reported as 47,635 mg/m^3; 95% confidence interval, 10,000 to 12,000 ppm) was determined by the Weil method. In another study, rats were exposed to concentrations of 1/32 to 1/2 of the LC_{50} value, and liver damage was assessed by measuring serum levels of sorbitol dehydrogenase activity or by histologic analysis of liver sections. Xylene exposure at these concentrations failed to induce any measurable hepatotoxicity.

Bonnet et al. (1982) exposed groups of 12 male Sprague-Dawley rats to various concentrations of *o*-xylene (98% purity), *m*-xylene (97% purity), or *p*-xylene (98% purity) for 6 h. Vapor concentrations were determined by gas chromatography. Animals were observed for mortality for 14 days after exposure, and LC_{50} values were calculated by the method of Bliss (1938). Individual exposure concentrations and mortalities were not provided in the study. Clinical signs reported for rats exposed to *m*-xylene and *o*-xylene consisted of loss of muscle tone and somnolence, while rats exposed to *p*-xylene also exhibited tremor, shaking, and repetitive movements. The 6-h LC_{50} values with 95% confidence limits were *m*-xylene, 5,984 (5,796 to 6,181); *o*-xylene, 4,330 ppm (4,247 to 4,432); and *p*-xylene, 4,591 ppm (4,353 to 5,049).

Groups of 15 or 16 male albino rats (Harlan-Wistar strain) approximately 5 weeks of age were exposed for 4 h to air containing measured concentrations of mixed xylenes at 580, 1,300, 2,800, 6,000, or 9,000 ppm (*p*-xylene, 7.84%; *m*-xylene, 65.01%; *o*-xylene, 7.63%; ethylbenzene, 19.27%) (Carpenter et al.

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1975b; methods are given by Carpenter et al. 1975a). Ten rats per group were used for the LC_{50} determination, while five rats per group were sacrificed after exposure and necropsied. Animals were observed continuously for the first 5 min of exposure, at 15 and 30 min, then at 30-min intervals until 1 and 2 h post-exposure, and daily thereafter. All organs were evaluated grossly at death or sacrifice. Histopathologic evaluation was done of the respiratory tract and liver from three animals (or fewer) per exposure concentration at the end of the 4-h exposure and after 2 days postexposure and of the respiratory tract, liver, kidney, brain, and bone marrow at sacrifice after the 14-day postexposure observation. Mortality results and clinical signs are reported in Table 6-5. A 4-h LC_{50} of 6,700 ppm (95% confidence interval, 5,100 to 8,500 ppm) was calculated by the Thompson method of moving averages. The only findings at necropsy ascribed to treatment were two cases each of pulmonary atelectasis, hemorrhage, and interlobular edema in rats that died after exposure to the highest concentration.

Male Long-Evans rats weighing between 150 and 300 g were exposed to a mixture of *o*-xylene, *m*-xylene, *p*-xylene, and ethylbenzene by inhalation for 4 h and were observed for 14 days for mortality for determination of a 4-h LC_{50} using the method of Litchfield and Wilcoxon (Hine and Zuidema 1970). Information not provided by the study authors included the number of rats per group, the percent of each component in the mixture, the exposure concentration, and whether the LC_{50} calculation was based on nominal or analytic concentrations. The 4-h LC_{50} was 6,350 ppm (confidence limits [not further defined], 4,670 to 8,640 ppm). The authors reported that all deaths occurred during exposure; survivors were comatose upon removal from the chamber but recovered shortly thereafter.

TABLE 6-5 Mortality of Male Rats Exposed to Xylene Vapor for 4 Hours

Concentration (ppm)	Mortality	Other Effects
580	0/10	None observed
1,300	0/10	Poor coordination (slight coordination loss) after 2 h, did not persist after exposure
2,800	0/10	Irritation (not described further), all rats prostrate between 2 and 3.5 h; recovered within 1 h, but coordination remained poor; return to normal following day
6,000	4/10 Died within 3.5 h	Rats prostrate within 30 min; all survivors prostrate but recovered promptly
9,900	10/10 Died within 2.25 h	None stated
6,700		4-h LC_{50}

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To determine the effects of xenobiotic interactions with *p*-xylene, adult female CD rats were pretreated with saline, phenobarbital at 75 mg per kg of body weight (mg/kg), chlorpromazine at 15 mg/kg, corn oil, or 3-methylcholanthrene at 20 mg/kg by intraperitoneal injection for three consecutive days before inhalation exposure to *p*-xylene for 4 h for determination of LC_{50} values (Harper et al. 1975). Methods for the xylene exposures were the same as those used for benzene in a previous study (Drew and Fouts 1974). Chamber concentrations were monitored at 30-min intervals by bubbling the air containing the vapor through methanol, and the vapor absorbed in the methanol was measured with a spectrophotometer. The concentrations used in calculating the 4-h LC_{50} are based on the arithmetic means of eight determinations over the 4-h exposure period. Animals were observed for 14 days. The LC_{50} values and the corresponding 95% confidence levels are presented in Table 6-6. Pretreatment with the xenobiotics resulted in minimal changes: phenobarbital increased the LC_{50} by ~20%, while 3-methylcholanthrene and chlorpromazine produced almost comparable LC_{50} values.

Cameron et al. (1938) exposed groups of 10 male and female albino rats or mice by inhalation to *p*-, *m*-, or *o*-xylene (source and purity not specified) at saturation or at one-half, one-fourth, or one-eighth of saturation and reported the resultant mortalities (length of observation not provided). Mortality results are presented in Table 6-7. No treatment-related changes were noted in organs of animals that died after exposure.

Groups of five male albino rats (Harlan-Wistar strain) were exposed to a measured concentration of mixed xylenes at 11,000 ppm for 2 h or for 15, 30, or 60 min (*p*-xylene, 7.84%; *m*-xylene, 65.01%; *o*-xylene, 7.63%; ethylbenzene, 19.27%) (Carpenter et al. 1975a; b). Animals were observed constantly during the exposure—at 0.5, 1, 2, and 4 h postexposure and then once daily for 7 days. Among rats exposed for 2 h, eye irritation was noted immediately at the start of exposure, with prostration present 20 min into the exposure and tremors observed at 45 min. Mortality was observed in 2/5 rats within 66 min and in 4/5 within 80 min. The rat that survived was said to have poor coordination, but the duration of that condition was not stated. No mortality was observed in rats exposed to mixed xylenes at 11,000 ppm for 15, 30, or 60 min. Eye irritation was again noted, and prostration was observed in rats exposed for 30 or 60 min, with full coordination returning 30 min or 2 h postexposure, respectively. The median lethal time was 92 min.

Smyth et al. (1962) reported that 2 h was the maximum exposure time resulting in no mortality within 14 days of exposure to a concentrated vapor of *m*-xylene in albino rats. Inhalation of a nominal concentration of *m*-xylene at 8,000 ppm for 4 h resulted in a mortality of 10/12 within the 14-day observation period.

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TABLE 6-6 Mortality of Male Rats Exposed to *p*-Xylene Vapor for 4 Hours After Pretreatment with Xenobiotics

Pretreatment[a]	Dose (mg/kg)	LC_{50} (ppm)	C.I.
Saline	–	4,740	4,520-4,960
Phenobarbital	75	5,810	5,460-6,160
Chlorpromazine	15	4,970	4,700-5,240
Corn oil	–	4,550	3,850-4,750
3-Methylcholanthrene	20	4,960	4,710-5,200

[a]Pretreatment by intraperitoneal injection for 3 days before exposure to xylenes.
Abbreviation: C.I., confidence interval.
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TABLE 6-7 Mortality of Rats Exposed to Xylene Vapor

Isomer	Concentration (ppm)	Length of Exposure (h)	Mortality
o-Xylene	12,250	12	2/10
	6,125	24	8/10
	3,062	24	1/10
	1,531	24	0/10
	1,531	8 × 14 h	0/10
m-Xylene	8,040	12	1/10
	2,010	24	0/10
	1,005	24	0/10
	1,005	8 × 14 h	0/10
p-Xylene	19,650	12	8/10
	4912	24-28	0/10
	2,451	24	0/10
	1,226	8 × 14 h	0/10

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3.1.3. Mice

Bonnet et al. (1979; 1982) exposed groups of 20 to 25 female mice (specific-pathogen-free of stock OF-1) to various concentrations of *o*-xylene (98% purity), *m*-xylene (97% purity), or *p*-xylene (98% purity) for 6 h. Vapor concentrations measured by gas chromatography were 90% to 100% of nominal concentrations. Animals were observed for mortality for 14 days after exposure and LC_{50} values were calculated by the method of Bliss (1938). Individual exposure concentrations and mortalities were not provided in the study; it was stated that mice exposed to *o*-xylene had incidences of delayed mortality between 5 and 10 days postexposure. The 6-h LC_{50} values with 95% confidence limits were *m*-xylene, 5,267 ppm (5,025 to 5,490); *o*-xylene, 4,595 ppm (4,468 to 4,744); and *p*-xylene, 3,907 ppm (3,747 to 4,015).

The concentrations of the xylene isomers required to produce narcosis in white mice were 3,500 to 4,600 ppm for *o*-xylene, 2,300 to 3, 500 ppm for *m*-xylene, and 2,300 ppm for *p*-xylene, while the lethal concentrations were 6,900 ppm for *o*-xylene, 11,500 ppm for *m*-xylene, and 3,500 to 8,100 ppm for *p*-xylene (Lazarew 1929).

Cameron et al. (1938) exposed groups of 10 male and female mice by inhalation to *p*-, *m*-, or *o*-xylene (source and purity not specified) at saturation or at one-half, one-fourth, or one-eighth of saturation and reported the resultant mortalities (length of observation not provided). Mortality results are presented in Table 6-8. No treatment-related changes were noted in organs from animals that died after exposure.

TABLE 6-8 Mortality of Mice Exposed to Xylene Vapor

Isomer	Concentration (ppm)	Length of exposure (h)	Mortality
o-Xylene	12,250	12	2/10
	6,125	24	9/10
	3,062	24	4/10
	1,531	24	0/10
m-Xylene	8,040	12	6/10
	2,010	24	6/10
	1,005	24	0/10
p-Xylene	19,650	12	9/10
	4,912	24-28	0/10
	2,451	24	0/10
	1,226	8 × 14 h	0/10

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3.2. Nonlethal Toxicity

3.2.1. Dogs

Lacrimation developed in male beagles (number of animals not provided) exposed to mixed xylenes at 1,200 ppm for 4 h, while a 4-h exposure to mixed xylenes at 530 ppm had no observable effects (xylene composed of *p*-xylene, 7.84%; *m*-xylene, 65.01%; *o*-xylene, 7.63%; ethylbenzene, 19.27%) (Carpenter et al. 1975b; methods reported by Carpenter et al. 1975a). It is not clear if the concentrations reported by the authors are nominal or were corrected for the 50% to 60% loss that occurred during exposure.

3.2.2. Rats

Flash evoked potentials were assessed in groups of adult male Long-Evans hooded rats after a 4-h exposure to air containing *p*-xylene at 0, 800, or 1,600 ppm (99.8% pure; number of animals not indicated) (Dyer et al. 1988). Exposure to *p*-xylene at 1,600 ppm resulted in a significant depression ($p < 0.003$) in the amplitude of peak N3 depending on time, with a return to control levels 75 min postexposure. The authors postulated that the depression in the N3 peak was the result of increased arousal from the *p*-xylene exposure. This idea is supported by a similar depression in the N3 peak observed after amphetamine administration in rats.

To assess the potential for *p*-xylene exposure to alter serum enzyme activity, groups of female Sprague-Dawley rats were exposed to air containing *p*-xylene at 0, 1,000, 1,500, or 2,000 ppm for 4 h (Patel et al. 1979). Blood samples were collected from the heart from four animals per group immediately after exposure and 24 h after initiation of exposure. By 24 h, increases were observed in the activities of serum glutamic pyruvic transaminase, oxaloacetic transaminase, and 5'-nucleotidase in all exposure groups and of glucose-6-phosphate dehydrogenase, isocitric dehydrogenase, glutathione reductase, and lactic dehydrogenase in the 1,500- and 2,000-ppm groups. In general, the activity of the enzymes was increased in a concentration-related manner. Pseudocholinesterase activity exhibited a concentration-related increase immediately after exposure, but activity returned to control levels 24 h later.

The toxicity of inhaled *p*-xylene was investigated in male Long-Evans rats using a conditioned flavor aversion paradigm, which operates on the premise that pairing the consumption of a novel compound, such as saccharin, with a toxic agent results in a conditioned aversion to the novel flavor (Bushnell and Peele 1988). After acclimation for 1 week, rats were placed on a restricted water schedule of 30 min/day for 10 days. Then rats were given a 0.1% saccharin solution for 30 min instead of water on day 11, followed 30 min later by exposure to *p*-xylene (99.7% pure). Rats were exposed for 4 h to 0, 50, 100, 200, 400, 800, or 1,600 ppm, or for 0.5, 1, 2, 4, or 8 h to *p*-xylene at 0 or 400 ppm. A third

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group of rats were exposed to *p*-xylene at 0, 200, or 800 ppm after a 24-h delay after saccharin exposure. After exposure to *p*-xylene, rats were kept on a restricted water schedule but were offered a choice between tap water and a 0.1% saccharin solution. A concentration-related decrease in relative saccharin consumption was observed in all groups exposed to *p*-xylene for 4 h, with maximal aversion occurring in the 800- and 1,600-ppm groups. In rats exposed to *p*-xylene at 400 ppm for various time periods, maximal aversion was noted at 2 to 8 h. Although exposure to *p*-xylene affected saccharin intake, total water consumption was not affected. Rats exposed to *p*-xylene following a 24-h delay after saccharin exposure did not exhibit any aversion to saccharin, demonstrating that there must be close temporal pairing of *p*-xylene and saccharin to produce conditioned flavor aversion.

Groups of eight male Long-Evans hooded rats exposed to *p*-xylene at 1,600 ppm for 4 h/day for 1 to 5 days had improved autoshaping compared with controls as assessed by retraction of a single response lever on a variable 35-second (s) schedule followed by delivery of a food pellet (Bushnell 1989). When the force of the lever was doubled, however, xylene exposure did not facilitate autoshaping compared with controls. Assessment of motor activity after daily exposure indicated that horizontally directed movement in xylene-exposed rats was increased by 30% for the first 15 min of testing, while vertically directed movement was not affected. The activity level of xylene-exposed animals returned to control levels every day, and no difference in activity level of xylene-exposed rats was observed 3.5 h postexposure compared with 0.5 h postexposure. Clinical signs in rats after exposure to *p*-xylene at 1,600 ppm included slight activation, fine tremor, and unsteadiness.

Ghosh et al. (1987) investigated the effects of xylene exposure on fixed-ratio responding in male Fischer 344 (F344) rats. The mixed xylenes comprised *m*-, *p*-, and *o*-xylene with ethylbenzene present as a contaminant; the percent composition was not provided. Xylene exposures and behavioral testing were both carried out in a dynamic exposure behavioral chamber. Chamber concentrations were measured by gas chromatography in 15-min increments. For 6 to 8 weeks, rats were first trained to push a lever 24 times (FR24), which resulted in receiving a 5% sucrose solution. After the initial training, the rats were divided into three groups for further training specific to the experimental protocol of interest. In all three experiments, the recording of the stabilized reinforcement rate for 3 to 4 days before xylene exposure served as the control. In the first group, four rats were further trained for 6.25-h sessions, where lever pressing was rewarded only in the last 15-min period of each hour. After training, rats were successively exposed to graded concentrations of xylenes at 113, 216, and 430 ppm for 2 h each. A concentration-related decrease in the reinforcement rate was observed during the first, third, and fifth hours, while no significant change was observed at the second, fourth, and sixth hours, indicating that tolerance had developed. In the second group, five rats were trained for 2.25-h sessions, where behavioral performance was restricted to the second and fourth 15-min periods of each hour (15 to 30, 45 to 60, 75 to 90, and 105 to 120 min). After training,

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rats were exposed for 2 h to xylenes at 114, 212, or 446 ppm, with a minimum of 7 days separating each of the exposures so that tolerance would not develop. A significant decrease in the reinforcement rate was noted at all three concentrations during the 45- to 60-min period (20%, 27%, and 23% decrease in the 114-, 212-, and 446-ppm groups, respectively). Although some decreases in performance were also present in the 212-ppm group at 75 to 90 min (11% decrease) and in the 446-ppm group at 75 to 90 and 105 to 120 min (19% and 17%, respectively), the differences were not statistically significant. In the last group, four rats were trained for 5.25-h sessions in which behavioral performance was limited to the last 15-min period of each hour. Rats were then exposed to xylenes at 98.5 ppm for 5 h. No effects on behavioral performance were observed at any of the time periods during the exposure. The study authors therefore concluded that the minimum effective concentration for xylenes to cause a decrease in reinforcement rate was 113 ppm.

In a similarly designed experiment, Wimolwattanapun et al. (1987) investigated the effect of xylene exposure on intracranial self-stimulation behavior in male F344 rats. The mixed xylenes comprised *m*-, *p*-, and *o*-xylene with ethylbenzene present as a contaminant; the percent composition was not provided. Xylene exposure and behavioral testing were carried out in a dynamic exposure behavioral chamber. Chamber concentrations were measured by gas chromatography in 15-min increments. To study the effects of xylene exposure on intracranial self-stimulation behavior in rats, groups of male F344 rats had bipolar electrodes surgically implanted into the ventral tegmental area of the rats. One week after surgery, rats were trained to press a lever to receive reinforcing electrical stimulation. After the initial training, the rats were divided into three groups for training specific to the experimental protocol of interest. In all three experiments, the recording of the stabilized reinforcement rate for 3 to 4 days before xylene exposure(s) served as the control, and rate of response during exposure was recorded for each 20-min period, with the first and last 20-min periods being control exposures. In the first group, five rats were further trained for 2.67-h periods. After training, rats were exposed to xylene at 102, 192, 419, or 613 ppm for 2 h, with a minimum of 7 days separating the exposures so that tolerance would not develop. Significant decreases ($p < 0.05$) in the rate of response were observed in rats exposed to at least 192 ppm; results are presented in Table 6-9. In the second experiment, no effects on self-stimulation behavior were observed in four rats trained for 4.67-h sessions followed by exposure to 106 ppm for 4 h. The third experiment consisted of exposure of four rats to 444 ppm for 2-h periods for five consecutive days. Results indicated the development of tolerance. Rate of response significantly decreased during the fourth, fifth, and sixth periods on the first day; during all periods on the third day; during the sixth period on the fourth day; and at no time on the fifth day.

To assess the effects of xylene exposure on motility, groups of eight CFY white male rats were exposed by inhalation for 4 h to at least six concentrations each of *m*-xylene (>96% pure), *o*-xylene (>99% pure), or *p*-xylene (>99% pure)

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TABLE 6-9 Effect of Xylene Exposure on Self-Stimulation Behavior in Rats

	Responses/20-min period (as percentage of control)							
Concentration (ppm)	−20-0 (control)	1st (0-20)	2nd (20-40)	3rd (40-60)	4th (60-80)	5th (80-100)	6th (100-120)	120-140 (control)
102	112	106	96	105	96	91	98	103
192	100	101	82	75	73*	94	75	90
419	103	98	87	85	61*	75*	76*	84
623	99	87*	81*	84*	89*	85*	87*	99

* Significant decrease ($p < 0.05$) in the rate of response.
Source: Wimolwattanapun et al. 1987. Reprinted with permission; copyright 1987, *Neuropharmacology*.

(individual concentrations not provided) (Molnár et al. 1986). Animals were exposed in a 30-L cylindrical glass chamber, with a solvent-saturated airstream diluted with clean air introduced at the top of the chamber and exhausted at the bottom of the chamber. Concentrations were determined every 30 min by use of an ultraviolet spectrophotometer. Motility during exposure was assessed by means of four electromechanical transducers housed in metal tubes placed perpendicularly throughout the exposure chamber. An electric counter continuously recorded the number of nose touches. The experiments required more than 1 day for completion, and it was not stated if rats were reused for the various exposures. It was stated that exposure to *m*-xylene at 130 to 1,500 ppm and to *p*-xylene at 400 to 1,500 ppm resulted in a concentration-related increase in group motility, while exposure to *o*-xylene at 150 to 1,800 ppm resulted in a slight depression of activity. At higher concentrations, activity decreased in all groups, with the minimum narcotic concentration for the three isomers reported as 2,180 ppm for *o*-xylene, 2,100 ppm for *m*-xylene, and 1,940 ppm for *p*-xylene.

To determine the median effective concentration (EC_{50}) of xylene on rotarod performance, groups of 10 male Wistar rats were exposed to 1,050, 2,030, 2,610, 2,710, 4,130, or 4,700 ppm (reagent grade *o*-, *m*-, *p*-xylene) for 4 h, with a parallel control group of 15 rats exposed to 0 ppm (Korsak et al. 1988). Chamber concentrations were analyzed by gas chromatography. The rotarod test was run both before and immediately after the exposure. All animals survived exposure. The EC_{50} for decreased rotarod performance with a 95% confidence interval was 4,520 ppm (3,800 to 5,390 ppm).

Korsak et al. (1990) exposed groups of 10 male Wistar rats for 6 h to *o*-, *m*-, or *p*-xylene at approximately 3,000 ppm to determine any potential differences in the toxicity of the individual isomers as measured by a rotarod test. Exposures were conducted in a dynamic inhalation chamber (1.3 m^3), where xylene concentrations were measured by gas chromatography. Rats were trained on the rotarod for at least 1 week before exposure. During testing, rotarod performance was measured before and after termination of exposure. The results of the testing given in terms of the number of failures/number of tested animals were as follows: *o*-xylene at an average concentration of 3,027 ppm, 19/20; *m*-

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xylene at an average concentration of 3,093 ppm, 6/20; *p*-xylene at an average concentration of 3,065 ppm, 1/20. From this limited experiment, it appeared that *o*-xylene was a more potent CNS depressant in rats than the other two isomers.

In a later experiment in which only *m*-xylene was tested, Korsak et al. (1993) exposed groups of 8 to 10 male Wistar rats to different concentrations of *m*-xylene in a dynamic inhalation chamber for 4 h immediately followed by rotarod testing or measurement of spontaneous motor activity by an actometer for 1 h postexposure. Xylene concentrations were measured in the exposure chamber in 30-min increments by gas chromatography. Exact exposure concentrations were not provided, but graphic representation of data showed *m*-xylene exposure concentrations of about 500, 1,000, 1,500, 2,000, or 3,000 ppm. The effect of exposure on spontaneous motor activity was biphasic, with lower concentrations (up to 2,000 ppm) resulting in increased motor activity and higher concentrations (3,000 ppm) resulting in decreased motor activity. The 4-h EC_{50} for *m*-xylene effects on rotarod performance was 1,982 ppm (95% confidence interval, 1,530 to 2,565 ppm). This concentration is lower than that used in the previous Korsak et al. (1990) experiment, in which the toxicity of the individual isomers was assessed in rats by comparing rotarod performance after exposure to 3,000 ppm for 6 h.

To assess erythrocyte fragility after exposure to xylene, groups of five male albino rats (Harlan-Wistar strain) were exposed to air containing mixed xylenes in metered concentrations of 0 or 15,000 ppm (64 mg/L) (corrected concentration approximately 8,800 ppm: composed of *p*-xylene, 7.84%; *m*-xylene, 65.01%; *o*-xylene, 7.63%; ethylbenzene, 19.27%) for 45 min (Carpenter et al. 1975a,b). The rats were killed after exposure, and their blood was collected. Erythrocyte fragility was determined by placing one drop of blood in a tube with different concentrations of saline to determine the concentration of saline causing initial and complete hemolysis. Hemolysis in xylene-exposed rats was comparable to that in the concurrent controls. No increase in erythrocyte fragility was observed in xylene-exposed rats compared with controls.

3.2.3. Mice

Groups of six male Swiss OF_1 mice inhaled air containing at least four different concentrations of *o*-xylene to determine the concentration of *o*-xylene associated with a 50% decrease in respiratory rate (RD_{50}) (de Ceaurriz et al. 1981). The test concentrations were not stated, and, although it was stated that xylene was of a high purity, the actual purity was not provided. Animals were exposed in a 200-L exposure chamber with adjustable air flow. The air concentration of *o*-xylene in the test chamber was determined by sweeping a sample loop through the cell atmosphere and analyzing the sample by gas chromatography. Respiratory rate was measured with a body plethysmograph. Recordings were made for 10 min before exposure, and then the mice were placed in an exposure cell with a predetermined concentration of *o*-xylene until the maximum

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decrease in respiration was reached. Exposure was generally for only 5 min. On the basis of results from these exposures, the RD_{50} for *o*-xylene was 1,467 ppm.

Korsak et al. (1988) determined the RD_{50} for mixed xylenes (reagent grade *o*-, *m*-, *p*-xylene; further details not provided), toluene, and a 50:50 (vol/vol) mixture in groups of two to four BALB/c male mice. For determination of the xylene RD_{50}, mice were exposed to xylene at 2,600, 4,000, 4,600, or 7,000 ppm, and their respiratory rates were measured with a body plethysmograph continuously before exposure, during 6 min of exposure, and 2 to 3 min after termination of exposure. The RD_{50} for mixed xylenes was 2,440 ppm.

Korsak et al. (1993) also determined the RD_{50} for *m*-xylene, *n*-butyl alcohol, and the 50:50 mixture (supplied by Reachim and the Polish Chemical Reagent company, further information not provided) in groups of 8 to 10 BALB/c male mice with a body plethysmograph. Actual test concentrations studied were not provided. The RD_{50} for *m*-xylene was 1,361 ppm. In a second paper, Korsak et al. (1990) exposed groups of six BALB/c male mice to *p*-, *o*-, or *m*-xylene at 3,000 ppm, and the respiratory rate was measured with a body plethysmograph continuously before exposure, during 6 min of exposure, and 3 min after termination of exposure. The maximum reductions in respiratory rate were measured during the first minute of exposure and were 54%, 46%, and 43% of controls for the mice exposed to *p*-xylene, *o*-xylene, and *m*-xylene, respectively.

Carpenter et al. (1975a,b) exposed Swiss-Webster male mice to air containing measured concentrations of mixed xylenes (*p*-xylene, 7.84%; *m*-xylene, 65.01%; *o*-xylene, 7.63%; ethylbenzene, 19.27%) for 1 min and then measured respiratory rate during a 15-min postexposure period. A 50% or greater decrease in respiratory rate was observed in 5/5 rats at 12,000 ppm, 4/6 rats at 6,500 ppm, 2/6 rats at 2,500 ppm, and 2/6 rats at 1,300 ppm. A 50% reduction in respiratory rate was not observed in any of the rats exposed to 460 ppm.

A functional observational battery (FOB) was adapted to mice to evaluate acute behavioral effects of alkylbenzenes (Tegeris and Balster 1994). Groups of eight adult male CFW albino mice inhaled air containing *m*-xylene (98% purity) at 0, 2,000, 4,000, or 8,000 ppm for 20 min under static conditions. After individual mice were placed into 29-L glass jars sealed with a lid, xylene was injected onto a filter paper in the jar, with a fan blade in the chamber distributing the vapors equally. Analysis of air by infrared spectrometry confirmed nominal vapor concentrations and demonstrated that maximal concentrations were reached within 3 min of fan activation and remained constant throughout exposure. Within 10 to 15 s after exposure, mice were removed from the jar and evaluated according to a complete FOB.

During the last 2 min of the exposure, concentration-related, statistically significant effects were observed including decreased arousal and rearing, abnormal posture, altered palpebral closure, and disturbances of gait (Tegeris and Balster 1994). Because the statistical significance of the effects was reported at two or more doses, it is not clear whether the changes were limited to the 4,000- and 8,000-ppm groups or extended to the 2,000-ppm group. The authors stated that exposure to all concentrations including 2,000 ppm resulted in decreased

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rearing. After exposure, the FOB revealed statistically significant reductions among the 8,000-ppm group in the percentage of animals with a successful inversion in the inverted screen test, in the percentage of animals with a normal ranking in righting reflex, and in mean forelimb grip strength. Statistically increased mean hindlimb foot splay was observed in all exposure groups. Exposure to xylene also resulted in decreased responsiveness to stimulus presentation. Because the purpose of the study was to qualitatively compare six alkylbenzenes, no attempt was made to determine minimally effective concentrations.

The effects of the individual xylene isomers and a commercial xylene mixture on operant responding and motor performance were assessed in CD-1 male albino mice (Moser et al. 1985). All exposures occurred in a 29-L glass chamber under static conditions, with measurements of chamber air by an infrared spectrophotometer confirming that solvent concentration remained stable throughout the exposure. To measure operant response after xylene exposure, 15 mice were feed-deprived throughout the study. The mice were tested in three squads, with the order of each isomer counterbalanced among the squads and a week separating the testing of each isomer. The xylene mixture was tested during the last week for all squads. Mice were exposed on Tuesday through Friday of each week to air or ascending xylene concentrations of 500, 800, 1,400, 2,400, 4,000, 5,000, or 7,000 ppm for 30 min. Immediately after exposure, mice were placed in an operant chamber. Before exposure, they were trained to lever-press during daily 15-min sessions, followed by a differential-reinforcement-of-low-rates 10-s schedule. Motor performance was assessed by measuring the performance of mice in an inverted screen test study after exposure to the solvent. Groups of 12 mice (two squads of six mice) were exposed to at least three concentrations producing between 0% and 100% effects (2,000 to 7,000 ppm). It is assumed that the exposure duration was 30 min, but it was not stated definitively in the paper.

The results of the operant studies indicated that the order of exposure to the xylene isomers or mixture had no effect on the outcome (Moser et al. 1985). The minimally effective concentration for disruption of operant performance was 1,400 ppm for all isomers, with an EC_{50} (concentration producing half-maximal decreases in response rate) of 6,176, 5,179, and 5,611 ppm for *m*-xylene, *o*-xylene, and *p*-xylene, respectively. The operant response was biphasic, with concentrations of 1,400 to 2,400 ppm producing increased rates of response, and a concentration of 7,000 ppm suppressing the response rate and producing gross ataxia and prostration. The minimally effective concentrations for the inverted screen test were 3,000 ppm for *m*- and *o*-xylene and 2,000 ppm for *p*-xylene, while the EC_{50} values for performance on the inverted screen test were 3,790, 3,640, and 2,676 ppm for *m*-xylene, *o*-xylene, and *p*-xylene, respectively. Motor ability was recovered 5 to 15 min after exposure. The study authors concluded that there was no consistent, significant difference in the potency of the individual isomers. While *o*-xylene exhibited increased potency on operant behavior, *p*-xylene reduced motor performance.

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3.3. Developmental and Reproductive Effects

In a one-generation reproduction study, groups of male and female CD rats were exposed to mixed xylenes at 0, 60, 250, and 500 ppm (Groups I, II, III, and IV, respectively; technical grade xylene: 2.4% toluene, 12.8% ethylbenzene, 20.3% *p*-xylene, 44.2% *m*-xylene, 20.4% *o*-xylene) by inhalation for 6 h/day, 5 days/week, for 131 days before mating (Bio/dynamics Inc. 1983). Exposure continued in the females during gestation days (GD) 1 to 20 and throughout lactation days 5 to 20. Two additional 500-ppm groups were included: only the males were exposed in Group V, and only the females were exposed in Group VI. Potential pup exposure to xylenes was only through milk. No definite, exposure-related adverse effects were noted in F_0 adults or pups. Although marginal reductions in pup body weight in exposed groups were observed, the changes were not considered adverse because the concurrent control group had an elevated mean pup body weight associated with a smaller mean litter size (mean number of live pups per litter: 9.6, 11.8, 12.5, 12.4, 10.8, and 11.8 for Groups I to VI, respectively). No decrease in pup body weight was observed in Group VI, in which dams were exposed to the same concentration of xylene for the same period of time as dams in Group IV.

To assess potential developmental toxicity, one-half of the Group I F_0 females (20 females; control group) and Group IV F_0 females (12 females; mixed xylenes at 500 ppm by inhalation for 6 h/day, 5 days/week, for 131 days before to mating and during GD 1 to 20) were killed on GD 21 (Bio/dynamics Inc. 1983). No exposure-related signs of maternal toxicity were observed. No statistically significant differences were noted between treated and control groups for mean number of corpora lutea, implantations, resorption sites, live fetuses, mean percentage of live fetuses/implants, or fetal sex ratios. No definitive treatment-related external, visceral, or skeletal malformations or variations were observed. The report stated that fetuses exposed to high doses had a slightly higher incidence of unossified sternebrae and incompletely ossified cervical vertebral transverse processes, but the data were provided in terms of fetal incidence instead of litter incidence. Mean fetal body weight on GD 21 was marginally but statistically reduced in female offspring from Group IV (93% of controls); however, male fetal body weight was comparable to that of controls. This marginal reduction in body weight in female offspring is difficult to assess because male fetal weight was unaffected.

No signs of maternal or developmental toxicity were observed after exposure of pregnant CRL: COBS CD (SD) BR rats to xylene at 0, 100, or 400 ppm (52% *m*-xylene, 11% *o*-xylene, 0.31% *p*-xylene, 36% ethylbenzene) for 6 h/day on GD 6 to 15 (Litton Bionetics 1978a). The no-observed-adverse-effect level (NOAEL) is therefore ≥400 ppm.

To evaluate the effects of prenatal xylene exposure on postnatal development, pregnant (Mol:WIST) rats were exposed by inhalation to xylene at 0 or 500 ppm (19% *o*-xylene, 45% *m*-xylene, 20% *p*-xylene, 15% ethylbenzene) 6 h/day, on GD 7 to 20 and were allowed to deliver (Hass et al. 1995). From each

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litter, two males and two females were kept for behavioral testing, one male and one female were kept in standardized housing and left undisturbed other than feeding and taking body weight measurements until 3 months of age when they were tested in the Morris water maze test (tests learning and memory). One male and one female were kept in enriched housing (cages contained various toys) and tested for rotarod, open field, and Morris maze performance at about 3 months of age. The only possible effect observed was that offspring from xylene-exposed rats raised in the standard housing had impaired performance in the Morris maze test compared with controls. Testing at 12 weeks showed a nonsignificant trend ($p = 0.059$) for increased latency for finding the platform at the beginning of the learning test. At 16 weeks, these rats required significantly more time to find a platform hidden in the center of the pool. Further analysis revealed the effect was limited to the female offspring, which had an increased swimming length (took a longer route to reach the platform), while swim speed was unaffected. Offspring from xylene-exposed rats raised in the enriched environment showed no difference in the Morris maze test compared with controls.

In a study designed to investigate the persistence of the decreased Morris water maze test performance of the offspring from the xylene-exposed (Mol:WIST) female rats, the female offspring raised in standard housing were also evaluated at 28 and 52 weeks (Hass et al. 1997). At 28 weeks, increased latency for finding a platform that was moved to a new position was observed in the female offspring from exposed rats during the first trial of a testing block. The next two trials resulted in similar latency in exposed but not control rats. The increased latency again corresponded to increased swimming distance. No significant differences were observed for other testing situations in the Morris maze test. At 55 weeks, no statistically significant differences were observed between groups.

The Hass et al. (1995, 1997) studies found that prenatal exposure to xylenes affected the performance of female offspring in the Morris water maze test: female offspring took longer to find the platform. While swim length increased, swim speed was unaffected, indicating a cognitive rather than motor effect. These studies are limited, however, in that a concentration response is lacking because only one concentration was tested. Furthermore, no clear effect was observed in any of the other neurologic tests.

Groups of 36 pregnant, female Wistar rats were exposed to technical grade xylene at 0 or 200 ppm (exact composition not provided) for 6 h/day during GD 6 to 20 (Hass and Jakobsen 1993). On GD 21, two-thirds of the dams were killed and used to assess developmental toxicity, and one-third of the dams were allowed to deliver and developmental milestones and rotarod performance were assessed in eight offspring (four males and four females) from each litter. No signs of maternal toxicity were observed in any of the exposed dams. The only effect noted in fetuses from xylene-treated dams was an increased incidence of delayed ossification of the os maxcillare in the skull, with 18/26 exposed litters affected versus 2/22 control litters. In the postnatal study, statistically decreased rotarod performance was observed in female pups on postnatal days 22 and 23

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and in male pups on postnatal day 23. The Hass and Jakobsen (1993) study is limited in that only one exposure concentration was tested and only a limited battery of behavioral tests was used. Hass et al. (1995) conceded that the investigators were not blind to the exposure status of the animals.

Exposure of pregnant Sprague-Dawley rats to *p*-xylene at 800 or 1,600 ppm (3,500 or 7,000 mg/m^3; 99% pure) on GD 7 to 16 failed to influence litter size or pup weights at birth or on postnatal day 3; CNS development as measured by the acoustic startle response on postnatal days 13, 17, 21, and 63 or the figure-eight maze activity evaluated on postnatal days 22 and 65; or the growth rate of the pups (Rosen et al. 1986). The only effect of exposure was a significant reduction in maternal body weight gain in the dams at 1,600 ppm (74% of controls).

To investigate the effect of xylene inhalation on the liver of pregnant and nonpregnant rats and pups of exposed litters, pregnant Wistar rats were exposed to xylenes at 2,600 ppm (11,284 mg/m^3) (purity and composition not stated) for 8 h/day on GD 6 until term (GD 21) (Kükner et al. 1997-1998). Nonpregnant female rats were exposed to xylenes at 2,600 ppm for the same period, and a control group of pregnant rats inhaled clean air (not stated if nonpregnant controls were also included). Biochemical analyses of the livers from pregnant rats exposed to xylene found minimal increases in aspartate aminotransferase (18%), alanine aminotransferase (19%), alkaline phosphatase (17%), and arginase (63%) activity. Electron microscopic evaluation of pregnant and nonpregnant rat liver tissue revealed mitochondria that concentrated near the periphery of hepatocytes and nuclei, increased numbers of lysosomes, and expanded smooth endoplasmic reticulum. In fetal liver from exposed litters, findings included expanded smooth endoplasmic reticulum, structurally deformed mitochondria, and granular endoplasmic reticulum. No structural defects were observed in the kidneys or pancreas from exposed pregnant or nonpregnant rats or from fetuses recovered from xylene-exposed litters.

A number of other developmental toxicity studies were identified in the literature but were limited by several factors: composition and purity of the tested xylenes was not stated, values for the toxicity end points were not provided, fetal instead of litter incidences were reported, or inadequate sample sizes were available (Hudák and Ungváry 1978; Ungváry et al. 1980; Mirkova et al. 1983; Ungváry and Tátrai 1985).

3.4. Genotoxicity

The genotoxicity of commercial xylene and all three individual isomers has been extensively tested and the results have generally been consistently negative. All studies evaluated by the GENETOX panel and cited in the GENETOX database were negative except for one study for which no conclusion could be drawn (GENETOX 1992). Xylene was not mutagenic in bacterial

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test systems with strains of *Salmonella typhimurium* (Florin et al. 1980; Bos et al. 1981; NTP 1986) and *Escherichia coli* (McCarroll et al. 1981) or in cultured mouse lymphoma cells (Litton Bionetics 1978b). Xylene failed to induce chromosomal aberrations or sister chromatid exchanges in cultured Chinese hamster ovary cells (Anderson et al. 1990) or cultured human lymphocytes (Gerner-Smidt and Friedrich 1978), chromosomal aberrations in rat bone marrow (Litton Bionetics 1978b), micronuclei in mouse bone marrow (Mohtashamipur et al. 1985), or sperm head abnormalities in rats (Washington et al. 1983). Technical grade xylene, but not *o*- and *m*-xylene, was weakly mutagenic in *Drosophila* recessive lethal tests (Donner et al. 1980).

3.5. Carcinogenicity

No studies were found in the published literature on the carcinogenic potential of inhaled xylene in animals.

In a National Toxicology Program (NTP 1986) chronic toxicity and carcinogenesis bioassay, groups of 50 male and 50 female F344 rats and 50 male and 50 female B6C3F1 mice were administered mixed xylenes (60% *m*-xylene, 13.6% *p*-xylene, 17.0% ethylbenzene, and 9.1% *o*-xylene) in corn oil by gavage at doses of 0, 250, or 500 mg/kg/day (rats) and 0, 500, or 1,000 mg/kg/day (mice) for 5 days/week for 103 weeks. Histopathologic examination of the rats revealed an increased incidence of interstitial cell tumors in the testis of high-dose males after survival-adjusted analysis, but this increase was believed to be a consequence of high-dose animals dying between weeks 62 and 92. The overall incidence of interstitial cell tumors between groups was comparable (43/50, 38/50, and 41/49 for the control, low-dose, and high-dose groups, respectively). Therefore, the marginal increase in this tumor type was not ascribed to treatment. The National Toxicology Program (NTP 1986) reported no significant nonneoplastic or neoplastic effects in male or female mice.

Maltoni et al. (1983, 1985) exposed groups of 40 male and 40 female Sprague-Dawley rats to xylene at 0 or 500 mg/kg (mix of *o*-, *p*-, and *m*-xylenes; proportion of each isomer not stated) in olive oil orally by gavage 4 to 5 days/week for 104 weeks, followed by discontinuation of dosing to study termination at 141 weeks. Although Maltoni et al. reported an increase in the overall number of malignant tumors in treated males (14/40 versus 11/50 for controls) and females (22/40 versus 10/50 for controls), further results and explanation were not provided.

IARC (1999) has concluded that evidence for the carcinogenicity of xylene in humans or in experimental animals is inadequate and therefore stated that xylene is not classifiable as to carcinogenicity in humans (Group 3). The EPA (2003) has not classified xylenes as to carcinogenicity because data are inadequate to assess their carcinogenic potential.

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3.6. Summary

Xylene is an anesthetic solvent that may cause narcosis and death at sufficiently high atmospheric concentrations. In rats and mice, 4-h LC_{50} values ranging from 3,907 to 11,000 ppm have been reported (see Table 6-10). At lower concentrations, CNS disturbances and irritation are evident (see Table 6-11). No indication of consistent developmental or reproductive signs of toxicity was documented in the available literature. Commercial xylene and the three individual isomers failed to demonstrate any evidence of genotoxicity. Xylenes are currently not classified as to carcinogenicity by IARC (1999) or the EPA (2003).

TABLE 6-10 Summary of Lethal Xylene Inhalation Data in Laboratory Animals

Concentration (ppm)	Duration (h)	Isomer	Mortality and Other Effects	Reference
Cat				
9,500	2	Mixed	Killed all 4 cats	Carpenter et al. 1975b
Rat				
6,700	4	Mixed	LC_{50}	Carpenter et al. 1975b
6,350	4	Mixed	LC_{50}	Hine and Zuidema 1970
4,645	4	*p*-	LC_{50}	Harper et al. 1975
11,000	4	Mixed	LC_{50}	Lundberg et al. 1986
5,984	6	*m*-	LC_{50} *m*-xylene	Bonnet et al. 1982
4,330	6	*o*-	LC_{50} *o*-xylene	
4,591	6	*p*-	LC_{50} *p*-xylene	
1,531	24	*o*-	Highest no-effect level for death	Cameron et al. 1938
2,010	24	*m*-		
4,912	24-28	*p*-		
Mouse				
5,267	6	*m*-	LC_{50} *m*-xylene	Bonnet et al. 1979, 1982
4,595	6	*o*-	LC_{50} *o*-xylene	
3,907	6	*p*-	LC_{50} *p*-xylene	
1,531	24	*o*-	Highest no-effect level for death	Cameron et al. 1938
1,005	24	*m*-		
4,912	24	*p*-		

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TABLE 6-11 Summary of Nonlethal Xylene Inhalation Data in Laboratory Animals

Concentration (ppm)	Duration (h)	Isomer	Effects	References
Dog				
530	4	Mixed	No effect level	Carpenter et al. 1975b
1,200	4	Mixed	Lacrimation	
Rat				
580	4	Mixed	No effect level	Carpenter et al. 1975b
1,300	4	Mixed	Poor coordination after 2 h, recovered postexposure	
2,800	4	Mixed	Irritation; rats prostrate within 2.5-3 h into exposure; recovered within 1 h, but poor coordination until following day.	
1,600	4	*p*-	Changes in flash evoked potential suggest increased arousal	Dyer et al. 1988
2,100	4	*m*-	Minimum narcotic concentration	Molnár et al. 1986
2,180	4	*o*-		
1,940	4	*p*-		
800, 1,600	4	*p*-	Induced flavor aversion	Bushnell and Peele 1988
1,600	4	*p*-	Hyperactivity, fine tremor, unsteadiness	Bushnell 1989
113	2	Mixed	Minimum effective concentration for decreased reinforcement rate	Ghosh et al. 1987

(Continued)

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TABLE 6-11 Continued

Concentration (ppm)	Duration (h)	Isomer	Effects	References
98.5	5	Mixed	No effect of reinforcement rate	Ghosh et al. 1987
192	2	Mixed	Lowest concentration resulting in decrease in the rate of response for self-stimulation behavior	Wimolwattanapun et al. 1987
4,520	4	Mixed	EC_{50} for rotarod performance	Korsak et al. 1988
1,982	4	*m*-	EC_{50} for rotarod performance	Korsak et al. 1993
Mouse				
1,467		*o*-	RD_{50}	de Ceaurriz et al. 1981
1,361		*m*-	RD_{50}; not recommended strain of mice	Korsak et al. 1993
2,440		Mixed	RD_{50}; not recommended strain of mice	Korsak et al. 1988
2,000, 4,000, 8,000	0.33 (static)	*m*-	Increased mean hindlimb foot splay, decreased rearing	Tegeris and Balster 1994
3,790	0.5 (static)	*m*-	EC_{50} for inverted screen test	Moser et al. 1985
3,640	0.5 (static)	*o*-		
2,676	0.5 (static)	*p*-		
6,176	0.5 (static)	*m*-	EC_{50} for disruption of operant performance	Moser et al. 1985
5,179	0.5 (static)	*o*-		
5,611	0.5 (static)	*p*-		

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4. SPECIAL CONSIDERATIONS

4.1. Metabolism and Disposition

Pulmonary retention of inhaled xylene in humans has been reported to range between 49.8% and 72.8% (ATSDR 2007). No difference in retention has been observed among the individual isomers (Sedivec and Flek 1976) or between sexes (Senczuk and Orlowski 1978), but systemic uptake is increased by exercise (Astrand et al. 1978; Gamberale et al. 1978). After uptake from the lungs, xylene is distributed by the circulation to the peripheral tissues. The values for the human blood-air partition coefficient (PB) are 26.4, 31.9, and 32.5 for *m*-xylene; 31.1, 35.2, and 34.9 for *o*-xylene; and 37.6, 39.0, and 44.7 for *p*-xylene (Sato and Nakajima 1979; Gargas et al. 1989; Pierce et al. 1996), and the values for the rat PB are 46.0 for *m*-xylene, 44.3 for *o*-xylene, and 41.3 for *p*-xylene (Gargas et al. 1989). In human blood, xylene is associated primarily with serum proteins (Riihimaki et al. 1979). Distribution to human adipose tissue has been estimated to represent 3.7% to 10% of total uptake after inhalation (Engstrom and Riihimaki 1979; Astrand 1982). Inhalation exposure of mice and rats to radiolabeled xylene demonstrated that xylene is rapidly taken up from the lungs by the blood and immediately distributed to the kidneys, brain, subcutaneous body fat, bone marrow, spinal cord and spinal nerves, liver, and nasal mucosa; it is rapidly eliminated from these tissues with the exception of fat (Carlsson 1981; Bergman 1983; Kumarathasan et al. 1997). Ghantous et al. (1990) reported accumulation of xylene metabolites, primarily methylhippuric acid, in the nasal mucosa and olfactory bulb of the brain in mice after inhalation of radiolabeled *p*-xylene. Xylene has also been detected in the placenta, fetus, and amniotic fluid after maternal exposure, but the concentrations in fetal tissues were much lower than those in the maternal tissues (Ungváry et al. 1980; Ghantous and Danielsson 1986).

The primary metabolic pathway in humans is side-chain dehydroxylation by hepatic mixed-function oxidases to toluic acids (see Figure 6-1). The toluic acids are then conjugated with glycine to form methylhippuric acid isomers and excreted in urine. The methylhippuric acid isomers are produced almost exclusively in humans, with urine accounting for elimination of 95% to 97% of the absorbed dose in humans (Sedivec and Flek 1976; Riihimaki et al. 1979; Engstrom et al. 1984). Less than 10% of the absorbed dose is excreted unchanged by the lungs or kidneys (Sedivec and Flek 1976; Riihimaki et al. 1979) or as minor metabolites including urinary xylenols (Sedivec and Flek 1976; Riihimaki et al. 1979; Engstrom et al. 1984), toluic acid glucuronides (Ogata et al. 1979, 1980), or mercapturic acid (Norström et al. 1988). Miller and Edwards (1999) found evidence that of the three xylene isomers, *m*-xylene is preferentially metabolized to methylhippuric acid in the presence of the other two isomers, regardless of the isomer composition. The relevance of this finding in assessing the toxicity of the individual isomers is not known at this time.

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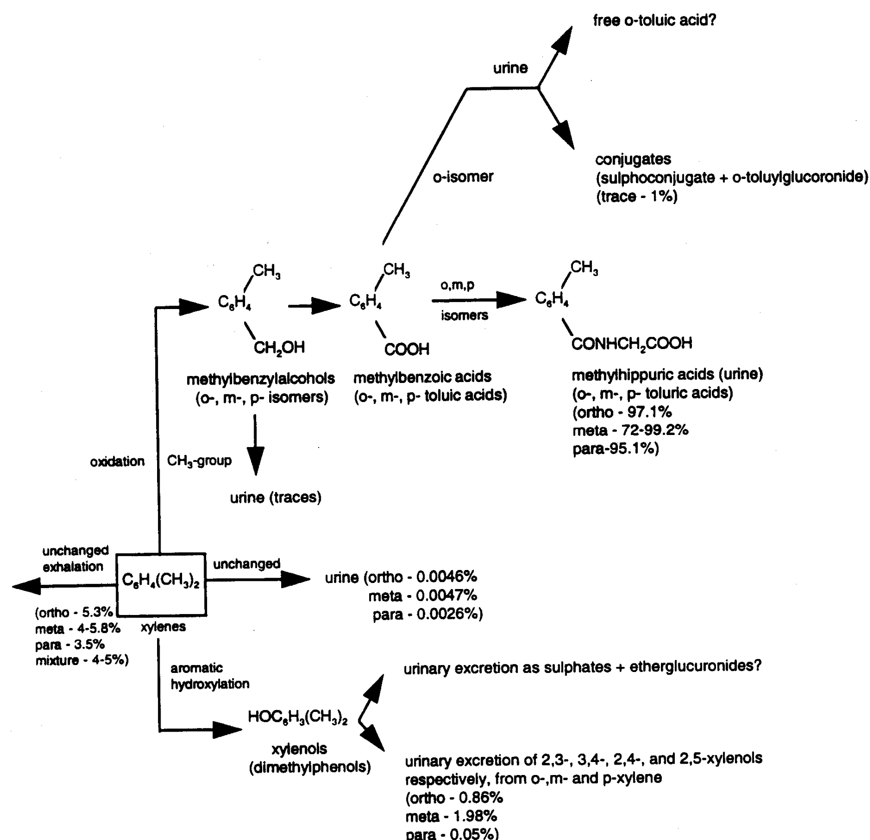

FIGURE 6-1 Metabolic scheme for xylenes in humans. Source: ATSDR 2007.

Metabolism of xylenes in common laboratory animals also proceeds via hepatic side-chain dehydroxylation by mixed function oxidases to toluic acids. Further metabolism depends on species and isomer composition, with fluctuations occurring primarily in the ratio of urinary methylhippuric acid and toluic acid glucuronides (Bray et al. 1949; Ogata et al. 1980; Tardif et al. 1989). It has been proposed that the differences observed between humans and animals in xylene metabolism may be due to differences in the size of the doses administered to each: the larger doses received by animals may saturate the glycine-conjugation pathway (Figure 6-1) (ATSDR 2007).

In humans, excretion of xylenes after inhalation is rapid and occurs almost exclusively as urinary methylhippuric acid isomers with a minor amount as toluic acid glucuronides (Ogata et al. 1970; Senczuk and Orlowski 1978; Riihimaki et al. 1979; Ogata et al. 1980; Engström et al. 1984). Only a minor amount (4% to 5%) of absorbed xylene is excreted unchanged by the lungs (Sedivec and

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Flek 1976; Riihimaki et al. 1979). Riihimaki et al. (1979) estimated that human excretion of xylene in air and urine has an initial half-life of 1 h, followed by a slow phase with an estimated half-life of 20 h. In general, a linear correlation has been found between the intensity of xylene exposure and the amount of methylhippuric acid isomers excreted in the urine (Lundberg and Sollenberg 1986; Imbriani et al. 1987; Kawai et al. 1991, 1992; Inoue et al. 1993).

4.2. Mechanism of Toxicity

Xylene exposure of humans and common laboratory animals can result in nervous system disturbance. CNS effects in humans after acute and chronic inhalation exposure to xylene include headache, vertigo, nausea, fatigue, irritability, dizziness, impaired concentration, and confusion (Carpenter et al. 1975b; Hipolito 1980; Klaucke et al. 1982). Case reports of individuals exposed to high concentrations of xylenes by inhalation, ingestion, or intravenous injection have reported severe respiratory effects including respiratory failure (Morley et al. 1970; Recchia et al. 1985; Abu Al Ragheb et al. 1986; Sevcik et al. 1992). The respiratory effects were most likely a secondary response to depression of the respiratory center of the brain. Nonlethal effects after exposure to high concentrations of xylenes (~10,000 ppm) include unconsciousness, slurred speech, and ataxia (Morley et al. 1970). Controlled acute inhalation exposure in human males yielded mixed results after neurobehavioral testing. A number of studies found that exposure to *p*- or *m*-xylene concentrations ranging from 70 to 400 ppm for up to 4 h either failed to affect the performance of subjects in neurobehavioral testing (Olson et al. 1985) or actually improved performance (Savolainen et al. 1981, 1985b; Laine et al. 1993). Other studies found correlations between acute exposure to *m*-xylene at concentrations ranging from 64 to 400 ppm for up to 4 h and impaired performance (Gamberale et al. 1978; Savolainen and Linnavo 1979; Savolainen et al. 1979b, 1980; Savolainen and Riihimaki 1981; Seppalainen et al. 1983; Savolainen et al. 1984, 1985a; Seppalainen et al. 1989; Dudek et al. 1990; Seppalainen et al. 1991). Some studies evaluating the effects of acute exposure to *m*-xylene have indicated the development of tolerance in exposed subjects (Savolainen et al. 1980; Savolainen and Riihimaki 1981).

Signs of CNS toxicity after acute exposure to xylenes were also reported in animals. Preanesthetic effects associated with acute exposure to high concentrations of xylenes include poor coordination and prostration (Carpenter et al. 1975b), increased hindlimb foot splay (Tegeris and Balster 1994), reduced performance on the inverted screen test (Moser et al. 1985) and rotarod (Korsak et al. 1988; 1993), and disrupted operant performance (Moser et al. 1985). CNS effects after exposure to lower concentrations of xylenes include changes in flash evoked potentials (Dyer et al. 1988), induced flavor aversion (Bushnell and Peele 1988), decreased reinforcement rates of fixed-ratio responding (Ghosh et al. 1987), decreased rates of response for self-stimulation behavior (Wimolwat-

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tanapun et al. 1987), and facilitated autoshaping (Bushnell 1989). Evidence of tolerance was also reported in animals (Ghosh et al. 1987; Wimolwattanapun et al. 1987).

The low molecular weight and lipophilic nature of xylenes allow the solvent to readily cross the blood-brain barrier. Studies investigating the distribution of radiolabeled xylenes after inhalation confirmed high concentrations of xylene in the brain and central and peripheral nervous systems immediately after exposure, with elimination often occurring by 1 h postexposure (Carlsson 1981; Bergman 1983; Ghantous and Danielsson 1986; Kumarathasan et al. 1997). The transient nature of many of the xylene-induced nervous system disturbances is likely due to the rapid elimination of xylene. The precise molecular mechanism by which xylenes affect the nervous system is not known. An in vitro study using human and rat cell membranes demonstrated that xylene and other solvents with anesthetic properties could bind in hydrophobic pockets in integral cell membrane proteins, thereby altering the properties of integral enzymes (Tahti 1992). Others found that xylene exposure affected the enkephalinergic neuromodulatory system (de Gandarias et al. 1995), catecholamine neurotransmission by altering levels of dopamine and noradrenaline (Andersson et al. 1981), and levels of brain acetylcholine and glutamine (Honma et al. 1983). Xylene exposure reduced transport of cellular materials to axons and nerve ending regions in rats (Padilla and Lyerly 1989), affected microsomal superoxide dismutase activity in the brain of rats (Savolainen et al. 1979a), and resulted in findings compatible with astrogliosis in gerbils (increased brain concentrations of glial fibrillary acidic protein, S-100 protein, and DNA) (Rosengren et al. 1986). Xylene exposure did not influence neutral or basic aminopeptidase activities in the brains of rats (de Gandarias et al. 1993).

Data demonstrating the hepatoxicity of xylene are limited. Human data were primarily limited to case reports. Morley et al. (1970) reported an accidental exposure to xylene at approximately 10,000 ppm. The autopsy of a worker who died revealed hepatic congestion with swelling and vacuolization of cells in the centrilobular areas. Two other xylene-exposed workers who survived had only slight hepatic impairment as indicated by a rise in serum transaminase activity over 48 h after exposure, after which the enzyme activity returned to normal levels. Hepatic changes in rats sometimes followed subchronic oral or inhalation exposure to xylenes and may be consistent with an adaptative response (Tátrai and Ungváry 1980; Tátrai et al. 1981; Ungváry 1990).

Developmental toxicity in animals has generally been observed only at doses or concentrations similar to or exceeding those resulting in maternal toxicity. Two studies were found investigating the potential mechanism for xylene-induced developmental toxicity (developmental retardation and death). In a study investigating the role of maternal sex steroid production and metabolism in *p*-xylene embryotoxicity, Ungváry et al. (1981) reported that exposure of pregnant rats to *p*-xylene at 690 ppm (3,000 mg/m^3) on GD 10 or GD 9 to 10 did not affect maternal ovarian and uterine circulation or ovarian hormone secretion rate as measured on GD 11 as compared with controls. However, exposure to *p*-

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xylene for 48 h (GD 9 to 10) did result in a statistically significant decrease in the peripheral levels of progesterone and β-estradiol and significantly decreased fetal body weight (actual data not provided). It was proposed that the hepatic enzyme induction by *p*-xylene was responsible for increased metabolism of the sex hormones, which in turn was responsible for fetotoxicity. Ungváry and Donáth (1984) found that exposure of pregnant rats to *p*-xylene at approximately 350 ppm resulted in hyperinnervation or degeneration of noradrenergic nerves of reproductive organs (uterus, ovaries). They proposed that damage to the peripheral noradrenergic nerves can result in altered control of uterine and ovarian blood flow and steroid production, resulting in fetal toxicity.

4.3. Other Relevant Information

4.3.1. Interspecies Differences

Physiologically based pharmacokinetic (PBPK) modeling was done for rats and humans (see Appendix C). PBPK modeling allows a comparison of the internal dose in both species receiving identical external exposures. As shown in Figure 6C-9 in Appendix C, rats achieve higher blood *m*-xylene concentrations than humans at the same atmospheric xylene levels. This effect is primarily due to a higher blood-air partition coefficient (PB) in rats (46) compared with humans (26.4, 31.9, and 32.5) (Sato and Nakajima 1979; Gargas et al. 1989; Pierce et al. 1996). Other factors include the rats' higher respiratory (alveolar ventilation) rate and higher cardiovascular output and tissue blood flow rates.

The total interspecies factor includes the pharmacodynamic component as well. The available data indicate relatively little difference in interspecies sensitivity to xylene. Lethality data for mice and rats were nearly identical (Cameron et al. 1938; Bonnet et al. 1982). Death was preceded by narcosis and was likely the result of depression of the CNS resulting in respiratory arrest. Nonlethal effects in both humans and animals are similar and consist primarily of mucus irritation and CNS depression.

4.3.2. Intraspecies Differences

Available data point to a 2- to 3-fold difference in interindividual sensitivity to xylenes.

At sufficiently high concentrations, xylene acts as an anesthetic (Fang et al. 1996). Studies indicate that children, particularly infants, are more resistant than adults to the pharmacologic actions of various volatile anesthetics (Gregory et al. 1969; Stevens et al. 1975; Lerman et al. 1983; LeDez and Lerman 1987; Katoh and Ikeda 1992; Chan et al. 1996). The susceptibility of different age groups has been extensively studied in the medical literature where the concentrations of various anesthetic gases in the lung that produce "anesthesia" (lack of movement) have been measured (NRC 2002). Values are usually reported as the

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minimum alveolar concentration (MAC) that produces lack of movement in 50% of persons exposed to that concentration. MACs for several anesthetic gases have been measured as a function of age. The results consistently show a pattern with maximal sensitivity (lowest MAC) in newborns, particularly prematures, pregnant women, and the elderly. The least sensitivity (highest MAC values) occurs in older infants, toddlers, and children as compared with adults. The total range of sensitivity is 2- to 3-fold. On the basis of this knowledge, it is not unreasonable to conclude that the same 2- to 3-fold difference in sensitivity exists among individuals exposed to xylenes.

4.3.3. Concentration-Exposure Duration Relationship

The two primary effects of xylene exposure are irritation and CNS effects. Irritation is considered a threshold effect and therefore should not vary over time. An AEGL value based on irritation is therefore not scaled across time but rather the same value is applied across all times.

The CNS effects of xylene are attributed to the low molecular weight and lipophilic nature, which allow the solvent to readily cross the blood-brain barrier (see Section 4.2). Distribution studies of xylene after inhalation have confirmed high concentrations of xylene in the brain and central and peripheral nervous system immediately after exposure, with substantial elimination often occurring by 1 h postexposure. The rapid onset and transient nature of CNS effects caused by xylene are likely due to direct interaction with molecular receptors in the CNS followed by the rapid elimination of xylene. Xylenes readily diffuse bidirectionally between the blood and brain, rapidly attaining and striving to maintain an equilibrium between the two compartments. The blood-brain partition coefficient is the ratio of the xylene concentrations in blood and brain under near-steady-state conditions. Thus, the arterial or venous blood concentration of xylene is a reliable index of the brain concentration and, in turn, the magnitude of the CNS depression that is due to the parent compound. Thus, the xylene-blood concentration is a key determinant of impaired CNS activity. Therefore, the venous xylene blood concentration (CV) after exposure would be expected to provide an internal dose measurement correlating with clinical signs. PBPK modeling (see Appendix C) was used to determine the internal dose (CV) producing poor coordination for the AEGL-2 and prostration for the AEGL-3 in rats. The human PBPK model of xylene was then run for each defined AEGL time period to determine the equivalent atmospheric exposure concentration producing the target CV.

4.3.4. PBPK Model

As discussed in Section 4.3.3, PBPK modeling was applied to derive the AEGL-2 and AEGL-3 values. The PBPK modeling process for xylenes is described in detail in Appendix C. Briefly, two research groups developed PBPK

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models for *m*-xylene: one group developed a six-compartment model in rats and a seven-compartment model in humans; the other group developed a four-compartment model in rats and humans (Kaneko et al. 1991a,b, Tardif et al. 1993, 1997; Haddad et al. 1999; Kaneko et al. 2000). The basic model generated by Tardif et al. (1993, 1997) and Haddad et al. (1999) was chosen for the AEGL derivations because it was more data rich. The main difference among the models was in the physiologic parameters used. The rat model from Haddad et al. (1999) was chosen because it was the more recent, was more data rich, and had a better fit. The Haddad et al. (1999) model was optimized by using the Tardiff et al. (1993) gas uptake data (500, 1,000, 2,000, and 4,000 ppm). The acute lethality critical study (AEGL-3) is based on an exposure concentration (2,800 ppm) that lies within the range of concentrations used in the gas uptake study. The model was then visually reoptimized for *m*- and *p*-xylene with the available human data. Finally, the internal dose (CV) producing the toxicity end point of concern in rats was determined. The human PBPK model of xylene was then run for each defined AEGL time period to determine the equivalent atmospheric exposure concentration producing the target CV.

4.3.5. Comparison of the Toxicity of Individual Xylene Isomers

Because xylene exists as a mixture or as any of three individual isomers, the question arises as to whether there are differences in toxicity among the individual isomers and the mixture. PBPK model predictions indicate that the internal dose (CV) after exposure does not vary significantly among the individual isomers (see Figure 6C-10 in Appendix C).

Fang et al. (1996) determined the MAC (the concentration that produces anesthesia, or lack of movement, in 50% of those exposed) of the individual isomers in rats. The MACs of *o*-, *m*-, and *p*-xylene were 0.00118 ± 0.00009, 0.00139 ± 0.00010, and 00.00151 ± 0.0007 atmospheres, respectively, with a difference in MAC values of less than 30% among the isomers.

Only a limited number of studies were found in the open literature comparing the toxicity of the individual xylene isomers. Although differences did exist among the studies, no consistent, significant differences in the toxicologic potency of the xylene isomers after oral or inhalation exposure were identified (Ungváry et al. 1980; Moser et al. 1985; Molnár et al. 1986; Condie et al. 1988; Korsak et al. 1990).

5. DATA ANALYSIS AND PROPOSED AEGL-1

5.1. Human Data Relevant to AEGL-1

Exposure to mixed xylenes at 100, 200 or 400 ppm for 30 min resulted in (nonstatistically) increased complaints of eye irritation; no increased nose or throat irritation was noted and no changes in behavioral tests or respiratory

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measurements were evident in controlled studies (Hastings et al. 1984). That mild degree of eye irritation is supported by observation that the number of eye blinks per minute was not affected by exposure. Exposure to *p*-xylene at 100 or 150 ppm for 7.5 h/day, 5 days/week resulted in mild eye irritation, most often in one male wearing contact lenses (irritation was noted on the first exposure day) (Hake et al.1981). No effects on performance tests were observed at these levels. Exposure to mixed xylenes at 110 ppm for 15 min resulted in intermittent, mild throat irritation in 1/6 individuals, while exposure to 230 ppm for 15 min resulted in eye irritation and mild dizziness in 1/7 individuals (Carpenter et al. 1975b).

A number of controlled human studies reported no adverse effects after exposure to xylenes. Exposure to *m*- or *p*-xylene at 100 or 200 ppm for 3 or 7 h failed to influence blood pressure, pulse rate, flicker value, or reaction time (Ogata et al. 1970). Olson et al. (1985) found exposure to *p*-xylene at 70 ppm for 4 h had no effect on choice reaction time, simple reaction time, short-term memory, heart rate, or subjective symptoms in exposed volunteers. No adverse effects on VEP, tapping speed, body sway, reaction time, or critical flicker fusion were measured in volunteers exposed to *m*-xylene at 200 ppm for 4 h (Savolainen et al. 1981; Seppalainen et al. 1983). Body sway, reaction time, and active or quiet sleep were not affected by exposure to 200 ppm for 5.5 h (Laine et al. 1993).

5.2. Animal Data Relevant to AEGL-1

No signs of toxicity were observed in dogs exposed to mixed xylenes at 530 ppm or in rats exposed to 580 ppm for 4 h, while lacrimation in dogs and poor coordination in rats were observed at 1,200 and 1,300 ppm, respectively (Carpenter et al. 1975b). In the study investigating conditioned flavor aversion, a concentration-related decrease in relative saccharin consumption was observed in all groups exposed to *p*-xylene for 4 h, with maximal aversion occurring in the 800- and 1,600-ppm groups (Bushnell and Peele 1988). The premise of the conditioned flavor aversion paradigm is combining exposure to a chemical with the introduction of something new, such as saccharine water. The animals then associate any negative effects they experience from exposure to the chemical with the consumption of the new food item and develop an aversion to the food item. Although it is a nonsensory effect, aversion represents an AEGL-1 effect.

5.3. Derivation of AEGL-1

The AEGL-1 is based on mild eye irritation in human subjects noted by Hastings et al. (1984) during a 30-min exposure to mixed xylenes at 400 ppm. The Hastings et al. (1984) results were considered most relevant because exposure was to mixed xylenes and represented a concentration at which an AEGL-1 effect was observed. An interspecies uncertainty factor was not applied because

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the key study used human data. An intraspecies uncertainty factor of 3 was applied because slight eye irritation is caused by a direct effect of the chemical and the response is not expected to vary greatly among individuals. Irritation is considered a threshold effect, which should not vary over time; therefore, the AEGL-1 value was not scaled across time, but rather the same value is applied across all times. AEGL-1 values are presented in Table 6-12.

The 130-ppm value is supported by several other studies, including the *p*-xylene 150-ppm exposure resulting in eye irritation in a contact lens wearer (represents sensitive population; Hake et al. 1981); the 15-min exposure to mixed xylenes at 230 ppm resulting in mild eye irritation and dizziness in one individual; and the 3-h exposure to *m*- or *p*-xylene at 200 ppm (Ogata et al. 1970), the 4-h exposure to *m*-xylene at 200 ppm (Savolainen et al. 1981), and the 5.5-h exposure to *m*-xylene at 200 ppm (Laine et al. 1993). All these values represent acute NOAELs in adult humans.

6. DATA ANALYSIS AND PROPOSED AEGL-2

6.1. Human Data Relevant to AEGL-2

One of six or seven individuals noted dizziness during a 15-min exposure to mixed xylenes at 230 ppm (during the last 2 min of exposure) or 460 ppm (starting at the 6th minute and continuing to the end of exposure in the same individual), while a 15-min exposure to 690 ppm resulted in dizziness and light-headedness in 4/6 individuals (Carpenter et al. 1975b). In the same study, a 15-min exposure resulted in eye irritation in 1/7, 4/6, and 4/6 individuals exposed to mixed xylenes at 230, 460, and 690 ppm, respectively.

6.2. Animal Data Relevant to AEGL-2

Exposure to mixed xylenes at 1,200 ppm for 4 h represents a threshold for lacrimation in dogs, while rats exposed to 1,300 ppm exhibited poor coordination (reversible) 2 h into a 4-h exposure (Carpenter et al. 1975b). The 4-h *m*-xylene EC_{50} for decreased rotarod performance in rats was 1,982 ppm (Korsak et al. 1993), and the 4-h minimum narcotic concentrations for the three xylene isomers in rats ranged from 1,940 to 2,180 ppm (Molnár et al. 1986). Exposure of rats to *p*-xylene at 1,600 ppm for 4 h resulted in hyperactivity, fine tremor, and unsteadiness (Bushnell 1989) and caused changes in the flash evoked potential suggestive of increased arousal (Dyer et al. 1988).

TABLE 6-12 AEGL-1 Values for Xylenes

10 min	30 min	1 h	4 h	8 h
130 ppm ($560 mg/m^3$)	130 ppm ($560 mg/m^3$)	130 ppm ($560 mg/m^3$)	130 ppm ($560 mg/m^3$)	130 ppm ($560 mg/m^3$)

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After 30-min static exposures in mice, Moser et al. (1985) determined that the EC_{50} for decreased performance on the inverted screen test was 3,790 ppm for *m*-xylene, 3,640 ppm for *o*-xylene, and 2,676 ppm for *p*-xylene, while the EC_{50} for disruption of operant performance was 6,176 ppm for *m*-xylene, 5,179 ppm for *o*-xylene, and 5,611 ppm for *p*-xylene.

6.3. Derivation of AEGL-2

The AEGL-2 is based on the threshold for reversible equilibrium disturbances and the no-effect level for the impaired ability to escape. Poor coordination was observed in rats 2 h into a 4-h exposure to mixed xylenes at 1,300 ppm (Carpenter et al. 1975b). This concentration and end point are consistent with the preponderance of available data for 4-h exposures in rats: the EC_{50} for decreased rotarod performance was 1,982 ppm (Korsak et al. 1993); the minimum narcotic concentrations for *m*-, *o*-, and *p*-xylene ranged from 1,940 to 2,180 ppm (Molnár et al. 1986); and exposure to *p*-xylene at 1,600 ppm resulted in hyperactivity, fine tremor, and unsteadiness (Bushnell 1989) and caused changes in the flash evoked potential suggestive of increased arousal (Dyer et al. 1988). It is assumed that the CNS response observed after xylene exposure is directly related to the concentration of parent material reaching the brain and that CV values correlate with brain concentrations. Therefore, the CV of xylene after a 2-h exposure to xylene at 1,300 ppm is expected to provide an internal dose measurement correlating with the clinical sign of poor coordination. The internal dose (CV) producing impaired coordination in rats was determined with the PBPK model (see Appendix C). Then, the human PBPK model was run for each defined AEGL time period to determine the equivalent exposure concentration producing the target CV.

A total uncertainty factor of 3 was applied to the AEGL-2 and AEGL-3 dose metrics. An intraspecies uncertainty factor of 3 was applied for the pharmacokinetic and pharmacodynamic uncertainty because the MAC for volatile anesthetics should not vary by more than 2- to 3-fold among humans (NRC 2002). An interspecies uncertainty factor of 3 would usually be applied. PBPK modeling reduced the toxicokinetic component of the uncertainty factor to 1, but the pharmacodynamic component would normally be retained and assigned a 3 (although it appears that similar CNS effects occur in humans and animals, it is not known if they occur at the same tissue dose). A total uncertainty factor of 10, however, drives the AEGL-2 value for 8 h (180 ppm) to an exposure concentration that humans are known to tolerate with minimal or no adverse effects. Humans exposed to *p*-xylene at 150 ppm for 7.5 h did not exhibit any effects on performance tests and noted only mild eye irritation (Hake et al. 1981). Therefore, the interspecies uncertainty factor is reduced to 1, and a total uncertainty factor of 3 is applied to the AEGL-2 values (NRC 2002).

AEGL-2 values are presented in Table 6-13.

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TABLE 6-13 AEGL-2 Values for Xylenes

10 min	30 min	1 h	4 h	8 h
2,500 ppm ($11,000 mg/m^3$)	1,300 ppm ($5,600 mg/m^3$)	920 ppm ($4,000 mg/m^3$)	500 ppm ($2,200 mg/m^3$)	400 ppm ($1,700 mg/m^3$)

The human data reported by Carpenter et al. (1975b) were not used for the AEGL-2 derivation as the exposure duration was brief (15 min) and it was not consistent with the preponderance of human data from other controlled human exposures. If one were to use the highest exposure concentration (690 ppm) that resulted in eye irritation and dizziness in 4/6 individuals, a threshold for equilibrium effects, and apply the intraspecies uncertainty factor of 3, one would obtain a value of 230 ppm. This concentration is supposed to represent a concentration at which exposed individuals could experience irreversible or other serious, long-lasting adverse health effects or have an impaired ability to escape. However, a number of studies demonstrated no adverse effects after exposure to *m*- or *p*-xylene at 100 or 200 ppm for 3 or 7 h (Ogata et al. 1970), to *m*-xylene at 200 ppm for 4 h (Savolainen et al. 1981), or to *m*-xylene at 200 ppm for 5.5 h (Laine et al. 1993). The Ogata et al. (1970) study found no effect on the ability to escape at 200 ppm for 3 or 7 h and thus supports the AEGL-2 value. However, it is not appropriate to use this study to derive the AEGL-2 value as 200 ppm is far below the threshold for CNS effects.

7. DATA ANALYSIS AND PROPOSED AEGL-3

7.1. Human Data Relevant to AEGL-3

Morley et al. (1970) reported the cases of three individuals exposed to xylene at approximately 10,000 ppm for about 18 h. One individual died, and the other two were found unconscious but experienced a full recovery.

7.2. Animal Data Relevant to AEGL-3

Two cats exposed to mixed xylenes at 9,500 ppm exhibited signs of CNS depression followed by death 2 h into the exposure (Carpenter et al. 1975b). In rats, 4-h LC_{50} values for mixed xylenes have been reported as 6,350 ppm (Hine and Zuidema 1970), 6,011 ppm (Carpenter et al. 1975b), and 11,000 ppm (Lundberg et al. 1986), and for *p*-xylene as 4,645 ppm (Harper et al. 1975). Six-hour LC_{50} values for the *m*-, *o*-, and *p*-isomers were 5,984, 4,330, and 4,591 ppm, respectively, in rats and 5,267, 4,595, and 3,907 ppm, respectively, in mice (Bonnet et al. 1979; 1982).

A no-effect level for death in rats after exposure to mixed xylenes for 4 h was 2,800 ppm (Carpenter et al. 1975b). Clinical signs observed during exposure to 2,800 ppm included prostration between 2 and 3.5 h into the exposure.

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Recovery occurred within 1 h postexposure, but coordination remained poor until the following day. At the next lower concentration of 1,300 ppm, poor coordination was noted 2 h into the exposure, with coordination returning to normal after the exposure. Molnár et al. (1986) reported 4-h minimum narcotic concentrations of 2,100, 2,180, and 1,940 ppm for the *m*-, *o*-, and *p*-xylene isomers, respectively.

RD_{50} values in mice were 1,467 ppm for *o*-xylene (de Ceaurriz et al. 1981), 1,361 ppm for *m*-xylene (Korsak et al. 1993), and 2,440 ppm for mixed xylenes (Korsak et al. 1988). Korsak et al. (1988, 1993) did not use the recommended strain of mice.

7.3. Derivation of AEGL-3

The AEGL-3 derivation is based on reversible prostration and a no-observed-effect level (NOEL) for death in rats exposed to 2,800 ppm for 4 h (Carpenter et al. 1975b). Although coordination initially remained poor, it returned to normal the following day. This concentration represents a threshold for marked CNS depression, which could lead to death. As for the AEGL-2, it is assumed that the CNS effects observed after xylene exposure are directly related to the concentration of parent material reaching the brain. Therefore, PBPK modeling (see Appendix C) was again used to calculate the internal dose (CV) correlating with an exposure of rats to 2,800 ppm for 4 h, which produced prostration. The human PBPK model was then run for each defined AEGL time period to determine the equivalent exposure concentration producing the target CV.

A total uncertainty factor of 3 was applied to the AEGL-2 and -3 dose metrics. An intraspecies uncertainty factor of 3 was applied for the pharmacokinetic and pharmacodynamic uncertainty because the MAC for volatile anesthetics should not vary by more than 2- to 3-fold among humans (NRC 2002). An interspecies uncertainty factor of 3 would usually be applied. PBPK modeling reduced the toxicokinetic component of the uncertainty factor to 1, but the pharmacodynamic component would normally be retained and assigned a 3 (although it appears that similar CNS effects occur in humans and animals, it is not known if they occur at the same tissue dose). A total uncertainty factor of 10, however, drives the 4-h AEGL-3 value to 447 ppm, an exposure concentration that humans are known to tolerate with minimal or no adverse effects. Numerous human studies investigated the effects of exposure to *m*-xylene at 130 to 200 ppm for 4 to 6 h, with 20-min peaks of 400 ppm with or without exercise (Savolainen and Linnavuo 1979; Savolainen et al. 1984, 1985a,b; Seppalainen et al. 1989, 1991; Laine et al. 1993) and found no effect or only minimal CNS effects. Therefore, the interspecies uncertainty factor is reduced to 1, and a total uncertainty factor of 3 is applied to the AEGL-3 values (NRC 2002).

AEGL-3 values are presented in Table 6-14.

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TABLE 6-14 AEGL-3 Values for Xylenes

10 min	30 min	1 h	4 h	8 h
7,200 ppm ($31,000 mg/m^3$)	3,600 ppm ($16,000 mg/m^3$)	2,500 ppm ($11,000 mg/m^3$)	1,300 ppm ($5,600 mg/m^3$)	1,000 ppm ($4,300 mg/m^3$)

8. SUMMARY OF PROPOSED AEGLs

8.1. AEGL Values and Toxicity End Points

The AEGL values for xylenes summarized in Table 6-15 apply to each of the individual xylene isomers or a mixture of xylene isomers. AEGL-2 and AEGL-3 values are greater than 10% of the lower explosive limit. As discussed in Section 4.3.3, no significant differences in the potency of the isomers after oral or inhalation exposure were identified and metabolism of each isomer proceeds along the same pathways.

A useful way to evaluate the AEGL values in the context of existing empirical data is presented in Figure 6-2. For this plot, the toxic response was placed in severity categories. The severity categories fit into definitions of the AEGL health effects: no effects, discomfort, disabling, some lethality (an experimental concentration at which some of the animals died and some did not), and lethal. The effects that place an experimental result in a particular category vary according to the spectrum of data available on a specific chemical and the effects from exposure to that chemical. The doses often span a number of orders of magnitude, especially when human data exist. Therefore, the concentration is on a logarithmic scale. Figure 6-2 plots the xylene AEGL values along with the existing acute human and animal toxicity data for xylene in terms of the categories assigned to them.

TABLE 6-15 Summary and Relationship of AEGL Values

Classification	10 min	30 min	1 h	4 h	8 h
AEGL-1 (Nondisabling)	130 ppm ($560 mg/m^3$)	130 ($560 mg/m^3$)	130 ($560 mg/m^3$)	130 ($560 mg/m^3$)	130 ($560 mg/m^3$)
AEGL-2 (Disabling)	2,500 ppm[a] ($11,000 mg/m^3$)	1,300 ppm[a] ($5,600 mg/m^3$)	920 ppm[a] ($4,000 mg/m^3$)	500 ppm ($2,200 mg/m^3$)	400 ppm ($1,700 mg/m^3$)
AEGL-3 (Lethal)	—[b]	3,600 ppm[a] ($16,000 mg/m^3$)	2,500 ppm[a] ($11,000 mg/m^3$)	1,300 ppm[a] ($5,600 mg/m^3$)	1,000 ppm[a] ($4,300 mg/m^3$)

[a]Concentrations are at or higher than 1/10th of the LEL for all forms of xylene (*o*-xylene LEL, 9,000 ppm; *m*- and *p*-xylene LEL, 11,000 ppm). Therefore, safety considerations against the hazard of explosion must be taken into account.
[b]10-min AEGL-3 = 7,200 ppm is ≥50% LEL. Therefore, extreme safety considerations against the hazard of explosion must be taken into account.

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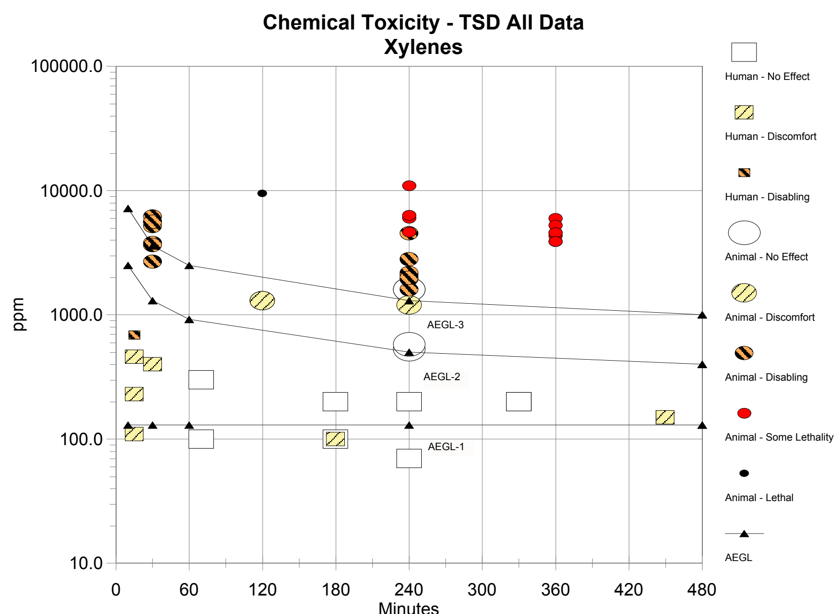

FIGURE 6-2 Category plot of human and animal toxicity data compared with AEGL values.

8.2. Comparisons with Other Standards

Standards and guidance levels for workplace and community exposures are listed in Table 6-16. The 130-ppm AEGL-1 is almost identical to the American Conference of Governmental Industrial Hygienists (ACGIH) and the National Institute for Occupational Safety and Health (NIOSH) 15-min short-term exposure limit (150 ppm), and it is close to the ACGIH, NIOSH, and Occupational Safety and Health Administration 8-h TWA (100 ppm). The 30-min AEGL-2 (1,300 ppm) and AEGL-3 (3,600 ppm) are higher than the 30-min concentration that is immediately dangerous to life or health (900 ppm).

8.3. Data Adequacy and Research Needs

Data appropriate for use in xylene AEGL derivations are robust. Numerous studies investigating controlled human exposures are available, but the effects noted are generally less serious than those defined by the AEGLs. Human lethality data are limited to a case report in which one of three individuals exposed to approximately 10,000 ppm for almost 19 h died (Morley et al. 1970). Animal data on nonlethal effects were available for dogs, cats, rats, and mice, although most of the data documenting CNS effects after acute exposure were

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on rats. Lethality data were available for rats and mice and indicated little difference in sensitivity between these species.

Because of inadequate evidence, xylene is currently not classifiable as to carcinogenicity by IARC or the EPA.

TABLE 6-16 Extant Standards and Guidelines for Xylenes

Guideline	Exposure Duration						
	10 min	15 min	30 min	1 h	4 h	8 h	24 h
AEGL-1	130 ppm		130 ppm	130 ppm	130 ppm	130 ppm	
AEGL-2	2,500 ppm		1,300 ppm	920 ppm	5,00 ppm	400 ppm	
AEGL-3	7,200 ppm		3,600 ppm	2,500 ppm	1,300 ppm	1,000 ppm	
EEL (NRC)[a]				200 ppm			100 ppm
SMAC (NRC)[b]				100 ppm			100 ppm
IDLH (NIOSH)[c]			900 ppm				
TLV-TWA (ACGIH)[d]						100 ppm	
PEL-TWA (OSHA)[e]						100 ppm	
REL-TWA (NIOSH)[f]						100 ppm	
TLV-STEL (ACGIH)[g]		150 ppm					
REL-STEL (NIOSH)[h]		150 ppm					
MAK (Germany)[i]						440 mg/m^3 100 ppm	
MAC (The Netherlands)[j]						210 mg/m^3 50 ppm	

[a]EEL (emergency exposure limit, National Research Council) (NRC 1984) is defined as a ceiling limit for an unpredictable single exposure, usually lasting 60 min or less, and never more than 24 h—an occurrence expected to be rare in the lifetime of any person. It reflects an acceptance of the statistical likelihood of the occurrence of a nonincapacitating reversible effect in an exposed population. It is designed to avoid any substantial decrements in performance during emergencies and might contain no uncertainty factor. The use of uncertainty factors depends on the compound in question and on the type of effect it produces.

[b]SMAC (spacecraft maximum allowable concentration, National Research Council) (Garcia 1996) provides guidance on chemical exposures during normal operations of spacecraft as well as emergency situations. The 1-h SMAC is a concentration of airborne substance that will not compromise the performance of specific tasks by astronauts during emergency conditions or cause serious or permanent toxic effects. Such exposure may cause reversible effects, such as skin or eye irritation, but they are not expected to impair judgment or interfere with proper responses to emergencies.

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[c]IDLH (immediately dangerous to life or health, National Institute for Occupational Safety and Health) (NIOSH 1996, 2005) represents the maximum concentration from which one could escape within 30 min without any escape-impairing symptoms or any irreversible health effects.
[d]TLV-TWA (Threshold Limit Value–time-weighted average, American Conference of Governmental Industrial Hygienists) (ACGIH 2003) is the time-weighted average concentration for a normal 8-h workday and a 40-h workweek, to which nearly all workers may be repeatedly exposed, day after day, without adverse effect.
[e]PEL-TWA (permissible exposure limit–time-weighted average, Occupational Health and Safety Administration) (29CFR Part 1910.1000 [1996]) is analogous to the ACGIH TLV-TWA but is for exposures of no more than 10 h/day, 40 h/week.
[f]REL-TWA (recommended exposure limit–time-weighted average, National Institute for Occupational Safety and Health) (NIOSH 2005) is analogous to the ACGIH TLV-TWA.
[g]TLV-STEL (Threshold Limit Value–short-term exposure limit, American Conference of Governmental Industrial Hygienists) (ACGIH 2003) is defined as a 15-min TWA exposure that should not be exceeded at any time during the workday even if the 8-h TWA is within the TLV-TWA. Exposures above the TLV-TWA up to the STEL should not be longer than 15 min and should not occur more than four times per day. There should be at least 60 min between successive exposures in this range.
[h]REL-STEL (recommended exposure limit–short-term exposure limit, National Institute for Occupational Safety and Health) (NIOSH 2005) is a 15-min TWA exposure that should not be exceeded at any time during a workday.
[i]MAK (maximale arbeitsplatzkonzentration [maximum workplace concentration]) (Deutsche Forschungsgemeinschaft [German Research Association] (DFG 2002) is analogous to the ACGIH TLV-TWA.
[j]MAC (maximaal aanvaarde concentratie [maximal accepted concentration], Ministerie van Sociale Zaken en Werkgelegenheid (MSZW 2004) is analogous to the ACGIH TLV-TWA.

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APPENDIX A

DERIVATION OF AEGL VALUES FOR XYLENE

Derivation of AEGL-1 Values

Key study:	Hastings et al. 1984
Toxicity end point:	Eye irritation in human volunteers exposed to mixed xylenes at 400 ppm for 30 min
Scaling:	Because irritation is considered a threshold effect and should not vary over time, the AEGL-1 value is not scaled across time but rather the threshold value is applied to all times.
Uncertainty factors:	1 for interspecies variability 3 for intraspecies variability
Modifying factor:	Not applicable
10-min, 30-min, 1-h, 4-h, 8-h AEGL-1:	Concentration producing effect was applied to all times: 400 ppm/3 = 130 ppm

Derivation of AEGL-2 Values

Key study:	Carpenter et al. 1975b
Toxicity end point:	Rats exposed 2 h into a 4-h exposure to 1,300 ppm exhibited poor coordination
Scaling:	It is assumed that the CNS effects observed after xylene exposure are directly related to parent material reaching the brain. Therefore, PBPK modeling (see Appendix C) was used to calculate the internal dose (CV) correlating to exposure to 1,300 ppm for 2 h, which produced poor coordination in rats. A human PBPK model was then run for each defined AEGL time period to determine the equivalent exposure concentration producing the target CV.

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Uncertainty factors: 3 for intraspecies variability
1 for interspecies variability

A total uncertainty factor of 10 would have driven the AEGL-2 value for 8 h (180 ppm) to an exposure concentration that humans are known to tolerate with minimal or no adverse effects: humans exposed to *p*-xylene at 150 ppm for 7.5 h did not exhibit any effects on performance tests and noted only mild eye irritation (Hake et al. 1981). Therefore, the interspecies uncertainty factor is reduced to 1, and a total uncertainty factor of 3 is applied to the AEGL-2 and AEGL-3 values (NRC 2002).

Modifying factor: Not applicable

10-min AEGL-2: Application of PBPK model: 2,500 ppm

30-min AEGL-2: Application of PBPK model: 1,300 ppm

1-h AEGL-2: Application of PBPK model: 920 ppm

4-h AEGL-2: Application of PBPK model: 500 ppm

8-h AEGL-2: Application of PBPK model: 400 ppm

Derivation of AEGL-3 Values

Key study: Carpenter et al. 1975b

Toxicity end point: Reversible prostration and a no-effect level for mortality in rats exposed to 2,800 ppm for 4 h

Scaling: It is assumed that the CNS effects observed after xylene exposure are directly related to parent material reaching the brain. Therefore, PBPK modeling (see Appendix C) was again used to calculate the internal dose (CV) correlating to exposure at 2,800 ppm for 4 h, which produced prostration in rats. A human PBPK model was then run for each defined AEGL time period to determine the equivalent exposure concentration producing the target CV.

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Uncertainty factors:	3 for intraspecies variability 1 for interspecies variability A total uncertainty factor of 10 drives the 4-h AEGL-3 value to 447 ppm, an exposure concentration that humans are known to tolerate with minimal or no adverse effects. Numerous human studies investigated the effects of exposure to *m*-xylene at 130 to 200 ppm for 4 to 6 h, with 20-min peaks of 400 ppm with or without exercise (Savolainen and Linnavuo 1979; Savolainen et al. 1984, 1985a,b; Seppalainen et al. 1989, 1991; Laine et al. 1993) and found no effect or minimal CNS effects. Therefore, the interspecies uncertainty factor is reduced to 1, and a total uncertainty factor of 3 is applied to the AEGL-2 and AEGL-3 values (NRC 2002).
Modifying factor:	Not applicable
10-min AEGL-3:	Application of PBPK model: 7,200 ppm
30-min AEGL-3:	Application of PBPK model: 3,600 ppm
1-h AEGL-3:	Application of PBPK model: 2,500 ppm
4-h AEGL-3:	Application of PBPK model: 1,300 ppm
8-h AEGL-3:	Application of PBPK model: 1,000 ppm

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APPENDIX B

ACUTE EXPOSURE GUIDELINE LEVELS FOR XYLENES

Derivation Summary for Xylenes

AEGL-1 VALUES

10 min	30 min	1 h	4 h	8 h
130 ppm	130 ppm	130 ppm	130 ppm	130 ppm

Key Reference:
Hastings, L., G.P. Cooper, and W. Burg. 1984. Human sensory response to selected petroleum hydrocarbons. Pp. 255-270 in Advances in Modern Environmental Toxicology, Vol. VI. Applied Toxicology of Petroleum Hydrocarbons, H.N. MacFarland, ed. Princeton, NJ: Princeton Scientific Publishers.

Test Species/Strain/Number: Volunteer human male

Exposure Route/Concentration
s/Durations: Subjects were exposed by inhalation via an olfactometer delivery hood to mixed xylenes at 0, 100, 200, or 400 ppm for 30 min

Effects: Mild eye irritation reported by 56%, 60%, 70%, and 90% of subjects exposed to mixed xylenes at 0, 100, 200, and 400 ppm, respectively; no effects observed on behavioral test results

End Point/Concentration/Rationale: Mild eye irritation was noted by 90% of the subjects exposed to 400 ppm

Uncertainty Factors/Rationale:
Total uncertainty factor: 3
Interspecies: 1 for human data used
Intraspecies: 3 because slight eye irritation is caused by a direct effect of the chemical and the response is not expected to vary greatly among individuals.

Modifying Factor: Not applied

Animal to Human Dosimetric Adjustment: Not applied, human data used

Time-Scaling: Irritation is considered a threshold effect and should not vary over time. The AEGL-1 value based on irritation is therefore not scaled across time, but rather the threshold value is applied to all times.

Data Adequacy: This study was acceptable but could have been improved had the number of volunteers been reported. However, the data are consistent with other human studies and represent a value consistent with exposure concentrations that might result in mild eye irritation.

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Acute Exposure Guideline Levels for Selected Airborne Chemicals: Volume 9

AEGL-2 VALUES

10 min	30 min	1 h	4 h	8 h
2,500 ppm	1,300 ppm	920 ppm	500 ppm	400 ppm

Key Reference:
Carpenter, C.P., E.R. Kinkead, D.L. Geary Jr., L.J. Sullivan, and J.M. King. 1975b. Petroleum hydrocarbon toxicity studies. V. Animal and human response to vapors of mixed xylenes. Toxicol. Appl. Pharmacol. 33(3): 543-558.

Test Species/Strain/Number: 10 male albino rats (Harlan-Wistar strain) approximately 5 weeks old per group

Exposure Route/Concentrations/Durations: Rats were exposed by inhalation to mixed xylenes at 580, 1,300, 2,800, 4,000, or 9,000 ppm for 4 h

Effects:

Concentration	Mortality	Other Effects
580 ppm	0/10	None observed
1,300 ppm	0/10	Poor coordination after 2 h, returned to normal
2,800 ppm	0/10	Irritation; all rats prostrate between 2 and 3.5 h recovered within 1 h;coordination returned to normal next day
6,000 ppm	4/10	Rats prostrate within 30 min; all survivors prostrate but recovered promptly
9,900 ppm	10/10	None stated

End Point/Concentration/Rationale: The AEGL-2 is based on the no-effect level for impaired ability to escape. During a 4-h exposure to mixed xylenes at 1,300 ppm, rats developed poor coordination (slight coordination loss) after 2 h of exposure, returning to normal coordination postexposure. The point of departure of 1,300 ppm for 2 h therefore represents the threshold for reversible equilibrium disturbances and the no-effect level for impaired ability to escape. It is assumed that the CNS effects observed after xylene exposure are directly related to parent material reaching the brain and that CV values correlate with brain concentrations. Therefore, the CV of xylene after a 2-h exposure to xylene at 1,300 ppm would be expected to provide an internal dose measurement correlating with the clinical sign of poor coordination. By using PBPK modeling (see Appendix C), the internal dose (CV) producing poor coordination in rats was determined. Then, a human PBPK model was run for each defined AEGL time period to determine the equivalent exposure concentration producing the target CV.

Uncertainty Factors/Rationale:
Total uncertainty factor: 3
Intraspecies: 3. An intraspecies uncertainty factor of 3 was applied for the pharmacokinetic and pharmacodynamic uncertainty because the MAC for volatile anesthetics should not vary by more than 2- to 3-fold among humans (NRC 2002).
Interspecies: 1. An interspecies uncertainty factor of 3 would usually be applied. PBPK modeling reduced the toxicokinetic component of the uncertainty factor to 1, but the

(Continued)

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Acute Exposure Guideline Levels for Selected Airborne Chemicals: Volume 9

AEGL-2 VALUES Continued

10 min	30 min	1 h	4 h	8 h
2,500 ppm	1,300 ppm	920 ppm	500 ppm	400 ppm

pharmacodynamic component would normally be retained and assigned a 3 (although it appears that similar CNS effects occur in humans and animals, it is not known if they occur at the same tissue dose). A total uncertainty factor of 10, however, drives the AEGL-2 value for 8 h (180 ppm) to an exposure concentration that humans are known to tolerate with minimal or no adverse effects. Humans exposed to *p*-xylene at 150 ppm for 7.5 h did not exhibit any effects on performance tests and noted only mild eye irritation (Hake et al. 1981). Therefore, the interspecies uncertainty factor is reduced to 1, and a total uncertainty factor of 3 is applied to the AEGL-2 values (NRC 2002).

Modifying Factor: Not applied

Animal to Human Dosimetric Adjustment: Not applied

Time Scaling: PBPK modeling predicted human exposure concentrations correlating with a target CV at each defined AEGL time period.

Data Adequacy: This was a well-designed and conducted study. The data are supported by numerous other studies in rats as well as a study in dogs. The AEGL-2 values are protective.

AEGL-3 VALUES

10 min	30 min	1 h	4 h	8 h
7,200 ppm	3,600 ppm	2,500 ppm	1,300 ppm	1,000 ppm

Key Reference:
Carpenter, C.P., E.R. Kinkead, D.L. Geary Jr., L.J. Sullivan, and J.M. King. 1975b. Petroleum hydrocarbon toxicity studies. V. Animal and human response to vapors of mixed xylenes. Toxicol. Appl. Pharmacol. 33(3):543-558.

Test Species/Strain/Number: 10 male albino rats (Harlan-Wistar strain) approximately 5 weeks old per group

Exposure Route/Concentrations/Durations: Rats were exposed by inhalation to mixed xylenes at 580, 1,300, 2,800, 4,000, or 9,000 ppm for 4 h

Effects:

Concentration	Mortality	Other Effects
580 ppm	0/10	None observed
1,300 ppm	0/10	Poor coordination after 2 h, returned to normal
2,800 ppm	0/10	Irritation; all rats prostrate between 2 and 3.5 h recovered within 1 h;coordination returned to normal next day
6,000 ppm	4/10	Rats prostrate within 30 min; all survivors prostrate but recovered promptly
9,900 ppm	10/10	None stated

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AEGL-3 VALUES Continued

10 min	30 min	1 h	4 h	8 h
7,200 ppm	3,600 ppm	2,500 ppm	1,300 ppm	1,000 ppm

End Point/Concentration/Rationale: The AEGL-3 derivation is based on reversible prostration and a NOEL for death in rats exposed to 2,800 ppm for 4 h. Although coordination initially remained poor, it returned to normal the following day. This concentration represents a threshold for marked CNS depression, which could lead to death. As for the AEGL-2, it is assumed that the CNS effects observed after xylene exposure are directly related to the concentration of parent material reaching the brain. Therefore, PBPK modeling (see Appendix C) was again used to calculate the internal dose (CV) correlating with an exposure to 2,800 ppm for 4 h, which produced prostration in rats. The human PBPK model was then run for each defined AEGL time period to determine the equivalent exposure concentration producing the target CV.

Uncertainty Factors/Rationale:
Total uncertainty factor: 3

Intraspecies: 3. An intraspecies uncertainty factor of 3 was applied for the pharmacokinetic and pharmacodynamic uncertainty because the MAC for volatile anesthetics should not vary by more than 2- to 3-fold among humans (NRC 2002).
Interspecies: 3. An interspecies uncertainty factor of 3 would usually be applied. PBPK modeling reduced the toxicokinetic component of the uncertainty factor to 1, but the pharmacodynamic component would normally be retained and assigned a 3 (although it appears that similar CNS effects occur in humans and animals, it is not known if they occur at the same tissue dose). A total uncertainty factor of 10, however, drives the 4-h AEGL-3 value to 447 ppm, an exposure concentration that humans are known to tolerate with minimal or no adverse effects. Numerous human studies investigated the effects of exposure to *m*-xylene at 130 to 200 ppm for 4 to 6 h, with 20-min peaks of 400 ppm with or without exercise (Savolainen and Linnavuo 1979; Savolainen et al. 1984, 1985a,b; Seppalainen et al. 1989, 1991; Laine et al. 1993) and found no effect or minimal CNS effects. Therefore, the interspecies uncertainty factor is reduced to 1, and a total uncertainty factor of 3 is applied to the AEGL-3 values (NRC 2002).

Modifying Factor: Not applied

Animal to Human Dosimetric Adjustment: Not applied

Time Scaling: PBPK modeling predicted human exposure concentrations correlating with a target CV at each defined AEGL time period.

Data Adequacy: This was a well-conducted study. The AEGL-3 levels are supported by human data demonstrating that exposure to 690 ppm for 15 min resulted in lightheadedness and dizziness and a 30-min exposure to 700 ppm resulted in nausea, vomiting, dizziness, or vertigo.

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APPENDIX C

PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELING OF XYLENE

Summary

Two research groups developed PBPK models for xylene, and both groups modeled the single isomer *m*-xylene. Kaneko et al. (1991ab, 2000) developed a six-compartment model in rats and a seven-compartment model in humans. The other research group developed a four-compartment model in rats and humans (Tardif et al. 1993, 1997; Haddad et al. 1999). The basic model generated by Tardif et al. (1993, 1997) and Haddad et al. (1999) was chosen for the AEGL derivations because it was more data rich. The main difference among the models was in the physiologic parameters used. The model was coupled with additional human data from five publications for verification (see Table 6C-1). Next, the rat model was run to determine CV at the exposure concentration producing the defined AEGL end point. Then the human model was run for each time period to determine the equivalent exposure concentration producing the same CV. The application of PBPK modeling to the derivation of the xylene AEGL values was based on guidance in the PBPK modeling White Paper (Dennison et al. 2009). All PBPK modeling was performed in Berkeley Madonna, version 8.0.2a8, a recent beta version that includes scripting capabilities (Macey and Oster 2002). A glossary of PBPK modeling abbreviations is provided at the end of this appendix.

Rat Model

The basis of the model was a standard four-compartment model that included richly perfused tissue, slowly perfused tissue, fat, and liver (Figure 6C-1), with the rate of change in the concentration in each tissue described by the equation shown below (Ramsey and Andersen 1984):

$$V_i dC_i/dt = Q_i(C_a - CV_i) - RAM,$$

where

V_i = tissue volume (L),
Q_i = tissue perfusion rate (L/h),
C_a = concentration of solvent in the systemic arterial blood (mg/L),
CV_i = concentration of solvent in venous blood leaving tissue, i (mg/L),
RAM = rate of change in the amount metabolized,
RAM = AMS + AML,
AMS = $V_{max} \times C_{VL}/(K_m + C_{VL})$,

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AML = KF × C_{VL}, and
KF = first-order rate constant for high-capacity low-affinity enzymes.

It was assumed that metabolism occurred exclusively in the liver. V_{max} was scaled to the body weight by using $V_{max}C \times BW^{0.75}$. The following data were used to develop the rat PBPK model:

Gas-uptake data from rats exposed at 500, 1,000, 2,000, or 4,000 ppm (Tardif et al. 1993).
CV in rats after a 4-h exposure to 100 or 200 ppm (Tardif et al. 1997).
CV in rats after a 4-h exposure to 50 ppm (Haddad et al. 1999).
Partition parameters from Gargas et al. (1989) (in vitro).
Standard parameters for tissue flows and volumes (see Table 6C-2).

TABLE 6C-1 CV in Humans Exposed to *m*-Xylene at 200 ppm

Time (h)	CV (μmol/L)	Reference
0.12	11.6[a]	Seppalainen et al. 1991
0.15	12.6[a]	Seppalainen et al. 1991
0.18	14.3[a]	Seppalainen et al. 1991
0.22	16.4 a	Seppalainen et al. 1991
0.25	16.6 ± 4.8	Laine et al. 1993
0.33	17.3 ± 5.5 17.5	Laine et al. 1993 Savolainen et al. 1985
0.5	17.5	Savolainen et al. 1984
0.67	21.3 ± 5.4	Laine et al. 1993
1	23.9*	Seppalainen et al. 1991
1.17	24.9 ± 2.1 (6)	Savolainen et al. 1981
1.5	26	Savolainen et al. 1984
2	28.5 ± 5.2 29	Laine et al. 1993 Savolainen et al. 1984
2.5	26.7 ± 3.4 (6) 30 30	Savolainen et al. 1981 Savolainen et al. 1984 Savolainen et al. 1985
3	31 31.4	Seppalainen et al. 1989 Seppalainen et al. 1991
3.75	28.6 ± 3.5 (6)	Savolainen et al. 1981

[a]These values read from a graph using digiMatic software (Digimatic 2004).

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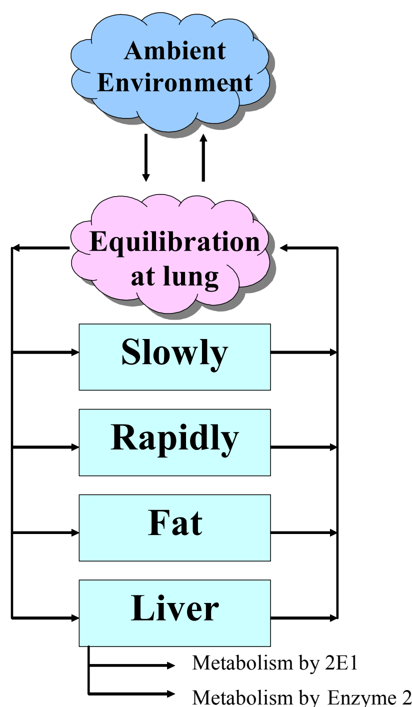

FIGURE 6C-1 Four-compartment rat model.

The rat model from Haddad et al. (1999) was chosen because it was more recent, was more data rich, and had a better fit. However, there was not a large difference among the models. The Haddad et al. (1999) model does have the limitation that it was created with data from Sprague-Dawley rats and had only postexposure data at xylene concentrations up to 200 ppm. In the more recent Haddad et al. (1999) model, slightly different parameters were used for tissue volumes and metabolism compared with the earlier model of Tardif et al. (1993). The 1999 model was run with the 1993 gas uptake data (500, 1,000, 2,000, and 4,000 ppm) (see Figure 6C-2). The results suggest that the 1993 and 1999 models are quite similar as the plot shown here is essentially the same as that in the 1993 paper. At the lower concentrations, the model would actually fit perfectly if the starting concentration were adjusted to the measured concentration. The acute lethality critical study (AEGL-3) is based on an exposure concentration (2,800 ppm) that lies within the range of concentrations used in the gas uptake study.

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TABLE 6C-2 Summary of Parameter Values Used in Rat and Human PBPK Model

Variable	Rat[a]	Human[b]	
Body weight (kg) (BW)	0.1678	70	
Alveolar ventilation rate (L/h/kg) (QPC)[c]	15	14.9	
Cardiac output (L/h/kg) (QCC)[c]	15	12.4	
Fraction of body weight (kg/kg BW)			
Fraction fat tissue (VFC)	0.07	0.19[d]	
Fraction liver tissue (VLC)	0.04	0.026[d]	
Fraction rapidly perfused (VRC)	0.05	0.05[d]	
Fraction slowly perfused (VSC)	0.75	0.62[d]	
Fraction of cardiac output corresponding to each compartment ((L/h)/QC)			
Fraction blood flow to fat (QFC)	0.09	0.05	
Fraction to rapidly perfused (QRC)	0.51	0.42	
Fraction to liver (QLC)	0.25	0.32	
Fraction to slowly perfused (QSC)	0.15	0.21	
Partition coefficients			
Blood-air (PB)			
m-xylene	46[e]	32.5[e]; 26.4[f]; 31.9[g];	Average = 30.3
o-xylene	44.3[e]	34.9[e]; 31.1[f]; 35.2[g];	Average = 33.7
p-xylene	41.3[e]	44.7[e]; 37.6[f]; 39.0[g];	Average = 40.4

(Continued)

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TABLE 6C-2 Continued

Variable	Rat[a]	Human[b]
Blood-fat (PFA)	1,859	1,859[a]
Slowly perfused-air (PSA)	41.9	41.9[a]
Rapidly perfused-air (PR)]	90.9	90.9[a]
Liver-air (PLA)	90.9	90.9[a]
Scaling coefficient (SF])	0.75	0.75
$V_{max}C$ (mg/h/kg)[g]	6.49	5.5
K_m (mg/L)	0.45	0.45[a]
KFC	0.1	0.1

[a]Haddad et al. 1999.
[b]Astrand 1983.
[c]QPC, QCC, and $V_{max}C$ were scaled to $BW^{0.75}$.
[d]Tardif et al. 1997.
[e]Gargas et al. 1989.
[f]Sato and Nakajima 1979.
[g]Pierce et al. 1996.

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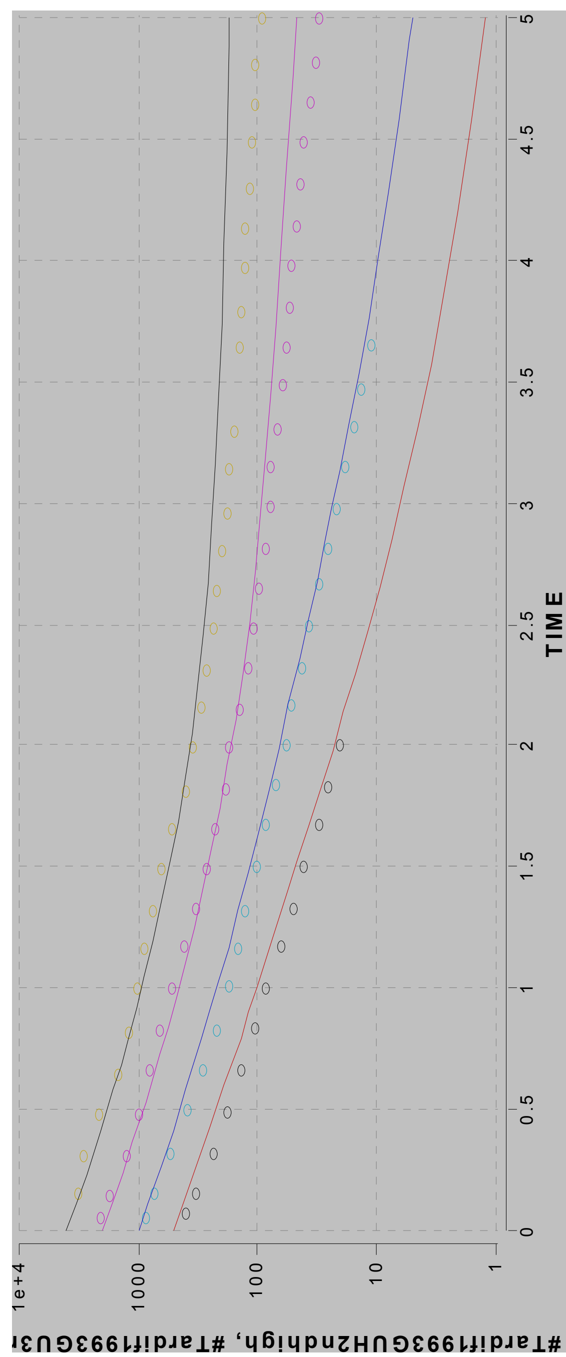

FIGURE 6C-2 The Haddad et al. (1999) model with the Tardif et al. (1993) gas uptake data (500, 1,000, 2,000, and 4,000 ppm).

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Using the Haddad et al. (1999) model, the starting concentrations were optimized to reflect the measured concentrations of the first data points. At the high concentrations of interest for xylene, enzymatic saturation of the primary metabolic pathway may have occurred. Therefore, a second pathway of metabolism (lumped metabolism by all the CYPs other than CYP2E1) was added to account for high-capacity low-affinity pathways of metabolism, which would occur at the much higher exposure concentrations (Clewell et al. 2001). The metabolism by the second series of CYPs is given as

$$\text{Rate of metabolism (RAM)} = KF \times C_{VL},$$

where

C_{VL} = concentration of xylenes in the venous blood leaving the liver and
$KF = 0.1/BW^{0.3}$.

The second pathway of metabolism was added and KF (first-order rate constant for high-capacity low-affinity enzymes) was determined. Figure 6C-3 shows the results of optimizing the starting concentrations and adding the second pathway of metabolism. Adding the second metabolic pathway resulted in a very close correspondence between the model and the data.

After optimizing the Haddad model with the Tardif et al. (1993) data, the model was run again with the same parameters against the Haddad data (Figure 6C-4). A good fit is obtained overall, although the 200-ppm experimental data are slightly underpredicted. However, the concern is primarily with estimating CV in rats at very high concentrations (1,000 to 3,000 ppm). Figure 6C-4 shows what the model does without the second metabolic pathway (perfect fit) and with it. There is no real difference at 50 or 100 ppm, but the second line from the top is the new model at 200 ppm.

Application of the Model to Humans

The optimized rat model can now be used to develop a xylene PBPK model for humans. The model was visually reoptimized for *m*- and *p*-xylene with the available human data. Multiple papers were available in which human *m*-xylene CV values were measured during exposure to *m*-xylene at 200 ppm (Savolainen et al. 1981, 1984, 1985; Seppalainen et al. 1989, 1991; Laine et al. 1993) (see Table 6C-1). Postexposure human CV were also reported by Hake et al. (1981) after exposure to *p*-xylene at 20, 100, or 150 ppm for 1, 3, or 7 h and by Tardif et al. (1997) after exposure to *m*-xylene at 33 ppm for 7 h.

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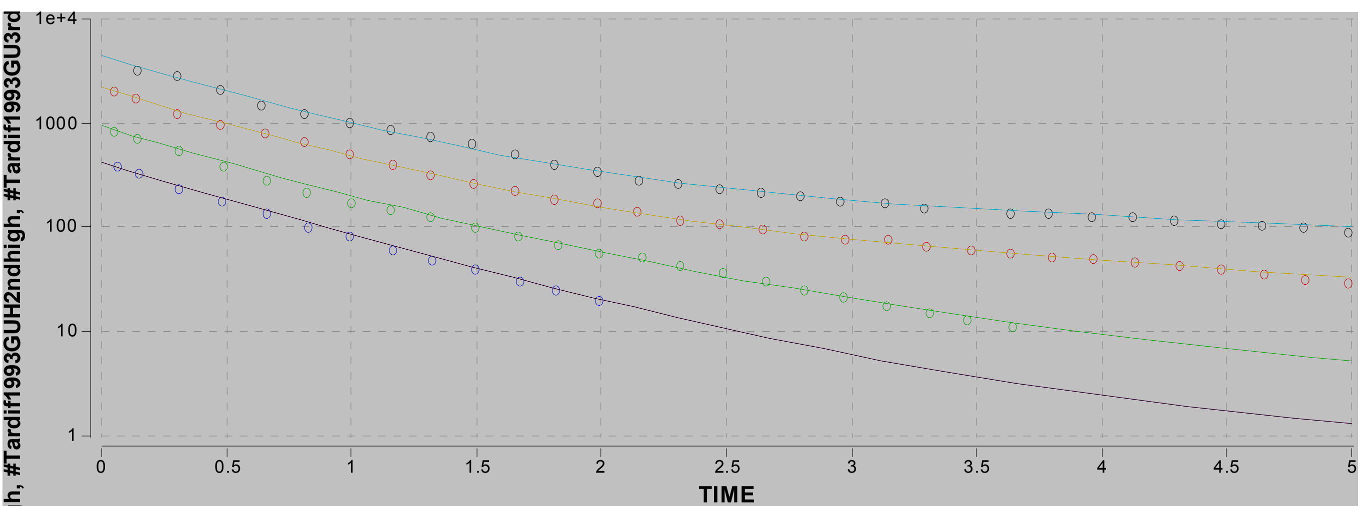

FIGURE 6C-3 The Haddad et al. (1999) model with the Tardif et al. (1993) gas uptake data (500, 1,000, 2,000, and 4,000 ppm), optimized for the starting concentration and inclusion of a second metabolism pathway.

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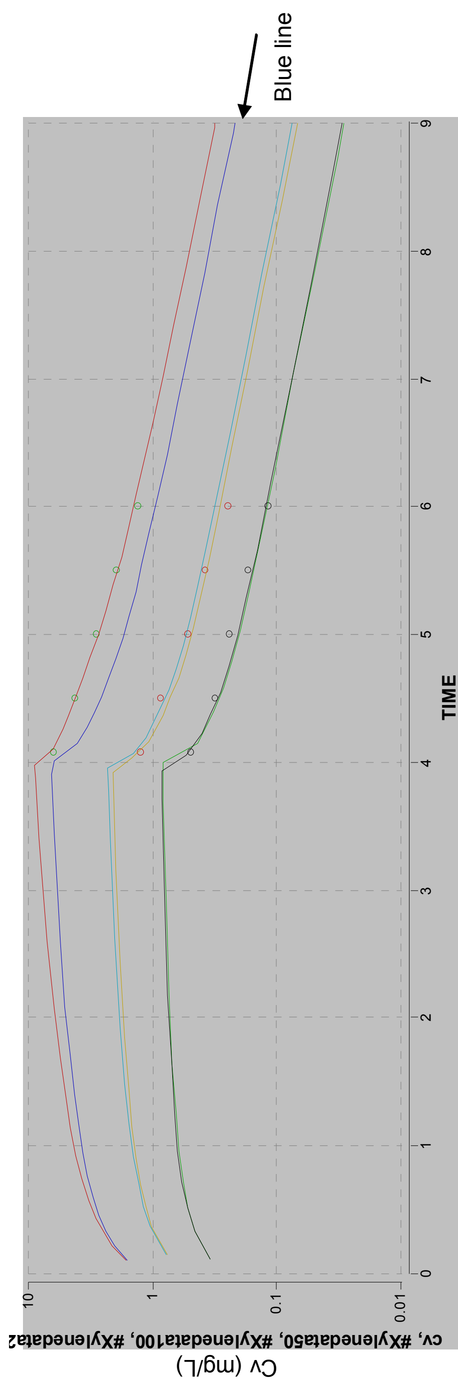

FIGURE 6C-4 Haddad et al. (1999) model after being optimized for the Tardif et al. (1993) gas uptake data.

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Human anatomic parameters were generally taken from Astrand (1983) (see Table 6C-2). Kinetics were scaled from the rat model except for $V_{max}C$, which was reoptimized and reduced to 5.5. Without the adjustment, the model tended to underpredict most of the data. Values for QCC or QPC were not optimized because these values came from part of a physiologic parameter set (Astrand 1983). Several human blood-air partition coefficients (PB) for the xylene isomers were reported in the literature and are presented in Table 6C-2. The average PB for the respective xylene isomer was used for modeling data. Figures 6C-5 to 6C-8 show the reoptimized human model predictions for CV for *m*- or *p*-xylene compared with the measured human CV values.

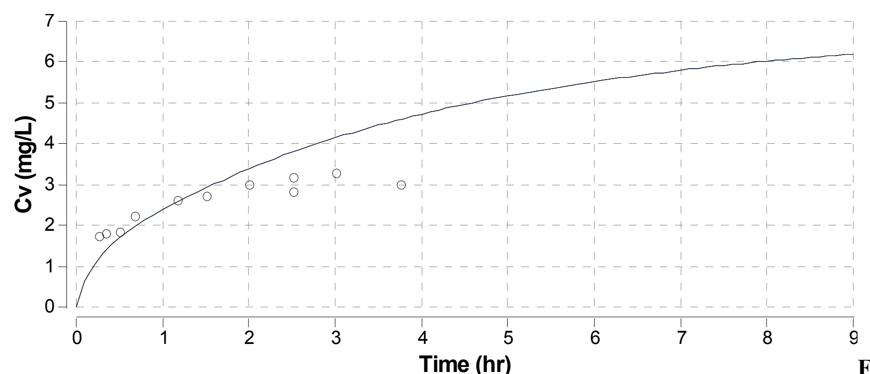

FIGURE 6C-5 Model predictions of CV (line; using human input parameters with PB of 30.3) compared with the actual measured human CV values during exposure to *m*-xylene at 200 ppm (open circles; combined data summarized in Table 6C-1).

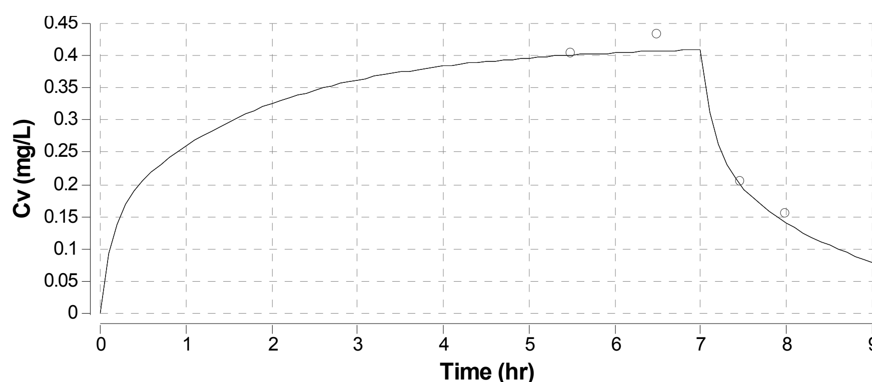

FIGURE 6C-6 Mmodel predictions of CV (line; using human input parameters with PB of 30.3) compared with the actual measured human CV values (open circles) during and after exposure to *m*-xylene at 33 ppm for 7 h (Tardif et al. 1997).

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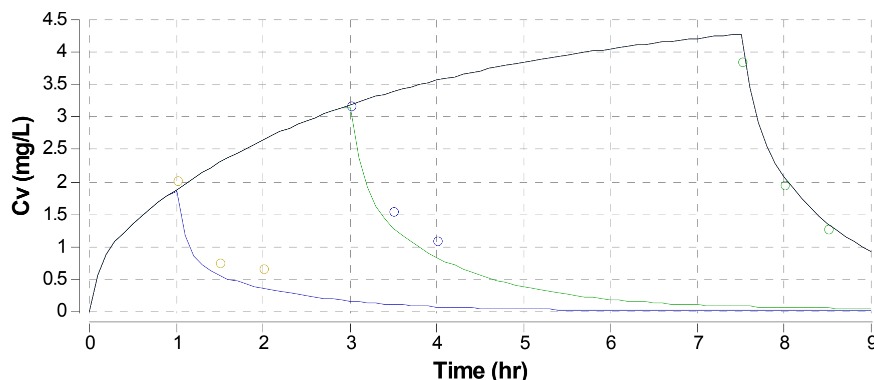

FIGURE 6C-7 Model predictions of CV (line; using male human input parameters with PB of 40.4) compared with the actual measured male human CV values (open circles) after exposure to *p*-xylene at 150 ppm for 1, 3, or 7.5 h (Hake et al. 1981).

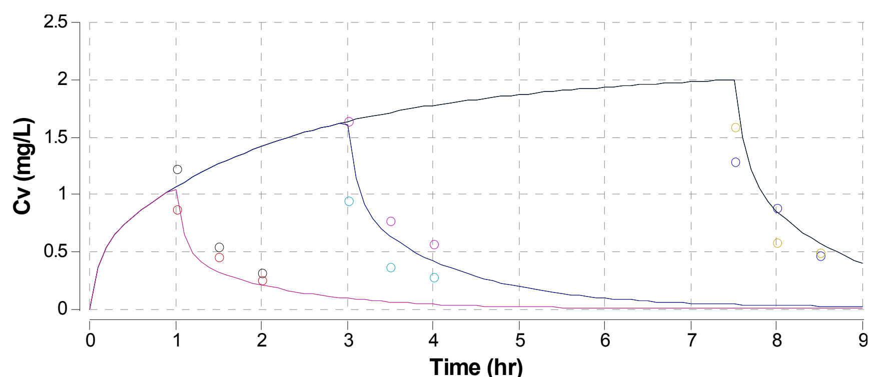

FIGURE 6C-8 Model predictions of CV (lines; using human female input parameters with PB of 40.4) compared with the actual measured human female CV values (open circles) after exposure to *p*-xylene at 100 ppm for 1, 3, or 7.5 h (Hake et al. 1981).

Comparison of Pharmacokinetics in Rats and Humans

Because the AEGL-2 and AEGL-3 key studies are based on rat data, extrapolation to humans is required. PBPK modeling allows a comparison of the internal dose that is received in both species receiving identical external exposures. As shown in Figure 6C-9, rats achieve higher blood *m*-xylene concentrations than humans. This is primarily due to a higher PB in rats (46) compared with humans (26 to 32 in humans). Figure 6C-9 plots CV for rats and humans using the validated models presented earlier at 200, 1,000, and 5,000 ppm.

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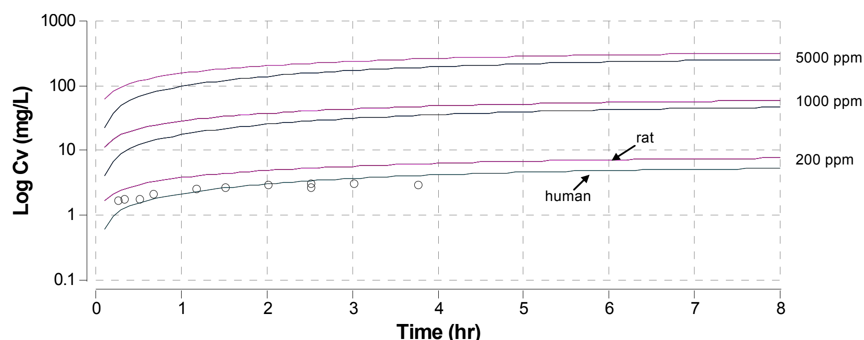

FIGURE 6C-9 Model predictions for CV in rats (top line of each pair of lines) and humans (bottom line of each pair of lines). Open circles are the actual measured human CV values for exposure to xylene at 200 ppm.

The y axis in Figure 6C-9 is on a logarithmic scale. By 8 h, steady state is still slowly increasing.

Application of Modeling to Derive AEGL Values

Because xylene can exist as a mixture or as any of three individual isomers, the question arises as to whether there are any differences in toxicity among the individual isomers and the mixture. No significant differences in the potency of the isomers after oral or inhalation exposure were identified and metabolism of each isomer proceeds via the same pathway. PBPK model predictions indicate that the internal dose (CV) after exposure does not vary significantly among the individual isomers (see Figure 6C-10).

The AEGL-2 and -3 values are based on a study in rats exposed to mixed xylenes for 4 h (Carpenter et al. 1975). The composition of the mixed xylenes used was provided as follows:

Component	Volume Percent
Nonaromatics	0.07
Toluene	0.14
Ethylbenzene	19.27
p-Xylene	7.84
m-Xylene	65.01
o-Xylene	7.63
C_9 + aromatics	0.04
Total	100.00

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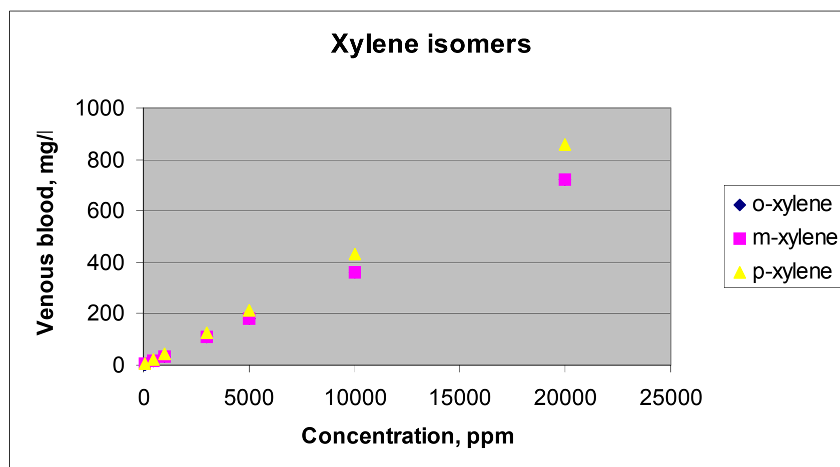

FIGURE 6C-10 The model predictions for CV in humans after exposure to the individual isomers (model parameters remain the same with the exception of PB values specific to the individual isomers; symbol for *m*-xylene is superimposed on symbol for *o*-xylene).

The amount of ethylbenzene is a typical amount seen in a xylene mixture. For the purpose of the modeling, it is known that ethylbenzene has the same spectrum of neurotoxic effects as xylenes, so assuming the exposure is to xylenes alone is reasonable. When considering only the amount of xylene isomers in the mixture and normalizing them to a total of 100%, 80% is the *m*-xylene isomer, while 10% is the *o*-xylene isomer and 10% is the *p*-xylene isomer. Therefore, the PB for *m*-xylene is used in the model.

The AEGL-2 derivation is based on poor coordination exhibited in rats 2 h into a 4-h exposure to mixed xylenes at 1,300 ppm (Carpenter et al. 1975). The rat PBPK model predicts that an exposure to xylenes at 1,300 ppm for 2 h would result in a CV of 48.9 mg/L. It is assumed that this internal dose of 48.9 mg/L is the dose resulting in the clinical sign of poor coordination. Therefore, it is assumed that the same internal dose of 48.9 mg/L would also result in adverse effects in humans. Using the human PBPK model, the model was run for each defined AEGL time point to determine the equivalent exposure concentration producing the same CV.

The AEGL-3 derivation is based on reversible prostration and a NOEL for death in rats exposed to 2,800 ppm for 4 h (Carpenter et al. 1975). The rat PBPK model predicts that an exposure to xylenes at 2,800 ppm for 4 h would result in a CV of 143.8 mg/L. Therefore, it is assumed that the same internal dose of 143.8 mg/L would also result in adverse effects in humans. Using the human PBPK model, the model was run for each defined AEGL time point to determine the equivalent exposure concentration producing the same CV.

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Recommended AEGL Values

A total uncertainty factor of 3 was applied to the AEGL-2 and -3 dose metrics.

An intraspecies uncertainty factor of 3 was applied for the pharmacokinetic and pharmacodynamic uncertainty because the MAC for volatile anesthetics should not vary by more than 2- to 3-fold among humans (NRC 2002).

An interspecies uncertainty factor of 3 would usually be applied. PBPK modeling reduced the toxicokinetic component of the uncertainty factor to 1, but the pharmacodynamic component would normally be retained and assigned a 3 (although it appears that similar CNS effects occur in humans and animals, it is not known if they occur at the same tissue dose). A total uncertainty factor of 10, however, drives the 8-h AEGL-2 value to 180 ppm and the 4-h AEGL-3 value to 447 ppm. These are exposure concentrations that humans are known to tolerate with minimal or no adverse effects. With regard to the AEGL-2, humans exposed to *p*-xylene at 150 ppm for 7.5 h did not exhibit any effects on performance tests and noted only mild eye irritation (Hake et al. 1981). With regard to the AEGL-3, numerous human studies investigated the effects of exposure to *m*-xylene at 130 to 200 ppm for 4 to 6 h, with 20-min peaks of 400 ppm with or without exercise (Savolainen and Linnavuo 1979; Savolainen et al. 1984, 1985; Seppalainen et al. 1989, 1991; Laine et al. 1993) and found no effect or minimal CNS effects. Therefore, the interspecies uncertainty factor is reduced to 1, and a total uncertainty factor of 3 is applied to the AEGL-2 and AEGL-3 values (NRC 2002).

GLOSSARY OF PBPK MODEL TERMS

Most used in the presentation:

CV	venous blood concentration
PB	Blood-air partition coefficient

Physiologic Parameters

BW	Body weight (kg)
QPC	Alveolar ventilation rate (L/h/kg)
QCC	Cardiac output (L/h/kg)
VFC	Fraction fat tissue (kg/(kg/BW))
VLC	Fraction liver tissue (kg/(kg/BW))
VRC	Fraction rapidly perfused (kg/(kg/BW))
QFC	Fractional blood flow to fat ((L/h)/QC)
QLC	Fractional blood flow to liver ((L/h)/QC)
QRC	Fractional blood flow to rapidly perfused ((L/h)/QC)
SF	Scaling coefficient

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Chemical-Specific Parameters

PLA =	Liver-air partition coefficient
PFA =	Fat-air partition coefficient
PSA =	Slowly perfused air partition coefficient
PRA =	Rapidly perfused air partition coefficient
PB =	Blood-air partition coefficient
PL = PLA/PB	Liver-blood partition coefficient
PF = PFA/PB	Fat-blood partition coefficient
PS = PSA/PB	Slowly perfused blood partition coefficient
PR = PRA/PB	Rapidly perfused blood partition coefficient
MW =	Molecular weight (g/mol)
$V_{max}C$ =	Maximum velocity of metabolism (mg/h/kg)
K_m =	Michaelis-Menten (mg/L)
KFC =	0.1
CONC =	Inhaled concentration (ppm)

Calculated Parameters

$QC = QCC \times BW^{SF}$	Cardiac output
$QP = QPC \times BW^{SF}$	Alveolar ventilation
$VS = VSC \times BW$	Volume slowly perfused tissue (L)
$VF = VFC \times BW$	Volume fat tissue (L)
$VL = VLC \times BW$	Volume liver (L)
$VR = VRC \times BW$	Volume rapidly perfused (L)
$QF = QFC \times QC$	Blood flow to fat (L/h)
$QL = QLC \times QC$	Blood flow to liver (L/h)
$QS = QC - QF - QL - QR$	Blood flow to non-fat tissue (L/h)
$QR = QRC \times QC$	Blood flow to rapidly perfused (L/h)
$CIX = CONC \times MW/24{,}450$	Exposure concentration (mg/L)
$V_{max} = V_{max}C \times BW^{SF}$	
$KF = KFC/BW^{0.3}$	First-order rate constant for high-capacity low-affinity enzymes

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7

PBPK Modeling White Paper: Addressing the Use of PBPK Models to Support Derivation of Acute Exposure Guideline Levels[1]

PREFACE

This White Paper describes the guidance that is proposed for use in the integration of physiologically based pharmacokinetic (PBPK) modeling in risk assessment in the EPA Acute Exposure Guideline Level (AEGL) program. After finalization, the guidance document will be added to the existing AEGL guidance for risk assessment activities. Therefore, the PBPK White Paper does not describe the entire methodology; rather, it describes the additional steps when PBPK modeling is undertaken within the existing risk assessment paradigm. As in any methodology, every facet of the method cannot be explicitly stated in a manner that is universally applicable to all chemicals. Where some details are not specified, the risk assessment process will be handled in accordance with the U.S. Environmental Protection Agency (EPA) document Approaches for the Application of Physiologically Based Pharmacokinetic (PBPK) Modeling and Supporting Data in Risk Assessment (EPA 2006).

[1]This White Paper was prepared by James E. Dennison, of Century Environmental Hygiene; Claudia Troxel, of Oak Ridge National Laboratory; and Robert Benson, of the U.S. Environmental Protection Agency (EPA), with the assistance of numerous scientists and risk assessors. Guidance from Ernest Falke, Marquea D. King, and Iris Camacho, of the AEGL Development Team, EPA; review by Robert Young, of Oak Ridge National Laboratory; review and discussions with William Boyes, Hugh Barton, Jane Ellen Simmons, Marina Evans, and Vernon Benignus, of the EPA National Health and Environmental Effects Research Laboratory, and Hisham El-Masri, Paul Schlosser, Robert Dewoskin, and George Woodall, of the EPA National Center for Environmental Assessment; and comments from international National Advisory Committee participants Ursula Gundert-Remy and Peter Griem, of Germany, were vital in its preparation.

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1. INTRODUCTION

AEGL values are developed in accordance with the Standing Operating Procedures for Developing Acute Exposure Guidelines Levels (AEGLs) for Hazardous Substances (NRC 2001). At the request of the AEGL/National Advisory Committee (NAC) and the AEGL Subcommittee of the Committee on Toxicology, National Academy of Sciences, this White Paper has been prepared to describe an approach for integrating the use of PBPK modeling into the development of AEGL values.

PBPK modeling serves as a useful adjunct to risk assessment of systemically acting chemicals by improving the basis of, or entirely allowing for, extrapolation of pharmacokinetics between animals and humans, extrapolation between various exposure scenarios (e.g., what exposure concentration for 10 minutes [min] results in the same internal dose produced from a 4-hour [h] exposure), and other types of extrapolation. As internal dose of a chemical agent is more closely associated with toxicity than is external exposure level of chemicals, extrapolating on the basis of internal dose is more reliable. In a sense, the use of PBPK models factors pharmacokinetic differences out of the extrapolation because they are handled by dose calculations instead of on the basis of an assumed equivalency followed by application of an uncertainty factor (UF) that is usually preset because of lack of knowledge about the true difference. As a result of using calculated doses, the overall uncertainty is reduced, and therefore the overall UFs may be reduced, allowing for more realistic exposure guidelines, which is the purpose in the advancement of the risk assessment process.

The risk assessment process includes identifying a point of departure (POD) from toxicity studies. The POD is usually the highest exposure concentration that did not result in the effect under consideration and may be a no-observed-adverse-effect level (NOAEL), a lowest-observed-adverse-effect level (LOAEL) if a NOAEL is not available, a level from a benchmark dose (BMD), or another value. The POD is then divided by UFs composed of estimated uncertainty in interspecies extrapolation, intraspecies variability, and other factors including weakness in the toxicologic database of information on a chemical.

Briefly, PBPK models are a description of the body and processes within the body (animal and human) that affect the disposition of a chemical. Disposition, or pharmacokinetics, includes the processes of absorption, distribution, metabolism, and excretion of chemicals. After development with necessary parameters and equations, the models calculate the concentration of the chemical (and metabolites, if necessary) in various parts of the body using exposure concentrations as the input.

The main function PBPK modeling serves in risk assessments is to provide a computational biology basis for some extrapolations that need to be made in the course of the risk assessment. This process is done by using PBPK models to determine the target tissue dose in humans or the test species (EPA 2006). Historically, in the AEGL program, types of extrapolations have included animal to human, within the human population, and for different periods of expo-

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sure. Animal-to-human extrapolation occurs when human studies are not available or cannot be used to determine the POD; therefore, the animal POD is used to estimate human risk. If an animal study is used, an interspecies UF is applied to the POD to guard against the likelihood that humans are more sensitive than other animals at a given exposure. The human variability issue is an extrapolation in the sense that the POD for a set of experimental subjects is a projection of the values that should protect most of the population. This extrapolation is offset by applying the intraspecies UF, which is intended to protect individuals who are more sensitive than those represented by the experimental data. The temporal extrapolation is performed when a POD is based on studies with different exposure durations than the AEGL value. Thus, the value for one period is extrapolated to another exposure period. This extrapolation is currently performed using the ten Berge empirical formula, by holding the value constant for all exposure durations, or possibly other approaches.

When PBPK modeling is used as an alternative method of extrapolation, associated UFs can be eliminated or reduced and other approaches can be supplanted. The animal-to-human extrapolation is made directly on the basis of internal dose, so the pharmacokinetic portion of the interspecies UF can be reduced. Temporal extrapolations, currently made by using empirical approaches, can be done with explicit calculations of the internal dose. Finally, PBPK modeling can be used to examine some types of intraspecies uncertainty.

Many toxicity studies are performed with the human volunteer or animal effectively in a resting condition. However, humans may be stressed, working, or otherwise in an altered physiologic state during an emergency event or other scenarios where the AEGLs may be applied. Altered physiologic states significantly affect the pharmacokinetics of some chemicals. The consequent alterations in pharmacokinetics are not commonly addressed in a traditional risk assessment.

PBPK modeling can be used to reduce both inter- and intraspecies uncertainties in human health risk assessments for chemicals. Risk assessments traditionally have been performed by using the external exposure concentration, as opposed to an internal exposure concentration, as the basis for the dose-response assessment that results in the POD selection. In recent years, there has been a movement to use internal measures of exposure calculated with a PBPK model instead of external measures. Risk assessments that rely on this general concept have been performed for many chemicals, often in the cancer, chronic noncancer, and developmental risk assessment areas. The rationale for using PBPK modeling in these other types of risk assessments applies as well in the assessment of acute exposure risks.

The difference between a PBPK-based and a traditional dose-response assessment is that the PBPK method relies on an internal measure of exposure rather than an external one. An internal measure of exposure can be thought of as the exposure of the target tissue to the chemical, or "dose." If the dose of chemical that reaches a target tissue can be determined with reasonable accuracy, then the pharmacokinetic issues described above can be dealt with by us-

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ing known biology rather than UFs and empirical techniques. PBPK approaches are further empowered through the use of different methods for integrating the measure of dose. Depending on the chemical, the best predictor of toxicity may be the average tissue concentration of chemical, the peak concentration, the area under the curve (AUC) (concentration × time), or some other expression of concentration. The specific integrated measure of dose is referred to as the dose metric (DM) and is selected based on the mode of toxicologic action of the chemical. PBPK models are used to determine the DM at the POD. This concentration would become, in effect, a pharmacokinetic POD. If the critical study involves humans, this target DM is used to determine the equivalent concentration for different exposure durations or physiologic conditions. If the critical study involves animals, the pharmacokinetic POD would be determined in an animal version of the model and a human version of the model would then be used to determine the exposure concentration that results in the same DM value in human tissue. Thus, extrapolating from an animal to a human is performed with uncertainty limited to model error that is assessed during evaluation of the model.

PBPK modeling can be utilized in quantifying the effect of workload (exercise) on toxicity. Values for physiologic properties of the human in the model can be adjusted to account for exercise. Exposure concentrations that yield the same target tissue DM value could be determined under the exercise condition. Likewise, extrapolating to other exposure periods can be performed by determining the exposure concentration under a different exposure duration that yields the same target tissue DM value. Thus, the PBPK model minimizes some sources of uncertainty by basing the risk assessment on an appropriate internal DM, so that species, temporal, and physiologic differences are explicitly taken into account.

PBPK modeling is advocated and frequently used in modern risk assessments, but there are times when it is not appropriate. There are no set criteria, but in general PBPK models can be used for AEGL risk assessment when:

- Existing PBPK models are available for a given chemical.
- Existing models can be used in their current form or can be readily adapted for use.
- Existing models can be adapted for the relevant species.
- The ability of the model to simulate DMs (evaluation) within the context of their use in AEGLs is reasonable.
- The PBPK models can calculate a DM that is appropriate, given the critical effect that is used in the risk assessment.

Different chemicals, exposure periods, and PODs may necessitate the use of different types of models. The criteria for deciding whether a model is acceptable for use in deriving AEGL values are provided in Section 4. When these criteria are not substantially met, PBPK models are not appropriate for use.

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When they are not appropriate and available for use in deriving AEGL values, the AEGL values should be derived with existing methodologies.

A mode of toxicologic action consists of both pharmacokinetic and pharmacodynamic processes. Pharmacokinetics is what the body does with the chemical, and pharmacodynamics is what the chemical does to the body. For example, if a chemical enters a tissue, binds to a receptor protein, and interferes with signal transduction, the entry into the tissue is a pharmacokinetic process and the effects are pharmacodynamic. As the two processes are often conceptually separate, different models can sometimes be developed for each aspect, and the models can be linked to produce a biologically based dose-response model. While PBPK models describe the relationship between exposure and tissue dose, physiologically based pharmacodynamic (PBPD) models describe the relationship between tissue dose and response. The linked PBPK and PBPD models are often referred to as PBPK/PD models. In some cases, it may not be possible to develop separate PBPK and PBPD models. Some examples of PBPK/PD models include those developed for acetylcholinesterase inhibition for chlorpyrifos (Timchalk et al. 2002) and other organophosphate pesticides, glutathione depletion (Frederick et al. 1992), and cytotoxic responses due to intracellular acidification (Andersen et al. 2000). If such models exist for an AEGL chemical and can be incorporated into derivation of AEGLs, these models would serve to further reduce uncertainty and may reduce the pharmacodynamic portion of the UF.

The methodology for using PBPK modeling in risk assessments has been described (Clewell et al. 2002). The methodology provided in this White Paper is consistent with the guidance provided by the current EPA document on the use of PBPK modeling in risk assessment (EPA 2006). This document describes the process and explores specific issues that arise in the context of AEGL development. Although not often used in the risk assessment context, under specific circumstances classical (i.e., non-physiologically based) pharmacokinetic modeling may be useful for performing the temporal extrapolations when a PBPK model is not available.

2. DESCRIPTION OF PBPK MODELING

In this section, PBPK models are described in a general manner. Additional detail may be found in various literature reviews of PBPK models (Krishnan and Andersen 1994; Leung and Paustenbach 1995; Bailer and Dankovic 1997; Reddy et al. 2005).

The pharmaceutical and medical sciences have studied and used pharmacokinetics for many years to determine appropriate doses of intentionally administered chemicals and drugs (pharmaceuticals) and, to a more limited extent, evaluate the effect of unintentional exposures (accidental overdoses, poisonings, narcotics usage). In these sciences, measures of dose such as the peak concentration (Cmax), time of peak concentration (Tmax), and AUC of concentration versus time have been of interest in determining the therapeutic dose. These

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efforts were made after it was recognized that internal dose was a better predictor of therapy or toxicity than external exposures. Mathematically, measures of dose were usually determined by using curve-fitting regression methods that fit a simple empirical model to the concentration-versus-time data. The data were usually fit with formulas that replicated either a one- or a two-compartment system that represents either whole body or tissue and body water constructs.

This approach served the intended pharmaceutical needs because they were usually based on relatively rich data sets, including human data from clinical trials. Thus, extrapolations to other exposure scenarios were not a major factor in their use, as a range of doses could be studied in experimental trials. If extrapolation were needed, it could be performed in an empirical manner. PBPK models first received attention in the medical literature. As far back as the 1920s, they were described for ether, an anesthetic gas. Unfortunately, the computation burden in these models is such that the model could be solved only at steady state. In the 1950s and 1960s, PBPK models were described for additional drugs, including the chemotherapeutic methotrexate. Later work by Fiserova-Bergerova and others in the 1970s returned to a series of other anesthetic gases. Starting in the 1980s, PBPK models largely turned to considering environmental risk assessment, starting with work on methylene chloride and other chemicals.

The classical pharmacokinetic modeling approaches used in pharmaceutics did not serve the needs of environmental risk assessments nearly as well, where the data are relatively less abundant. In environmental risk assessment, intentional dosing studies that cover a range of exposures are often not available. High-dose studies could be associated with morbidity and are therefore not permissible. Experiments designed to evaluate effects of low-dose toxicity would require doses much lower than typical therapeutic doses and generally do not have large enough study populations to detect effects. Thus, risk assessments are enhanced when supported by estimates of internal tissue dose (EPA 2006). Extrapolating to low or high doses could be performed using proportional methods or classical pharmacokinetic methodologies. Proportional methods rely on the assumption that dose is proportional to exposure. This assumption is not the case for many exposures because of nonlinear physiologic processes such as saturable metabolism. This issue is also a limitation of classical pharmacokinetics; that is, Cmax at a dose of 3x is often not three times the Cmax at a dose of x. Tissue responses are more closely related to the internal target tissue dose versus the external chemical compound.

The PBPK model mitigates this dilemma and reduces the uncertainty in the dose-response assessment. The use of mathematical representation of the body based on first principles, meaning that the underlying construct of the body is true to life rather than entirely empirical, allows for full utilization of available data. Each compartment in the model represents an actual portion of the body, and the more important physiologic and biochemical processes are explicitly included in the mathematics of the model. However, there is simplification, such as considering major metabolic processes while ignoring minor ones. This sim-

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plification is justified by the assumption, which can be tested, that the minor processes not included do not have a significant effect on model outcome. When all significant biologic processes are included in a model with equations that reflect the biology of the actual process, the outcome of the model will be a true representation of pharmacokinetics even when the doses are changed, so such models are a sounder basis for extrapolation. An example of the impact of using a PBPK model rather than empirical methods is provided in Figure 7-1. In this figure, the blood concentration is not directly proportional to exposure level. For example, at 8 h, the concentration of toluene in blood is about 1 milligram per liter (mg/L) after exposure to 100 parts per million (ppm); after a 1,000-ppm exposure, it exceeds 20 mg/L.

The internal concentration of a chemical or a chemical's metabolite has been referred to as tissue dose, which is considered a more salient measure of dose (DM) for a POD than the external exposure. The ultimate tissue dose versus time profile is a composite event that results from all pharmacokinetic processes that occur, broadly divided into the processes of absorption, distribution, metabolism, and elimination. When a chemical such as an anesthetic gas is inhaled, it is taken up through the upper respiratory passages into the deep lung. More water-soluble chemicals may be absorbed into the upper respiratory tract and may even cause toxicity in those tissues. Chemicals that persist into the deep lung are presented to the lung cells, perhaps after absorption into mucous layers. In accordance with chemical equilibrium partitioning and diffusion characteristics, the chemical is absorbed into lung tissue cells several layers thick and eventually diffuses out of the tissue and into the blood, which perfuses that tissue. In the blood (and the lymph), the chemical may remain as a free compound or may bind with macromolecules and be transported to other parts of the body.

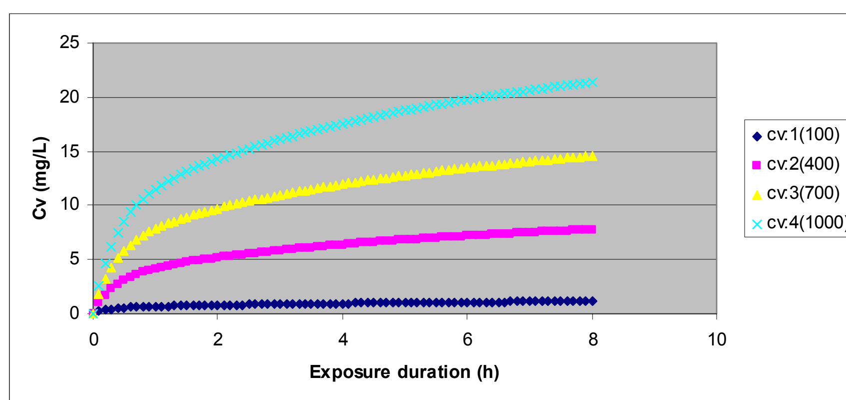

FIGURE 7-1 Plot of venous blood concentration (CV) of toluene (mg/L) versus time for four exposure levels (100, 400, 700, and 1,000 ppm) for up to 8 h. Based on PBPK model for toluene used for setting AEGL values for toluene.

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When reaching other tissues of the body, chemicals again diffuse into cells in accordance with rates of diffusion and equilibrium partitioning. For chemicals that diffuse relatively rapidly, it is usually assumed that diffusion rates are unimportant and that the concentration of the chemical in the blood leaving the tissue will be in equilibrium with the concentration in the tissue. When this assumption has been tested for small molecular weight hydrophobic chemicals, it has been found to be reasonable. In other cases, the rate of diffusion must be explicitly incorporated in the model.

Metabolism may occur in various tissues. For some chemicals, the liver is the major metabolic organ, but a significant degree of metabolism may occur in other tissues as well. These processes are incorporated in a PBPK model by inserting the Michaelis-Menten equation into the rate expression for concentration of chemical in the tissue. At low concentrations, the rate expression compresses to the linear rate of metabolism with tissue concentration as the variable parameter; at high concentrations, a zero order rate of metabolism occurs. For example, many small molecular weight organic molecules that are substrates for low-affinity constant enzyme cytochrome P-450 (CYP) 2E1 can begin to saturate the enzyme at exposure levels that are relevant to the AEGL risk assessment process. As many parallel or sequential metabolic steps as needed can be included. If the toxic agent is the parent chemical, the models are usually not set up to trace the pharmacokinetics of metabolites. However, some models are constructed to evaluate the pharmacokinetics of metabolites by including a submodel with the necessary equations and parameter values for partitioning, absorption, metabolism, and other biologic processes for the metabolite. A chemical may be eliminated via exhalation, excretion through the kidney (urine) or liver (bile), or, in a sense, metabolized. Rate expressions for any significant elimination process would be included, such as in models that have successfully simulated the appearance of a metabolite in urine or feces (Gearhart et al. 1993).

While the body undergoes many thousands of simultaneous processes on a macro or molecular basis, when chemical concentrations are measured in tissues, their pharmacokinetics are often dominated by a selected few macroscale processes. Absorption of airborne chemicals is dominated by breathing rates and equilibrium between the lung air and lung tissue blood. Distribution is dominated by rates of blood flow to various tissues and equilibrium in those locations. Metabolism of inhaled chemicals occurs in metabolically active tissues such as the liver and can involve multiple CYP enzymes and others as well. In some cases, the data indicate that one enzyme in one principal tissue, often the liver, predominates and that an adequate model can be developed in which the contribution of other isoforms or enzymes in the principal tissue or the same or other enzymes in other tissues can reasonably be lumped with the activity of the major enzyme in the predominant tissue. In other cases, multiple enzymes and multiple metabolic tissues are sufficiently important that they should be incorporated in the PBPK model. The determination of how complex the model should be must be guided by the available data for each chemical during model development. For many chemicals, PBPK models can be constructed with only a few

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rate expressions. Likewise, the anatomy of the body can be represented simply as well.

The use of PBPK modeling has been compared with results of using the ten Berge empirical equation for inhalation exposure to toluene. The specific results of this analysis are presented in Appendix A. The PBPK model was developed and then used to calculate the AEGL values at each exposure duration, based on achieving the same target tissue dose at all durations (toluene in brain or equivalently in blood). The target tissue dose was derived from the key study for that AEGL. In the toluene example, the PBPK model was able to determine the AEGL value for each duration that would yield the same expected tissue dose, while the ten Berge equation yielded tissue doses that varied from the target dose by a factor of 2-3.

The structure of a PBPK model has anatomic and kinetic elements. Anatomically, the body is represented as a system of compartments connected via blood flow. Typically, compartments are established for target tissue, a lung, blood, fat tissue, and the liver. Other tissues are usually grouped into rapidly perfused and slowly perfused tissues, and some tissues are combined when the processes that occur in them are relatively similar. For example, the gastrointestinal tract and kidney can be classified as rapidly perfused tissues, while muscle and bone can be considered slowly perfused tissues. Alternatively, any of the lumped-compartment tissues can be separated into its own compartment. Measured anatomic values for the size (volume or weight) of the tissue compartment are physiologic parameters. The sum of the tissue compartments is usually 80% to 90% of total body mass, as 10% to 20% of the body is not perfused with blood. A simple four-compartment model is shown in Figure 7-2.

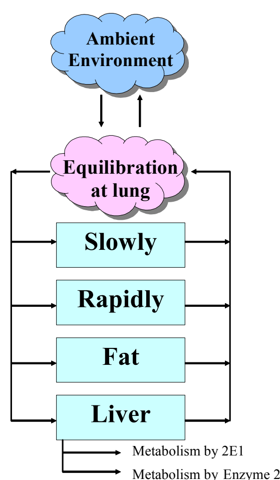

FIGURE 7-2 Four-compartment model.

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Kinetic elements of the model structure include ventilation, blood flow, and biochemical expressions for metabolism, excretion, and other processes. Unless lymph, bile, or other fluids are included in the model, the only flow rates that need to be included are alveolar ventilation and blood flows. Alveolar ventilation is the fraction of pulmonary ventilation that reaches the gas-exchanging tissue in the deep lung. Total ventilation may be relevant for some types of models. Blood flows include cardiac output, arterial and venous blood flow, and blood flow to tissue compartments. Each of these values is taken from standard physiology literature (Brown et al. 1997) as model inputs.

Biochemical expressions depend on the chemical in question. If the metabolic rate is significant, equations are included representing metabolism as saturable (Michaelis-Menten), first order, or second order, as indicated by experimental data. Excretion of parent chemical or metabolites through the lung is handled by lung equilibration. The model does not need to compute the time course of metabolites if the DM relates to the parent compound, but if metabolites need to be included, excretion to feces or urine may be relevant and in some cases for the parent compound. These processes can be represented as first-order rates or by other appropriate kinetic mechanisms.

This description of a simple four-compartment model is often used for lipophilic chemicals. Many other model structures have been developed to describe various types of chemicals. For example, some models have more detailed descriptions of the lung or skin compartment (McDougal et al. 1986; Frederick et al. 1992) and some models have descriptions of biochemical processes such as protein binding, diffusion-limited kinetics, or enterohepatic recirculation. In practice, the concept of modeling parsimony should be exercised. This concept states that the model should be kept as simple as possible yet still provide the information needed for the analysis. In the AEGL program, PBPK models considered may often be more complex than the four-compartment model and should be used with due regard for the parsimony principle.

The PBPK model consists of a series of equations that include differential equations for the rate expressions and algebraic equations that compute other quantities. The equations were originally developed using the mass balance concept, which means that the amount of chemical entering a compartment equals the amount leaving or cleared from the same compartment plus the amount retained in the compartment. These values are expressed as a function of time. During acute exposure, the tissue concentrations are often not at steady state and therefore are significantly affected by the duration of exposure.

The typical mass balance equation for a compartment is

$$\text{Rate of change of amount in tissue} = Qi \times (CA - CVi) - \text{clearance},$$

where
Qi = blood flow to tissue i,
CA = arterial blood concentration,
CVi = chemical concentration in the venous blood leaving tissue i, and

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clearance is an additional rate expression describing clearance processes, such as metabolism in the tissue.

The equation determines the rate of change in amount of the chemical in the ith compartment. The mass of chemical in the compartment is determined by integrating the equation. This normally has to be done by using a numerical method for integration. In other words, the mass balance equation can be restated:

Rate of change in the chemical amount in the tissue (mg/h) = tissue blood flow rate (L/h) × (concentration in arterial blood [mg/L] – concentration in venous blood leaving the tissue [mg/L]) – rate of change in chemical amount due to metabolism in the tissue (mg/h).

Additional quantities are then calculated:

CT = AT/VT concentration in each tissue compartment and
CVi = CT/PT concentration in venous blood leaving tissue,

where
CT = chemical concentration in each tissue,
AT = amount in each tissue,
VT = volume of each tissue, and
PT = partition coefficient between the tissue and blood.

Metabolism is computed by another rate equation. For Michaelis-Menten kinetics in the liver,

$$\text{Rate of metabolism} = V_{max} \times CVL/(Km + CVL),$$

where
V_{max} = maximum rate of metabolism,
CVL = concentration in venous blood leaving the liver, and
Km = affinity constant for the chemical.

Other rate equations describe the uptake of chemical into lung blood by equilibration. Full versions of model codes have been provided for typical models in the literature (Clewell et al. 2000). Models developed for AEGLs should be scientifically supported and documented when possible.

3. RECOMMENDATIONS FOR USE OF PBPK MODELS IN RISK ASSESSMENT

The EPA and other risk assessment organizations and practitioners have advocated the use of PBPK models to support risk assessment. These recom-

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mendations go back to at least 1987, when the National Research Council (NRC) stated that "relevant PBPK data can be used to reduce uncertainty in extrapolation and risk assessment" (NRC 1987). In another recommendation specifically addressing community emergency exposure levels, the predecessor to AEGLs, the NRC stated "If PBPK models for calculating delivered dose and cross-species extrapolation have been developed, the pharmacokinetic information should be incorporated into the quantitative risk assessments" (NRC 1993).

The EPA has strongly endorsed the use of PBPK modeling in risk assessment. In 2002, the EPA stated "The optimal approach for extrapolating from one dose-duration response situation to another is the use of a physiologically based pharmacokinetic model (PBPK) model" (EPA 2002a). The EPA has made similar recommendations in its "Draft Final Guidelines for Carcinogen Risk Assessment" and in the context of cumulative risk assessment (EPA 2001, 2003) and reference concentration development methodology for Category 3 gases (EPA 1994). Moreover, the EPA has recently developed a report on the use of PBPK modeling in risk assessment (EPA 2006). The proposed methodology for use of PBPK in AEGL risk assessments has been informed by and is consistent with this EPA methodology.

The EPA has recently used PBPK modeling in risk assessments for perchlorate (EPA 2002b), vinyl chloride (EPA 2000), and other compounds. The most recent permissible exposure limit promulgated by the Occupational Safety and Health Administration (OSHA) for methylene chloride was derived with a PBPK model (OSHA 1997). Risk assessments based on PBPK modeling have also been developed by groups on a variety of chemicals, including trichloroethylene (Fisher and Allen 1993), vinyl acetate (Bogdanffy et al. 1999), formaldehyde (Schlosser et al. 2003), ethyl acrylate (Sweeney et al. 2004), and methylene chloride (Andersen et al. 1987a). Several authors as well as the National Academy of Sciences have advocated using PBPK modeling in AEGL development (Bruckner et al. 2004; Krewski et al. 2004). An initial example for trichloroethylene has been described (Boyes et al. 2000, 2002, 2003, 2005; Simmons et al. 2002, 2005). Thus, guidance from both the EPA and input from the scientific community have clearly established that PBPK models should be considered for use in supporting risk assessment when such models are available and capable of predicting tissue dose of the chemical under conditions similar to environmental exposure or experimental studies.

The specific types of risk assessment applications for which PBPK modeling can be useful in the AEGL context have been previously demonstrated in other risk assessment applications, often for cancer and chronic noncancer risks. Many papers have illustrated the value of PBPK modeling for interspecies scaling (Ramsey and Andersen 1984; Clewell et al. 2001; Timchalk et al. 2002).

PBPK models have been previously used to time-scale dose from exposure regimens in chronic bioassays (e.g., 6 h/day, 5 days/week) to 24 h/day, 7 days/week (Clewell et al. 1997). PBPK models have also been used to time-scale worker exposure levels for long and short exposures (Andersen et al. 1987b). Their use in AEGL derivation is an extension of these previous uses.

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For each AEGL level, the critical study that determines the POD usually provides an estimate of the POD only at one time point—for example, a median lethal concentration (LC_{50}) study for 4 h. Thus, one task in the AEGL development process is to extrapolate from one exposure duration to others. It may be necessary to extrapolate from longer to shorter durations or vice versa. It has been done in the past by using various approaches, including the ten Berge equation ($k = C^n \times t$) where k = a constant, C = exposure concentration, n = an empirical constant, and t = time (ten Berge et al. 1986). The exponent n can be derived from available data; often, the default assumptions are used that n = 1 when extrapolating from shorter to longer periods and n = 3 when extrapolating from longer to shorter periods. However, as with any empirical approach, these assumptions do not always reflect the underlying pharmacokinetics in the animal and can lead to errors when extrapolating to humans.

PBPK models have also been used for workload physiology extrapolation (Johanson 1986; Dankovic and Bailer 1994; Kumagai et al. 1998; Jonsson et al. 2001). Workload physiology extrapolation is important because the tissue DMs for some chemicals can be significantly affected by increased ventilation, and altered blood flow can affect the distribution of the chemical. Studies have demonstrated that the tissue dose of several organic solvents were found to be increased with increasing workload (Carlsson 1982; Pezzagno et al. 1988), particularly with chemicals that have a relatively high blood-air partition coefficient (PB). Csanady and Filser noted that workload was a significant factor in the pharmacokinetics of chemicals with a PB greater than 6 (Csanady and Filser 2001). Numerous chemicals on the target list of AEGLs have higher PB values. However, in a non-steady-state environment, this issue can be addressed only with a pharmacokinetic model.

PBPK modeling can be used to develop AEGL-1, AEGL-2, and AEGL-3 values. Depending on the availability of appropriate models and DMs, the PBPK models can be used for some or all of the AEGL level values, with other methods used for the values not derived by PBPK modeling.

4. CRITERIA FOR USE OF PBPK MODELING IN AEGL DEVELOPMENT

Several issues must be addressed when PBPK models are being considered for use in AEGL development. Using PBPK modeling in AEGL development has three stages: initial determination of feasibility, in-depth determination of adequacy, and implementation. The initial determination of feasibility is a screening process in which the use of PBPK modeling for a particular chemical can be evaluated based on a priori criteria that are simple to evaluate. The in-depth determination of adequacy is a second required stage, because a number of problems that are not immediately obvious can appear during model development or evaluation. For example, a published model may be developed with one data set, but evaluation with additional data could indicate that the model

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should not be used for AEGL value development because its overall performance is judged to be inadequate. Implementation involves using the models to determine AEGL values. These assessments should be conducted in a manner consistent with EPA guidance (EPA 2006).

4.1. Initial Determination of PBPK Modeling Feasibility

When a chemical is being considered for the development of AEGLs or revisions to existing AEGLs are being made, the applicability of PBPK modeling should be considered. The AEGL development team should include someone with PBPK modeling experience to help in this evaluation. The determination should weigh the following factors:

- Is there a basis to expect that PBPK modeling may yield more reliable and realistic AEGL values than other approaches?
- Is there an existing PBPK model for the chemical? If the model is not in a strain or species of interest, it may still be viable, as modification of species may not be overly time-consuming if data are available. At times, development of a new PBPK model may be warranted. For example, if AEGLs are being developed for a chemical similar to another with an existing model, and data are available for adjusting the parameters, the model development process may be worthwhile.
- Can the model provide data on appropriate DMs? A tentative DM should be determined early in the process, based on the mode of toxicologic action of the chemical.

4.2. In-Depth Determination of Adequacy

This stage is a continuation of the screening analysis but denotes a more in-depth analysis of the models and data available and consequently is more time-consuming. PBPK model adequacy for use in AEGL value determination rests on a number of factors that relate to the ability of the model to calculate the DM.

During development and evaluation of the model, some factors that should be considered in determining whether the model is sufficient include the following:

- Is an existing model established for humans? If not, can an animal model be reliably modified to apply to humans?
- If animal models are required, are existing models available for the species of interest? If not, can existing models be adapted for the species?
- If no existing models are available, can one be developed with a reasonable amount of effort?

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- How well do these data project to the conditions relevant to AEGLs? Do they include exposures at reasonably high concentrations and short durations? Do they include pharmacokinetic data for workload conditions (for human models where applicable)?
- Are the existing models sufficiently well described in publication to be usable?
- Are parameters available for all needed inputs?
- Do the models calculate appropriate DMs or can they be modified to do so?
- Are sufficient data available to evaluate the models? How well evaluated are the models?

4.3. Model Selection

In some cases, more than one existing model is available for use. The possibility exists that modification of an existing model may improve it for use in AEGL development. Therefore, a model has to be selected during the development process. The following procedures should be used in this regard.

Published literature should be thoroughly reviewed to identify existing models. All available models should be reviewed for potential use in AEGL development. During the review, the following questions should be considered:

- Was the model fully documented in terms of equations and parameter values?
- How was the model evaluated? What kinds of data were used?
- Was the model published in the peer-reviewed literature?
- Is the model appropriate for AEGL development? Will it support computation of the DM relevant for AEGL end points?

In general, the use of existing models is preferable to revising models or developing new ones. The existing models should be reviewed by comparing model performance. This review is done by running each model against a group of data sets that are chosen a priori for the purpose of model evaluation. Data sets that are representative and relevant for the AEGL development process and that include data from laboratories other than those connected with the model's developers should be used and justified. Other considerations for data set selection include the following:

- Do the data involve exposures in the range of interest (likely range of AEGL values)?
- Do the data provide multiple concentrations in one set of studies?
- Are data from time-course studies rather than a single time point?
- Are there data for more than one tissue?

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- Are the data collected from the species of interest?
- Are there PBPK model parameters for the experimental species?
- Were body weights reported?
- Are exposure conditions clearly defined?
- Is the route of exposure appropriate?
- Do the data relate to the DM?
- Are there data from more than one laboratory?
- Are there data for exercising humans?

Selection of the most appropriate model depends on many of these factors as well as professional judgment. Evaluation of model performance depends heavily on the ability of the model to describe pharmacokinetic data, especially those data in the region of interest. This evaluation can be performed by visual observation of the plots of model predictions or by statistical analysis. Use of visual observation is consistent with EPA guidance at this time (EPA 2006), although implementation of statistical procedures to evaluate a particular model or to select from multiple models is also consistent with current guidance. If the results of the model performance indicate that model improvement is worthwhile, models can then be revised and compared with the existing models. The best overall model should then be selected for use based on considerations described by the EPA (EPA 2006).

4.4. Considerations for Model Modifications

When modifications are being considered, sensitivity analysis should be used to guide the process. For new, modified, or existing models, sensitivity analysis should be performed and reported to help reviewers understand the relative importance of parameters.

Optimization, the statistical process of modifying a parameter's value until the best fit to a calibration data set is achieved, should be performed numerically after a best visual fit is obtained. Optimized parameter values should be within the range of existing measurements or estimates and should be reasonable when compared with values for similar compounds.

Human parameter values for partition coefficients and metabolism are preferred to animal values when using the human versions of the models. Although these parameters are often similar, the differences that occur can have a significant impact on DMs. Parameters that were determined experimentally rather than through optimization are preferred. However, optimization is acceptable, particularly in the range of experimental results. All resulting parameter values should be reasonable and should be compared with parameter values reported in the literature.

Generally, models published in the peer-reviewed literature would be used in an unmodified manner for AEGLs. In some instances, modified models offer significant improvement to warrant the investment required to modify them. For

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example, available models might have been developed for exposure levels far lower than the range from which AEGL values will be set. When a significant improvement can be made, these modified models may be used for setting AEGLs, but peer review of the modified model is required before such use.

4.5. Model Evaluation and Verification

Model evaluation is one the most critical aspects in selection and use of a PBPK model. Evaluation of PBPK models has been discussed elsewhere (Clark et al. 2004) and pertinent points are discussed below. The evaluation should be performed using as much data as practicable. In particular, data in the strain and species for which the model is developed, for the exposure route of interest, in the concentration range of interest, and for the DM of interest should be used. Data from as many different laboratories as possible will strengthen the model. Data for pharmacokinetics during workload conditions should also be used if workload is an appropriate consideration. Data for more than one tissue, even for tissues that are not target tissues, will strengthen the model.

Models should be evaluated with the following considerations:

- Are the deviations between simulations and experimental data large or small?
- Do the deviations have a systematic component; for example, does the model consistently over- or underpredict portions of the data such as early time points or high exposures?
- How does the magnitude of the deviations compare among the model undergoing evaluation and other models that have been used for risk assessment?
- How well does the model perform in the exposure and duration range of interest?
- How rich were the animal and particularly the human data?

Results of model evaluation should be reported, generally in graphic format.

4.6. Model Quality Assurance and Documentation

As with other parts of the AEGL risk assessment, the PBPK model should be formulated into a report for inserting into a technical support document (TSD). Before issuance, it should go through a quality assurance review. This review would have two parts: editorial review and technical review that would include reproduction of some model output and calculations to check for accuracy.

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To facilitate the review process, the model and all related calculations should be fully documented in the model report. This report should include true copies of all model codes, parameter lists, data sets, and outputs. They should also be made available electronically. Standardized software should be used so that model runs can be repeated with minimal effort. If any scripts are used, they should be designed to take the user through the steps required to reproduce all evaluations and calculation processes. For example, a statement could be provided such as “Fig. X is produced by running Script A, then B with a concentration of C.” Additional guidance on evaluation and documentation of PBPK models is available (Clark et al. 2004).

5. APPLICATION OF PBPK MODELING TO THE AEGL DEVELOPMENT PROCESS

Figure 7-3 describes the process by which PBPK modeling can be used in AEGL development as a series of sequential steps.

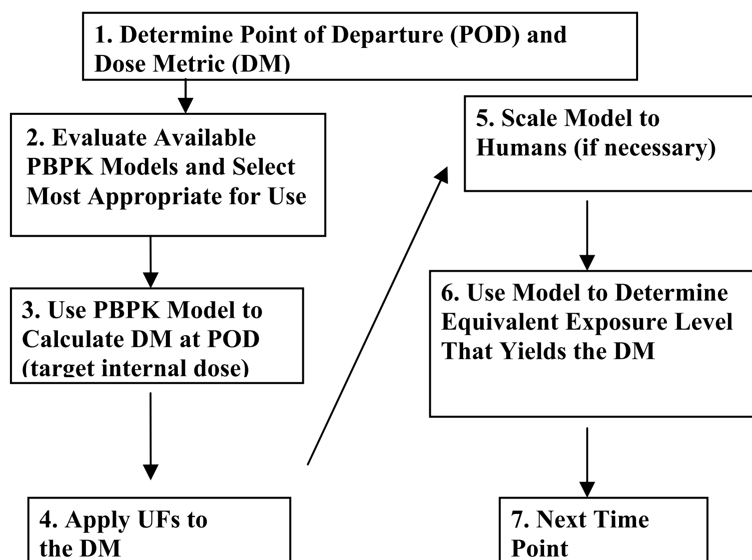

FIGURE 7-3 Use of PBPK models in AEGL development.

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Step 1. Determine POD and DM. The key study and effect are determined through a review of all available literature. If the mode of toxicologic action is understood so that a DM can be determined through a PBPK (or PBPD) model, then modeling will be undertaken. For example, the AEGL-2 POD for toluene was based on a study in which the NOAEL for toluene exposure was 700 ppm for 20 min.

Step 2. Evaluate available PBPK models and select most appropriate for use. Available PBPK models will be evaluated. The most appropriate model will be selected for use, as described in Section 4. This step is often the most time-consuming step of the process, but it is critical. Returning to the example of the toluene AEGL PBPK model, existing models poorly described high-exposure-level data, so a modified model was selected for AEGL development.

Step 3. Use PBPK model to calculate DM @ POD (target internal dose). Use the selected model to calculate the DM at the POD. In the case of toluene, the AEGL-2 critical study involved an experimental design that made it difficult to determine the POD for two reasons. First, it required extrapolation from the very short exposure to longer exposure periods. Second, the 700-ppm exposure was preceded by exposures at 100, 300, and 500 ppm and a break, confounding the assessment of the POD. PBPK modeling was used to determine the internal DM for the exposure at the NOAEL. This yielded a DM of toluene in venous blood of 6.5 mg/L.

Step 4. Apply uncertainty factors to the DM. UFs for toluene consisted of a total of 3 for intraspecies. This DM would be applied to the internal DM but in some instances might be applied to final values instead (see discussion in Section 5.1). Thus, the final target dose of toluene in venous blood was 6.5/3, or 2.16 mg/L.

Step 5. Scale model to humans (if necessary). The model is scaled to humans if the original was an animal model. For example, in the toluene AEGL, no scaling was required because the human model was used to determine the internal DM level in the critical study, which involved human subjects. On the other hand, the AEGL-3 for toluene was based on animal data. In either case, the model selected for use had been evaluated against human data and accepted for use.

Step 6. Use model to determine equivalent exposure level that yields the DM. The model is then used to determine the human equivalent concentration for one exposure duration, such as 10 min. This method is referred to as “bootstrapping” because the model is iteratively run until the concentration input that leads to the targeted internal dose is found. For example, with toluene, the model was bootstrapped until the concentration was found that yielded a venous blood concentration of 2.16 mg/L after 10 min of exposure.

Step 7. Next time point. The bootstrapping method is repeated for each exposure period. The process is repeated for each AEGL level where modeling will be used.

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5.1. Application of UFs

In the AEGL process that does not generally incorporate modeling, two UFs are frequently applied to the POD: the interspecies UF when the POD is obtained from an animal study and the intraspecies UF that adjusts for human variability. The intraspecies UF may be reduced in some cases by PBPK modeling, although the methods for doing so are not as readily accepted by the scientific community. For instance, if data were available to allow modeling of the most sensitive human subpopulation, a reduction in the intraspecies UF may be justified. Other UFs can be considered but are used less often in the AEGL development process, including LOAEL to NOAEL and database UFs. PBPK modeling can be a useful tool in replacing some of these UFs, but its most frequent applications are in the interspecies context and in temporal extrapolation.

The interspecies UF default of 10 can be subdivided into a pharmacokinetic component and a pharmacodynamic component according to the existing standard operating procedures for AEGLs (NRC 2001). Typically, the practice is to split the interspecies UF into 3 for pharmacokinetics and 3 for pharmacodynamics (EPA 2006). When PBPK modeling is used to perform the internal dose calculations for extrapolation, the EPA supports an appropriate reduction in the pharmacokinetic portion of the interspecies UF (EPA 2006). Similar reductions in other UFs should be considered if PBPK (or PBPD) modeling can be used to reduce uncertainty in the extrapolation.

During AEGL development without PBPK modeling, the UFs are generally applied only to the POD. When PBPK modeling is used, the UFs can reasonably be applied in two places. First, they can be applied to the target DM level, the internal dose that corresponds to the POD. For example, if the POD corresponds to a tissue dose of 20 mg/L and the UF is 3, the target tissue dose would become 6.67 mg/L. The PBPK model would then be run again to determine the human exposure that yields that target dose level. The second option is to apply the UFs after the human equivalent concentration is determined via the model. Thus, for the example above, the model would determine the human equivalent concentration that yields an internal dose of 20 mg/L and that concentration would be divided by 3. Given that there often are UFs pertaining to inter- and intraspecies extrapolations, a third choice of application is also present: one of the UFs could be applied to the DM and the other to the final values.

Whether there is a difference in the final AEGL value when the UF is applied to the internal DM or the POD depends on the shape of the exposure-dose curves. The exposure-dose curve may be quite linear, depending on the chemical and the concentration range. In these cases, there is no difference in the resulting AEGL value determined by either method. However, when there are significant nonlinearities in the concentration-dose curve, differences can occur.

To assess the potential magnitude of these differences, AEGL values were calculated both ways for two chemicals: toluene and xylene. In the proposed AEGLs for toluene and xylene, under standard AEGL derivation the comparison was made with UFs of 3, 10, and 30. For these two chemicals, no appreciable

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difference between the approaches was found when the total UF was assumed to be 3. More frequently, an appreciable difference was found when the total UF was 10 or 30, particularly the latter. The differences were greater for the 8-h AEGLs than for the shorter time periods. Either method could yield the larger AEGL value at different times. When a difference was found, most of the time the two values were within a factor of 2 and occasionally were as high as 2.5.

The argument for applying the UF to the DM is that this approach results in a more reliable reduction of risk as the internal dose is more closely correlated with risk than the external exposure level (Clewell and Jarnot 1994; Clewell et al. 1997, 2002). Stated another way, if the UF is applied to the final values, the reduction in internal dose will not exactly equal the intended UF reduction based on the net reduction in the internal dose, so applying the UF to the internal dose is more scientifically defensible. On the other hand, it has been observed that default UFs represent policy decisions rather than clear estimates of uncertainty and thus should be applied to final values. There is some consensus indicating that the interspecies UF (pharmacodynamics) is best applied to the DM, while there is less consensus indicating that the intraspecies UF should be applied to the DM. While the EPA position on this matter is being established, the AEGL program will need to implement one of the following options:

- Option 1: Apply all UFs to the DM.
- Option 2: Apply all UFs to the final values.
- Option 3: If both intraspecies (PD) and interspecies (PD/PK) UFs are to be applied, apply the interspecies to the DM and the intraspecies to the final values.

As the best approach is undecided, another factor should be considered. In many cases in the AEGL program, human pharmacokinetic data are at lower levels than the POD, especially for AEGL-3, which is usually obtained from animal studies. When the UFs are applied to the DM, they reduce the target tissue dose before modeling is used to determine human equivalent concentrations , thereby reducing the extent of the high-dose extrapolation of the human model. For these reasons, Option 1 is the default choice of method. However, the NAC at its discretion may select one of the alternative methods on a case-by-case basis where indicated by scientific data.

5.2. Use of Benchmark Dose Statistical Modeling

An alternative to using a NOAEL as the POD is the use of a BMD. If a BMD is to be calculated and a PBPK model is available, the model should be used to calculate the DM for each exposure level in the critical study. The DM values should be used as inputs into the BMD calculation rather than the exposure levels. For example, for the toluene AEGL-3, the BMD can be determined by using the PBPK model to determine the peak blood concentration of toluene

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at each exposure level that goes into the BMD calculation, and these blood concentrations are used for the dose side of the BMD input. This approach is preferable because the closer relationship between the DM and toxicity subjects the statistical model to less confounding by the nonlinear relationship between exposure and internal dose (Clewell et al. 2002). Examples of the use of BMD and PBPK modeling were developed for acute and longer-term exposure guidelines at the Agency for Toxic Substances and Disease Registry (Clewell et al. 1997).

5.3. Inclusion of Exercise Physiology

The current AEGL standard operating procedure does not recommend adjusting AEGL values based on activity levels. Therefore, the NAC has decided not to adjust values for activity levels even when possible by using the PBPK model.

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APPENDIX A

PBPK Modeling-Based Derivation of AEGL Values for Toluene

SUMMARY

The method used in this study to determine human equivalent AEGL values is similar to that previously reported (Bruckner et al. 2004; Krewski et al. 2004). The method reduces the uncertainty inherent in extrapolating rat toxicity data to humans and extrapolating toxicity data across time-scales by using validated PBPK models to perform the extrapolation based on an internal measure of dose. This reduces the uncertainty in the pharmacokinetic component of the extrapolation. Uncertainty in the pharmacodynamic component of the rat-to-human extrapolation is handled with standard UFs.

The end points found in the critical studies for all three AEGLs can be reasonably associated with the blood concentration of toluene. The blood concentration is superior to the applied concentration (exposure concentration) as a measure of dose because, as an internal measure of dose, pharmacokinetic alterations in tissue dosimetry are addressed in extrapolation by explicit quantification. In extrapolation, for example, of a 1-h AEGL to an 8-h AEGL, the increase in blood concentration over time is explicitly compensated for by reducing the 8-h AEGL to the point where blood concentrations are equivalent. This process obviates the need for algorithms such as the ten Berge (ten Berge et al. 1986) equation, which can result in corresponding errors when the empirical parameters are unknown.

Fundamentally, the PBPK-based AEGL values are based on the same critical studies as the AEGL values established in the TSD; only the method of extrapolating from rat to human (dosimetry replaces pharmacokinetic uncertainty factors) and over time (dosimetry replaces empirical formulas) differs. When the PBPK-based approach replaces pharmacokinetic UFs, the resulting AEGL value may be higher, potentially avoiding issues with AEGLs that are close to occupational exposure limits. Thus, this approach may avoid the problem of overconservatism in setting AEGLs caused by extrapolative uncertainty.

The PBPK risk assessment method involves the following specific steps:

Step 1. At the NOAEL found in the critical study for setting AEGL-1 (of X ppm in rats), the CV is determined. This CV is the target internal dose for any time period at that AEGL. If the AEGL is based on human data, the CV is computed directly in the human model (skip to Step 2).

Step 2. The human version of the model is used to determine the exposure level that yields the same internal dose for each exposure period (10 and 30 min and 1, 4, and 8 h). This value is then divided by applicable pharmacodynamic or intraspecies UFs to yield the final AEGL value.

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As humans may be under exertion during emergency events, the human model has been run using physiologic parameters corresponding to each of four states: resting state, 50 watts (W) of workload, 75 W of workload, and 100 W of workload.

Step 3. Repeat for AEGL-2 and AEGL-3.

This appendix has three parts. First, the structure and parameterization of the toluene PBPK model are described. Second, the model is validated by showing model performance against rat and human data sets obtained from the literature. Third, recommended AEGL values are derived.

The AEGLs based on PBPK dosimetry are often quite different from those based on the ten Berge equation (as described in the TSD document). When extrapolated to shorter timeframes, the toluene PBPK-based AEGLs tend to be much higher than the ones derived with the ten Berge equation. Conversely, the PBPK-based AEGLs are much reduced if the exercise scenario is considered. From the 10-min to 8-h AEGLs, the PBPK-based approach yields values that decrease quickly, as toluene takes longer to reach steady state in this PBPK model than previously thought.

INTRODUCTION

The critical studies that provide the NOAEL used in this analysis are the same as those used in the TSD (Table A-1) to calculate AEGL values. As in the TSD, supporting studies were not used in any of the AEGL calculations.

Additional information and justification of these choices of critical studies are available in the TSD. The target tissue dose (CV) was determined from these studies.

Selection of Dose Metric

The DM used for the PBPK-based risk assessment is the CV of toluene. The critical effect of toluene for setting AEGLs is depression of the central nervous system (CNS), based on the analysis of toxicity studies presented in the TSD. It has been generally suggested that CNS depression caused by organic solvents such as toluene is mediated by the action of the parent chemical and not metabolites (Bruckner and Warren 2001). The concentration of toluene in the target tissue, such as brain, is proportional to the CV (van Asperen et al. 2003), so AEGL values determined using either DM should be equivalent. Furthermore, although substantial data are available to validate a model for blood toluene, only limited data for brain concentrations exist. Therefore, CV has been selected as the DM for the analysis. It has previously been used as a surrogate DM for CNS effects (Haddad et al. 1999a; Dobrev et al. 2001), although arterial concentrations (CA) have also been used (Benignus et al. 1998; Bruckner et al.

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2004). The PBPK models have been optimized to provide CV as model output under the exposure conditions indicated for this assessment.

Model Selection

The current approach requires a validated PBPK model for rats and humans. Three options exist for developing or selecting a model to use: develop a new model, modify an existing model, or select an existing model and use it in its current form. If an existing model would serve the needs of this risk assessment, option 3 is the preferred choice and was the first approach to be used. Ultimately, an existing model was used with minor modifications for the current risk assessment.

An evaluation of all existing models can be performed in principle to determine the best available model. However, this process is time-consuming and can be arbitrary to some extent. Therefore, the method of selecting a model was to screen models for good candidates by using specific criteria and evaluate models one by one until an acceptable model was identified. The criteria used to screen models included the following: (1) the model should include the inhalation route of exposure (primarily), (2) development of the model should incorporate validation against venous blood data, (3) the model should be reported in the peer-reviewed literature, and (4) the model should have as a primary purpose the goal of rat-to-human extrapolation.

A number of PBPK models have been developed for inhalation of toluene (Purcell et al. 1990; Tardif et al. 1993; Pierce et al. 1996a; Tardif et al. 1997; Benignus et al. 1998; Pierce et al. 1998; Ali and Tardif 1999; Haddad et al. 1999a; Pierce et al. 1999; Vicini et al. 1999; Jonsson and Johanson 2001; Tardif et al. 2002; van Asperen et al. 2003). Several of them are quite similar. Some were developed for rats, others for humans, and some for both (with modification of appropriate parameter values). The purpose of some models was to evaluate mixture interactions, although in each case a model was first developed for toluene as a single chemical.

TABLE A-1 Critical Studies for Toluene AEGLs

AEGL	Study	Species	NOAEL	Duration
AEGL-1	Weight-of-evidence	Human	200 ppm	8 h
AEGL-2	Gamberale and Hultengren 1972	Human	700 ppm[a]	20 min
AEGL3	Mullin and Krivanek 1982	Rat	6,250 ppm	2 h

[a]After initial exposures at 100 to 500 ppm.

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The first models of this group that generally met the criteria expressed above were the models published by Tardif et al. (Tardif et al. 1993, 1997; Haddad et al. 1999a). It is quite possible, although outside the scope of this work to determine, that other models may perform similarly or better.

The models described in these publications (Tardif et al. 1993, 1997; Haddad et al. 1999a) are “essentially equivalent” models with some minor changes from one to another. The Tardif et al. (1993, 1997) models were essentially the same. Haddad et al. (1999a) used a slightly different set of physiologic parameters, and that led to reoptimization of metabolic parameters, which then varied slightly from the earlier versions. The two models by Tardif et al. (1993, 1997) addressed rats and humans, while the Haddad et al. (1999a) model addressed only rats. However, the human version of the final model could be easily inferred from the earlier work. The final version of this model (Haddad et al. (1999a)) was therefore selected for initial validation studies.

Most experimental data in rats, and more so in humans, are at moderate exposures of less than 500 ppm, and often less than 100 ppm. However, the purpose of this PBPK model is to perform high-dose extrapolations. Therefore, special attention was paid to validating the model with all available data sets for high-level exposure. In addition, attention was paid to validating the model for work and exercise.

Model Structure

A four-compartment PBPK model was used in this analysis, similar to that used in the past (Figure A-1).

In this model, the four compartments (fat, liver, and slowly and rapidly perfused tissues) are linked by the arterial and venous blood supply. The CA is set equal to the concentration in a small volume of lung blood, which is assumed to be in equilibrium with the exhaled air concentration. All metabolism is assumed to occur in the liver, because only slight differences were noted when a proportion of the metabolism occurs in extrahepatic tissues (data not shown). In tissues, equilibrium is assumed to exist between the venous blood returning from the tissues and the tissue itself, according to the tissue-blood partition coefficient. This equilibrium requires that diffusional resistance to mass transfer of the chemical is insignificant, which has ordinarily been observed in PBPK models for small molecular weight organic solvents (Ramsey and Andersen 1984; Dennison et al. 2003). All structural model details are identical to those of Haddad et al. (1999a) except for (1) the lung blood compartment, the use of which is a slightly better approach than the use of a steady-state assumption, although differences are not noted for most chemicals; and (2) the incorporation of a second metabolic pathway, as described below.

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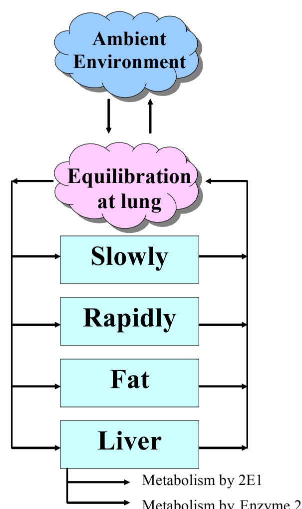

FIGURE A-1 Structure of the PBPK model used in this analysis. The four-compartment model includes fat, liver, and slowly and rapidly perfused tissue groups. A small lung blood compartment is present where equilibration occurs between the arterial blood and the exhaled air. All metabolism is assumed to occur in the liver.

PHYSIOLOGIC PARAMETER VALUES

Physiologic parameter values used in the current model for rats and for humans at different levels of exercise are listed in Tables A-2 and A-3.

In summary, all parameter values were set to be the same as those in the Haddad et al. (1999a) study except as noted here. The Haddad et al. (1999a) study did not include a PBPK model for humans. Therefore, the PBPK model parameters for human tissue volumes were taken from the earlier version of the model (Tardif et al. 1997). These parameter values are similar to values used in similar studies. The PB was also taken from another study, for reasons described below.

The body weight parameter depends on the context of the model. For validation studies, actual or assumed body weights are used. Validation studies include relevant studies in which venous blood and other data are provided. In some of these studies, actual body weight ranges are provided. In these cases, the arithmetic average of the weight range is used when simulating the blood data with the model. In some cases, the age and strain of the animal is provided without body weight. In these cases, the average weight of the species or closest species of rat was used, as provided by a laboratory animal supplier (Harlan 2004). In human studies, if no body weights are provided, 70 kg is assumed. It was further verified that model output was generally insensitive to the body

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weight (see below). Therefore, this assumption was justifiable. When AEGL values were calculated, 70 kg was also used as the human body weight.

Several studies have shown that the blood concentrations of several small molecular weight organic solvents are highly dependent on physiologic parameters, which in turn are highly dependent on workload (Droz and Fernandez 1977; Johanson 1986; Kumagai et al. 1998; Jonsson et al. 2001). In an assessment of the effect of the PB on blood concentrations, modified by exercise level, Csanady et al. recommended that exercise be incorporated in risk assessments if the PB is greater than ~6 (Csanady and Filser 2001). As the PB of toluene is ~15, it is relevant for derivation of toluene AEGL values.

TABLE A-2 Summary of Parameter Values Used in Rat and Human PBPK Model

Variable	Rat	Human
Body weight (BW) (kg)	[a]	70[b]
Tissue compartment weight (kg/(kg/BW)):		
Fraction fat tissue (VFC)	0.07[c]	0.19[d]
Fraction liver tissue (VLC)	0.04[c]	0.026[d]
Fraction rapidly perfused (VRC)	0.05[c]	0.05[d]
Fraction slowly perfused (VSC)	0.75[c]	0.62[d]
Fraction lung blood (VBC)	0.0005[e]	0.0005[e]
Partition coefficients		
Blood-air (PB)	18[f]	13.9[g]
Fat-air (PFA)	1021[f]	1021[f]
Slowly perfused air (PSA)	27.7[f]	27.7[f]
Rapidly perfused air (PRA)	83.6[f]	83.6[f]
Liver-air (PLA)]	83.6[f]	83.6[f]
Maximum velocity of metabolism (before scaling for body weight) (mg/h/kg$^{0.75}$) $V_{max}C$	3.44[c,h]	3.44[c,h]
Affinity constant (mg/L) (Km)	0.13[c]	0.13[c]
Linear metabolism rate constant (mg/L/kg$^{0.3}$ (KFC)	0.05[e]	0.05[e]

Note: Names of parameters are provided in the left column, followed by the units used, and acronym (if applicable).
[a]Body weights were set equal to those reported or inferred from applicable studies.
[b]Human body weight is 70 kg in the AEGL analysis and was 70 kg in validation studies unless indicated otherwise in the study description.
[c]Haddad et al. 1999a.
[d]Tardif et al. 1997.
[e]This study.
[f]Used by Haddad et al. (1999a) and Tardif et al. (1997), as originally determined by Gargas et al. (1989).
[g]Thrall et al. (2002).
[h]Scaled to L/h/kg$^{0.75}$, as done previously (Haddad et al. 1999a).

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TABLE A-3 Summary of Blood Flow Parameter Values and Alveolar Ventilation at Rest and Workloads

	Rat	Human				
Variable	Rest	Rest	50 W	75 W	100 W	150 W
Alveolar ventilation (QPC) ($L/h/kg^{0.75}$)	15[a]	18[b]	53[c]	70[d]	87[c]	100[e]
Cardiac output (QCC) ($L/h/kg^{0.75}$)	15[a]	18[b]	50[f]	59[d]	68.5[f]	79[f]
Fraction of QCC to liver (QLC) (%)	25[a]	26[b]	13[f]	10[d]	7.6[f]	4.2[f]
Fraction of QCC to Fat (QFC) (%)	9[a]	9	3.1[f]	3[d]	3.4[f]	2.4[f]
Fraction of QCC to Rapidly perfused (QRC) (%)	51[a]	55	60[f]	59[d]	58[f]	58[f]
Fraction of QCC to Slowly perfused (QSC) (%)	15[a]	10	23.9[f]	28[d]	31[f]	35.4[f]

[c](Jonsson et al. 2001).
[f]Recalculated from Johanson 1986.
[a](Haddad et al. 1999a).
[b](Tardif et al. 1997).
[d]Average of 50- and 100-W values.
[e]This study.

The principal effects of exercise on an organic solvent's pharmacokinetics involve alveolar ventilation, cardiac output, and blood flow to tissues. Data are incomplete on these rates in human exercise conditions. Therefore, the approach used should be improved in the future, when possible. For the current analysis, parameters were taken from the literature, as listed in Table A-3.

The model was coded into the ordinary differential equation solver, Berkeley Madonna, 8.0.2a8. The model code is included in Attachment A-1. Data sets were electronically read with Digimatic (Digimatic 2004). Numerical optimization was performed by visually estimating the best fit, as Berkeley Madonna is not capable of optimizing against multiple experiments at the same time.

RAT PBPK MODEL

Model Calibration

Two aspects of the Haddad et al. (1999a) model were reoptimized during the evaluation process: metabolism and the PB. These aspects of the model were selected after observation of the model's performance versus data sets from other laboratories. The error structure in model deviations and other literature on toluene suggested that these parameters should be reconsidered.

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The PB is an important parameter in this toluene PBPK model (see Sensitivity Analysis section below). However, there is uncertainty about the correct parameter value. Various studies have reported different values for the PB of toluene (Table A-4).

Many PBPK models have used a value of 18 for PB in rats (Purcell et al. 1990; Haddad et al. 1999a). van Asperen et al. (2003) used a PB of 13; however, it was based on optimization and not on in vitro data. The value selected for the rat PB in this model (18) has been used in numerous toluene PBPK models for rats and lies in the middle of three published values; it appears to allow a successful description of rat blood data (see below).

Human PB values tend to be lower than those of rats (Gargas et al. 1989). For example, Thrall et al. (2002) measured a toluene PB in humans of 13.9 and in rats of 21, although Sato and Nakajima (1979a,b) measured a toluene PB in humans of 15.6 and in rats of 15.2. Gargas et al. (1989) examined the relationship between human and rat (Fischer 344) PBs for 36 organic solvents. Their regression equation predicts a human PB for toluene of 11.0. The value of 13.9 published by Thrall et al. (2002) was used in this model because it is closest to the arithmetic average of the four published values (Table A-4), it is more consistent with the empirical relationship published by Gargas et al. (1989), and it permitted a reasonable description of the human blood data (see below). This value does not work well with the data from Tardif et al. (1997) (not shown), but this data set consisted of blood data after exposure of four male volunteers at a single low concentration. The concentration used by Tardif et al. (1997) (17 ppm) was much lower than concentrations of concern in this model, and additional verification at other exposure levels is not available. The value of 15.6 used by Tardif et al. (1997) was not substantially higher than the value used here.

TABLE A-4 Reported Blood-Air Partition Coefficients for Toluene in Rats and Humans

PB Value (unitless)	Species	Study
18	F344 rats	(Gargas et al. 1989)
21	F344 rats	(Thrall et al. 2002)
15.2	Wistar rats	(Sato and Nakajima 1979b)
13	WAG/RijCrlBR rats	(van Asperen et al. 2003)
13.9	Human	(Thrall et al. 2002)
15.6	Human	(Sato and Nakajima 1979b)
10.0	Human	(Fiserova-Bergerova and Diaz 1986)
18.3	Human	(Pierce et al. 1996b)
11.0	Human	(Gargas et al. 1989)

Abbreviation: F344 rats, Fischer rats.

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At high exposure levels, previous models have sometimes incorporated a second metabolic pathway (Leavens and Bond 1996; Clewell et al. 2001). The initial step of toluene metabolism is oxidation by CYP. The primary isozyme responsible for toluene metabolism is CYP2E1 but other CYP isozymes are also involved, particularly at higher substrate concentrations (Kim et al. 1997; Nakajima et al. 1997). Thus, CYP2E1 is the high-affinity, low-capacity isozyme, and a second metabolic pathway can be used to represent the total metabolism by high-capacity, low-affinity isozymes. In this model, it is represented by saturable (Michaelis-Menten) metabolism (V_{max}, K_m) for CYP2E1 and by linear metabolism for the total metabolism by other CYPs.

The parameter value for the linear metabolic pathway rate constant (KFC) was determined by using data sets from Kishi et al. (1988). The Kishi et al. data (Figure A-2) were overpredicted by the Haddad et al. (1999a) model (data not shown) and the degree of overprediction increased at higher exposure concentrations, suggesting that a low-affinity enzyme was involved. Increasing the maximum rate of metabolism (V_{max}) or decreasing the affinity constant (Km) improved the model at some exposure levels but did not achieve a reasonable fit at others. Adding the second metabolic pathway with a rate constant of 0.05/h to increase metabolism at the higher concentrations allowed a reasonable fit to the Kishi et al. data at all exposures (Figure A-2).

Tardif et al. (1993, 1997) and Haddad et al. (1999a) incorporated a single saturable enzyme in the model. The Tardif et al. (1993) gas uptake pharmacokinetic data (Figure A-3) were reasonably well represented with the single enzyme (upper curves). However, adding a second enzyme with a metabolic constant of 0.05/h (lower curves) improved the fit of the second and third exposure levels. At the highest exposure level, the model with the second enzyme tended to underpredict by a greater margin. Possible reasons for this underprediction include suppression of ventilation and blood flow (Dennison et al. 2003). In addition, including the second enzyme provided a better fit to other data sets. Therefore, the second metabolic pathway was retained.

Rat Model Validation

The following data sets were used to evaluate the model with parameters listed above. In other words, these data sets were not used to further optimize parameter values but served as model validation.

In two publications, Tardif and co-workers published blood data on Fischer 344 rats (Tardif et al. 1997; Haddad et al. 1999a). The data were collected for 2 h in the postexposure period after a 4-h exposure to toluene at 50, 100, and 200 ppm. A reasonable correspondence was obtained between the revised model and these data (see Figure A-4).

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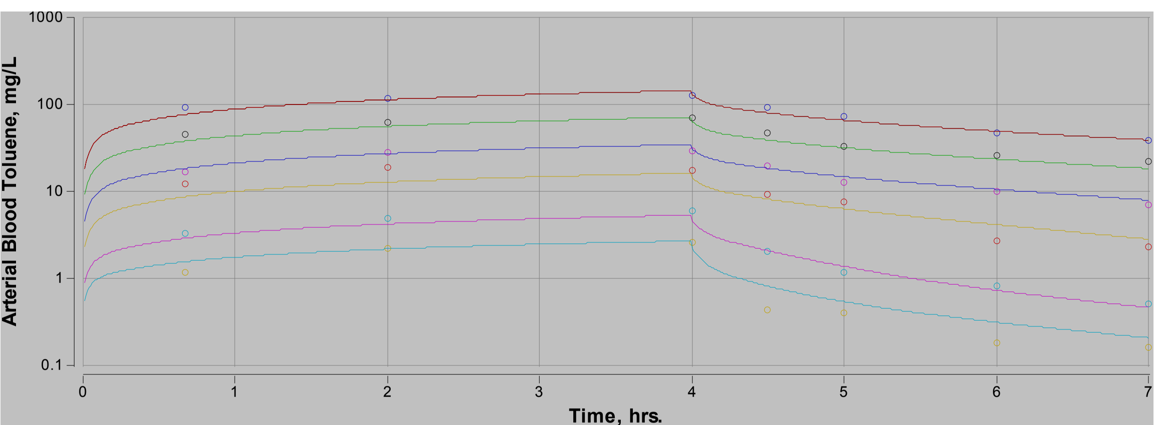

FIGURE A-2 PBPK model and Kishi et al. data. Output from the PBPK model with the second linear metabolic pathway included, based on six exposure levels (4 h and 3 h postexposure) in Wistar rats (7 weeks, estimated weight 200 g). Without the second pathway, the model consistently overpredicted the blood data (not shown). In all figures, symbols represent experimental data and curves are output of the model as described.

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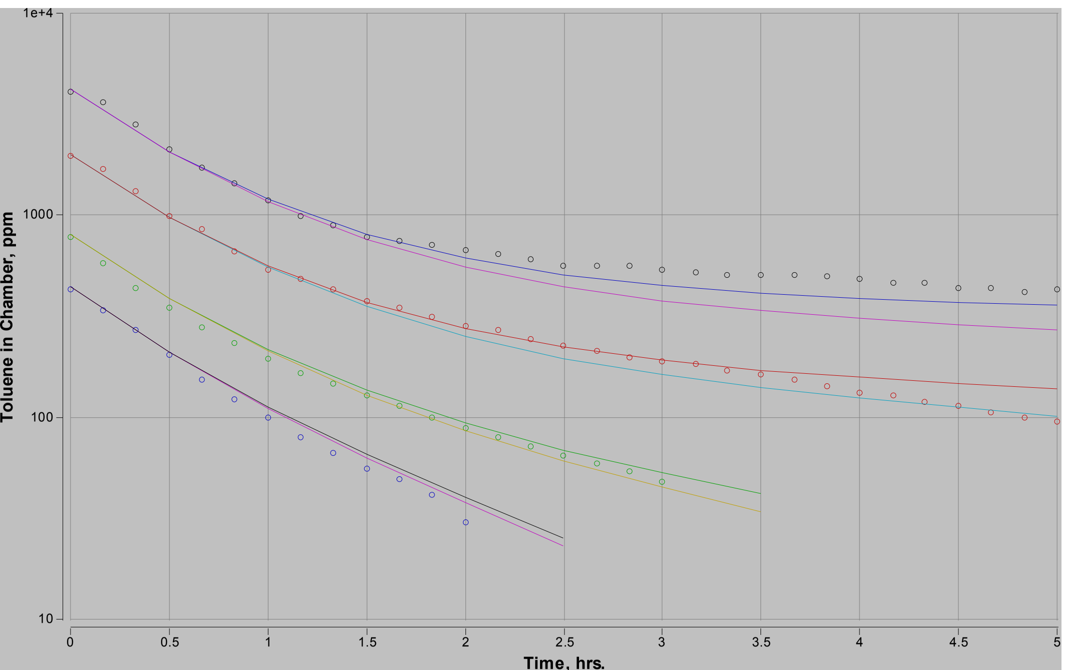

FIGURE A-3 PBPK model and Tardif et al. (1993) gas uptake data. Output from the PBPK model described above was plotted against data from Tardif et al. (1993) at four exposure concentrations. The upper curve in each group included a single enzyme and the lower curve also included a linear pathway representing other CYPs.

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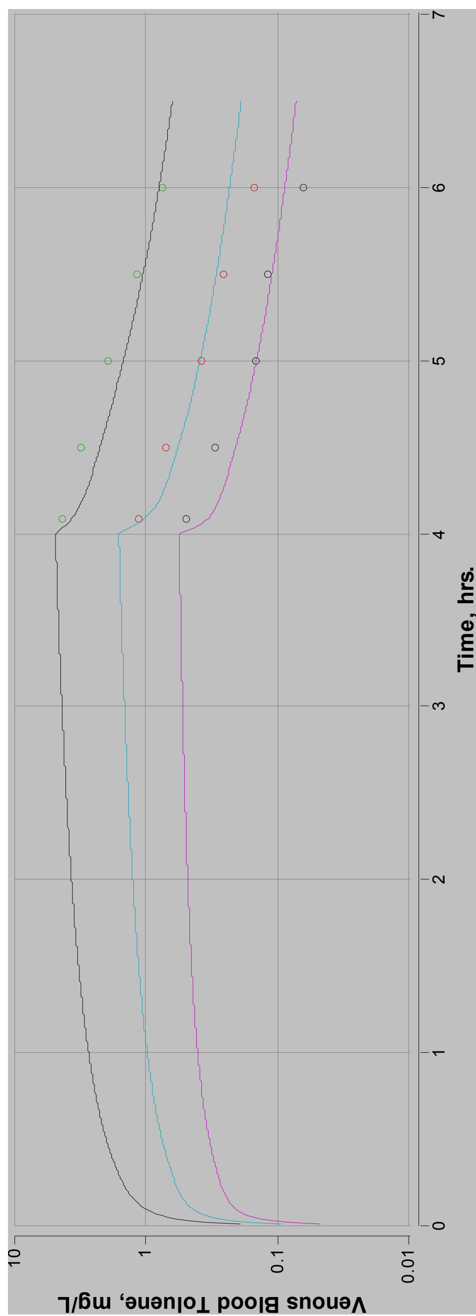

FIGURE A-4 PBPK model and data from Tardif et al. (1997) and Haddad et al. (1999a). Using the same model as described above, a reasonable fit was obtained for CV in rats after 4 h of exposure to lower levels of toluene.

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Another group conducted pharmacokinetic experiments with toluene (van Asperen et al. 2003). These studies used WAG/RijCrlBR rats, which are slowly growing rats produced in the Netherlands, derived from the Wistar strain. At an age of 14 weeks, this rat weighs only about 212 g (J.H. Lammers, TNO Nutrition and Food Research, personal commun., August 11, 2004), compared with Wistar and other strains, which weigh 300 to 400 g at that age.

In the model by van Asperen et al. (2003), the PB was set at 13 after the authors observed that the model overpredicted the data when a PB of 18 was used. This value is much lower than other published values and could imply that the human value would need to be lower yet. At the same time, with PB at 13, the data for the lower concentration were slightly underpredicted, suggesting that the PB may not be the only parameter that should be considered.

A reasonable fit between the present PBPK model with a rat PB of 18 and the van Asperen et al. (2003) experimental data from exposure at 2,667 ppm for 7.5 h was obtained (Figure A-5). Possible reasons why this model described the data without a reduction in the rat PB include the fact that the models differed structurally in some ways, and the current model included a second metabolic pathway. Interstrain differences in the rats are also possible. Given the reasonable fit with the existing model, the current value of PB appears to be justifiable.

Human Model Validation

After development and validation of the model using rat data and parameters, the model was scaled up to humans and revalidated against additional human data. The scaling was done by changing the values of anatomic and physiologic parameters to human values as listed in Tables A-2 and A-3. No equations in the model were altered.

After scaling to humans, the model was run in a manner corresponding to various experiments in which data were available for validation. On the basis of the reported information in each paper from which data were taken, the body weight was altered (or assumed to be 70 kg if not reported), and the concentration and duration of exposure were used in the model. Of the large number of potential data sets, key data sets were selected based on the following primary criteria: (1) exposure concentration and duration were clearly reported; (2) emphasis was placed on data sets that included exposure during exercise; (3) emphasis was placed on high exposure levels. Several of the data sets selected according to these criteria were unique in that exposure concentrations and workloads both varied or were varied several times during a single pharmacokinetic experiment, challenging the model from several perspectives. In the figures, when complex variations in exposure concentration or workload occurred, the model incorporated the changes as step functions that are displayed on the right axis to help clarify the experimental protocol.

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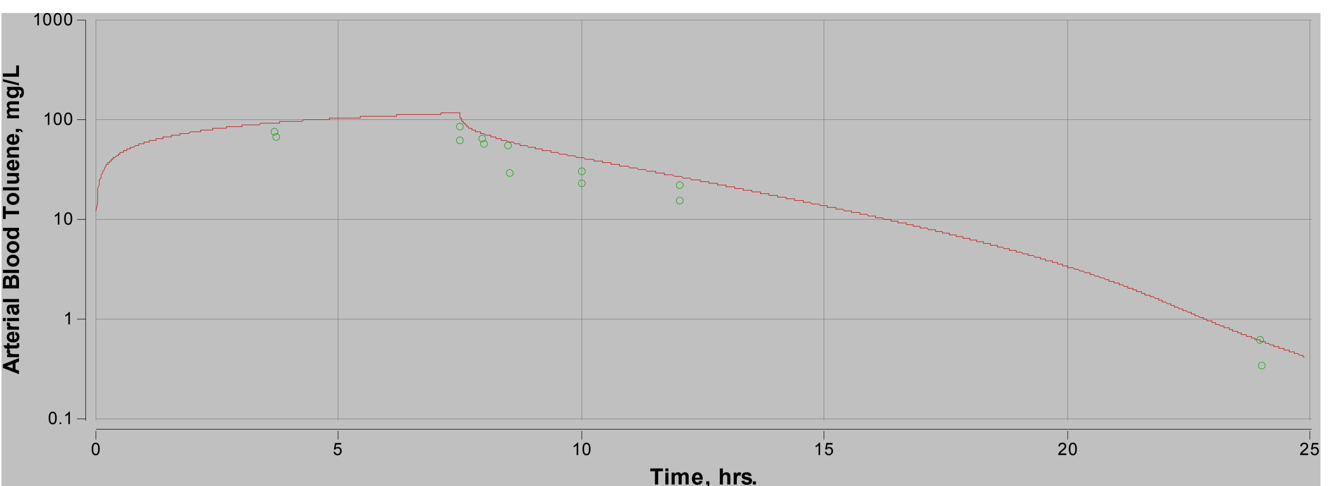

FIGURE A-5 PBPK Model and data from van Asperen et al. (2003). The PBPK model is slightly higher than the data from this experiment at 2,667 ppm, but a different very small-sized rat strain was used in these studies.

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Astrand et al. (1972) exposed a subject to toluene at 0, 95, or 175 ppm at rest or a workload of 50 W (Figure A-6). While the model somewhat underpredicted the CV, it closely predicted the exhaled air concentration except at the end of the experiment. During the final segment of the experiment, the volunteer ceased exercising at 50 W and rested. However, the relevant physiologic functions do not immediately step down to basal rates as specified in this model. The actual rate of the decrease to the basal state is unknown, but in principle it could be included in the model. Ultimately, if the increases were gradually relaxed to basal levels, both exhaled air and venous blood levels would be lower than the model currently predicts. Therefore, a model coded with a more realistic parameter set for the postexercise period would better simulate the last-stage data. This phenomenon could affect other model outputs at cessation of exercise.

Astrand et al. (1972) exposed a volunteer to toluene at 105 ppm for 1 h at 75 and 150 W of workload (Figure A-7). While the model predicted the uptake phase reasonably well, it grossly overpredicted the postexposure phase (data not shown). Because of the extreme change in physiologic parameters, the current model was recoded to extend the exercise (but not exposure) for 10 min after it was ceased in the experiment and then immediately decreased to a basal state. This revision resulted in better correspondence between the data and the model (Figure A-7). A closer fit might occur if the actual parameters were known.

This issue raises the question of whether the noninstantaneous rise in parameter values at the onset of exercise would have any effect. Most likely, the current approach of simulating work as an instantaneous rise overstates the uptake of chemical into the body by some amount. However, the error introduced at the onset of exercise should be smaller than at the cessation of exercise because physiologic function reaches the new steady state faster at the onset of a higher workload than at the onset of a lower workload.

Astrand et al. (1972) also exposed a volunteer to toluene at 200 ppm at rest and at 50 W of workload (Figure A-8). A reasonable correspondence was obtained between the model and data during uptake, although the venous blood prediction was high during the first segment. In the postexposure phase, the fit was not good. However, the venous blood data do not track well with the exhaled air data in the final stage of the experiment, so experimental issues may be present with this part of the data.

The model performs reasonably well against these three Astrand et al. (1972) data sets, although only a single volunteer was used in all three experiments.

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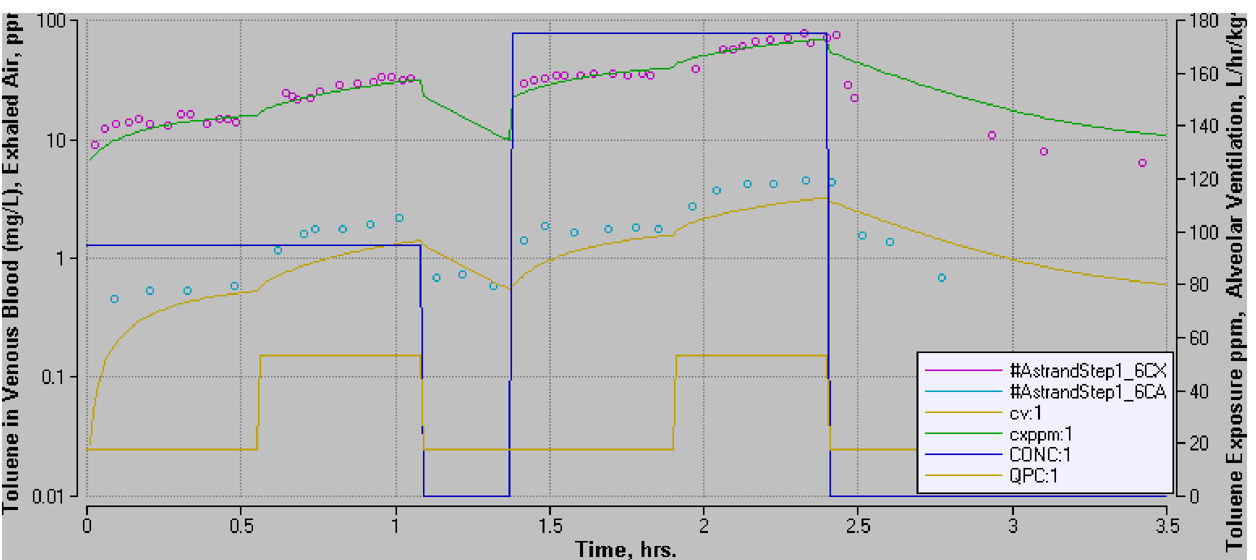

FIGURE A-6 PBPK model and data from Astrand et al. (1972, Figure 3). One subject was exposed to toluene at 0, 95, and 175 ppm at rest or at 50 W of work (right axis). The upper curve and data are toluene concentrations in exhaled air and the lower curve is toluene CV.

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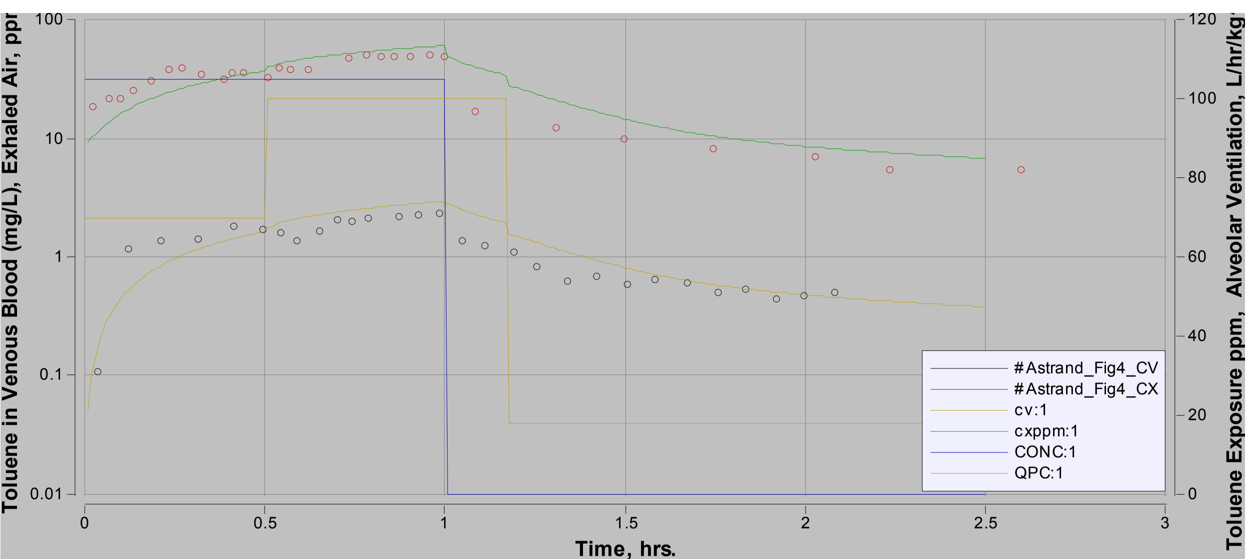

FIGURE A-7 PBPK model and data from Astrand et al. (1972, Figure 4). One subject was exposed to toluene at 105 ppm for 1 h. The first half hour was at 75 W and the second half hour was at 150 W of work. The upper curve is exhaled air and the lower curve is CV. The rapid decrease in toluene concentrations after exposure ended was not simulated well when the simulated work level decreased to resting levels immediately. Here, it was assumed that exercise continued for 10 min after exposure ended.

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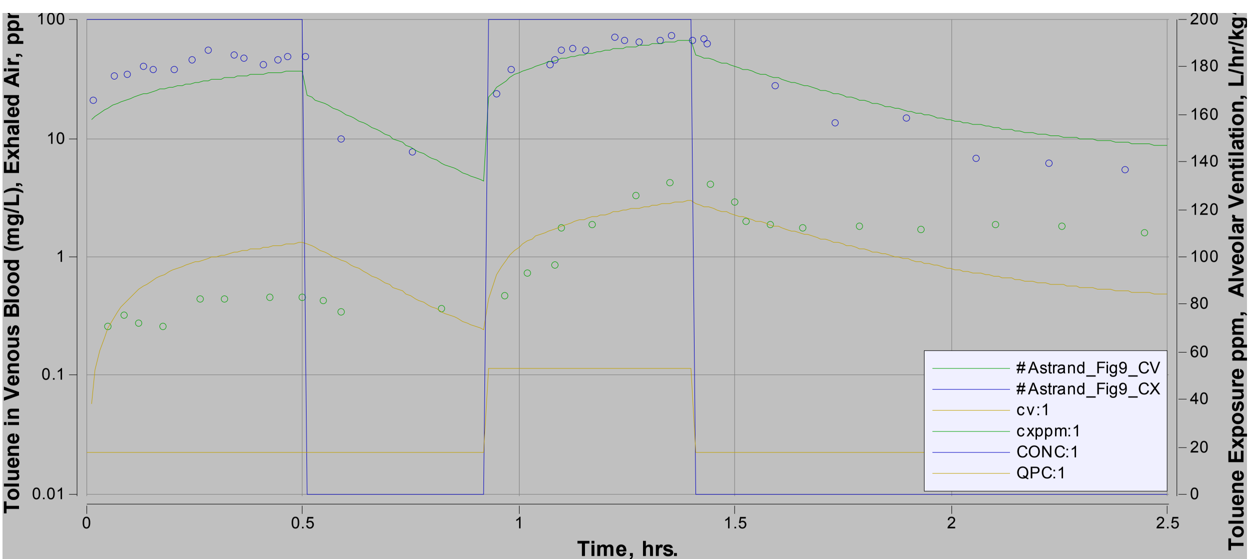

FIGURE A-8 PBPK model and data from Astrand et al. (1972) (Figure 9). One subject was exposed to toluene at 200 ppm at rest and at 50 W of workload, as indicated on the right axis. The upper curve is the concentration of toluene in exhaled air and the lower curve is CV.

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Carlsson (1982) exposed volunteers to toluene at about 80 ppm for 2 h at rest, 50 W of workload, and at workloads of rest, 50, 100, and 150 W in equal segments (Figures A-9 to A-11). A good correspondence between the data for venous blood and exhaled air was obtained at rest and at 50 W (Figures A-9 and A-10). In the third experiment, the workload was increased from resting to 150 W in four equal segments of 30 min each (Figure A-11). An excellent description of the blood data was obtained at all workloads and a good fit to the exhaled air data was obtained except during the resting period. Because of the cumulative nature of the error, the model performs well at all three levels of exercise but underpredicts at the end of the resting period.

To check the validity of the model against the data set with the highest available human exposures, the data of Gamberale and Hultengren (1972) were used. These investigators exposed a group of volunteers to variable concentrations of toluene. Twenty-minute sequential exposures to toluene at 100, 300, 500, and 700 ppm with a brief break in the middle were conducted (Figure A-12). The model with the resting parameter set (lower curve) provided an excellent description of the exhaled air data at all concentrations. The upper curve (modeled at 50 W) underscores the fact that exercise is an important determinant of the dosimetry.

Time to Steady State

The time it takes toluene to reach steady state in the blood can be easily calculated with the PBPK model. As the concentration of toluene in blood approaches the pharmacokinetic limit asymptotically, it is common to speak of benchmarks such as 95% of steady state or 99% of steady state. The time to steady state was plotted (Figure A-13) over time for exposures to 200 ppm at 50 W and at rest. The time to steady state for other exposure levels is almost identical (not shown). As shown in Figure A-13, the approach to steady state is governed in part by exercise conditions; as the workload increases, steady state is approached faster. The current model varies in its ability to describe different data sets in the approach to the steady-state period. For example, data from Carlsson (1982) and Gamberale and Hultengren (1972) are reasonably well predicted in the first few minutes of exposure, while data from Astrand et al. (1972) are not as well predicted. The reason for this discrepancy is not clear, but evidently the data sets are not consistent with each other. Which data better represent actual conditions is unknown. A possible explanation is that the subjects in the studies that exhibit higher initial blood concentration data were not fully at rest or were at a higher workload than reported (stress). If data such as those of Astrand et al. (1972) are more representative of normal conditions, then the current PBPK model will overstate the AEGL values for short timeframes, especially the 10-min values. A previous model explored this issue for a series of anesthetic gases (Vinegar et al. 1998). For these chemicals, it was shown that a

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simple model of lung structure did not adequately describe data in the very early stages of uptake. However, the deviations for these chemicals were observed only in the period up to 2 min, and thereafter the simple model (similar to the one used here) performed adequately.

Sensitivity Analysis

A sensitivity analysis was conducted on the model with the parameter values listed in Tables A-2 and A-3. A sensitivity analysis is performed by assessing the relative impact of a small change in a parameter value on model output. The parameters that exhibit the most sensitivity are ones that should be given the most careful consideration. The sensitivity of a given parameter, however, varies with experimental settings, such as exposure concentration, exposure duration, and workload. Therefore, the sensitivity analysis was conducted on the human model at two exposures—one at rest and one at 50 W for an 8-h period. The output variable used for the analysis was venous blood concentration, as it is the DM used for the risk assessment.

The analysis results in a sensitivity coefficient (S) that is computed according to $S = 100 \times (CV1 - CV2)/CV1$, where CV1 is the venous blood concentration predicted by the base model (no parameter change) and CV2 is the venous blood concentration predicted when one parameter was increased by 1% of its normal value in the model. The absolute values of the changes are plotted in Figure A-14 *a* and *b*. Under both conditions, the model was sensitive to PB and the alveolar ventilation rate (QPC). These parameters were given careful consideration during model validation.

Overview of Validation Data

For the rat model validations, the model reasonably predicts blood data from three different labs at toluene exposures ranging from 50 to 4,000 ppm. The lower limit of this range is below the range of exposures considered in the AEGL extrapolations and the upper limit is close to the upper limit of the extrapolations. The gas uptake data are reasonably well predicted by the model. Therefore, the rat version of the model is deemed adequate for risk assessment extrapolation in or near these ranges.

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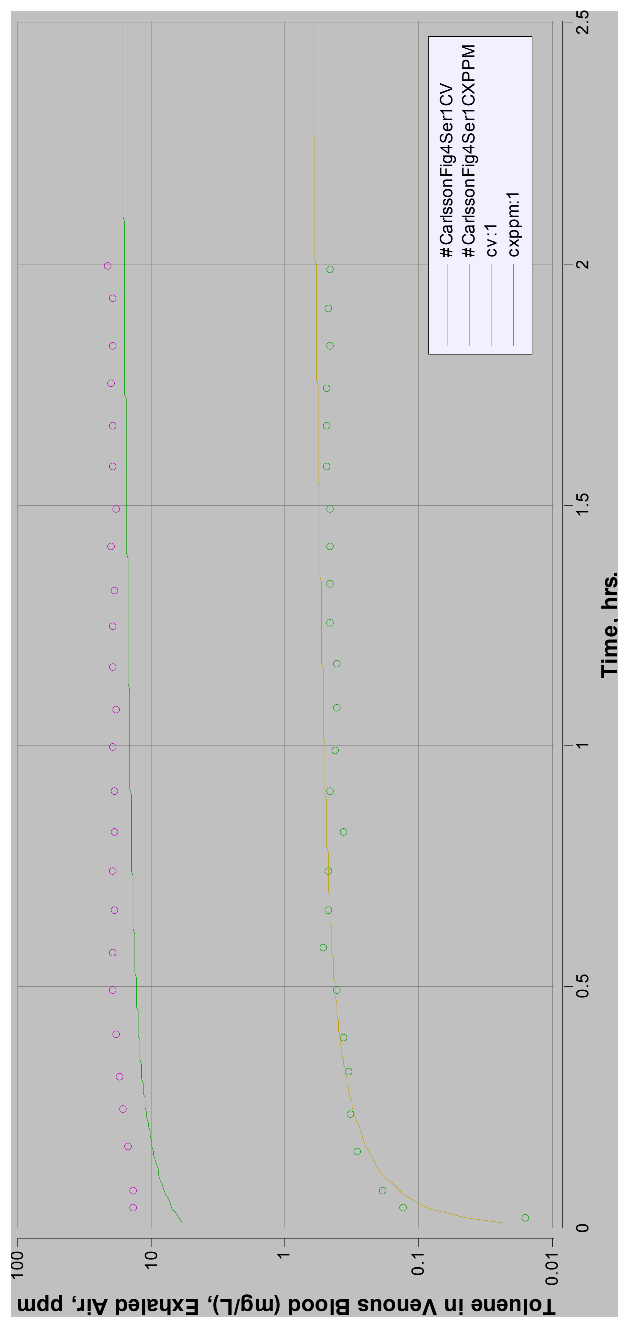

FIGURE A-9 PBPK model and data from Carlsson (1982, Figure 4, Series 1). Mean values for four subjects exposed to toluene at 81.2 ppm.

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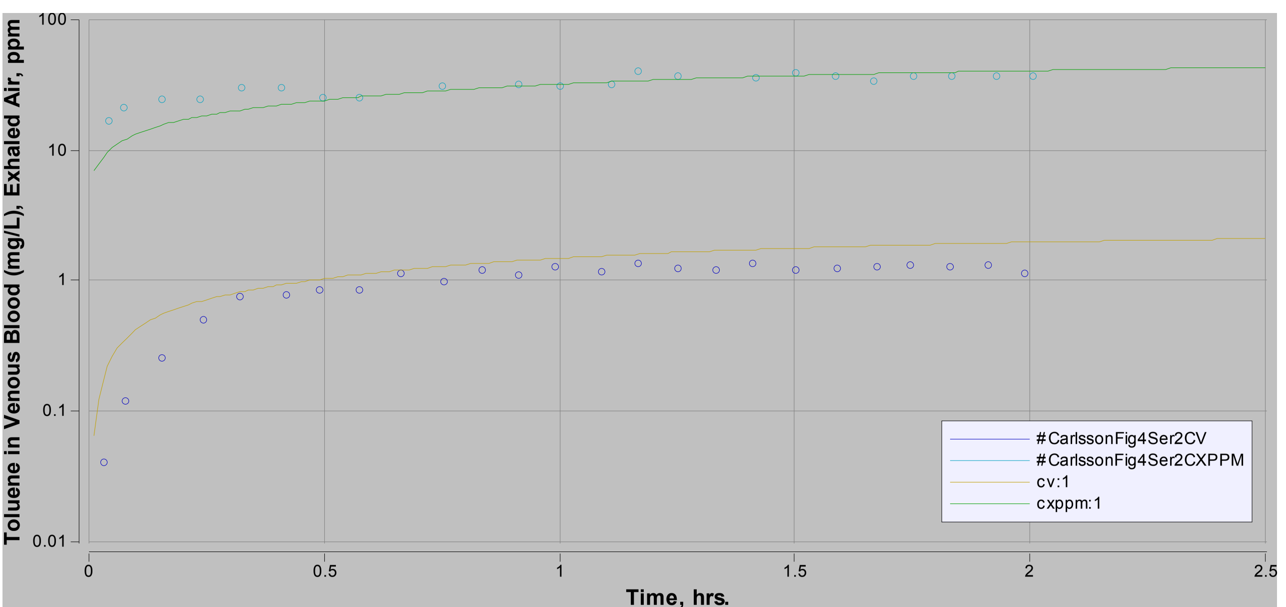

FIGURE A-10 PBPK model and data from Carlsson (1982) (Figure 4, Series 2). Mean values for three subjects at a workload of 50 W exposed to toluene at 81.2 ppm.

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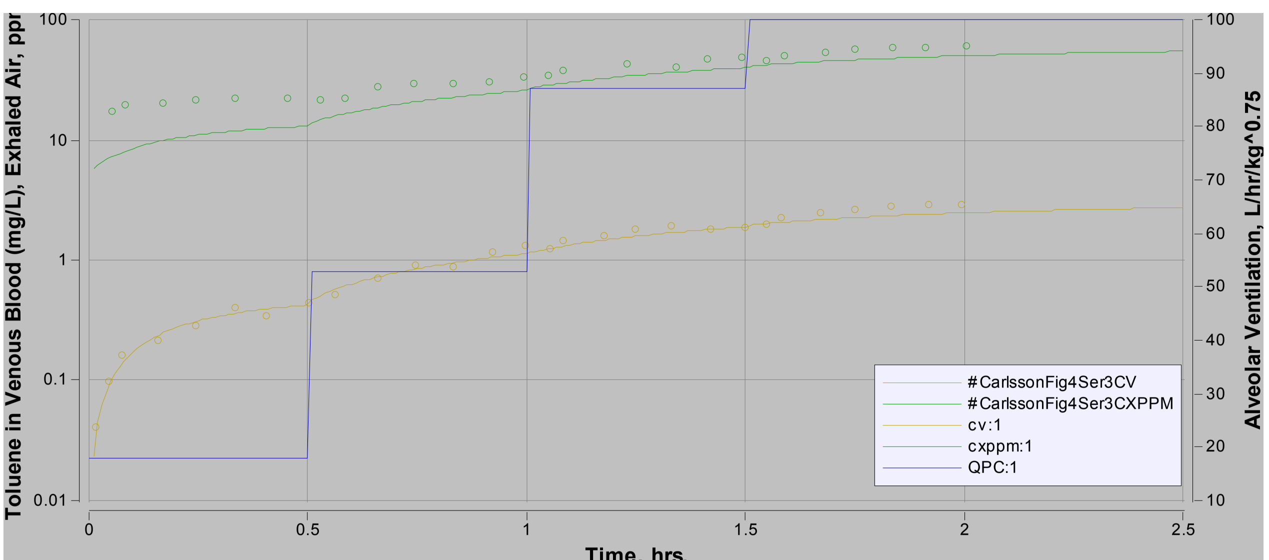

FIGURE A-11 PBPK model and data from Carlsson (1982) (Figure 4, Series 3). Mean values for several subjects shown after exposure to toluene at 81.2 ppm at rest and at 50, 100, and 150 W of workload for 30 min each. Upper curve is exhaled air and lower curve is CV.

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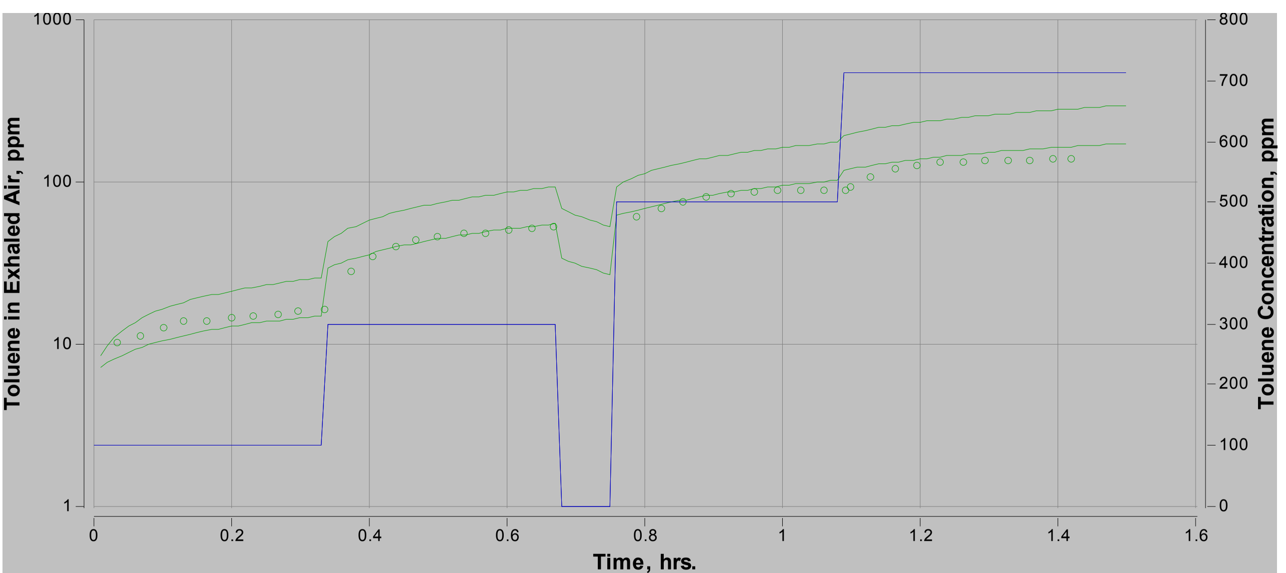

FIGURE A-12 PBPK model and data from Gamberale and Hultengren (1972, Figure 1). Mean values for toluene in exhaled air for 12 subjects exposed to different levels of toluene at rest. Toluene concentrations were approximately 100, 300, 0, 500, and 700 ppm as indicated on the right axis. Lower curve is simulation at rest and upper curve is simulation at 50 W.

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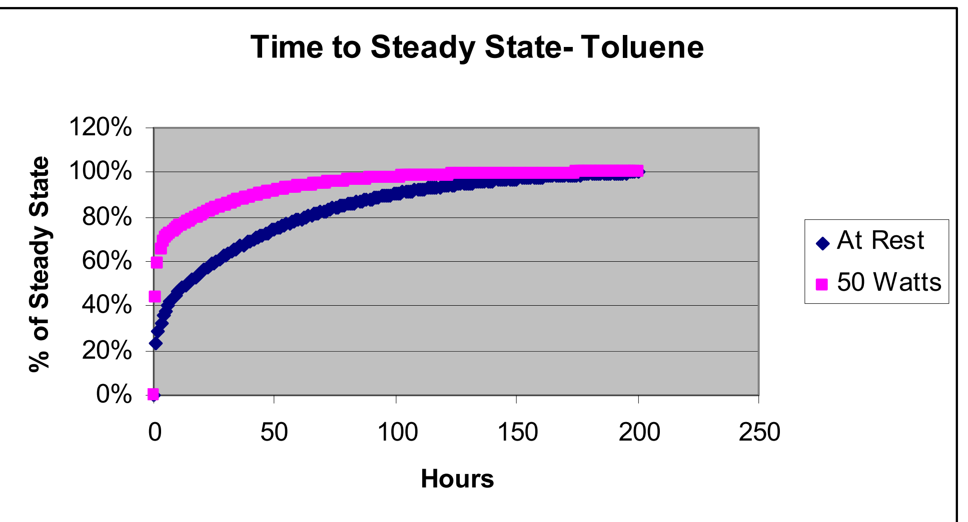

FIGURE A-13 Time to steady state as determined by the current PBPK model. Simulations were conducted for exposures to toluene at 200 ppm at rest and at 50 W of workload. At higher workloads, the approach to steady state is much faster. Results of the simulation for higher concentrations were identical.

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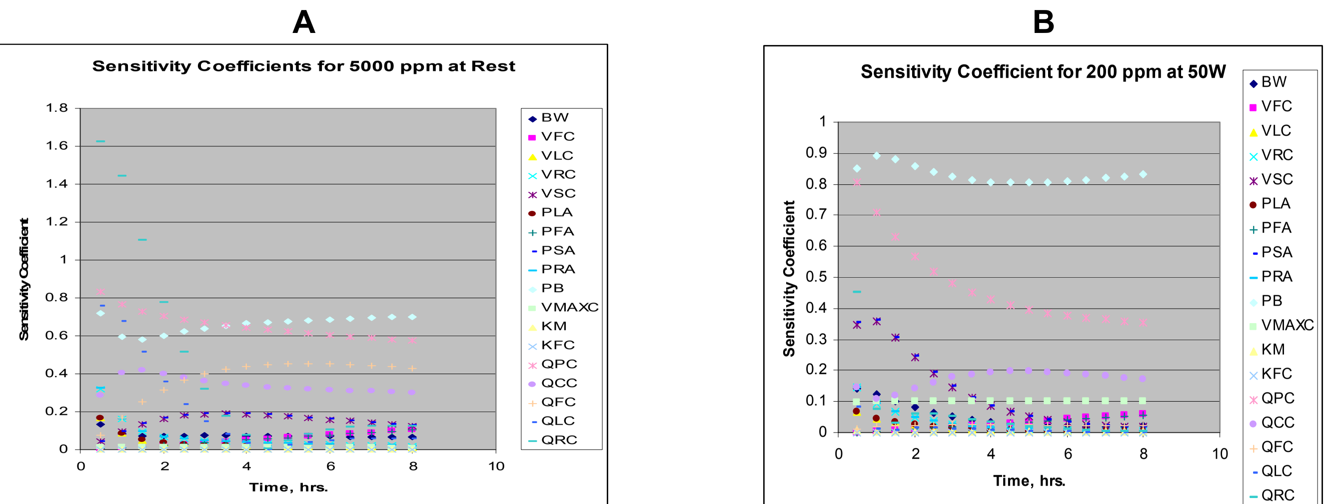

FIGURE A-14 *a* and *b*. Sensitivity coefficients for the toluene PBPK model. Sensitivity analysis at 5,000 ppm (rest) and 200 ppm (50 W) were calculated. At 50 W and 200 ppm, the model is primarily sensitive to PB and QPC. At rest and 5,000 ppm, the model is sensitive to these parameters and also moderately sensitive to QFC (percent of blood flow going to fat) and QCC (cardiac output). The model is also differentially sensitive to some other parameters in the period of initial uptake, especially to QRC (percent of blood flow going to rapidly perfused tissue) in *a*.

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The human version of the model also reasonably represents the data sets used for validation. These data sets include data from seven experiments from three labs and include both venous blood and exhaled air data. Where available, modeling of arterial blood concentrations (CA) provided a similar fit to arterial data, although not shown because of overlap on the figures. The range of toluene exposures was 80 to 700 ppm. The lower bound of this range is less than the lower bound of the AEGL extrapolations, although the upper bound of validation is lower than some of the AEGL values. The quality of the fit in many cases was very close, and the deviations did not tend to show an overall low or high bias, so the model could be viewed as evening out differences from lab to lab.

The model appears to perform well at resting and exercise levels. The sensitivity of the model to the exercise parameters is shown by the upper curve in Figure A-12. The large differences between the exhaled air concentrations at rest and work support the observation that the model deals well with exercise, as the differences are large compared with the errors between the model and the validation data sets. Furthermore, the numerous changes in exposure levels and workload in some of the data sets provide a comparatively demanding test of the model's validity. Therefore, the human version of the model is also deemed adequate for the AEGL risk assessment.

DERIVATION OF AEGLS WITH PBPK-BASED RISK APPROACH

For AEGL-1, no specific critical study was identified in the TSD. However, based on numerous studies (weight of evidence), a critical effect level of 200 ppm for 8 h was selected. This value is shown in parentheses in Table A-5. With the PBPK model, this value was time-scaled back to 10 min. Increasing the workload greatly increases the CV, thereby decreasing the exposure needed to produce the critical effect.

The AEGL-2 critical study was by Gamberale and Hultengren (1972), in which subjects were exposed to toluene vapor at 100, 300, 500, and 700 ppm for 20 min at each level and with a short break in the middle. The CV calculated by the PBPK model is much greater when the full exposure regimen was simulated, compared with the concentration after only a 20-min exposure (~6.5 versus 4.5 mg/L). The exposures were roughly equivalent to a 20-min exposure to ~1,000 ppm. Therefore, the CV determined for the actual experimental conditions was used to derive AEGL-2 values. Because of the prior exposure to lower levels of toluene, the 30-min AEGL (at rest) is actually more than 700 ppm. The 20-min value was time-scaled down to 10 min and up to 8 h and was extrapolated to higher workloads (Table A-6).

The AEGL-3 was based on a rat NOAEL for lethality. The CV of toluene during this experimental scenario (6,250 ppm for 2 h) was determined and served as the target concentration for setting the AEGLs. The human model was then used to determine the exposure levels in humans that yield the same con-

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centrations. This exposure level (top section of Table A-7) was then divided by the same intraspecies UF that was used in the TSD (3).

TABLE A-5 AEGL-1 Values Determined with PBPK Model, ppm

Workload	10 min	30 min	1 h	4 h	8 h
Rest	820	420	330	230	(200)
50 W	410	230	160	110	100
75 W	360	190	140	100	100
100 W	320	170	120	100	90

Notes: Based on a weight-of-evidence determination that 200 ppm for 8 h constitutes the AEGL-1 target concentration of 200 ppm (8 h) in human studies. Target CV is 3.27 mg/L.

TABLE A-6 AEGL-2 Values Determined with PBPK Model, ppm

Workload	10 min	30 min	1 h	4 h	8 h
Rest	1,580	780	590	410	350
50 W	810	430	300	200	190
75 W	700	370	260	190	180
100 W	630	330	240	180	170

Notes: Based on Gamberale and Hultengren (1972). CV was 6.54 mg/L after exposure of humans to toluene at100 to 700 ppm (stepped).

TABLE A-7 AEGL-3 Values Determined with PBPK Model, ppm

Workload	10 min	30 min	1 h	4 h	8 h
Rest	38,420	18,200	13,470	8,890	7,320
50 W	20,020	10,480	7,190	4,580	4,300
75 W	17,450	8,950	6,190	4,310	4,100
100 W	15,740	8,060	5,710	4,300	4,060
AEGL-3	AEGL recommendation after application of UF (3), ppm				
Workload	10 min	30 min	1 h	4 h	8 h
Rest	12,800	6,070	4,490	2,960	2,440
50 W	6,670	3,490	2,400	1,530	1,430
75 W	5,820	2,980	2,060	1,440	1,370
100 W	5,250	2,690	1,900	1,430	1,350

Notes: Based on Mullin and Krivanek (1982), NOAEL for lethality, 6,250 ppm (2 h). Target CV of toluene is 165 mg/L. Upper section of the table is the equivalent human exposure to produce this target concentration at various workloads. Bottom portion of the table is the AEGL recommendation after application of an intraspecies UF of 3. As the critical study lasted 2 h, the AEGLs were time-scaled up and down. While the PBPK-based AEGLs at rest were somewhat higher than those determined in the TSD, at 50 W the two sets of values were similar.

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Order of Application of Uncertainty Factors

The uncertainty factor for intraspecies (3) can be applied before or after the PBPK dosimetric adjustment is made. In a previous PBPK-based determination of AEGLs for xylene, the UF was applied after dosimetric adjustment (see the TSD for xylene). However, in a case example, the UF was applied first (Bruckner et al. 2004). The argument can be made that, as the dosimetric adjustment is being applied to "real data," in a biologically plausible manner (using a PBPK model), and the UF is more loosely determined, dividing the final values is more appropriate. However, for toluene, the order of application was inconsequential. To evaluate this, the AEGL-3 at 50 W was also determined by dividing the rat NOAEL by 3 to obtain a target CV, and the human model was then used to determine the equivalent human concentration for the five time points (Table A-8). The results were quite similar, although not identical. For example, the 1-h AEGL determined above was 2,397 ppm, while applying the UF first led to an AEGL of 2,360.

Comparison of PBPK-Based AEGL Values with ten Berge Approach

A useful comparison can be made between the AEGL values determined using the ten Berge approach (ten Berge et al. 1986) and the PBPK model. In Figure A-15, the CV of toluene was calculated using the PBPK model for three scenarios: the AEGL values determined with the PBPK model, the AEGL values recommended with the ten Berge time-scaling equation assuming the subject is at rest during the emergency event, and the AEGL values recommended with the ten Berge time-scaling equation assuming the subject is at work, 50 W, during the emergency event. As the AEGL values were reverse-calculated with the PBPK model, the PBPK model predicted a consistent CV of 6.54 mg/L. The CVs based on the ten Berge approach were highly variable. If the subject is at rest during an emergency event, the ten Berge values may be below or above the target dose. However, if the subject is exercising, the ten Berge values are consistently above the target dose; in other words, the ten Berge AEGL values were underprotective.

Advantages of the PBPK Model Approach

The PBPK model has several distinct advantages that make the modeling effort worthwhile. First, the AEGL values for different timeframes determined by using the PBPK model are all equally protective, assuming that peak CV is an appropriate DM for a given AEGL level toxicity end point. The ten Berge-derived AEGL values can vary in protectiveness because peak CV can vary by a factor of 2 to 3. Whether the calculated target tissue dose is appropriate depends primarily on appropriate selection of the critical data and not on the validity of the PBPK model used.

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TABLE A-8 AEGL-3 Based on Target CV of 53.8 (rat CV for NOAEL of 6,250 ppm divided by UF of 3), ppm

Workload	10 min	30 min	1 h	4 h	8 h
50 W	6,540	3,430	2,360	1,520	1,410

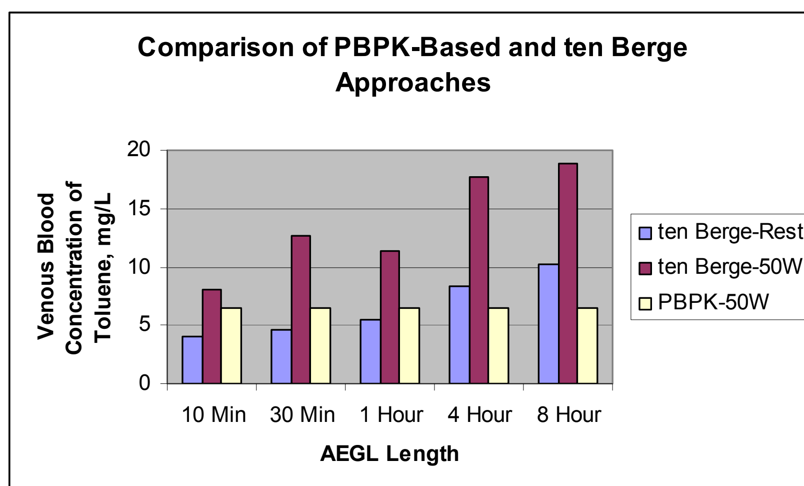

FIGURE A-15 Comparison of CVs of toluene resulting from exposure for each AEGL time period at the values resulting from the PBPK-based approach and the ten Berge equation. The CV for toluene was determined with the PBPK model for each AEGL-2 value determined by using the ten Berge equation (as listed in the TSD) or with the PBPK model. The PBPK model, for obvious reasons, predicts a constant CV of 6.54 mg/L. The CVs from the AEGLs derived using the ten Berge equation were determined both at rest and at a workload of 50 W. If the exposed subject is at rest, the CVs associated with the ten Berge-derived AEGLs are lower or higher than those derived by using the PBPK approach. At 50 W, the CVs associated with the ten Berge-derived AEGLs are consistently higher than those corresponding to the human NOAEL, by a factor of up to ~3.

Second, the existing ten Berge approach does not permit consideration of exertion, which has a pronounced impact on tissue dosimetry. Recent research has indicated that exercise is an important factor in determining internal dose related to toxicity for toluene and similar chemicals (Csanady and Filser 2001). Therefore, if exercise during emergency events is the probable physiologic mode for exposed persons, the ten Berge approach will not be adequately protective.

Third, the PBPK modeling approach is uniquely suited for use when a critical study had a complex exposure scenario, as in the case of the Gamberale and Hultengren (1972) model. The ten Berge approach used a DM of 700 ppm (the final experimental concentration). It was separately determined that the ex-

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perimental concentration was equivalent to an exposure to 1,000 ppm (for 20 min), but this could not be accounted for without the PBPK model.

Fourth, the PBPK-based approach allows an improvement in the basis for the animal-to-human extrapolation. While this advantage was not relevant at all levels, the AEGL-3 for toluene was based on a rat-to-human extrapolation of lethality data.

Some concern may exist over setting AEGLs at less than the existing permissible exposure limits (PEL) from OSHA (200 ppm). However, the current PEL for toluene was derived from toxicologic assessment performed in the mid-1940s. The Threshold Limit Value (TLV) was 200 ppm until adoption as a PEL in 1970 by OSHA, but the TLV was reduced to 100 ppm in the early 1970s and to 50 ppm in 1991-1992. Thus, the studies that are the basis for the AEGLs had not even been conducted when the current PEL was established, the organization that set the value that eventually became the PEL has since lowered the value two times, and the current PEL is effectively a 60-year-old standard.

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Abbreviations

AEGL	acute exposure guideline level
AT	amount of chemical in each tissue
AUC	area under the curve
BMD	benchmark dose
BW	body weight
CA	arterial blood concentration
C_{max}	maximum concentration
CNS	central nervous system
CT	chemical concentration in each tissue
CV	venous blood concentration
CVi	chemical concentration in the venous blood leaving tissue i
CVL	concentration of chemical in venous blood leaving the liver
CYP	cytochrome P-450
DM	dose metric
EPA	U.S. Environmental Protection Agency
h	hour
KFC	linear metabolism rate constant
Km	affinity constant for the chemical
LC_{50}	median lethal concentration
LOAEL	lowest-observed-adverse-effect level
mg/L	milligram per liter
min	minute
NAC	National Advisory Committee
NOAEL	no-observed-adverse-effect level
NRC	National Research Council
OSHA	Occupational Safety and Health Administration
PB	blood-air partition coefficient
PBPD	physiologically based pharmacodynamic
PBPK	physiologically based pharmacokinetic
PEL	permissible exposure limits
PFA	fat-air coefficient
PLA	liver-air coefficient

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POD	point of departure
ppm	parts per million
PRA	rapidly perfused air coefficient
PSA	slowly perfused air coefficient
PT	partition coefficient between the tissue and blood
QCC	cardiac output
QFC	percentage of blood flow going to fat
Qi	blood flow to tissue i
QLC	fraction of QCC to liver
QPC	alveolar ventilation rate
QRC	percentage of blood flow going to rapidly perfused tissues
QSC	percentage of blood flow going to slowly perfused tissues
S	sensitivity coefficient
TLV	Threshold Limit Value
T_{max}	time (of maximum concentration)
TSD	technical support document
UF	uncertainty factor
VBC	fraction lung blood
VFC	fraction fat tissue
VLC	fraction liver tissue
V_{max}	maximum rate of metabolism
$V_{max}C$	maximum velocity of metabolism
VRC	fraction rapidly perfused
VSC	fraction slowly perfused
VT	volume of each tissue
W	watt

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ATTACHMENT 1

PBPK MODEL EQUATIONS FOR TOLUENE AEGL MODEL[2]

This is a four-compartment model for toluene inhalation in the rat and human.

```
;Physiologic parameters
 BW = 70                        ;Body weight (kg)
 VFC = 0.19                     ;Fraction fat tissue (kg/(kg/BW))
 VLC = 0.026                    ;Fraction liver tissue (kg/(kg/BW))
 VRC= 0.05                      ;Fraction rapidly perfused (kg/(kg/BW))
 VSC = 0.62                     ;Fraction slowly perfused (kg/(kg/BW))
 SF =    .75                    ;Scaling coefficient
 QPC = 18                       ;Alveolar ventilation rate (L/h/kg)
 QCC = 18                       ;Cardiac output (L/h/kg)
 QFC = 0.09                     ;Fractional blood flow to fat ((L/h)/QC)
 QLC = 0.26                     ;Fractional blood flow to liver ((L/h)/QC)
 QRC= 0.55                      ;Fractional blood flow to rapidly perfused
                                ((L/h)/QC)

;Chemical-specific parameters
 PLA = 83.6                     ;Liver-air partition coefficient
 PFA = 1021                     ;Fat-air partition coefficient
 PSA = 27.7                     ;Slowly perfused air partition coefficient
 PRA = 83.6                     ;Rapidly perfused air partition coefficient
 PB =   18                      ;Blood-air partition coefficient
 PL = PLA/PB                    ;Liver-blood partition coefficient
 PF = PFA/PB                    ;Fat-blood partition coefficient
 PS = PSA/PB                    ;Slowly perfused blood partition coefficient
 PR = PRA/PB                    ;Rapidly perfused blood partition coefficient
 MW = 92.13                     ;Molecular weight (g/mol)
 VMAXC  = 3.44                  ;Maximum velocity of metabolism
                                (mg/h/kg)
 KM = 0.13                      ;Michaelis-Menten (mg/L)
 KFC = 0.05                     ;First-order rate constant

;Calculated parameters
 QC = QCC × BW^SF               ;Cardiac output
 QP = QPC × BW^SF               ;Alveolar vent
 VS = VSC × BW                  ;Volume slowly perfused tissue (L)
```

[2]PROGRAM: Toluene, last Revision 08-11-04; J Dennison.

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```
VF = VFC × BW                      ;Volume fat tissue (L)
VL = VLC × BW                      ;Volume liver (L)
VR= VRC × BW                       ;Volume rapidly perfused (L)
VB = 0.0005 × BW                   ;Lung blood volume (L)
QF = QFC × QC                      ;Blood flow to fat (L/h)
QL = QLC × QC                      ;Blood flow to liver (L/h)
QS = QC – QF – QL – QR             ;Blood flow to nonfat tissue (L/h)
QR = QRC × QC                      ;Blood flow to rapidly perfused (L/h)
VMAX  = VMAXC × BW^SF              ;Maximum rate of metabolism (mg/h)
KF = KFC/BW^0.3                    ;Linear metabolic rate

;Parameters for simulated experiment

CONC = 500                         ;Inhaled concentration (ppm)

;Parameters for exercise (50 W, 75 W, 100 W, 150 W)
QPC50 = 53
QCC50 = 50
QLC50 = 0.13
QFC50 = 0.031
QRC50 = 0.60

QPC75 = 70
QCC75 = 59
QLC75 = 0.10
QFC75 = 0.030
QRC75 = 0.28

QPC100 = 87
QCC100 = 68.5
QLC100 = 0.076
QFC100 = 0.034
QRC100 = 0.58

QPC150 = 100
QCC150 = 79
QLC150 = 0.042
QFC150 = 0.024
QRC150 = 0.58

;The following IF THEN statements implement the Carlsson Stage 3 exercise
        scenario (rest, 50 W, 100 W, 150 W)
;QPC = IF TIME >= 1.5 THEN QPC150 ELSE IF TIME >= 1.0 THEN QPC100
        ELSE IF TIME >= .5 THEN QPC50 ELSE 18
```

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```
;QCC = IF TIME >= 1.5 THEN QCC150 ELSE IF TIME >= 1.0 THEN
        QCC100 ELSE IF TIME >= 0.5 THEN QCC50 ELSE 18
;QLC = IF TIME >= 1.5 THEN QLC150 ELSE IF TIME >= 1.0 THEN
        QLC100 ELSE IF TIME >= 0.5 THEN QLC50 ELSE 0.26
;QFC = IF TIME >= 1.5 THEN QFC150 ELSE IF TIME >= 1.0 THEN QFC100
        ELSE IF TIME >= 0.5 THEN QFC50 ELSE 0.09
;QRC = IF TIME >= 1.5 THEN QRC150 ELSE IF TIME >= 1.0 THEN
        QRC100 ELSE IF TIME >= 0.5 THEN QRC50 ELSE 0.55

;The following IF THEN statements implement the Carlsson Stage 3 exercise
        scenario (rest, 50 W, 100 W, 150 W) with QPC and QCC from
        QCP2004 calculations
;QPC = IF TIME >= 1.5 THEN 129 ELSE IF TIME >= 1.0 THEN 88.4 ELSE
        IF TIME >= .5 THEN 45 ELSE 14.7
;QCC = IF TIME >= 1.5 THEN 46.6 ELSE IF TIME >= 1.0 THEN 37.1 ELSE
        IF TIME >= 0.5 THEN 26 ELSE 14.4
;QLC = IF TIME >= 1.5 THEN 0.05 ELSE IF TIME >= 1.0 THEN .076 ELSE
        IF TIME >= 0.5 THEN 0.13 ELSE 0.26
;QFC = IF TIME >= 1.5 THEN 0.03 ELSE IF TIME >= 1.0 THEN 0.03 ELSE
        IF TIME >= 0.5 THEN 0.03 ELSE 0.09
;QRC = IF TIME >= 1.5 THEN 0.58 ELSE IF TIME >= 1.0 THEN 0.58 ELSE
        IF TIME >= 0.5 THEN 0.60 ELSE 0.55

;The following IF THEN statements implement the Astrand et al. (1972) Figure
        3, Steps 1 and 2, exercise scenario (rest, 50 W)
;QPC = IF TIME >= 0.5 THEN QPC50 ELSE 18
;QCC = IF TIME >= 0.5 THEN QCC50 ELSE 18
;QLC = IF TIME >= 0.5 THEN QLC50 ELSE 0.26
;QFC = IF TIME >= 0.5 THEN QFC50 ELSE 0.09
;QRC = IF TIME >= 0.5 THEN QRC50 ELSE 0.55

;The following IF THEN statements implement the Astrand et al. (1972) Figure
        3, Steps 1 to 4, exercise scenario (rest, 50 W)
;QPC = IF TIME >= 1.08 THEN 18 ELSE IF TIME >= .55 THEN 53 ELSE 18
;QCC = IF TIME >= 1.08 THEN 18 ELSE IF TIME >= .55 THEN 50 ELSE 18
;QLC = IF TIME >= 1.08 THEN 0.26 ELSE IF TIME >= .55 THEN .13 ELSE
        0.26
;QFC = IF TIME >= 1.08 THEN 0.09 ELSE IF TIME >= .55 THEN 0.03 ELSE
        0.09
;QRC = IF TIME >= 1.08 THEN 0.55 ELSE IF TIME >= .55 THEN 0.6 ELSE
        0.55
;CONC = IF TIME >= 1.37 THEN 175 ELSE IF TIME >= 1.08 THEN 0 ELSE
        95
```

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```
;The following IF THEN statements implement the Astrand et al. (1972) Figure
        3, Steps 1 to 6, exercise scenario (rest, 50 W)
;QPC = IF TIME >= 2.4 THEN 18 ELSE IF TIME >= 1.9 THEN QPC50 ELSE
        IF TIME>= 1.08 THEN 18 ELSE IF TIME >= .55 THEN QPC50
        ELSE 18
;QCC = IF TIME >= 2.4 THEN 18 ELSE IF TIME >= 1.9 THEN QCC50 ELSE
        IF TIME >= 1.08 THEN 18 ELSE IF TIME >= .55 THEN QCC50
        ELSE 18
;QLC = IF TIME >= 2.4 THEN .26 ELSE IF TIME >= 1.9 THEN QLC50 ELSE
        IF TIME >= 1.08 THEN 0.26 ELSE IF TIME >= .55 THEN QLC50
        ELSE 0.26
;QFC = IF TIME >= 2.4 THEN .09 ELSE IF TIME >= 1.9 THEN QFC50 ELSE
        IF TIME >= 1.08 THEN 0.09 ELSE IF TIME >= .55 THEN QFC50
        ELSE 0.09
;QRC = IF TIME >= 2.4 THEN .55 ELSE IF TIME >= 1.9 THEN QRC50
        ELSE IF TIME >= 1.08 THEN 0.55 ELSE IF TIME >= .55 THEN
        QRC50 ELSE 0.55
;CONC = IF TIME >= 2.4 THEN 0 ELSE IF TIME >= 1.37 THEN 175 ELSE
        IF TIME >= 1.08 THEN 0 ELSE 95

;The following IF THEN statements implement the Astrand et al. (1972) Figure
        4 exercise scenario (75 W, 150 W, rest)
;QPC = IF TIME >= 1.17 THEN 18 ELSE IF TIME >= .5 THEN QPC150
        ELSE QPC75
;QCC = IF TIME >= 1.17 THEN 18 ELSE IF TIME >= .5 THEN QCC150
        ELSE QCC75
;QLC = IF TIME >= 1.17 THEN 0.26 ELSE IF TIME >= .5 THEN QLC150
        ELSE QLC75
;QFC = IF TIME >= 1.17 THEN 0.09 ELSE IF TIME >= .5 THEN QFC150
        ELSE QFC75
;QRC = IF TIME >= 1.17 THEN 0.55 ELSE IF TIME >= .5 THEN QRC150
        ELSE QRC75
;CONC = IF TIME >= 1 THEN 0 ELSE 105

;The following IF THEN statements implement the Astrand et al. (1972) Figure
        9 exercise scenario
;QPC = IF TIME >= 1.4 THEN 18 ELSE IF TIME >= 0.92 THEN 53 ELSE IF
        TIME >= .5 THEN 18 ELSE 18
;QCC = IF TIME >= 1.4 THEN 18 ELSE IF TIME >= 0.92 THEN 50 ELSE IF
        TIME >= 0.5 THEN 18 ELSE 18
;QLC = IF TIME >= 1.4 THEN 0.26 ELSE IF TIME >= 0.92 THEN .13 ELSE
        IF TIME >= 0.5 THEN 0.26 ELSE 0.26
;QFC = IF TIME >= 1.4 THEN 0.09 ELSE IF TIME >= 0.92 THEN 0.03 ELSE
        IF TIME >= 0.5 THEN 0.09 ELSE 0.09
```

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```
;QRC = IF TIME >= 1.4 THEN 0.55 ELSE IF TIME >= 0.92 THEN 0.6 ELSE
        IF TIME >= 0.5 THEN 0.55 ELSE 0.55
;CONC = IF TIME >= 1.4 THEN 0 ELSE IF TIME >= .92 THEN 200 ELSE IF
        TIME >= .5 THEN 0 ELSE 200

;The following IF THEN statements implement the Gamberale and Hultengren
        (1972) experiment (Figure 1)
;CONC = IF TIME >= 1.08 THEN 714 ELSE IF TIME >= .75 THEN 501
        ELSE IF TIME >= .67 THEN 0 ELSE IF TIME >= .33 THEN 300
        ELSE 100


CIX = CONC × MW/24,450          ;Exposure concentration (mg/L)


LENGTH = 4                      ;Length of inhalation exposure (h)
INTERVAL = 8
CI = CIX × (mod(time,interval)<=length)

method RK4                      ;Rosenbrock stiff solver
starttime = 0                   ;start integration
stoptime = 8                    ;end integration
dtmin = 0.0001                  ;minimum (and initial) step size
dtmax = 1                       ;maximum step size
tolerance = 0.0001              ;error tolerance for stiff solver
dtout = 0.1                     ;communication interval (optional)
deltaT = stepsize               ;allows plotting step sizes used as deltaT
                                  (optional)

display cv, ca, vlc, vrc, vfc, vsc, qfc, qlc, qrc, sf, dose, mass, massbal, pfa, pla,
        psa, pra
display length, bw, qpc, qcc, pb, vfc, km, vmaxc, interval, kfc
display cl, cr, cxppm, ci, conc, af, as, ar, al

;INTEGRATIONS

;Chemical in blood
AB' = QP × (CI – CX) + QC × (CV– CA)
INIT AB = 0
CA = AB/VB
CV = (QF × CVF + QR × CVR + QL × CVL + QS × CVS)/QC
                                        ;Mixed venous (mg/L)

;Exhaled chemical
```

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 CX = CA/PB                              ;Alveolar (mg/L)
 CXPPM = CX × 24,450/MW

;Chemical in slowly perfused compartment
 AS' = QS × (CA – CVS)                   ;(mg/h)
 init AS = 0. ;(mg)
 CS = AS/VS ;(mg/L)
 CVS = CS/PS                             ;Venous blood (mg/L)

Chemical in fat compartment
 AF' = QF × (CA – CVF)                    ;(mg/h)
 init AF = 0                             ;(mg)
 CF = AF/VF                              ;(mg/L)
 CVF = CF/PF                             ;Venous blood (mg/L)

;Chemical in rapidly perfused compartment
 AR' = QR × (CA – CVR)                   ;(mg/h)
 init AR = 0                              ;(mg)
 CR = AR/VR                              ;(mg/L)
 CVR = CR/PR                             ;Venous blood (mg/L)

;Chemical in liver compartment
 AL' = QL × (CA – CVL) – AM'             ;(mg/h)
 init AL = 0                             ;(mg)
 CL = AL/VL                              ;(mg/L)
 CVL = CL/PL                             ;Venous blood (mg/L)

;Metabolism
 AMS' = VMAX × CVL/(KM + CVL)            ;Saturable metabolism (mg/h)
 init AMS = 0
 AML' = KF × CVL                         ;Linear metabolism (mg/h)
 init AML = 0
 AM' = AMS' + AML'                       ;Total metabolism
 init AM = 0

;Mass balance
 DOSE' = QP × (CI – CX)                  ;Net absorption (mg/h)
 init DOSE = 0                           ;Net absorption (mg)
 MASS = AF + AS + AL + AM + AR + AB ;In tissues + metabolized (mg)
 MASSBAL = DOSE – MASS + 1
```

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