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Acute Exposure Guideline Levels for Selected Airborne Chemicals

VOLUME 12

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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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 more than 270 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 twelfth volume in

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

that series. AEGL documents for butane, chloroacetaldehyde, chlorobenzene, chloroform, methyl bromide, methyl chloride, and propane are each published as an appendix in this report. 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.

The committee's review of the AEGL documents involved both oral and written presentations to the committee by the 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 five 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 five committee interim reports, which summarize the committee's conclusions and recommendations for improving NAC's AEGL documents for butane (interim reports 17 and 20a), chloroacetaldehyde (interim report 17), chlorobenzene (interim report 17), chloroform (interim reports 13, 14, and 18), methyl bromide (interim reports 18 and 20a), methyl chloride (interm reports 18 and 10a), and propane (interim reports 17 and 20a): Deepak Bhalla (Wayne State University), Harvey Clewell (The Hamner Institutes for Health Sciences), Jeffrey Fisher (U.S. Food and Drug Administration), David Gaylor (Gaylor and Associates, LLC), A. Wallace Hayes (Harvard School of Public Health), Sam Kacew (University of Ottawa), Kenneth Still (Occupational Toxicology Associates), Joyce Tsuji (Exponent, Inc.), and Judith Zelikoff (New York University).

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 interim report 13 was overseen by Sidney Green, Jr. (Howard University), and interim reports 14, 17, 18, and 20a were overseen by Robert Goyer (University of Western Ontario [retired]). 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 pro-

cedures 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 and Iris A. Camacho (both from EPA) and George Rusch (Risk Assessment and Toxicology Services). The committee also acknowledges Susan Martel, the project director for her work this project. Other staff members who contributed to this effort are James J. Reisa (director of the Board on Environmental Studies and Toxicology), Radiah Rose (manager, editorial projects), Mirsada Karalic-Loncarevic (manager of the Technical Information Center), 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

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

This report is the twelfth 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

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:

[1]NAC completed its chemical reviews in October 2011. The committee was composed of members from EPA, DOD, many other federal and state agencies, industry, academia, and other organizations. From 1996 to 2011, the NAC discussed over 300 chemicals and developed AEGLs values for at least 272 of the 329 chemicals on the AEGLs priority chemicals lists. Although the work of the NAC has ended, the NAC-reviewed technical support documents are being submitted to the NRC for independent review and finalization.

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 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 extrapola-

tion 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 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 were initially prepared by ad hoc AEGL development teams consisting of a chemical manager, chemical reviewers, and a staff scientist of the NAC contractors—Oak Ridge National Laboratory and subsequently Syracuse Research Corporation. The draft documents were then reviewed by NAC and elevated from "draft" to "proposed" status. After the AEGL documents were approved by NAC, they were published in the *Federal Register* for public comment. The reports were 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 recommenda-

tions 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 committee 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 AEGL reports. Thus far, the committee has prepared eleven reports in the series *Acute Exposure Guideline Levels for Selected Airborne Chemicals* (NRC 2001b, 2002b, 2003, 2004, 2007b, 2008b, 2009, 2010a,b, 2011, 2012). This report is the twelfth volume in that series. AEGL documents for butane, chloroacetaldehyde, chlorobenzene, chloroform, methyl bromide, methyl chloride, and propane are each published as an appendix in this report. 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

1

Butane[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 are defined as follows:

AEGL-1 is the airborne concentration (expressed as parts per million or milligrams per cubic meter [ppm or 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 Peter Bos (RIVM, The Dutch National Institute of Public Health and the Environment), Julie M. Klotzbach (Syracuse Research Corporation), Chemical Managers Jonathan Borak and Larry Gephart (National Advisory Committee [NAC] on Acute Exposure Guideline Levels for Hazardous Substances), and Ernest V. Falke (U.S. Environmental Protection Agency). 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 guidelines reports (NRC 1993, 2001).

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 concentrations 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 AEGL values represent threshold concentrations 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

Butane is a colorless gas with a faint disagreeable odor, although it is considered to be odorless by some. It is poorly soluble in water. The lower explosive limit is 1.9%. Butane is produced from natural gas. Its main uses are in the production of chemicals like ethylene and 1,3-butadiene, as a refrigerant, as an aerosol propellant, as a constituent in liquefied petroleum gas, and as the main component of gas lighter refills. Because it is easily accessible, butane is often used in inhalant abuse.

The toxicity of butane is low. Huge exposure concentrations can be assumed in butane abuse. The predominant effects observed in abuse cases are central nervous system (CNS) and cardiac effects. Case studies also reveal that serious brain damage and underdeveloped organs can occur in fetuses in case of high single exposures during the week 27 or 30 of pregnancy. Quantitative data for setting AEGL values are sparse. Quantitative human data include an old study with human volunteers focused on the warning properties of butane.

Mortality from butane in mice and rats is preceded by CNS effects. Some data are available on cardiac effects in dogs, but they are insufficient for setting AEGL values. Data on CNS effects are available for mice and guinea pigs. Butane was negative in the bacterial reverse-mutation assay (Ames test). Carcinogenicity studies and studies on reproductive toxicity are lacking.

The AEGL-1 values for butane are based on observations in a study with volunteers on the warning properties of short exposures to butane (Patty and Yant 1929). It was concluded that 10,000 ppm (10-min exposure) was a boundary for drowsiness. An intraspecies uncertainty factor of 1 is considered adequate because the concentration-response curve for CNS-effects appears to be very steep; thus, interindividual variability will be relatively small. Also, no noticeable irritation was reported at concentrations up to 100,000 ppm (probably for a few min), and a larger uncertainty factor of 3 would lead to unrealistically low AEGL-1 values. Available data suggest a relatively high value for n (Stoughton and Lamson 1936), so time extrapolation was performed using n = 3. Data on butane (Gill et al. 1991) and propane (Stewart et al. 1977) indicate that steady-state plasma concentrations for butane will be reached within 30 min. By analogy to other CNS-depressing substances, the effects of butane are assumed to be solely concentration dependent. Therefore, time extrapolation was performed from 10 min to 30- and 60-min exposures, where the steady-state concentration was calculated. The calculated values for AEGL-1 are presented in Table 1-1. The values are considered protective of the irregular breathing observed in guinea pigs exposed to butane at 21,000-28,000 ppm for up to 2 h (Nuckolls 1933). The calculated 10-min AEGL-1 value is greater than 50% of the lower explosive limit for butane, and the other AEGL-1 values are greater than than 10% of the lower explosive limit.

The AEGL-2 values for butane are based on a study with guinea pigs exposed to butane for 2 h at concentrations between 50,000 and 56,000 ppm (Nuckolls 1933). Animals had a "dazed appearance," but were able to walk. Therefore, the effects were considered not to be serious enough to impair escape and the lower value in this range (50,000 ppm) was used as starting point for the derivation of AEGL-2 values. Small interindividual differences are expected because the effects are attributed to butane itself and no relevant differences in kinetics are assumed. However, a large uncertainty factor is not necessary considering the steep concentration-response curve; a large factor also would lead to unrealistically low AEGL-2 values that would be similar to the AEGL-1 values. Thus, a total uncertainty factor of 3 is considered sufficient. Time extrapolation was performed using n = 3 for similar reasons as for AEGL-1. No increase in effect from longer durations of exposure is expected for concentration-dependent effects after reaching a steady state. For the same reasons as for AEGL-1, steady-state plasma concentrations will be reached within 30 min of exposure. Thus, the AEGL-2 values for 30-min and 1, 4, and 8 h will be set equal to the 2-h concentration. The AEGL-2 values for the 10-min exposure is derived by time scaling according to the dose-response regression equation $C^n \times t = k$, using n = 3. The calculated 10-min AEGL-2 value is greater than the lower explosive limit and that the other AEGL-2 values are greater than 50% of the lower explosive limit.

The AEGL-3 values for butane are based on an acute exposure study with rats and mice (Shugaev 1969). Mice and rats were exposed to butane for 2 and 4 h, respectively. The reported data allowed the calculation of LC_{01}s (lethal con-

centrations, 1% lethality). The 2-h LC_{01} for mice was 160,000 ppm and the 4-h LC_{01} for rats was 172,000 ppm. The 2-h LC_{01} for mice was chosen as starting point for AEGL-3 derivation, because mice appear to be the more susceptible species and 160,000 ppm was the lowest concentration tested. A total uncertainty factor of 3 is considered sufficient to account for toxicokinetic and toxicodynamic differences between individuals and interspecies differences for the following reasons. The effects are attributed to butane itself and no relevant differences in kinetics are assumed. The data are from a species with a relatively high susceptibility to butane. The concentration-response curve appears to be very steep indicating that a large uncertainty factor is unnecessary. Further, a larger factor would lead to unrealistically low values that would be similar to the AEGL-2 values. Time scaling was conducted similar to that performed for AEGL-2 values. The AEGL-3 values for 30 min and for 1, 4 and 8 h of exposure were set equal to that for the 2-h AEGL value. The AEGL-3 values for the 10-min exposure were derived by time scaling according to the dose-response regression equation $C^n \times t = k$, using n = 3. The calculated 10-min value of 77,000 ppm is supported by the data from Patty and Yant (1929). They reported that exposure to slowly increasing concentrations of butane up to 50,000 ppm (total exposure duration at least 10 min) and a short exposure (duration not specified) to 100,000 ppm on the same day did not result in serious complaints (Patty and Yant 1929). All of the AEGL-3 values are greater than the lower explosive limit for butane.

The AEGL values for butane are presented in Table 1-1.

TABLE 1-1 Summary of AEGL Values for Butane

Classification	10 min	30 min	1 h	4 h	8 h	End Point (Reference)
AEGL-1 (nondisabling)	See below[a]	6,900 ppm[b] (16,000 mg/m^3)	5,500 ppm[b] (13,000 mg/m^3)	5,500 ppm[b] (13,000 mg/m^3)	5,500 ppm[b] (13,000 mg/m^3)	Drowsiness in humans (Patty and Yant 1929)
AEGL-2 (disabling)	See below[c]	See below[d]	See below[d]	See below[d]	See below[d]	Dazed appearance in guinea pigs (Nuckolls 1933)
AEGL-3 (lethal)	See below[e]	See below[e]	See below[e]	See below[e]	See below[e]	LC_{01} in mice (Shugaev 1969)

[a]The 10-min AEGL-1 value is 10,000 ppm (24,000 mg/m^3), which is greater than 50% of the lower explosive limit for butane in air of 19,000 ppm. Therefore, extreme safety considerations against the hazard of explosion must be taken into account.

[b]The AEGL-1 value is greater than 10% of the lower explosive limit for butane in air of 19,000 ppm. Therefore, safety considerations against the hazard of explosion must be taken into account.

[c]The 10-min AEGL-2 value is 24,000 ppm (57,000 mg/m^3), which is greater than the lower explosive limit for butane in air of 19,000 ppm. Therefore, extreme safety considerations against the hazard of explosion must be taken into account.

[d]The AEGL-2 values for 30 min and 1, 4, and 8 h are 17,000 ppm (40,000 mg/m^3), which is greater than 50% of the lower explosive limit for butane in air of 19,000 ppm. There-

fore, extreme safety considerations against the hazard of explosion must be taken into account.

[e]The 10-min AEGL-3 value is 77,000 ppm (180,000 mg/m^3). The AEGL-3 values for 30 min and 1, 4, and 8 h are 53,000 ppm (130,000 mg/m^3). These values are greater than the lower explosive limit for butane in air of 19,000 ppm). Therefore, extreme safety considerations against the hazard of explosion must be taken into account.

1. INTRODUCTION

Butane is produced from raw natural gas and from petroleum streams obtained by catalytic cracking, catalytic reforming, and other refining processes. Butane is used in the production of ethylene and 1,3-butadiene, in the synthesis of a number of chemicals, as a refrigerant and an aerosol propellant, in the blending of gasoline or motor fuel, as a constituent in liquefied petroleum gas, and as an extraction solvent in deasphalting processes (Low et al. 1987). Butane used in gas lighter refills consists of butane with small amounts of isobutane and propane.

Chemical and physical data for butane are presented in Table 1-2.

2. HUMAN TOXICITY DATA

Most reports of butane intoxication are from cases of butane abuse or suicide attempts. These data are only briefly described because they provide no clear dose-response data and, for abuse cases, subjects generally have a history of repeated exposure, so tolerance to butane could have developed (Evans and Raistrick 1987). In addition, abuse of other volatile organic solvents cannot be excluded. Data on intoxication by liquefied petroleum gas (a mixture of predominantly propane and butane in varying proportions) were not considered.

2.1. Acute Lethality

2.1.1. Case Reports

Substance abuse is one of the predominant causes of death from butane intoxication. Fuel gases containing butane appeared to be responsible for about 30% of deaths from solvent abuse in the United Kingdom and aerosol propellants for about 20% (Adgey et al. 1995). In 2000, 64 deaths were associated with abuse of volatile substances; over 50% of the deaths were attributed to gas fuel inhalation, mainly butane lighter refills (Chaudhry 2002). In Virginia, 39 cases of people who likely died as a direct consequence abusing an inhalant were found between 1987 and 1996. Thirteen of these cases were associated with butane (Bowen et al. 1999). Clear central nervous system (CNS) effects were reported by butane abusers, including disturbed behavior, slow speech, elated mood, hallucinations, and illusionary experiences.

TABLE 1-2 Chemical and Physical Properties for Butane

Parameter	Value	Reference
Synonyms	Diethyl; methylethylmethane	Lewis 1999
CAS registry no.	106-97-8	
Chemical formula	C_4H_{10}	
Molecular weight	58.14	Lewis 1999
Physical state	Gas	Lewis 1999
Color	Colorless	Lewis 1999
Odor	Odorless Faint disagreeable odor[a]	Lewis 1999
Melting point	-138.35°C	Cavender 1994
Boiling point	-0.5°C	Cavender 1994
Density Vapor Liquid	 2.07 (air = 1) 0.601 g/cm^3 at -0.5°C (water = 1) 0.573 g/cm^3 at -25°C (water = 1)	Low et al. 1987; Lide 1999
Solubility	61 mg/L in water at 25°C	Low et al. 1987
Vapor pressure	243 kPa (25°C)	ECB 2000
Flammability	Extremely flammable	ECB 2000
Explosive	Lower explosive limit = 1.9%	Lewis 1999
Conversion factors	1 mg/m^3 = 0.422 ppm 1 ppm = 2.37 mg/m^3	Low et al. 1987

[a]Although butane is considered odorless by some, it has been reported that the odor of butane can be detected at concentrations of 1.2-6.2 ppm (2.85-14.63 mg/m^3) (Ruth 1986).

Graefe et al. (1999) described a fatal case of a 19-year-old male. He had a history of butane abuse. Froth was present in the trachea and bronchi; pulmonary edema was also reported. The highest concentrations of butane were found in the liver (310 µg/g), brain (282 µg/g), blood (129 µg/mL), and kidneys (54 µg/g).

A 14-year-old boy was found unconsciousness as a result of butane abuse; he died 34 h after the exposure despite resuscitation efforts (Rieder-Scharinger et al. 2000). Death was attributed to multiple organ failure involving the CNS (brain edema), cardiovascular system, pulmonary system, and the liver.

A 13-year-old boy died from butane abuse (Nishi et al. 1985). The cause of death was cardiac arrhythmia and lung edema. The boy had undergone an operation for a cardiac ventricular septal defect at the age of 10. Butane concentrations in his tissues were highest in fat (4.5 µL/g) and brain (3.9 µL/g), followed by kidney (2.1 µL/g), liver (2.0 µL/g), spleen (1.5 µL/g), heart (1.2 µL/g), and blood (0.9 µL/g). Propane and isobutane were also detected.

2.2. Nonlethal Toxicity

2.2.1. Case Reports

In nonfatal cases, butane appears to have frequently affected the heart and brain. Most of these cases involved inhalant abusers with repetitive exposure to butane.

Severe encephalopathy was observed in a 15-year-old girl as the result of abusive butane inhalation. She had been inhaling butane repeatedly for 4 weeks until an acute incident occurred. Cardiopulmonary resuscitation was needed. Repeated magnetic-resonance-imaging scans revealed disintegration of gray matter, increasing cerebral atrophy, and destruction of basal ganglia. Electroencephalography showed strongly diminished basal activity with flat amplitude (Döring et al. 2002). A 15-year-old boy, who was known to inhale butane from a plastic bag, had bilateral hemispheric infarcts (Bauman et al. 1991). Another 15-year-old boy suffered from right-sided hemiparesis after butane abuse; he did not lose consciousness. A computed tomography (CT) head scan on the day following admission to the hospital was normal. At the time of discharge (after 5 days), he still had pronounced upper limb, proximal muscle weakness and a hemiplegic gait (Gray and Lazarus 1993). In another case of butane abuse, a swollen brain was observed in a 15-year-old girl without a history of butane abuse (Williams and Cole 1998), while a CT head scan showed no abnormalities in a 17-year-old male with a 3-year history of butane abuse (Edwards and Wenstone 2000).

Ventricular tachycardia and ventricular fibrillation were noted in a 15-year-old boy, who was found unresponsive and cyanotic. He was known to inhale butane from a plastic bag. During his hospitalization his cardiac status improved but brain functions remained disturbed (Bauman et al. 1991). A 17-year-old male with a 3-year history of butane abuse was found collapsed and showing ventricular fibrillation. He was resuscitated during which he received epinephrine. An electrocardiogram showed an acute anterolateral infarction. Recovery was slow and complicated by acute renal failure and recurrent pulmonary edema (Edwards and Wenstone 2000). Roberts et al. (1990) described a 16-year-old boy who was found unconscious. He had been abusing lighter fuel for two months. The boy suffered from asystolic cardiac arrest and cardiopulmonary resuscitation was commenced. The patient was discharged 10 days after admittance to the hospital. Gunn et al. (1989) described ventricular fibrillation in a 15-year-old boy with a habit of lighter-fuel abuse. A few moments after one such episode of abuse he experienced severe anterior chest pain. He ran downstairs where he collapsed. An ambulance arrived within 5 min. The boy suffered from sinus tachycardia and a widespread ST-segment elevation was noted. A 15-year-old girl, without history of butane abuse, had been inhaling butane intermittently over a period of 2 h. She collapsed when running away from the police (Williams and Cole 1998). On admission to the hospital, there was no spontaneous respiration; an electrocardiogram showed sinus tachycardia with marked T-wave

inversion in the anterolateral leads. A CT scan showed a very tight swollen brain. For 5 days she remained cardiovascularly stable with persistent T-wave inversion on the electrocardiogram. It was concluded that the most plausible cause was a direct effect of butane on the myocardium. Butane could have caused cardiac sensitization, and a surge of adrenaline would have caused the arrhythmia rather than hypoxic arrest. Adgey et al. (1995) describe a case of a 16-year-old boy who collapsed following inhalation of butane from a cigarette lighter refill. The initial cardiac rhythm was ventricular asystole. Cardiopulmonary resuscitation was commenced. The electrocardiogram showed T-wave inversion across the anterior chest leads.

Cartwright et al. (1983) reported pleural effusions and pulmonary infiltrates in a 19-year-old man who had been "fire-breathing." He had filled his oral cavity with butane from a cigarette lighter and forcefully expelled it over a flame. Because butane is heavier than air, the pulmonary effects were considered to be the result of descending butane gas into the tracheobronchial tree by gravity.

2.2.2. Experimental Studies

Patty and Yant (1929) studied the warning properties of several alkanes, including butane. In a continuous exposure test, subjects were exposed to butane at slowly increasing concentrations up to 50,000 ppm for an unknown duration (but at least 10 min). In an intermittent exposure test, subjects were exposed at fixed concentration for a short, unspecified duration. The concentrations of butane in the intermittent exposure test were approximately 1,000, 2,000, 5,000, 7,000, 10,000, 20,000, and 100,000 ppm. Exposure groups consisted of 3-6 laboratory or clerical personnel (males and females, 20-30 years of age). Subjects first underwent the continuous exposure test, followed on the same day by the intermittent exposure test after a recovery period. The chamber concentration was periodically analyzed. Odor detection was rated by means of an odor scale ranging from 0 (no detectable odor) to 5 (intense effect, may bite or irritate). Individual scores did not differ much from the average scores. Butane could not be detected in the continuous exposure test at concentrations up to 50,000 ppm. In the intermittent exposure test, butane at 18,000 ppm was described as having a "weak odor, readily perceptible" (mean score of 2). The score for odor detection was below 4 (cogent, forcible odor) at 100,000 ppm. The physiologic responses were very briefly reported. Although a table in the report indicated that exposure to butane at 10,000 ppm for 10 min caused drowsiness, this was contradicted by a statement in the text that 10-min exposure to butane 10,000 ppm caused no symptoms.

2.2.3. Occupational and Epidemiological Studies

No data were available.

2.3. Neurotoxicity

Several case reports of intentional butane exposure indicate that butane induces neurotoxicity. Severe encephalopathy was observed in a 15-year-old girl as the result of butane abuse. She had been inhaling butane repeatedly for 4 weeks, until an acute incident occurred that required cardiopulmonary resuscitation. Repeated magnetic resonance imaging over the following weeks revealed disintegration of gray matter, increasing cerebral atrophy, and destruction of basal ganglia. An electroencephalogram showed strongly diminished basal activity with flat amplitude (Döring et al. 2002). A 15-year-old boy who was known to inhale butane from a plastic bag had bilateral hemispheric infarcts (Bauman et al. 1991). Another 15-year-old boy suffered from right-sided hemiparesis after butane abuse; he did not lose consciousness. A CT head scan on the day following admission to the hospital was normal. At the time of discharge (5 days later), he still had pronounced upper limb, proximal muscle weakness and a hemiplegic gait (Gray and Lazarus 1993). In another case, a swollen brain was observed in a 15-year-old girl without a history of butane abuse (Williams and Cole 1998), whereas a CT head scan showed no abnormalities in a 17-year-old male with a 3-year history of butane abuse (Edwards and Wenstone 2000).

A 15-year-old boy was found unresponsive and cyanotic after reportedly inhaling butane from a plastic bag. Ventricular tachycardia and ventricular fibrillation were noted. Cardiac status improved during hospitalization, but brain functions remained disturbed (Bauman et al. 1991). A 15-year-old girl without a history of butane abuse inhaled butane intermittently over a 2-h period. She collapsed when running away from the police (Williams and Cole 1998). On admission to the hospital, there was no spontaneous respiration; a CT scan showed a very tight, swollen brain and an electrocardiogram showed sinus tachycardia with marked T-wave inversion in the anterolateral leads.

2.4. Developmental and Reproductive Toxicity

A pregnant 34-year-old woman accidentally inhaled butane in during week 27 of her pregnancy. She was found unconscious and required mechanical ventilation for 5 h. The exposure duration and concentration of butane were not reported, nor was the amount of time that elapsed before resuscitation commenced. She gradually regained consciousness and was discharged 48 h after admission. An ultrasound at week 39 of the pregnancy showed an almost complete absence of brain tissue in the fetus. The female child was delivered normally and appeared in good condition. A CT scan at 7 days post-partum revealed an almost complete absence of both cerebral hemispheres in the newborn. The thalamus, brainstem, and cerebellum were preserved (Fernàndez et al. 1986).

A 25-year-old pregnant woman tried to commit suicide by inhaling butane at 30-weeks gestation. She was found comatose and needed resuscitation. The

duration and concentration of butane exposure were not reported, nor was the amount of time that elapsed before resuscitation commenced. Spontaneous labor occurred at 36 weeks. The infant did not breathe spontaneously; he was resuscitated, intubated, and ventilated artificially, but died 11 h after birth (Gosseye et al. 1982). The infant's brain weighed 99 g (mean normal weight is 308 g), and the general appearance of the convolutions corresponded to about 30 weeks of maturation. A severe encephalomalacia was noted. The kidneys were underdeveloped, and the heart showed some foci of fibrosis in the subendocardial myocardium. The lungs were poorly aerated and the alveoli contained a number of squamous cells. Other viscera were reported to be unremarkable.

2.5. Genotoxicity

No data were available.

2.6. Carcinogenicity

No data were available.

2.7. Summary

A number of fatal and nonfatal cases related to butane abuse or suicide attempts have been described. Quantitative exposure estimates are lacking for all cases. In the case of butane abuse, most of the health effects described in case reports are thought to be induced by repeated exposures and abuse of other chemicals cannot be ruled out. Organs that were most often seriously affected in these cases were the brain and heart.

A 10-min exposure to butane at 10,000 ppm caused drowsiness in human volunteers. These were probably rather minor effects. Butane was reported to be "readily perceptible" at a concentration of 18,000 ppm. No irritation was noted at 100,000 ppm (exposure duration not specified but was probably for a few minutes).

Inhalation of butane during pregnancy (weeks 27 and 30 of gestation) at concentrations that produced unconsciousness in the mother caused clearly underdeveloped brains in two fetuses. In both cases, the effects were attributed intrauterine anoxia.

3. ANIMAL TOXICITY DATA

3.1. Acute Lethality

3.1.1. Monkeys

No data were available.

3.1.2. Dogs

Butane at concentrations of 200,000-250,000 ppm produced "relaxation" in dogs (number and sex not specified), but caused death after a short time (Stoughton and Lamson 1936). No further details were given.

3.1.3. Rats

Shugaev (1969) exposed rats (sex and strain not specified) to varying concentrations of butane for 4 h. The number of animals was not specified but the results suggest that the exposure groups consisted of 6 animals. Exposure concentrations were reported to be controlled by gas chromatography, but no information about the concentrations of butane tested or the duration of the post-exposure observation period was provided. The experimental data were analyzed by probit analysis. A 4-h LC_{50} (lethal concentration 50% lethality) of 278,000 ppm (658 g/m^3) was reported, with 95% confidence limits of 252,000-302,000 ppm. Most rats died during the third or fourth hour of exposure. The LC_{16} was calculated to be 227,000 ppm (537 g/m^3) and the LC_{84} to be 333,000 ppm (790 g/m^3). Mean butane concentrations in organs at the LC_{50} were 7.5 µg/g in the brain, 4.9 µg/g in the liver, 4.4 µg/g in the kidneys, 5.2 µg/g in the spleen, and 20.9 µg/g in perinephric fat.

3.1.4. Mice

Shugaev (1969) exposed mice (sex and strain not specified) to various butane concentrations for 2 h. The number of animals was not specified but the results suggest that exposure groups consisted of 6 animals. Exposure concentrations were reported to be controlled by gas chromatography, but no information about the concentrations of butane tested or the duration of the post-exposure observation period was provided. The experimental data were analyzed by probit analysis. A 2-h LC_{50} of 287,000 ppm (680 g/m^3) was reported, with 95% confidence limits of 252,000-327,000 ppm. Most of the mice died during the second hour of exposure. The LC_{16} was calculated to be 224,000 ppm (530 g/m^3) and the LC_{84} to be 363,000 ppm (860 g/m^3). The mean butane concentration in the brain of dead mice at the LC_{50} was 7.8 µg/g.

Groups of mice (sex and strain not specified) were exposed to butane at concentrations of 130,000, 220,000, 270,000, or 310,000 ppm; 6 mice were tested at the lowest concentration, and 10 mice at each of the higher concentrations (Stoughton and Lamson 1936). The animals were observed for 24-48 h after exposure. Although it was not clearly described, the study description suggests that the animals were exposed under static conditions in a closed-chamber setting. The animals were observed for 48 h after exposure. Effects observed were "light anesthesia," "loss of posture" (complete anesthesia), and death. Exposure to butane at 270,000 ppm for 2 h was lethal to 4 of 10 mice; the average

time of death was 84 min. Exposure at 310,000 ppm was lethal to 60% of the mice, and the average time of death was 65 min. No deaths occurred in mice exposed for 2 h at 130,000 or 220,000 ppm. All deaths occurred during exposure. Surviving mice recovered rapidly, within 5 min after exposure ended (Stoughton and Lamson 1936).

A summary of data on lethality from acute inhalation of butane is provided in Table 1-3.

3.2. Nonlethal Toxicity

3.2.1. Dogs

Six dogs were exposed to butane for various durations to study its potency as a cardiac sensitizer (Chenoweth 1946). Anesthetized dogs were exposed to butane by a tracheal cannula, and epinephrine (0.01 or 0.02 mg/kg) was injected intravenously at different intervals during exposure. Of the 15 trials with individual dogs, three resulted in ventricular fibrillation. The lowest concentration at which ventricular fibrillation occurred was approximately 35,000 ppm (estimated from a graph); epinephrine was injected after 2 min. Other dogs showed no ventricular fibrillation after injection with epinephrine when exposed to butane at about 50,000 ppm. Because details of the study were lacking and because the tests were conducted in anesthetized dogs, no clear conclusions can be drawn from this study. Krantz et al. (1948) also reported cardiac sensitization by butane in dogs, but the exposure conditions were not specified.

TABLE 1-3 Lethality in Laboratory Animals after Acute Inhalation of Butane

Species	Concentration (ppm)	Exposure Duration	Lethality	Reference
Rat	227,000	4 h	LC_{16}	Shugaev 1969
	278,000		LC_{50}	
	333,000		LC_{84}	
Mouse	224,000	2 h	LC_{16}	Shugaev 1969
	287,000		LC_{50}	
	363,000		LC_{84}	
Mouse	130,000	2 h	0/6 deaths	Stoughton and Lamson 1936
Mouse	220,000	2 h	0/10 deaths	Stoughton and Lamson 1936
Mouse	270,000	2 h	4/10 deaths	Stoughton and Lamson 1936
Mouse	310,000	2 h	6/10 deaths	Stoughton and Lamson 1936

Abbreviation: $LC_{\%}$, lethal concentration, % lethality.

The hemodynamic effects of butane were studied in groups of 7 anesthetized (pentobarbital at 30-35 mg/kg) adult mongrel dogs. Dogs were artificially ventilated via an endotracheal cannula and several parameters of cardiac function (e.g., pulmonary arterial pressure, atrial pressure, ventricular pressure, heart rate, stroke volume) were studied (Zakhari 1977). Each dog was exposed butane at nominal concentrations of 0.5, 1.0, 2.5, 5.0, and 10.0% (5,000, 10,000, 25,000, 50,000, and 100,000 ppm, respectively) via the respirator for 5 min; each exposure immediately following the preceding one. No further details were presented on actual exposure concentrations. Concentration-related decreases in cardiac output and left ventricular pressure were observed starting at 5,000 ppm. Myocardial contractility (the rate of rise in left ventricular pressure) and mean aortic pressure showed a concentration-related decrease starting at 25,000 ppm. The individual contribution of butane exposure (as opposed to coexposure with anesthesia) to produce these effects is unclear.

3.2.2. Guinea Pigs

Nuckolls (1933) exposed groups of three guinea pigs to butane at 21,000-28,000 ppm or 50,000-56,000 ppm for 5, 30, 60, or 120 min. The animals were observed during exposure and for 10 days after exposure. The concentrations were analyzed periodically and adjustments made to maintain the predetermined concentrations. Guinea pigs exposed at 21,000-28,000 ppm showed occasional chewing movements and irregular or rapid breathing, but the effects did not worsen as the exposure duration increased. Animals recovered quickly and appeared normal after exposure ended. Guinea pigs exposed for 5 min at 50,000-56,000 ppm showed no significant effects. Continuation of exposure resulted in irregular breathing, occasional retching, and chewing movements, and the animals showed a "dazed appearance" in the second hour of exposure but were able to walk. The description of the results suggests that the effects did not increase in severity with continuation of exposure. One guinea pig exposed for 2 h at 50,000-56,000 ppm was examined histopathologically 7 days after exposure; no effects were found.

3.2.3. Mice

Groups of mice (sex and strain not specified) were exposed to butane at concentrations of 130,000, 220,000, 270,000, or 310,000 ppm; 6 mice were tested at the lowest concentration and 10 mice at each of the higher concentrations (Stoughton and Lamson 1936). The study description suggests that the concentrations reported were initial concentrations and that the animals were exposed in a closed-chamber setting. The animals were observed for 48 h after exposure. Effects observed were "light anesthesia" and "loss of posture" (complete anesthesia). Light anesthesia was defined as being unable to maintain an upright position in a rotating bottle (2 mice in a 2-L bottle). Complete anesthesia

(loss of posture) was defined as the inability to regain an upright position after shaking the bottle in which the mice were placed (5 mice in a 20-L bottle). Exposure to butane at 130,000 ppm induced light anesthesia within 25 min (on average). Light anesthesia was observed within 1 min of exposure to butane at 220,000 ppm, and loss of posture was observed within 15 min. Loss of posture occurred within 4 min at 270,000 ppm and within 3 min at 310,000 ppm. Butane at concentrations of 270,000 and 310,000 ppm caused mortality (see Section 3.1.5). Surviving mice recovered within a few min after exposure ended.

A summary of nonlethal effects from acute inhalation of butane is provided in Table 1-4.

3.3. Neurotoxicity

No data other than that described in Sections 3.2.2 and 3.2.3 were available.

3.4. Developmental and Reproductive Toxicity

No were data available.

3.5. Genotoxicity

Butane appears to be negative in the Ames test, with and without metabolic activation (citation of an unpublished report in Moore 1982). Shimizu et al. (1985) reported that butane at concentrations up to 25,000 ppm was negative in tests with *Salmonella typhimurium* TA98, TA100, TA1535, TA1537, and TA1538, and in *Escherichia coli* (WP2uvrA), with and without metabolic activation.

TABLE 1-4 Nonlethal Effects in Laboratory Animals after Inhalation of Butane

Species	Concentration (ppm)	Exposure Duration	Effect	Reference
Guinea pigs	21,000-28,000	Up to 2 h	Increased respiration rate; increased sniffing and chewing behavior.	Nuckolls 1933
Guinea pigs	50,000-56,000	Up to 2 h	Increased respiration rate; increased retching and chewing behavior; dazed appearance.	Nuckolls 1933
Mice	130,000	2 h	Light anesthesia within 25 min.	Stoughton and Lamson 1936
Mice	220,000	2 h	Light anesthesia within 1 min; complete anesthesia within 15 min.	Stoughton and Lamson 1936
Mice	270,000	2 h	Complete anesthesia within 4 min.	Stoughton and Lamson 1936
Mice	310,000	2 h	Complete anesthesia within 3 min.	Stoughton and Lamson 1936

3.6. Carcinogenicity

No were data available.

3.7. Summary

Butane was reported to cause cardiac sensitization in dogs, but the studies did not provide detailed information on exposure concentrations and duration or were performed on anesthetized dogs. Because of these limitations, no clear conclusions can be drawn. Butane caused hemodynamic effects in anesthetized dogs but considering the exposure conditions of the study it cannot be used as a basis for setting AEGL values.

Slight effects on the respiratory rate were reported in guinea pigs exposed to butane at 21,000-28,000 ppm for up to 2 h. Guinea pigs exposed at 50,000-56,000 ppm for 2 h showed a "dazed appearance" but were able to walk. Light and complete anesthesia occurred in mice exposed to initial concentrations of butane at 130,000 ppm (within 25 min) and 220,000 ppm (within 15 min). Light anesthesia was defined as "being unable to maintain an upright position in a rotating bottle."

A steep concentration-response curve for mortality was observed in mice and rats; the LC_{84}:LC_{16} ratio was 1.6 for mice (2-h exposure) and 1.5 for rats (4-h exposure). The response in mice and rats were remarkably comparable at similar concentrations, despite the difference in exposure duration. In another study, no deaths occurred in mice exposed to initial concentrations of butane at 130,000 or 220,000 ppm for 2 h; mortality was 40 and 60% at concentrations of 270,000 and 310,000 ppm, respectively. No deaths occurred in guinea pigs exposed to butane at 50,000-56,000 ppm for 2 h.

Butane was negative in the bacterial reverse mutation (Ames) test with and without metabolic activation.

4. SPECIAL CONSIDERATIONS

4.1. Metabolism and Disposition

4.1.1. Absorption

Four human subjects (3 male, 1 female; 20-21-years of age) were exposed to butane at 600 ppm for 4 h. The chamber concentration was monitored continuously. Pulmonary uptake of butane appears to increase quickly within the first 5 min of exposure and reaches a plateau within the first 30 min. Pulmonary uptake remained fairly constant during the remainder of the exposure, ranging from 30% to 50% (Gill et al. 1991). Butane concentration in exhaled breath decreased rapidly to less than 5 ppm, 20 min after exposure ended. Blood concentrations of butane also decreased rapidly to less than 0.02 μg/mL after 20 min (data were estimated from graphs).

4.1.2. Metabolism

Tsukamoto et al. (1985) exposed male ICR mice (number of animals not specified) for 1 h to a mixture of butane, air, and oxygen (in the proportion of 2:1:1; thus, the butane concentration was 500,000 ppm). Animals were killed immediately after exposure. In addition to butane, methyl ethyl ketone and *sec*-butanol were detected in blood and tissues as metabolites. Tissue concentrations of methyl ethyl ketone were 2.9-4 μg/g, with highest concentrations in blood, followed by the liver, kidneys, and brain. The concentration of *sec*-butanol in these tissues was 30-35 μg/g, with highest concentrations in blood and brain. Exposure to the butane mixture killed 40% of the animals despite the high oxygen content (>25%).

In vitro studies with rat liver microsomes showed that hydroxylation results for nearly 100% in 2-butanone over 1-butanone (Frommer et al. 1970).

4.1.3. Species Variability

Shugaev (1969) determined LC_{50} values for butane for 2-h exposures in mice and 4-h exposures in rats. The different exposure duration for the two species was based on a comparison of the ratio of minute ventilation (volume of gas exchanged from the lungs per minute) to body weight, which is approximately two-fold greater in mice. The 2-h LC_{16}, LC_{50}, and the LC_{84} for the mouse were similar to the corresponding 4-h values for the rat (see Table 1-3). Most of the mice died during the second hour of exposure, whereas rats mainly died during the third and fourth hour of exposure. Mean brain concentrations of butane in dead rats (7.5 μg/g) and mice (7.8 μg/g) were similar. However, tissue concentrations of butane in rats were not determined after a 2-h exposure and the reported brain concentration could already have been reached with a shorter exposure duration. Results of lethality studies suggest that mice might be more sensitive than rats; mice had a shorter time to death and their 2-h LC_{50} values were close to the 4-h LC_{50} values in rats.

5. DATA ANALYSIS FOR AEGL-1

5.1. Summary of Human Data Relevant to AEGL-1

Case reports of butane exposure do not provide any quantitative data that could be used for deriving AEGL-1 values. Patty and Yant (1929) studied the warning properties of short-term exposures to butane. Physiologic responses were only briefly described, and a discrepancy was noted. It was stated in a table that exposure to butane at 10,000 ppm for 10 min produced drowsiness in volunteers (n = 3 or 6), but the text indicated that no symptoms occurred. Several alkanes (C_3 to C_7) were studied in this experiment and more severe effects were reported for hexane and heptanes, suggesting that if effects were observed with butane they would have been described more explicitly. It is therefore concluded

that any drowsiness associated with butane was of a very minor severity. Odor detection was assessed by means of an odor scale ranging from 0 (no detectable odor) to 5 (intense effect, may bite or irritate). No noticeable irritation was reported at concentrations up to 100,000 ppm for a short but undefined exposure period; the odor detection score was below 4 (cogent, forcible odor).

5.2. Summary of Animal Data Relevant to AEGL-1

Nuckolls (1933) exposed groups of three guinea pigs to butane at 21,000-28,000 ppm or 50,000-56,000 ppm for 5, 30, 60, or 120 min. The animals were observed during exposure and for 10 days after exposure. Guinea pigs exposed at 21,000-28,000 ppm for up to 2 h showed occasional chewing movements and irregular or rapid breathing, while animals exposed at 50,000-56,000 ppm also had a "dazed appearance" in the second hour of exposure but were still able to walk. The description of the results suggests that the effects did not increase in severity with continuation of exposure. Animals recovered quickly and appeared normal after exposure ended. One guinea pig exposed for 2 h at 50,000-56,000 ppm was examined histopathologically 7 days after exposure; no effects were found.

5.3. Derivation of AEGL-1

The human data presented by Patty and Yant (1929) form the basis of the AEGL-1 values. Butane at a concentration of 10,000 ppm (10-min exposure) can be regarded as a boundary for the drowsiness reported; although some drowsiness may be noticed, it will not be experienced as discomfort. Further, no noticeable irritation was reported at concentration up to 100,000 ppm for a short exposure duration (exact duration unknown). Although the study was performed with small groups of volunteers (3 or 6 people) of a relatively young age (20-30 years), an intraspecies uncertainty of 1 is considered adequate for the following reasons. First, the concentration-response curve for CNS-effects appears to be very steep (see Section 6.3) and, thus, interindividual variability will be relatively small. Second, no noticeable irritation was reported at concentrations up to 100,000 ppm for a short duration (exact duration unknown). Third, the use of an intraspecies factor of 3 would lead to AEGL-1 values that are unrealistically low (e.g., in comparison with the occupational standards, see Section 8.2). For similar reasons and because subjects exposed at slowly increasing concentrations of butane up to 50,000 ppm for at least 10 min did not experience any significant adverse effects, a modifying factor is not considered necessary.

The rationale for time scaling in the derivation of AEGL-1 values and the choice of n in the dose-response regression equation is similar to that for AEGL-2 (see Section 6.3). By analogy to other CNS-depressing substances, the effects of butane are assumed to be solely concentration dependent. Thus, after reaching steady state (within 30 min of exposure), no increase in effect size is expected at

4 and 8 h. The other exposure duration-specific values were derived by time scaling according to the dose-response regression equation $C^n \times t = k$, using n = 3. Time extrapolation was performed from 10 min to 30- and 60-min exposures. The resulting values for AEGL-1 are presented in Table 1-5. These values are considered protective of the irregular breathing observed in guinea pigs exposed to butane at 21,000-28,000 ppm for up to 2 h.

6. DATA ANALYSIS FOR AEGL-2

6.1. Summary of Human Data Relevant to AEGL-2

No adequate human data relevant to AEGL-2 effects were found. Two case reports indicate that single exposure to high concentrations of butane might cause severe brain damage in the fetus, but these studies are not suitable as basis for AEGL-2 values because exposure data are lacking.

6.2. Summary of Animal Data Relevant to AEGL-2

Nuckolls (1933) exposed groups of three guinea pigs for 5, 30, 60, or 120 min to 50,000-56,000 ppm for 2 h. Guinea pigs had an increase in respiratory rate and sniffing and chewing behavior and showed a "dazed appearance" in the second hour of exposure, but the animals were still able to walk. The description of the effects appears to indicate that the effects did not increase in severity with continuation of exposure. Animals recovered quickly and appeared normal after exposure ended. One guinea pig exposed for 2 h was examined histopathologically 7 days after exposure; no effects were found.

Stoughton and Lamson (1936) exposed mice to butane at various concentrations for 2 h. Light anesthesia, defined as "being unable to maintain an upright position in a rotating bottle," occurred within 25 min (on average) at 130,000 ppm, the lowest concentration tested, and within 1 min at 220,000 ppm. Complete anesthesia, defined as "the inability to regain an upright position after shaking the bottle in which the mice were placed," was observed within 15, 4, and 3 min in mice exposed at 220,000, 270,000, and 310,000 ppm, respectively. Light anesthesia can be considered serious enough to impair escape, and could be used as basis for AEGL-2. However, the experimental procedure is poorly described but suggests that reported concentrations are probably initial concentrations in a closed-chamber setting. Butane concentration will have decreased during exposure; thus, the effects observed cannot be related to a specific exposure concentration.

6.3. Derivation of AEGL-2

Case reports indicate that single exposure to high concentrations of butane might cause severe brain damage in the fetus, but no adequate human or animal

data are available for a quantitative evaluation of this end point. Because human data are lacking, the AEGL-2 values are based on animal data. Two animal studies are available, a study with guinea pigs (Nuckolls 1933) and a study with mice (Stoughton and Lamson 1936). In the latter study, "light anesthesia" was observed after a mean exposure duration of 25 min to butane at 130,000 ppm and after 1 min at 220,000 ppm. The use of this study is hampered by the possibility that exposure concentration decreased during the study.[2]

The only available starting point adequate for AEGL-2 values is provided by the study of Nuckolls (1933), in which guinea pigs were exposed for 2 h to butane at concentration of 50,000-56,000 ppm. Because the animals were able to walk, their "dazed appearance" is considered not to be sufficiently serious to impair escape. A concentration of 50,000 ppm is considered an appropriate starting point for the derivation of AEGL-2 values. Because the anesthetic effects of butane are considered to be predominantly concentration dependent, a total uncertainty factor of 3 is considered sufficient for toxicokinetic and toxicodynamic differences between individuals and interspecies differences. The effects are attributed to butane itself and no relevant differences in kinetics are assumed, so only small interindividual differences are expected. The concentration-response curve appears to be very steep, indicating that a large uncertainty factor is unnecessary. Further, a larger uncertainty factor would lead to unrealistically low

TABLE 1-5 AEGL-1 Values for Butane

10 min	30 min	1 h	4 h	8 h
See below[a]	6,900 ppm[b] (16,000 mg/m^3)	5,500 ppm (13,000 mg/m^3)	5,500 ppm[b] (13,000 mg/m^3)	5,500 ppm[b] (13,000 mg/m^3)

[a]The 10-min AEGL-1 value is 10,000 ppm (24,000 mg/m^3), which is greater than 50% of the lower explosive limit for butane in air of 19,000 ppm. Therefore, extreme safety considerations against the hazard of explosion must be taken into account.

[b]The AEGL value is greater than 10% of the Lower explosive limit for butane in air of 19,000 ppm. Therefore, safety considerations against the hazard of explosion must be taken into account.

[2]During the evaluation of "light anesthesia," two mice were placed in a 2-L bottle. Assuming a minute volume for mice of 25 mL/min per animal (Paulussen et al. 1998), it can be calculated that the two animals breathe 2.5% (50/2,000) of the available air per minute. Assuming that the pulmonary retention in mice is comparable to the approximately 50% reported in humans (Gill et al. 1991), about 1.25% of the butane present in the air is retained by the two animals per minute. Thus, the butane concentration will have decreased to approximately 73% of the initial concentration (a decrease from 130,000 to 95,000 ppm) after 25 min. Although the reduction in concentration can be regarded as negligible for short-exposure durations because of general variation in actual exposure concentrations, a mean exposure duration of 1 min is inadequate as starting point for time scaling to 8 h of exposure. Therefore, the study by Stoughton and Lamson (1936) does not provide an adequate starting point for AEGL-2 values.

values for AEGL-2 and would be similar to the AEGL-1 values. 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 between n = 1 and n = 3. Consequently, these values were selected as the reasonable lower and upper bounds of n to use when data are not available to derive an empirical value for n. An indication for the value of n for CNS-depressing effects can be obtained from the study by Stoughton and Lamson (1936). Complete anesthesia from butane was reported to occur at an initial concentration of 220,000 ppm after 15 min, 270,000 ppm after 4 min, and 310,000 ppm after 3 min. On the basis of these data, n is estimated to be greater than 4 (after accounting for a small decrease in concentration during the 15-min exposure[3]). Although the data cannot be used to provide an estimate for n, it can be concluded that n will be relatively high and that the upper bound of n = 3 is an appropriate estimate for time scaling. This is consistent with other anesthetics for which effects are assumed to be concentration dependent rather than time dependent.

No increase in the severity of response by duration is expected for concentration-dependent effects after reaching a steady state. Although no appropriate kinetic data are available on butane to assess the duration needed to reach a steady state, it can be estimated from the pulmonary-uptake data from Gill et al. (1991) that a steady-state uptake, and hence, steady-state plasma values, will be reached within 30 min. In addition, it has been stated that gases which are relatively insoluble in blood increase rapidly toward equilibrium with the inhaled concentration and the less soluble in blood the faster the narcotic action of the gas (Drummond 1993). The increase to a quick equilibrium has been confirmed for propane, which has properties comparable to butane. Concentrations of propane were approximately similar in blood samples taken 15 min before the end of 1-, 2-, and 8-h exposures to propane at 250 and 500 ppm (Stewart et al. 1977).

Because of the poor solubility of butane in water (61 mg/L), it is expected that exposure to butane will lead to a rapid equilibrium and that there will be no increase in the severity of response for duration of 30 min to 8 h. Therefore, the AEGL-2 values for 30 min and 1, 4 and 8 h are set equal to the 2-h concentration. The AEGL-2 value for the 10-min exposure is derived by time scaling according to the dose-response regression equation $C^n \times t = k$, using n = 3.

[3]"Complete anesthesia" was evaluated with five mice in a 20-L bottle. Using the assumptions made in footnote 1, approximately 0.3% of the butane will be retained by the five animals per min. The concentration of butane will have decreased to about 95% of the initial concentration (from 220,000 to 209,000 ppm) after 15 min.

The AEGL-2 values for butane are presented in Table 1-6.

7. DATA ANALYSIS FOR AEGL-3

7.1. Summary of Human Data Relevant to AEGL-3

Case reports indicate that butane's effects on the brain (e.g., encephalopathy) or effects on the heart (either direct cardiotoxicity or cardiac sensitization) might cause death. It is difficult to distinguish between direct toxicity of butane and the effects caused by hypoxia. The case reports do not provide adequate quantitative data to derive AEGL-3 values. Exposure to butane at slowly increasing concentrations up to 50,000 ppm (total exposure duration was at least 10 min) or at 100,000 ppm (short exposure, exact duration unknown) on the same day did not result in serious complaints (Patty and Yant 1929).

7.2. Summary of Animal Data Relevant to AEGL-3

Experiments with dogs (Chenoweth 1946; Krantz et al. 1948) support the cardiac sensitization potency of butane, but neither study provides an adequate basis for deriving AEGL-3 values.

Two relevant animal studies are available, a study with rats and mice by Shugaev (1969) and a study in mice by Stoughton and Lamson (1936). Shugaev (1969) reported a 2-h LC_{50} of 287,000 ppm (680 g/m^3) in mice and a 4-h LC_{50} of 278,000 ppm (658 g/m^3) in rats. The concentration-response curve was very steep, with LC_{84}:LC_{16} ratios of approximately 1.5 for both species. The 2-h lethality data in mice obtained by Stoughton and Lamson (1936) were remarkably similar to those obtained by Shugaev (1969). Stoughton and Lamson (1936) observed no mortality in mice after a 2-h exposure to butane at 130,000 or 220,000 ppm, whereas 4/10 and 6/10 mice died during a 2-h exposure at 270,000 and 310,000 ppm, respectively. The experimental procedure in the study by Stoughton and Lamson (1936) is poorly described, but suggests that the reported concentrations of butane are probably initial concentrations in a closed-chamber setting. Thus, the concentration of butane will have decreased during exposure.[4]

7.3. Derivation of AEGL-3

There are no adequate human data for derivation of AEGL-3 values. Although case reports indicate that humans exposed to butane at high concentra-

[4]Lethality was studied with five mice in a 20-L bottle. The concentration of butane will have decreased to about 83% of the original concentration after 60 min and to 69% after 120 min (see footnote 1). Deaths occurred after 84 min (on average) at an initial butane concentration of 270,000 ppm and after 65 min (on average) at an initial concentration of 310,000 ppm.

tions might develop cardiac arrhythmias, which are potentially fatal, the data were inadequate to evaluate this end point. Therefore, AEGL-3 values are based on animal data.

7.3. Derivation of AEGL-3

There are no adequate human data for derivation of AEGL-3 values. Although case reports indicate that humans exposed to butane at high concentrations might develop cardiac arrhythmias, which are potentially fatal, the data were inadequate to evaluate this end point. Therefore, AEGL-3 values are based on animal data.

The study by Stoughton and Lamson (1936) provides a no-observed-adverse-effect level for lethality in mice exposed to butane for 2 h. However, the mice were probably exposed in a closed-chamber setting and the reported butane concentrations might refer to initial concentrations. Hence, this study does not provide an adequate starting point for deriving AEGL-3 values. Shugaev (1969) exposed mice and rats to butane for 2 and 4 h, respectively. The reported data (LC_{16}, LC_{50}, and LC_{84}) indicate that the concentration-response curve for a 2-h exposure in mice and a 4-h exposure in rats are very similar (see Table 1-3). Further, brain concentrations of butane in dead mice and rats exposed at the LC_{50} appeared to be comparable. This might be explained by the difference in the ratio of minute ventilation to body weight, which is approximately two-fold greater in mice. However, it might be an indication that mice are more susceptible to butane toxicity, because a steady state is expected to be reached rapidly with butane (see Section 6.2). Because no further data are available, it is assumed that mice are more susceptible than rats. Because the study by Shugaev (1969) reports only LC_{16}, LC_{50}, and the LC_{84} values obtained by probit analysis and not the individual experimental data, benchmark dose-response modeling is not possible. However, the LC_{01} can be calculated because the mean is known and the standard deviation of the underlying lognormal distribution can be derived from these data. The 2-h LC_{01} for mice is 160,000 ppm and the 4-h LC_{01} for rats is 172,000 ppm. The 2-h LC_{01} for mice is chosen as the starting point for the AEGL-3 values, because it is the lowest concentration tested in what appears to be a more susceptible species.

TABLE 1-6 AEGL-2 Values for Butane

10 min	30 min	1 h	4 h	8 h
See below[a]	See below[b]	See below[b]	See below[b]	See below[b]

[a]The 10-min AEGL-2 value is 24,000 ppm (57,000 mg/m^3), which is greater than the lower explosive limit for butane in air of 19,000 ppm. Therefore, extreme safety considerations against the hazard of explosion must be taken into account.

[b]The AEGL-2 values for 30-min and 1-, 4-, and 8-h are 17,000 ppm (40,000 mg/m^3), which is greater than 50% of the lower explosive limit for butane in air of 19,000 ppm. Therefore, extreme safety considerations against the hazard of explosion must be taken into account.

A total uncertainty factor of 3 is considered sufficient for toxicokinetic and toxicodynamic differences between individuals and interspecies differences for the following reasons. The effects are attributed to butane itself and no relevant differences in kinetics are assumed. A species with a relatively high susceptibility is used as starting point. The concentration-response curve appears to be very steep indicating that a large uncertainty factor is unnecessary. Further, a larger uncertainty factor would lead to unrealistically low values for AEGL-3, which would be similar to the AEGL-2 values.

As indicated by the study by Stoughton and Lamson (1936), mortality is preceded by CNS-depression. Hence, the rationale described in Section 6.2 for determining the value of n for time scaling to derive AEGL-2 values is considered also appropriate for AEGL-3 values. After a steady state has been reached, no increase in effect severity by exposure duration is expected. Therefore, the AEGL-3 values for 30 min and for 1, 4 and 8 h of exposure will be set equal to that for the 2-h exposure. The AEGL-3 values for the 10-min exposure are derived by time scaling according to the dose-response regression equation $C^n \times t = k$, using n = 3. The 10-min AEGL of 77,000 ppm is supported by the data from Patty and Yant (1929). They reported that exposure to butane at slowly increasing concentrations up to 50,000 ppm (total exposure duration at least 10 min) and to 100,000 ppm (short exposure, exact duration unknown) on the same day did not result in serious complaints (Patty and Yant 1929).

The AEGL-3 values for butane are presented in Table 1-7.

8. SUMMARY OF AEGLS

8.1. AEGL Values and Toxicity End Points

The AEGL values for butane are summarized in Table 1-8.

8.2. Comparison with Other Standards and Guidelines

Standards and guidelines for short-term exposures to butane are presented in Table 1-9.

8.3. Data Quality and Research Needs

The database for butane is poor and important studies date back to the 1920s or 1930s. Although case reports indicate that butane might cause arrhythmias in humans exposed at high concentrations, no adequate human or animal data are available to evaluate this end point in a quantitative way. Similarly, case reports indicate that single exposure to high concentrations of butane might cause severe brain damage in the fetus, but no adequate data are available for a quantitative evaluation.

TABLE 1-7 AEGL-3 Values for Butane

10 min	30 min	1 h	4 h	8 h
See below[a]	See below[a]	See below[a]	See below[a]	See below[a]

[a]The 10-min AEGL-3 value is 77,000 ppm (180,000 mg/m^3), and the 30-min and 1-, 4-, and 8-h AEGL-3 values are 53,000 ppm (130,000 mg/m^3). All of these values are greater than the lower explosive limit for butane in air of 19,000 ppm. Therefore, extreme safety considerations against the hazard of explosion must be taken into account.

TABLE 1-8 Summary of AEGL Values for Butane

Classification	10 min	30 min	1 h	4 h	8 h
AEGL-1 (nondisabling)	See below[a]	6,900 ppm[b] (16,000 mg/m^3)	5,500 ppm[b] (13,000 mg/m^3)	5,500 ppm[b] (13,000 mg/m^3)	5,500 ppm[b] (13,000 mg/m^3)
AEGL-2 (disabling)	See below[c]	See below[d]	See below[d]	See below[d]	See below[d]
AEGL-3 (lethal)	See below[e]	See below[e]	See below[e]	See below[e]	See below[e]

[a]The 10-min AEGL-1 value is 10,000 ppm (24,000 mg/m^3), which is greater than 50% of the lower explosive limit for butane in air of 19,000 ppm. Therefore, extreme safety considerations against the hazard of explosion must be taken into account.
[b]The AEGL-1 value is greater than 10% of the lower explosive limit for butane in air of 19,000 ppm. Therefore, safety considerations against the hazard of explosion must be taken into account.
[c]The 10-min AEGL-2 value is 24,000 ppm (57,000 mg/m^3), which is greater than the lower explosive limit for butane in air of 19,000 ppm. Therefore, extreme safety considerations against the hazard of explosion must be taken into account.
[d]The AEGL-2 values for 30 min and 1, 4, and 8 h are 17,000 ppm (40,000 mg/m^3), which is greater than 50% of the lower explosive limit for butane in air of 19,000 ppm. Therefore, extreme safety considerations against the hazard of explosion must be taken into account.
[e]The 10-min AEGL-3 value is 77,000 ppm (180,000 mg/m^3). The AEGL-3 values for 30 min and 1, 4, and 8 h are 53,000 ppm (130,000 mg/m^3). These values are greater than the lower explosive limit for butane in air of 19,000 ppm). Therefore, extreme safety considerations against the hazard of explosion must be taken into account.

TABLE 1-9 Extant Standards and Guidelines for Butane

	Exposure Duration				
Guideline	10 min	30 min	1 h	4 h	8 h
AEGL-1	See below[a]	6,900 ppm[b] (16,000 mg/m^3)	5,500 ppm[b] (13,000 mg/m^3)	5,500 ppm[b] (13,000 mg/m^3)	5,500 ppm[b] (13,000 mg/m^3)
AEGL-2	See below[c]	See below[d]	See below[d]	See below[d]	See below[d]
AEGL-3	See below[e]	See below[e]	See below[e]	See below[e]	See below[e]
TLV-TWA (ACGIH)[f]					1,000 ppm

(Continued)

TABLE 1-9 Continued

Guideline	Exposure Duration				
	10 min	30 min	1 h	4 h	8 h
REL-TWA (NIOSH)[g]					800 ppm
MAK (Germany)[h]					1,000 ppm
MAK Peak Limit (Germany)[i]			2,000 ppm		
MAC (The Netherlands)[j]					600 ppm

[a]The 10-min AEGL-1 value is 10,000 ppm (24,000 mg/m^3), which is greater than 50% of the lower explosive limit for butane in air of 19,000 ppm. Therefore, extreme safety considerations against the hazard of explosion must be taken into account.

[b]The AEGL-1 value is greater than 10% of the lower explosive limit for butane in air of 19,000 ppm. Therefore, safety considerations against the hazard of explosion must be taken into account.

[c]The 10-min AEGL-2 value is 24,000 ppm (57,000 mg/m^3), which is greater than the lower explosive limit for butane in air of 19,000 ppm. Therefore, extreme safety considerations against the hazard of explosion must be taken into account.

[d]The AEGL-2 values for 30 min and 1, 4, and 8 h are 17,000 ppm (40,000 mg/m^3), which is greater than 50% of the lower explosive limit for butane in air of 19,000 ppm. Therefore, extreme safety considerations against the hazard of explosion must be taken into account.

[e]The 10-min AEGL-3 value is 77,000 ppm (180,000 mg/m^3). The AEGL-3 values for 30 min and 1, 4, and 8 h are 53,000 ppm (130,000 mg/m^3). These values are greater than the lower explosive limit for butane in air of 19,000 ppm). Therefore, extreme safety considerations against the hazard of explosion must be taken into account.

[f]TLV-TWA (threshold limit value - time weighted average, American Conference of Governmental Industrial Hygienists) (ACGIH 2007) 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.

[g]REL-TWA (recommended exposure limit - time weighted average, National Institute for Occupational Safety and Health) (NIOSH 2010) is defined analogous to the ACGIH TLV-TWA.

[h]MAK (maximale arbeitsplatzkonzentration [maximum workplace concentration]) (Deutsche Forschungsgemeinschaft [German Research Association] (DFG 2007) is defined analogous to the ACGIH TLV-TWA.

[i]MAK spitzenbegrenzung (peak limit,German Research Association (DFG 2007) constitutes the maximum concentration to which workers can be exposed for a period up to 60 min with no more than three exposure periods per work shift; total exposure may not exceed the 8-h MAK.

[j]MAC (maximaal aanvaaarde concentratie [maximal accepted concentration]) (Dutch Expert Committee for Occupational Standards, The Netherlands (MSZV 2004) is defined analogous to the ACGIH TLV-TWA.

The case reports do not provide an adequate basis for AEGL values. The only study with human volunteers (Patty and Yant 1929) is rather old and focused on the warning properties of butane.

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APPENDIX A

DERIVATION OF AEGL VALUES FOR BUTANE

Derivation of AEGL-1 Values

Key study:	Patty, F.A., and W.P. Yant. 1929. Odor Intensity and Symptoms Produced by Commercial Propane, Butane, Pentane, Hexane, and Heptane Vapor. U.S. Bureau of Mines Report of Investigation. No 2979. Washington, DC: U.S. Department of Commerce, Bureau of Mines.
Toxicity end point:	10-min exposure to 10,000 ppm is the no-observed-adverse-effect level for CNS depression.
Time scaling:	$C^3 \times t = k$ for extrapolation to 30 min and 60 min; flatlining assumed from 60 min to 4- and 8-h exposure (based on 60-min steady-state concentration). $k = (10{,}000 \text{ ppm})^3 \times 10 \text{ min} = 10^{13} \text{ ppm}^3\text{-min}$
Uncertainty factors:	1 for interindividual variability
Calculations:	
10-min AEGL-1:	10,000 ppm[a] (24,000 mg/m^3)
30-min AEGL-1:	$C^3 \times 30 \text{ min} = 10^{13} \text{ ppm}^3\text{-min}$ C = 6,900 ppm[b] (rounded) (16,000 mg/m^3)
1-h AEGL-1:	$C^3 \times 60 \text{ min} = 10^{13} \text{ ppm}^3\text{-min}$ C = 5,500 ppm[b] (rounded) (13,000 mg/m^3)
4-h AEGL-1:	Set equivalent to 1-h AEGL-1 of 5,500 ppm[b] (13,000 mg/m^3)
8-h AEGL-1:	Set equivalent to 1-h AEGL-1 of 5,500 ppm[b] (13,000 mg/m^3)

[a]The AEGL-1 value is greater than 10% of the lower explosive limit for butane in air of 19,000 ppm. Therefore, safety considerations against the hazard of explosion must be taken into account.
[b]The 10-min AEGL-1 value is greater than 50% of the lower explosive limit for butane in air of 19,000 ppm. Therefore, extreme safety considerations against the hazard of explosion must be taken into account.

Derivation of AEGL-2 Values

Key study:	Nuckolls, A.H. 1933. Underwriters' Laboratoris Report on the Comparative Life, Fire, and Explosion Hazards of Common Refrigerants. Miscellaneous Hazards No. 2375. Chicago, IL: National Board of Fire Underwriters.
Toxicity end point:	CNS depression, no effects consistent with definition of AEGL-2 in guinea pigs exposed to butane at 50,000 ppm for 2 h.
Time scaling:	$C^3 \times t = k$ for extrapolation to 10 min, flatlining assumed from 30 min to 8-h exposure (based on 2-h steady-state concentration). $k = (50{,}000 \text{ ppm})^3 \times 30 \text{ min} = 3.8 \times 10^{15}$ $\text{ppm}^3\text{-min}$
Uncertainty factors:	Total uncertainty factor of 3 for differences between species and individuals.
Calculations:	
10-min AEGL-2:	$C^3 \times 10 \text{ min} = 3.8 \times 10^{15}$ $\text{ppm}^3\text{-min}$ C = 72,112 ppm 72,112 ÷ 3 = 24,000 ppm[a] (rounded) (= 57,000 mg/m^3)
30-min AEGL-2:	C = 50,000 ppm (2-h steady state concentration) 50,000 ppm ÷ 3 = 17,000 ppm[b] (rounded) (40,000 mg/m^3)
1-h AEGL-2:	Set equivalent to the 30-min AEGL-2 of 17,000 ppm[b] (40,000 mg/m^3)
4-h AEGL-2:	Set equivalent to the 30-min AEGL-2 of 17,000 ppm[b] (40,000 mg/m^3)
8-h AEGL-2:	Set equivalent to the 30-min AEGL-2 of 17,000 ppm[b] (40,000 mg/m^3)

[a]The 10-min AEGL-2 value is greater than the lower explosive limit for butane in air of 19,000 ppm. Therefore, extreme safety considerations against the hazard of explosion must be taken into account.
[b]The AEGL-2 value is greater than 50% of the lower explosive limit for butane in air of 19,000 ppm. Therefore, extreme safety considerations against the hazard of explosion must be taken into account.

Derivation of AEGL-3 Values

Key study:	Shugaev, B.B. 1969. Concentrations of hydrocarbons in tissues as a measure of toxicity. Arch. Environ. Health 18(6):878-882.
Toxicity end point:	Lethality study in mice exposed for 2 h. The calculated 2-h LC_{01} is 160,000 ppm.
Time scaling:	$C^3 \times t = k$ for extrapolation to 10 min, flatlining assumed from 30 min to 8-h exposure (based on 2-h steady-state concentration). $k = (160{,}000\ ppm)^3 \times 30\ min = 1.2 \times 10^{17}$ ppm^3-min
Uncertainty factors:	Total uncertainty factor of 3 for differences between species and individuals.
Calculations:	
10-min AEGL-3:	$C^3 \times 10\ min = 1.2 \times 10^{17}$ ppm^3-min C = 230,760 ppm 230,760 ÷ 3 = 77,000 ppm (rounded) (18,000 mg/m^3)
30-min AEGL-3:	C = 160,000 ppm (2-h steady state concentration) 160,000 ppm ÷ 3 = 53,000 ppm[a] (rounded) (130,000 mg/m^3)
1-h AEGL-3:	Set equivalent to the 30-min AGEL-3 of 53,000 ppm[a] (130,000mg/m^3)
4-h AEGL-3:	Set equivalent to the 30-min AGEL-3 of 53,000 ppm[a] (130,000mg/m^3)
8-h AEGL-3:	Set equivalent to the 30-min AGEL-3 of 53,000 ppm[a] (130,000mg/m^3)

[a]The AEGL-3 values are greater than the lower explosive limit for butane in air of 19,000 ppm. Therefore, extreme safety considerations against the hazard of explosion must be taken into account.

APPENDIX B

CATEGORY GRAPH FOR BUTANE

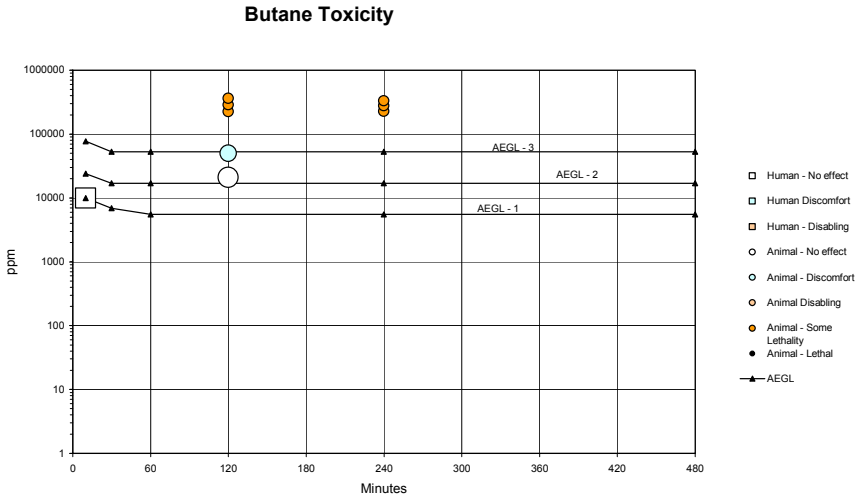

FIGURE B-1 Category graph of toxicity data and AEGLs values for butane.

APPENDIX C

ACUTE EXPOSURE GUIDELINE LEVELS FOR BUTANE

Derivation Summary for Butane

AEGL-1 VALUES

10 min	30 min	1 h	4 h	8 h
See below[a]	6,900 ppm[b] (16,000 mg/m^3)	5,500 ppm[b] (13,000 mg/m^3)	5,500 ppm[b] (13,000 mg/m^3)	5,500 ppm[b] (13,000 mg/m^3)

Key reference: Patty, F.A., and W.P. Yant 1929. Odor Intensity and Symptoms Produced by Commercial Propane, Butane, Pentane, Hexane, and Heptane Vapor. U.S. Bureau of Mines Report of Investigation. No 2979. Washington, DC: U.S. Department of Commerce, Bureau of Mines.

Test species/Strain/Number: Groups of 3- 6 human subjects (male and female, ages 20-30 years). The study was on the warning properties of several alkanes.

Exposure route/Concentrations/Durations: Subjects were exposed at slowly increasing concentrations up to 50,000 ppm (continuous exposure test, total exposure was at least 10 min), followed by exposure to fixed exposure concentrations for a short duration (exact duration unknown) on the same day (intermittent exposure test). The fixed exposure concentrations were approximately 1,000, 2,000, 5,000, 7,000, 10,000, 20,000, and 100,000 ppm.

Effects: No odor detection and no irritation were reported during the continuous exposure test. Drowsiness reported after a 10-min exposure to butane 10,000 ppm was considered to be of minor severity. No details on CNS effects were presented for the higher exposure concentrations. No irritation was reported at 100,000 ppm for 10 min.

End point/Concentration/Rationale: No AEGL-1 effects at 10,000 ppm for 10 min.

Uncertainty factors/Rationale:
Total uncertainty factor: 1
Interspecies: 1, test subjects were humans.
Intraspecies: 1, concentration-response curve appears to be very steep indicating small interindividual variability; no irritation at 100,000 ppm for 10 min; larger factor will result in unrealistically low values compared with occupational standards.

Modifying factor: Not applicable

Animal-to-human dosimetric adjustment: Not applicable

Time scaling: n = 3 for time scaling from 10 to 60 min (animal data suggest high value of n); because steady state is reached within 30 min, the values for the 4- and 8-h exposures were set equivalent to the 60-min value.

Data adequacy: Database is relatively poor but the values are supported by the available animal data.

[a]The AEGL-1 value is 10,000 ppm (23,700 mg/m^3), which is greater than 50% of the lower explosive limit for butane in air of 19,000 ppm. Therefore, extreme safety considerations against the hazard of explosion must be taken into account.

[b]The AEGL value is greater than 10% of the lower explosive limit for butane in air of 19,000 ppm. Therefore, safety considerations against the hazard of explosion must be taken into account.

AEGL-2 VALUES

10 min	30 min	1 h	4 h	8 h
See below[a]	See below[b]	See below[b]	See below[b]	See below[b]

Key reference: Nuckolls, A.H. 1933. Underwriters' Laboratoris Report on the Comparative Life, Fire, and Explosion Hazards of Common Refrigerants. Miscellaneous Hazards No. 2375. Chicago, IL: National Board of Fire Underwriters.

Test species/Strain/Number: Guinea pigs, 3 animals per every concentration-time combination.

Exposure route/Concentrations/Durations: Inhalation for 5, 30, 60, or 120 min at 21,000-28,000 ppm or 50,000-56,000 ppm.

Effects: At 21,000-28,000 ppm, occasional irregular and rapid breathing, did not worsen during exposure, and rapid recovery after exposure ended. At 50,000-56,000 ppm, irregular breathing and dazed appearance during second hour of exposure, but animals were able to walk

End point/Concentration/Rationale: Animals had dazed appearance but were able to walk at 50,000 ppm (lower concentration in the exposure range).

Uncertainty factors/Rationale:
Total uncertainty factor: A total uncertainty factor of 3 is considered sufficient for toxicokinetic and toxicodynamic differences between individuals and interspecies differences. The effects are attributed to butane itself and no relevant differences in kinetics are assumed, so only small interindividual differences are expected. The concentration-response curve appears to be very steep indicating that a large uncertainty factor is unnecessary. Further, a larger uncertainty factor would lead to unrealistically low values for AEGL-2 that would be similar to the AEGL-1 values.

Modifying factor: Not applicable

Animal-to-human dosimetric adjustment: Not applicable

Time scaling: A steady state is reached within 30 min, and the effects are considered to be concentration dependent. Therefore, the starting point for the 30-min and the 1-, 4-, and 8-h values were the 2-h steady-state value of 50,000 ppm. For extrapolation from 30 to 10 min, n = 3.

Data adequacy: Although case reports of butane exposure indicate potential for cardiac sensitization (analogous to propane), this end point has not been studied.

[a]The 10-min AEGL-2 value is 24,000 ppm (57,000 mg/m^3), which is greater than the lower explosive limit for butane in air of 19,000 ppm. Therefore, extreme safety considerations against the hazard of explosion must be taken into account.
[b]The AEGL-2 value for 30-min and 1-, 4-, and 8-h exposures is 17,000 ppm (40,000 mg/m^3), which is greater than 50% of the lower explosive limit for butane in air of 19,000 ppm. Therefore, extreme safety considerations against the hazard of explosion must be taken into account.

AEGL-3 VALUES

10 min	30 min	1 h	4 h	8 h
See below[a]	See below[a]	See below[a]	See below[a]	See below[a]

Key reference: Shugaev, B.B. 1969. Concentrations of hydrocarbons in tissues as a measure of toxicity. Arch. Environ. Health 18(6):878-882.

Test species/Strain/Number: Mice, strain and number not specified.

Exposure route/Concentrations/Durations: Inhalation for 2 h, butane concentrations not specified.

Effects: LC_{16} = 224,000 ppm; LC_{50} = 287,000 ppm; LC_{84} = 363,000 ppm
A 2-h LC_{01} was calculated to be 160,000 ppm.

End point/Concentration/Rationale: Lethal concentration, 1% lethality

Uncertainty factors/Rationale:
Total uncertainty factor: A total uncertainty factor of 3 is considered sufficient because the effects are attributed to butane itself, and no relevant differences in kinetics are assumed. A species with a relatively high susceptibility is used. The concentration-response curve appears to be very steep indicating that a large factor s unnecessary. Further, a larger uncertainty factor would lead to unrealistically low values for AEGL-3, which would be similar to the AEGL-2 values.

Modifying factor: Not applicable

Animal-to-human dosimetric adjustment: Not applicable

Time scaling: A steady state is reached within 30 min, and the effects are considered to be concentration dependent. Therefore, the starting point for the 30-min and the 1-, 4-, and 8-h values were the 2-h steady-state value of 160,000 ppm. For extrapolation from 30 to 10 min, n = 3.

Data adequacy: The results of the key study in mice are comparable with the results from a second study in mice. The 10-min value is supported by human data. Exposure to slowly increasing concentrations of butane up to 50,000 ppm (total exposure duration at least 10 min) and a short exposure (exact duration unknown) at 100,000 ppm on the same day did not result in serious complaints.

[a]The 10-min AEGL-3 values is 77,000 ppm (180,000 mg/m^3), and the AEGL-3 value for 30 min and 1, 4, and 8 h is 53,000 ppm (130,000 mg/m^3). All of the AEGL-3 values are greater than the lower explosive limit for butane in air of 19,000 ppm. Therefore, extreme safety considerations against the hazard of explosion must be taken into account.

2

Chloroacetaldehyde[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 are defined as follows:

AEGL-1 is the airborne concentration (expressed as parts per million or milligrams per cubic meter [ppm or 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 Peter Bos (RIVM, The Dutch National Institute of Public Health and the Environment), Julie M. Klotzbach (Syracuse Research Corporation), Chemical Manager Marinelle Payton (National Advisory Committee [NAC] on Acute Exposure Guideline Levels for Hazardous Substances), and Ernest V. Falke (U.S. Environmental Protection Agency). 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 guidelines reports (NRC 1993, 2001).

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 concentrations 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 AEGL values represent threshold concentrations 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

Chloroacetaldehyde is a colorless, volatile liquid with an acrid, penetrating odor. It evaporates easily and dissolves in water. It is not flammable, but vapor/air mixtures may be explosive at temperatures above 88°C. Chloroacetaldehyde can exist in combinations of four forms: monomer, monomer hydrate, dimer hydrate, and cyclic trimer. Commercial aqueous solution of chloroacetaldehyde (45%) contains a 50:50 mixture of the monomer and dimer hydrates. Chloroacetaldehyde is predominantly used as a chemical intermediate in the manufacture of 2-aminothiazole and other compounds, in the control of algae, bacteria, and fungi in water, and in a spinning solution of poly β-alanine.

The toxicity database on chloroacetaldehyde is poor. Apart from a brief statement indicating that a concentration of 10 ppm produced lacrimation and nasal irritation in humans, no information was available on human toxicity. Chloroacetaldehyde is known to be a strong corrosive agent. The predominant effect of chloroacetaldehyde in animals is direct, strong irritation of the eyes, nose, and lungs (resulting in pulmonary edema and death), and has a very steep concentration-response relationship. The best studies of these effects are by Dow Chemical Company (1952) and Arts (1987). The first study exposed mice, rats, and guinea pigs to chloroacetaldehyde at several concentrations and exposure duration, ranging from 400 ppm for 6 min to 10 ppm for 7 h. Rats, mice, and guinea pigs were also exposed repeatedly (eight exposures in 10 days) to

chloroacetaldehyde at 5 ppm for 7 h (Dow Chemical Company 1952). In the second study, rats were exposed at concentrations of 44-2,643 ppm for 1 h (Arts 1987). Both studies focused on mortality and reported nonlethal effects in a general manner. In rats, the lowest concentration-time combinations that induced lethality ranged from 25 ppm for 7 h (19 of 20 rats died) to 400 ppm for 0.1 h (1 of 20 rats died). No deaths were reported at concentration-time combinations ranging from 10 ppm for 7 h to 100 ppm for 12 min. Lethality increased both with concentration and with duration. A 1-h LC_{50} (lethal concentration, 50% lethality) for rats was estimated to fall between 203 and 243 ppm. Guinea pigs were less sensitive to chloroacetaldehyde than rats.

Nonlethal effects observed in these studies included ocular and nasal irritation. Irritation was slight after repeated exposure to chloroacetaldehyde at 5 ppm, and was more pronounced after single exposures at concentrations greater than 10 ppm. Pulmonary edema was found in some rats 2 weeks after being exposed to chloroacetaldehyde at 44 ppm for 1 h. Animals in this study also exhibited closed eyes and salivation. Pulmonary effects became more severe with increasing concentrations in some animals that died.

Studies of the neurotoxicity of chloroacetaldehyde were not found; however, indirect evidence from experiments with the anticancer drugs ifosfamide and cyclophosphamide (chloroacetaldehyde is a main metabolite of these drugs) suggests that it may have neurotoxic effects. In addition, chloroacetaldehyde was found to be mutagenic in several stains of *Salmonella typhimurium*, *Aspergillus nidulans*, *Streptomyces coelicolor*, and Chinese hamster V79 cells. Little information was available on the carcinogenicity of chloroacetaldehyde.

AEGL-1 values are based on nasal and ocular irritation observed in rats after a single exposure to chloroacetaldehyde at concentrations of 10 ppm and higher. Slight irritation was also observed in rabbits, rats, and mice, but not guinea pigs, after repeated exposure to chloroacetaldehyde at 5 ppm (7 h/day) (Dow Chemical Company 1952). Irritation was reported to be related to both concentration and exposure duration. A concentration of 5 ppm was chosen as the point of departure for the AEGL-1 values. A modifying factor of 2 was applied to reduce that concentration to a no-effect level. With the exception of the 10-min AEGL value, time scaling was performed using the equation $C^n \times t = k$. The value of n was determined to be 1.2 based on mortality data. A total uncertainty factor of 10 (two factors of 3) was considered sufficient for toxicokinetic and toxicodynamic differences between species and for individual variability, and no relevant differences in kinetics were assumed (the effects are attributed to direct interaction of chloroacetaldehyde with the mucous membranes of the nose and eyes). The 10-min AEGL-1 was set equal to the 30-min value, because extrapolation from a 7-h exposure to a 10-min value had too much uncertainty. The report of lacrimation and nasal irritation in humans within a few minutes of exposure to chloroacetaldehyde at 10 ppm (Dow Chemical Company 1952) provided supporting data to derive AEGL-1 values on the basis of the rat data.

AEGL-2 values are based on impaired pulmonary function in rats. Data from a well-performed and adequately documented study in rats (Arts 1987)

were chosen for the point of departure for the 1-h AEGL value. A 1-h exposure at 44 ppm (the lowest concentration tested) resulted in pulmonary edema in some animals that were killed at the end of a 2-week observation period. A modifying factor of 2 was applied to derive a no-effect level. A larger modifying factor was considered unnecessary because of the steep concentration-response curve of chloroacetaldehyde. Analogous to AEGL-1 values, a total uncertainty factor of 10 was used to derive the AEGL-2 values; a larger uncertainty factor would lead to unrealistically low values. AEGL-2 values for other time periods were derived by time scaling.

For AEGL-3 values, two studies of the acute lethality of chloroacetaldehyde (Dow Chemical Company 1952; Arts 1987) were considered. The mortality data showed a steep concentration-response curve; mortality shifted from 0% to close to 100% when concentration or exposure duration was doubled. Mortality data from the Arts (1987) study, in which rats were exposed to chloroacetaldehyde at 44-2,643 ppm for 1 h, was modeled using EPA benchmark dose software (version 1.3.2) (EPA 2005). A benchmark concentration associated with a 5% response (BMC_{05}) of 136 ppm was calculated, with a lower 95% confidence limit ($BMCL_{05}$) of 99 ppm. Application of a total uncertainty factor of 10, on the same basis as it used in deriving AEGL-1 and AEGL-2 values results, in a 1-h AEGL value of 9.9 ppm. AEGL-3 values for the other time periods were derived by time scaling.

The AEGL values for chloroacetaldehyde are presented in Table 2-1.

TABLE 2-1 Summary of AEGL Values for Chloroacetaldehyde

Classification	10 min	30 min	1 h	4 h	8 h	End Point (Reference)
AEGL-1 (nondisabling)	2.3 ppm (7.4 mg/m^3)	2.3 ppm (7.4 mg/m^3)	1.3 ppm (4.2 mg/m^3)	0.40 ppm (1.3 mg/m^3)	0.22 ppm (0.71 mg/m^3)	Ocular and nasal irritation (Dow Chemical Company 1952)
AEGL-2 (disabling)	9.8 ppm (31 mg/m^3)	3.9 ppm (13 mg/m^3)	2.2 ppm (7.1 mg/m^3)	0.69 ppm (2.2 mg/m^3)	0.39 ppm (1.5 mg/m^3)	Pulmonary edema (Arts 1987)
AEGL-3 (lethal)	44 ppm (140 mg/m^3)	18 ppm (57 mg/m^3)	9.9 ppm (32 mg/m^3)	3.1 ppm (10 mg/m^3)	1.8 ppm (5.6 mg/m^3)	Mortality, $BMCL_{05}$ (Dow Chemical Company 1952; Arts 1987)

1. INTRODUCTION

Chloroacetaldehyde is a colorless, volatile liquid with an acrid, penetrating odor. It evaporates easily and dissolves in water. Chloroacetaldehyde is not flammable, but vapor/air mixtures may be explosive at temperatures above 88°C (see Table 2-2).

Chloroacetaldehyde can exist in combinations of four forms depending on how it was prepared: monomer, monomer hydrate, dimer hydrate, and cyclic trimer (Elmore et al. 1976). The monomer and dimer hydrates are formed instantly when the anhydrous monomer is added to water. The cyclic trimer is formed from the anhydrous monomer upon standing under dry conditions. The cyclic trimer is only slightly soluble in water, but the monomer and dimer hydrates are formed when the cyclic trimer is heated in water. The anhydrous monomer is obtained by cracking the cyclic trimer.

Commercial aqueous solution of chloroacetaldehyde (45%) contains a 50:50 mixture of the monomer and dimer hydrates (Elmore et al. 1976). The two hydrates are dehydrated and converted to the monomer under gas-liquid phase chromatography conditions. Chloroacetaldehyde is sufficiently stable to permit its direct collection on silica gel and subsequent storage in a freezer.

TABLE 2-2 Chemical and Physical Properties for Chloroacetaldehyde

Parameter	Value	Reference
CAS registry no.	107-20-0	HSDB 2009
Synonyms	Monochloroacetaldehyde; 2-chloro-1-ethanal	Budavari et al. 1989
Chemical formula	C_2H_3ClO	Budavari et al. 1989
Molecular weight	78.50	Budavari et al. 1989
Physical state	Liquid	Budavari et al. 1989
Color	Colorless	HSDB 2009
Odor	Acrid, penetrating	Budavari et al. 1989
Melting point	-16.3°C (40% aqueous solution)	IPCS 2005
Boiling point	85-86°C (pure) 85-100°C 90-100°C (40% aqueous solution)	Budavari et al. 1989 IPCS 2005 OSHA 1989
Vapor density (air = 1)	2.7	IPCS 2005
Liquid density (water = 1)	1.19 (40% aqueous solution)	IPCS 2005
Solubility in water	Yes	Budavari et al. 1989
Vapor pressure	100 mm Hg at 45°C (40% aqueous solution) 110 mm Hg at 20°C	NIOSH 1991 HSDB 2009
Explosive	Vapor/air mixtures may be explosive (40% aqueous solution) above 88°C	IPCS 2005
Conversion factors	1 mg/m^3 = 0.312 ppm 1 ppm = 3.21 mg/m^3	NIOSH 2011

No current information was found on the chemical production of chloroacetaldehyde. The amount of chloroacetaldehyde manufactured or imported in the United States in 1977 was reported to be 1-10 million pounds (EPA 1987).

Chloroacetaldehyde is used primarily as a chemical intermediate (EPA 1987) in the manufacture of 2-aminothiazole and other compounds (ACGIH 1991). It is also used in the control of algae, bacteria, and fungi in water, and in a spinning solution of poly β-alanine (ACGIH 1991). Furthermore, it has its application in tree-trunk debarking operations and in analytical chemistry as a fluorescent label (McCann 1975).

2. HUMAN TOXICITY DATA

2.1. Acute Lethality

No case reports on human deaths from acute exposure to chloroacetaldehyde were found.

2.2. Nonlethal Toxicity

Case reports of nonlethal toxicity in humans were not found, nor were occupational or epidemiologic studies available. A report of the Dow Chemical Company (1952) on acute mortality in experimental animals stated the following: "Every concentration employed including the lowest (10 ppm) produced lacrimation and nasal irritation in humans within a few minutes." No additional details were provided.

Several studies have investigated the toxicity of the antineoplastic agent ifosfamide which indicate a causative role for chloroacetaldehyde (the main metabolite of ifosfamide) in the development of nephrotoxicity (Loebstein et al. 1999; Skinner et al. 2000; Aleksa et al. 2001; Yaseen et al. 2008; Hanly et al. 2009). Additional information on ifosfamide-induced nephrotoxicity is reviewed in Section 4.2.

2.3. Neurotoxicity

No reports on neurotoxicity induced by chloroacetaldehyde were found. Goren et al. (1986), however, suggested a causative role for chloroacetaldehyde (a main metabolite of the anticancer drug ifosfamide) in the development of neurotoxic side-effects of ifosfamide chemotherapy. Cerebellar dysfunction, seizures, and changes in mental status were reported in as many as 30% of patients on high-dose treatment with ifosfamide. Blood concentrations of chloroacetaldehyde were 88 micromoles per liter (μmol/L) at 6 h and 109 μmol/L at 24 h in two patients with neurotoxic effects (somnolescence, urinary incontinence, and inappropriate behavior), compared with 45 μmol/L at 6 h and 22 μmol/L at 24 h

in four patients without such effects. Rieger et al. (2004) conducted a retrospective trial of 60 cancer patients receiving ifosfamide as part of multiple drug chemotherapy regimens to evaluate potential risk factors for ifosfamide-induced encephalopathy. Sixteen patients (26.6%) developed neurologic symptoms; the effects were not correlated with age, sex, hepatic function, or renal function.

2.4. Developmental and Reproductive Toxicity

No studies on developmental or reproductive toxicity of chloroacetaldehyde in humans were found.

2.5. Genotoxicity

No studies on the genotoxicity of chloroacetaldehyde in humans or on human cells were found.

2.6. Carcinogenicity

No carcinogenicity studies of chloroacetaldehyde in humans were found.

2.7. Summary of Human Data

No information on chloroacetaldehyde toxicity in humans was available, other than a brief statement that chloroacetaldehyde at 10 ppm produced lacrimation and nasal irritation in humans (Dow Chemical Company 1952). Studies with ifosfamide suggest a causative role for chloroacetaldehyde (a main metabolite of the drug) in neurologic and renal effects.

3. ANIMAL TOXICITY DATA

3.1. Acute Lethality

A summary of the data on acute lethality in laboratory animals exposed to chloroacetaldehyde is presented in Table 2-3.

3.1.1. Guinea Pigs

Guinea pigs (10 animals/group; sex and strain not specified) were exposed to chloroacetaldehyde at target concentrations of 25 (for 7 h), 50 (for 4 h), 100 (for 2 h), or 400 ppm (for 0.5 h). No details were provided on the purity the chloroacetaldehyde or the exposure conditions. Target concentrations were monitored during the experiment, but the method and measurements were not specified. It was not clear whether an unexposed control group was used. Mortality was observed only at 400 ppm; seven of 10 guinea pigs died (Dow Chemical Company 1952).

3.1.2. Rats

Rats (19 or 20 animals/group; sex and strain not specified) were exposed to chloroacetaldehyde at target concentrations of 10 (for 7 h), 25 (for 7 h), 50 (for 1, 3.5, or 4 h), 100 (for 0.2 or 2 h), or 400 ppm (for 0.1, 0.25, or 0.5 h). No details were provided on the purity of the chloroacetaldehyde or the exposure conditions. Target concentrations were monitored during the experiment, but the method and measurements were not specified. It was not clear whether an unexposed control group was used. Mortality was related to the concentration and exposure duration (see Table 2-3). No mortality was occurred at concentrations (and durations) of 10 ppm (for 7 h), 50 ppm (for 1 h), and 100 ppm (for 0. 2 h). One animal died at 400 ppm (0.1 h). Almost all of the rats died at 25 ppm (for 7 h), 50 ppm (for 3.5 h and 4 h), 100 ppm (for 2 h), and 400 ppm (for 0.25 and 0.5 h) (Dow Chemical Company 1952).

TABLE 2-3 Acute Lethality in Animals Exposed to Chloroacetaldehyde

Species	Concentration (ppm)	Exposure Duration	Mortality	Reference
Guinea pig (n = 10)	25	7 h	0/10	Dow Chemical Company 1952
	50	4 h	0/10	
	100	2 h	0/10	
	400	0.5 h	7/10	
Rat (n = 19-20)	10	7 h	0/20	Dow Chemical Company 1952
	25	7 h	19/20	
	50	1 h	0/20	
		3.5 h	20/20	
		4 h	18/20	
	100	0.2 h	0/19	
		2 h	20/20	
	400	0.1 h	1/20	
		0.25 h	20/20	
		0.5 h	19/20	
Rat (n = 10)	44	1 h	0/10	Arts 1987
	159		3/10	
	203		4/10	
	243		10/10	
	309		10/10	
	596		10/10	
	2,643		10/10	
	203-243	1 h	LC_{50}	

3.2. Nonlethal Toxicity

SPF-reared Borr:WISW rats (five animals/sex/group) were exposed (whole body; individually housed) for 1 h to mean concentrations of chloroacetaldehyde (45.4% (w/w) at 44, 159, 203, 243, 309, 596, or 2,643 ppm (0.14, 0.51, 0.65, 0.78, 0.99, 1.91, or 8.47 g/m^3, respectively). Concentrations of chloroacetaldehyde were continuously monitored during exposure. No unexposed control animals were used. Relative humidity was high (51-91%) during exposure, due, in part, to the large amount of water in the test material. The animals were observed for up to 2 weeks. Mortality rates of 0% (44 ppm), 30% (159 ppm), 40% (203 ppm), and 100% (≥243 ppm) were found (see Table 2-3). Deaths were observed during exposure (at the two highest concentrations) or within several hours or 1-2 days after exposure. A 1-h LC_{50} value of 203-243 ppm was estimated. Because of the steep concentration-effect curve and natural variability between groups, it was not possible to determine an LC_{50} value for chloroacetaldehyde with 95% confidence intervals. The LC_{50} value was estimated to be closer to 203 ppm, because a considerable decrease in body weight that would probably have lead to death was observed in some animals exposed at 159 and 203 (Arts 1987).

A summary of the nonlethal effects of chloroacetaldehyde in laboratory animals is presented in Table 2-4.

3.2.1. Guinea Pigs and Rabbits

In the acute inhalation experiment with guinea pigs describe earlier (see Section 3.1.1.), ocular and nasal irritation was found very early during exposure at all concentrations tested (25-400 ppm) (Dow Chemical Company 1952). The degree of irritation was related to concentration. Labored breathing was also observed at the higher concentrations, and slight drowsiness was apparent at some concentrations (not specified).

In a repeated-exposure study by Dow Chemical Company (1952), groups of five male guinea pigs and one female rabbit (strains not specified) were exposed to chloroacetaldehyde at 0 or 5 ppm for 7 h/day, 5 days/week, for a total of eight exposures in 10 days. No details were provided on the purity of chloroacetaldehyde, actual or nominal concentrations, or exposure conditions. Slight ocular irritation was observed in the rabbit, but it was unclear from the report whether nasal irritation was also present. No irritating effects were reported in the guinea pigs. No effect on growth, organ weights, and gross pathology were found in either species.

3.2.2. Rats

Rats (19 or 20 animals/group; sex and strain not specified) were exposed to chloroacetaldehyde at target concentrations of 10 (for 7 h), 25 (for 7 h), 50 (for 1, 3.5, or 4 h), 100 (for 0.2 or 2 h), or 400 ppm (0.1, 0.25, or 0.5 h). No

details were provided on the purity of the chloroacetaldehyde or the exposure conditions. Target concentrations were monitored during the experiment, but the method and measurements were not specified. It was not clear whether an unexposed control group was used. Ocular and nasal irritation was observed very early during exposure at all concentrations. Degree of irritation was related to concentration and duration of exposure. Labored breathing was also observed at the higher concentrations, and slight drowsiness was apparent at some concentrations (not specified) (Dow Chemical Company 1952).

Groups of five male and five female rats (strain not specified) were exposed to chloroacetaldehyde at 0 or 5 ppm for 7 h/day, 5 days/week, for a total of eight exposures in 10 days. No details were provided on the purity of chloroacetaldehyde, actual or nominal concentrations, or exposure conditions. Exposed rats exhibited slight nasal irritation and very slight ocular irritation. Growth of the male rats was slightly depressed, while the growth of the female rats was comparable to that of the control animals. No effects on organ weight or gross pathology were found (Dow Chemical Company 1952).

SPF-reared Borr:WISW rats (five animals/sex/group) were exposed (whole body; individually housed) for 1 h to mean concentrations of chloroacetaldehyde (45.4% (w/w) at 44, 159, 203, 243, 309, 596, or 2,643 ppm (0.14, 0.51, 0.65, 0.78, 0.99, 1.91, or 8.47 g/m^3, respectively). Concentrations of chloroacetaldehyde were continuously monitored during exposure. No unexposed control animals were used. Relative humidity was high (51-91%) during exposure, due, in part, to the large amount of water in the test material. The animals were observed for up to 2 weeks. Descriptions of the observations were generally reported and did not always specify the number of animals affected or the exposure concentrations. Rats were restless and showed signs of discomfort (closed eyes, salivation, and, at the higher concentrations, wet nares, nasal discharge, and wet and soiled heads and breasts). At the highest concentration of 2,643 ppm, all rats exhibited labored respiration, accompanied by dyspnea and mouth breathing. Mortality was observed at all concentration, except the lowest of 44 ppm (see Table 2-3). Many of the rats that died had bloodstains around the nose and mouth. Rats exposed at the highest concentrations that did not die immediately were reported to have breathed "wheezingly". Two rats exposed at 596 ppm became blind. No chloroacetaldehyde-induced effects on body weight we found, although two animals in the 159- and 203-ppm groups lost a considerable amount of weight. Animals that died during exposure or within the first 2 days of observation had pulmonary edema, which was accompanied in some cases by atelectasis and in most cases by hydrothorax. The investigators suggested that the latter finding could be explained by induced hypertension, although no information was provided to support that conclusion. Pulmonary edema was also observed in some animals exposed at the three lowest concentrations. The investigators concluded that the pulmonary effects suggested an impairment of pulmonary function. The stomachs and intestines were often filled with air because of mouth breathing, and an occasional thrombus was detected in the heart area (Arts 1987).

3.2.3. Mice

Groups of five female mice (strain not specified) were exposed to chloroacetaldehyde at 0 or 5 ppm for 7 h/day, 5 days/week for a total of eight exposures in 10 days. No details were provided on the purity of the chloroacetaldehyde, actual or nominal concentrations, or the exposure conditions. Mice exhibited slight nasal irritation. No effects on growth, organ weights, or gross pathology were found.

TABLE 2-4 Nonlethal Toxicity in Animals Exposed to Chloroacetaldehyde

Species	Concentration (ppm)	Exposure Duration	Effects	Reference
Guinea pig (n = 10)	25 50 100 400	7 h 4 h 2 h 0.5 h	Concentration-related ocular and nasal irritation; labored breathing at the higher concentrations; slight drowsiness (concentrations not specified)	Dow Chemical Company 1952
Guinea pig (n = 5)	5	7 h/d, 5 d/wk, 8 exposures in 10 d	No effects reported	Dow Chemical Company 1952
Rabbit (n = 1)	5	7 h/d, 5 d/wk, 8 exposures in 10 d	Slight ocular irritation	Dow Chemical Company 1952
Rat (n = 19 or 20)	10 25 50 100 400	7 h 7 h 1, 3.5, 4 h 0.2, 2 h 0.1, 0.25, 0.5 h	Concentration- and duration-related ocular and nasal irritation; labored breathing at higher concentrations; slight drowsiness (concentrations not specified)	Dow Chemical Company 1952
Rat (n = 10)	5	7 h/d, 5 d/wk, 8 exposures in 10 d	Slight nasal irritation, very slight ocular irritation	Dow Chemical Company 1952
Rat (n = 10)	44 159 203 243 309 596 2,643	1 h	At all concentrations, closed eyes, salivation, and decreased pulmonary function (e.g., pulmonary edema [with some atelectasis and hydrothorax], labored breathing). At higher concentrations, wet nares, nasal discharge, wet and soiled heads and breasts.	Arts 1987
Mouse (n = 5)	5	7 h/d, 5 d/wk, 8 exposures in 10 d	Slight nasal irritation	Dow Chemical Company 1952

3.3. Neurotoxicity

No neurotoxicity studies on experimental animals exposed to chloroacetaldehyde were found. However, a study on metabolism in rats found that chloroacetaldehyde specifically affected mitochondrial long-chain fatty acid metabolism. The rate of palmitic-acid oxidation, but not that of succinic-acid or octanoic-acid oxidation, was affected. Such changes in fatty-acid metabolism might play a role in encephalopathy and chronic fatigue observed after treatment with the antitumor alkylating agents ifosfamide and cyclophosphamide, which form chloroacetaldehyde as a main reactive metabolite (Visarius et al. 1999).

3.4. Developmental and Reproductive Toxicity

No studies on developmental or reproductive toxicity of chloroacetaldehyde in experimental animals were found.

3.5. Genotoxicity

Early indications of the genotoxicity of chloroacetaldehyde came from studies on the highly genotoxic compound vinyl chloride. The genotoxicity of vinyl chloride has been attributed to its metabolite chloroacetaldehyde, among others. The genotoxicity of chloroacetaldehyde was reviewed by Bartsch et al. (1976). Mutagenic responses were seen with *Salmonella typhimurium* TA100, TA1530, and TA1535. Relatively high toxicity in *S. typhimurium* TA1530 was found for chloroacetaldehyde compared with other mutagenic metabolites of vinyl chloride. A post-mitochondrial mouse-liver fraction decreased the mutagenic effect of chloroacetaldehyde on *S. typhimurium* TA100. Chloroacetaldehyde was further shown to be a strong mutagen in the Chinese hamster V79 cell system, inducing 8-azaguanine-resistant mutants. The chemical was also high cytotoxicity to those cells. Chloroacetaldehyde reacted covalently with adenine and cytidine in vitro. ACGIH (1991) reported that chloroacetaldehyde was mutagenic in the forward mutation system of *Aspergillus nidulans*, and in the forward and back mutation system of *Streptomyces coelicolor*.

3.6. Carcinogenicity

No carcinogenicity studies on inhalation exposure to chloroacetaldehyde were found. For other routes of exposure (skin application, skin initiation-promotion [promoter: phorbol myristate acetate], repeated subcutaneous injections, and intragastric feeding), Van Duuren et al. (1979) found that chloroacetaldehyde was not carcinogenic in male and female Ha:ICR Swiss mice, despite the fact that chloroacetaldehyde was mutagenic in microorganisms and Chinese hamster V-79 cells and was strongly implicated as the proximal carcinogenic

metabolite formed from the animal and human carcinogen vinyl chloride (see Bartsch et al. 1976; Goldschmidt 1984; NRC 2012). However, chloroacetaldehyde was not hepatocarcinogenic in male $B6C3F_1$ mice (Daniel et al. 1992). In that study, chloroacetaldehyde was administered in drinking water at a target concentration of 0.1 g/L (mean measured dose: 0.095 ± 0.006 g/L) for 104 weeks. The mean daily-ingested dose was calculated to be 17 mg/kg/day. The only obvious target organ was the liver, as indicated by an increase in the absolute and relative organ weight. A significant increase in the prevalence of hepatic tumors was found. Prevalence rates for carcinomas, adenomas, and combined tumors (adenomas and carcinomas) were 31, 8, and 38% in treated rats, compared with 10, 5, and 15% among controls. Furthermore, hepatocellular hyperplastic nodules (a preneoplastic lesion) occurred in the liver in the chloroacetaldehyde group (8%) but not in the control group. Histotologic effects in the liver were mild and included cytomegaly, necrosis, and chronic active inflammation.

3.7. Summary of Animal Data

The database on chloroacetaldehyde is poor. Most of the available data are on the lethal and irritant effects of chloroacetaldehyde. In rats, the lowest concentration-duration combinations found to induce lethality ranged from 25 ppm for 7 h (19 of 20 rats died) to 400 ppm for 0.1 h (1 of 20 rats died). No deaths were reported in rats at concentration-duration combinations ranging from 10 ppm for 7 h to 100 ppm for 0.2 h. Lethality increased both with concentration and with duration. A 1-h LC_{50} for rats was estimated to be between 203 and 243 ppm. Guinea pigs were less sensitive than rats to chloroacetaldehyde.

Chloroacetaldehyde is a strong corrosive agent, and has been shown to be very irritating to the eyes and nose of laboratory animals. These effects occur soon after onset of exposure and are related to concentration and duration of exposure. Repeated exposure to chloroacetaldehyde at 5 ppm induced slight nasal and ocular irritation. Irritation became more pronounced with single exposures to chloroacetaldehyde at concentrations greater than 10 ppm. Pulmonary edema was observed in some rats 2 weeks after exposure to chloroacetaldehyde at 44 ppm for 1-h. Closed eyes and salivation were also observed at that concentration. Pulmonary effects became more severe with increasing concentrations. Animals that died had pulmonary edema, often accompanied by atelectasis and hydrothorax. Although no neurotoxicity studies of chloroacetaldehyde were found, some indirect indications of such effects are suggested by experiments with ifosfamide and cyclophosphamide.

Chloroacetaldehyde was found to be mutagenic in several stains of *S. typhimurium*, *A. nidulans*, *S. coelicolor*, and Chinese hamster V79 cells.

Little information was available on the carcinogenicity of chloroacetaldehyde. However, the target organ for chloroacetaldehyde appears to the liver, as

evidenced by increased absolute and relative liver weights, increased hepatic tumors and preneoplastic lesions, and mild histologic effects.

4. SPECIAL CONSIDERATIONS

4.1. Metabolism and Disposition

Absorption, Distribution, and Excretion

No reports on absorption, distribution, and excretion of chloroacetaldehyde were found.

Metabolism

Joqueviel et al. (1997) summarized information on the metabolic pathways of chloroacetaldehyde. Little information was available on the metabolism of chloroacetaldehyde. What information was available was from in vitro and in vivo studies of rat hepatocytes. The metabolic pathways of chloroacetate and chloroethanol were better documented, and were used to develop a metabolism scheme. In brief, chloroacetaldehyde can be oxidized to chloroacetic acid by aldehyde dehydrogenase, followed by conjugation with glutathione, and finally the formation of the urinary metabolite thiodiglycolic acid. Chloroacetaldehyde may also be directly conjugated with glutathione. This scheme was in close agreement with the metabolic pathways for vinyl chloride (ATSDR 1997; NRC 2012).

4.2. Mechanism of Toxicity

No in vivo studies on the mechanism of toxicity of chloroacetaldehyde following inhalation exposure in humans or animals were found. Studies of other routes of exposure included a study of rats exposed to chloroacetaldehyde by intraperitoneal injection (Visarius et al. 1999) and in vitro studies using isolated rat hepatocytes (Sood and O'Brien 1993, 1994) and liver enzymes (Sharpe and Carter 1993), human kidney tubules (Dubourg et al. 2001, 2002), and perfused rabbit hearts (Joqueviel et al. 1997). In addition, studies of two human lung-cancer cell lines were available (Manzano et al. 1996). In all of these studies, chloroacetaldehyde was investigated as a metabolite of vinyl chloride or the alkylating antitumor agents ifosfamide and cyclophosphamide to understand the mechanism of hepatoxicity, nephrotoxicity, cardiotoxicity, encephalopathy, and chronic fatigue observed after exposure or treatment with those agents. These studies are summarized below. Collectively, they indicate that chloroacetaldehyde has the potential to cause serious effects in different organs once it becomes systemically available. However, because chloroacetaldehyde is very

reactive, effects on the respiratory tract are likely to be predominant following inhalation exposure. Although the potential for chloroacetaldehyde to produce systemic effects cannot be ruled out, it is probable that the respiratory tract will be most sensitive to inhalation exposure; this is supported by results of studies in animals (see Section 3.2).

Hepatoxicity

Chloroacetaldehyde induced a loss in viability of isolated rat hepatocytes in a concentration- and time-dependent manner. Chloroacetaldehyde was metabolized rapidly and the cytotoxic effects were irreversible (Sood and O'Brien 1993). Cytosolic and mitochondrial rat-liver aldehyde dehydrogenases seemed to play a significant role in the metabolism of chloroacetaldehyde (Sharpe and Carter 1993; Sood and O'Brien 1994). Cytotoxicity in isolated rat hepatocytes was enhanced markedly if hepatocyte alcohol or aldehyde dehydrogenase was inhibited before exposure to chloroacetaldehyde. Furthermore, the metabolites chloroacetate and chloroethanol were far less toxic than chloroacetaldehyde. Sood and O'Brien (1993, 1994) found that the concentration of glutathione, reversible thiol protein adduct formation (such as hemithioacetals or thioacetals), mitochondrial toxicity, and lipid peroxidation were involved in chloroacetaldehyde-induced hepatocyte cytotoxicity. Hepatocytes from fasted rats were more susceptible to chloroacetaldehyde. These in vitro results were confirmed in vivo by Visarius et al. (1999).

Nephrotoxicity

Dubourg et al. (2001) investigated the mechanism of nephrotoxicity on isolated tubular (mainly proximal) fragments of human kidney cortex. Chloroacetaldehyde was highly toxic to human kidney tubules. A dramatic decrease in cellular adenosine triphosphate (ATP) concentrations occurred. Concentrations of CoA (substrate of pyruvate dehydrogenase) and acetyl-CoA (activator of pyruvate carboxylase) were virtually depleted. The correlation between the cellular depletion of CoA and acetyl-CoA and nephrotoxic effects demonstrated the importance of thiol compounds in the mechanism of the nephrotoxicity. Chloroacetaldehyde was metabolized at high rates, presumably by oxidation via aldehyde dehydrogenase (a very active enzyme in human kidneys). Chloroacetate, which is less toxic than chloroacetaldehyde, was the only major product of chloroacetaldehyde metabolism by human kidney tubules. Results of this study strongly suggest that chloroacetaldehyde is detoxified by metabolism at high rates to the non-nephrotoxic metabolite chloroacetate and by binding to thiol compounds in isolated human kidney tubules. Dubourg et al. (2002) demonstrated that pediatric tubules are not more sensitive than adult tubules to the toxic effects of chloroacetaldehyde and the rate of chloroacetaldehyde uptake.

Several studies have investigated the mechanism of toxicity of ifosfamide, and indicate a causative role for chloroacetaldehyde in the development of nephrotoxicity (Loebstein et al. 1999; Skinner et al. 2000; Aleksa et al. 2001; Yaseen et al. 2008; Hanly et al. 2009). Ifosfamide is a pro-drug that undergoes intracellular oxidization to its active form by cytotochome P450 monooxygenases in the liver and kidneys; in the process, chloroacetaldehyde and acrolein are formed. Ifosfamide-induced nephrotoxicity might occur in any section of the nephron, but most commonly occurs in the proximal tubule (Hanly et al. 2009). Several mechanisms are probably involved in the development of chloroacetaldehyde-induced nephrotoxicity, including ATP depletion, inhibition of ATPase, collapse of the cellular protein gradient though alterations of mitochondria, the generation of reactive oxygen species, and inhibition of endocytosis (Yaseen et al. 2008; Hanly et al. 2009). Results of a clinical study by Loebstein et al. (1999) suggest that age is a risk factor in the development of ifosfamide-induced nephrotoxicity, with younger patients at greater risk possibly because they have a greater rate of renal metabolism (Loebstein et al. 1999). However, Skinner et al. (2000) did not find an association between age and ifosfamide-induced nephrotoxicity. Differences in study designs and other confounding factors (e.g., concomitant therapy with other antineoplastic agents) might have contributed to conflicting results regarding age dependence of ifosfamide-induced nephrotoxicity (Aleksa et al. 2001).

Cardiotoxicity

Lawrence et al. (1972) reported that intravenous injections of chloroacetaldehyde produced lethal cardiotoxicity in less than 90 min in rabbits; the greater the dose, the earlier the cardiac arrest. The mechanism of cardiotoxicity was studied in more detail in an isolated perfused-rabbit-heart model by Joqueviel et al. (1997). Numerous arrhythmias followed by a decrease in the number and strength of ventricular contractions and swelling of the heart were observed after chloroacetaldehyde treatment. The major metabolite formed was chloroacetate, which was far less toxic than chloroacetaldehyde and did not result in cardiotoxic symptoms. Their findings suggest that the mechanism of cardiotoxicity might be similar to that found in rat hepatocytes and in a rat renal model (lipid peroxidation, glutathione depletion, reversible thiol-protein-adduct formation, and mitochondrial toxicity with ATP depletion).

Lung

Manzano et al. (1996) demonstrated in two human lung-cancer cell lines (large-cell carcinoma cell line COR-L23/R and adenocarcinoma cell line MOR/R04) that chloroacetaldehyde depleted intracellular glutathione concentrations in the lung.

4.3. Other Relevant Information

4.3.1. Irritation and Sensitization

Chloroacetaldehyde vapor was highly irritating to the nose and eyes of guinea pigs, rabbits, rats, and mice (5-400 ppm for 6 min to 7 h; single or repeated exposure) (Dow Chemical Company 1952). Irritation was observed very early in exposure and at low concentrations (single exposure at ≥10 ppm; repeated exposure at ≥5 ppm) (see Section 3.2.). The degree of irritation increased with concentration and exposure duration. Lacrimation and nasal irritation were also reported in humans at 10 ppm within a few minutes (no further details provided). In addition, the following signs of discomfort that might be related to nasal or ocular irritation were reported in rats exposed at 44-2,643 ppm for 1 h: closed eyes, wet nares, nasal discharge (at the higher concentrations), blindness (at 596 ppm), and bloodstains around the nose and mouth of the rats that died (≥159 ppm) (Arts 1987)]. Salivation and labored respiration (at 2,643 ppm), accompanied by dyspnea and mouth breathing, suggest that chloroacetaldehyde was irritating to the throat and respiratory tract.

In the European Union, chloroacetaldehyde has been labeled as C; R34 (corrosive; causes burns) (EC/JRC 2012).

5. DATA ANALYSIS FOR AEGL-1

5.1. Summary of Human Data Relevant to AEGL-1

No information is available on chloroacetaldehyde toxicity in humans other than a brief statement that a concentration of 10 ppm produced lacrimation and nasal irritation in humans within a few minutes of exposure (Dow Chemical Company 1952).

5.2. Summary of Animal Data Relevant to AEGL-1

The database on chloroacetaldehyde is poor. The few studies available indicate that its predominant effect is direct, strong irritation of the eyes, nose, and lungs (resulting in pulmonary edema and death) (Dow Chemical Company 1952; Arts 1987). Effects appeared to be related to concentration and exposure duration, and the studies indicate a very steep concentration-response relationship. The effects occur at concentrations of 10 ppm and higher in rats and guinea pigs and appear soon after onset of exposure. Repeated exposure at 5 ppm (7 h/day, eight exposures in 10 days) induced slight nasal or ocular irritation in rabbits, rats, and mice. Guinea pigs were less sensitive (Dow Chemical Company 1952).

5.3. Derivation of AEGL-1

The predominant effect of acute exposures to chloroacetaldehyde is irritation of the eyes and respiratory tract. Because no direct information is available on chloroacetaldehyde toxicity in humans, the AEGL-1 values are based on animal data. A single 7-h exposure to chloroacetaldehyde at concentrations of 10 ppm and higher in rats and at 25 ppm and higher in guinea pigs induced ocular and nasal irritation. However, effects were very slight to slight in rats, mice, and one rabbit exposed daily at 5 ppm (the lowest concentration tested) for 7 h per day for up to eight exposures in 10 days. No effects were reported in guinea pigs (Dow Chemical Company 1952). No effects were found during gross pathologic exams or on organ weights. A concentration of 5 ppm was chosen as the point of departure for calculating the AEGL-1 values, and a modifying factor of 2 was applied to obtain a no-observed-adverse-effect level of 2.5 ppm. Although the critical effect is local irritation, the irritation was related to both concentration and exposure duration. Therefore, it was considered inappropriate to set the same values for all time periods. Instead, time scaling was performed using the equation $C^n \times t = k$. The value of n was determined to be 1.2 based on mortality data (see Section 7.3).

A total uncertainty factor of 10 (two factors of 3) was considered sufficient for toxicokinetic and toxicodynamic differences between species and in individual variability. Irritant effects were attributed to direct interaction of chloroacetaldehyde; therefore, no relevant differences in kinetics were assumed. The resulting AEGL-1 values are presented in Table 2-5. The 10-min AEGL-1 value was set equal to the 30-min value because extrapolation from a 7-h exposure to a 10-min value had too much uncertainty. The report of lacrimation and nasal irritation in humans within a few minutes of exposure to chloroacetaldehyde at 10 ppm (Dow Chemical Company 1952) provides support for deriving AEGL-1 values on the basis of the rat data.

6. DATA ANALYSIS FOR AEGL-2

6.1. Summary of Human Data Relevant to AEGL-2

No human data relevant to effects defined by AEGL-2 were found.

6.2. Summary of Animal Data Relevant to AEGL-2

Decreased pulmonary function, pulmonary edema, closed eyes, and blindness were the relevant effects for deriving AEGL-2 values for chloroacetaldehyde. Effects on the lungs were found in guinea pigs and rats (Dow Chemical Company 1952). Chloroacetaldehyde at nominal concentrations of 10-400 ppm for durations ranging from 7 h to 6 min caused labored breathing in rats at the

TABLE 2-5 AEGL-1 Values for Chloroacetaldehyde

10 min	30 min	1 h	4 h	8 h
2.3 ppm (7.4 mg/m^3)	2.3 ppm (7.4 mg/m^3)	1.3 ppm (4.2 mg/m^3)	0.40 ppm (1.3 mg/m^3)	0.22 ppm (0.71 mg/m^3)

higher concentrations tested. Furthermore, at some concentrations (not specified), slight drowsiness was apparent. No effects on organ weights or gross pathology findings were observed in rats, mice, guinea pigs, or one rabbit exposed to chloroacetaldehyde at 5 ppm for 7 h/day, for eight exposures in 10 days. Pulmonary toxicity was confirmed in a well-performed and adequately reported study of rats exposed to chloroacetaldehyde at concentrations of 44-2,643 ppm (analytically determined) for 1 h (Arts 1987). Shortly after the start of the experiment, labored respiration accompanied by dyspnea and mouth breathing was detected in all animals at the highest concentration (2,643 ppm). Closed eyes were also observed (concentrations not specified). Rats exposed at the highest concentrations that did not die immediately were described as breathing "wheezingly". The animals that died during exposure or within the first 2 days of observation had pulmonary edema accompanied in some cases by atelectasis and in most cases by hydrothorax. The latter finding could be explained by induced hypertension. Pulmonary edema was also observed in some animals in the three lowest-concentration groups (number of animals affected was not reported) that were killed at the end of the 2-week observation period. Effects indicated impairment of pulmonary function.

6.3. Derivation of AEGL-2

Impaired pulmonary function was the most relevant adverse effect for deriving AEGL-2 values for chloroacetaldehyde. A concentration of 44 ppm was the lowest-observed-adverse-effect level for this effect. A modifying factor or 2 was applied to obtain a point of departure of 22 ppm, because of an incomplete database; specifically, a no-effect level for AEGL-2 effects could not be identified, and effects were more severe than those that define AEGL-2 values. According to NRC (2001), application of an additional modifying factor may be necessary when an incomplete database exists. The modifying factor represents an adjustment for uncertainties in the overall database. It "reflects professional judgment on the entire database available for the specific agent" and is applied on a case-by-case basis. Data from Tables 2-3 and 2-4 indicate that the concentration-response curve for chloroacetaldehyde is very steep (e.g., about a two-fold difference in exposure duration or concentration between 0% or 100% mortality). Therefore a relatively small modifying factor of 2 was considered sufficient to derive a no-effect level.

A total uncertainty factor of 10 (two factors of 3) was considered sufficient for toxicokinetic and toxicodynamic differences between species and for individual variability for the following reasons. The effects were attributed to direct interaction of chloroacetaldehyde; therefore, no relevant differences in kinetics were assumed. The concentration-response curve appears to be very steep, indicating that a larger factor is unnecessary. Considering the effects at the lower exposure concentrations, a higher uncertainty factor would lead to unrealistically low values for AEGL-2. For time periods other than 1 h, time scaling was performed using the equation $C^n \times t = k$, with n = 1.2 based on mortality data (see Section 7.3). The resulting AEGL-2 values are presented in Table 2-6.

7. DATA ANALYSIS FOR AEGL-3

7.1. Summary of Human Data Relevant to AEGL-3

No adequate human data that address the level of effects defined by the AEGL-3 were found.

7.2. Summary of Animal Data Relevant to AEGL-3

Two studies of the acute lethality of chloroacetaldehyde were available (Dow Chemical Company 1952; Arts 1987). One study provided mortality data in rats and guinea pigs for exposures varying both in concentration and exposure duration (Dow Chemical Company 1952). At the concentrations tested, mortality was either 0% or close to 100%. The concentration-response curve was very steep, and rats were more susceptible than guinea pigs to chloroacetaldehyde. Slight nasal irritation was observed in rats exposed at 5 ppm for 7 h/day for eight exposures in 10 days, but nearly all of the rats exposed once at 25 ppm for 7 h died (Dow Chemical Company 1952). Arts (1987) studied mortality in rats exposed to chloroacetaldehyde for 1 h at several concentrations. Because of the steep concentration-response curve and the natural variability between the groups, it was not possible to determine an exact LC_{50} with a 95% confidence interval. The LC_{50} was between 203 and 243 ppm (mortality rates of 4/10 and 10/10, respectively).

7.3. Derivation of AEGL-3

Mortality data from Dow Chemical Company (1952) show a steep concentration-response curve for chloroacetaldehyde. Doubling of the concentration or the exposure duration shifted mortality from 0% to close to 100%. Concentration-response modeling of the data resulted in confidence intervals that were too

large and was, therefore, inappropriate. An additional problem was a lack of study details (e.g., only nominal concentrations of chloroacetaldehyde were reported). Inclusion of the Arts (1987) data in the modeling only slightly improved the analyses. Using the "Doseresp"-software developed by ten Berge, a value for n of 1.2 (with a 95% confidence interval of 0.88-1.53) for time scaling was derived from the Dow Chemical Company (1952) study in rats (see Table 2-3). Inclusion of the Arts (1987) data did not change the outcome significantly.

Mortality data from the Arts (1987) study (see Table 2-3) were analyzed using EPA benchmark dose software (version 1.3.2) (EPA 2005). Benchmark concentrations for a 1% response (BMC_{01}) and for a 5% response (BMC_{05}) were 118 ppm and 136 ppm, respectively. The lower 95% confidence limit for the BMC_{05} ($BMCL_{05}$) for a 1-h exposure was 99 ppm.

A total uncertainty factor of 10 (two factors of 3) was considered sufficient for toxicokinetic and toxicodynamic differences between species and for individual variability for the following reasons. The effects were attributed to direct interaction of chloroacetaldehyde and, therefore, no relevant differences in kinetics were assumed. The concentration-response curve appeared to be very steep, indicating that a larger factor is unnecessary. Doubling the exposure duration or concentration increased the mortality from 0% to 100%. Considering the response at the lower exposure concentrations, a larger uncertainty factor would lead to unrealistically low AEGL-3 values (see Tables 2-3 and 2-4).

The point of departure for calculating the AEGL-3 1-h value was the 1-h $BMCL_{05}$ of 99 ppm. Application of a total uncertainty factor of 10 results in 9.9 ppm for a 1-h exposure. AEGL-3 values for the other time periods were derived by time scaling according to the dose-response regression equation $C^n \times t = k$, with n = 1.2 based on mortality data (Dow Chemical Company 1952). The resulting AEGL-2 values are presented in Table 2-7.

8. SUMMARY OF AEGLS

8.1. AEGL Values and Toxicity End Points

The AEGLs values for chloroacetaldehyde are summarized in Table 2-8.

TABLE 2-6 AEGL-2 Values for Chloroacetaldehyde

10 min	30 min	1 h	4 h	8 h
9.8 ppm (31 mg/m^3)	3.9 ppm (13 mg/m^3)	2.2 ppm (7.1 mg/m^3)	0.69 ppm (2.2 mg/m^3)	0.39 ppm (1.5 mg/m^3)

TABLE 2-7 AEGL-3 Values for Chloroacetaldehyde

10 min	30 min	1 h	4 h	8 h
44 ppm (140 mg/m^3)	18 ppm (57 mg/m^3)	9.9 ppm (32 mg/m^3)	3.1 ppm (10 mg/m^3)	1.8 ppm (5.6 mg/m^3)

TABLE 2-8 Summary of AEGL Values for Chloroacetaldehyde

Classification	Exposure Duration				
	10 min	30 min	1 h	4 h	8 h
AEGL-1 (nondisabling)	2.3 ppm (7.4 mg/m^3)	2.3 ppm (7.4 mg/m^3)	1.3 ppm (4.2 mg/m^3)	0.40 ppm (1.3 mg/m^3)	0.22 ppm (0.71 mg/m^3)
AEGL-2 (disabling)	9.8 ppm (31 mg/m^3)	3.9 ppm (13 mg/m^3)	2.2 ppm (7.1 mg/m^3)	0.69 ppm (2.2 mg/m^3)	0.39 ppm (1.5 mg/m^3)
AEGL-3 (lethal)	44 ppm (140 mg/m^3)	18 ppm (57 mg/m^3)	9.9 ppm (32 mg/m^3)	3.1 ppm (10 mg/m^3)	1.8 ppm (5.6 mg/m^3)

TABLE 2-9 Extant Standards and Guidelines for Chloroacetaldehyde

Guideline	Exposure Duration				
	10 min	30 min	1 h	4 h	8 h
AEGL-1	2.3 ppm (7.4 mg/m^3)	2.3 ppm (7.4 mg/m^3)	1.3 ppm (4.2 mg/m^3)	0.40 ppm (1.3 mg/m^3)	0.22 ppm (0.71 mg/m^3)
AEGL-2	9.8 ppm (31 mg/m^3)	3.9 ppm (13 mg/m^3)	2.2 ppm (7.1 mg/m^3)	0.69 ppm (2.2 mg/m^3)	0.39 ppm (1.5 mg/m^3)
AEGL-3	44 ppm (140 mg/m^3)	18 ppm (57 mg/m^3)	9.9 ppm (32 mg/m^3)	3.1 ppm (10 mg/m^3)	1.8 ppm (5.6 mg/m^3)
IDLH (NIOSH)[a]		45 ppm			
TLV-C (ACGIH)[b]	1 ppm				
PEL-C (OSHA)[c]	1 ppm				
REL-C (NIOSH)[d]	1 ppm				
MAC (The Netherlands)[e]					1 ppm

[a]IDLH (immediately dangerous to life or health, National Institute for Occupational Safety and Health) (NIOSH 1994) represents the maximum concentration from which one could escape within 30 min without any escape-impairing symptoms, or any irreversible health effects.
[b]TLV-C (threshold limit value-ceiling, American Conference of Governmental Industrial Hygienists) (ACGIH 2010) is a value that must not be exceeded during any part of the workday.
[c]PEL-C (permissible exposure limit-ceiling, Occupational Safety and Health Administration) (29 CFR 1910.1000 [2006]) is defined analogous to the ACGIH TLV-C.
[d]REL-C (recommended exposure limit-ceiling, National Institute for Occupational Safety and Health) (NIOSH 2011) is defined analogous to the ACGIH TLV-C.
[e]MAC (maximaal aanvaaarde concentratie [maximal accepted concentration]), (Dutch Expert Committee for Occupational Standards, The Netherlands (MSZV 2004) is defined analogous to the ACGIH TLV-TWA (the time weighted average concentration for a normal 8-h workday and a 40-ho workweek, to which nearly all workers may be repeatedly exposed, day after day, without adverse effect).

8.2. Comparison with Other Standards and Guidelines

The Immediately Dangerous to Life or Health (IDLH) value for chloroacetaldehyde of 45 ppm is based on an analogy with crotonaldehyde. Crotonaldehyde at 45 ppm was disagreeable to human subjects and caused conjunctival irritation. The IDLH might be a conservative value because acute toxicity data at concentrations greater than 45 ppm are lacking.

The general standards for chloroacetaldehyde are based on the prevention of ocular and nasal irritation. Because of the corrosive properties of chloroacetaldehyde, the standards are set as ceiling values. The 10- and 30-min AEGL-1 values are about two-fold greater than the ceiling value of 1 ppm. However, considering the severity of irritation in relation to the exposure concentrations, as observed in the animal experiments (single and repeated exposure), exposure to chloroacetaldehyde at 2.3 ppm for up to 30 min will probably not lead to significant ocular or nasal irritation in humans.

8.3. Data Quality and Research Needs

No relevant, adequately-documented human data on chloroacetaldehyde were available. AEGL-1 values were based on data from repeated-exposure studies in animals, because no adequate acute-exposure studies of relevant AEGL-1 end points in animals were found. However, one of the repeated-exposure studies had inadequate details and both studies mainly focused on lethality and described nonlethal effects in a general manner without relating them to specific concentrations of chloroacetaldehyde. AEGL-2 values were based on a clear effect level; a no-observed-adverse-effect level could not be determined. However, because the critical end points for chloroacetaldehyde are fairly well understood, the uncertainties in the AEGL values are not expected to be large. An adequate animal experiment focusing on AEGL-1 and AEGL-2 end points after single exposure might be helpful to reduce uncertainties.

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APPENDIX A

DERIVATION OF AEGL VALUES FOR CHLOROACETALDEHYDE

Derivation of AEGL-1 Values

Key study:	Dow Chemical Company. 1952. Toxicity of Chloroacetaldehyde. Document No. 8EHQ-0392-28338. EPA Document No. 88920001475. Microfiche No. OTS0536151.
Toxicity end point:	5 ppm for 7 h, lowest-observed-adverse-effect level for nasal and ocular irritation. The point of departure was 2.5 ppm after a modifying factor of 2 was applied (see below).
Time scaling:	$C^n \times t = k$; n = 1.2 based on lethality data $k = (2.5 \text{ ppm})^{1.2} \times 420 \text{ min} = 1{,}261 \text{ ppm-min}$
Uncertainty factors:	3 for interspecies differences 3 for intraspecies variability Total uncertainty factor of 10
Modifying factor:	A modifying factor of 2 was applied to reduce the lowest-observed-adverse-effect level to a no-effect level.
Calculations:	
10-min AEGL-1:	Set equal to the 30-min AEGL of 2.3 ppm (= 7.4 mg/m^3)
30-min AEGL-1:	$C^{1.2} \times 30 \text{ min} = 1{,}261 \text{ ppm-min}$ C = 22.5 ppm 22.5 ÷ 10 = 2.3 ppm (rounded) (= 7.4 mg/m^3)
1-h AEGL-1:	$C^{1.2} \times 60 \text{ min} = 1{,}261 \text{ ppm-min}$ C = 12.7 ppm 12.7 ÷ 10 = 1.3 ppm (rounded) (= 4.2 mg/m^3)
4-h AEGL-1:	$C^{1.2} \times 240 \text{ min} = 1{,}261 \text{ ppm-min}$ C = 4.0 ppm 4.0 ÷ 10 = 0.40 ppm (rounded) (= 1.3 mg/m^3)
8-h AEGL-1:	$C^{1.2} \times 480 \text{ min} = 1{,}261 \text{ ppm-min}$ C = 2.2 ppm 2.2 ÷ 10 = 0.22 ppm (rounded) (= 0.71 mg/m^3)

Derivation of AEGL-2 Values

Key study:	Arts, J.H.E. 1987. Acute (One-Hour) Inhalation Toxicity Study of Chloroacetaldehyde in Rats. Report No. V 87.094/261236. Organization for Applied Scientific Research (TNO), Zeist, The Netherlands [online]. Available: http://yosemite.epa.gov/oppts/epatscat8.nsf/by+Service/731D9542E140E7DC85256F2600655CA2/$File/88870000029.pdf [accessed Feb. 10, 2012].
Toxicity end point:	44 ppm for 1 h, lowest-observed-adverse-effect level for impaired pulmonary function. The point of departure was 22 ppm after a modifying factor of 2 was applied (see below).
Time scaling:	$C^n \times t = k$; n = 1.2 based on lethality data $k = (22\ ppm)^{1.2} \times 60\ min = 2,449\ ppm\text{-}min$
Uncertainty factors:	3 for interspecies differences 3 for intraspecies variability Total uncertainty factor of 10
Modifying factor:	A modifying factor of 2 was applied to reduce the lowest-observed-adverse-effect level, because the pulmonary effects were more severe than those that define AEGL-2 values.
Calculations:	
10-min AEGL-2:	$C^{1.2} \times 10\ min = 2,449\ ppm\text{-}min$ C = 97.9 ppm 97.9 ÷ 10 = 9.8 ppm (rounded) (= 31 mg/m^3)
30-min AEGL-2:	$C^{1.2} \times 30\ min = 2,449\ ppm\text{-}min$ C = 39.2 ppm 39.2 ÷ 10 = 3.9 ppm (rounded) (= 13 mg/m^3)
1-h AEGL-2:	22 ppm ÷ 10 = 2.2 ppm (= 7.1 mg/m^3)
4-h AEGL-2:	$C^{1.2} \times 240\ min = 2,449\ ppm\text{-}min$ C = 6.9 ppm 6.9 ÷10 = 0.69 ppm (= 2.2 mg/m^3)
8-h AEGL-2:	$C^{1.2} \times 480\ min = 2,449\ ppm\text{-}min$ C = 3.9 ppm 3.9 ÷10 = 0.39 ppm (= 1.5 mg/m^3)

Derivation of AEGL-3 Values

Key studies:	Arts, J.H.E. 1987. Acute (One-Hour) Inhalation Toxicity Study of Chloroacetaldehyde in Rats. Report No. V 87.094/261236. Organization for Applied Scientific Research (TNO), Zeist, The Netherlands [online]. Available: http://yosemite.epa.gov/oppts/epatscat8.nsf/by+Service/731D9542E140E7DC85256F2600655CA2/$File/88870000029.pdf [accessed Feb. 10, 2012]. Dow Chemical Company. 1952. Toxicity of Chloroacetaldehyde. Document No. 8EHQ-0392-28338. EPA Document No. 88920001475. Microfiche No. OTS0536151.
Toxicity end point:	Lethality in rats exposed for 1 h. The 1-h BMC_{05} is 136 ppm, with a lower 95% confidence limit of 99 ppm (the point of departure).
Time scaling:	$C^n \times t = k$; n = 1.2 based on lethality data $k = (99\ ppm)^{1.2} \times 60\ min = 14{,}891\ ppm\text{-}min$
Uncertainty factors:	3 for interspecies differences 3 for intraspecies variability Total uncertainty factor of 10
Calculations:	
10-min AEGL-3:	$C^{1.2} \times 10\ min = 14{,}891\ ppm\text{-}min$ C = 441 ppm 441 ÷ 10 = 44 ppm (rounded) (= 140 mg/m^3)
30-min AEGL-3:	$C^{1.2} \times 30\ min = 14{,}891\ ppm\text{-}min$ C = 176 ppm 176 ÷ 10 = 18 ppm (rounded) (= 57 mg/m^3)
1-h AEGL-3:	99 ppm ÷ 10 = 9.9 ppm (= 32 mg/m^3)
4-h AEGL-3:	$C^{1.2} \times 240\ min = 14{,}891\ ppm\text{-}min$ C = 31 ppm 31 ÷ 10 = 3.1 ppm (= 10 mg/m^3)
8-h AEGL-3:	$C^{1.2} \times 480\ min = 14{,}891\ ppm\text{-}min$ C = 18 ppm 18 ÷ 10 = 1.8 ppm (= 5.6 mg/m^3)

APPENDIX B

ACUTE EXPOSURE GUIDELINE LEVELS FOR CHLOROACETALDEHYDE

Derivation Summary for Chloroacetaldehyde

AEGL-1 VALUES

10 min	30 min	1 h	4 h	8 h
2.3 ppm (7.4 mg/m^3)	2.3 ppm (7.4 mg/m^3)	1.3 ppm (4.2 mg/m^3)	0.40 ppm (1.3 mg/m^3)	0.22 ppm (0.71 mg/m^3)

Key reference: Dow Chemical Company. 1952. Toxicity of Chloroacetaldehyde. Document No. 8EHQ-0392-28338. EPA Document No. 88920001475. Microfiche No. OTS0536151.

Test species/Strain/Number:
Guinea pigs (strain not specified): 5
Rabbit (strain not specified): 1
Rats (strain not specified): 10
Mice (strain not specified): 5

Exposure route/Concentrations/Durations: Inhalation, chloroacetaldehyde at 0 or 5 ppm for 7 h/d, 5 d/wk for a total of eight exposures in 10 d.

Effects at 5 ppm:
Guinea pigs: no effects
Rabbit: slight ocular irritation
Rats: slight nasal irritation, very slight ocular irritation
Mice: slight nasal irritation

End point/Concentration/Rationale: Rabbit, rats, and mice had slight nasal and ocular irritation after 7 h of exposure at 5 ppm (lowest-observed-adverse-effect level).

Uncertainty factors/Rationale:
Total uncertainty factor: 10 was considered sufficient for toxicokinetic and toxicodynamic differences between species and individual variability. The effects were attributed to direct interaction of chloroacetaldehyde and, therefore, no relevant differences in kinetics between species and between humans were assumed.
Interspecies: 3
Intraspecies: 3

Modifying factor: A modifying factor of 2 was applied to reduce 5 ppm to a no-effect level of 2.5 ppm.

Animal-to-human dosimetric adjustment: Not applied

Time scaling: $C^n \times t = k$; n = 1.2 based on lethality data

(Continued)

AEGL-1 VALUES Continued

10 min	30 min	1 h	4 h	8 h
2.3 ppm (7.4 mg/m^3)	2.3 ppm (7.4 mg/m^3)	1.3 ppm (4.2 mg/m^3)	0.40 ppm (1.3 mg/m^3)	0.22 ppm (0.71 mg/m^3)

Data adequacy: No human data were available. Lacrimation and nasal irritation reported in humans within a few minutes of exposure to chloroacetaldehyde at 10 ppm (Dow Chemical Company 1952) provides supporting data for deriving AEGL-1 values on the basis of rat data. No adequate animal data identifying a no-effect level for ocular and nasal irritation were available.

AEGL-2 VALUES

10 min	30 min	1 h	4 h	8 h
9.8 ppm (31 mg/m^3)	3.9 ppm (13 mg/m^3)	2.2 ppm (7.1 mg/m^3)	0.69 ppm (2.2 mg/m^3)	0.39 ppm (1.5 mg/m^3)

Key reference: Arts, J.H.E. 1987. Acute (One-Hour) Inhalation Toxicity Study of Chloroacetaldehyde in Rats. Report No. V 87.094/261236. Organization for Applied Scientific Research (TNO), Zeist, The Netherlands.

Test species/Strain/Number: SPF-reared Borr:WISW rats (5 animals/sex/group)

Exposure route/Concentrations/Durations: Inhalation, mean actual concentrations of chloroacetaldehyde at 44, 159, 203, 243, 309, 596, and 2,643 ppm for 1 h.

Effects:

44 ppm: closed eyes, salivation; pulmonary edema (still presented 2 weeks after exposure)

159 ppm: 3/10 deaths

203 ppm: 4/10 deaths

243 ppm: 100% mortality

309 ppm: 100% mortality

596 ppm: 100% mortality

2,643 ppm: 100% mortality

End point/Concentration/Rationale: Pulmonary edema at 44 ppm, the lowest concentration tested.

Uncertainty factors/Rationale:

Total uncertainty factor: 10 was considered sufficient for toxicokinetic and toxicodynamic differences between species and individual variability. The effects were attributed to direct interaction of chloroacetaldehyde and, therefore, no relevant differences in kinetics between species and between humans were assumed.

Interspecies: 3

Intraspecies: 3

Modifying factor: A modifying factor of 2 was applied because of an incomplete database (a no-effect level was not identified). A factor of 2 was considered sufficient because of the steep concentration-response curve.

(Continued)

AEGL-2 VALUES Continued

10 min	30 min	1 h	4 h	8 h
9.8 ppm (31 mg/m^3)	3.9 ppm (13 mg/m^3)	2.2 ppm (7.1 mg/m^3)	0.69 ppm (2.2 mg/m^3)	0.39 ppm (1.5 mg/m^3)

Animal-to-human dosimetric adjustment: Not applied

Time scaling: $C^n \times t = k$; n = 1.2 based on lethality data

Data adequacy: No human data were available. A no-effect level for effects defined by the AEGL-2 could not be determined. However, because of the steep concentration-response curve and the additional animal data, the uncertainties in the AEGL-2 values probably not very large.

AEGL-3 VALUES

10 min	30 min	1 h	4 h	8 h
44 ppm (140 mg/m^3)	18 ppm (57 mg/m^3)	9.9 ppm (32 mg/m^3)	3.1 ppm (10 mg/m^3)	1.8 ppm (5.6 mg/m^3)

Key references: (1) Dow Chemical Company. 1952. Toxicity of Chloroacetaldehyde. Document No. 8EHQ-0392-28338. EPA Document No. 88920001475. Microfiche No. OTS0536151. (2) Arts, J.H.E. 1987. Acute (One-Hour) Inhalation Toxicity Study of Chloroacetaldehyde in Rats. Report No. V 87.094/261236. Organization for Applied Scientific Research (TNO), Zeist, The Netherlands.

Test species/Strain/Number: SPF-reared Borr: WISW rats (5 animals/sex/group)

Exposure Route/Concentrations/Durations: Inhalation, mean actual concentrations of 44, 159, 203, 243, 309, 596, and 2,643 ppm for 1 h

Effects:

44 ppm: 0/10 deaths
159 ppm: 3/10 deaths
203 ppm: 4/10 deaths
243 ppm: 100% mortality
309 ppm: 100% mortality
596 ppm: 100% mortality
2,643 ppm: 100% mortality

End point/Concentration/Rationale: A $BMCL_{05}$ of 99 ppm for 1 h was calculated using EPA benchmark dose software (EPA 2005).

Uncertainty factors/Rationale:
Total uncertainty factor: 10 was considered sufficient for toxicokinetic and toxicodynamic differences between species and individual variability. The effects were attributed to direct interaction of chloroacetaldehyde and, therefore, no relevant differences in kinetics between species and between humans were assumed.

Interspecies: 3
Intraspecies: 3

(Continued)

AEGL-3 VALUES Continued

10 min	30 min	1 h	4 h	8 h
44 ppm (140 mg/m^3)	18 ppm (57 mg/m^3)	9.9 ppm (32 mg/m^3)	3.1 ppm (10 mg/m^3)	1.8 ppm (5.6 mg/m^3)
Modifying factor: Not applied				
Animal-to-human dosimetric adjustment: Not applied				
Time scaling: $C^n \times t = k$; n = 1.2 based on lethality data				
Data adequacy: Sufficient for deriving AEGL-3 values.				

APPENDIX C

CATEGORY GRAPH OF TOXICITY DATA AND AEGL VALUES FOR CHLOROACETALDEHYDE

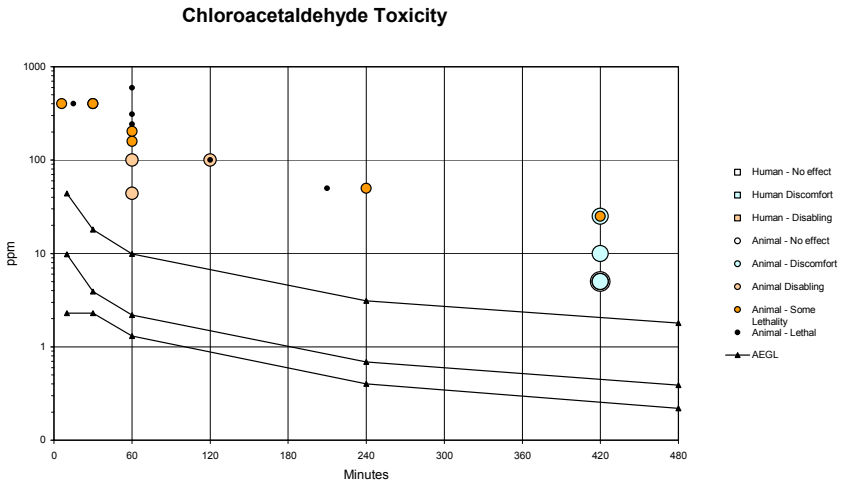

FIGURE C-1 Category graph of toxicity data and AEGLs values for chloroacetaldehyde.

3

Chlorobenzene[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 are defined as follows:

AEGL-1 is the airborne concentration (expressed as parts per million or milligrams per cubic meter [ppm or 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 J.J.A. Muller and Peter Bos (both from RIVM, The Dutch National Institute of Public Health and the Environment), Julie M. Klotzbach (Syracuse Research Corporation), Chemical Manager Marinelle Payton (National Advisory Committee [NAC] on Acute Exposure Guideline Levels for Hazardous Substances), and Ernest V. Falke (U.S. Environmental Protection Agency). 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 guidelines reports (NRC 1993, 2001).

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 concentrations 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 AEGL values represent threshold concentrations 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

Chlorobenzene is a flammable liquid with a high vapor pressure and a water solubility of 50 milligrams per liter (mg/L) at 20°C. It is used as a solvent and in the production of nitrochlorobenzene and intermediates for the synthesis of dyestuffs, pharmaceuticals, and products for the rubber and plastic industries. Chlorobenzene has an aromatic, almond-like odor. The odor threshold is 0.050 mg/L in water and is 0.2-1.8 ppm in air, although a value of 62 ppm has also been reported for air.

The toxicity database on chlorobenzene is poor. Information often had to be obtained from descriptions in reviews and summaries, and some older literature could not be obtained (e.g., Rozenbaum et al. [1947]). Human data include to two kinetic studies with volunteers. Animal data included studies on teratogenicity, reproductive toxicity, and mortality. A few studies with experimental animals addressing central nervous system (CNS) depression were reviewed, but were difficult to interpret.

AEGL-1 values are based on kinetic studies with volunteers. Effects in subjects exposed to chlorobenzene at 60 ppm for 7 h (with a 1-h break after 3 h) are indicative of slight CNS depression (drowsiness, heavy head, and headache) and local irritation (Ogata et al. 1991), and are considered evidence of discomfort. These effects were not observed is subjects exposed at 10 ppm for 8 h

(Knecht and Woitowitz 2000). Thus, 10 ppm was chosen as a conservative point of departure for the derivation of AEGL-1 values. Because human data are used, an interspecies uncertainty factor of 1 was used. Despite the fact that only a few subjects were tested, an uncertainty factor of 1 for intraspecies variability was considered appropriate because of the conservatism of the point of departure already provides a margin of safety. (The point of departure of 10 ppm was obtained from a repeated-exposure study, and effects observed at 60 ppm were rather slight.) No information about the time dependency of the effects at 10 or 60 ppm is available. Because the effects at 60 ppm include irritation and CNS effects, the 8-h AEGL-1 value of 10 ppm is considered appropriate for all time points. Furthermore, Knecht and Woitowitz (2000) reported that chlorobenzene concentrations in blood reached a steady-state level within 1 h.

There are no adequate human data for deriving AEGL-2 values. Some studies with experimental animals report subtle CNS effects, but the relevance of these effects to humans is difficult to interpret. The effects reported by Frantik et al. (1994) and De Ceaurriz et al. (1983) are considered effects below those defined by AEGL-2. A more appropriate study is the one by UBTL (1978), in which rats and guinea pigs experienced narcosis and effects that would impair ability to escape. A no-effect concentration of 2,990 ppm for 30 min was selected as the point of departure for calculating AEGL-2 values. An interspecies uncertainty factor of 3 was applied, because data were comparable for rats and guinea pigs, suggesting no large interspecies differences, and the critical effect is CNS depression. The concentration of chlorobenzene in the brain is probably related directly to inhalation rate. Therefore, humans probably require higher external exposures than rodents to obtain a similar concentration of chlorobenzene in the blood or brain. Experience with anesthetic gases shows that interindividual variability in CNS depression caused by these gases is generally not greater than a factor of 2 or 3. Therefore, an intraspecies uncertainty factor of 3 was used. A combined uncertainty factor of 10 was considered appropriate because a larger factor would result in AEGL-2 values below 60 ppm, which a concentration shown to cause only minor effects in humans. The 30-min AEGL-2 was 300 ppm. The 30-min value was extrapolated to 10-min and 1-h values using the equation $C^n \times t = k$, with default values of n = 1 for extrapolation to 1 h and n = 3 for extrapolation to 10 min. The 4- and 8-h AEGL-2 values were set equal to the 1-h value because chlorobenzene concentrations in blood reach a steady-state within 1 h and elimination is rapid. Furthermore, time scaling would result in 4- and 8-h AEGL-2 values that conflict with human data (Ogata et al. 1991).

For the derivation of AEGL-3 values, several mortality studies were found, but most were only available as summaries in other publications and could not be judged on their merits. Bonnet et al. (1979, 1982) reported a 6-h LC_{50} (lethal concentration, 50% lethality) of 2,965 ppm for male rats and a 6-h LC_{50} of 1,886 ppm for mice. No deaths were reported in rats or guinea pigs exposed to chlorobenzene at concentrations of up to 7,970 ppm for 30 min (UBTL 1978). Data in rats and guinea pigs reported by UBTL (1978) provide the most

appropriate point of departure for AEGL-3 derivation. A total uncertainty factor of 10 was applied on the same basis it was applied in the derivation of the AEGL-2 values, and time scaling was performed the same as was done for the AEGL-2 values. AEGL-3 values are consistent with the AEGL-2 values and are supported by the 6-h LC_{01} of 1,873 ppm calculated from the probit equation reported by Bonnet et al. (1982). AEGL values for chlorobenzene are presented in Table 3-1.

1. INTRODUCTION

Chlorobenzene is a flammable liquid with a high vapor pressure and a water solubility of 50 mg/L at 20°C. It is commercially produced by the chlorination of benzene in the presence of a catalyst (ATSDR 1990). Chlorobenzene is used as a solvent and in the production of nitrochlorobenzene and intermediates for the synthesis of dyestuffs, pharmaceuticals, and products for the rubber and plastic industries (BUA 1990). The production volume of chlorobenzene in 1992 was 231 million pounds in the United States (EPA 1995). More current information on production volumes was not available.

Chlorobenzene has an aromatic, almond-like odor. The odor threshold for chlorobenzene in water is 0.050 mg/L and in air is 0.2-1.8 ppm (Verschueren 1983). Odor thresholds for chlorobenzene have been reported as low as 0.2 ppm and as high as 62 ppm (Ruth 1986). Chemical and physical properties for chlorobenzene are presented in Table 3-2.

2. HUMAN TOXICITY DATA

2.1. Acute Lethality

No data were available.

TABLE 3-1 Summary of AEGL Values for Chlorobenzene

Classification	10 min	30 min	1h	4h	8h	End Point (Reference)
AEGL-1 (nondisabling)	10 ppm (47 mg/m^3)	10 ppm (47 mg/m^3)	10 ppm (47 mg/m^3)	10 ppm (47 mg/m^3)	10 ppm (47 mg/m^3)	No irritant or CNS effects (Ogata et al. 1991; Knecht and Woitowitz 2000)
AEGL-2 (disabling)	430 ppm (2,021 mg/m^3)	300 ppm (1,410 mg/m^3)	150 ppm (705 mg/m^3)	150 ppm (705 mg/m^3)	150 ppm (705 mg/m^3)	Narcosis (UBTL 1978)
AEGL-3 (lethal)	1,100 ppm (5,170 mg/m^3)	800 ppm (3,760 mg/m^3)	400 ppm (1,880 mg/m^3)	400 ppm (1,880 mg/m^3)	400 ppm (1,880 mg/m^3)	No mortality in rats or guinea pigs (UBTL 1978)

TABLE 3-2 Chemical and Physical Properties for Chlorobenzene

Parameter	Value	Reference
CAS registry no.	108-90-7	
Synonyms	Monochlorobenzene; benzene chloride; phenylchloride; MCB; chlorobenzol	
Chemical formula	C_6H_5Cl	
Molecular weight	112.56	
Physical state	Liquid	ATSDR 1990
Color	Colorless	ATSDR 1990
Odor	Aromatic, almond-like	ATSDR 1990
Melting point	-45.6°C	ATSDR 1990
Boiling point	132°C	ATSDR 1990
Liquid density (water = 1)	1.1058 g/cm^3	ATSDR 1990
Solubility in water	500 mg/L at 20°C	ATSDR 1990
Vapor pressure	8.8 mm Hg at 20°C	ATSDR 1990
Flammability	1.8-9.6%	ATSDR 1990
Lower explosive limit	1.3%	NIOSH 2011
Conversion factors	1 mg/m^3 = 0.22 ppm 1 ppm = 4.7 mg/m^3	ATSDR 1990

2.2. Nonlethal Toxicity

2.2.1. Case Reports

Several reviews including those of ACGIH (1991) and Hellman (1993) cited reports in which inhalation and oral exposure to chlorobenzene are described as having caused drowsiness, incoordination, and unconsciousness, as well as irritation of the eyes and respiratory tract. However, exposure concentrations were not specified.

Ruth (1986) reported that 205 ppm was an irritating concentration of chlorobenzene, but the source of that information was not provided.

2.2.2. Experimental Studies

In a study investigating urinary metabolites of chlorobenzene, subjects were asked to report subjective effects of the exposure (Ogata et al. 1991). Volunteers were exposed to chlorobenzene at 60.2 ± 3.9 ppm for 3 h in the morning

and 4 h in the afternoon, with a 1-h break between exposures. The concentrations were determined by gas chromatography and were reported to be constant within a 5% range. All of the volunteers complained of a disagreeable odor and drowsiness. Three had a heavy feeling in the head or headache, two had a throbbing pain in the eyes, and one had a sore throat. No information was given about the onset of these complaints. Chlorobenzene did not affect pulse rates or systolic and diastolic pressure. Flicker fusion frequency values (frequency at which successive flashes are seen as continuous) were reduced significantly from 39.1 to 35.9 cycles/second at the end of the 3-h exposure. No further effect was seen in the afternoon. The significance of this finding is difficult to interpret.

Eight volunteers were exposed to chlorobenzene at 10 ppm for 8 h per day for five consecutive days to determine the relationship between chlorobenzene and urinary concentrations of its metabolites 4-chlorocatechol and chlorophenols (Knecht and Woitowitz 2000). None of the subjects complained of irritant or CNS effects (U. Knecht, Justus Liebig University Giessen, Germany, personal commun., 2005).

2.2.3. Occupational and Epidemiologic Studies

The potential consequences of occupational exposure to chlorobenzene are described in a report by Izmerov et al. (1988). These cases are not included in the chapter because concentrations of chlorobenzene in those studies were unclear and coexposure to other chemicals was possible.

2.3. Neurotoxicity

Izmerov et al. (1988) described changes in electroencephalogram (EEG) readings as "evident on an individual basis" during exposure to chlorobenzene and as near-term and long-term effects. The specific changes were not described. On the basis of changes in electrical brain activity, 0.2 mg/m^3 (0.044 ppm) appeared to be a threshold concentration (exposure duration unknown), and 0.1 mg/m^3 (0.022 ppm) was a no-effect concentration. No further details were provided in the Izmerov report, and the original publications were not available. Therefore, these results are considered supplementary information.

2.4. Summary

No information is available on the acute lethality of chlorobenzene in humans. Chlorobenzene can be irritating to the eyes and respiratory tract, and signs of CNS effects (drowsiness, heavy feeling in the head, and headache) have been report in people exposed at 60 ppm for 7 h. Odor might have interfered with subjective complaints of irritation. No complaints of irritation were described in another study in which volunteer were exposed to chlorobenzene at 10 ppm for 8 h/day for 5 days.

3. ANIMAL TOXICITY DATA

3.1. Acute Lethality

The acute lethality data on chlorobenzene in laboratory animals is presented in Table 3-3.

TABLE 3-3 Acute Lethality Data on Chlorobenzene in Laboratory Animals

Species (sex)	Concentration (ppm)	Exposure Duration	Effect	Reference
Single exposure				
Rats (male)	2,965	6 h	LC_{50}	Bonnet et al. 1982
Rats (or mice)	4,400	2 h	LC_{100}	Rozenbaum et al. 1947
Guinea pigs	7,970	30 min	No mortality	UBTL 1978
Rats	22,000	3.5 h	2 of 3 died	Eastman Kodak Co. 1994
Rats	9,000	6 h	2 of 3 died	Eastman Kodak Co. 1994
Rats	7,970	30 min	No mortality	UBTL 1978
Mice (female)	1,886	6 h	LC_{50}	Bonnet et al. 1979
Mice	7,832	2 h	LC_{84}	Sanotsky and Ulanova 1975
	4,070	2 h	LC_{50}	
	2,244	2 h	LC_{16}	
Related exposures				
Rat (two-generation study)	450	6 h/d, 7 d/wk for up to 17 wk	No mortality	Nair et al. 1987
Rabbits (pregnant)	3,000	6 h/d for 13 d	Mortality	John et al. 1984
	1,000		No mortality	
Rats (pregnant)	3,000	6 h/d for 10 d	Mortality	John et al. 1984
	1,000		No mortality	
Rats	248	7 h/d, 5 d/wk for 24 wk	No mortality	Dilley 1977

3.1.1. Rabbits

A description of a study by Rozenbaum et al. (1947) was obtained from a report by ATSDR (1990), because the original publication could not be obtained. Rabbits (sex and number not specified) exposed to chlorobenzene (head only or whole body) at 550-660 ppm for 4 h died after 2 weeks, but no effects were observed at 110-220 ppm. Rabbits were also reported to have died 2 weeks after exposure to chlorobenzene at 537 ppm for 2 h. These results contrast with findings in other studies. For example, repeated exposure of 32 male rabbits to chlorobenzene at 248 ppm for up to 24 weeks did not increase mortality (Dilley 1977). In addition, no mortality was observed in a teratogenicity study of rabbits exposed at 1,000 ppm (6 h/day for 10 days), but deaths were observed at 3,000 ppm (John et al. 1984) (see Section 3.3 for further details of this study).

3.1.2. Guinea Pigs

Groups of five guinea pigs per sex were exposed (whole body) to chlorobenzene at mean (± standard deviation [SD]) analytic concentrations of 2,990 ± 53, 5,850 ± 1,350, or 7,970 ± 355 ppm for 30 min, and were observed for 14 days. No deaths were observed at any concentration (UBTL 1978).

3.1.3. Rats

Bonnet et al. (1982) determined the 6-h LC_{50} for chlorobenzene in male Sprague-Dawley rats. Twelve rats per concentration were exposed (whole body) and observed for 14 days. Nominal test concentrations were not provided. Actual concentrations were determined using gas chromatography, but information on the exposure concentrations was limited to a graph on log scale. It was estimated that the lowest concentration tested in rats was approximately 2,000 ppm and was associated with 8% mortality. The LC_{50} was 2,965 ppm (95% confidence interval [CI]: 2,787-3,169 ppm), with a regression line of probit = -33 + 10.9 logC (the paper presented a positive intercept [+33] but the data indicate that it should be -33). Hypotony, stereotypy, somnolence, tremor, and muscle contractions were observed during exposure.

A 2-h LC_{100} value of 4,400 ppm for rats was determined by Rozenbaum et al. (1947, as reported by BUA 1990). However, according to ATSDR (1990), this study was performed in mice. The original publication could not be retrieved to clarify the discrepancy.

The following statement was found in a submission to the U.S. Environmental Protection Agency (Eastman Kodak Co 1994): "Acute exposure to 22,000 ppm for 3½ h killed 2/3 rats in 2½ h while 9,000 ppm for 6 h killed 2/3 rats in 3 h." A reference to unpublished data from the Eastman Kodak Company was cited, but the original study was not available.

Groups of five rats per sex were exposed (whole body) to chlorobenzene at mean (± SD) analytic concentrations of 2,990 ± 53, 5,850 ± 1,350, or 7,970 ± 355 ppm for 30 min, and animals were observed for 14 days. No deaths were observed at any concentration (UBTL 1978).

Repeated exposure of 32 male rats to chlorobenzene at 248 ppm for up to 24 weeks did not result in mortality (Dilley 1977). In addition, no mortality was observed in a two-generation study (450 ppm, 6 h/day, 7 days/week for 17 weeks) (Nair et al. 1987) or in a rat developmental toxicity study (1,000 ppm, 6 h/day for 10 days) (John et al. 1984). However, in the latter study, increased mortality was observed at 3,000 ppm (John et al. 1984).

3.1.4. Mice

Bonnet et al. (1979) determined the 6-h LC_{50} of chlorobenzene in female mice (OF_1). Groups of 25 mice were exposed to chlorobenzene (whole body) and observed for 14 days. Nominal test concentrations were not provided. Actual concentrations were determined using gas chromatography. The analytic concentrations were 90-100% of the nominal concentrations. No details on the exposure concentrations were provided other than a graph on log scale. It was estimated that the lowest concentration tested was approximately 1,500 ppm and caused approximately 20% mortality. The LC_{50} was 1,886 ppm (95% CI: 1,781 -1,980 ppm), with a regression line of probit = -17.06 + 6.734 logC (the paper presented a positive intercept [+17.06] but the data indicate that it should be -17.06).

Izmerov et al. (1988) described a study by Sanotsky and Ulanova (1975) that found a 2-h LC_{50} of 4,070 ppm, an LC_{16} of 2,244 ppm, and an LC_{84} of 7,832 ppm for chlorobenzene in mice. Izmerov also reported that another study reported that exposure to chlorobenzene at 2,200 ppm (duration unknown) failed to kill mice, but that at 4,400 ppm three of four mice died. Neither of the primary studies could be obtained.

A 2-h LC_{100} value of 4,400 ppm for mice was reported by Rozenbaum et al. (1947, as cited by ATSDR 1990). However, according to BUA (1990), this study was performed in rats. The original publication could not be retrieved to clarify the discrepancy.

3.2. Nonlethal Toxicity

The acute nonlethal effects of chlorobenzene in laboratory animals are summarized in Table 3-4.

3.2.1. Guinea Pigs

Groups of five guinea pigs per sex were exposed (whole body) to chlorobenzene at mean (± SD) analytic concentrations of 2,990 ± 53, 5,850 ± 1,350, or

7,970 ± 355 ppm for 30 min, and were observed for 14 days. No deaths were observed at any concentration. At 2,990 ppm, slight ocular and nasal irritation was observed, but none of the animals were judged to have an impaired ability to escape. At the next higher concentration of 5,850 ppm, all guinea pigs suffered from narcosis and were judged to have impaired ability to escape. No deaths occurred at the highest concentration but ataxia occurred within 10 min and narcosis was evident after 15 min (UBTL 1978).

TABLE 3-4 Acute Nonlethal Effects of Chlorobenzene in Laboratory Animals

Species (sex)	Concentration (ppm)	Exposure Duration	Effect	Reference
Guinea pigs	2,990	30 min	Slight ocular and nasal irritation; no impaired ability to escape.	UBTL 1978
	5,850	30 min	Narcosis in all guinea pigs.	
	7,970	30 min	Ataxia within 10 min and narcosis within 15 min.	
Rats	2,990	30 min	Slight ocular and nasal irritation; no impaired ability to escape.	UBTL 1978
	5,850	30 min	Narcosis in most rats.	
	7,970	30 min	Ataxia at 10 min and narcosis within 25 min.	
Rats (male)	1,500	8 h/d for 5 d	Reduction in auditory-evoked response.	Rebert et al. 1995
	1,000		No effect.	
Rats (male)	611	4 h	Shortening of the tonic extension of the hind limbs by 37.5% after electrical stimulation.	Frantik et al. 1994
Mice (male)	1,054	5 min	RD_{50} for sensory irritation.	De Ceaurriz et al. 1981
Mice	75	3 h, once or for 5 d	No effect on host defense.	Aranyi et al. 1986
Mice (female)	610	2 h	Increased velocity of the tonic extension of the hind limbs by 30% after electrical stimulation.	Frantik et al. 1994
Mice (male)	650	4 h	Decrease in immobility in the "behavioral despair" swimming test by 2.	De Ceaurriz et al. 1983

3.2.2. Rats

Groups of five rats per sex were exposed (whole body) to chlorobenzene at mean (± SD) analytic concentrations of 2,990 ± 53, 5,850 ± 1,350, or 7,970 ± 355 ppm for 30 min, and were observed for 14 days. No deaths were observed at any concentration. At 2,990 ppm, slight ocular and nasal irritation was observed but none of the animals were judged to suffer from impaired ability to escape. At the next higher concentration of 5,850 ppm, most rats suffered from "narcosis" and were judged to have impaired ability to escape; the animals recovered quickly after exposure ended. No deaths occurred at the highest concentration, but ataxia was present at 10 min and narcosis was evident in all animals after 25 min of exposure (UBTL 1978).

Frantik et al. (1994) investigated the relative neurotoxicity of several solvents. Groups of four adult male rats (albino, specific pathogen free) were exposed at least three concentrations of chlorobenzene (analytic purity) or to ambient air. Inhalation exposure was performed in a dynamic system for 4 h, and concentrations were measured by gas chromatography. The actual exposure concentrations were not specified. Most animals were tested three or four times at intervals of 3 weeks. Immediately after exposure, the animals received a short electrical pulse through ear electrodes. The duration of subsequent tonic extension of the hind limbs was determined. This parameter was shown to be the most sensitive and consistent. The study authors calculated the concentration required to induce a 37.5% change in the neurologic response (decrease in duration of the tonic extension from 8 to 5 seconds). A 37.5%-effect concentration of 611 ppm (90% CI: 538-684 ppm) was reported for chlorobenzene. The slope was 0.061%/ppm. A 37.5% response corresponds, according to the study authors, to a concentration that does not influence normal locomotor activity or induce behavioral excitation, so it may be considered a sensitive neurologic end point.

Rebert et al. (1995) studied the effect of chlorobenzene on the auditory system of rats. Groups of eight or nine male Long Evans rats were exposed (whole body) at target concentrations of chlorobenzene of 500-2,400 ppm for 8 h per day for 5 days. Analytic concentrations determined by gas chromatography were within 10% of the target concentrations. Auditory function was assessed 3-13 days after exposure using the brainstem auditory-evoked response (integrated amplitude) elicited by 16-kilohertz (kHz) tone pips over a range of 25-95 decibels (dB), with 10 dB increments. The average response over 55-85 dB was compared with controls. A reduction in the integrated amplitude of the response was found in animals exposed at 2,000 ppm or 2,400 ppm in one experiment and at approximately 1,500 or 2,000 ppm in another (estimated from a figure) but not at 500 or 1,000 ppm (estimated from a figure). For one of the experiments, the effect was still present 4 weeks after exposure. Although it was not a subject in the Rebert et al. (1995) study, it is known that exposure to other organic solvents can result in permanent hearing loss from the destruction of cochlear hair cells. A reduction in body weight gain was observed at 2,000 and 2,400 ppm. No information was available on body weights of animals exposed at 1,500 ppm or less. Other effects

are not described in this study. The highest concentration of 2,400 ppm is close to the 6-h LC_{50} of 2,965 ppm in the rat study by Bonnet et al. (1982).

3.2.3. Mice

Frantik et al. (1994) investigated the relative neurotoxicity of several solvents. Groups of eight female H-strain mice were exposed to at least three concentrations of chlorobenzene (analytic purity) or to ambient air. Inhalation exposure was performed in a dynamic system for 2 h and concentrations were measured by gas chromatography. The concentrations of chlorobenzene were not defined. Most animals were used three or four times at intervals of 3 weeks. Immediately after inhalation, the animals received a short electrical pulse through ear electrodes. The velocity of tonic extension from toxicity was determined. This parameter was shown to be the most sensitive and consistent. The authors calculated the concentration needed to induce a 30% change in the neurologic response (decrease in velocity of the tonic extension). For chlorobenzene, a 30%-effect concentration of 610 ppm was reported (90% CI: 320-900 ppm). The slope was 0.041%/ppm. This 30% response level corresponds, according to the study authors, to a concentration that does not influence normal locomotor activity or induced behavioral excitation.

De Ceaurriz et al. (1983) tested the effect of chlorobenzene on the duration of immobility during a 3-min "behavioral despair" swimming test. Groups of 10 male Swiss OF_1 mice were exposed (whole body) for 4 h to chlorobenzene at 0, 650, 785, 875, or 1,000 ppm. The analytic concentrations were determined using gas-liquid chromatography but the results were not provided, so it was assumed that the stated concentrations were the analytic concentrations. After exposure, mice were placed in water, and duration of immobility was determined over 3 min and compared with that of control animals. A significant and concentration-dependent decrease in immobility of -28, -45, -53, and -82% was found at 650, 785, 875, and 1,000 ppm, respectively. The concentration at which there was a 50% decrease in immobility was estimated to be 804 ppm (95% CI: 718-887 ppm).

De Ceaurriz et al. (1981) determined the concentration of chlorobenzene that reduced the respiratory rate by 50% (RD_{50}) in mice. Groups of six male Swiss OF_1 mice were exposed (head only) for 5 min to at least four different concentrations of chlorobenzene. Respiratory rate was determined during exposure with a plethysmograph. The analytic concentration was determined using gas chromatography, but it is unclear whether the RD_{50} of 1,054 ppm was based on target, nominal, or analytic concentrations.

Aranyi et al. (1986) examined the effect of chlorobenzene on murine host defenses. Groups of approximately 150 female mice were exposed to chlorobenzene at 75 ppm for 3 h once or five times on 5 consecutive days. Analytic concentrations were determined using gas chromatography and were in close agreement with the target concentrations. Host defense status was determined by challenge with *Streptococcus zooepidemicus* and *Klebsiella pneumonia*. Deaths

were recorded daily over a 14-day observation period. No effect on mortality from streptococcus challenge or on bactericidal activity was found.

3.3. Developmental and Reproductive Toxicity

Groups of 32-33 pregnant female F344 rats were exposed by inhalation (whole body) to chlorobenzene at 0, 75, 210, or 590 ppm (99.982% pure, nominal concentrations) for 6 h per day on gestation days 6-15 (Hayes et al. 1982; John et al. 1984). Animals were killed on day 21 of gestation and the fetuses examined. Chlorobenzene concentrations in the chamber were determined with infrared spectrophotometry. The time-weighted average analytic concentrations were within 7-8% of the target concentrations. The exposure conditions of this study were chosen on the basis of a preliminary range-finding study in which test atmospheres of 0, 300, 1,000, and 3,000 ppm were generated, and 10 rats per concentration were exposed 6 h/day on gestation days 6-5 and sacrificed on day 16.

In the range-finding study, 3,000 ppm induced severe irritation of the eyes and nasal area, signs of narcosis, and mortality (or a moribund state). The time of onset of these effects was not specified. Effects observed at 1,000 ppm included a reduction in absolute body weight, reduced food consumption, internal and external lesions, an increase in relative kidney and liver weights, reduction in thymus size, and an increase in the number of resorptions. At 300 ppm, only a small decrease in body weight gain (on days 6-8) and an increase in relative liver weight were observed.

In the main study, maternal toxicity observed only at the highest concentration of 590 ppm, and consisted of a significant reduction in weight gain on days 6-8 and a significant increase in absolute and relative liver weights. No effects were found on pregnancy rate, litter size, resorptions, fetal body weights, or the incidence of external or soft-tissue alterations. At the highest concentration some increases in skeletal variations, such as a delay in ossification, were found (Hayes et al. 1982; John et al. 1984).

In another study, groups of 30 pregnant New Zealand white rabbits were exposed (whole body) to chlorobenzene at 0, 75, 210, or 590 ppm (99.982% pure, nominal concentrations) for 6 h/day on gestation days 6-18 (Hayes et al. 1982; John et al. 1984). Animals were killed on day 29 of gestation and the fetuses were examined. Chlorobenzene concentrations in the chamber were determined with infrared spectrophotometry. The time-weighted average concentrations were within 7-8% of the target concentrations. These exposure conditions were chosen on the basis of a preliminary range-finding study in which groups of seven rabbits were exposed at 0, 300, 1,000, and 3,000 ppm for 6 h/day on gestation days 6-18, and sacrificed on day 19.

The effects observed at 3,000 ppm in the range-finding study with rabbits were mortality (or moribund state) during exposure, severe systemic toxicity and hepatotoxicity, reduced weight gain, and macroscopic changes of the liver. Effects observed at 1,000 ppm included reduced bodyweight gain on days 6-8 and

macroscopic changes of the liver. Slight liver effects (not described) were found at 300 ppm.

Maternal toxicity observed only in the 210- and 590-ppm groups, and consisted of a significant increase in absolute and relative liver weights. No effects were found on pregnancy rate, litter size, resorptions, or fetal body weights. A small but not significant increase in head and facial anomalies and heart defects was found in the 210- and 590-ppm groups. The incidence of an extra (thoracic) rib (variation) was significantly increased in the offspring from does exposed at 580 ppm (Hayes et al. 1982; John et al. 1984).

Because of the small increase in malformations, the rabbit study was repeated using concentrations of chlorobenzene at 10, 30, 75, and 590 ppm. Maternal toxicity was found only in the 590-ppm group, and consisted of an increase in liver weight. The percentage of litters with resorptions was significantly increased at 590 ppm, but this observation was within the historical-control range. No effects were found on pregnancy rate, litter size, fetal body weights, or the incidence of external, skeletal, or soft-tissue alterations (Hayes et al. 1982 John et al. 1984). It was concluded from these studies in rats and rabbits that chlorobenzene does not induce teratogenic or embryolethal effects at up to maternally-toxic concentrations.

Nair et al. (1987) performed a two-generation reproduction study in rats exposed to chlorobenzene by inhalation. Groups of 30 male and 30 female CD rats were exposed (whole body) to target concentrations of 0, 50, 150, or 450 ppm for 6 h/day, 7 days per week. The analytic concentrations of chlorobenzene were determined using a MIRAN 1A organic vapor analyzer, and were found to be approximately 10% higher than target concentrations. No effects on mortality, body weight, food consumption, reproductive parameters, pup viability, or survival were found. Hepatic toxicity was mainly seen at 150 and 450 ppm. Increases in the incidence of small flaccid testes and in the incidence and severity of unilateral or bilateral degeneration (minimal to severe) of the germinal epithelium were found at 450 ppm in both generations. Three of six affected rats exposed at the highest concentration in each generation sired litters. Small increases in these effects were also seen at 150 ppm. An increased incidence of dilated renal pelvis was observed in males of the F_0 generation exposed at the highest concentration and in all treated males of the F_1 generation. Microscopically, an increase in renal degeneration and inflammatory lesions was found at the two highest concentrations.

The transfer of chlorobenzene to the fetus of pregnant mice after inhalation exposure at 500 ppm for 1 h was shown by Shimada (1988b). It can be concluded that chlorobenzene does not affect fertility in rats at concentrations up to those that induce over maternal toxicity.

3.4. Genotoxicity

The genotoxicity of chlorobenzene has been evaluated in several in vitro and in vivo models, as reviewed by NTP (1985), ATSDR (1990), BUA (1990),

IPCS (1991), and Hellman (1993), reported in more recent studies. A summary of the information presented in these reviews are presented in the sections below.

3.4.1. In Vitro Studies

Results of several in vitro genotoxicity studies in nonmammlian test systems, including *Salmonella typhimurium* (Lawlor et al. 1979; Haworth et al. 1983; Shimizu et al. 1983; Simmon et al.1979, NTP 1985), *Escherichia coli* (Lawlor et al. 1979), *Aspergillus nidulans* (Prasad 1970; Prasad and Pramer 1968), and *Saccharomyces cerevisiae* (Monsanto Company 1976), indicate that chlorobenzene does not induce DNA damage or gene mutations. However, chlorobenzene did induce mutations in *Actinomycetes antibioticus* (Keskinova 1968) and in another study of *S. cerevisiae* (Simmon et al. 1979).

Results of in vitro studies in mammalian cells have yielded conflicting results regarding the genotoxic potential of chlorobenzene. Chlorobenzene did not induce gene mutations in mouse lymphoma cells (Monsanto Company 1976), unscheduled DNA repair in rat liver cells (Shimada et al. 1983); Williams et al. 1989), or chromosome aberrations (Loveday et al. 1989). However, chlorobenzene induced gene mutations in mouse lymphoma cells (McGregor et al. 1988) and sister-chromatid exchange (Loveday et al. 1989). Chlorobenzene also produced decreases in cell proliferation and mitotic indices and an increase in sister-chromatid exchange rat bone-marrow cells (Khalil and Odeh 1994).

3.4.2. In Vivo Studies on Animals

Results of in vivo studies indicate that chlorobenzene has some potential to induce genotoxicity, although conflicting results have been reported. In studies of interactions with DNA, chlorobenzene has been reported to bind DNA in mouse liver, kidneys, and lungs (Grilli et al. 1985), and a guanine DNA adduct was found in the urine of rats after exposure to chlorobenzene (Krewet et al. 1989). Chlorobenzene also inducted DNA damage in peripheral lymphocytes, but not bone-marrow cells from mice following in vivo exposure (Vaghef and Hellman 1995). Chlorobenzene induced a dose-related increase in the formation of micronucleated polychromatic erythrocytes in the femoral bone marrow of mice (Mohtashamipur et al. 1987). In contrast, in vivo exposure to chlorobenzene did not induce mirconucleus formation in erythrocytes of the bone marrow of mice (Shelby et al. 1993), dominant lethal mutations or sister-chromatid exchange in mice (Feldt 1985), or recessive lethal mutations in *Drosophila melanogaster* (Valencia 1982).

3.4.3. Conclusion

The available information indicates that chlorobenzene has some potential to induce DNA damage, which is further underpinned by the formation of

epoxide-containing metabolites. However, because of conflicting results from mutagenic tests in vitro and in vivo, it is unclear whether the genotoxic activity could represent a risk to human health.

3.5. Carcinogenicity

No inhalation carcinogenicity studies are available for chlorobenzene. However, a gavage study in rats and mice was performed by NTP (1985). That study concluded that chlorobenzene increased the occurrence of neoplastic nodules of the liver at high dose (120 milligrams per kilogram of body weight per day) in male F344/N rats, providing some, but not clear evidence, of carcinogenicity in male rats. Carcinogenic effects of chlorobenzene were not observed in female F344/N rats or in male or female $B6C3F_1$ mice.

3.6. Summary

Only a few animal studies provide LC_{50} values. Bonnet et al. (1979) reported 6-h LC_{50} values of 2,965 ppm and 1,886 ppm for male rats and female mice, respectively. The concentration-response curve in rats was steep; the extrapolated concentration range between 0-100% was covered by a factor of approximately 3. However, in mice the concentration-response curve was comparatively shallow and was covered by a factor of approximately 10. No deaths were observed in rats or guinea pigs exposed at concentrations up to 7,970 ppm for 30 min (UBTL 1978). Other studies were had limitations in their reporting or were only available as a summary.

An RD_{50} for sensory irritation of 1,054 ppm in mice was assessed for chlorobenzene (De Ceaurriz et al. 1981). Chlorobenzene was moderately irritating to the skin and not irritating to the eye in standard tests (Mihail 1984). Slight ocular and nasal irritation was observed in rats and guinea pigs exposed at 2,990 ppm for 30 min (UBTL 1978). Ocular irritation in the rat was also observed after repeated exposure to chlorobenzene at 3,000 ppm (John et al. 1984).

Similar to many other volatile organic compounds, exposure to sufficiently high concentrations of chlorobenzene can induce signs of CNS depression. CNS effects are concentration-related in a continuum of slight effects (lightheadedness) to narcosis, and eventually lead to death from paralysis of the respiratory center.

Animal data related to neurotoxicity are available include clinical effects observed in standard studies and as measurements of specific parameters in specific studies. Ataxia and narcosis were reported in most rats and all guinea pigs exposed to chlorobenzene at 5,850 ppm for 30 min; the effects occurred earlier in guinea pigs than in rats (UBTL 1978). Several clinical effects indicative of neurotoxicity were reported in the acute toxicity study on rats by Bonnet et al. (1982). However, no information on the concentrations at which those effects were observed was provided. Frantik et al. (1994) studied chlorobenzene exposure in relation to the duration of the tonic extension of the hind limbs after a

short electrical pulse in rats and mice. Neurologic responses (decrease in velocity of the tonic extension) of 30% and 37.5% were found in rats and mice, respectively, exposed at 610 ppm for 4 h. This effect is considered a relatively mild end point. Rebert et al. (1995) determined that the concentration threshold for auditory changes in rats was 1,500 ppm (exposed for 8 h/ day for 5 days) and that the no-observed effect level was 1,000 ppm. The concentration of chlorobenzene that caused a 50% decrease in the duration of immobility in a "behavioral despair" swimming test in mice was 804 ppm for 4 h (De Ceaurriz et al. 1983). This effect is considered a relatively mild end point.

The developmental studies of chlorobenzene in rats and rabbits (John et al. 1984) indicates that chlorobenzene does not induce irreversible structural effects in animals when tested at up to maternally toxic concentrations of 590 ppm (for 6 h). In rats, an increase in fetal skeletal variations, such as delayed ossification, was found at 590 ppm.

A two-generation test of chlorobenzene in rats (Nair et al. 1987) indicates that chlorobenzene does not influence fertility at up to concentrations inducing systemic toxicity in the parents of 450 ppm (for 6 h).

In vitro studies indicate that chlorobenzene does not induce DNA damage or gene mutations in bacterial tests. Contradicting results were found in gene mutation tests on yeast and DNA damage and gene-mutation tests using mammalian cells. Results of an in vitro test showed that chlorobenzene did not induce chromosome damage. Based on the in vitro tests, DNA damage and gene mutations cannot be excluded. In vivo studies indicate that chlorobenzene has some potential for DNA damage. The results of the chromosome mutation tests were contradictory. A mutagenic potential in vivo for chlorobenzene cannot be excluded. Overall, the available information indicates that chlorobenzene has some potential to induce DNA damage, perhaps associated with the formation of epoxide-containing metabolites. However, because of conflicting results of the in vitro and in vivo mutagenic tests, it is unclear whether the potential measured in some of the studies represents a risk to human health.

The carcinogenicity of chlorobenzene was only tested in an oral study in rats and mice (NTP 1985). NTP concluded that chlorobenzene increased the occurrence of neoplastic nodules of the liver at 120 mg/kg/day in male F344/N rats, but not in female F344/N rats or in male or female $B6C3F_1$ mice.

4. SPECIAL CONSIDERATIONS

4.1. Metabolism and Disposition

4.1.1. Absorption

Ogata and Shimada (1983) determined the metabolites excreted in the urine of two workers exposed to chlorobenzene at 0.84 ppm for 415 min or 0.5 ppm for 228 min and compared it with their estimated intake. The main chlorobenzene metabolites recovered in the urine were *p*-chlorobenzenemercaptic acid

(MA) and 4-chlorocatechol (4-CC). Comparison of combined urinary metabolites to the estimated dose of chlorobenzene ([MA + 4-CC] ÷ estimated dose of chlorobenzene expressed as mmole/kg body weight) were 0.38 and 0.45 for the 0.84 ppm (415 min) and 0.5 ppm (228 min) exposures, respectively.

Knecht and Woitowitz (2000) exposed eight volunteers to chlorobenzene at10 ppm for 8 h/day, with an interruption of 45 min, for 5 successive days to determine a biologic tolerance level. Chlorobenzene concentrations in the blood were determined at the end of each day and every 10 min after the last exposure for 3.5 h. In one volunteer, the blood concentration was determined every hour up to 4 h during exposure. Urinary concentrations of metabolites were collected daily at before, during, and after exposure, and for 24 h after the last exposure. Chlorobenzene in blood reached a steady-state level within 1 h during a 4-h exposure at 10 ppm. No estimate of pulmonary absorption was provided.

4.1.2. Distribution

Sullivan et al. (1983) determined the distribution of ^{14}C-labeled chlorobenzene in rats after single or repeated 8-h exposures. Radioactivity in all tissues, except fat, increased in proportion to the exposure concentration. The amount of radiolabel in fat increased 30-fold between 100 and 700 ppm. Immediately after exposure, radioactivity was highest in fat tissues. The preferential distribution of chlorobenzene to adipose tissue reflects the lipophilic nature of this compound, and is confirmed in a study with mice by Shimada (1988a).

4.1.3. Metabolism

The metabolism of chlorobenzene has been investigated in several species (summarized in ATSDR 1990; BUA 1990; IPCS 1991). The first step in chlorobenzene metabolism is hepatic oxidation by cytochrome P450 to mainly chlorobenzene-3,4-epoxide, but also to chlorobenzene-2,3-epoxide and 3-chlorophenol. The epoxides are converted enzymatically by glutathione-transferase to water-soluble mercapturic-acid derivates and by epoxide hydratase via dihydrodihydroxychlorobenzene to chlorocathechols. Nonenzymatic rearrangement of the epoxides results in the formation of chlorophenols. The chlorophenols and chlorocathechols can be eliminated in the urine directly or after conjugation with glucuronic acid or sulfate. There was some indication that metabolism was saturated in rats exposed at 400 ppm. Metabolism can be affected by pretreatment with microsomal enzyme-inducing agents and by glutathione depletion. Binding of chlorobenzene to protein, RNA, and DNA was shown in several studies and were probably caused by the reactivity of the epoxide (BUA 1990).

4.1.4. Excretion

Chlorobenzene is eliminated unchanged via the lungs and as metabolites principally in the urine and to a smaller extent in the feces. The main urinary

metabolites are 4-chlorocatechol conjugates and 4-chlorophenylmercapturic acid. The percentage eliminated via the lungs increases with concentration. The elimination of inhaled chlorobenzene consists of a quick first phase (probably exhalation of chlorobenzene) and a second slower phase (probably metabolism and urinary excretion) (ATSDR 1990).

4.2. Mechanism of Toxicity

Acute inhalation exposure to chlorobenzene results in contact irritation and CNS depression. Repeated also results in toxicity to several organs including the liver, kidneys, and white blood cells.

As with many volatile organic compounds, the concentration of chlorobenzene in the brain is probably the pivotal factor for CNS depression. According to De Jongh et al. (1998), the calculated concentration of chlorobenzene in the lipid phase of the brain shows a good correlation with acute mortality data for several volatile organic compounds. So, the concentration of chlorobenzene in the brain lipid phase probably determines the potential for CNS depression and, eventually, death from paralysis of the respiratory center.

No information is available on the mechanism of the toxicity to several organs including liver, kidneys, and white blood cells after inhalation exposure. However, studies (Reid 1973; Reid and Krishna 1973) with intraperitoneal exposure to radioactive chlorobenzene show covalent binding to proteins. Also, pretreatment with cytochrome-P450 inhibitors or inducers reduced or potentiated the binding and the renal and hepatic necrosis. This suggests that toxic effects in the liver and kidneys and probably also white blood cells (or bone marrow) are from reactive metabolites. The reactive epoxides are normally removed by the enzymatic reaction with glutathione or by epoxide hydratase. Repeated exposure can, therefore, result in glutathione depletion. A decrease in glutathione can result in an increase in covalent binding and toxicity.

The mechanism of action for the toxicity of chlorobenzene is unknown and, therefore, the appropriate dose metric cannot be assessed. Physiologically-based pharmacokinetic (PBPK) modeling could provide some additional insight by evaluating correlations between selected dose metrics and dose-response data from toxicity studies. However, not all metabolites can be modeled in sufficient detail currently and the available data are scarce for appropriate dose-response modeling. Therefore, the uncertainty was judged to be too large for using the PBPK-modeling to derive AEGL values.

4.3. Structure-Activity Relationships

No quantitative structure-activity relationships were found for chlorobenzene and its congeners. However, chlorobenzene shares its CNS-depressing action with other aromatic compounds like benzene, toluene, and xylene. A comparison of LC_{50} values for several aromatic compounds (Bonnet et al. 1979,

1982), indicated that chlorobenzene was one of most toxic compounds. Only dichlorobenzene was more toxic than chlorobenzene.

4.4. Other Relevant Information

4.4.1. Irritation and Sensitization

Chlorobenzene was considered moderately irritating to the skin and nonirritating to the eye in the OECD 404 and 405 tests (Suberg 1983). A maximization test gave no indication of a sensitizing potential (Mihail 1984).

5. DATA ANALYSIS FOR AEGL-1

5.1. Summary of Human Data Relevant to AEGL-1

Ogata et al. (1991) exposed four volunteers to a chlorobenzene at 60 ppm for 3 h in the morning and 4 h in the afternoon, with a 1-h break between exposures. All volunteers complained of a disagreeable odor and of drowsiness after exposure ended. Three subjects had a heavy feeling in the head or had headaches, two reported throbbing pain in the eyes, and one complained of sore throat. No information was given about the time of onset of these complaints. No effect on pulse rate or systolic and diastolic pressure was found. Flicker fusion frequency values were reduced significantly at the end of the 3-h exposure period in the morning, indicating lowered perception, but no further effect was seen in the afternoon. The significance of this finding is difficult to interpret.

Knecht and Woitowitz (2000) exposed eight volunteers to chlorobenzene at 10 ppm for 8 h/day for 5 days to determine the relationship between exposure and urinary concentrations of 4-chlorocatechol and chlorophenols. None of the subjects complained of irritant or CNS effects (U. Knecht, Justus Liebig University Giessen, Germany, personal commun. 2005).

5.2. Summary of Animal Data Relevant to AEGL-1

No adequate animal data relevant to the type of effects defined by the AEGL-1 were found.

5.3. Derivation of AEGL-1

Effects in subjects exposed to chlorobenzene at 60 ppm are indicative of slight CNS depression and local irritation (Ogata et al. 1991), and are considered evidence of discomfort. These effects were not observed is subjects exposed at 10 ppm for 8 h (Knecht and Woitowitz 2000). Thus, 10 ppm was chosen as a conservative point of departure for the derivation of AEGL-1 values. Because human data are used, an interspecies uncertainty factor of 1 was used. Despite

the fact that only a few subjects were tested, an uncertainty factor of 1 for intraspecies variability was considered appropriate because of the conservatism of the point of departure already provides a margin of safety. (The point of departure of 10 ppm was obtained from a repeated-exposure study, and effects observed at 60 ppm were rather slight.) No information about the time dependency of the effects at 10 or 60 ppm is available. Because the effects at 60 ppm include irritation and CNS effects (drowsiness, heavy feeling in the head, and headache), the 8-h AEGL-1 value of 10 ppm is considered appropriate for all time points. Furthermore, Knecht and Woitowitz (2000) reported that chlorobenzene concentrations in blood reached a steady-state level within 1 h. AEGL-1 values for chlorobenzene are presented in Table 3-5.

6. DATA ANALYSIS FOR AEGL-2

6.1. Summary of Human Data Relevant to AEGL-2

No human data were available that adequately address toxicity end points as defined by AEGL-2.

6.2. Summary of Animal Data Relevant to AEGL-2

Slight ocular and nasal irritation was found in guinea pigs exposed to chlorobenzene at a mean (± SD) analytic concentration of 2,990 ± 53 ppm, but none of the animals was judged to suffer from impaired ability to escape. At the next higher concentration tested of 5,850 ± 1,350 ppm, all guinea pigs and most rats suffered from narcosis and were judged to have impaired ability to escape (UBTL 1978). Frantik et al. (1994) determined that a 37.5% decrease in the duration of tonic extension of the hind limbs of rats after a short electrical pulse was induced after a 4-h exposure at 611 ppm. A comparable study in mice found a 30% effect level at 610 ppm. The investigators noted that these concentrations were not expected to influence normal locomotor activity or to induce behavioral excitation, based on extrapolation from concentration-response data. Rebert et al. (1995) determined a no-observed-effect level of 1,000 ppm for auditory effects in rats exposed for 8 h/day for 5 days. Because organic solvents can induce permanent hearing loss, this was considered a relevant end point for AEGL-2 derivation. However, whether permanent hearing loss can be induced by single exposure is unclear. De Ceaurriz et al. (1983) conducted a study to evaluate behavioral response to swimming in an enclosed space (escape behavior followed by immobility), and estimated the concentration of chlorobenzene to elicit a 50% decrease in immobility to be 804 ppm. However, no signs of toxicity, such as motor impairment, were reported in response to chlorobenzene exposure. Thus, this effect was considered a subtle change in neurobehavior.

Repeated exposure of rats and rabbits to chlorobenzene resulted in severe effects at concentrations of 1,000 ppm and greater, in limited effects around 500 ppm, and no effects at 200 ppm (Hayes et al. 1982; John et al. 1984).

TABLE 3-5 AEGL-1 Values for Chlorobenzene

10 min	30 min	1 h	4 h	8 h
10 ppm (47 mg/m^3)	10 ppm (47 mg/m^3)	10 ppm (47 mg/m^3)	10 ppm (47 mg/m^3)	10 ppm (47 mg/m^3)

6.3. Derivation of AEGL-2

There are no adequate human data for deriving AEGL-2 values, so animal data were used. The threshold for AEGL-2 effects (impaired ability to escape) in rats and guinea pigs appears to be between 2,900 ppm, which produced slight ocular and nasal irritation, 5,980 ppm, which produced narcosis (UBTL 1978).

Behavioral and neurophysiologic effects were observed in rats and mice at concentrations below 2,990 ppm (De Ceaurriz et al. 1983; Frantik et al. 1994; Rebert et al. 1995); however, these observations were not considered suitable bases for a point of departure for AEGL-2 values for the following reasons. Effects on brainstem auditory-evoked potential in rats were associated with repeated exposures at >1,000 ppm (8 h/day for 5 days) (Rebert et al. 1995), and represent changes in sensitivity of the brain (or cochlear hair cells) to detect sound at specific frequencies. The effects cannot be associated reliably with concentrations would cause hearing loss sufficient to impair escape, nor can they be extrapolated to single exposures as the effect observed might reflect cumulative exposures over 5 days (Rebert et al. 1995).

Inhibition of electrically-evoked seizures in rats and mice (Frantik et al. 1994) was observed with chlorobenzene at 611 and 610 ppm, respectively. Suppression of such seizure activity has been shown to be a predictor of CNS depressing activity (e.g., narcosis) at higher concentrations for a variety of substances (Frantik et al. 1994). However, the CNS effects observed occurred at concentrations below the levels that would be expected to produce CNS depression of sufficient magnitude to impair mobility or escape (based on results of UBTL 1978). In addition, narcosis was not reported in the Frantik et al. (1994) study.

Shortening the duration of the immobility response in mice occurred in association with a 4-h exposure to chlorobenzene at 650 ppm (De Ceaurriz et al. 1983). This effect has been reported in rats and mice after treatment with antidepressant drugs (De Ceaurriz et al. 1983). The mechanism for the response to chlorobenzene is not understood, although a CNS effect is suggested (De Ceaurriz et al. 1983). The effects in the De Ceaurriz et al. (1983) study would not be sufficient to affect mobility (or escape) in the absence of a panic stimulus. In mice, the effect was to shorten the duration of immobility, not to produce immobility or lengthen the period of immobility. In addition, narcosis was not reported in the study.

Given that the neurosensory and behavioral and effects observed at lower concentrations are not directly relevant to AEGL-2 effects, the most appropriate point of departure is the absence of AEGL-2 related effects in rats and guinea

pigs exposed at 2,990 ppm for 30 min (UBTL 1978). An interspecies uncertainty factor of 3 was applied because data were comparable for rats and guinea pigs, suggesting no large interspecies differences, and the critical effect is CNS depression. The concentration of chlorobenzene in the brain is probably related directly to inhalation rate. Therefore, humans probably require higher external exposures that rodents to obtain a similar concentration of chlorobenzene in the blood or brain. Experience with anesthetic gases shows that interindividual variability in CNS depression is generally not greater than a factor of 2 or 3. Therefore, an intraspecies uncertainty factor of 3 was used. A combined uncertainty factor of 10 was considered appropriate because a larger factor would result in AEGL-2 values that would conflict with human data (values would be lower than 60 ppm, a concentration shown to cause only minor effects in humans). With a combined uncertainty actor of 10, the 30-min AEGL-2 is 300 ppm.

The 30-min value was extrapolated across time periods using the equation $C^n \times t = k$, with default values of n = 1 for extrapolation to 10-min and n = 3 for extrapolation to 1-h values. The 4- and 8-h values were set equal to the 1-h value because a steady-state chlorobenzene concentration in blood is reached within 1 h and its elimination is rapid. Furthermore, time scaling would result in 4- and 8-h AEGL-2 values (37 and 19 ppm, respectively) that conflict with human data (Ogata et al. 1991). AEGL-2 values are presented in Table 3-6.

7. DATA ANALYSIS FOR AEGL-3

7.1. Summary of Human Data Relevant to AEGL-3

No human mortality data on chlorobenzene were available.

7.2. Summary of Animal Data Relevant to AEGL-3

Several animal studies relevant to deriving AGEL-3 values were available, but most were available only from descriptions in secondary sources or were from older literature that lacked adequate details. Mortality data on rabbits exposed to chlorobenzene at 550-660 ppm (Rozenbaum et al. 1947 could not be used because the results were from a secondary source and the findings conflicted with a developmental-toxicity study in which no mortality occurred in rabbits exposed at 1,000 ppm (John et al. 1984). No deaths occurred in guinea pigs exposed at 2,990, 5,850, or 7,970 ppm for 30 min (UBTL 1978).

Bonnet et al. (1982) estimated a 6-h LC_{50} of 2,965 ppm for chlorobenzene in male Sprague-Dawley rats. This estimate is consistent with the results of a range-finding developmental-toxicity study in rats, in which deaths were observed at 3,000 ppm but not at 1,000 ppm (John et al. 1984). However, the results of Bonnet et al. (1982) conflict with those of Rebert et al. (1995), who reported no deaths in male Long Evans rats exposed at 1,000-2,400 ppm for 8 h/day for 5 days. Strain differences might have contributed to these conflicting results.

There are several studies of acute mortality in mice exposed to chlorobenzene, but most descriptions lack adequate detail. A 6-h LC_{50} of 1,886 ppm in mice was reported by Bonnet et al. (1979). Mice appear to be more sensitive than rats to chlorobenzene.

7.3. Derivation of AEGL-3

Data in rats and guinea pigs reported by UBTL (1978) provide the most appropriate point of departure for AEGL-3 derivation. No mortality occurred in either species after exposure to chlorobenzene at 7,970 ppm for 30 min. Interspecies and intraspecies factors of 3 were applied on the same basis they were applied in the derivation of AEGL-2 values. Time scaling was also performed in the same fashion as for the AEGL-2 calculations, and the 4- and 8-h AEGL-3 values were set equal to the 1-h value. Time scaling with n = 1 would result in an 8-h AEGL-3 value of 50 ppm, which would not be consistent with the observation that only slight CNS depression and irritation occurred in humans exposed at 60 ppm for 7 h. Table 3-7 summarizes the AEGL-3 values. These values are consistent with the AEGL-2 values (see Table 3-6) and are supported by the 6-h LC_{01} of 1,873 ppm calculated from the probit equation reported by Bonnet et al. (1982).

8. SUMMARY OF AEGLS

8.1. AEGL Values and Toxicity End Points

AEGL values for chlorobenzene are presented in Table 3-8.

8.2. Comparison with Other Standards and Guidelines

The immediate danger to life or health (IDLH) value of 1,000 ppm was based on acute inhalation toxicity studies. With exception of the 10-min AEGL-3 value, all AEGL-values are below the IDLH value. The 8-h AEGL-2 value is about twice as high as the PEL-TWA (permissible exposure limits - time weighted average) established by the Occupational Safety and Health Administration. The TLV-TWA (threshold limit value - time weighted average) of the American Conference of Governmental Industrial Hygienists and analogous German and Dutch standards are equal to the 8-h AEGL-1 value. A summary of extant standards and guidelines for chlorobenzene is provided in Table 3-9.

TABLE 3-6 AEGL-2 Values for Chlorobenzene

10 min	30 min	1h	4h	8h
430 ppm (2,021 mg/m^3)	300 ppm (1,410 mg/m^3)	150 ppm (705 mg/m^3)	150 ppm (705 mg/m^3)	150 ppm (705 mg/m^3)

TABLE 3-7 AEGL-3 Values for Chlorobenzene

10 min	30 min	1h	4h	8h
1,100 ppm (5,170 mg/m^3)	800 ppm (3,760 mg/m^3)	400 ppm (1,880 mg/m^3)	400 ppm (1,880 mg/m^3)	400 ppm (1,880 mg/m^3)

TABLE 3-8 Summary of AEGL Values for Chlorobenzene

Classification	Exposure Duration				
	10 min	30 min	1h	4h	8h
AEGL-1 (nondisabling)	10 ppm (47 mg/m^3)	10 ppm (47 mg/m^3)	10 ppm (47 mg/m^3)	10 ppm (47 mg/m^3)	10 ppm (47 mg/m^3)
AEGL-2 (disabling)	430 ppm (2,021 mg/m^3)	300 ppm (1,410 mg/m^3)	150 ppm (705 mg/m^3)	150 ppm (705 mg/m^3)	150 ppm (705 mg/m^3)
AEGL-3 (lethal)	1,100 ppm (5,170 mg/m^3)	800 ppm (3,760 mg/m^3)	400 ppm (1,880 mg/m^3)	400 ppm (1,880 mg/m^3)	400 ppm (1,880 mg/m^3)

TABLE 3-9 Extant Standards and Guidelines for Chlorobenzene

Guideline	Exposure Duration				
	10 min	30 min	1h	4h	8h
AEGL-1	10 ppm (47 mg/m^3)	10 ppm (47 mg/m^3)	10 ppm (47 mg/m^3)	10 ppm (47 mg/m^3)	10 ppm (47 mg/m^3)
AEGL-2	430 ppm (2,021 mg/m^3)	300 ppm (1,410 mg/m^3)	150 ppm (705 mg/m^3)	150 ppm (705 mg/m^3)	150 ppm (705 mg/m^3)
AEGL-3	1,100 ppm (5,170 mg/m^3)	800 ppm (3,760 mg/m^3)	400 ppm (1,880 mg/m^3)	400 ppm (1,880 mg/m^3)	400 ppm (1,880 mg/m^3)
ERPG-1 (AIHA)[a]			30 ppm		
ERPG-2 (AIHA)			500 ppm		
ERPG-3 (AIHA)			1,000 ppm		
IDLH (NIOSH)[b]		1,000 ppm			
TLV-TWA (ACGIH)[c]					10 ppm
PEL-TWA (OSHA)[d]					75 ppm
MAK (Germany)[e]					10 ppm
MAC (The Netherlands)[f]					10 ppm

[a]ERPG (emergency response planning guidelines, American Industrial Hygiene Association) (AIHA 2010)
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 protection 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 1994) represents the maximum concentration from which one could escape within 30 min without any escape-impairing symptoms, or any irreversible health effects.

[c]TLV-TWA (threshold limit value – time weighted average, American Conference of Governmental Industrial Hygienists) (ACGIH 2010) 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.

[d]PEL-TWA (permissible exposure limits - time weighted average, Occupational Safety and Health Administration) (29 CFR 1910.1000 [2006]) is defined analogous to the ACGIH TLV-TWA, but is for exposures of no more than 10 h/day, 40 h/week. .

[e]MAK (maximale arbeitsplatzkonzentration [maximum workplace concentration]) (Deutsche forschungsgemeinschaft [German Research Association] (DFG 2005) is defined analogous to the ACGIH TLV-TWA.

[f]MAC (maximaal aanvaaarde concentratie [maximal accepted concentration]) Dutch Expert Committee for Occupational Standards,, The Netherlands (MSZW 2004) is defined analogous to the ACGIH TLV-TWA.

8.3. Data Quality and Research Needs

Human data are scarce. Only two kinetic studies of chlorobenzene in humans were found, and no adequate studies of toxic effects in human were available. Of the studies relevant to AEGL-2 values, only one study was of sufficient quality, in which rats and guinea pigs were exposed for 30 min to nonlethal concentrations.

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APPENDIX A

DERIVATION OF AEGLS VALUES FOR CHLOROBENZENE

Derivation of AEGL-1 Values

Key studies:	Ogata, M., and Y. Shimada. 1983. Differences in urinary monochlorobenzene metabolites between rats and humans. Int. Arch. Occup. Environ. Health 53(1):51-57. Knecht, U., and H.J. Woitowitz. 2000. Human toxicokinetics of inhaled monochlorobenzene: Latest experimental findings regarding re-evaluation of the biological tolerance value. Int. Arch. Occup. Environ. Health 73(8):543-554.
Toxicity end point:	Slight CNS effects (drowsiness, heavy feeling in the head, and headache) and local irritation at 60 ppm (7 h with a 1-h break after 3 h) and no effects at 10 ppm (8 h/day for 5 days). The latter concentration was used as the point of departure.
Time scaling:	None, because chlorobenzene concentrations in blood reach a steady-state level within 1 h.
Uncertainty factors:	1 for interspecies differences 1 for intraspecies variability
Calculations:	
10-min AEGL-1:	Set equal to 8-h AEGL-1 value of 10 ppm
30-min AEGL-1:	Set equal to 8-h AEGL-1 value of 10 ppm
1-h AEGL-1:	Set equal to 8-h AEGL-1 value of 10 ppm
4-h AEGL-1:	Set equal to 8-h AEGL-1 value of 10 ppm
8-h AEGL-1:	10 ppm

Derivation of AEGL-2 Values

Key study:	UBTL (Utah Biomedical Test Laboratory). 1978. Utah Biomedical Test Laboratory Report on NIOSH Sponsored Inhalation Study for IDLH

	Values (Final Report) with Cover Letter Dated 102291. Submitted by Shell Oil Company to U.S. Environmental Protection Agency, Washington, DC. EPA Document No. TSCATS 88-920000156. Microfiche No. OTS 0534605.
Toxicity end point:	Narcosis in rats and guinea pigs.
Time scaling:	2,990 ppm for 30 min was extrapolated across time periods using the equation $C^n \times t = k$, with default values of n = 1 for extrapolation to 10-min and n = 3 for extrapolation to 1-h values. The 4- and 8-h values were set equal to the 1-h value because a steady-state chlorobenzene concentration in blood is reached within 1 h and for reasons of consistency with human data. $C^3 \times t = k$: $k = (2{,}990 \text{ ppm})^3 \times 30 \text{ min} = 8.02 \times 10^{11}$ ppm-min $C^1 \times t = k$: $k = 2{,}990 \text{ ppm} \times 30 \text{ min} = 89{,}700$ ppm-min
Uncertainty factors:	3 for interspecies differences 3 for intraspecies variability
Calculations:	
10-min AEGL-2:	$[(8.02 \times 10^{11} \text{ ppm-min}) \div 10 \text{ min}]^{1/3} \div 10 = 430$ ppm (rounded)
30-min AEGL-2:	2,990 ppm ÷ 10 = 300 ppm (rounded)
1-h AEGL-2:	(89,700 ppm-min ÷ 60 min) ÷ 10 = 150 ppm
4-h AEGL-2:	Set equal to 1-h AEGL-2 value of 150 ppm
8-h AEGL-2:	Set equal to 1-h AEGL-2 value of 150 ppm

Derivation of AEGL-3 Values

Key study:	UBTL (Utah Biomedical Test Laboratory). 1978. Utah Biomedical Test Laboratory Report on NIOSH Sponsored Inhalation Study for IDLH Values (Final Report) with Cover Letter Dated 102291. Submitted by Shell Oil Company to U.S. Environmental Protection Agency, Washington,

	DC. EPA Document No. TSCATS 88-920000156. Microfiche No. OTS 0534605
Toxicity end point:	No mortality in rats or guinea pigs.
Time scaling:	7,970 ppm for 30 min was extrapolated across time periods using $C^n \times t = k$, with default values of n = 1 for extrapolation to 10-min and n = 3 for extrapolation to 1-h values. The 4- and 8-h values were set equal to the 1-h value because a steady-state chlorobenzene concentration in blood is reached in 1 h and for reasons of consistency with AEGL-2 values. $C^3 \times t = k$: $k = (7{,}970 \text{ ppm})^3 \times 30 \text{ min} = 1.52 \times 10^{13}$ ppm-min $C^1 \times t = k$: k = 7,970 ppm × 30 min = 239,100 ppm-min
Uncertainty factors:	3 for interspecies differences 3 for intraspecies variability
Calculations:	
10-min AEGL-3:	$[(1.52 \times 10^{13} \text{ ppm-min}) \div 10 \text{ min}]^{1/3} \div 10 = 1{,}100$ ppm (rounded)
30-min AEGL-3:	7,970 ppm ÷ 10 = 800 ppm (rounded)
1-h AEGL-3:	(239,100 ppm-min ÷ 60 min) ÷ 10 = 400 ppm (rounded)
4-h AEGL-3:	Set equal to 1-h AEGL-3 value of 400 ppm
8-h AEGL-3:	Set equal to 1-h AEGL-3 value of 400 ppm

APPENDIX B

CATEGORY PLOT FOR CHLOROBENZENE

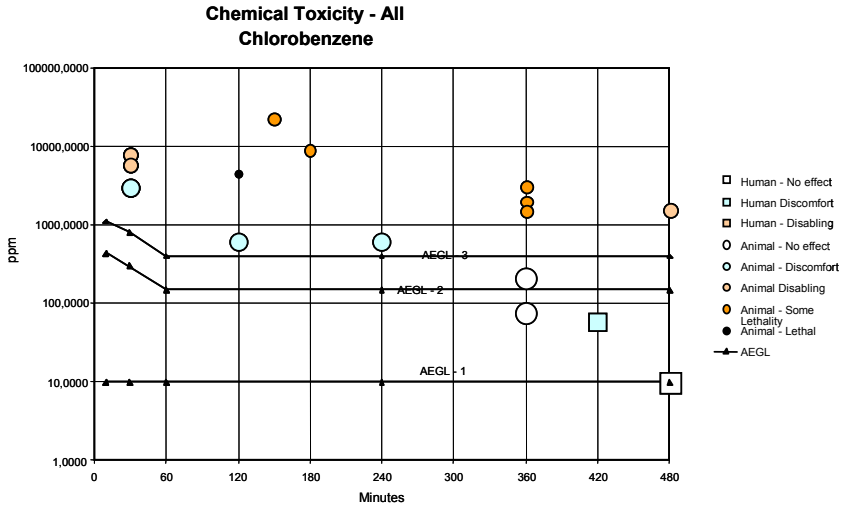

FIGURE B-1 Category plot of animal and human data and AEGL values for chlorobenzene.

APPENDIX C

ACUTE EXPOSURE GUIDELINE LEVELS FOR CHLOROBENZENE

Derivation Summary for Chlorobenzene

AEGL-1 VALUES

10 min	30 min	1h	4h	8h
10 ppm (47 mg/m^3)	10 ppm (47 mg/m^3)	10 ppm (47 mg/m^3)	10 ppm (47 mg/m^3)	10 ppm (47 mg/m^3)

Key references:
Study 1: Ogata, M., and Y. Shimada. 1983 Differences in urinary monochlorobenzene metabolites between rats and humans. Int. Arch. Occup. Environ. Health 53(1):51-57.
Study 2: Knecht, U., and H.J. Woitowitz. 2000. Human toxicokinetics of inhaled monochlorobenzene: Latest experimental findings regarding re-evaluation of the biological tolerance value. Int. Arch. Occup. Environ. Health 73(8):543-554.

Test species/Strain/Number: Humans, 4 subjects (study 1), 8 subjects (study 2)

Exposure route/Concentrations/Durations: Study 1: inhalation, 60 ppm, 7-h exposure with a 1-h break after 3 h; Study 2: inhalation, 10 ppm, 8 h/day for 5 days

Effects: Study 1: slight CNS effects (drowsiness, heavy feeling in the head, and headache) and local irritation at 60 ppm; Study 2: no effects at 10 ppm.

End point/Concentration/Rationale: No discomfort effects at 10 ppm

Uncertainty factors/Rationale:
Total uncertainty factor: 1
Interspecies: 1, data are from a human study
Intraspecies: 1, effects at 60 ppm were very slight

Modifying factor: None

Animal-to-human dosimetric adjustment: Not relevant

Time scaling: None. No information on time dependency is available in the studies, but the observed effects do not indicate a strong time dependency; this is also supported by absorption data.

Data adequacy: The key studies were evaluations of the kinetics of chlorobenzene, and were not designed to determine toxicity.

AEGL-2 VALUES

10 min	30 min	1h	4h	8h
430 ppm (2,021 mg/m^3)	300 ppm (1,410 mg/m^3)	150 ppm (705 mg/m^3)	150 ppm (705 mg/m^3)	150 ppm (705 mg/m^3)

Key reference: UBTL (Utah Biomedical Test Laboratory). 1978. Utah Biomedical Test Laboratory Report on NIOSH Sponsored Inhalation Study for IDLH Values

(Continued)

AEGL-2 VALUES Continued

10 min	30 min	1h	4h	8h
430 ppm (2,021 mg/m^3)	300 ppm (1,410 mg/m^3)	150 ppm (705 mg/m^3)	150 ppm (705 mg/m^3)	150 ppm (705 mg/m^3)

(continued)

(Final Report) with Cover Letter Dated 102291. U.S. EPA/OPTS Public Files OTS0534605. Submitted by Shell Oil Company to U.S. Environmental Protection Agency, Washington, DC. EPA Document No. TSCATS 88-920000156. Microfiche No. OTS 0534605.

Test species/Strain/Number: Rats and guinea pigs, strains unknown, five per sex per species.

Exposure route/Concentrations/Durations: Inhalation, 2,990, 5,850, or 7,970 ppm for 30 min; 14-day observation.

Effects:
2,990 ppm: Slight ocular and nasal irritation
5,850 ppm: Narcosis and impaired ability to escape
7,970 ppm: No deaths; ataxia and narcosis

End point/Concentration/Rationale: No narcosis at 2,990 ppm for 30 min; no animals suffered from impaired ability to escape.

Uncertainty factors/Rationale:
Total uncertainty factor: 10 (a larger uncertainty factor would lead to AEGL-2 values that conflict with human data)
Interspecies: 3, data were comparable for rats and guinea pigs suggesting no large interspecies differences, and the critical effect is CNS depression.
Intraspecies: 3, interindividual variability for CNS depression by comparable gases generally will not be greater than a factor of 2 or 3.

Modifying factor: None

Animal-to-human dosimetric adjustment: None

Time scaling: $C^n \times t = k$; default values of n = 3 for 10-min value and n = 1 for 60-min value. AEGL-2 values for 4 and 8 h are set equal to the 1-h value because chlorobenzene concentrations in blood reach a steady-state within 1 h and its elimination is rapid. Time scaling would result in 4- and 8-h AEGL-2 values that would conflict with human data.

Data adequacy: Only one study (30-min exposures at three concentrations) aimed at identifying an immediately dangerous to life and health value.

AEGL-3 VALUES

10 min	30 min	1h	4h	8h
1,100 ppm (5,170 mg/m^3)	800 ppm (3,760 mg/m^3)	400 ppm (1,880 mg/m^3)	400 ppm (1,880 mg/m^3)	400 ppm (1,880 mg/m^3)

Key reference: UBTL (Utah Biomedical Test Laboratory). 1978. Utah Biomedical Test Laboratory Report on NIOSH Sponsored Inhalation Study for IDLH Values

(Continued)

AEGL-3 VALUES Continued

10 min	30 min	1h	4h	8h
1,100 ppm (5,170 mg/m^3)	800 ppm (3,760 mg/m^3)	400 ppm (1,880 mg/m^3)	400 ppm (1,880 mg/m^3)	400 ppm (1,880 mg/m^3)

(Final Report) with Cover Letter Dated 102291. Submitted by Shell Oil Company to U.S. Environmental Protection Agency, Washington, DC. EPA Document No. TSCATS 88-920000156. Microfiche No. OTS 0534605

Test species/Strain/Number: Rats and guinea pigs, strains unknown, five per sex per species.

Exposure route/Concentrations/Durations: Inhalation, 2,990, 5,850, or 7,970 ppm for 30 min; 14-day observation.

Effects:
2,990 ppm: Slight ocular and nasal irritation
5,850 ppm: Narcosis and impaired ability to escape
7,970 ppm: No deaths; ataxia and narcosis

End point/Concentration/Rationale: No deaths after 30-min exposure at 7,970 ppm.

Uncertainty factors/Rationale:
Total uncertainty factor: 10 (a larger factor would lead to AEGL-3 values that would conflict with AEGL-2 values)
Interspecies: 3, data were comparable for rats and guinea pigs suggesting no large interspecies differences, and the critical effect is CNS depression.
Intraspecies: 3, interindividual variability for CNS depression by comparable gases generally will not be greater than a factor of 2 or 3.

Modifying factor: None

Animal-to-human dosimetric adjustment: None

Time scaling: $C^n \times t = k$; default values of n = 3 for 10-min value and n = 1 for 1-h value. AEGL-3 values for 4- and 8-h are set equal to the 1-h value because chlorobenzene concentrations in blood reach a steady-state within 1 h and its elimination is rapid. Furthermore, time scaling would result in 4- and 8-h AEGL-3 values that would conflict with AEGL-2 values and human data.

Data adequacy: Sufficient

4

Chloroform[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 minute (min) to 8 hour (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 are defined as follows:

AEGL-1 is the airborne concentration (expressed as parts per million or milligrams per cubic meter [ppm or 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 Robert Young (Oak Ridge National Laboratory), Gary Diamond (Syracuse Research Corporation), Chemical Manager Steven Barbee (National Advisory Committee [NAC] on Acute Exposure Guideline Levels for Hazardous Substances), and Ernest V. Falke (U.S. Environmental Protection Agency). 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 guidelines reports (NRC 1993, 2001).

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 concentrations 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 AEGL values represent threshold concentrations 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

Chloroform is a volatile liquid with a pleasant, nonirritating odor. The chemical is miscible with organic solvents but is only slightly soluble in water. Chloroform is produced and imported in large quantities for use in chemical syntheses, as a solvent, and in the manufacture of some plastics. It was used in the past as an anesthetic and in pharmaceutical preparations, but such uses are no longer allowed in the United States.

Human data on acute exposure to chloroform are from older studies that tested various exposure regimens (680-7,200 ppm for 3-30 min); effects included detection of a strong odor, headaches, dizziness, and vertigo. Published reports of surgical patients anesthetized with chloroform lack precise exposure details, but suggest that exposure to high concentrations (generally greater than 13,000 ppm) might produce cardiac arrhythmias and transient hepatic and renal toxicity. Quantitative data on human fatalities after acute inhalation exposure to chloroform were not available.

Only a few animal studies on the lethality from acute exposure to chloroform were available. Quantitative data include a 4-h LC_{50} (lethal concentration, 50% lethality) of 9,780 ppm in rats and a 7-h LC_{50} of 5,687 ppm in mice. Other data indicate notable lethality after exposures ranging from 5 min at “saturated” concentration (approximately 25,000 ppm) to 12 h at 726 ppm. Nonlethal effects of chloroform in laboratory animals include biochemical (elevated serum-

enzyme activity) and histopathologic indices of hepatic toxicity. Data on the reproductive and developmental toxicity of chloroform in animals are equivocal. One study reported evidence of fetotoxicity in rats after gestational exposure to chloroform at 30 ppm, but another study found no evidence of such toxicity with gestational exposures at 2,232 ppm.

There are no inhalation exposure studies demonstrating carcinogenic responses to chloroform, but oral exposure has been shown to cause tumors in rats (kidney tumors in male) and mice (hepatocarcinomas in male and female mice). Data on the mechanism of toxicity and tumorigenicity of chloroform suggest that the tumorigenic response occurs at concentrations that cause cell death and proliferative cellular regeneration. Thus, a linear low-dose extrapolation for cancer risk might not be appropriate. For this reason and because the inhalation slope factor for chloroform is based on oral-exposure studies, the AEGL values for chloroform are based on noncarcinogenic effects.

Metabolism and disposition studies have affirmed that metabolism of chloroform to phosgene is mediated by the enzyme cytochrome P-450 IIE1, and that phosgene along with the depletion of glutathione and the formation of trichlorocarbon-radical intermediates are responsible for the toxicity of chloroform. Data from several studies indicate that the metabolism and, therefore, the rate of production of reactive metabolites are greater in rodents than in humans.

AEGL-1 values for chloroform were not recommended. Attempts to identify a critical effect consistent with the AEGL-1 definition were considered tenuous and uncertain. Exposures of humans to chloroform at concentrations approaching those inducing narcosis or possibly causing hepatic and renal effects (AEGL-2 effects) are not accompanied by overt signs or symptoms. Furthermore, chloroform is not irritating and its odor is not unpleasant.

AEGL-2 values for chloroform were based on embryotoxicity and fetotoxicity observed in rats when dams were exposed to chloroform at 100 ppm for 7 h/day on gestation days 6-15 (Schwetz et al. 1974). An assumption was made that the effects could be caused by a single 7-h exposure. Because data on the metabolism and kinetics of chloroform indicate that rodents are more sensitive than humans to the toxic effects of chloroform, an uncertainty factor for interspecies differences was not applied. An intraspecies uncertainty factor of 3 was applied to account for variability in metabolism and disposition among individuals and to protect more susceptible individuals (e.g., individual exposed to other inducers of P-450 monooxygenase, such as alcohol). Additional reduction of the AEGL-2 values was not warranted because the critical effect and the assumption of a single-exposure scenario provided a conservative point of departure. The concentration-time relationship for many irritant and systemically acting vapors and gases may be described by the equation $C^n \times t = k$, where the exponent n ranges from 0.8 to 3.5 (ten Berge et al. 1986). In the absence of data with which to empirically derive a chloroform-specific scaling exponent (n), temporal scaling was performed using default values of n = 3 when extrapolating to shorter-exposure durations or n = 1 when extrapolating to longer-exposure durations.

AEGL-3 values for chloroform were based on a mouse 560-min LCt_{50} of 4,500 ppm. Because no data were available for estimating a lethality threshold, the LC_{50} was reduced by a factor of 3 to 1,500 ppm, a concentration unlikely to cause lethality based on comparisons with other human and animal data. An uncertainty factor of 3 to protect sensitive individuals was applied. As with the AEGL-2 derivations, an intraspecies uncertainty factor of 3 was selected because it is unlikely that induction of metabolism would increase toxic effects by an order of magnitude. Rodents appear to metabolize chloroform at a greater rate than humans, resulting in the production of reactive, toxic intermediates at a greater rate. Results of physiologically-based pharmacokinetic (PBPK) model studies have shown that rodents, especially mice, are considerably more susceptible to the lethal effects of chloroform than humans. Therefore, the AEGL-3 values were increased 3-fold by a weight-of-evidence adjustment factor of 1/3. This adjustment is further justified by data on the use of chloroform as a surgical anesthesia, which show that cumulative exposures to chloroform at concentrations >675,000 ppm/min or at 22,500 ppm for up to 120 min resulted in surgical anesthesia and cardiac irregularities but not death. Time scaling was performed using n = 3 to extrapolate from the 560-min duration (the point of departure) to the shorter AEGL-time periods. To minimize uncertainties with extrapolating a 560-min exposure to a 10-min exposure, the 30-min AEGL-3 value of 4,000 ppm was adopted for the 10-min AEGL value.

Carcinogenic potential after a single, acute exposure to chloroform was assessed, and AEGLs values calculated. AEGL-2 values based on noncancer toxicity were slightly greater than those based on cancer risks. However, the carcinogenic response to chloroform appears to be a function of necrosis and subsequent regenerative cellular proliferation, which are not relevant to a single acute exposure.

TABLE 4-1 Summary of AEGL Values for Chloroform

Classification	10 min	30 min	1 h	4 h	8 h	End Point (Reference)
AEGL-1 (nondisabling)	NR[a]	NR[a]	NR[a]	NR[a]	NR[a]	
AEGL-2 (disabling)	120 ppm (580 mg/m^3)	80 ppm (390 mg/m^3)	64 ppm (312 mg/m^3)	40 ppm (195 mg/m^3)	29 ppm (141 mg/m^3)	Embryotoxicity and fetotoxicity in rats exposed for 7 h/day on gestation days 6-15 (Schwetz et al. 1974); single exposure assumed.
AEGL-3 (lethal)	4,000 ppm (19,000 mg/m^3)	4,000 ppm (19,000 mg/m^3)	3,200 ppm (16,000 mg/m^3)	2,000 ppm (9,700 mg/m^3)	1,600 ppm (7,800 mg/m^3)	Estimated lethality threshold for mice; 3-fold reduction of 560-min LC_{50} of 4,500 ppm to 1,500 ppm (Gehring 1968).

[a]Not recommended; data were insufficient to develop AEGL-1 values and AEGL-1 effects unlikely to occur in the absence of notable toxicity.

1. INTRODUCTION

Chloroform is a volatile liquid with a pleasant, nonirritating odor. The chemical is miscible with organic solvents but is only slightly soluble in water. Chloroform is produced and imported in large quantities (93-350 million pounds/year) and used in chemical syntheses, for refrigeration, as a solvent, and in the manufacture of polytetrafluoroethylene plastics (DeShon 1978; Li et al. 1993). It was used in the past as an anesthetic and in pharmaceutical preparations, but such uses are no longer allowed in the United States. Chloroform is also a byproduct of wood-pulp chlorination for production of paper products. Chemical and physical data on chloroform are presented in Table 4-2.

AIHA (1989) reported an odor threshold for chloroform of 192 ppm based on the geometric mean of acceptable values (133-276 ppm). An odor detection concentration of 6.1 ppm was reported by EPA (1992).

2. HUMAN TOXICITY DATA

2.1. Acute Lethality

Quantitative data on acute inhalation exposures to chloroform resulting in death were not available.

TABLE 4-2 Chemical and Physical Data for Chloroform

Parameter	Value	Reference
Synonyms	Trichloromethane; methenyl chloride; methyl trichloride	DeShon 1978
CAS registry no.	67-66-3	Budavari et al. 1996
Chemical formula	$CHCl_3$	Budavari et al. 1996
Molecular weight	119.39	Budavari et al. 1996
Physical state	Liquid	Budavari et al. 1996
Vapor pressure	159.6 mm Hg at 20°C	DeShon 1978
Density	1.484 at 20°C	Budavari et al. 1996
Boiling/melting point	61-62°C/-63.5°C	Budavari et al. 1996
Solubility	1 mL/200 mL water at 20°C	Budavari et al. 1996
Conversion factors in air	1 ppm = 4.88 mg/m^3 1 mg/m^3 = 0.21 ppm	NIOSH 2011

2.2. Nonlethal Toxicity

Several reports are available to qualitatively characterize the human health effects from acute inhalation exposure to chloroform. Hutchens and Küng (1985) reported nausea, appetite loss, transitory jaundice, cardiac arrhythmias, arterial hypotension, mild intravascular hemolysis, and unconsciousness in an individual after intentional, nonsuicidal inhalation of chloroform.

Lehmann and Hasegawa (1910) conducted controlled exposure studies on human subjects. The results of this study showed that a 3-min exposure to chloroform at 920 ppm induced vertigo and dizziness and a 30-min exposure at 680 ppm produced a moderately strong odor. A 30-min exposure at 1,400 ppm produced lightheadedness, giddiness, lassitude, and headache; at 3,000 ppm, gagging and pounding of the heart occurred. Chloroform at 4,300-5,100 ppm for 20 min or at 7,200 ppm for 15 min produced light intoxication and dizziness. These data appeared to be from only three subjects, and the methods of exposure and measurements were unavailable. The signs and symptoms of exposure described in this report appear to be consistent with early stages of narcosis.

Lehmann and Flury (1943) reported that chloroform at 389 ppm for 30 min was tolerated in humans without complaint, but that a concentration of 1,030 ppm caused dizziness, intracranial pressure, and nausea within 7 min and headache that persisted for several hours.

Whitaker and Jones (1965) analyzed the clinical effects of chloroform anesthesia in 1,502 surgery patients. Although the duration of anesthesia varied from <30 min to over 2 h, chloroform concentration never exceeded 2.25% (22,500 ppm). For most of the cases (1,164), anesthesia was for less than 30 min. Clinical observations included tachypnea, bradycardia, cardiac arrhythmias, hypotension, one case of transient jaundice, and one death (this case was complicated by renal insufficiency and could not necessarily be attributed to chloroform). The duration required to attain anesthesia was not specified, but it probably occurred within a few minutes. These observations demonstrate that a short exposure to chloroform at 22,500 ppm will induce a surgical plane of anesthesia concurrent with various physiologic responses.

The clinical effects associated with chloroform-induced anesthesia were also studied by Smith et al. (1973). However, the use of these data for AEGL development is compromised by confounders, including the use of premedication with diazepam and pentobarbital or with hydroxyzine and pentobarbital. The concentration of chloroform inspired appeared to vary between 0.85% (8,500 ppm) and 1.3% (13,000 ppm), and the average duration of anesthesia was 112.0 ± 60.38 min among the 58 surgical patients. Forty-six percent of the patients receiving chloroform experienced nausea and vomiting. Clinical assessment of liver function and toxicity indicated transient alterations. One ventricular tachycardia occurred that necessitated pharmacologic correction. Data from a single patient indicated that chloroform at 8,500 ppm would induce anesthesia.

McDonald and Vire (1992) examined the possible health hazards associated with chloroform use in endodontic procedures. Two industrial hygiene monitors were used to sample the air in the treatment operatory and additional sampling devices were attached to the dentist and the dental assistant. The concentrations of chloroform in the operatory area samples were <0.57 ppm for a 5.5-h period, and concentrations in individual air samples were <0.88 ppm over a 150-min period. Health screening tests of the dentist and assistant revealed no signs of liver, kidney, or lung damage 5 h postexposure or 1 year after the study.

Although specific data were not presented, Snyder and Andrews (1996) reported that humans might tolerate chloroform at up to 400 ppm for 30 min without complaint, but might experience dizziness and gastrointestinal upset at 1,000 ppm for 7 min and narcosis at 14,000 ppm (no duration specified).

2.2.1. Epidemiologic Studies

Several epidemiologic studies on occupational exposure to chloroform have been conducted. These studies involve worker populations exposed to chloroform for periods of time in excess of what would be considered acute exposure, and are not directly applicable to developing AEGL values. They do, however, provide some insight regarding the relationship between the AEGL values and health effects that might be associated with long-term exposures.

Challen et al. (1958) evaluated workers in a pharmaceutical manufacturing process that involved exposure to chloroform vapor. The exposure groups were described as eight "long-service operators" (3- to 10-year exposures) exposed at 77-237 ppm; nine "short-service operators" (10- to 24-month exposures), who were replacements for the long-service operators and were exposed at 23-71 ppm; and five controls, who were not exposed to processes involving chloroform. All of the workers were women whose ages ranged from 34 to 60 years. Some long-service operators had been observed staggering about the work area. All long-service workers experienced alimentary effects (e.g., nausea, flatulence, thirst), increased micturition and urinary discomfort, and behavioral effects (e.g., depression, irritability, poor concentration ability, motor deficiencies) during employment. All experienced nausea and stomach upset on smelling chloroform after leaving their employment. Two of nine short-service operators reported no effects from chloroform exposure, five reported dryness of the mouth and throat while at work, two had similar experiences as the long-service operators, and several reported lassitude.

Bomski et al. (1967) studied workers in a Polish pharmaceutical factory and gave special emphasis to chloroform-induced susceptibility to viral infection. Chloroform exposures were 2-205 ppm, but the frequency of sampling was not specified. The incidence of viral hepatitis was greater in chloroform-exposed workers than in nonexposed subjects, so the authors postulated that chloroform-induced hepatic damage might have predisposed the workers to the viral infec-

tion. Increased incidences of spleen and liver enlargement were also found in the chloroform-exposed workers.

Li et al. (1993) conducted surveys of chloroform-producing facilities in Shanghai, China. Most of the workers exposed to chloroform were involved in the production of perspex (polymethylmethacrylate) and chemical synthesis. In the three facilities sampled (where no effective preventive equipment or measures were in place), chloroform concentrations were 4.27-147.91 mg/m^3 (0.88-31.06 ppm), with a geometric mean of 21.38 mg/m^3 (4.49 ppm) for 119 samples. Chloroform concentrations were <20 mg/m^3 (4.20 ppm) in 45.5% of the samples. Exposure groups were classified as Exposure I (13.49 mg/m^3 [2.83 ppm]; 1-15 years of exposure) and Exposure 2 (29.51 mg/m^3 [6.20 ppm]; 1-15 years of exposure). The exposure groups and control group (no obvious chloroform or other hazardous exposures) included males and females as well as smokers and nonsmokers; all groups had an average age of approximately 36 years. The investigators concluded that long-term exposure to chloroform at 29.51 mg/m^3 (6.20 ppm) resulted in functional liver damage, as determined by changes in various serum enzymes (alanine aminotransferase [ALT], gamma-glutamyl transferase, and adenosine deaminase), prealbumin, serum transferrin, and blood urea nitrogen.

2.3. Reproductive and Developmental Toxicity

Wennborg et al. (2000) studied a cohort of Swedish women who had worked in laboratory or nonlaboratory jobs for 1 year or more in 1990-1994. The investigators obtained data from questionnaires sent to 763 women (66 were excluded for various reasons) that assessed reproductive history, health status, time-to-pregnancy, personal habits, specific work, and exposure to various agents and specific times at which those exposures occurred. The data were compared with respective birth information from the Swedish Medical Register. Parameters examined included spontaneous abortion, birth weight, preterm delivery, small-for-gestation age, large-for-gestation age, and congenital deformities. A number of confounding variables were considered (e.g., high blood pressure, smoking, gynecologic and chronic disease, sexually transmitted infectious diseases, father's work and potential exposures during time of conception, previous abortions). Information about consumption of alcohol, tea, and coffee and stress levels was not included. The analysis included 869 pregnancies but did not involve specific-exposure concentrations, and did not account for exposures to other chemicals. There was no association between laboratory work and spontaneous abortions. A weak association between women who had worked with chloroform before conceiving and spontaneous abortions was found, but there was no significant association between chloroform exposure and small-for-gestational age or body weight.

2.4. Genotoxicity

No studies were found on the genotoxicity of chloroform in humans.

2.5. Carcinogenicity

Although epidemiology studies have been conducted to assess the carcinogenic potential of chloroform in drinking water, no studies are available on the carcinogenic potential of chloroform in humans following inhalation exposure. In 1987, EPA (2012) developed an inhalation slope factor of 6.1×10^{-3} per mg/kg/day based on an increased incidence of renal tumors in male rats after long-term exposure to chloroform in drinking water (Jorgenson et al. 1985). Route-to-route extrapolation was required for calculating the slope factor because inhalation data were not available.

2.6. Summary

Quantitative data on human lethality after acute exposure to chloroform are unavailable. Although they lack quantitative data and often pertain to oral exposures, clinical reports affirm the hepatotoxicity and renal toxicity of chloroform, as well as its neurologic effects. The available data on nonlethal responses indicate that acute inhalation of chloroform might result in narcosis and might be preceded by signs and symptoms characteristic of early stages of anesthesia. Early reports in which the effects of chloroform inhalation were observed in human subjects have uncertainties related to the concentration measurements but do provide information on the human experience that does not appear to be inconsistent with other data. A summary of data relevant to acute, nonlethal exposure of humans to chloroform is presented in Table 4-3.

3. ANIMAL TOXICITY DATA

3.1. Lethal Toxicity

3.1.1. Rats

Results of preliminary range-finding experiments for a large number of chemicals were reported by Smyth et al. (1962). Chloroform vapor (concentration not specified but presumably a saturated concentration of approximately 25,000 ppm) killed all 6 of the albino rats (strain not specified) exposed for 5 min. A 4-h exposure at 8,000 ppm (nominal concentration; no analytical determination) killed 5 of 6 albino rats.

TABLE 4-3 Nonlethal Effects of Chloroform in Humans after Acute Inhalation Exposure

Number of Subjects	Concentration, ppm	Duration, min	Effect	Reference
3	920	3	Vertigo	Lehmann and Hasegawa 1910
3	680	30	Strong odor	Lehmann and Hasegawa 1910
3	1,400	30	Light headedness, lassitude, headache	Lehmann and Hasegawa 1910
3	3,000	30	Pounding heart, gagging	Lehmann and Hasegawa 1910
NA	4,300-5,100	20	Intoxication, dizziness	Lehmann and Hasegawa 1910
NA	7,200	15	Intoxication, dizziness	Lehmann and Hasegawa 1910
NA	389	30	No complaints	Lehmann and Flury 1943
NA	1,030	7	Dizziness, intracranial pressure, nausea, persistent headache	Lehmann and Flury 1943
1,502	22,500	<30 - >120 (most <30)	Surgical anesthesia, cardiac irregularities	Whitaker and Jones 1965
58	8,500-13,000	113 (mean duration)	Surgical anesthesia	Smith et al. 1973
2	<0.5	330	No effects[a]	McDonald and Vire 1992
2	<0.88	150	No effects[a]	McDonald and Vire 1992

[a]Health screening conducted at 5 h postexposure and at one year after exposure.
Abbreviations: NA, not available

The results of an inhalation study in rats were briefly described in report to E. I. du Pont de Nemours and Co. (Haskell Laboratory 1964). The study, designed to assess the toxicity of Freon TC® and Freon-113®, also included experiments with chloroform (a component of Freon TC®). Mortality in rats (sex and strain not specified) exposed to chloroform at concentrations of 5,000, 3,700, or 3,000 ppm for 4 h was 3/4, 3/4, and 0/4, respectively. Deaths occurred 2-3 days after exposure; the four rats in the 3,000-ppm group were killed 14-days postexposure. No information was provided about the methods for measuring chloroform concentrations (atmosphere produced by heating chloroform and injection into the chamber via a nebulizer); only nominal exposure concentrations were reported. No histopathology data were provided on the chloroform-treated rats.

In experiments of the effect of chloroform on barbiturate metabolism and narcosis, Puri et al. (1971) exposed male Sprague-Dawley rats at 726 ppm for up to 48 h (continuous exposure). One group of rats was exposed to chloroform alone. On the basis data presented in graphs, continuous 12-h exposure to chloroform resulted in at least 10 deaths. It is unclear if any deaths occurred before 12 h.

Lundberg et al. (1986) reported a 4-h LC_{50} of 47,702 mg/m^3 (9,780 ppm) for female Sprague-Dawley rats. Groups of 10 rats were exposed to a series of chloroform concentrations (specific-exposure concentrations for the series were not provided but were reported as being equivalent to 1/2, 1/4, 1/8, 1/16, or 1/32 of the LC_{50} or the saturation concentration). Mortality was determined 24 h after exposure. The exposure concentrations were measured by infrared detection in a suitably designed apparatus.

3.1.2. Mice

The results of studies with mice exposed to chloroform were reported by Fühner (1923). Groups of mice (sex and strain not reported; 30 mice total) were exposed to chloroform at 12-38 mg/L (2,458-7,782 ppm). Each mouse was exposed in a 10-L bottle in which chloroform was vaporized to achieve the desired concentration. Concentrations were not determined analytically. Five mice exposed at 2,458-5,120 ppm exhibited reflex loss after 48-215 min, but no deaths occurred. Exposure at 4,710-5,529 ppm resulted in reflex loss after 30-90 min; 12 of 18 animals recovered and 6 died. Deaths occurred within 71-175 min of exposure. Six of seven mice exposed to chloroform at 6,758-7,782 ppm exhibited reflex loss after 13-46 min and one mouse died after a 35-min exposure (reflex loss occurred at 8 min). The absence of validated exposure concentrations limits the quantitative validity of these data. Four additional mice were exposed at 5,585 ppm for 120 or 135 min. For the three mice exposed for 120 min, death occurred 105, 130, and 140 min after the start of exposure, and the one mouse exposed for 135 min died 95 min after exposure. Under the conditions of these experiments, the findings suggest that exposure concentrations in the vicinity of 4,710 ppm might represent a lethal threshold for mice after 1-2 h of exposure.

A 7-h LC_{50} of 5,687 ppm for mice was reported by von Oettingen et al. (1949). These experiments used 20 adult white mice (strain and sex not specified) exposed to chloroform in a bell jar. Chloroform concentrations were calculated on the basis of the amount of test material volatilized over time and the volume of air passed through the chamber. The concentrations were also determined by chemical analysis. The graphic presentation of the experimental results indicated an LC_{30} of 5,529 ppm and an LC_{90} of 6,963 ppm. At the concentrations tested (4,915-7,372 ppm), the mice exhibited progressive central nervous system depression followed by rapidly occurring narcosis. Deaths started occurring after 3-5 h.

In a study by Deringer et al. (1953), the nephrotoxic and lethal effects of inhaled chloroform were examined using male and female C3H mice. Groups of 2-month old mice (six of each sex) were exposed to chloroform at concentrations of 3.38-5.4 mg/L (693-1,106 ppm) for 1, 2, or 3 h. Groups of 8-month old mice (six of each sex) were also exposed similarly. Twenty-two male and 20 female mice served as untreated controls. Mice were observed daily for deaths or morbidity, and were examined weekly for tumors or other abnormal conditions. Necropsies were performed on all moribund or dead mice and any female mice with mammary tumors. Regardless of the exposure duration or concentration, all of the male mice (except one) exposed to chloroform exhibited evidence of kidney damage. Within 11 days after exposure, 15 of 18 8-month old males and 7 of 18 2-month old males died. The remainder of the 8-month old males survived 5-7 months, and the remainder of the 2-month old male mice survived 14-18 months. Generally, the deaths occurred earlier in mice exposed for 2-3 h than in those exposed for only 1 h; specific data, however, were not provided. Histologic findings in mice that died included necrosis and calcification of the proximal and distal convoluted tubules of the kidney. Necrosis appeared to be more severe with earlier deaths. Additionally, hepatic necrosis was also observed in mice exposed at 942-1,106 ppm that died within 6 days. For male mice surviving longer and in all female mice, hepatic damage was not notable. The results of this study show that a 3-h exposure of male C3H male mice to chloroform at 692 ppm or a 1-h exposure at 921 ppm resulted in severe renal damage and death.

The influence of sex-hormone status on gender-specific chloroform-induced nephrotoxicity in mice was studied by Culliford and Hewitt (1957). Although the primary objective of the study was to verify the influence of androgens on chloroform-induced nephrotoxicity, the initial results of the study provided evidence of nearly complete tubular necrosis in two strains of male mice after a 2-h inhalation exposure. Male Westminster Hospital (in-house, uniform heterozygous) mice exposed to chloroform at 3.3-7.0 mg/L (676-1,434 ppm) and male CBA mice exposed at 1.2-5 mg/L (246-1,024 ppm) all exhibited complete tubular necrosis 24 h after exposure. Female mice of these strains did not exhibit any evidence of renal damage. The study also showed that administration of estrogen to male mice abolished the susceptibility to the nephrotoxic response, and that the administration of testosterone to female mice increased susceptibility. The chloroform concentrations were calculated on the basis of the amount of chloroform added to the 6-L exposure chamber, and the assumption of complete vaporization at 80°F and uniform dispersal. No analytic measurements were made, thereby imparting some uncertainty about the chamber concentrations.

In studying the hepatotoxicity of chlorinated hydrocarbons, Gehring (1968) calculated a 4,500-ppm LCt_{50} for chloroform of 560 min (540-585 min, 95% confidence interval [CI]) for female Swiss-Webster mice, a 4,500-ppm ECt_{50} of 35 min (31.0-39.6 min, 95% CI) for narcosis, and a 4,500-ppm ECt_{50} of

2.3 min (1.9-2.8 min, 95% CI) for increased serum glutamic pyruvic transaminase (SGPT) activity. Groups of mice (10/group for narcosis determination and 20/group for lethality determination) were exposed to chloroform at 4,500 ppm. The control group consisted of 254 mice representing a composite group of controls for all of the chlorinated hydrocarbons tested. Chloroform concentrations were attained by metering it into a heated tube for vaporization. Actual concentrations were measured by continuous flow of the atmosphere through an infrared spectrophotometric cell. The experiment was repeated if the chloroform concentration varied by more than 7%. Mortality at 4,500-ppm ranged from approximately 5% after 400 min to 80% after 700 min. The exposure-response relationship for narcosis exhibited the same slope. These data suggest that, at a chloroform concentration of 4,500 ppm, there is approximately a 16-fold difference between the time-to-narcosis (35 min) and the time-to-death (560 min) for mice exposed under the conditions of this study. Increased SGPT was also reported but exhibited a notably different exposure-response relationship (see Section 3.2.2.).

3.1.3. Dogs

The effect of chloroform-induced anesthesia in dogs was studied by Whipple and Sperry (1909). Details of the exposure concentrations are limited to notation of the amount of chloroform (in ounces) used on each dog. Anesthesia duration varied from 1.5 to 2.5 h and chloroform amounts varied from <1 to 3 ounces. Some of the dogs died. It was not possible to ascertain a definitive dose-response relationship from the data.

von Oettingen et al. (1949) studied the effects of chloroform (15,000 ppm nominal; 14,376 ppm determined) in 10 beagles (sex not specified) that had been surgically prepared with a tracheal cannula and carotid- and femoral-artery cannulae to which pressure transducers were attached. After recovering from the pentothal-induced surgical anesthesia (beginning of voluntary movement and "lively" reflex), the dogs were exposed continuously to the chloroform. The average survival time was 202 min with a range of 60-285 min.

3.1.4. Summary of Lethal Toxicity in Animals

The lethality of inhaled chloroform in laboratory animals is summarized in Table 4-4. With the exception of a rat 4-h LC_{50} value (9,780 ppm) reported by Lundberg et al. (1986) and a mouse LCt_{50} (4,500 ppm; 560 min) reported by Gehring (1968), the data are more qualitative in nature. Data from older studies lack details about the generation and measurement of exposure atmospheres of chloroform. The available data do not present a clear delineation of the lethality of acute inhalation exposure to chloroform.

TABLE 4-4 Lethal Toxicity of Chloroform in Laboratory Animals after Acute Inhalation Exposure

Species	Exposure Concentration (ppm)	Exposure Duration (min)	Effect	Reference
Rat	9,780	240	4-h LC_{50}[a]	Lundberg et al. 1986
Rat	3,000	240	100% mortality	Haskell Laboratory 1964
Rat	3,700	240	75% mortality[b]	Haskell Laboratory 1964
Rat	5,000	240	75% mortality[b]	Haskell Laboratory 1964
Rat	8,000	240	≈80% mortality	Smyth et al. 1962
Rat	"Saturated concentration"	5	100% mortality	Smyth et al. 1962
Rat	726	720	Lethality (no specifics provided)	Puri et al. 1971
Mouse	5,529	420	7-h LC_{30}	von Oettingen et al. 1949
Mouse	5,687	420	7-h LC_{50}	von Oettingen et al. 1949
Mouse	6,963	420	7-h LC_{90}	von Oettingen et al. 1949
Mouse	4,710-5,529	71-175	66% mortality	Fühner 1923
Mouse	6,758-7,782	35	14% mortality	Fühner 1923
Mouse	2,458-5,120	48-215	No deaths	Fühner 1923
Mouse	5,585	120	75% mortality[c]	Fühner 1923
Mouse	4,500	560 min	LCt_{50}	Gehring 1968

[a]Mortality occurred 24-h postexposure.
[b]Deaths determined 2-3 days postexposure.
[c]Deaths occurred 105-140 min postexposure.

3.2. Nonlethal Toxicity

3.2.1. Rats

In experiments by Brown et al. (1974b), groups of 3-9 male Sprague-Dawley rats were used to assess the effect of P-450 induction by phenobarbital on chloroform-induced reductions in glutathione (GSH). Both induced and non-induced (control) rats were exposed for 2 h to chloroform at concentrations of 0.5 or 1.0% (5,000 or 10,000 ppm). Induced rats in the 5,000- and 10,000-ppm groups had a decrease in GSH of approximately 70% and 83%, respectively. Control rats exhibited no decrease in GSH concentrations, which suggests that GSH concentrations in rats are more than sufficient for scavenging reactive intermediates of chloroform metabolism at the concentrations tested.

Brondeau et al. (1983) examined the effect of chloroform on serum-enzyme activities in rats. Groups of eight male Sprague-Dawley rats were exposed (whole-body) to chloroform at concentrations of 0, 137, 292, 400, 618, 942, or 1,075 ppm for 4 h. Chamber atmospheres were analyzed by gas chromatography (sample loop was compared with a known concentration standard) and

by analysis of a solid absorbent (activated charcoal or silica gel subjected to appropriate solvent extraction and gas-liquid chromatography). Exposure to the lowest concentration failed to significantly alter the activity levels of any the tested enzymes (glutamate dehydrogenase [GLDH], glutamic oxaloacetic transaminase [GOT], glutamic pyruvic transaminase [GPT], and sorbitol dehydrogenase [SDH]). Even at the highest concentration there was only a <2- to 7-fold increase in serum-enzyme activities. Statistically significant increases in GLDH and SDH were observed in rats exposed at 292 ppm. GLDH and SDH appeared to be most affected, although none of the changes in activity levels demonstrated a definitive exposure-response relationship. Although some of the increases were statistically significant ($p < 0.05$ for GLDH; $p < 0.02$ for SDH), the toxicologic relevance of these changes is uncertain. In a second phase of the study, rats were exposed to chloroform at 301 ppm (considered by the investigators to be a threshold for alteration in serum-enzyme activity based on the 4-h experiments) for two 6-h or four 6-h exposures. GLDH and SDH activities were somewhat greater after four 6-h exposures than after a single 4-h exposure or two 4-h exposures.

Statistically significant increases in serum SDH activity were also reported in female Sprague-Dawley rats exposed to chloroform for 4 h at concentrations as low as 153 ppm (1/64 of the LC_{50} for chloroform) (Lundberg et al. 1986). Although useful as biomarkers of exposure, increases in serum-enzyme activity in the absence of clinical correlates is limited use as an end point for AEGL derivation.

Ikatsu and Nakajima (1992) studied the interaction between carbon tetrachloride and chloroform in ethanol-treated rats. Controls groups of four rats were exposed only to chloroform at concentrations of 0, 50, or 100 ppm for 8 h. Concentrations in the dynamic air flow chamber were monitored every 15 to 30 min by gas chromatography. Hepatotoxicity was determined by assessing changes in serum glutamic oxaloacetic transaminase (SGOT), SGPT, liver malondialdehyde (MDA), and plasma MDA. Only marginal and statistically insignificant changes were detected for these indices in chloroform-treated rats, thereby indicating that an 8-h exposure at 50 or 100 ppm was without appreciable effect. Histopathologic examination revealed only negligible fat deposits in the liver of rats exposed at 100 ppm. These findings are consistent with those of Brondeau et al. (1983). In rats pretreated with ethanol (2 g ethanol/80 mL liquid diet fed daily for 6 weeks), only SGOT levels were increased significantly (3-fold; $p < 0.05$) after exposure to chloroform at 50 ppm. Exposure of ethanol-treated rats to chloroform at 100 ppm chloroform, however, resulted in significant ($p < 0.05$) increases in SGOT (7.5-fold) and SGPT (14-fold). There was no effect on liver MDA or plasma MDA. In ethanol-treated rats, ballooned hepatocytes in midzonal areas of the liver were observed only in rats exposed to chloroform at 100 ppm. The results of this study indicate that 8-h exposure of rats to chloroform at 50 or 100 ppm produce only minor effects that are more indicative of exposure rather than toxicity. Ethanol pretreatment followed by an 8-h exposure to chloroform at 100 ppm produced notable signs of toxicity.

In a study of the hepatotoxicity and renal toxicity of inhaled chloroform, male F344 rats (5 animals per group) were exposed at 1, 3, 10, 30, 100, or 300 ppm for 6 h/day for 7 days (Larson et al. 1994). Effects on nasopharyngeal tissue were also examined (Méry et al. 1994). Cage-side observations indicated no signs of toxicity during the exposure period, although there was a significant dose-dependent decrease in body weight gain at concentrations of 10 ppm and greater. Mild centrilobular vacuolation was observed only in the 300-ppm group, and histopathologic changes (hyperplasia) were found in the groups exposed at 10 ppm and greater. Two treatment-related lesions were observed in the nasal region of the chloroform-exposed rats. An increase in the size of goblet cells of the nasopharyngeal meatus was observed in rats exposed at 100 or 300 ppm. Also, region-specific changes were observed in the olfactory mucosa and bone of the ethmoid turbinates of rats exposed to chloroform at 10 ppm or greater. Although these data are not appropriate for deriving AEGL values, they may be used to evaluate the protectiveness of the AEGL values.

In studies to assess the effect of ethanol on the metabolism and toxicity of chloroform by various routes of administration, Wang et al. (1994) described nonlethal effects in male Wistar rats exposed by inhalation to chloroform alone (50, 100, or 500 ppm for 6 h). Indices of hepatotoxicity (GOT, GPT, and GSH) were evaluated in groups of five rats. Rats exposed to chloroform at 50 or 100 ppm exhibited no significant changes in any serum-enzyme activities. Both GOT and GPT were significantly ($p < 0.05$) increased after a 6-h exposure to chloroform at 500 ppm (about 1.6- and 1.2-fold, respectively), but were not considered indicative of severe hepatotoxicity. Ethanol pretreatment resulted in enzyme activities that were approximately 2-fold greater than from chloroform alone, and failed to alter GSH levels.

3.2.2. Mice

As described in Section 3.1.2, Fühner (1923) exposed mice to chloroform at 12-38 mg/L (2,458-7,782 ppm). In addition to lethality, nonlethal effects were observed. Mice exposed at 2,458-5,120 ppm exhibited reflex loss after 48-215 min of exposure, but no deaths occurred. Exposure at 4,710-5,529 ppm resulted in reflex loss after 30-90 min; 12 of 18 animals recovered and 6 died. Deaths occurred with 71-175 min of exposure. For mice exposed at 6,758-7,782 ppm, six mice exhibited reflex loss after 13-46 min and one mouse died after a 35-min exposure (reflex loss occurred after 8 min). The absence of validated exposure concentrations limits the quantitative validity of these data.

Kylin et al. (1963) reported on hepatotoxicity in mice after a single inhalation exposure to chloroform. A pilot study to determine the exposure duration needed to reach a maximum increase in serum ornithine carbamyl transferase (OCT) was conducted using groups of five female albino mice exposed to chloroform at 3,000 ppm for 4 h. Mice were killed after 1, 2, 4, 8, or 16 days. In the main study, groups of 10 female albino mice were exposed to chloroform at 0,

100, 200, 400, or 800 ppm for 4 h. Histopathologic examination of the liver and measurements of serum OCT were conducted 24 and 72 h after exposure and used as indices of effect. Chloroform was vaporized before injection into the constant-flow chamber, but no information was provided about whether concentrations were measured in the chamber. In the pilot study, serum OCT concentrations reach a maximum 4 days postexposure. In the main study, fatty infiltration of the liver was observed 1 day after exposure to chloroform at 100 ppm. At higher concentrations, the extent and severity of fatty degeneration increased. The authors concluded that the minimum concentration of chloroform to produce fatty infiltration of the liver in mice after a 4-h exposure was <100 ppm. Histologic changes (fatty infiltration and necrosis) also appeared to be greater after 24 h than after 72 h.

Gehring (1968), in addition to examining indices of lethality (see Section 3.1.2), determined 4,500-ppm ECt values for narcosis and for significant increases in SGPT in female Swiss-Webster mice. Groups of mice (10/group for narcosis determination and 8-10/group for SGPT determination) were exposed to chloroform at 4,500 ppm and the response rate was evaluated relative to exposure duration. The control group consisted of 254 mice representing a composite group of controls for all of the chlorinated hydrocarbons tested. SGPT increases greater than 54 Reitman-Frankel (R-F) units were considered statistically significant (control values were 24.4 ± 14.7 R-F units). Chloroform concentrations were attained by metering the chloroform into a heated tube for vaporization. Actual concentrations were measured by continuous flow of the atmosphere through an infrared spectrophotometric cell. The experiment was repeated if the chloroform concentration varied by more than 7%. The 4,500-ppm ECt_{50} for narcosis was 35 min (31.0-39.6 min, 95% CI), with a 10% response occurring after 15 min and 80% response occurring after 40 min. The 4,500-ppm ECt_{50} for a significant increase in SGPT was 13.5 min (10.1-18.1 min, 95% CI). A 20% response was observed after about 6 min, and a 90% response was observed after 20 min. The exposure-response relationship for SGPT increases was notably different than that observed for narcosis and lethality. The authors noted that elevation of SGPT activity occurred much earlier than narcosis or lethality; therefore, chloroform was induced liver damage before the onset of narcosis.

The hepatotoxicity and renal toxicity of inhaled chloroform was studied in female $B6C3F_1$ mice (5 animals per group) exposed to chloroform at 1, 3, 10, 30, 100, or 300 ppm for 6 h/day for 7 d (Larson et al. 1994). The effects on nasopharyngeal tissue were also examined (Méry et al. 1994). Centrilobular hepatocyte necrosis and severe vacuolation in centrilobular hepatocytes were observed in mice exposed at 100 and 300 ppm. Mild to moderate vacuolar changes were observed in the 10-ppm and 30-ppm groups. Notable renal toxicity was observed only in the 300-ppm group. Histologic changes in the nasal region of female mice included increased cell proliferation at concentrations of 10 ppm and greater, and a slight indication of new bone growth in the endoturbinate of one mouse in the 300-ppm group. In a later report, however, it was noted that

the nasal lesions induced in female mice after exposure to chloroform at 10, 30, or 90 ppm for 6 h/day were transient and not sustained in mice similarly exposed for up to 13 weeks (Larson et al. 1996).

3.2.3. Dogs

Renal toxicity in dogs exposed by inhalation to chloroform was reported by Whipple and Sperry (1909). Details of the experimental protocol, including the exposure conditions, were lacking. The report provided only qualitative information regarding clinical signs (vomiting, diarrhea, and lassitude). Gross pathologic and histopathologic evidence of hepatotoxicity and renal toxicity was reported for dogs on successive days after being exposed to 1-2 ounces of chloroform over 1-2 h.

von Oettingen et al. (1949) described the effects of chloroform on various physiologic functions in dogs surgically prepared for monitoring of respiration and blood pressure (see Section 3.1.3.). Continuous exposure to chloroform at 15,000 ppm resulted in the death of all 10 dogs (6-285 min). The dogs exhibited notable cardiovascular responses (decreased arterial blood pressure), decreased respiratory rate and body temperature, and depression of voluntary and involuntary reflexes within 35 min. Although it is uncertain whether deaths would have been prevented by ending the exposure at or before 35 min, the data provide a qualitative description of the response in dog to very high concentrations of chloroform.

3.2.4. Cats

Nonlethal effects in cats exposed to chloroform were described by Lehmann and Schmidt-Kehl (1936). In this study, adult cats were exposed to chloroform at concentrations of 7,200 or 22,000 ppm. Concentrations were determined by chemical reaction (hydrolysis with alkali in alcohol). At 7,500 ppm, cats exhibited light narcosis after 78 min and deep narcosis after 93 min. Light and deep narcosis were induced after 10 min and 13 min, respectively, at 22,000 ppm. Irritation of the eyes, mouth, and nose were also found at that concentration.

3.2.5. Summary of Nonlethal Toxicity in Animals

The nonlethal toxicity of chloroform in laboratory animals (rats, mice, and cats) after acute inhalation exposure is summarized in Table 4-5. As would be expected of a hepatotoxicant, many of the nonlethal effects reported were indices of liver damage. Acute exposures (1-4 h) to chloroform at concentrations of 100-292 ppm have resulted in some degree of hepatic injury, as indicated by increased serum-enzyme activities and histopathologic examination. Without

histopathologic correlates, however, marginal increases (although statistically significant) in serum enzyme activities might not be indicative of a serious toxic response. Renal toxicity also has been demonstrated in mice at exposures that are relatively low (e.g., 246-665 ppm for 2 h or 693 ppm for 1 h) compared with those inducing narcosis (e.g., 4,500 ppm for 35 min). Data in cats are from studies that involved high, narcosis-inducing exposures.

TABLE 4-5 Nonlethal Effects of Chloroform in Laboratory Animals after Acute Inhalation Exposure

Species	Exposure Concentration (ppm)	Exposure Duration	Effect	Reference
Rat	500	6 h	Statistically significant increase in serum-enzyme activity	Wang et al. 1994
Rat	10	6 h/d for 7 d	Histopathologic changes in the liver	Larson et al. 1994
Rat	50	8 h	No increase in liver weight	Ikatsu and Nakajima 1992
Rat	100	8 h	Marginal, biologically insignificant increase in serum-enzyme activity	Ikatsu and Nakajima 1992
Rat	153	4 h	Increased serum-enzyme activity	Lundberg et al. 1986
Rat	292	4 h	Increased serum-enzyme activity	Brondeau et al. 1983
Rat	10,000	2 h	No effect on hepatic GSH[a]	Brown et al. 1974b
Mouse	2,458-5,120	48 min	Reflex loss	Fühner 1923
Mouse	100	4 h	Fatty infiltration of liver	Kylin et al. 1963
Mouse	693	1 h	Renal toxicity	Deringer et al. 1953
Mouse	246	2 h	Renal tubular necrosis	Culliford and Hewitt 1957
Mouse	665	2 h	Renal necrosis in males	Culliford and Hewitt 1957
Mouse	4,500	35 min	50% narcosis (ECt_{50})	Gehring 1968
Mouse	4,500	13.5 min	50% significantly increased SGPT (ECt_{50})[b]	Gehring 1968
Cat	7,500	78 min	Light narcosis	Lehmann and Schmidt-Kehl 1936
Cat	22,000	10 min	Narcosis; irritation of eyes, mouth, and nose	Lehmann and Schmidt-Kehl 1936

[a]Narcosis and significant reduction in glutathione was found in phenobarbital-induced rats exposed to chloroform at 5,000 ppm for 2 h.
[b]Approximately 2.2-fold increase relative to unexposed controls; considered by investigators to be statistically significant.

3.3. Developmental and Reproductive Toxicity

3.3.1. Rats

The embryotoxicity and fetotoxicity of chloroform in Sprague-Dawley rats was studied by Schwetz et al. (1974). Pregnant rats were exposed to chloroform at 30 ppm (22 dams), 100 ppm (23 dams), or 300 ppm (3 dams) for 7 h/day on gestation days 6-15; control rats (68) were exposed to filtered air (see Table 4-6). The exposure concentrations were subanesthetic and varied <5% from the target concentrations. Concentrations were monitored three times per day using an infrared spectrophotometer. The 300-ppm group had marked anorexia at the end of the treatment period, although a comparison with a pair-fed control group (8 dams) later showed that inanition was not a contributor to the observed embryotoxicity and fetotoxicity. Chloroform at 30 ppm induced some evidence of embryotoxicity and fetotoxicity, while the 100- and 300-ppm exposures caused significant toxicity (see Table 4-6).

The investigators concluded that chloroform produced minor effects on the embryo and fetus at 30 ppm, was highly embryotoxic and fetotoxic at 100 ppm, and was embryocidal, embryotoxic, and fetotoxic at 300 ppm. At 100 ppm, frank teratogenic effects (imperforate anus and acaudia [missing tail]) were observed in three litters. The observed effects could not be correlated with maternal toxicity or inanition.

TABLE 4-6 Embryotoxicity and Fetotoxicity of Chloroform in Rats Exposed During Gestation

Parameter	Control	Pair-fed Control	30 ppm	100 ppm	300 ppm
% Pregnancy (pregnant/bred)	88 (68/77)	100 (8/8)	71 (22/31)	82 (23/28)	15 (3/20)[a]
Corpora lutea/dam[b]	14 ± 2	14 ± 2	16 ± 3[b]	14 ± 2	14 ± 1
Live fetuses/litter[b]	10 ± 4	10 ± 4	12 ± 2	11 ± 2	4 ± 7[a]
% Reabsorptions/implantations	8 (63/769)	7 (6/87)	8 (24/291)	6 (16/278)	61 (20/33)[a]
Fetal body weight (g)[c]	5.69 ± 0.36	5.19 ± 0.29[a]	5.51 ± 0.20	5.59 ± 0.24	3.42 ± 0.02[a]
Fetal crown-rump length (mm)[c]	43.5 ± 1.1	42.1 ± 1.1[a]	42.5 ± 0.6[a]	43.6 ± 0.7	36.9 ± 0.2[a]
Total gross anomalies[d]	1/68	0/8	0/22	3/23[a]	0/3
Total skeletal anomalies[d]	46/68	3/8	20/22[a]	17/23	2/3
Total soft-tissue anomalies[d]	33/68	3/8	10/22	15/23	1/3

[a]Significantly different from control; $p < 0.05$.
[b]Mean ± standard deviation.
[c]Mean of litter means ± standard deviation.
[d]Litters affected/litters examined.
Source: Adapted from Schwetz et al. 1974.

Newell and Dilley (1978) conducted experiments in which Sprague-Dawley rats were exposed to chloroform at 942, 2,232, or 4,117 ppm for 1 h/day on gestation days 7-14. Controls were exposed to clean air. The number of resorptions was increased (45% relative to controls) and the average fetal body weight was decreased in the high-exposure group. No notable effects were found in the low- or mid-exposure groups. No evidence of teratogenic effects was found.

A series of experiments (two preliminary studies and one main study) to assess developmental toxicity of chloroform in Wistar rats were conducted by Baeder and Hoffman (1988). In one preliminary study, time-mated Wistar rats (4-6/group) were exposed to chloroform for 6 h/day at concentrations of 0, 10, 30, or 100 ppm on gestation days 7-11 and 14-16. At 10 ppm, two dams had no fetuses and a single implantation site. At 30 ppm, one dam had only one fetus and three empty implantation sites. No such effects were reported for the 100-ppm group. In the second preliminary experiment, Wistar rats exposed at 100 and 300 ppm (6 h/day) on gestation days 7-16 exhibited decreased feed consumption and body weight loss. Fetal weights in two litters in the 100-ppm group were slightly decreased. The 300-ppm group had three dams with normally developed fetuses, one dam with totally resorbed fetuses, and one dam had only empty implantation sites. In the main study, groups of 20-23 time-mated Wistar rats were exposed to chloroform at concentrations of 0, 30, 100, or 300 ppm (7 h/day, gestation days 7-16). During exposure, chloroform-exposed rats exhibited decreased feed consumption and body-weight gain ($p < 0.05$ for all exposure groups, except for body-weight gain for 30-ppm group exposed on gestation day 21) relative to controls. Litter data for the main study are summarized in Table 4-7. Although fetal weight was significantly decreased in the 300-ppm group and crown-rump length was significantly decreased in all chloroform-exposed groups, these effects might be a function of maternal feed consumption and body weight effects. Incidences of external and internal malformations and skeletal abnormalities were not statistically significant.

TABLE 4-7 Litter Data from Study of Wistar Rats Exposed to Chloroform During Gestation

Parameter	Concentration (ppm)			
	0	30	100	300
No. pregnant/no. sperm plugs	20/20	20/20	20/21	20/23
No. lost litters	0	2	3	8
No. live litters	20	18	17	12
Resorptions/live litter (mean)	0.75	0.22	0.53	0.92
Live fetuses/litter (mean)	12.4	12.8	12.8	13.4
Fetal weight (g)[b]	3.19 ± 0.30	3.16 ± 0.19	3.13 ± 0.21	3.00 ± 0.19[a]
Fetal crown-rump length (cm)[b]	3.52 ± 0.17	3.38 ± 0.12[a]	3.39 ± 0.10[a]	3.39 ± 0.12[a]

[a]Significantly different from control group; $p < 0.5$.
[b]Mean ± standard deviation.
Source: Baeder and Hoffman 1988.

A follow-up study was conducted by Baeder and Hoffman (1991) in which groups of 20 time-mated Wistar rats were exposed to chloroform (0, 3, 10, or 30 ppm, 7 h/day) on gestation days 7-16. Feed consumption during the first week of exposure was significantly decreased ($p < 0.05$) and remained so for the 30-ppm group to the end of the study. Body weight of the 3-ppm group was unaffected but an exposure-dependent decrease was detected by gestation day 17. Body weights remained lower than controls on gestation day 21 for the 10-ppm and 30-ppm groups. Analysis of litter data by the investigators revealed a significant decrease in fetal weight and crown-rump length in the 30-ppm group (see Table 4-8). Significantly increased incidences of ossification variations were observed, especially for the 30-ppm group (see Table 4-9).

TABLE 4-8 Litter Data from Follow-up Study of Wistar Rats Exposed to Chloroform during Gestation

	Concentration (ppm)			
Parameter	0	3	10	30
Number pregnant	20	20	20	20
Number lost litters	0	0	0	1
Number live litters	20	20	20	19
Resorptions/live litter[a]	0.55 ± 0.89	0.40 ± 0.60	0.75 ± 1.02	0.84 ± 1.42
Live fetuses/litter[a]	12.4 ± 2.4	12.4 ± 3.5	12.9 ± 3.0	12.5 ± 1.9
Fetal weight (g)[a]	3.4 ± 0.3	3.2 ± 0.3	3.2 ± 0.3	3.2 ± 0.3[b]
Fetal crown-rump length (mm)[a]	35.8 ± 2.0	35.5 ± 2.1	34.4 ± 2.6	34.0 ± 1.9[b]

[a]Litter mean ± standard deviation.
[b]Significantly different from control group; $p < 0.05$.
Source: Baeder and Hoffman 1991.

TABLE 4-9 Skeletal and Ossification Variations in Wistar Rats Exposed to Chloroform During Gestation

	Concentration (ppm)			
Parameter	0	3	10	30
Number live litters	20	20	20	19
Poorly ossified cranial bones[a]	42/14	47/17	48/16	60[b]/17
Ossification of less than 2 caudal vertebrae[a]	4/3	14[b]/5	16[b]/6	14[b]/8
Non- or weakly ossified sternebrae[a]	7/3	32[b]/13[b]	35[b] /14[b]	18[b]/11[b]
Wavy or thickened ribs[a]	10/6	11/5	22[b] /10	15/4

[a]Number of affected fetuses/number of litters with affected fetuses.
[b]Significantly different from control group; $p < 0.05$.
Source: Baeder and Hoffman 1991.

3.3.2. Mice

Murray et al. (1979) examined the developmental toxicity of chloroform in CF-1 mice after gestational exposure. Groups of 34-40 pregnant mice were exposed to chloroform at 100 ppm for 7 h/day on gestation days 6-15, 1-7, or 8-15. Controls were exposed to filtered room air. Chloroform concentrations were monitored by infrared spectrophotometry and varied by <3% from the target concentration. Maintenance of pregnancy was significantly decreased ($p < 0.05$) in the dams exposed on gestation days 1-7 (44% vs. 74% in controls) and 6-15 (43% vs. 91% in controls), but not for those exposed on days 8-15 (decreased, but not significantly). The significant developmental toxicity findings are shown in Table 4-10. It was reported that delayed ossification of the skull bones was significantly increased in all of the chloroform-treated groups and that, with the exception of the group treated on days 6-16 of gestation, delays in the ossification of sternebrae were significantly more frequent in the treated groups compared with controls. However, these data were not presented in the report's tables. There was also evidence of hepatotoxicity in chloroform-exposed dams as demonstrated by significantly increased absolute and relative liver weights and by elevated SGPT activity. The investigators concluded that exposure of pregnant mice to chloroform at 100 ppm (7 h/day) on gestation days 1-7 or 6-15 decreased the ability to maintain pregnancy but was not teratogenic. Exposure on gestation days 8-15 did not affect pregnancy maintenance but resulted in an increased incidence of cleft palate.

TABLE 4-10 Developmental Toxicity of Chloroform in Mice Exposed During Gestation

	Days 1-7		Days 6-15		Days 8-15	
Parameter	Control	100 ppm	Control	100 ppm	Control	100 ppm
Litters examined	22	11	29	12	24	18
Resorptions/litter[a]	2 ± 2	4 ± 5[b]	2 ± 2	1 ± 1	2 ± 2	2 ± 2
Fetal body weight (g)[c]	1.02 ± 0.10	0.92 ± 0.07[b]	0.99 ± 0.11	0.95 ± 0.13	1.00 ± 0.12	0.85 ± 0.17[b]
Fetal crown-rump length (mm)[c]	24.7 ± 1.0	23.6 ± 1.2[a]	23.7 ± 1.3	23.2 ± 1.1	24.1 ± 1.1	22.9 ± 2.2[a]
Cleft palate (number fetuses [no. litters])	3 [1]	0	0	0	1 [1]	10 [4][d]

[a]Mean ± standard deviation.
[b]Significantly different from control ($p < 0.05$).
[c]Mean of the litter means ± standard deviation.
[d]Six fetuses in one litter exhibited cleft palate.
Source: Adapted from Murray et al. 1979.

Land et al. (1981) studied the morphologic changes in spermatozoa of C57B1/C3H mice exposed to chloroform. The mice were observed 28 days after exposure to chloroform at 0.1 or 0.05 of the minimal alveolar concentration (4 h/day for 5 days). Chloroform was delivered via calibrated vaporizers and the concentration was monitored by gas chromatography. Mice were killed 28 days after the last exposure and spermatozoa (1,000/slide) were examined independently by two pathologists. On the basis of data from groups of five mice, the percentage of abnormal spermatozoa was 1.42 ± 0.08, 2.76 ± 0.31, and 3.48 ± 0.66 for the control (clean air), 0.5- and 1.0-ppm groups, respectively. Both treatment groups were significantly different ($p < 0.01$) from the controls. Abnormalities included flattened spermatozoa, amorphous spermatozoa, and spermatozoa with abnormal hook formation.

3.4. Genotoxicity

Numerous genotoxicity assays have been performed with chloroform (ATSDR 1997). Generally, the results of these bioassays indicate chloroform to be a weak mutagen with low potential for interaction with DNA.

3.5. Carcinogenicity

Renal and hepatic tumors have been reported in rodents following chronic oral administration of chloroform (reviewed in ATSDR 1997). The results of cancer bioassays appear to be substantially influenced by the method of administration (gavage vs. drinking water) and by the vehicle (corn oil vs. water). Inhalation exposure studies of the tumorigenic potential of chloroform include a 90-day study in F344 rats by Templin et al. (1996a), a short-term exposure study by Templin et al. (1996b), and a long-term inhalation study by Yamamoto et al. (1994).

In the 90-day study by Templin et al. (1996a), male and female F344 rats were exposed to chloroform at 0, 2, 10, 20, 30, 90, or 300 ppm for 6 h/day for 7 days/week. Groups of rats (15-60/group) were subjected to different exposure protocols: male rats were exposed for 4 days or 3, 6, or 13 weeks, and female rats were exposed for 3 or 13 weeks. Exposure atmospheres were monitored by infrared gas analysis. Average analytically-determined concentrations were always within 4.5% of the target concentration. Results of the study indicate that the primary targets of toxicity are the liver, kidneys, and nasal passages. Cytolethality and regenerative cell proliferation were significant at 300 ppm. Although long-term exposure at 300 ppm would probably induce a tumorigenic response, this concentration was considered by the investigators to be highly cytotoxic (in excess of the maximum tolerated dose [MTD]) and not relevant for extrapolating to low-dose responses. Statistically significant body-weight loss was observed in male rats exposed for 4 days but kidney lesions were seen only in rats exposed at 30 (1 of 5 rats), 90 (3 of 5 rats), or 300 ppm (5 of 5 rats).

Templin et al. (1996b) conducted studies in BDF_1 mice to affirm the role of cytotoxicity and regenerative cell proliferation in the tumorigenic response to chloroform. Groups of male and female mice were exposed to chloroform at 0, 0.3, 5, 30, or 90 ppm 6 h/day for 4 days. Bromodeoxyuridine (BrdU) was administered by osmotic pumps implanted 3.5 days before necropsy and served to provide a labeling index for S-phase cells. Additional groups of mice were exposed to chloroform at 30 or 90 ppm for 5 days/week for 2 weeks. Degenerative lesions and a 7- to 10-fold increase in the labeling index were observed in the kidneys of male but not female mice exposed at 30 or 90 ppm. Liver lesions and an increased hepatocyte labeling index were observed in male mice exposed at 30 and 90 ppm and in female mice exposed at 90 ppm. Lethality was 40 and 80% in the 30- and 90-ppm groups, respectively, exposed for 2 weeks; severe kidney damage was evident in the animals. These findings show that in the two-year assays, chloroform exposures actually exceeded the MTD and were tolerated only because of the step-wise exposure protocol that allowed the animals to accommodate metabolically to the high concentrations. Templin et al. (1996b) questioned the validity of low-dose extrapolation from tumor data of this type (e.g., nongenotoxic-cytotoxic mechanism that is secondary to organ-specific toxicity).

In a preliminary report of a 2-year cancer bioassay, Yamamoto et al. (1994) observed no increase in tumor incidences in male and female F344 rats exposed to chloroform at 10, 30, or 90 ppm for 5 days/week. No further details are available on this study.

Several issues, however, are relevant to the carcinogenic potential of chloroform. These are especially relevant regarding the estimation of carcinogenic risk after a single acute exposure. As reviewed by Conolly (1995) and Golden et al. (1997), the tumorigenic dose-response of mice and rats to chloroform appears to be nonlinear and is secondary to cytotoxicity (i.e., cell necrosis and subsequent cellular regeneration) following exposures that induce frank toxicity in tissues that are tumor sites and at concentrations that often exceed the MTD. Additionally, both in vivo and in vitro genotoxicity data indicate the absence of a genotoxic mechanism for chloroform.

The significance of regenerative cell proliferation in chloroform-induced cancer was also examined by Butterworth et al. (1995) and Wolf and Butterworth (1997). An analysis of the available data indicates that chloroform acts through a nongenotoxic, cytotoxic mechanism. In rodent studies, toxicity is observed only when chloroform is metabolized to reactive metabolites at a rate sufficient to cause cytolethality. As such, a linearized extrapolation from high concentrations that produce tumors to very low concentrations is considered inappropriate. Additionally, the current inhalation cancer risk is 2.3×10^{-5} $(\mu g/m^3)^{-1}$ (EPA 2012) and is based on a tumorigenic response (hepatocellular carcinomas) in $B6C3F_1$ mice administered chloroform by gavage (NCI 1976) and, therefore, involves the uncertainties associated with route-to-route extrapolation.

Butterworth et al. (1995) and Wolf and Butterworth (1997) compared the results of cancer risk assessments performed using the linearized multistage model for low-dose extrapolation with the results based on a threshold response (cytolethality and cellular regeneration). The resulting outcomes are remarkably different. Application of the linearized multistage model to tumor incidence data from a gavage study with mice (NCI 1976) resulted in a virtually-safe concentration (relative to a 1×10^{-6} cancer risk) of 8×10^{-6} ppm. However, a virtually-safe concentration of 0.01 ppm is obtained when uncertainty factors are applied (three factors of 10 for interspecies differences, intraspecies variability, and use of a subchronic study) to 10 ppm, a concentration that did not produce cytolethality or cellular regeneration in inhalation studies with rodents. The investigators justify their approach by citing the apparent need for cytolethality and cellular regeneration in the tumorigenic response.

Melnick et al. (1998) provided data and alternate interpretations regarding the relevance of cytolethality and proliferative cellular regeneration to the tumorigenic response observed in rodents following oral administration of chloroform in corn oil. Following gavage dosing of female $B6C3F_1$ mice (10/group) with chloroform (5 times/week for 3 weeks at doses of 55, 110, 238, or 477 mg/kg), biochemical indices of toxicity (ALT, SDH) and labeling index (BrdU) for S-phase hepatocytes were measured and histopathologic examination were performed to ascertain the relationship between regenerative hyperplasia and tumor induction. As expected, a dose-related response was observed for liver-to-body weight ratio, increase in ALT and SDH activity, severity and incidence of hepatocyte hydropic degeneration, and labeling index. The investigators compared the dose-response curves for tumor incidence (using data from previous cancer bioassays) and hepatocyte labeling index and reported that the processes are not causally related. In other words, an elevated labeling index resulting from cellular proliferation is not required for a tumorigenic response.

4. SPECIAL CONSIDERATIONS

4.1. Metabolism and Disposition

The metabolism of chloroform has been thoroughly studied (reviewed in ATSDR 1997). Although metabolism via cytochrome P-450 IIE1 is well-established, a minor anaerobic pathway also exists resulting in a dichloromethyl radical intermediate. Phosgene, formed by P-450-mediated dehydrochlorination, may react with cellular proteins or be converted to hydrochloric acid and carbon dioxide (Pohl et al. 1981). Phosgene may also react with GSH to form diglutathionyl dithiocarbonate which is then metabolized to 2-oxothiazolidine-4-carboxylic acid (Mansuy et al. 1977; Pohl et al. 1977; Branchflower et al. 1984).

Brown et al. (1974a) studied the metabolism of orally administered [^{14}C]-chloroform (60 mg/kg) in male Sprague-Dawley rats; male CBA, CF/LP, and C57 mice; and squirrel monkeys. In all test species, $^{14}CO_2$ was a major excretory

product but species-dependent variability was observed in its elimination. Eighty percent of the administered dose was excreted as $^{14}CO_2$ in all three strains of mice, whereas 60% and 20% was eliminated in rats and squirrel monkeys, respectively.

Fry et al. (1972) reported that 17.8-66.6% of an oral dose of radiolabeled chloroform (500 mg) was expired unchanged by eight human volunteers over an 8-h period. Maximum excretion of chloroform occurred 40 min to 2 h after administration. Carbon-dioxide excretion was measured in one male and one female volunteer. Over a 450-min period, 48% (woman) and 50% (man) of the dose was expired as carbon dioxide. The investigators also reported decreased excretion of chloroform by obese subjects and suggested that resulted from uptake of chloroform by greater amounts of adipose tissue. Peak blood concentrations (≈1 µg/mL) occurred about 45 min after dosing. Elimination of chloroform from the blood appeared to be biphasic: an initial rapid clearance within an hour and a slower clearance over the next 6 h. As chloroform concentration in the blood increased, pulmonary excretion increased.

Corley et al. (1990) developed a PBPK model for chloroform based on a kinetic constant from in vivo studies with rats and mice, in vitro enzymatic studies with human tissue samples, and physiologically-based estimates for absorption, distribution, metabolism, and excretion processes. Macromolecular binding was considered a measure of internal dose. The model was validated by comparing predicted values with experimental data from mice, rats, and humans. Human metabolic and macromolecular-binding constants for $V_{max}C$ (15.7 mg/hr/kg) and K_m (0.448 mg/L) were derived. It was also shown that metabolic activation of chloroform to reactive intermediates, such as phosgene, was greatest in mice. Metabolic activation was less in rats and lowest in humans. Therefore, it was estimated that exposure to equivalent concentrations of chloroform would result in a lower delivered dose in humans than in laboratory animals. Species variability was also reported by Brown et al. (1974a), who reported that conversion of chloroform to carbon dioxide was highest in mice (80%) and lowest in squirrel monkeys (18%). In rats and mice, [^{14}C]-urea was detected in the urine along with two unidentified metabolites, and parent compound was found in the bile of the squirrel monkeys. In mice, radioactivity in the blood peaked 1 h after dosing and decreased gradually over the next 24 h.

The chloroform PBPK model developed by Corely et al. (1990) was used by Delic et al. (2000) to develop models for humans and rats to compare rates of metabolism in the context of assessing the validity of uncertainty factors used to determine occupational exposure limits. The study also utilized Monte Carlo analysis to determine the extent of variability within human and animal-model populations. The results demonstrated that even at the most extreme ranges within the human population, concentrations of toxic metabolites necessary to induce a toxic response would not be generated at rates comparable to that in rats. Specifically, the model showed that the mean peak rate of metabolism of inhaled chloroform (at the mouse no-observed-adverse-effect level of 10 ppm) is approximately 78-fold lower in humans and that the chloroform concentration

required to achieve a peak metabolism rate in humans would be 65-fold higher than that in mice. Monte Carlo analysis of population variability also indicated that chloroform metabolism rates between mice and humans varied by 25- to 50-fold. Overall, the work clearly demonstrated that considerably higher concentrations of chloroform are required to induce a toxic response in humans compared with mice.

Data regarding the distribution of chloroform among brain, lung, and liver tissue of humans was obtained by Gettler and Blume (1931) from suicide victims or deaths from chloroform anesthesia. The brain and lungs consistently had the highest concentrations of chloroform (60-480 mg/g in brain; 24-485 mg/g in lung), whereas liver tissue tended to have lower concentrations (24-238 mg/g). These values reflect tissue burdens after high exposures to chloroform.

The distribution of [^{14}C]-chloroform in pregnant C57BL mice after a single 10-min inhalation exposure (approximately 16 mmoles based on specific activity) was studied by Danielsson et al. (1986). Assessments were conducted at 0.5, 4, and 24 h. At all time points, radioactivity was greatest in the lungs, liver, and kidneys. Radioactivity in the respiratory tract was associated with epithelial tissue (nasal mucosa, trachea, and bronchi). Radioactivity was also found in the fetus and placenta at all time points, peaking at 0.5 h and gradually decreasing over the 24-h time frame. In addition to total radioactivity, the investigators also determined bound radioactivity in various tissues and found that the respiratory tract and centrilobular portion of the liver contained bound radioactivity, which possibly indicates on-site production of reactive metabolites.

Wang et al. (1997) reported on the effects of ethanol pretreatment (2 g/rat/day for 3 weeks) on the metabolism and hepatotoxicity of chloroform in rats following administration of chloroform by various routes (intraperitoneal, perioral, and inhalation). Ethanol pretreatment increased cytochrome P-450 from 0.74 nmol/mg to 1.10 nmol/mg and increased the metabolism of inhaled chloroform 7-fold in rats exposed to chloroform at 500 ppm for 6 h, but did not increase the metabolism of chloroform in rats exposed at 50 ppm for 6 h. Hepatotoxicity, as determined by GPT, GOT, and GSH activity, was unaffected in the 50-ppm group and increased approximately 6-fold in the 500-ppm group.

4.2. Mechanism of Toxicity

The noncarcinogenic and carcinogenic mechanisms of chloroform have been previously reviewed (Butterworth et al. 1995; Conolly 1995; Templin et al. 1996a,b; ATSDR 1997; Golden et al. 1997; Wolf and Butterworth 1997). Chloroform toxicity may be generally categorized as effects on the central nervous system, liver, kidneys, and heart (primarily the result of myocardial sensitization to epinephrine).

The precise mechanism of chloroform on neural activity is unknown. It is generally assumed that general anesthetics act by influencing synaptic transmission (e.g., potentiating transmitter release at inhibitory synapses or inhibiting

release at excitatory synapses). These actions may be the result of interaction with protein-lipid interfaces (Kennedy and Longnecker 1996).

The underlying mechanism of chloroform's hepatic and renal toxicity is the binding of reactive intermediates, such as phosgene (Pohl et al. 1977), to cellular macromolecules, the depletion of these macromolecules, and subsequent cell death.

Brown et al. (1974b) exposed phenobarbital-treated rats for 2 h to chloroform at 0.5% (5,000 ppm) or 1.0% (10,000 ppm) and found a 70% and 83% reduction in hepatic GSH ($p < 0.001$), respectively. At these concentrations, however, noninduced rats exhibited no significant change in GSH activity.

The importance of GSH depletion was also demonstrated by Docks and Krishna (1976), who showed that administration of chloroform (80 mg/kg, intraperitoneal) to phenobarbital-treated rats decreased GSH and resulted in massive liver necrosis. Docks and Krishna (1976) postulated that chloroform-mediated decreases in GSH was not from the trichlorocarbon radical, because depletion of GSH was greater from chloroform than by halomethanes known to be metabolized to the trichlorcarbon radical.

The mechanism of chloroform toxicity in isolated rat hepatocytes was studied by el-Shenawy and Abdel-Rahman (1993). The results support the contention of Docks and Krishna (1976) that depletion of GSH is a causative precursor for cytotoxicity. Isolated rat hepatocytes exposed to chloroform at concentrations of 1, 10, 100, or 1,000 ppm exhibited a concentration-dependent decrease in viability ($p < 0.05$ at all concentrations). Leakage of serum aspartate aminotransferase (AST) occurred at all the concentrations, but was significant only at 1 ppm after 60 min and at 10 ppm after 30 min. Leakage of ALT was significant at 100 and 1,000 ppm after 60 min and 30 min, respectively. GSH was significantly decreased between 15-120 min after hepatocytes were incubated with chloroform at 1,000 ppm. At 100 ppm and 10 ppm, GSH depletion became significant at 30 min and 120 min, respectively.

4.3. Structure-Activity Relationships

Assessment of structure-activity relationships was not instrumental is deriving AEGL values for chloroform.

4.4. Other Relevant Information

4.4.1. Species Variability

Strain, species, and gender variability in the metabolism and toxicity of chloroform has been demonstrated. As previously noted, male mice exhibit both renal toxicity and hepatotoxicity after exposure to chloroform, whereas female mice exhibit only hepatotoxicity. This has been shown to be from hormone-specific cytochrome P-450 in the kidneys of male mice. By examining differences in the biotransformation of chloroform to phosgene, Pohl et al. (1984)

demonstrated strain and sex differences in chloroform-induced renal toxicity. The differences could be attributed to strain- and gender-dependent differences in the rate of phosgene production by microsomal and mitochondrial fractions from the kidneys. A notable difference was observed between sensitive male DBA/2J mice and less sensitive C57BL/6J mice. Male mice formed phosgene at a rate nearly an order of magnitude more rapid than female mice. Additionally, based on results of PBPK model studies using metabolism and disposition data, humans appear to be less sensitive than rodent species, and the mouse appears to be the most sensitive.

4.4.2. Concurrent Exposure Issues

Because the biotransformation of chloroform to reactive intermediates is mediated by cytochrome P-450 IIE1, exposures to chemicals that induce P-450 might increase the toxic response of chloroform. From a practical standpoint, alcohol consumption would be a special concern.

5. DATA ANALYSIS FOR AEGL-1

5.1. Summary of Human Data Relevant to AEGL-1

Human exposure data on chloroform consistent with AEGL-1 effects include studies by Lehmann and Hasegawa (1910) of human volunteers and by McDonald and Vire (1992) of dental workers. Lehmann and Hasegawa (1910) reported that exposure to chloroform at 920-1,100 ppm for 2-3 min resulted in vertigo and that concentrations as high as 1,400 ppm for 15-30 min produced lassitude, vertigo, and headache. Some individuals exposed at 620 ppm for 30 min reported only the sensation of a not unpleasant odor and no neurologic symptoms. Because vertigo could affect escape from a potentially hazardous condition, concentrations of chloroform inducing this condition are inappropriate for developing AEGL-1 values. The study by Lehmann and Hasegawa (1910) lacks details on exposure methods and validation of exposure measurements. The McDonald and Vire (1992) study involved exposure at low concentrations during endodontic procedures (<0.57 ppm for 5.5 h and <0.88 ppm for over 150 min). These exposures did not result in any signs or symptoms even after clinical screening at 4 h and 1 year after exposure. No additional human data consistent with the AEGL-1 definition were available.

5.2. Summary of Animal Data Relevant to AEGL-1

Animal data consistent with AEGL-1 effects include alterations in clinical chemistry determinations (specifically serum ALT, AST, GLDH, and SDH activity) and minor histopathologic findings in the liver and kidneys of rats and mice. Increased serum-enzyme activities were observed in rats exposed to chlo-

roform at 153 ppm for 4 h (Lundberg et al. 1986) or at 292 ppm (Brondeau et al. 1983). Exposure of rats to chloroform at 500 ppm for 6 h produced statistically significant increases in serum-enzyme activity (Wang et al. 1994). Rats exposed at 50 ppm for 8 h had no increase in liver weight, but rats exposed at 100 ppm had a slight increase in serum-enzyme activity (Ikatsu and Nakajima 1992). Although statistically significant increases in serum-enzyme activities were reported in several studies, they were not necessarily indicative of biologically-relevant hepatic damage (some of the enzyme activities were increased only 2-fold and histologic correlates were negligible) and, therefore, would not be appropriate as AEGL-1 end points.

5.3. Derivation of AEGL-1

Human data sets for determining AEGL-1 values have poorly described methodology and inadequate characterizations of exposure. Animal data consistent with AEGL-1 effects have better defined exposure data, but are limited to clinical chemistry findings that are more indicative of biologic indices of exposure than overt toxicity. Concentrations of chloroform that do not produce overt signs of toxicity in humans are neither irritating nor have an unpleasant odor. Thus, it would be difficult to identify exposures that would produce notable discomfort or mild sensory irritation without approaching concentrations that might be near a threshold for narcosis. As a result, AEGL-1 values are not recommended.

6. DATA ANALYSIS FOR AEGL-2

6.1. Summary of Human Data Relevant to AEGL-2

In an assessment of 1,502 surgical patients anesthetized with chloroform (concentrations never greater than 22,500 ppm) for <30 min to >120 min, Whitaker and Jones (1965) reported cardiac irregularities in some patients (bradycardia in 8.1%; arrhythmias in 1.3%). Protection against narcosis even in the absence of toxic effects would appear to be at least one goal of the AEGL-2 values, so these data would be an inappropriate basis for AEGL-2 values. Lehmann and Hasegawa (1910) reported “intoxication and dizziness” in human subjects exposed to chloroform at 4,300-5,100 ppm for 20 min or at 7,200 ppm for 15 min. Three volunteers reported pounding heart and experienced gagging during a 30-min exposure at 3,000 ppm, and “light-headedness” and lassitude after 30 min at 1,400 ppm. Smith et al. (1973) evaluated surgical patients anesthetized with chloroform (8,500-13,000 ppm; concentration never exceeded 2% [20,000 ppm]) for a mean duration of 112.96 min. Cardiac arrhythmias of various types were detected in 1-17 of the patients. With the exception of a slight elevation of lactate dehydrogenase, serum enzyme values (SGPT, SGOT, and alkaline phosphatase) were not altered by chloroform. Nausea and vomiting occurred in 46% of the patients.

6.2. Summary of Animal Data Relevant to AEGL-2

Several studies in rats indicate that signs of hepatotoxicity (fatty infiltration) and renal damage (tubular necrosis) might occur at cumulative exposures of 400-1,330 ppm-h that encompass exposure durations of 1-4 h and concentrations of 100-693 ppm (Deringer et al. 1953; Culliford and Hewitt 1957; Kylin et al. 1963). Exposure of pregnant rats during gestation (7 h/day on gestation days 6-15) to chloroform at 30 ppm produced minor effects in the embryo and fetus, and exposure at 100 ppm was significantly embryotoxic and fetotoxic (Schwetz et al. 1974). Newell and Dilley (1978), however, found that gestational exposure of rats to chloroform at concentrations as high as 2,232 ppm (1 h/day on gestation days 7-14) did not cause developmental effects, although exposure at 4,117 ppm increased resorptions by 45% and decreased fetal body weight.

6.3. Derivation of AEGL-2

Severe hepatic toxicity, renal toxicity, and narcosis appear to be critical effects for the development of AEGL-2 values for chloroform. Human data suggest that exposures to chloroform at 8,500 ppm will induce anesthesia. The duration of exposure required is unknown, but is assumed to be on the order of a minute. Human data reported by Lehmann and Hasegawa (1910) suggest that exposure to chloroform at 7,500 ppm for 15 min or at 4,300-5,100 ppm for 20 min approached narcosis-inducing concentrations, as determined by signs and symptoms of dizziness and "intoxication." These data and the anesthesia data of Whitaker and Jones (1965) are, however, compromised by the uncertainties with the determination of exposure concentrations and the specific concentration-duration relationships. Alternatively, the fetotoxicity reported by Schwetz et al. (1974) in rats exposed to chloroform at 100 ppm (7 h/day) on gestation days 6-15 was considered a sensitive critical effect and was selected as the point of departure for developing AEGL-2 values. It was assumed that the reported fetotoxic effects could result from a single 7-h exposure. This assumption is not without precedent, as has been shown by analyses of developmental toxicity data for other chemicals (van Raaij et al. 2003). An intraspecies uncertainty factor of 3 was applied to account for individual variability in metabolism and disposition of chloroform. No adjustment was made for interspecies variability because metabolism and kinetics data and PBPK models (Corley et al. 1990) indicate that humans are less sensitive than laboratory species to chloroform. The attenuated uncertainty factors were justified by the sensitive end point selected for AEGL-2 development and the results of another study (Newell and Dilley 1978) that showed gestational exposure of rats to chloroform at concentrations as high as 2.232 ppm (1 h/day on gestation days 7-14) was without effect.

The concentration-time relationship for many irritant and systemically-acting vapors and gases may be described by the equation $C^n \times t = k$, where the

exponent n ranges from 0.8 to 3.5 (ten Berge et al. 1986). In the absence of chemical-specific data to determine an empirical value for n, default values of n = 1 for extrapolation from shorter to longer durations and n = 3 for extrapolation from longer to shorter durations were used. AEGL-2 values for chloroform are presented in Table 4-11 and Appendix A.

7. DATA ANALYSIS FOR AEGL-3

7.1. Summary of Human Data Relevant to AEGL-3

Definitive lethality data for humans are not available. Although the weight of evidence indicates that acute exposure to high concentrations of chloroform might result in narcosis and subsequent death, the precise exposure concentrations and durations for such exposures are unavailable. Human data generally suggest that concentrations greater than 10,000 ppm are required for an unspecified, although short, exposure duration for surgical anesthesia. In an analysis of surgical patients anesthetized with chloroform, Whitaker and Jones (1965) reported that a concentration of 22,500 ppm also produced evidence of potentially serious cardiovascular effects. Although these data may appear compelling for development of AEGL values, it is not possible to quantify an exposure-duration relationship. Additionally, chloroform concentrations probably varied during anesthesia. This is not unexpected; anesthesia procedures with chloroform start with very high concentrations (25,000-30,000 ppm) of very short duration (2-3 min) for the purpose of inducing unconsciousness, but then lower concentrations are used to maintain surgical anesthesia (NRC 1984; ATSDR 1997). Therefore, it is unlikely that patients were exposed at the highest concentrations for AEGL-specific durations. Arrhythmias were also reported by Smith et al. (1973) in some patients anesthetized for 113 min at concentrations (at least initially) of 8,500-13,000 ppm. The available data suggest that surgical narcosis would occur at or above 8,500 ppm after short-duration exposure. It is not feasible to extrapolate to an exposure duration that would result in death.

7.2. Summary of Animal Data Relevant to AEGL-3

Data on lethality in animals after acute inhalation exposure to chloroform include studies of rats and mice. Exposure to chloroform at 3,000-8,000 ppm for 4 h resulted in 75-100% mortality in rats (lethality determined 2-3 days postexposure) (Smyth et al. 1962; Haskell Laboratory 1964), and a 4-h LC_{50} of 9,780 ppm was reported by Lundberg et al. (1986). For mice, mortality was 75% at 5,585 ppm for 120-min, 66% at 4,710-5,529 ppm for durations of 71-175 min, and 14% at 6,758-7,782 ppm for 35 min (Fühner 1923). However, no deaths occurred with chloroform at 2,458-5,120 ppm for 48-215 min (Fühner 1923). If

TABLE 4-11 AEGL-2 Values for Chloroform

10 min	30 min	1 h	4 h	8 h
120 ppm (580 mg/m^3)	80 ppm (390 mg/m^3)	64 ppm 312 mg/m^3)	40 ppm 195 mg/m^3)	29 ppm 141mg/m^3)

the aforementioned results are converted to consider cumulative exposures, inconsistencies in the data become apparent. For example, no deaths were observed at concentrations of 2,458-5,120 ppm for 48-215 min (a maximum of 1,100,800 ppm-min); yet 66% mortality was observed at concentrations of 4,710-5,529 ppm for durations of 71-175 min (a minimum of 334,410 ppm-min). A well-conducted study by Gehring (1968) reported a 4,500-ppm LCt_{50} of 560 min (540-585 min, 95% CI) for female Swiss-Webster mice.

7.3. Derivation of AEGL-3

The available data do not identify a definitive lethality threshold in humans from acute exposure to chloroform. Data regarding chloroform as an anesthetic for humans suggest that very high concentrations (greater than 8,500 ppm) are tolerated for brief durations, although quantitative concentration-time data are lacking. These limitations preclude the use of the human data in the estimation of a lethality threshold for humans.

Animal data are inconsistent regarding the lethality from acute inhalation exposure to chloroform. Data on mice are highly variable, but this species appears to be the most sensitive and is affirmed by PBPK models. Exposure to chloroform at 3,000-8,000 ppm for 4 h reportedly produced 75-100% mortality in rats (Smyth et al. 1962; Haskell Laboratory 1964). Assuming the mouse to be the most sensitive species, the 560-min LC_{50} of 4,500 ppm reported by Gehring (1968) appears to be a valid basis for development of the AEGL-3 values. A 3-fold reduction in this value for an estimate of the lethality threshold for mice results in a point of departure of 1,500 ppm. Consistent with the Standing Operating Procedures (SOP) for developing AEGLs (NRC 2001), an exponent of 3 was applied for time scaling ($C^n \times t = k$) because data were insufficient for empirically deriving a value for n. Because the point of departure was based on a 560-min exposure duration, the 10-min AEGL-3 value was set equivalent to the 30-min AEGL-3 value to avoid uncertainties inherent in extrapolating from 560 min to 10 min. An uncertainty factor of 3 was applied to account for potentially sensitive individuals, such as those exposed to inducers of cytochrome P-450 IIE1 (e.g., consumers of ethanol). No interspecies uncertainty factor was applied because laboratory species metabolize chloroform more rapidly than humans and are, therefore, more susceptible to the toxic effects of the more rapidly formed toxic intermediates. PBPK models (Corley et al. 1990) support this contention. Further, human anesthesia data show that cumulative exposures considerably greater than those associated with the AEGL-3 values are not lethal. A more recent study using the PBPK model to compare the metabolism of chloro-

form in mice and humans demonstrated the overwhelmingly greater sensitivity of mice (primarily from a 25- to 50-fold difference in the rate of metabolism of chloroform) and the overly protective nature of typically applied uncertainty factors. These findings and the overall weight of evidence indicating the greater sensitivity of rodents to chloroform-induced toxicity justified further adjustment of the AEGL-3 values. This adjustment, applied as a weight-of evidence factor of 1/3, effectively increases the AEGL-3 values. The resulting AEGL-3 values are shown in Table 4-12 and Appendix A.

8. SUMMARY OF PROPOSED AEGLS

8.1. AEGL Values and Toxicity End Points

The AEGL values for chloroform are presented in Table 4-13.

AEGL-1 values for chloroform were not recommended because an exposure consistent with the AEGL-1 definition could not be determined. The properties of chloroform are such that the odor is not unpleasant and it is not irritating even at concentrations approaching levels inducing narcosis.

AEGL-2 values were developed using embryotoxicity and fetotoxicity in rats as the critical effect. These were considered very sensitive end points, especially with the assumption of a single-exposure response (fetotoxic effects resulting from 7-h exposures on gestation days 6-15 were assumed possible following only one 7-h exposure).

AEGL-3 values were based on an estimate of the lethality threshold for chloroform in mice.

TABLE 4-12 AEGL-3 Values for Chloroform

10 min	30 min	1 h	4 h	8 h
4,000 ppm (19,000 mg/m^3)	4,000 ppm (19,000 mg/m^3)	3,200 ppm (16,000 mg/m^3)	2,000 ppm (9,700 mg/m^3)	1,600 ppm (7,800 mg/m^3)

TABLE 4-13 AEGL Values for Chloroform

Classification	10 min	30 min	1 h	4 h	8 h
AEGL-1 (nondisabling)	NR[a]	NR	NR	NR	NR
AEGL-2 (disabling)	120 ppm (580 mg/m^3)	80 ppm 390 mg/m^3)	64 ppm (312 mg/m^3)	40 ppm (195 mg/m^3)	29 ppm (141 mg/m^3)
AEGL-3 (lethal)	4,000 ppm (19,000 mg/m^3)	4,000 ppm (19,000 mg/m^3)	3,200 ppm (16,000 mg/m^3)	2,000 ppm (9,700 mg/m^3)	1,600 ppm (7,800 mg/m^3)

[a]Not recommended.

AEGL values were developed using an uncertainty factor of 3 for protection of sensitive individuals. Because chloroform is metabolized to toxic intermediates (phosgene) by cytochrome P-450 IIE1, induction of this enzyme (by inducers such as ethanol) potentially increases susceptibility, although it does not appear to do so by an order of magnitude (e.g., Brown et al. [1974b] reported a 2.6-fold increase in P-450 levels after induction by phenobarbitol, a more effective P-450 inducer than ethanol). Furthermore, dose rate appears to be a relevant factor in toxicity outcomes following exposure to halogenated hydrocarbons such as chloroform, a factor that might justify the application of an intraspecies uncertainty factor of less than an order of magnitude. Because of effects on P-450 and GSH levels, single exposures result in toxic outcomes that are different from those for repeated exposures. Available data and application of pharmacokinetic modeling indicate that rodents metabolize chloroform more rapidly than humans. Therefore, the application of an interspecies uncertainty factor was minimized. Furthermore, human data indicate that cumulative exposures of >675,000 ppm-min and exposures at 22,500 ppm for up to 120 min resulted in surgical anesthesia and cardiac irregularities but not death. These data suggest that the AEGL-3 values represent a no-observed-adverse-effect level for lethality.

When compared with occupational exposure data reported by Challen et al. (1958) for pharmaceutical workers, the AEGL values appear to be sufficiently protective. Workers exposed to chloroform at 71 ppm (4 h/day for 10-24 months) experienced mild symptoms (dryness of mouth and throat) whereas workers exposed at 77-232 ppm over a period of 3-10 years exhibited notable signs of exposure (staggering). These findings are the result of repeated exposures to chloroform, and the study did not specify if any of the workers represented a sensitive population.

8.2. Comparison with Other Standards and Guidelines

Standards and guidance values for workplace and community exposures to chloroform are presented in Table 4-14. The cancer notation for some of the criteria was not considered appropriate for AEGL values.

8.3. Data Quality and Research Needs

Much of the human data on chloroform are from older studies that lacked information on the analytic techniques used to determine exposure concentrations. Human anesthesia data focus on initial concentration and duration of anesthesia, and were not sufficient for developing AEGL values.

The most obvious data deficiency regarding development of AEGL values for chloroform is the lack of data with which to determine a lethality threshold. There is also a paucity of reliable data demonstrating definitive concentration-response relationships. Human data are deficient in exposure-time relationships

or are unreliable and difficult to validate. The animal data are variable. Acute exposure studies providing exposure-response data for specific toxicity end points (e.g., hepatotoxicity, renal toxicity, narcosis threshold, lethality) in two or more species would be desirable.

TABLE 4-14 Extant Standards and Guidelines for Chloroform

Guideline	Exposure Duration				
	10 min	30 min	1 h	4 h	8 h
AEGL-1 (Nondisabling)	NR	NR	NR	NR	NR
AEGL-2 (Disabling)	120 ppm	80 ppm	64 ppm	40 ppm	29 ppm
AEGL-3 (Lethal)	4,000 ppm	4,000 ppm	3,200 ppm	2,000 ppm	1,600 ppm
ERPG-1 (AIHA)[a]			NA		
ERPG-2 (AIHA)			50 ppm		
ERPG-3 (AIHA)			5,000 ppm		
EEL (NRC)[b]			200 ppm (30 ppm, 24 h)		
IDLH (NIOSH)[c]		500 ppm			
TLV-TWA (ACGIH)[d]					10 ppm
REL-TWA (NIOSH)[e]			2 ppm (60min)		
PEL-C (OSHA)[f]					50 ppm
MAK (Germany)[g]					0.5ppm
MAC (the Netherlands)[h]	5 ppm (15 min)				1 ppm

[a]ERPG (emergency response planning guideline, American Industrial Hygiene Association (AIHA 2010)

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.

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 protection action.

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] EEL (emergency exposure guidance level, National Research Council) (NRC 1984) is a ceiling concentration that will not cause irreversible harm or prevent performance of essential tasks, such as closing a hatch or using a fire extinguisher, during a rare emergency situation usually lasting 1-24 h.

[c]IDLH (immediately dangerous to life or health, National Institute for Occupational Safety and Health, NIOSH 1994) represents the maximum concentration from which one could escape within 30 min without any escape-impairing symptoms, or any irreversible health effects. IDLH carries a cancer notation.
[d]TLV-TWA (threshold limit value - time weighted average, American Conference of Governmental Industrial Hygienists) 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 2011).
[e]REL-TWA (recommended exposure limit - time weighted average, National Institute for Occupational Safety and Health) is defined analogous to the TLV-TWA, with cancer notation (NIOSH 2011).
[f]PEL-C (permissible exposure limit-ceiling, Occupational Health and Safety Administration) is a value that must not be exceeded during any part of the workday (NIOSH 2011).
[g]MAK (maximale argeitsplatzkonzentration [maximum workplace concentration], German Research Association) (DFG 2005) is defined analogous to the ACGIH TLV-TWA. Cancer category 4 noted.
[h]MAC (maximaal aanvaaarde concentratie [maximal accepted concentration]), Dutch Expert Committee for Occupational Standards, The Netherlands(MSZW 2004) is defined analogous to the ACGIH TLV-TWA.

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APPENDIX A

DERIVATION SUMMARIES OF AEGL VALUES FOR CHLOROFORM

Derivation of AEGL-1 Values

AEGL-1 values were not recommended because it was not possible to identify a definitive effect consistent with the AEGL-1 definition. Concentrations of chloroform approaching those inducing narcosis or hepatic and renal effects are not accompanied by overt signs or symptoms. Furthermore, chloroform is not irritating and its odor is not unpleasant.

Derivation of AEGL-2 Values

Key study:	Schwetz, B.A., B.K. Leong, and P.J. Gehring. 1974. Embryo- and fetotoxicity of inhaled chloroform in rats. Toxicol. Appl. Pharmacol. 28(3):442-451.
Toxicity end point:	No developmental effects in rats.
Time scaling:	$C^n \times t = k$ (default n = 3 for longer to shorter exposure durations; n = 1 for shorter to longer exposure durations) $(100 \text{ ppm})^1 \times 7 \text{ h} = 700 \text{ ppm-h}$ $(100 \text{ ppm})^3 \times 7 \text{ h} = 7000 \text{ ppm-h}$
Uncertainty factors:	An interspecies uncertainty factor was not applied because the available metabolism and kinetics data and PBPK models (Corley et al. 1990) indicate that humans may be less sensitive than laboratory animals to chloroform. Additional adjustments were considered unnecessary because a single 7-h exposure was assumed to produce effects rather than the full-exposure period specified in the study protocol (7 h/day on gestation days 6-15). 3 for intraspecies variability in metabolism and disposition of chloroform. Additional adjustment was not made because the point of departure and the assumption of a single-exposure effect were considered conservative.

Total uncertainty factor of 3

10-min AEGL-2:	$C^3 \times 0.1667$ h = 7,000,000 ppm-h C = 348 ppm 348 ppm ÷ 3 = 120 ppm (rounded)
30-min AEGL-2:	$C^3 \times 0.5$ h = 7,000,000 ppm-h C = 241 ppm 241 ppm ÷ 3 = 80 ppm (rounded)
1-h AEGL-2:	$C^3 \times 1$ h = 7,000,000 ppm-h C = 191 ppm 191 ppm ÷ 3 = 64 ppm (rounded)
4-h AEGL-2:	$C^3 \times 4$ h = 7,000,000 ppm-h C = 121 ppm 121 ppm ÷ 3 = 40 ppm (rounded)
8-h AEGL-2:	$C^1 \times 8$ h = 700 ppm-h C = 87.5 ppm 87.5 ppm ÷ 3 = 29 ppm (rounded)

Derivation of AEGL-3 Values

Key study:	Gehring, P.J. 1968. Hepatotoxic potency of various chlorinated hydrocarbon vapours relative to their narcotic and lethal potencies in mice. Toxicol. Appl. Pharmacol. 13(3):287-298.
Toxicity end point:	Lethality; 3-fold reduction in a 560-min LC_{50} of 4,500 ppm in mice was assumed to be a threshold for lethality (4,500 ppm ÷ 3 = 1,500 ppm).
Scaling:	$C^n \times t = k$ (default n = 3 for longer to shorter exposure durations; n = 1 for shorter to longer exposure durations) $(1{,}500 \text{ ppm})^3 \times 9.3 \text{ h} = 3.1 \times 10^{10} \text{ ppm}^3\text{-h}$
Uncertainty factors:	An interspecies uncertainty factor was not applied because the available metabolism and kinetics data and PBPK models (Corley et al. 1990) indicate that humans may be less sensitive than laboratory animals to chloroform.

	3 for intraspecies variability in metabolism and disposition of chloroform (e.g., induction of P-450 enzymes and subsequent enhancement of toxicity). Comparison with available anesthesia data in humans precluded incorporation of additional uncertainty factor adjustment. Because results of PBPK models (Corley et al. 1990; Delic et al. 2000) show that mice are considerably more sensitive (25- to 50-fold difference in rate of metabolism of chloroform) to the toxic effects of inhaled chloroform than are humans, an additional adjustment factor of 1/3 was applied and resulted in an overall net adjustment of 1.
10-min AEGL-3:	Set equivalent to the 30-min value of 4,000 ppm to minimize uncertainty associated with extrapolating a 560-min exposure duration to 10 min.
30-min AEGL-3:	$C^3 \times 0.5\ h = 3.1 \times 10^{10}\ ppm^3\text{-}h$ C = 4,000 ppm (rounded)
1-h AEGL-3:	$C^3 \times 1\ h = 3.1 \times 10^{10}\ ppm^3\text{-}h$ C = 3,200 ppm (rounded)
4-h AEGL-3:	$C^3 \times 4\ h = 3.1 \times 10^{10}\ ppm^3\text{-}h$ C = 2,000 (rounded)
8-h AEGL-3:	$C^3 \times 8\ h = 3.1 \times 10^{10}\ ppm^3\text{-}h$ C = 1,600 (rounded)

APPENDIX B

ACUTE EXPOSURE GUIDELINE LEVELS FOR CHLOROFORM

Derivation Summary for Chloroform

AEGL-1 VALUES

AEGL-1 values were not recommended because it was not possible to identify a definitive effect consistent with the AEGL-1 definition. Concentrations of chloroform approaching those inducing narcosis or hepatic and renal effects are not accompanied by overt signs or symptoms. Furthermore, chloroform is not irritating and its odor is not unpleasant.

AEGL-2 VALUES

10 min	30 min	1 h	4 h	8 h
120 ppm	80 ppm	64 ppm	40 ppm	29 ppm

Reference: Schwetz, B.A., B.K. Leong, and P.J. Gehring. 1974. Embryo- and fetotoxicity of inhaled chloroform in rats. Toxicol. Appl. Pharmacol. 28(3):442-451.

Test species/Strain/Number: Sprague Dawley rats; 68, 8, 22, 23, and 3 dams for the control, pair-fed control, low-, mid-, and high-concentration groups, respectively.

Exposure route/Concentrations/Durations: Inhalation (whole body); 0, 30, 100, or 300 ppm, 7 h/day on gestation days 6-15.

Effects:

Effect (litters affected/litters examined)	Control	Pair-fed	30 ppm	100 ppm[a]	300 ppm
Total gross anomalies	1/68	0/8	0/22	3/23[b]	0/3
Total skeletal anomalies	46/68	3/8	20/22[b]	17/23	2/3
Total soft-tissue anomalies	33/68	3/8	10/22	15/23	1/3
Fetal body weight (g)	5.69	5.19	5.51	5.59	3.42[b]
Fetal crown-rump length (mm)	43.5	42.1	42.5[b]	43.6	36.9[b]

[a]Determinant for AEGL-2 (100 ppm); although the effects reported in the study were the result of 7-h exposures on gestation days 6-15, it was assumed that the effects were the result of a single 7-h exposure.

[b]$p < 0.05$.

End point/Concentration/Rationale: Fetotoxicity (total gross anomalies), 7-h exposure at 100 ppm. It was assumed that a single 7-h exposure would produce the same effects as the 10-day exposure used in the study. Fetotoxicity was considered a sensitive indicator of potential serious and irreversible effects in a susceptible population.

(Continued)

AEGL-2 VALUES Continued

10 min	30 min	1 h	4 h	8 h
120 ppm	80 ppm	64 ppm	40 ppm	29 ppm

Uncertainty factors/Rationale:
Total uncertainty factor: 3
Interspecies: None; metabolism and kinetics data and PBPK models (Corley et al. 1990) indicate that humans are less sensitive than rats to chloroform.
Intraspecies: 3 for individual variability in metabolism and disposition of chloroform and protection of individuals with altered metabolism and disposition (e.g., consumers of alcohol); the fetuses are a sensitive population but a larger uncertainty factor is unwarranted because the critical study involved effects on the fetus.

Modifying factor: None

Animal-to-human dosimetric adjustments: Insufficient data.

Time scaling: The concentration-time relationship for many irritant and systemically acting vapors and gases may be described by $C^n \times t = k$, where the exponent n ranges from 0.8 to 3.5 (ten Berge et al. 1986). In the absence of chemical-specific data, temporal scaling was performed using n = 3 when extrapolating to shorter durations and n = 1 when extrapolating to longer durations.

Data adequacy: A conservative approach to select the point of departure was used by assuming that a single 7-h exposure would result in fetotoxicity. The values are considered to be protective of human health consistent with the AEGL-2 definition.

AEGL-3 VALUES

10 min	30 min	1 h	4 h	8 hr
4,000 ppm	4,000 ppm	3,200 ppm	2,000 ppm	1,600 ppm

Reference: Gehring, P.J. 1968. Hepatotoxic potency of various chlorinated hydrocarbon vapours relative to their narcotic and lethal potencies in mice. Toxicol. Appl. Pharmacol. 13(3):287-298.

Test species/Strain/Number: Female Swiss-Webster mice (20/group)

Exposure route/Concentrations/Durations: Inhalation, various concentrations and durations

Effects: Lethality, 4,500-ppm LCt_{50} of 560 min (540-585 min, 95% CI)

End point/Concentration/ Rationale: Lethality threshold estimated by reducing the 560-min LC_{50} of 4,500 ppm by a factor of 3.

Uncertainty Factors/Rationale:
Total uncertainty factor: 1
Interspecies: None; laboratory animals metabolize chloroform more rapidly than humans and are, therefore, probably to be more susceptible to the toxic effects of the more rapidly formed toxic intermediates. PBPK models (Corley et al. 1990) also support not applying an uncertainty factor.

(Continued)

AEGL-3 VALUES Continued

10 min	30 min	1 h	4 h	8 hr
4,000 ppm	4,000 ppm	3,200 ppm	2,000 ppm	1,600 ppm

Intraspecies: 3 to account for individual variability in the sensitivity to chloroform-induced toxicity (e.g., alcohol-potentiated hepatotoxicity). An additional adjustment (weight-of-evidence factor of 1/3) was applied to account for the PBPK findings indicating that the mouse is more susceptible to chloroform.

Modifying factor: None applied.

Animal-to-human dosimetric adjustments: Insufficient data.

Time scaling: 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), where the exponent n ranges from 0.8 to 3.5. In the absence of chemical-specific data, temporal scaling was performed using the default of n = 3 when extrapolating to shorter durations.

Data adequacy: Human lethality data are lacking and lethality data in laboratory animals have limitations. However, when compared with human anesthesia data, the AEGL-3 values appear to be sufficiently protective. PBPK models affirm that rodents, especially mice, are a considerably more sensitive species than humans to chloroform.

APPENDIX C

CATEGORY GRAPH FOR CHLOROFORM

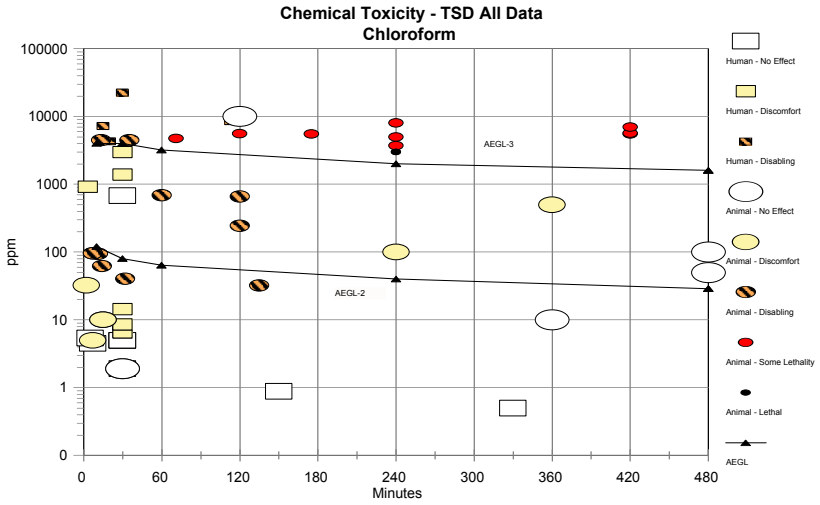

FIGURE C-1 Category graph of toxicity data and AEGLs values for chloroform.

APPENDIX D

CARCINOGENICITY ASSESSMENT FOR CHLOROFORM

Cancer Assessment of Chloroform

The cancer inhalation unit risk for chloroform is 2.3×10^{-5} per ($\mu g/m^3$) (EPA 2001, 2012), and is based on a tumorigenic response (hepatocellular carcinomas) in $B6C3F_1$ mice administered chloroform by gavage (NCI 1976). On the basis of this unit risk, the upper-bound unit risks of 10^{-4} to 10^{-7} are 4×10^{-3} to 4×10^{-6} mg/m^3, assuming an inhalation rate of 20 m^3/day for a 70 kg individual. At the 10^{-4} risk level, the virtually safe dose (d) is 4 $\mu g/m^3$.

A 70-year exposure may be converted to a 24-h exposure by the following calculation:

24-h exposure = d × 25,600 days; where d = 4 $\mu g/m^3$
= (4 $\mu g/m^3$) × 25,600 days
= 102,400 $\mu g/m^3$ (102.4 mg/m^3)

To account for uncertainty in the variability in the stage at which chloroform or its metabolites may act on the cancer process, a multistage factor of 6 is applied (Crump and Howe 1984):

$$(102.4\ mg/m^3) \div 6 = 17.07\ mg/m^3$$

Therefore, based on the potential carcinogenicity of chloroform, an acceptable 24-h exposure would be 17.07 mg/m^3 (3.58 ppm). If the exposure is limited to a fraction (*f*) of a 24-h period, the fractional exposure becomes 1/f × 24 h (NRC 1984), resulting in the following values:

24-h exposure	= 17.07 mg/m^3 (3.58 ppm)
8 h	= 51.21 mg/m^3 (11 ppm)
4 h	= 102.42 mg/m^3 (22 ppm)
1 h	= 409.68 mg/m^3 (86 ppm)
0.5 h	= 819.36 mg/m^3 (172 ppm)

The AEGL-2 values based on acute toxicity were somewhat greater than the values derived based on potential carcinogenicity. However, the data are compelling that the carcinogenic response to chloroform has a threshold, such that repeated exposures are needed that result in tissue necrosis and regeneration.

A virtually safe dose of 0.01 ppm (48.7 $\mu g/m^3$) was derived by Butterworth et al. (1995) and Wolf and Butterworth (1997) based on a no-observed-adverse-effect level of 10 ppm in mice and the assumption that the tumorigenic response was secondary to necrosis and regenerative cell proliferation (a threshold response). Cancer risk based on this approach is 12-fold less than those derived from the 10^{-4} unit risk number.

5

Methyl Bromide[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 are defined as follows:

AEGL-1 is the airborne concentration (expressed as parts per million or milligrams per cubic meter [ppm or 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 (Summitec Corporation), Julie M. Klotzbach (Syracuse Research Corporation), Chemical Manager George Rodgers (National Advisory Committee [NAC] on Acute Exposure Guideline Levels for Hazardous Substances), and Ernest V. Falke (U.S. Environmental Protection Agency). 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 guidelines reports (NRC 1993, 2001).

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 concentrations 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 AEGL values represent threshold concentrations 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

Methyl bromide is a colorless, nonflammable gas, with no taste or odor properties at low concentrations. Methyl bromide is currently used as a fumigant for buildings and soil and as a methylation agent in industry. Methyl bromide is an effective herbicide, rodenticide, nematicide, insecticide, bactericide, and fungicide. In the past, it was used as a refrigerant and fire extinguisher. Worldwide consumption of methyl bromide in 1996 was approximately 68 thousand metric tons. It is available as a liquefied gas in steel cylinders or cans.

Although numerous reports of accidental exposure of humans to methyl bromide that resulted in neurotoxicity or deaths are available in the literature, reliable information on exposure concentrations was not available. Acute, repeat-exposure, subchronic, and chronic studies, primarily with rats and mice, were available. Human case reports and controlled animal studies document that the central nervous system (CNS) is the primary target of methyl bromide. Neurotoxic symptoms can be delayed for several hours. Extremely high concentrations also produce lung edema. The mechanism-of-action of monohalomethanes is not completely understood, but could involve metabolism via the glutathione-*S*-transferase (GST) pathway to products that alkylate or inactivate cellular proteins. Species with higher cellular concentrations of GST appear to be more sensitive to the effects of methyl bromide than those with lower concentrations. The same is true for humans because of genetic polymorphisms of GST in the human population.

Data from animal studies were available on lethal and sublethal concentrations, neurotoxicity, developmental and reproductive effects, genotoxicity, and potential carcinogenicity. Although genotoxicity studies show that alkylation of DNA and proteins occurs, carcinogenicity has not been established in chronic studies with rats and mice. The dose-response curve for lethality is steep and the margin of safety between no-effect and lethal values is small. Data from rat and mouse studies show that the concentration-response curve for 50% lethality (LC_{50} values) over exposure durations of 3.5-480 min can be determined with the equation $C^{1.2} \times t = k$.

Methyl bromide has no odor or irritation properties at concentrations below those that define the AEGL-2 values. Therefore, AEGL-1 values were not established.

The AEGL-2 values are based on the no-observed-adverse-effect level (NOAEL) for neurotoxicity, as evidenced by a lack of clinical signs in studies with rats and dogs. The weight-of-evidence from those studies indicates that 200 ppm of methyl bromide for 4 h is the threshold concentration for neurotoxicity (Hurtt et al. 1988; Hastings 1990; Japanese Ministry of Labour 1992; Newton 1994a). Reversible impairment of olfactory function (lesions of the olfactory epithelium) was observed in the rat (Hurtt et al. 1988; Hastings 1990; Japanese Ministry of Labour 1992). These lesions are specific to the nasal olfactory epithelium of the rat, based on nasal air flow patterns (Bush et al. 1998; Frederick et al. 1998), so it is unlikely that such lesions would occur in primates at the same exposure concentration and duration. Because uptake of methyl bromide is greater in rodents than in humans (based on comparative respiratory rates and comparisons with methyl chloride) and because GST concentrations in rodents are greater than in humans (resulting in more rapid production of toxic metabolites), an interspecies uncertainty factor of 1 was applied. Humans differ in their capacity to metabolize the related chemical methyl chloride; but, toxicologically, the difference is thought to be less than 3-fold (Nolan et al. 1985). Therefore, an intraspecies uncertainty factor of 3 was applied. The resulting 4-h value of 67 ppm was time scaled to the other exposure durations using the equation $C^n \times t = k$, with n = 1.2. The value of n was based on lethality studies in rats. The mechanism for delayed neurotoxic effects (AEGL-2) and death (AEGL-3) are assumed to be the same. Because the time-scaled 8-h AEGL-2 value of 37 ppm is close to the chronic NOAEL of 33 ppm for mice (NTP 1992), is less than the 4-day NOAEL of 55 ppm for clinical signs and tissue lesions in dogs (Newton 1994a), and less than the 36-week NOAEL of 55 ppm for neurobehavioral parameters and nerve conduction velocity in rats (Anger et al. 1981), the 8-h value was set equal to the 4-h AEGL-2 value of 67 ppm.

Because of differences in methyl-halide metabolism between mice and other rodents and the greater sensitivity of mice to the structurally-similar chemical methyl chloride (metabolism is also by the glutathione [GHS] pathway), the mouse was not considered an appropriate model from which to derive AEGL values for methyl bromide. The AEGL-3 values were based on the $BMCL_{05}$ (benchmark concentration, 95% lower confidence limit with 5% re-

sponse) of 701 ppm in a 4-h exposure study of rats (Kato et al. 1986). The $BMCL_{05}$ was also the highest nonlethal value in the study. An interspecies uncertainty factor of 1 and an intraspecies uncertainty factor of 3 were applied, as was done in the calculation of AEGL-2 values. For time scaling ($C^n \times t = k$), n was set equal to 1.2, based on lethality data in the rat. Because uptake of methyl bromide is greater in rodents than in humans (based on comparative respiratory rates and comparisons with methyl chloride) and because GST concentrations in rodents are higher than in humans (resulting in more rapid production of toxic metabolites), an interspecies uncertainty factor of 1 was considered sufficient. Humans differ in their capacity to metabolize methyl bromide, but toxicologically the difference is not thought to be greater than 3-fold (Nolan et al. 1985). An intraspecies uncertainty factor of 3 is supported by the steep dose-response curve for lethality by methyl bromide, which indicates that there might be little intraspecies variation. Furthermore, a larger uncertainty factor would result in values that would be near the AEGL-2 values. Therefore, an intraspecies uncertainty factor of 3 was considered sufficient. The 8-h AEGL-3 value of 130 ppm is supported by a repeat-dose study in which dogs exposed to methyl bromide at 156 ppm for 7 h/day did not exhibit severe clinical signs until the third day of exposure (Newton 1994a). There were no remarkable histopathologic lesions at autopsy.

The AEGL values for methyl bromide are presented in Table 5-1.

TABLE 5-1 Summary of AEGL Values for Methyl Bromide

Classification	10 min	30 min	1 h	4 h	8 h	End Point (Reference)
AEGL-1 (nondisabling)	NR[a]	NR[a]	NR[a]	NR[a]	NR[a]	
AEGL-2 (disabling)	940 ppm (3,657 mg/m^3)	380 ppm (1,478 mg/m^3)	210 ppm (817 mg/m^3)	67 ppm (261 mg/m^3)	67 ppm (261 mg/m^3)	NOAEL for clinical signs in rats and dogs (Hurtt et al. 1988; Hastings 1990; Japanese Ministry of Labour 1992; Newton 1994a)
AEGL-3 (lethal)	3,300 ppm (12,837 mg/m^3)	1,300 ppm (5,057 mg/m^3)	740 ppm (2,879 mg/m^3)	230 ppm (895 mg/m^3)	130 ppm (506 mg/m^3)	$BMCL_{05}$ in rats (Kato et al. 1986)

[a]Numerical values are not recommended because the data indicate that toxic effects might occur below the odor threshold for methyl bromide. Absence of an AEGL-1 value does not imply that exposure below the AEGL-2 value is without adverse effects.
Abbreviations: $BMCL_{05}$, benchmark concentration, 95% lower confidence limit with 5% response; NOAEL, no-observed-adverse-effect level; NR, not recommended.

1. INTRODUCTION

Methyl bromide is a colorless, highly volatile gas that exists as a liquid below 3.6°C. It is heavier than air. Methyl bromide is nonflammable over a wide range of concentrations in air, and poses practically no fire hazard. These physical properties result in excellent penetration properties and make it a good fumigant. Additional chemical and physical properties are listed in Table 5-2.

Methyl bromide is ubiquitous in the environment, because it is generated naturally by oceans, biomass burning, and plants. For industrial purposes, methyl bromide is produced by direct bromination of methane and by the hydrobromination of methanol (Davis et al. 1977; O'Neil et al. 2001; Ioffe and Kampf 2002). Sulfur or hydrogen sulfide may be added as reducing agents to the methanol and sodium bromide. Anthropogenic methyl bromide is used mainly as a fumigant. It is an effective herbicide, rodenticide, nematicide, insecticide, bactericide, and fungicide, and has been used commercially in the United States for most of the twentieth century for the fumigation of soil, structures (such as warehouses), and food commodities, as well as for quarantine purposes (Duafala and Gillis 1999). Approximately 77% is used in preplanting fumigation of soil (IPCS 1995). In 1995, between 25,000 and 27,000 tons of methyl bromide were applied as a fumigant in the United States. Methyl bromide is also used as an intermediate for the manufacture of pharmaceuticals, in ionization chambers, for degreasing wool, and for extracting oils from nuts, seeds, and flowers (O'Neil et al. 2001; Ioffe and Kampf 2002). In the past, methyl bromide was used in fire extinguishers, as a refrigerant, and even as an anesthetic agent in dentistry (Alexeeff and Kilgore 1983).

In 1996, world consumption of methyl bromide was 68.4 thousand metric tons (Ioffe and Kampf 2002). The U.S. Environmental Protection Agency (EPA 2011) lists the production range of methyl bromide as 16.4 million pounds in 2003 and 1.8 in 2010. Production has decreased because of environmental concerns about depletion of the ozone layer by such chemicals.

Methyl bromide is easily liquefied, and is commercially available as a liquefied gas contained in steel cylinders or cans, usually under its own pressure of about two atmospheres (Braker and Mossman 1980; IPCS 1995; Duafala and Gillis 1999). Nitrogen or carbon dioxide may be added before shipment to permit rapid ejection at low temperatures. Formulations for soil fumigation contain chloropicrin (2%) or amyl acetate (0.3%) as warning agents.

2. HUMAN TOXICITY DATA

2.1. Odor Threshold

Methyl bromide has almost no odor or irritating effect, even at physiologically hazardous concentrations (Reid 2001). Reported odor thresholds vary from 20 to 1,000 ppm (Van den Oever et al. 1982; Sittig 1985; Ruth 1986). The odor of methyl bromide has been described as sweetish and similar to chloroform

(O'Neil et al. 2001), musty or fruity at concentrations above 1,000 ppm (Marraccini et al. 1983; ATSDR 1992), or faintly acrid at around 500 ppm (Hustinx et al. 1993). Methyl bromide has a burning taste, and contact with the skin may cause frostbite (O'Neil et al. 2001). When methyl bromide is used as a structural fumigant, it may react with sulfur-containing materials in buildings to produce a persistent odor (Anger et al. 1986).

The addition of 2% chloropicrin as a warning agent (a potent lacrimator sensed at 1.3 ppm) to some preparations of methyl bromide intended for fumigation (IPCS 1995; Reid 2001) is of limited safety efficacy, because chloropicrin vapor typically disappears before the methyl bromide vapor dissipates.

2.2. Toxicity and Neurotoxicity

The toxicity of methyl bromide has been reviewed by EPA (1980, 1992), Alexeeff and Kilgore (1983), ATSDR (1992), IPCS (1995), Yang et al. (1995), Reid (2001), OECD SIDS (2002), and HSDB (2010). A review of the literature published in 1983 documented 115 fatalities, 523 systemic illnesses, and 242 skin and eye injuries on a worldwide basis (Alexeeff and Kilgore 1983).

TABLE 5-2 Chemical and Physical Properties of Methyl Bromide

Parameter	Value	Reference
Synonyms	Bromomethane, monobromomethane, methyl fume, isobrome	O'Neil et al. 2001
CAS registry no.	74-83-9	O'Neil et al. 2001
Chemical formula	CH_3Br	O'Neil et al. 2001
Molecular weight	94.95	O'Neil et al. 2001
Physical state	Colorless gas (above 4°C)	O'Neil et al. 2001
Melting point	-93.7°C	Reid 2001
Boiling point	3.56°C	Reid 2001
Density		O'Neil et al. 2001
Vapor	3.97 g/L at 20°C (air = 1)	
Liquid	1.73 g/mL at 4°C (water = 1)	
Solubility	1.75 g/100 g in water at 20°C, 748 mm Hg	O'Neil et al. 2001
Vapor pressure	1,420 mm Hg at 20°C	O'Neil et al. 2001
Flammability limits	Practically nonflammable; flame propagation range is 13.5-14.5% by volume in air; ignition temperature is 537°C; burns in O_2	Reid 2001
Conversion factors	1 ppm = 3.89 mg/m^3 at 25°C 1 mg/m^3 = 0.257 ppm	Reid 2001

Most cases of accidental exposures have involved manufacturing or packaging operations, use of fire extinguishers containing methyl bromide, or fumigation activities. Exposures at high concentrations may occur during fumigation activities, especially when methyl bromide is first released to the environment after fumigation ends, or when fumigated areas are not properly ventilated. When methyl bromide is used as a storage fumigant, its concentrations usually range from 4,112 to 25,700 ppm for 2-3 days; higher concentrations are required to kill eggs and pupae. Accidental inhalation exposure incidents have occurred during atmospheric inversions, which prevent methyl bromide gas from rising and dispersing into the troposphere, or when children, adults, or animals enter sealed, fumigated structures. Most human exposure data on methyl bromide are from its use as an agricultural fumigant. It is applied to soil under plastic sheets or used in space fumigation under tarpaulins. It is also applied to a variety of agricultural commodities in specially designed fumigation chambers. Worker exposure may result from leaks in the plastic sheets or tarpaulin or from failure to allow adequate time for the methyl bromide to dissipate following fumigation (NIOSH 1984). The data from these accidental exposures are generally old, and concentration measurements were either not made or conducted using outdated analytic techniques. Regardless, estimates of concentrations leading to human deaths range from 1,600 to 60,000 ppm, depending on duration of exposure (ATSDR 1992).

The primary target of toxicity in humans accidentally or occupationally exposed to methyl bromide is the CNS (Alexeeff and Kilgore 1983; O'Neil et al. 2001). Symptoms of overexposure by inhalation to methyl bromide are headache, visual disturbance, vertigo, nausea, vomiting, anorexia, irritation of the respiratory system, abdominal pain, malaise, muscle weakness, incoordination, slurring of speech, staggering gait, hand tremor, convulsions, mental confusion, dyspnea, pulmonary edema, coma, and death from respiratory or circulatory collapse (O'Neil et al. 2001). Severe exposures may result in bronchial or pulmonary inflammation and pulmonary edema, which may not appear for 24 h or more after exposure. Death may occur from respiratory or cardiovascular failure. Exposure to methyl bromide has been known to adversely affect the kidneys, eyes, liver, and skin. Methyl bromide is an insidiously-acting chemical because of its lack of odor or immediate irritating properties at low concentrations (Reid 2001), and because signs of exposure are often delayed. In severe cases of poisoning, recovery can be protracted, with persisting neurologic problems.

Inhalation is the most significant route of exposure to methyl bromide, although skin absorption does occur. Standard protective clothing did not protect fumigators wearing respirators from developing skin lesions during two 20-min exposures at concentrations estimated to be about 9,000 ppm (Zwaveling et al. 1987; Hezemans-Boer et al. 1988). Absorption of methyl bromide was indicated by bromide concentrations in the blood.

Numerous case reports of methyl bromide exposure are described in the literature. In these reports, concentrations are either unknown or were measured or calculated after the incident. Analytic methods used in older studies, such as

colorimetry, have limited sensitivity. For example, Watrous (1942) describes both mild symptoms and more severe symptoms of nausea, vomiting, headache, and skin lesions in workers exposed for up to 2 weeks. Measurements of methyl bromide were generally less than 35 ppm, but exposures were based on a color detection method (methanol torch) with a lower detection limit of 35 ppm. Analytic methods for detecting higher concentrations involved flame colorimetry, an imprecise method. The exposures were complicated by accidents and routine dermal contact with the cooled liquid. In a factory where fire extinguishers were filled with methyl bromide, one death occurred (accompanied by convulsions) and another employee suffered less severe effects (Tourangeau and Plamondon 1945). Measurements taken at 30 min and 1 h had methyl bromide at concentrations of 297-390 ppm in front of the hood, where filling took place. Three additional nonfatal cases are described as examples below. Two of these cases also measured or estimated concentrations after the event. These cases are followed by a description of study of neurologic changes in methyl bromide applicators.

Because over 50 cases of methyl-bromide poisoning were reported in date processing and packaging plants in Southern California, Ingram (1951) and Johnstone (1945) conducted a series of surveys in 40 plants. Fumigation took place in a chamber that opened directly into the employee's workroom. Appropriate amounts of methyl bromide were released from 50-pound drums or by using 2-pound or 1-pound cans. Exhaust systems were generally inadequate to dissipate the fumes following fumigation. Tests in these plants showed methyl bromide at concentrations up to 100 ppm in the general workroom air, up to 500 ppm near the walls of ineffectively sealed chambers, and over 1,000 ppm at the breathing zone of workers entering the fumigation chamber. Semiquantitative measurements were made with a halide torch, and average concentrations over time were measured colorimetrically with a halogenated-hydrocarbon apparatus.

Hustinx et al. (1993) described an accidental exposure during greenhouse fumigation. Nine individuals were inadvertently exposed while working in an enclosed area adjacent to the area being fumigated. The areas were separated by a poorly sealed partition. Three weeks earlier, the portion of the greenhouse in which the accident occurred had been fumigated with methyl bromide at 200 g/m^2 (five times greater than the legally allowable concentration of 40 g/m^2). At that time, two of the five workers in the nonfumigated section experienced nausea, vomiting, and dizziness. During fumigation, the highest methyl bromide concentration (25 ppm) was measured near the partition in the nonfumigated portion of the greenhouse. Measurements were made with Drager gas detectors (lower detection limit of 3-5 ppm). On the day before the accident (3 weeks after fumigation of the first greenhouse section), all nine workers were in the nonfumigated portion of the greenhouse for an average of 6 h (range of 4-8 h). Most workers experienced nausea and headache that day, and two of them stayed home the following day. The next day, fumigation was carried out in the previously nonfumigated section of the greenhouse, while the laborers worked in the section that had been fumigated 3 weeks earlier. After spending 2 h at work, all but one of the remaining seven workers experienced sudden and almost simulta-

neous nausea, dizziness, and vomiting (the one exception experienced only a slight burning sensation in the throat). All seven workers left the greenhouse and went home. Within 2 h, two workers developed twitching of all limbs followed by generalized and continuous seizure activity, necessitating the induction of a sodium thiopental coma to stop the seizures. Methyl bromide concentrations ranged from 200 ppm near the partition to 150 ppm at the far end of the nonfumigated section 5 h after the accident, suggesting that the actual exposure was ≥200 ppm. Three days after admission to the hospital, chest x-rays revealed unilateral infiltration and pleural effusion, which subsided over the next 10-14 days. The thiopental coma was withdrawn after 3 weeks from the two severely affected patients, who then manifested persistent signs of axonal neuropathy. These signs improved only slightly over 6 months. Both workers had exhibited similar rises in serum alanine aminotransferase, aspartate aminotransferase, and lactic acid dehydrogenase activities, which peaked on the sixth day after admission and returned to normal before the thiopental treatment was discontinued, suggesting the increased activities reflected a methyl-bromide-related hepatic effect. The other seven patients experienced remarkably uniform signs, which included headache, nausea, and a "floating" sensation. Within 19 days after the accident, all residual complaints had disappeared in these seven patients. An unused, dry set of drainage pipes that crossed the entire length of both greenhouse sections was identified as the most likely major cause of the spread of methyl bromide to the nonfumigated section.

In the third case report, two fumigation workers entered a fumigated building in which the measured concentration (gas chromatography) was 4,370 ppm (Deschamps and Turpin 1996; Garnier et al. 1996). The workers wore cartridge respirators, which are saturable within a few minutes at that concentration (autonomous air flow masks are obligatory under these circumstances). The workers failed to wait until the concentration had decreased to the recommended level of 5 ppm. Both workers opened windows and doors in the nine-floor building over a period of 45 min to 1 h. During the 100-mile journey home, both workers experienced dizziness, fatigue, nausea, vomiting, chest pain, and shortness of breath. They were admitted to a hospital where the condition of the one improved rapidly. The other patient experienced convulsions, ataxia, and kidney failure. His tremors and ataxia were still present 5 months later (he experienced permanent neurologic damage). Bromide concentrations in the blood measured 40-48 h after admittance to the hospital were 47 and 156 mg/L in the first and second patient, respectively. Inspection of the charcoal cartridges of the respirators showed a concentration bromide greater than 10 mg/g; the highest concentration was found in the cartridge of the most injured worker.

Verberk et al. (1979) described bromine in the blood, electroencephalographic (EEG) disturbances, liver function (serum transaminases), serum proteins, and neurologic changes in 33 men engaged in soil disinfection inside greenhouses. Duration of employment ranged from a few months to 11 years. The amount of methyl bromide applied within the past year ranged from 1,500 to 6,000 kg. The relationship between different factors was based on a product-

moment correlation coefficient or Student's t-test. No relationship was found between bromine concentration in blood and subjective symptoms, general neurologic deficits, or serum proteins. Slight EEG changes and a small increase in serum transaminases were related to blood concentrations of bromine. The authors considered the effects marginal.

2.3. Developmental and Reproductive Toxicity

No studies were found on reproductive or developmental effects in humans after inhalation of methyl bromide.

2.4. Genotoxicity

Liquid methyl bromide tested positive for sister chromatid exchanges (SCE) in in vitro tests with human lymphocytes (Tucker et al. 1986; Garry et al. 1990). When people who are GSH conjugators and nonconjugators were tested, methyl bromide tested positive for SCE in lymphocytes from GSH nonconjugators but not in lymphocytes from GSH conjugators (Hallier et al. 1990). See Section 4.4.2 for an explanation of human variability in GSH conjugation.

2.5. Carcinogenicity

EPA has classified methyl bromide as a Group D carcinogen, "not classifiable as to human carcinogenicity" (EPA 1992). On the basis of animal studies, the National Institute for Occupational Safety and Health characterizes methyl bromide as a "potential occupational carcinogen" (NIOSH 2010). The International Agency for Research on Cancer (IARC 1999) has determined that there is limited evidence for carcinogenicity in animals and inadequate evidence in humans. The overall evaluation states that methyl bromide "is not classifiable as to its carcinogenicity to humans" (Group 3). The American Conference of Governmental Industrial Hygienists (ACGIH 2004) classifies methyl bromide as A4, "not classifiable as a human carcinogen."

Alavanja et al. (2003) investigated the link between exposure to 45 common agricultural pesticides and the eventual development of prostate cancer in a cohort of 55,332 initially healthy male pesticide applicators in Iowa and North Carolina. The data were collected by self-administered questionnaires that were completed at enrollment (1993-1997). The incidence of cancer in the general population was determined through cancer registries between the time of enrollment through the end of 1999, and a prostate cancer standardized incidence ratio was computed for the cohort. Odds ratios were determined for individual pesticides and for pesticide use patterns identified by the use of factor analysis. Over a period of 4 years, 566 of the men developed prostate cancer, a number greater than the total number of expected prostate cancer cases (494.5; odds ra-

tio of 1.14), based on state age-adjusted incidence rates. Among the 45 pesticides studied, only methyl bromide use showed a statistically significant exposure-response trend. The data suggested that if methyl bromide is responsible for the increased incidence of prostate cancer, this effect occurs only in those individuals with relatively frequent exposure. Limitations of this study acknowledged by the authors include the fact that the method of data collection was subject to significant recall bias, particularly in participants who had been exposed to the pesticides many years prior to the study. In addition, no direct measurements of pesticide exposure were obtained for the study. The follow-up period for the study was relatively short (an average of 4.3 years), precluding the evaluation of time-dependent exposures and risk. Finally, the authors acknowledged that the finding of increased risk of prostate cancer from the combined effect of exposure to several pesticides and a family history of prostate cancer was somewhat unexpected, and that the study must be replicated before any recommendations can be made.

2.6. Summary

Methyl bromide has been responsible for many occupational poisoning incidents, reflecting its wide use as a fumigant. Although many occupational and accidental exposures to methyl bromide have occurred, few cases have accurately documented exposure concentrations or durations. Methyl bromide is practically odorless, even at lethal concentrations. Descriptive symptoms indicate methyl bromide acts on the CNS (e.g., headache, visual disturbance, mental disturbance, nausea, vomiting) and directly on the lungs (lung edema). Case reports indicate that daily exposure to methyl bromide at 35 ppm (with possible dermal contact) and acute exposures to several hundred ppm can cause mild to severe symptoms.

3. ANIMAL TOXICITY DATA

3.1. Acute Lethality

Early studies with several species of mammals were carried out by Irish et al. (1940) and Sayers et al. (1929). These reports lack details, used obsolete analytic methods, and used visual inspection rather than standard neurotoxicity tests to assess behavioral deficits. The studies are described here for completeness, but were not considered in the determination of AEGL values. Rats and rabbits were given single exposures to methyl bromide at a series of concentrations which resulted in either 100% mortality or 100% survival (Irish et al. 1940). The postexposure observation period was 4 weeks. Exposure of rats to methyl bromide at 13,000, 5,200, 2,600, 520, 260, 220, or 100 ppm resulted in 100% mortality in 6, 24, and 42 min and 6, 22, 26, and >26 h, respectively. Survival was 100% when exposures at the respective concentrations were 3, 6, and 25 min

and 2, 8, 12, and 22 h. For 8-h exposures, survival was 100% at 240 ppm and 0% at 470 ppm. Survival times for rabbits exposed at the same concentrations were longer by a factor of 2-3. Neurotoxicity was evident in rats exposed at concentrations below 260 ppm, and lung congestion and edema was found at 260-5,200 ppm. Rats withstood repeated exposures to methyl bromide for up to 6 months at 66 ppm. Guinea pigs also survived without demonstrable effects. Rabbits, however, became paralyzed. Results of repeated exposures in monkeys were complicated by deaths from pneumonia and tuberculosis. Similar observations in guinea pigs were reported by Sayers et al. (1929). In addition, Balander and Polyak, (1962) report a 2-h LC_{50} in mice of 397 ppm; the same data appear to be reported by Izmerov et al. (1982). This value is considerably lower than those reported in more recent studies.

Recent, well-conducted studies with acute exposure durations are discussed below and are summarized in Table 5-3.

TABLE 5-3 Acute Lethality in Laboratory Animals Exposed to Methyl Bromide by Inhalation

Species	Concentration (ppm)	Exposure Duration	Effect	Reference
Rat	19,460	3.5 min	LC_{50}	Zwart et al. 1992;
	1,880	1 h	LC_{50}	Zwart 1988
	334	8 h	LC_{50}	
Rat	2,830	30 min	LC_{50}	Bakhishev 1973
Rat	832	4 h	100% mortality	Kato et al. 1986
	780	4 h	LC_{50}	
	701	4 h	No deaths	
Rat	1,140, 760	4 h	100% mortality	Japanese Ministry of
	506	4 h	No deaths	Labour 1992
Rat	402	8 h	100% mortality	Honma et al. 1985
	302	8 h	LC_{50}	
	268	8 h	No deaths	
Mouse	1,700	30 min	LC_{50}	Bakhishev 1973
Mouse	1,200	1 h	LC_{50}	Alexeeff et al. 1985
	900	1 h	No deaths	
Mouse	760	4 h	100% mortality	Japanese Ministry of
	506	4 h	90% mortality	Labour 1992
	338	4 h	No deaths	
Mouse	500	2 h	No deaths	Yamano 1991
	405	4 h	LC_{50}	
	312	4 h	No deaths	

3.1.1. Rats

A concentration-time mortality method was used to estimate LC_{50} values in male SPF-Wistar rats (Zwart 1988; Zwart et al. 1992). The scheme use approximately 50 rats tested in groups of two at seven exposure durations (3.5-480 min) and various concentrations. Probit analysis allowed calculation of a time-scaling value (see Section 4.4.3). LC_{50} values ranged from 19,460 ppm at 3.5 min to 334 ppm at 480 min. The 1-h LC_{50} was 1,880 ppm. Most animals showed some clinical signs, such as incoordination immediately after exposure. All mortalities occurred during the first week; these animals exhibited red, discolored lungs. Examination of the remaining rats at 2 weeks after exposure showed clear or light-red fluid in the lungs of some (exposure groups not explained).

Bakhishev (1973) exposed several species of mammals (number and strains not reported) to methyl bromide for 30 min. The 30-min LC_{50} for rats was 2,830 ppm. Although the details of this study are lacking, the value is similar to that predicted by Zwart et al. (1992) above.

In two separate experiments, groups of 5 male Sprague-Dawley rats were exposed to measured concentrations of methyl bromide at 502, 622, 667, 799, or 896 ppm by inhalation for 4 h, and groups of 10 male Sprague-Dawley rats were exposed at 701, 767, 808, 817, or 832 ppm for 4 h (Kato et al. 1986). The post-exposure observation period was 1 week. The 4-h LC_{50} from the combined studies was 780 ppm (95% confidence limits of 760-810 ppm). Mortalities (estimated from a graph) were 0% at 502-701 ppm, 30% at 767 ppm, 60% at 799 ppm, 70% at 808 ppm, 80% at 817 ppm, and 100% at 832 and 896 ppm. Clinical signs were not described.

Groups of 10 male and 10 female F344 rats inhaled methyl bromide at 150, 225, 338, 506, 760, or 1,140 ppm for 4 h (Japanese Ministry of Labour 1992). At concentrations of 338 ppm or greater, there was decreased locomotor activity, ataxia, nasal discharge, lacrimation, diarrhea, irregular breathing, and bradycardia. All rats exposed at 760 and 1,140 ppm died. Necropsy revealed pulmonary congestion, hepatic degeneration, renal necrosis, myocardial hemorrhages, hemorrhage and necrosis of the adrenal glands, and congestion of the thymus. Rats in the 225-, 338-, and 506-ppm groups exhibited metaplasia of the olfactory epithelium, and rats exposed at 760 and 1,140 ppm (no deaths) exhibited necrosis of the olfactory epithelium. Sublethal effects are summarized in Table 5-4.

Groups of 5 male Sprague-Dawley rats inhaled methyl bromide at 268, 335, 402, 469, or 536 ppm for 8 h (Honma et al. 1985). Atmospheres were monitored with a gas chromatograph fitted with a flame ionization detector. The postexposure observation period was not specified, but it was stated that no deaths occurred later than 6 h after exposure. The 8-h LC_{50} was 302 ppm (95% confidence limits of 267-340 ppm). All rats survived in the 268-ppm exposure group, and all rats died in the 402-ppm exposure group. Severe hemorrhage was found in the lungs of dead rats. Death was preceded by convulsions in some rats.

Sedation was observed in a concentration-dependent manner. No further details of sedation were provided.

3.1.2. Mice

Bakhishev (1973) reported a 30-min LC_{50} in mice (number and strain not reported) of 1,700 ppm. Groups of 6 male Swiss-Webster mice were exposed by nose only to methyl bromide at 0, 224, 443, 566, 700, 900, 984, 1,200, 1,486, or 1,527 ppm for 1 h (Alexeeff et al. 1985). Atmospheres were measured with gas chromatography. The mice were observed for 1 week after exposure. The 1-h LC_{50} was approximately 1,200 ppm (95% confidence limits of 1,058-1,370 ppm). No deaths occurred at ≤900 ppm. Clinical observations were made after exposure. Mice exposed at 700 and 984 ppm exhibited transient hyperactivity during the first 20 h after exposure. At ≥900 ppm, signs of abnormal gait, passivity, lack of grooming, increased respiratory depth, decreased respiratory rate, and tremors appeared that were dose-dependent in number and time of onset. Signs that preceded death included fasciculations, loss of righting reflex, splayed limbs, tonic seizures, muscular rigidity, and rectal bleeding, with the latter effect appearing at the two highest concentrations. The behavior of mice exposed at 224, 443, or 566 ppm was not different from that of the controls (see Table 5-4). Transient weight loss was observed in all treatment groups. One week after exposure, weight loss was observed only at ≥900 ppm. Kidney lesions were observed grossly at concentrations above 900 ppm. Liver congestion and hemorrhage were observed at 1,200 ppm. Cerebral hemorrhage and congestion was observed at ≥984 ppm. Compared with the control group, brain weight to bodyweight ratios were decreased at 443 ppm and increased at 700, 900, and 982 ppm. Liver GSH was reduced at 1,200 and 1,527 ppm. Bromide ion was not detected in tissues at 1 week after exposure to methyl bromide at concentrations up to 700 ppm.

Groups of 10 male and 10 female BDF1 mice inhaled methyl bromide at 100, 150, 225, 338, 506, or 760 ppm for 4 h (Japanese Ministry of Labour 1992). All mice in the 760 ppm group died, and all but two male mice died in the 506 ppm group. Mice in these groups exhibited decreased locomotor activity, tremor, convulsions, diarrhea, irregular breathing, and bradypnoea. Mice in the 100-, 150-, 225-, and 338-ppm groups did not exhibit any clinical signs. Necropsy of the two highest dose groups revealed pulmonary congestion, hepatic degeneration and necrosis, renal tubular necrosis, karyorrhexis of the thymus and lymph nodes, and necrosis of the olfactory epithelium. A single female mouse exposed at 338 ppm exhibited metaplasia of the olfactory epithelium. Sublethal effects are summarized in Table 5-4.

Groups of 6 or 10 ICR-SPF male mice inhaled methyl bromide at 312, 357, 377, 449, or 464 ppm for 4 h (Yamano 1991). No deaths occurred at 312 ppm. Mortality was 10% at 357 and 377 ppm, 90% at 449 ppm, and 100% at 464 ppm. The 4-h LC_{50} was 405 ppm (95% confidence limits of 386-425 ppm).

The mortality rates of mice exposed at 500 ppm for 105, 120, 130, 140, 150, and 180 min were 0, 0, 11, 15, 85, and 90%, respectively. The post-exposure observation period was not specified. Mortality in mice exposed at 500 ppm for 150 min that had been injected with GSH (500 mg/kg) previously was only 5.3% compared with 85% in mice that were not injected with GSH.

3.2. Acute Nonlethal Toxicity

Many of the acute studies addressed neurotoxicity. These studies are summarized in Section 3.3 (Neurotoxicity) and are listed in Table 5-4. Four studies with rats addressed acute effects on the olfactory epithelium.

Groups of male F344 rats were exposed by inhalation to methyl bromide at 0, 90 (6 rats), or 200 ppm (15 rats) for 6 h (Hurtt et al. 1988). Damage to the olfactory epithelium was assessed in 3 rats/day on day 0 and post-exposure day 1 (90 ppm) or day 0 and post-exposure days 1, 3, 5, and 7 (200 ppm). An additional group of 40 rats were exposed at 200 ppm for 6 h/day for up to 5 days. There were no treatment-related clinical signs during the exposures. Exposure at 90 ppm caused no observable effect on olfactory function or nasal morphology (examined microscopically). Rats exposed at 200 ppm gained less weight than the control group. Exposure at 200 ppm for 6 h resulted in extensive destruction of the olfactory epithelium; however, the basal cells were generally unaffected. A single 6-h treatment with 200 ppm rendered rats unable to find a hidden food pellet; the ability to locate a food pellet returned within 4-6 days. Cellular repair began by day 3 and was essentially complete 10 weeks after the last exposure. At 10 weeks, only small areas of residual damage remained. The absence of lesions in the more anterior respiratory epithelium (where most irritant gases induce damage) indicates specific sensitivity of the olfactory epithelium to methyl bromide. Exposure at 90 ppm for 5 days was also a no-effect level for damage to the olfactory epithelium in an earlier study (Hurtt et al. 1987; see Section 3.3).

Hastings (1990) studied the effect of methyl bromide on olfactory function in rats. A group of 30 rats (sex and strain not identified) were exposed at 200 ppm for 4 h/day, 4 days/week for 2 weeks. After the initial 4-h exposure, rats were unable to locate a hidden food pellet. However, with additional exposures, the rats showed improvement until their performance was equal to that of the control group by day 4. There were no clinical signs or body weight changes. Damage to the olfactory epithelium was extensive and required more than 30 days for repair to near-normal appearance.

Groups of three male Wistar-derived rats were exposed nose-only to methyl bromide at 200 ppm for 6 h, and then killed 25 h later (Reed et al. 1995). There was marked degeneration of over 95% of the olfactory epithelium, with only one or two layers of cells remaining. The lesion did not reach the transitional or respiratory epithelium. No further details of the study were available.

Schwob et al. (1999) studied the reinnervation of the olfactory bulb after near-complete destruction with methyl bromide (330 ppm for 6 h) in male Long-Evans hooded rats. Repair was evaluated for up to 8 weeks after exposure. Repair and reinnervation was nearly complete at 8 weeks in rats that had no diet restrictions.

3.3. Acute Neurotoxicity

Studies of acute exposures to methyl bromide are discussed here and summarized in Table 5-4. Repeat-exposure studies are discussed under Section 3.4.

3.3.1. Dogs

In a one-day range-finding study, beagles were exposed to methyl bromide at 233, 314, 345, 350, or 394 ppm (measured concentrations) for 7 h (Newton 1994a). Two dogs were test at 345 ppm, and one dog was tested at each of the other concentrations. Neurotoxicity (tremors, hunched appearance, and labored breathing) was observed by the seventh hour at all concentrations, with the first signs appearing at 3 h at the three highest concentrations, and during the last 2 and 3 h in the dogs exposed at 233 and 314 ppm, respectively. Postexposure observation of the dog exposed at 233 ppm revealed no remarkable effects.

In the same study, groups of 2-3 dogs inhaled methyl bromide at 55, 156, 268, or 283 ppm, 7 h/day for 4 days (see Table 5-5). At 55 ppm, there were no clinical signs. By the third day at 156 ppm, lacrimation, labored breathing, prostration, and decreased activity were observed. Because toxic effects appeared after repeat exposure at 156 ppm, the authors considered the effects cumulative. For both exposures, postmortem findings, including microscopic examination of brain tissue, were unremarkable. Dogs exposed at the two higher concentrations exhibited decreased activity, labored breathing, and excessive salivation during the exposures and irregular gait, ataxia, emesis, rales, white nasal discharge, and general traumatized behavior postexposure. These effects were not observed during the first day of exposure at 268 ppm. Postexposure examination of the medulla/pons, cerebrum, and cerebellum of these animals showed no methyl-bromide-related lesions (Newton 1994a).

3.3.2. Rats

Groups of 15 male and 15 female CD (Sprague-Dawley) rats inhaled methyl bromide at 0, 30, 100, or 350 ppm for 6 h (Driscoll and Hurley 1993). Concentrations were verified by a gas chromatograph equipped with a flame ionization detector. Animals were assessed for clinical signs and changes in body weight. EPA's functional observational battery of neurotoxicity tests and an automated assessment of motor activity test were conducted 3 h and 7 and 14

TABLE 5-4 Nonlethal Effects of Methyl Bromide in Laboratory Animals Exposed by Inhalation

Species	Concentration (ppm)	Exposure Duration	Effect	Reference
Dog	233, 314, 345, 350, 394	7 h	Concentration- and time-dependent increase in tremors, hunched appearance, and labored breathing.	Newton 1994a
Rat	150	4 h	No clinical signs.	Japanese Ministry of Labour 1992
	225	4 h	Metaplasia of the olfactory epithelium.	
	338, 506	4 h	Decreased motor activity, ataxia, nasal discharge, lacrimation, diarrhea, irregular breathing, bradycardia, metaplasia of the olfactory epithelium.	
Rat	200	4 h	No clinical signs or effect on body weight; transient impairment of olfactory function.	Hastings 1990
Rat	0, 30, 100	6 h	No neurotoxicity or tissue lesions.	Driscoll and Hurley 1993
	350	6 h	Changes in neurobehavioral battery 3 h after exposure; no tissue lesions.	
Rat	90	6 h	No clinical signs or effect on olfactory epithelium.	Hurtt et al. 1988
	200	6 h	No clinical signs; extensive olfactory-epithelium degeneration and reduced olfactory function, followed by repair.	
Rat	200	6 h	Marked degeneration of the olfactory epithelium.	Reed et al. 1995
Rat	330	6 h	Near complete destruction of the olfactory epithelium, with repair and reinnervation at 8 wk postexposure.	Schwob et al. 1999
Rat	63	8 h	No effect on body temperature or locomotor activity.	Honma et al. 1985
	125	8 h	Transient decrease in body temperature.	
	188	8 h	Transient decrease in body temperature and body weight gain.	
Mouse	224, 443, 566	1 h	No clinical signs; transient weight loss; no gross pathologic lesions; no effect on brain weight; no reduction in liver glutathione at 224 ppm; no detectable tissue bromide at 1 wk postexposure.	Alexeeff et al. 1985
	700	1 h	Transient hyperactivity.	
Mouse	100, 150, 225, 338	4 h	No clinical signs; metaplasia of the olfactory epithelium at 338 ppm.	Japanese Ministry of Labour 1992

days postexposure. Nasal tissue, brain, spinal cord, and peripheral nerves were examined microscopically at necropsy performed 16-19 days after exposure. There were no effects on mortality, body weight, or organ weights, including brain weights. At 350 ppm exposure, clinical signs consisted of drooping eyelids, decreased arousal, piloerection, decreased rearing, depressed body temperatures, and markedly decreased motor activity. These signs were transient; they occurred only at the 3-h postexposure observation period. No treatment-related histologic findings were seen in nervous-system or nasal tissues. The lowest-observed-adverse-effect level (LOAEL) and NOAEL for neurotoxicity were 350 and 100 ppm, respectively.

Locomotor activity and body temperature of male Sprague-Dawley rats exposed to methyl bromide at 63, 125, 188, or 250 ppm for 8 h were measured (Honma et al. 1985). These end points were unaffected by methyl bromide at 63 ppm, but activity was decreased and strongly inhibited at 188 and 250 ppm, respectively, and body temperature was lowered by 2°C. These effects were reversed by the next day.

3.3.3. Mice

Passive-avoidance and motor-coordination tests were administered to mice following 1-h exposures to methyl bromide (Alexeeff et al. 1985). Concentrations of 224-984 ppm did not affect the ability of mice to recall a single-task passive avoidance test. Results were variable in the rotorod test, but performances were significantly different from the control group, particularly at 1,486 and 1,527 ppm.

3.4. Repeat-Dose Studies

Studies with repeated exposures are summarized in Table 5-5 and discussed below.

3.4.1. Dogs

In a repeat-exposure study with methyl bromide at 5 ppm for 7 h/day for 30 exposures, equivocal evidence of neurotoxicity was reported at the thirtieth exposure (Newton 1994b). A small number of dogs were tested in this study (one per exposure), there was no dose-response relationship, the observations were not part of a standardized protocol, and some of the dogs had been used in a previous study with methyl bromide.

A 6-week study was undertaken to resolve the issues in the Newton (1994a) study (see Table 5-5). Groups of 4 male and 4 female beagles were exposed (whole body) to methyl bromide at concentrations of 0, 5, 10, and 20 ppm (measured by gas chromatography) for 7 h/day, 5 days/week (Schaefer 2002). Potential neurotoxic effects were evaluated with EPA's functional observational

battery of neurotoxicity tests and an automated motor-activity evaluation during the second, fourth, and sixth week of exposure. Tissues of the nervous system were examined at the end of the study. There were no mortalities. Clinical observations, body weights, food consumption, body temperatures, and the functional-observational-battery and motor-activity parameters were unaffected by exposures. No tissue lesions were observed at necropsy.

Groups of 4 male and 4 female beagles inhaled methyl bromide at 0, 5, 10, 25, 50, or 100 ppm for 7 h/day, 5 days/week for 4-5 weeks (Newton 1994b). During the fifth day of week 5, dogs in the 10-ppm group were exposed at 150 ppm for 6 exposures (analytical concentration of 158 ppm). Physical observations, ophthalmoscopic examinations, neurologic examinations, body weight and food consumption measurements, hematology and clinical chemistry parameters, and urinalysis were performed pretest and during the exposures. At the end of the study, organs were weighed and examined microscopically. No deaths occurred. No treatment-related clinical signs were observed in the 5-, 10-, or 15-ppm groups. Decreased activity was noted in two dogs in the 50-ppm group beginning on exposure day 14, in 3 of 8 dogs in the 100 ppm group beginning on exposure day 9, and in all dogs in the 158-ppm group beginning on exposure day 2. Clinical signs increased in the 158-ppm group as exposure to methyl bromide continued, and included irregular gait, opisthotonos, and convulsions in 3 of 8 dogs. Depression, tremor, and ataxia were observed in the remaining dogs after their sixth exposure. Hematology and clinical chemistry parameters were generally unaffected at ≤100 ppm, and body weight was unaffected at ≤50 ppm. There was no effect on absolute or relative organ weights. Although the signs in the 158-ppm group (examined 2 days after the sixth exposure) suggested diffuse CNS dysfunction, the dominant signs indicated cerebellar or vestibular dysfunction. At autopsy, microscopic examination of tissues revealed lesions in only the group exposed at both 10 and 158 ppm. The lesions included minimal vacuolation of the cerebellum, vacuolation of the adrenal gland, and moderate to moderately severe degeneration of the olfactory epithelium of the nasoturbinates.

3.4.2. Rats

Groups of 10 male F-344 rats were exposed by inhalation to methyl bromide at 0, 90, 175, 250, or 325 ppm for 6 h/day for 5 days (Hurtt et al. 1987). At 250 and 325 ppm, animals developed diarrhea (day 2), hemoglobinuria, and, in a few cases, gait disturbances and convulsions (day 3). Rats in the 325-ppm group died or were sacrificed in extremis before exposure on the fifth day. At ≥175 ppm, vaculolar degeneration of the zona fasciculata of the adrenal glands, cerebellar granule cell degeneration, and nasal olfactory sensory-cell degeneration occurred in a dose-dependent manner. Cerebral degeneration was seen only in the 325-ppm group. Hepatocellular degeneration was seen in the 250- and 325-ppm groups. The 5-day NOAEL for all tissue lesions, including the olfactory epithelium, was 90 ppm.

TABLE 5-5 Repeat-Dose Studies of Methyl Bromide

Concentration (ppm)	Exposure Duration	Effects	Reference
Dog			
0, 55, 156, 268, 283	7 h/d, 4 d	55 ppm: no clinical signs or lesions. 156 ppm: lacrimation, labored breathing, irregular gait by day 3; no brain lesions. 268 and 283: Severe signs; exposure stopped after day 2.	Newton 1994a
0, 5, 10, 25, 50, 100, 158[a]	7 h/d, 5 d/wk, 4-5 wk	5-25 ppm: no clinical signs or tissue lesions. 50 ppm: decreased activity on exposure day 14. 100 ppm: decreased activity on exposure day 9. 158 ppm[a]: decreased activity by exposure day 2, followed by neurotoxic signs, tremors, convulsions on succeeding days; histopathologic examination indicated minimal cerebellar vacuolation, adrenal gland vacuolation, and moderate to moderately severe degeneration of the olfactory epithelium of the nasal passages.	Newton 1994b
0, 5, 10, 20	7 h/d, 5 d/wk, 6 wk	No neurotoxicity or tissue lesions.	Schaeffer 2002
Rat			
0, 90, 175, 250, 325	6 h/d, 5 d	90 ppm: no tissue lesions, including olfactory. 175 ppm: degeneration of adrenal glands. ≥250 ppm: diarrhea, hepatocellular degeneration, cerebral degeneration; death at 325 ppm.	Hurtt et al. 1987
0, 150	6 h/d, 5 d	No clinical signs, weight differences, or brain lesions.	Davenport et al. 1992
0, 190, 300	4 h/d, 5 d/wk, 3 wk	Compared with controls: minimal body weight gains; difference in spontaneous activity; no brain or nerve tissue lesions; death in 2/12 rats at 300 ppm.	Ikeda et al. 1980
0, 200, 300, 400	4 h/d, 5 d/wk, 6 wk	200 ppm: no clinical signs or deaths, heart lesions. 300 ppm: paralysis in 3/12 rats, early necropsy, heart lesions 400 ppm: ataxia and paralysis; early deaths, heart and brain lesions.	Kato et al. 1986

150	4 h/d, 5 d/wk, 11 wk	No clinical signs or deaths; heart lesions.	
0, 160	6 h/d, 5 d/wk, 6 wk	Early deaths, numerous tissue and organ lesions.	Eustis et al. 1988; NTP 1992
0, 30, 70, 140	6 h/d, 5 d/wk, 13 wk	30 ppm: no significant effects or lesions 70 ppm: decreased motor activity at week 13 and decreased body weight by week 13 140 ppm: early deaths, nerve lesions.	Norris et al. 1993
0, 55	7.5 h/d, 4 d/wk, 36 wk	No effect on neurobehavioral parameters or nerve conduction velocity.	Anger et al. 1981
Mouse			
0, 160	6 h/d, 5 d/wk, 6 wk	Early deaths; numerous tissue and organ lesions.	Eustis et al. 1988; NTP 1992
0, 10, 33, 100	6 h/d, 5 d/wk, 2 y	Neurotoxicity, brain lesions, and early deaths at 100 ppm; no clinical signs or brain lesions at 10 and 33 ppm.	NTP 1992
Rabbit			
0, 65	7.5 h/d, 4 d/wk, 4 wk	Weight loss by week 3, eyeblink response and nerve conduction velocity significantly reduced; partial recovery.	Anger et al. 1981
0, 27	7.5 h/d, 4 d/wk, 8 mon	No changes in neurobehavioral tests; no weight loss.	Russo et al. 1984

[a]Starting with the last day of exposure of the fifth week, the 10-ppm group was exposed to methyl bromide at 150 ppm (analytical concentration = 158 ppm) for six exposures.

Groups of 8 male and 8 female F-344 rats were exposed to methyl bromide at 0 or 150 ppm for 6 h/day for 5 days (Davenport et al. 1992). Treated animals exhibited no clinical signs, no differences in body weights, and no histologic evidence of brain lesions.

Groups of 12 male Wistar rats were exposed to methyl bromide at 0, 190, or 300 ppm for 4 h/day, 5 days/week for 3 weeks (Ikeda et al. 1980). Body weights were monitored and physiologic responses, equilibrium on the rotorod, and spontaneous activity in an automated activity cage were measured before treatment and at various times up to 29 days after treatment. Brain and nerve tissue of two rats in the 300-ppm group were examined 29 days after treatment. During the exposures, body-weight gains were minimal in the exposure groups compared with the control group (data presented graphically). During the postexposure period, body-weight gains in the treated groups increased, but did not reach that of the control group. Two of the rats in the 300-ppm group died and one exhibited convulsions (time not stated). The remaining rats in the 300-ppm group showed decreased spontaneous motor activity. Physiologic responses (rearing in the open field, defecation) did not differ among groups. Time on the rotorod and the circadian rhythm of spontaneous activity (activity during dark and light periods) were affected in the two treatment groups. Spontaneous activity returned to control values by postexposure day 21. Histologic examinations of the CNS and peripheral nerves revealed no abnormalities.

Kato et al. (1986) conducted repeat inhalation studies with male Sprague-Dawley rats. Groups of 10-12 rats were exposed to methyl bromide by inhalation at 0, 200, 300, or 400 ppm for 4 h/day, 5 days/week for 6 weeks. Another group was exposed at 150 ppm for 11 weeks under the same conditions. Animals were killed 5 days after exposure. No deaths occurred in rats exposed at 150 or 200 ppm, and no clinical signs were observed, although body-weight gains were slightly depressed. Three of 12 rats exposed at 300 ppm developed paralysis and were killed after 4 weeks. Rats in the 400-ppm group exhibited clinical signs of ataxia and paralysis after 2 weeks. Six of 10 rats died or were killed after 5 weeks. At concentrations of 300 ppm and greater, serum-enzyme activities and lipids were affected. Bromide ion accumulated in the kidney and spleen at all concentrations, but there was no clear dose-response relationship. There was no clear dose-response effect on organ weights. Microscopic necrotic lesions were observed in the brain only at 400 ppm, but heart lesions were found at all concentrations.

Target organ toxicity studies were carried out to determine test concentrations for chronic studies by the National Toxicology Program (Eustis et al. 1988; NTP 1992). Groups of 20 F-344 rats/sex/concentration were exposed to methyl bromide at 160 ppm for 6 h/day, 5 days/week for up to 6 weeks. Animals were killed after 3, 10, or 30 exposures or when 50% mortality was reached. Mortality rates exceeded 50% in male rats after 14 exposures. Female rats survived the 30 exposures with less than 50% mortality. The brain, kidneys, nasal cavity, heart, adrenal glands, liver, and testes were the primary target organs. In rats, neuronal necrosis occurred in the cerebral cortex, hippocampus, and thalamus of the

brain. Necrosis of the olfactory epithelium was more severe and extensive in rats than in mice exposed at the same concentrations. Myocardial degeneration was more frequent and severe in male and female rats than in male mice. Cytoplasmic vacuolation of the adrenal cortex was observed in rats. Testicular and thymic degeneration occurred in rats and mice. The authors noted that the effects of methyl bromide were similar to those of methyl chloride.

In a subchronic study, groups of 15 male and 15 female CD rats inhaled methyl bromide at 0, 30, 70, or 140 ppm for 6 h/day, 5 days/week for 13 weeks (Norris et al. 1993). Sacrifice took place 15 days later. At 140 ppm, two male rats died during the first month. Two other males exposed at 140 ppm exhibited salivation, rapid breathing, hyperactivity, and convulsions, and one subsequently died. Males and females in the 140-ppm group had significant depressed body weights and body-weight gain by the end of the study. Body weights of females in the 70-ppm group were also significantly reduced. The body weights of females in the 30-ppm group were lower than those of the controls, but the difference was not statistically significant. Absolute brain weights were reduced in association with the reduced body weights, but brain weights relative to body weights were not affected. The two males that died in the 140-ppm group exhibited moderate to severe brain hemorrhage. Microscopically, neuronal necrosis was observed in several brain areas except the cerebellum. Another male in this group had edema of the hippocampus. No brain lesions were found in females in this group or in males or females exposed at lower concentrations. Slight lesions of the peripheral nerves were observed, primarily in the 140-ppm group, but incidences in the other exposure groups were not concentration related. In the functional observational battery of tests, forelimb grip strength was slightly reduced in males in the 140-ppm group at week 13, and motor activity was decreased in females in the 70-ppm group during week 13, and in the 140 ppm group at most intervals.

3.4.3. Mice

In a 14-day study, groups of 10 male and 10 female B6C3F_1 mice were exposed to methyl bromide at 0, 12, 25, 50, 100, or 200 ppm for 6 h/day, 5 days/week (NTP 1992). Neurobehavioral signs of trembling, jumpiness, and paralysis were observed at all test concentrations, but primarily at the three highest concentrations. The time of onset of clinical signs was not described. Nine male and 6 female mice in the 200-ppm group died, with the first death occurring on day 11. Bloody urine was observed on day 6 in mice exposed at 200 ppm. Minimal hyperemia of the lung, liver, and kidneys was seen in females in the 200-ppm group. There were no other lesions, including lesions of the brain and nervous system, attributable to treatment.

Target organ toxicity studies were carried out to determine text concentrations for chronic studies by the National Toxicology Program (Eustis et al. 1988;

NTP 1992). Groups of 20 B6C3F$_1$ mice/sex/concentration were exposed to methyl bromide at 160 ppm for 6 h/day, 5 days/week for up to 6 weeks. Animals were killed after 3, 10, or 30 exposures or when 50% mortality was reached. Mortality rates exceeded 50% in male mice after eight exposures and in female mice after six exposures. The brain, kidneys, nasal cavity, heart, adrenal glands, liver, and testes were the primary target organs. In the brain, neuronal necrosis occurred primarily in the cerebellum. Nephrosis was the likely cause of deaths. Necrosis of the olfactory epithelium was not severe compared with the lesion in rats. Myocardial degeneration, observed in rats at the same concentrations, was not severe in male mice. Atrophy of the adrenal cortex was observed in female mice. Testicular and thymic degeneration occurred in rats and mice. The authors noted that the effects of methyl bromide were similar to those of methyl chloride.

In a chronic study of B6C3F$_1$ mice administered methyl bromide at 0, 10, 33, or 100 ppm (described in Section 3.6), NTP (1992) identified a LOAEL for neurotoxicity of 100 ppm and a NOAEL of 33 ppm. Mice were tested at 3-month intervals throughout the study. Cerebellar and cerebral degeneration were observed in 11/60 and 2/60 mice, respectively, in the 100 ppm group. These lesions were not observed at lower concentrations or in the control groups. Clinical signs of neurotoxicity, tremors, abnormal posture, tachypnea, and hind leg paralysis were observed in mice exposed at 100 ppm.

3.4.4. Rabbits

Sixteen Sprague-Dawley rats and 6 New Zealand white rabbits were exposed to methyl bromide at 65 ppm for 7.5 h/day, 4 days/week for 4 weeks (Anger et al. 1981). Control groups were composed of four rats and two rabbits. Neurobehavioral testing of nerve conduction, eyeblink reflex, activity, and grip/coordination were administered weekly. Another group of 32 rats were exposed to methyl bromide at 55 ppm for 6 h/day, 5 days/week for 36 weeks. Neurobehavioral testing of this group was conducted monthly. Rabbits exposed at 65 ppm began to lose weight by the third week of exposure. By week 4, eyeblink responses and nerve conduction velocity in rabbits were significantly reduced, but rats were unaffected. The symptoms in rabbits partially subsided within 6-8 weeks after exposure ended (Russo et al. 1984). No effects on nerve conduction velocity, open-field activity, or coordination were found in rats exposed at 55 ppm for 36 weeks.

To identify the threshold for chronic neurotoxicity in rabbits, Russo et al. (1984) exposed adult male New Zealand white rabbits to methyl bromide at 27 ppm for 7.5 h/day, 4 days/week, for 8 months. Eyeblink and nerve conduction tests were administered biweekly. The neurobehavioral tests were negative, and rabbits gained weight and appeared healthy.

3.5. Reproductive and Developmental Toxicity

Hurtt and Working (1988) evaluated reproductive parameters of male F-344 rats exposed by inhalation to methyl bromide at 200 ppm for 6 h/day for 5 consecutive days (see Table 5-6). Compared with a control group, plasma testosterone concentration was significantly decreased during the 5-day exposure, but returned to control concentrations by day 8 (3 days postexposure). Concentrations of GSH in the testes and liver were also depressed but returned to control concentrations by day 8. There was no effect on testicular weight or sperm production and motility.

TABLE 5-6 Reproductive Toxicity of Methyl Bromide in Animal Models

Concentration (ppm)	Exposure Duration	Effects	Reference
Rat			
0, 200	6 h/d for 5 d	Transient decrease in plasma testosterone and testicular and liver glutathione; no effect on testicular weight or sperm production and motility.	Hurtt and Working 1988
0, 3, 30, 90	6 h/d, 5 d/wk, 2 generations	Body and brain weights of parental (F_0) males depressed at 30 and 90 ppm; at 90 ppm, brain weights of F_1 offspring decreased without histologic correlates; no effect on litter size, sex ratio, or survival; no gross abnormalities in either generation.	Mayhew 1986
0, 20, 70	7 h/d, 5 d/wk, before mating and through day 19 of gestation	No maternal toxicity; no adverse developmental effects.	Hardin et al. 1981; Sikov et al. 1981
Rabbit			
0, 20, 70	7 h/d, 5 d/wk, before mating and through gestation	Increased maternal mortality at 70 ppm; no maternal mortalities or clinical signs at 20 ppm; no adverse developmental effects.	Hardin et al. 1981; Sikov et al. 1981
0, 20, 40, 80	6 h/d, gestation days 7-19	First study: maternal toxicity at 80 ppm; increase in fetal variations, partially attributed to sire; no effects at lower concentrations. Second study: less maternal toxicity and incidence of fetal variations not statistically significant at 80 ppm.	Breslin et al. 1990

In a two-generation study, Sprague-Dawley rats were exposed by whole-body inhalation to methyl bromide at 0, 3, 30, or 90 ppm for 6 h/day, 5 days/week (Mayhew 1986). Exposures were for at least 8 weeks before mating and continued over the production of two litters (temporarily suspended in F_0 dams from day 21 of gestation until day 5 of lactation). Two litters were produced by both the F_0 and F_1 generations. There were no clinical signs and no effect on survival in treated animals. In parental animals (F_0 generation), body and brain weights of males were decreased in the 30- and 90-ppm groups. Brain weights of F_1 parental males and females exposed at 90 ppm were also reduced. No histologic correlates were observed, and no pathologic changes were found in other organs or tissues. At 30 and 90 ppm, pups from the F_1 parental generation had reduced body weights compared with controls. Absolute, but not relative, organ weights were also reduced, probably reflecting smaller body sizes. There were no treatment-related effects on litter size, sex ratio, survival through lactation, or grossly observable abnormalities.

No adverse developmental effects in fetuses and no significant maternal toxicity, other than transient lower body weight, compared with controls were noted when female Wistar rats were exposed to methyl bromide at nominal concentrations of 0, 20, or 70 ppm (Hardin et al. 1981; Sikov et al. 1981). Exposures were for 7 h/day, 5 days/week, before mating and through 19 days of gestation. There were no differences in pregnancy rates, embryotoxicity, or fetal viability, and no effect on soft-tissue or skeletal anomalies.

In the same study (Hardin et al. 1981; Sikov et al. 1981), methyl bromide at 70 ppm was highly toxic to pregnant New Zealand rabbits. Exposure was terminated on day 15, but 24 of 25 rabbits died by gestation day 30. There were no deaths or clinical signs in the control or 20-ppm groups. No adverse developmental effects were observed in offspring of dams exposed at 20 ppm or in the offspring of the surviving rabbit in the 70-ppm group.

New Zealand white rabbits were exposed to methyl bromide at 0, 20, 40, or 80 ppm for 6 h/day during gestation days 7-19 (Breslin et al. 1990). No clinical signs of toxicity were observed at the lower concentrations. Clinical signs of maternal toxicity in the 80-ppm group included decreased feces (decreased food intake), lethargy, head tilt, ataxia, and lateral recumbency. Terminal body weight and body weight gain were decreased by 5 and 50%, respectively. Neurotoxicity was observed in 3/26 does after 12 exposures. Severe weight loss in two of dams in the 80-ppm group (464 and 604 g), indicates that decreased feeding began earlier in the study. Fetuses from the 80-ppm group showed decreased weights (4%) and an increased incidence of fused sternebrae, which the authors attributed to maternal stress. Fetuses in the 80-ppm group also had a higher incidence of missing gall bladder and missing caudal lobe of the lung (considered variations). Because these findings were associated with a sire that had a missing gall bladder, the study was repeated to test methyl bromide at 80 ppm. Maternal toxicity appeared to be less severe in this study and the incidences of

missing gall bladder and lung lobe, although increased, were not statistically significant compared with the control group. No maternal or fetal effects were found at the lower concentrations tested in the first study.

3.6. Genotoxicity

Methyl bromide, tested as a gas in sealed desiccators, was mutagenic in *Salmonella typhimurium* TA100 with and without metabolic activation, but no mutagenic response was observed in TA98 (NTP 1992). Methyl bromide induced sister-chromatid exchanges in bone-marrow cells and micronuclei in peripheral erythrocytes of female $B6C3F_1$ mice exposed at concentrations up to 200 ppm for 6 h/day for 14 days. Results were equivocal in male mice. When exposure duration was lengthened to 4, 8, or 12 weeks, no significant increase in sister-chromatid exchanges or micronuclei was observed in male or female mice.

The mutagenicity and genotoxicity of methyl bromide was reviewed by IPCS (1995) and IARC (1999). Methyl bromide was positive for reverse gene mutation in *S. typhimurium* TA100 and TA1535, but not in TA98 or TA1538. Metabolic activation was not required for positive results. Methyl bromide was also positive in tests of forward and reverse mutations in *Escherichia coli*. It bound covalently to DNA in vitro and in vivo in various organs of rats and mice, and induced micronuclei in bone marrow and peripheral blood cells of rats and mice. The frequency of bone marrow cells with chromosomal aberrations was not increased in rats exposed at 70 ppm for 5 days. Methyl bromide did not induce unscheduled DNA synthesis in cultured rat hepatocytes. Assays for dominant and recessive lethal mutations were negative in mice and rats.

3.7. Chronic Toxicity and Carcinogenicity

Several chronic inhalation studies were available to assess the chronic toxicity and oncogenicity of methyl bromide. In one study (Gotoh et al. 1994), groups of 50 male and 50 female F-344.DuCrj rats were exposed to methyl bromide at 0, 4, 20, or 100 ppm (99.9% purity) whole body for 6 h/day, 5 days/week for 104 weeks. Survival in males of the control, 4-, 20-, and 100-ppm groups was 68%, 68%, 62%, and 66%, respectively. Survival in females was 86%, 76%, 78%, and 82%, respectively. The incidence of pituitary adenomas was significantly increased in males exposed at 100 ppm (60%) compared with controls (32%). No increase in treatment-related tumors was observed in female rats. In the same study, 50 male and 50 female BDF_1 mice exposed to methyl bromide at 4, 16, or 64 ppm under the same exposure conditions. At 104 weeks, survival was unaffected by treatment and there was no increased incidence of tumors related to treatment.

Reuzel et al. (1987, 1991) exposed male and female Wistar rats to methyl bromide at concentrations of 0, 3, 30, or 90 ppm for 6 h/day, 5 days/week for 29

months. Each group had 90 males and 90 females; 10 rats/sex/group were killed after 13, 52, and 104 weeks. Body weights, clinical signs, hematology, biochemistry, and gross and microscopic effects were examined at those time points. Exposure to methyl bromide at 90 ppm was clearly toxic; early deaths were reported (but were not statistically significant at the end of the study) and body weights were significantly lower than those of respective control groups throughout most of the study. At the end of the study, effects on the heart were apparent in the 90-ppm group. Statistically significant increases in cartilaginous metaplasia (males), moderate to severe myocardial degeneration (females), and thrombi (males and females) were found. Myocardial degeneration also occurred in aged control rats. Therefore, when total incidences of myocardial degeneration were considered, incidences in the control and 90-ppm groups were similar for both sexes. At the end of the study, 3 ppm was the NOAEL for decreases in body weight and absolute and relative brain weight.

Basal-cell hyperplasia of the olfactory epithelium was present in both males and females in a dose-related manner after 29 months, but not at 13, 52, or 104 weeks. The incidence was statistically significant in the 3-ppm group when total incidence was considered (13/48 in males and 19/58 in females compared with 4/46 and 9/58 in the respective control groups). The majority of lesions were characterized as "very slight" in the 3-ppm group, and as "slight" to "moderate" in the higher exposure groups. These lesions were not present in either males or females in the 3-ppm group at 52 weeks and were not significantly increased over those of the respective control groups at 104 weeks. However, these lesions were found in the female control group after 104 weeks (40%), at the incidence observed in females exposed to methyl bromide at 3 ppm for 29 months (40%). At 29 months, the incidence of total olfactory lesions in males exposed at 3 ppm was 27% compared with 9% in males of the control group.

Nasal lesions increased in controls in an age-dependent manner. All but one of the lesions in the 3-ppm group were classified as slight or very slight, and one moderate lesion of the nasal mucosa was observed in controls at 24 months (accompanied by a 40% incidence of total lesions in control females). The incidence in the control males at 24 months was 30%. Thus, the effect in the 3-ppm groups at the end of the study, although dose-related and statistically significant, must be considered slight or equivocal. This study was well-conducted, used a relevant route of administration, used an adequate number of rats of both sexes, and examined all relevant end points of methyl bromide toxicity. The study shows that the nasal lesions occur in aged rats. There was no indication of carcinogenic activity.

A two-year inhalation study of methyl bromide in $B6C3F_1$ mice was conducted by NTP (1992). Groups of 70 male and 70 female mice were exposed to methyl bromide at 0, 10, 33, or 100 ppm. Neurotoxicity tests were performed on 16 mice (8 males and 8 females) per group. Ten animals per group were killed at 6 and 15 months. The exposure at 100 ppm was discontinued after 20 weeks because of neurotoxicity and early deaths. The same organs and tissue that were affected in the Reuzel et al. (1987, 1991) study were targets in this study,

namely the nose, heart, and brain. The bone was additionally affected. Aside from increased mortality in the 100-ppm dose group, statistically significant differences in LOAELs and NOAELs were found for the following end points: cerebellar and cerebral degeneration, 100 and 33 ppm; myocardial degeneration and chronic cardiopathy, 100 ppm and 33 ppm; sternal dysplasia, 100 ppm and 33 ppm, increased but not statistically significant for either males or females in the 33-ppm group compared with controls); and olfactory metaplasia/necrosis, 100 ppm and 33 ppm. Similar to results in the Reuzel et al. (1987, 1991) study, no olfactory lesions were found in the 3-ppm group at the end of 24 months. No increase in tumor incidence occurred. NTP concluded there was no evidence of carcinogenic activity of methyl bromide in male or female $B6C3F_1$ mice exposed at 10, 33, or 100 ppm.

Danse et al. (1984) showed that orally-administered methyl bromide is carcinogenic in the forestomach of Wistar rats. Doses were 0, 0.4, 2, 10, or 50 mg/kg for 13 weeks. At 50 mg/kg, severe hyperplasia of the stratified squamous epithelium in the forestomach was found. A dose of 10 mg/kg resulted in slight epithelial hyperplasia in the forestomach. Adverse effects were not observed at 0.4 or 2 mg/kg. Extrapolation of forestomach cancers to humans is problematic because methyl bromide, a volatile, reactive chemical, was introduced directly into the stomach by gavage and because humans lack a forestomach (Lu and Coulston 1996). Furthermore, Boorman et al. (1986) showed regression of the tumors after discontinuation of exposure.

3.8. Summary

The concentration-response relationship for mortality is steep in all animal species tested, as shown by the margin between LC_{50}s and nonlethal concentrations (Alexeeff et al. 1985; Kato et al. 1986). LC_{50} values for the rat ranged from 19,460 ppm for 3.5 min to 302-334 ppm for 8 h (Honma et al. 1985; Zwart 1988; Zwart et al. 1992). In rats, no deaths occurred after exposure to methyl bromide at 506 or 700 ppm for 4 h (Kato et al. 1986; Japanese Ministry of Labour 1992) or at 268 ppm for 8 h (Honma et al. 1985). The mouse was a more susceptible species, having LC_{50} values ranging from 1,200 ppm for 1 h (Alexeeff et al. 1985) to 405 ppm for 4 h (Yamano 1991). Nonlethal concentrations of methyl bromide in mice were 900 ppm for 1 h (Alexeeff et al. 1985) and 312 and 338 ppm for 4 h (Yamano 1991; Japanese Ministry of Labour 1992).

Toxicity to the CNS was evident as clinical and neurotoxic signs and tissue lesions. The nasal olfactory epithelium was also a target of methyl bromide. Clinical or neurotoxic signs were absent in rats exposed at 150 ppm for 4 h (Japanese Ministry of Labour 1992), in rats exposed at 90 or 100 ppm for 6 h (Hurtt et al. 1988; Driscoll and Hurley 1993), in rats exposed at 63 ppm for 8 h (Honma et al. 1985), in mice exposed at 225 ppm for 4 h (Japanese Ministry of Labour 1992), and in mice exposed at 566 ppm for 1 h, although there was a transient weight loss in the mice (Alexeeff et al. 1985). A 6-h exposure of rats to

methyl bromide at 90 ppm was a NOAEL for damage to the olfactory epithelium (Hurtt et al. 1988), whereas the 4-h NOAEL in mice was 150 ppm (Japanese Ministry of Labour 1992). Dogs exhibited clinical signs of tremors, hunched appearance, and labored breathing during the last 2 h of a 7-h exposure at 233 ppm (Newton 1994a). Rats exhibited transient changes in standard neurobehavioral tests after a 6-h exposure at 350 ppm (Driscoll and Hurley 1993). In contrast to the olfactory lesions observed in rats exposed at 200 ppm for 6 h (Hurtt et al. 1988), no lesions were found after a 6-h exposure at 350 ppm (Driscoll and Hurley 1993).

In 5-day repeat-exposure studies with rats, NOAELs were 90 ppm for tissue lesions, including damage to the olfactory epithelium (Hurtt et al. 1987), and 150 ppm for lesions in the brain (Davenport et al. 1992). The 6-week NOAEL for clinical and neurotoxic signs and tissue lesions in dogs was 20 ppm, the highest concentration tested (Schaeffer 2002). The NOAEL for neurobehavioral effects in a 36-week exposure study with of rats was 55 ppm, but there were severe effects on rabbits exposed at 65 ppm for 4 weeks (Anger et al. 1981). In a subchronic study that examined body and organ weights, clinical signs, neurotoxicity, and microscopic tissue lesions, the lowest LOAEL was 30 ppm in females, based on slightly reduced body weight (Norris et al. 1993). The NOAEL for neurotoxicity was 70 ppm for exposures up to week 13 (both sexes), and the NOAEL for motor activity was 30 ppm for females and 140 ppm for males. In a chronic study with mice, 33 ppm was the NOAEL for behavioral neurotoxicity, cerebellar and cerebral degeneration, myocardial lesions, and olfactory metaplasia/necrosis (NTP 1992).

No reproductive effects were observed in animals after acute exposure to methyl bromide, although plasma testosterone was transiently decreased in rats exposed at 200 ppm for 5 days (Hurtt and Working 1988). Studies with rats and rabbits indicate that inhalation of methyl bromide at up to 70 ppm during gestation does not result in any significant developmental effects, although there was severe maternal toxicity in rabbits (Hardin et al. 1981; Sikov et al. 1981).

Methyl bromide tested positive in numerous mutagenicity and genotoxicity tests. Mutagenicity did not require metabolic activation, which is consistent with direct-acting alkylation of DNA. Alkylation suggests that methyl bromide might be carcinogenic, but carcinogenicity has not been established in chronic studies with rats and mice (Reuzel et al. 1991; NTP 1992; Gotoh et al. 1994).

4. SPECIAL CONSIDERATIONS

4.1. Metabolism and Disposition

Inhalation studies with rats and dogs have demonstrated that [^{14}C]methyl bromide is rapidly absorbed in the lower respiratory tract and quickly distributed to all tissues, with the lungs, liver, and kidneys being the major organs of distribution immediately after exposure (Bond et al. 1985; Medinsky et al. 1985; Jas-

kot et al. 1988). Appreciable amounts of ^{14}C are also found in the nasal turbinates. This deposition, however, might indicate metabolites. Methyl bromide readily crosses cell membranes, whereas the bromide ion does not.

Uptake of methyl bromide has been measured in the rat, dog, and man. Lung uptake in rats is directly proportional to the concentration in air. Andersen et al. (1980) determined the kinetic constants for metabolism of inhaled methyl bromide in male F-344 rats. Concentrations were 100, 1,000, 3,000, and 10,000 ppm. Rats were placed in vacuum chambers and chamber depletion measurements were taken every 10 min for 180 min (chamber atmospheres were recirculated). Methyl bromide exhibited rapid, first-order uptake, with the first-order rate constant decreasing only at concentrations that caused death during the exposure (10,000 ppm). The rate plot was a straight line that was fitted by unweighted linear least squares to estimate the rate constant (0.44/kg/h). This constant was later recalculated as 0.55/kg/h (Gargas and Andersen 1982). The rapid, first-order uptake was considered to be nonenzymatic metabolism. Because methyl bromide had no measurable saturable uptake component, a K_m, the ambient concentration at which uptake proceeds at half the maximum rate, and a V_{max}, the maximum rate of uptake, could not be calculated.

In contrast to the work of Andersen et al. (1980), Medinsky et al. (1985) observed uptake saturation at higher concentrations. Their 6-h, nose-only inhalation study with male F-344 rats found uptake of 48% at low concentrations (1.6 and 9.0 ppm) which decreased to 38% at 170 ppm and 27% at 310 ppm. Medinsky et al. (1985) proposed that the availability of GSH might be the rate-limiting variable on the pulmonary absorption of methyl bromide at high concentrations. The observation that the rate of pulmonary absorption decreases with increased exposure concentrations suggests there is a saturation point for inhaled methyl bromide. Measurements of respiratory parameters during the study indicated the tidal volume and minute volume were significantly decreased at 310 ppm. Uptake was also measured in the beagle and man. Steady-state uptake in beagles exposed at 174-361 parts per billion (ppb) was 39.5% during a 3-h exposure (Raabe 1986); the clearance half-time was 41 h. Uptake in humans during nasal or oral breathing of methyl bromide at 18 ppb were similar, 52-55% (Raabe 1988).

Metabolism may take place by two pathways. The presence of methanol and bromide ion in tissues implies nucleophilic displacement of the bromide ion (Gargas and Andersen 1982; Honma et al. 1985). However, the primary pathway is probably conjugation with the tripeptide GSH. By analogy with methyl chloride, outlined by Kornbrust and Bus (1983), conjugation of methyl bromide with GSH yields S-methylglutathione cleavage of the glutamic acid and glycine moieties of GSH yields S-methylcysteine, and transamination and decarboxylation yields the mercapturic acid, methylthioacetic acid. The latter sulfur-containing compounds can be excreted in the urine. Methylthioacetic acid may be further metabolized to methanethiol which may yield, via P-450 metabolism, formaldehyde and formic acid; the latter compounds can enter the one-carbon pool or be excreted as carbon dioxide. The reaction with GSH appears to be

primarily enzyme catalyzed, probably by GST. Formation of formaldehyde appears to be a minor pathway. Additional studies of the metabolism of methyl bromide provide strong evidence that GSH conjugation is the primary metabolic pathway for methyl bromide (Peter et al. 1989; Hallier et al. 1990; Bonnefoi et al. 1991). Several studies have shown that administration of methyl bromide decreases the nonprotein sulfhydryl content (GSH) and, depending on tissue and administered concentration, increases or decreases the GST content of tissues (Roycroft et al. 1981; Alexeeff et al. 1985; Davenport et al. 1992). The concentration of GSH in liver is extremely high (about 10 mM), hence nonenzymatic conjugation of some xenobiotics with GSH can be significant. GSTs are abundant cellular components, accounting for up to 10% of the total cellular protein (Parkinson 2001).

Excretion occurs mainly by expiration of CO_2 and urinary excretion. After inhalation of ^{14}C-methyl bromide by rats (1.6-310 ppm), 43-50% of elimination of the radiolabel was pulmonary ($^{14}CO_2$) and 20% was renal (Bond et al. 1985; Medinsky et al. 1985; Jaskot et al. 1988). Less than 4% was expired as methyl bromide. Only small amounts are excreted in the feces. A significant amount (25-39%) remains in the tissues after 24-72 h, and is excreted slowly. This fraction presumably represents adducts and incorporation into metabolic pools.

Blood bromide concentrations in humans normally range up to 15-30 mg/L (EPA 1980; ATSDR 1992; Fuortes 1992). Concentrations of 25-250 mg/L were reported in severe poisoning cases and 83-2,116 mg/L in fatal poisoning cases. When these measurements were taken is unknown in some of these cases. Bromine concentrations in blood are generally lower with poisoning by methyl bromide than by inorganic bromide, indicating that free bromide might not be an indicator of the severity of effects.

Honma et al. (1985) measured methyl bromide and bromine concentrations in blood and tissues of rats after a 2-h exposure at 0, 250, 500, 750, or 1,000 ppm. Methyl bromide in tissues reached maximum values within 1 h, and was rapidly eliminated (half-life of about 30 min). There was a linear relationship between methyl-bromide concentrations in air and blood; the blood concentrations ranged from 0 μg/g at 0 ppm to 0.374 μg/g at 1,000 ppm (measured immediately after exposure). A linear relationship was also found between bromine in tissues and exposure concentrations. Blood bromine ranged from 7 μg/g at 0 ppm to 90 μg/g at 1,000 ppm. In contrast with tissues concentrations of methyl bromide, tissue concentrations of bromine peaked 4-8 h after exposure, and the half-life of elimination was about 5 days. Methanol concentrations remained below those considered responsible for CNS effects.

4.2. Mechanism of Toxicity

The mechanism of methyl-bromide-induced CNS toxicity has not been establish, although it is known that methyl-bromide toxicity results from the methylation of crucial sulfhydryl containing enzymes and proteins in mammalian

tissues and that CNS toxicity might be mediated by CNS glutathione depletion and inhibition of GST activity (Davenport et al. 1992). Methylation of proteins in addition to reduced GSH concentrations might cause cellular disruption. Cellular disruption, especially in the CNS, results in progressive dysfunction. Immunochemical studies of the rat brain show that GST isozymes are present in the cytoplasm, nuclei, and nucleolei of neurons, and the glia of the brainstem, forebrain, and cerebellum. The pattern of GST isozyme distribution throughout regions of the brain is not uniform, which might result in regional susceptibility or resistance to chemical-induced damage (Johnson et al. 1993). In contrast to this proposed mechanism of action, GSH depletion has been found to inhibit toxicity by methyl bromide in some species (Peter et al. 1989). In addition to inhibition of sulfhydryl enzymes, methyl bromide reversibly breaks down adenosine triphosphate (Reigart and Roberts 1999). Another suggested mechanism of toxicity is the formation of methanethiol and formaldehyde as neurotoxic metabolites of methyl bromide (Garnier et al. 1996). In such a case, individuals with greater glutathione-transferase activity might be predisposed to the neurotoxic effects if methyl bromide. However, with the related chemical, methyl chloride, no increase in formaldehyde concentration was observed in the liver or kidneys of mice exposed at 1,000 ppm for 8 h (Jager et al. 1988). Hydrolysis of methyl bromide, resulting in bromide ion, does not appear to be the toxic mechanism. Blood concentrations of bromide after methyl-bromide poisoning are not as high as those associated with intoxication by bromide salts (Gosselin et al. 1984).

In rats, methyl bromide specifically damages the olfactory mucosa lining the posterior regions of the nose, but does not adversely affect the respiratory epithelium (Hurtt et al. 1987, 1988). The reason for the specific sensitivity of the olfactory epithelium is unknown, although methyl bromide is known to inhibit GST activity of olfactory nasal epithelial cells (Thomas et al. 1989). This is a reversible lesion; near complete destruction of the rat olfactory epithelium by methyl bromide at 330 ppm for 6 h was followed by reinnervation and repair after exposure ended (Schwob et al. 1999).

On the basis of chemical similarity, the mechanism of action of methyl bromide is predicted to be the same as that of methyl chloride. Although the exact mechanism of action of methyl chloride is unclear, it appears to involve GSH depletion in target tissues or the production of toxic metabolites, such as formaldehyde or methanethiol, and lipid peroxidation. A lack of GSH could impair the ability of tissues to suppress lipid-peroxidation reactions (Kornbrust and Bus 1983). The buildup of leukotrienes also has been suggested as a toxic mechanism of action. Under conditions of GSH depletion, 5-hydroperoxicosotetraenoic acid, the immediate precursor of leukotrienes, accumulates in the tissues. Leukotrienes are potent vasoconstrictors, and cause increased capillary permeability and tissue edema (AIHA 1997). In contrast with tests of methyl chloride, depletion of GSH before administration of methyl bromide enhanced toxicity (Thomas and Morgan 1988).

Honma (1987; Honma et al. 1991) investigated the effect of methyl bromide on brain neurotransmitters, particularly changes in catecholamine production in the hypothalmus of rats in relation to CNS effects. Rats were exposed to methyl bromide at 0, 16, 31, 63, 125, 188, or 250 ppm for 8 h, and were killed immediately after exposure. There were no effects on catecholamine activity at 16 ppm. At 31-250 ppm, there was a dose-dependent decrease in the neurotransmitter norepinephrine (13-30%), an increase in 3-methoxy-4-hydroxyphenylglycol, a metabolite of norepinephrine, and a decrease in tyrosine hydroxylase activity. As tyrosine hydroxylase catalyzes hydroxylation of tyrosine to L-dopa, the precursor of the catecholamines, the decrease in norepinephrine appears to result from the inhibition of tyrosine hydroxylase. These changes were linked changes in body temperature and appetite noted in Honma et al. (1987).

Two studies show that exogenously-administered GSH protects against the acute lethal effects of methyl bromide. Mortality in mice injected with GSH (500 mg/kg) and exposed to methyl bromide at 500 ppm for 150 min was 5.3%, compared with 85% in mice that were not pretreated with GSH (Yamano 1991). Glutathione injected intraperitoneally (750 mg/kg) resulted in 100% survival of mice exposed at a lethal concentration of 1,850 ppm for 1 h (Kawai and Ueda 1972).

4.3. Structure-Activity Relationships

Toxicity of the monohalomethanes—methyl chloride, methyl bromide, and methyl iodide—appears to be related to atomic weight. All produce similar toxic effects in humans (Gosselin et al. 1984), with the greatest toxicity from methyl iodide, followed by methyl bromide and methyl chloride. Bakhishev (1975) reported 30-min inhalation LC_{50} values in the rat for methyl chloride, methyl bromide, and methyl iodide of 39,000 ppm, 2,800 ppm, and 2,300 ppm, respectively. The target organs of methyl bromide and methyl chloride in the rat are the same—the brain, liver, adrenal glands, olfactory epithelium, and the testes (Morgan et al. 1982; Hurtt et al. 1987).

4.4. Other Relevant Information

4.4.1. Species Variability

The available data allowed comparisons between rats and mice. Where data were available for the same time periods, mice were more susceptible to the lethal effects of methyl bromide than rats. On the basis of 30-min (Bakhishev 1973), 1-h (Alexeeff et al. 1985; Zwart 1988), and 4-h LC_{50} values (Kato et al. 1986; Yamano 1991), the mouse is two-fold more sensitive to methyl bromide than the rat. For example, the rat 1-h LC_{50} of 1,880 ppm (Zwart 1988) is 1.6 times greater than the 1-h mouse LC_{50} of 1,200 ppm (Alexeeff et al. 1985). This greater sensitivity might be related to the higher concentrations of GST found in

mouse tissues (Griem et al. 2002). Greater sensitivity might also be a reflection of the greater respiratory rate of mice compared with rats. Hori et al. (2002) reported that at methyl bromide concentrations of 500-10,000 ppm for up to 8 hours, survival times of rats were greater than those of mice. The difference in survival times between the species was large at low concentrations, but decreased substantially with increasing concentrations. The animals were killed immediately after the exposures, limiting the usefulness of the data.

The available data also indicate that rabbits are more sensitive than rats or guinea pigs. Guinea pigs and rats withstood 6-month exposures to methyl bromide at 66 ppm without demonstrable effects, but rabbits became paralyzed (Irish et al. 1940). A similar result was reported in a more recent study (Anger et al. 1981). Rabbits exposed at 65 ppm began to lose weight by the third week of exposure and eyeblink responses and nerve-conduction velocity in rabbits were significantly reduced. Rats were unaffected under the same exposure regime. Maternal toxicity was greater in pregnant rabbits exposed to methyl bromide at 70 ppm before to mating and through gestation, whereas no maternal toxicity was evident in rats under the same exposure scenario (Hardin et al. 1981; Sikov et al. 1981).

Both in vitro and in vivo comparisons of different species indicate that concentrations of GST enzymes are much lower in human tissues (liver and lung) than in mice or rats (Andersen et al. 1987; Reitz et al. 1989). The data are consistent with the hypothesis that the rate of activation of mono- and dihalomethanes to toxic metabolites by the GST pathway occurs much more slowly in humans than in rodents. Jager et al. (1988) investigated the concentrations of GSH in rodent tissues. Activities of GSH in the liver were 2-3 times greater in male $B6C3F_1$ mice than in female mice and F-344 rats of both sexes. Griem et al. (2002) compiled ratios of GST activity in rodents to humans in various tissues. The ratios of rat:human and mouse:human GST activity in the liver are 3.95 and 7.64, respectively. On the other hand, nonprotein sulfhydral content (primarily GSH) is similar among human, monkey, and rat tissues on a μmol/mL of tissue basis (Frederick et al. 2002). This was true for major organs, but not nasal tissue. Rat nasal tissue had more nonprotein sulfhydral content than human tissue.

For the related chemical, methyl chloride, blood concentrations of humans, dogs, and rats exposed to 50 ppm for 6 h reached a plateau during the first hours of exposure; elimination was rapid once the exposures were terminated (Landry et al. 1983; Nolan et al. 1985). Blood concentrations of methyl chloride in slow human metabolizers plateaued at 60% of those found in the rat and 70% of those found in the dog. Postexposure elimination was most rapid in the rat ($t_{1/2}$ = 15 min). The dog and rapid human metabolizers had the same elimination rate ($t_{1/2}$ = 50 min), and the slow human metabolizers eliminated at the slowest rate ($t_{1/2}$ = 90 min). At 50 ppm, the rat absorbed 10 μg/min/kg whereas the rapid and slow human metabolizers absorbed 3.7 and 1.4 μg/min/kg, respectively. According to Nolan et al. (1985), differences in the pharmacokinetics between these three species were adequately explained by the differences in respiratory

minute volume and basal metabolic rates (rat > dog > man). Similar comparative studies were not available for methyl bromide, so it should be noted that uptake kinetics of methyl bromide and methyl chloride could be different. Andersen et al. (1980) found uptake of methyl chloride to be saturable, being associated with enzymatic metabolism, whereas the rapid, first-order uptake of methyl bromide was considered to be nonenzymatic metabolism.

Monohalomethanes are not metabolized by the erythrocyte cytoplasm of rats, mice, Rhesus monkeys, cows, pigs, and sheep, but are metabolized by approximately 60% of humans (Redford-Ellis and Gowenlock 1971; Peter et al. 1989). Lack of erythrocytic metabolism might explain the rapid equilibrium between the gas phase of methyl chloride and methyl bromide and whole blood of rats observed in pharmacokinetic studies.

Inhalation studies conducted on various mammalian species have demonstrated clear sex-related differences in susceptibility to methyl bromide. In the 6-week repeat-exposure study of Eustis et al. (1988), survival was much greater in females than in males.

Methyl bromide was toxic only to olfactory epithelium and not the other nasal epithelia of rats (Hurtt et al. 1988). Histochemical techniques revealed degeneration of the sensory and sustentacular cells but not the basal cells from which the former cells are regenerated. This was a reversible effect as the basal cells were not affected. The olfactory region (dorsal meatus) of rats is highly exposed to chemicals because of air-flow characteristics in the nasal turbinates. In rodents, an inhaled vapor traverses a few millimeters of resistant respiratory epithelium before reaching sensitive olfactory tissue; whereas, in humans, an inhaled vapor has to traverse several centimeters and a much larger surface area of respiratory epithelium to reach the olfactory tissue. A mathematical model based on a combination of computational fluid dynamics and physiologically-based pharmacokinetics showed that the dorsal meatus region of the rat nose receives 12-20% of the inhaled air (Bush et al. 1998; Frederick et al. 1998). A comparison with airflow patterns in the human nose shows that the olfactory epithelium in the dorsal meatus region of the nasal cavity of the rat is exposed to two- to three-fold greater concentrations of chemicals. Therefore, higher concentrations of methyl bromide would likely be required to induce this lesion in humans.

4.4.2. Susceptible Populations

Interindividual variation in the rate of metabolism of methyl halides has been observed in humans. At least two distinct populations of humans with different metabolism rates of the structurally-similar methyl chloride have been identified (Nolan et al. 1985; ATSDR 1992; WHO 2000). The differences in metabolism rate are attributed to a genetic polymorphism of GST. Depending on the presence or absence of GST, humans may be “fast metabolizers” or “slow metabolizers” of methyl chloride. There may be a third phenotype, non-conjugators

(Warholm et al. 1994; Lof et al. 2000). Fast metabolism may lead to the formation of toxic metabolites that can exert their action before they can be eliminated. Slow metabolizers would be expected to be less susceptible to the toxic effects of methyl halides as formation of S-methylglutathione is limited to the nonenzymatic reaction of methyl bromide with GSH. Garnier et al. (1996) reported that of two fumigation workers similarly exposed to methyl bromide during fumigation activities, neurologic and systemic symptoms were greater in the "conjugator." GST activity was undetectable in the erythrocytes of the nonconjugator. The nonconjugator also had greater concentrations of S-methylcysteine adducts in blood albumin and globin.

For the related chemical, methyl chloride, uptake differed by less than 3-fold between slow and fast metabolizers exposed at 50 ppm (Nolan et al. 1985). Elimination was rapid in both groups after the exposure ended. Elimination was more rapid in volunteers with lower blood and expired-air concentrations. The authors explained the difference in the two groups by a two-fold difference in the rate at which they metabolized methyl chloride. They considered the difference of questionable toxicologic significance.

Among Caucasians, the majority of individuals possess at least one copy of the GST gene; 10-25% are nonconjugators (Nelson et al. 1995; Warholm et al. 1994; Kempkes et al. 1996). Approximately 60% of Asians lack the gene (Nelson et al. 1995).

As noted by ATSDR (1992), people that have kidney or liver disease, anemia, or neurologic deficits might be more susceptible to the toxic effects of methyl chloride. Persons with deficient glucose-6-phosphate dehydrogenase might have reduced concentrations of GSH (Bloom and Brandt 2001). Additionally, accidental exposures suggest that infants are more susceptible than adults (Langard et al. 1996). However, death of an infant was from acute pneumonia resulting from vomiting and aspiration after inhaling methyl bromide.

4.4.3. Concentration-Exposure Duration Relationship

Using approximately 50 male SPF-Wistar rats tested in groups of two at 23 different combinations of time (seven exposure durations, ranging from 3.5 to 480 min) and concentrations, Zwart et al. (1992; Zwart 1988) established a concentration-time–mortality relationship. The probit equation was -30.5 + 6.6 ln C + 5.5 ln T, where C = concentration and T = time. Using the relationship $C^n \times t = k$, the value of n was 1.2. Using all of the LC_{50} data from rat studies with exposure durations of 3.5 min to 8 h, the best fit of the concentration × time curve results in an n value of 1.23 (see Appendix A). Approximately the same value (1.3) is generated when the mouse data are graphed or when the mouse and rat data are graphed together (graph not shown). Because the mouse is slightly more susceptible than the rat, the graph line for mouse lethality data is parallel to, but just slightly below, the graph line for the rat lethality data.

From the studies summarized in Tables 5-3 to 5-5, it is apparent that there is a threshold concentration at which effects occur or fail to occur. For example, 90 ppm was a NOAEL for olfactory lesions in the rat, both during a 1-day and a 5-day repeat-exposure study, whereas a single exposure at 200 ppm produced such lesions (Hurtt et al. 1987). No neurotoxicity was observed in dogs exposed at 20 ppm for 7 h/day, 5 days/week for 6 weeks (Schaeffer 2002) or in rats exposed at 55 ppm for 7.5 h/day for 36 weeks (Anger et al. 1981). Concentrations of 90 and 175 ppm were the 5-day NOAEL and LOAEL, respectively, for nasal lesions in rats (Hurtt et al. 1988). Concentrations of 33 and 100 ppm were the chronic NOAEL and LOAEL for tissue lesions and neurotoxicity in mice (NTP 1992). There is also a time element. Both rats and mice withstood single exposures to methyl bromide at 140-300 ppm, but deaths occurred when these exposures were repeated (Ikeda et al. 1980; Kato et al. 1986; Hurtt et al. 1987; NTP 1992; Norris et al. 1993).

4.4.4. Concurrent Exposure Issues

No concurrent exposure issues were identified.

5. DATA ANALYSIS FOR AEGL-1

5.1. Summary of Human Data Relevant to AEGL-1

No reliable data relevant to derivation of AEGL-1 values were found. Methyl bromide is not detectable by odor or irritation at concentrations that are thresholds for tissue lesions or neurotoxicity. Lacrimation, an AEGL-1 level effect, was observed in rats exposed for 4 h to methyl bromide concentrations ≥338 ppm, with a no-effect level of 225 ppm (Japanese Ministry of Labour 1992). However, the no-effect level for lacrimation is not an appropriate basis for the derivation of AEGL-1 values, because signs of neurotoxicity (decreased locomotor activity and ataxia) were also observed at concentrations producing lacrimation. Furthermore, lacrimation was observed at methyl bromide concentrations above the point-of-departure used to derive AEGL-2 values (200 ppm). Therefore, derivation of AEGL-1 values would not be appropriate for methyl bromide, because it has no warning properties (e.g., odor or irritation) at concentrations below those that produce neurotoxicity. At one time, the American Conference of Governmental Industrial Hygienists had a threshold limit value-time weighted average (TLV-TWA) for methyl bromide of 20 ppm. The current ceiling standard of the Occupational Safety and Health Administration (OSHA) is 20 ppm (see Section 8.2).

5.2. Summary of Animal Data Relevant to AEGL-1

Only repeat-exposure studies were available on methyl bromide at low concentrations. Several examples of repeat-exposure studies are cited here. No

neurotoxic signs or tissue lesions were observed in dogs exposed at 20 ppm (the highest concentration tested), 7 h/day, 5 days/week for 6 weeks (Schaeffer 2002). In an 8-month study with rabbits, a particularly sensitive species, 27 ppm was the NOAEL for neurobehavioral effects (Russo et al. 1984). In a well-conducted carcinogenicity study with mice, 33 ppm was a NOAEL for any type of effect, including neurotoxicity, tissue lesions, carcinogenicity, and early mortality (NTP 1992). Exposures in that study were for 6 h/day, 5 days/week for 2 years. Finally, in developmental toxicity studies, 20 ppm was a NOAEL for both maternal toxicity and developmental effects in rats and rabbits (Breslin et al. 1990; Hardin et al. 1981).

5.3. Derivation of AEGL-1

Because methyl bromide is not detectable by odor or sensory irritation at concentrations below the AEGL-2 values (see below), AEGL-1 values were not derived. Absence of AEGL-1 values does not imply that exposures below the AEGL-2 values are without adverse effects.

6. DATA ANALYSIS FOR AEGL-2

6.1. Summary of Human Data Relevant to AEGL-2

No reliable human data that address the threshold for neurotoxicity or tissues lesions were available.

6.2. Summary of Animal Data Relevant to AEGL-2

In dogs, neurotoxicity, apparent as tremors, hunched appearance, and labored breathing, was not observed during the first 5 h of exposure to methyl bromide at 233 ppm, during the first 2 days of exposure at 156 ppm for 7 h, during the first day of exposure at 158 ppm, or after 4 daily 7-h exposures at 55 ppm (Newton 1994a). Because signs of neurotoxicity might be delayed, the 233-ppm exposure for 4 h could induce signs at a later time and, therefore, could be considered the threshold for neurotoxicity in dogs. A 4-h exposure at 200 or 225 ppm was a NOAEL for clinical signs in rats during one day, 4-h exposures (Hastings 1990; Japanese Ministry of Labour 1992). In 6-h studies, the NOAELs for neurotoxicity in rats were 200 ppm (Hurtt et al. 1988) and between 100 and 350 ppm (Driscoll and Hurley 1993). No clinical signs were observed in mice after exposure for 1 h at 224 ppm (Alexeeff et al. 1985) or after 4 h at 225 ppm (Japanese Ministry of Labour 1992). In most cases, the exposures were also NOAELs for tissue lesions; the exception being the reversible olfactory lesions in the rat. Because of the differences in the amount and placement of olfactory

epithelium in humans and rodents and the differences in air flow patterns, olfactory lesions in rats might not be relevant to humans.

The developmental study of methyl bromide in rabbits by Breslin et al. (1990) was not considered appropriate for developing AEGL-2 values. The decreased weight of fetuses born to does exposed to methyl bromide at 80 ppm is not considered the result of a single exposure. Furthermore, signs of maternal stress were observed at concentrations below those of other species, such as the rat (Hardin et al. 1981). The reason for the greater sensitivity of the rabbit is unknown.

6.3. Derivation of AEGL-2

For methyl bromide, neurotoxicity leading to an inability to escape is the most relevant end point for AEGL-2 values. Because of the steep dose-response curve for such effects, a NOAEL for neurotoxicity is the appropriate starting point for calculating AEGL-2 values. On the basis of data from three studies in rats (Hurtt et al. 1988; Hastings 1990; Japanese Ministry of Labour 1992) and one study in dogs (Newton 1994a), 200 ppm for 4 h was selected as the point-of-departure for derivation of AEGL-2 values. Studies in rats identified the following values as no-effect levels for neurotoxic effects for single exposures: 200 ppm for 6 h in 15 rats/group (Hurtt et al. 1988), 200 ppm for 4 h in 30 rats/group (Hastings 1990), and 225 ppm for 4 h in 20 rats/group (Japanese Ministry of Labour 1992). Thus, the database for rats is robust, with consistent results in all three studies. The study in dogs evaluated effects of a single 7-h exposure to methyl bromide in 1-2 dogs/group and of repeated exposures (7 h/day for 4 days) in 2-3 dogs/group (Newton 1994a). Results of the single exposure study in dogs did not identify a no-effect level for neurotoxicity. Signs of neurotoxicity were observed at all concentrations tested (≥233 ppm); however, no signs of neurotoxicity were observed during the first 5 h of exposure at 233 ppm. Results of the repeated-exposure study in dogs (2-3/group) showed the following: no effects at 55 ppm, clinical signs of neurotoxicity on day 3 of exposure at 156 ppm, and severe signs of neurotoxicity on the second day of exposure at 268 ppm. A small number of dogs were tested, so the rat data were considered a stronger basis for selecting a NOAEL for neurotoxicity. The dog data support the selection of 200 ppm for a single 4-h exposure in rats as the point-of-departure.

Because uptake of methyl bromide is greater in rodents than in humans (based on comparative respiratory rates and comparisons with methyl chloride) and because GST concentrations in rodents are greater than in humans (allowing more rapid production of toxic metabolites), an interspecies uncertainty factor of 1 was applied. Although humans differ in their capacity to metabolize methyl bromide, the difference is considered to be less than 3-fold from a toxicologic stand point (Nolan et al. 1985). An intraspecies uncertainty factor of 3 was applied. The resulting 4-h value of 67 ppm was time scaled to the other exposure

durations using the equation $C^n \times t = k$, with n = 1.2. Because the time-scaled 8-h value of 37 ppm is close to the chronic NOAEL of 33 ppm for mice (NTP 1992) and is less than the 5-day NOAEL of 55 ppm for clinical signs and tissue lesions in dogs (Newton 1994a) and the 36-week NOAEL of 55 ppm for neurobehavioral parameters and nerve conduction velocity in rats (Anger et al. 1981), the 8-h value was set equal to the 4-h value. AEGL-2 values are presented in Table 5-7, and the calculations are shown in Appendix B. A category graph of AEGL values in relation to animal toxicity data is provided in Appendix C.

7. DATA ANALYSIS FOR AEGL-3

7.1. Summary of Human Data Relevant to AEGL-3

ATSDR (1992) estimates that the concentrations of methyl bromide that lead to death in humans range from 1,600 to 60,000 ppm, depending on the duration of exposure. These estimates are based on older studies with concentrations that were either estimated or measured with techniques of limited sensitivity. Although the human data are not robust, the values can be considered as support for values derived from recent, well-conducted animal studies.

7.2. Summary of Animal Data Relevant to AEGL-3

Data relevant to deriving AEGL-3 values for methyl bromide were available from studies in the rat and mouse. The highest nonlethal concentrations and exposure durations for methyl bromide in the rat were 700 ppm for 4 h (Kato et al. 1986) and 268 ppm for 8 h (Honma et al. 1985). For the mouse, the highest nonlethal concentrations were 900 ppm for 1 h (Alexeeff et al. 1985) and 312 ppm for 4 h (Yamano 1991). For each species, data from different laboratories provide a good fit to a dose-response curve with a time-scaling exponent of 1.2. On the basis of 30-min and 4-h LC_{50} values (Bakhishev 1975; Kato et al. 1986; Yamano 1991), it appears that the mouse is approximately two-fold more sensitive to methyl bromide than the rat. This greater sensitivity might be related to the higher concentrations of GST in mouse tissues (Griem et al. 2002) and a higher respiratory rate. The mouse was also more sensitive than rats to the related chemical, methyl chloride (see Chapter 6).

7.3. Derivation of AEGL-3

Dividing the highest nonlethal values by any of the commonly used combination of interspecies and intraspecies uncertainty factors of 100, 30, or 10 results in values that are close to the ACGIH TLV-TWA values (currently 1 ppm, but up to 20 ppm in previous years) in the first two cases and below the AEGL-2 values in the latter case.

TABLE 5-7 AEGL-2 Values for Methyl Bromide

10 min	30 min	1 h	4 h	8 h
940 ppm (3,657 mg/m^3)	380 ppm (1,478 mg/m^3)	210 ppm (817 mg/m^3)	67 ppm (261 mg/m^3)	67 ppm (261 mg/m^3)

Because of differences in methyl halide metabolism between mice and other rodents and because of the greater sensitivity of mice to the structurally-similar chemical methyl chloride (metabolism is by the same GSH conjugation pathway), the mouse was not considered an appropriate model for deriving AEGL values for methyl bromide. The AEGL-3 values were based on the $BMCL_{05}$ (benchmark concentration, 95% lower confidence limit with 5% response) of 701 ppm, calculated using data from a 4-h exposure study in rats (Kato et al. 1986). That concentration was also the highest nonlethal value in the study. As in the derivation of AEGL-2 values, uncertainty factors of 1 and 3 were applied to adjust for interspecies and intraspecies differences, respectively. Time scaling was conducted using the equation $C^n \times t = k$, with n= 1.2, based on lethality data in the rat. Because uptake of methyl bromide is greater in rodents than in humans (based on comparative respiratory rates and comparisons with methyl chloride) and because GST concentrations are higher in rodents than in humans (resulting in more rapid production of toxic metabolites), an interspecies uncertainty factor of 1 was considered sufficient. Humans differ in their capacity to metabolize methyl bromide, but the difference is not considered to be greater than 3-fold from a toxicologic stand point (Nolan et al. 1985). In addition, use of an intraspecies uncertainty factor of 3 is supported by the steep dose-response curve for lethality, which indicates little intraspecies variation (see Chapter 6). Furthermore, larger reduction of the AEGL-3 values would result in values close to the AEGL-2 values. Therefore, an intraspecies uncertainty factor of 3 was considered sufficient. The AEGL-3 values are presented in Table 5-8. The 8-h AEGL-3 value of 130 ppm is supported by repeat-exposure studies, in which dogs exposed to methyl bromide at 156 or 158 ppm for 7 h/day did not exhibit severe clinical signs until the second or third day of exposure (Newton 1994a,b). There were no remarkable histopathologic lesions in the dogs at necropsy after a 4-day exposure. Cerebellar lesions were observed in dogs that were first exposed to methyl bromide at 10 ppm for 7 h/day for 4 weeks, and then exposed at 158 ppm for 6 days.

8. SUMMARY OF AEGLS

8.1. AEGL Values and Toxicity End Points

AEGL values for methyl bromide are presented in Table 5-9, and derivation summaries are provided in Appendix D.

TABLE 5-8 AEGL-3 Values for Methyl Bromide

10 min	30 min	1 h	4 h	8 h
3,300 ppm (12,837 mg/m^3)	1,300 ppm (5,057 mg/m^3)	740 ppm (2,879 mg/m^3)	230 ppm (895 mg/m^3)	130 ppm (506 mg/m^3)

TABLE 5-9 Summary of AEGL Values for Methyl Bromide

Classification	10 min	30 min	1 h	4 h	8 h
AEGL-1 (nondisabling)	NR[a]	NR[a]	NR[a]	NR[a]	NR[a]
AEGL-2 (disabling)	940 ppm (3,657 mg/m^3)	380 ppm (1,478 mg/m^3)	210 ppm (817 mg/m^3)	67 ppm (261 mg/m^3)	67 ppm (261 mg/m^3)
AEGL-3 (lethal)	3,300 ppm (12,837 mg/m^3)	1,300 ppm (5,057 mg/m^3)	740 ppm (2,879 mg/m^3)	230 ppm (895 mg/m^3)	130 ppm (506 mg/m^3)

[a]Numerical values are not recommended because the data indicate that toxic effects might occur below the odor threshold for methyl bromide. Absence of an AEGL-1 value does not imply that exposure below the AEGL-2 value is without adverse effects.
Abbreviations: NR, not recommended.

8.2. Comparison with Other Standards and Guidelines

Guidelines and standards for methyl bromide are presented in Table 5-10. The American Industrial Hygienists Association (AIHA 1997) did not establish a level 1 Emergency Response Planning Guideline (ERPG-1) for methyl bromide because the chemical is not detectable by odor or irritation at concentration below its level 2 guideline (ERPG-2). AEGL-1 values were not derived for the same reason. The 1-h ERPG-2 of 50 ppm is based on animal and human data that suggest no significant respiratory irritation or CNS dysfunction at that concentration. The 1-h ERPG-3 of 200 ppm is based on the observation of Clarke et al. (1945) that exposures of relatively short duration (≤2 h), which might have been as low as 250 ppm, have been lethal to animals and humans. However, the observations of Clarke et al. are based on older, poorly cited sources. The AEGL-2 and AEGL-3 values are larger than the corresponding ERPG values and are based on more recent animal data.

The immediately dangerous to life or health (IDLH) standard established by the National Institute for Occupational Safety and Health (NIOSH 1994) is comparable to the corresponding AEGL-2 value. The IDLH of 250 ppm is based on acute inhalation toxicity data from Clarke et al. (1945), which indicated that methyl bromide at 220 ppm can be endured for several hours without serious effects. NIOSH acknowledges that 250 ppm might be a conservative value because there are no data for workers exposed above 250 ppm.

From 1948-1962, the TLV-TWA for methyl bromide was 20 ppm (ACGIH 2004). A skin notation was added in 1961. The TLV-TWA was reduced to 15 ppm in 1973. That standard was subsequently reduced in 1979 to 5 ppm, and a short-term exposure limit (STEL) of 15 ppm was established. In 1996, based on uncertain results in the Reuzel et al. (1991) study, the TLV-TWA was lowered to 1 ppm. The 1 ppm concentration protects against mild irritation of the nasal mucosa. ACGIH notes that extensive experience in occupational exposures did not indicate adverse health effects at the previous TLV-TWA of 5 ppm.

TABLE 5-10 Extant Standards and Guidelines for Methyl Bromide

	Exposure Duration				
Guideline	10 min	30 min	1 h	4 h	8 h
AEGL-1	NR[a]	NR[a]	NR[a]	NR[a]	NR[a]
AEGL-2	940 ppm	380 ppm	210 ppm	67 ppm	67 ppm
AEGL-3	3,300 ppm	1,300 ppm	740 ppm	230 ppm	130 ppm
ERPG-1 (AIHA)[b]			NA		
ERPG-2 (AIHA)			50 ppm		
ERPG-3 (AIHA)			200 ppm		
IDLH (NIOSH)[c]		250 ppm			
TLV-TWA (ACGIH)[d]					1 ppm (skin)[e]
REL-TWA (NIOSH)[f]					Lowest feasible concentration
PEL-TWA (OSHA)[g]					None
PEL-C (OSHA)[h]					20 ppm (skin)[e]
MAK (Germany)[i]					Skin[e]
MAC (The Netherlands)[j]					0.3 ppm

[a]Numerical values are not recommended because the data indicate that toxic effects might occur below the odor threshold for methyl bromide. Absence of an AEGL-1 value does not imply that exposure below the AEGL-2 value is without adverse effects.
[b]ERPG (emergency response planning guidelines, American Industrial Hygiene Association (AIHA 1997).
The ERPG-1 is the maximum airborne concentration below which it is believed nearly all individuals could be exposed for up to 1h 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 irreversi-

ble or other serious health effects or symptoms that could impair an individual's ability to take protection 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.
[c]IDLH (immediately dangerous to life or health, National Institute for Occupational Safety and Health) (NIOSH 1994) originally represented the maximum concentration from which one could escape within 30 min without any escape-impairing symptoms or any irreversible health effects in the event that protective respiratory equipment failed. NIOSH is currently assessing the diverse uses of IDLHs and determining whether the original criteria used to derive the IDLH values are valid, or if other criteria should be used. Currently, NIOSH considers an IDLH exposure condition as one "that poses a threat of exposure to airborne contaminants when that exposure is likely to cause death or immediate or delayed permanent adverse health effects or prevent escape from such an environment." The original purpose of the IDLH remains (to ensure worker escape in the event of protective respiratory-equipment failure). The IDLH of 250-ppm is based on acute inhalation toxicity data in humans (Clarke et al. 1945). However, the value is considered possibly "conservative" because there was a lack of relevant acute toxicity data for workers exposed at concentrations above 220 ppm.
[d]TLV-TWA (threshold limit value - time weighted average, American Conference of Governmental Industrial Hygienists) (ACGIH 2004) 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. Certain sensitive populations might not be adequately protected from adverse health effects at or below this concentration. This TLV differs from the ceiling of 20 ppm established by OSHA because of ACGIH considered the potential carcinogenicity methyl bromide, its capacity to be absorbed through the skin, its marked neurotoxicity, and its significant nasal and dermal irritation, which warrant a greater degree of caution and a reduction in the previously recommended TLV for occupational exposure. The classification of A4 indicates that methyl bromide is considered "not classifiable as a human carcinogen." Data were insufficient to recommend a TLV-STEL for methyl bromide.
[e]Skin notation indicates the danger of cutaneous absorption.
[f]REL-TWA (recommended exposure limit - time weighted average, National Institute for Occupational Safety and Health). There is no REL-TWA for methyl bromide; NIOSH considers methyl bromide a potential occupational carcinogen, as defined by the OSHA carcinogen policy [29 CFR 1990 [1980].[g] PEL-TWA (Permissible Exposure Limits - Time Weighted Average, Occupational Safety and Health Administration) (NIOSH 2010 is defined analogous to the ACGIH TLV-TWA, but is for exposures of no more than 10 h/day, 40 h/week.
[h]PEL-C (permissible exposure limit-ceiling) (NIOSH 2010) is a value that must not be exceeded during any part of the workday. The PEL-TWA of 5 ppm established by OSHA in 1989 was vacated in 1993, and the ceiling limit of 20 ppm, with a skin notation, has been retained.
[i]MAK (maximale arbeitsplatzkonzentration [maximum workplace concentration]) (Deutsche Forschungsgemeinschaft [German Research Association] (DFG 1999) is analogous to the ACGIH TLV-TWA, but there is no current MAK value for methyl bromide; there is a skin notation and it is considered a Group IIIB substance ("justifiably suspected of having carcinogenic potential") because of its potential carcinogenicity.

[j]MAC (maximaal aanvaarde concentratie [maximal accepted concentration]) (Dutch Expert Committee for Occupational Standards, The Netherlands (MSZW 2004) is defined analogous to the ACGIH TLV-TWA.
Abbreviations: NA, not appropriate; NR, not recommended.

8.3. Data Adequacy and Research Needs

Data on human exposures to known concentrations of methyl bromide are lacking. The database of animal studies is robust, containing information on multiple species (dog, rat, mouse, rabbit, and guinea pig) and involving acute and longer-term exposure studies that address lethal and sublethal effects, reproductive and developmental toxicity, genotoxicity, and chronic toxicity and carcinogenicity. Information on metabolism and mechanism of action are supported by studies, including clinical studies, with the related chemical, methyl chloride.

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APPENDIX A

TIME-SCALING CALCULATION FOR METHYL BROMIDE

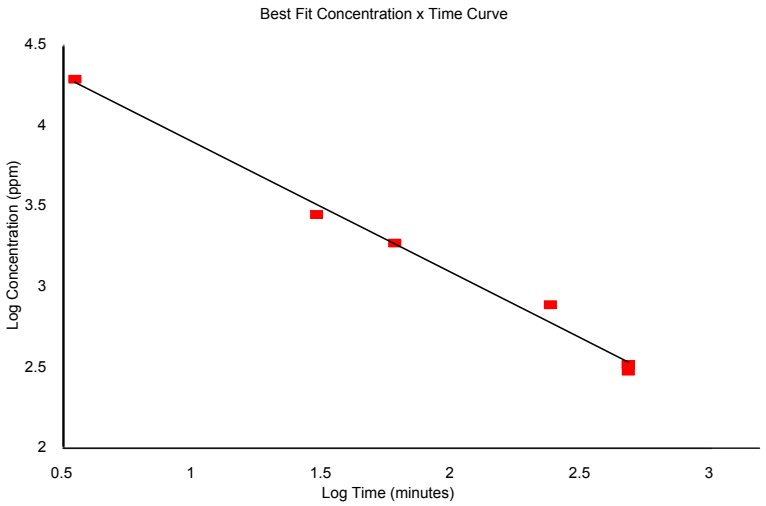

FIGURE A-1 Regression line of LC_{50} data in rats. Source: Data from Bakhishev 1973; Honma et al. 1985; Kato et al. 1986; Zwart 1988).

Data:

Time (min)	Concentration (ppm)	Log time	Log concentration
3.5	19,460	0.5441	4.2891
30	2,830	1.4771	3.4518
60	1,880	1.7782	3.2742
240	780	2.3802	2.8921
480	302	2.6812	2.4800
480	334	2.6812	2.5237

Regression Output:

Intercept	4.7129
Slope	-0.8115
R Squared	0.9914
Correlation	-0.9957
Degrees of Freedom	4
Observations	6

n = 1.23
k = 642,119

APPENDIX B

DERIVATION OF AEGL VALUES FOR METHYL BROMIDE

Derivation of AEGL-1 Values

AEGL-1 values are not recommended because toxic effects might occur below the odor threshold for methyl bromide. Absence of AEGL-1 values does not imply that exposures below the AEGL-2 values are without adverse effects.

Derivation of AEGL-2 Values

Key studies:	Hurtt, M.E., D.A. Thomas, P.K. Working, T.M. Monticello, and K.T. Morgan. 1988. Degeneration and regeneration of the olfactory epithelium following inhalation exposure to methyl bromide: pathology, cell kinetics, and olfactory function. Toxicol. Appl. Pharmacol. 94(2):311-328. Hastings, L. 1990. Sensory neurotoxicology: Use of the olfactory system in the assessment of toxicity. Neurotoxicol. Teratol. 12(5):455-459. Japanese Ministry of Labour. 1992. Toxicology and Carcinogenesis Studies of Methyl Bromide in F344 Rat and BDF Mice (Inhalation Studies). Industrial Safety and Health Association, Japanese Bioassay Laboratory, Tokyo (as cited in IPCS 1995). Newton, P.E. 1994a. An Up-and-Down Acute Inhalation Toxicity Study of Methyl Bromide in the Dog. Study No. 93-6067. Pharmaco LSR, East Millstone, NJ.
Toxicity end points:	Clinical signs of neurotoxicity, NOAEL is 200 ppm for 4 h
Time scaling:	$C^{1.2} \times t = k$, based on rat lethality data $k = (200 \text{ ppm} \div 3)^{1.2} \times 240 \text{ min}$ $k = 37{,}059.7 \text{ ppm-min}$
Uncertainty factors:	1 for interspecies differences 3 for intraspecies differences

Modifying factor: Not applied

Calculations:

10-min AEGL-2: $C^{1.2}$ = 37,059.7 ppm-min ÷ 10 min
C = 940 ppm

30-min AEGL-2: $C^{1.2}$ = 37,059.7 ppm-min ÷ 30 min
C = 380 ppm

1-h AEGL-2: $C^{1.2}$ = 37,059.7 ppm-min ÷ 60 min
C = 210 ppm

4-h AEGL-2: $C^{1.2}$ = 37,059.7 ppm-min ÷ 240 min
C = 67 ppm

8-h AEGL-2: Set equal to the 4-h AEGL-2 of 67 ppm (If based on a chronic NOAEL of 33 ppm for mice (NTP 1992), a 4-day NOAEL of 55 ppm for clinical signs and tissue lesions in dogs (Newton 1994a), and the 36-week NOAEL of 55 ppm for neurobehavioral parameters and nerve conduction velocity in rats (Anger et al. 1981), the time-scaled value would be 37 ppm.)

Derivation of AEGL-3 Values

Key study: Kato, N., S. Morinobu, and S. Ishizu. 1986. Subacute inhalation experiment for methyl bromide in rats. Ind. Health 24(2):87-103.

Toxicity end point: $BMCL_{05}$ of 701 ppm in the rat

Time scaling: $C^{1.2} \times t = k$, based on rat lethality data.
$k = (701 \text{ ppm} \div 3)^{1.2} \times 240 \text{ min}$
k = 166,927.3 ppm-min

Uncertainty factors: 1 for interspecies differences
3 for intraspecies variability

Modifying factor: Not applied

Calculations:

10-min AEGL-3: $C^{1.2}$ = 166,927.3 ppm-min ÷ 10 min
C = 3,300 ppm

30-min AEGL-3:	C = 166,927.3 ppm-min ÷ 30 min C = 1,300 ppm
1-h AEGL-3	C = 166,927.3 ppm-min ÷ 60 min C = 740 ppm
4-h AEGL-3:	C = 166,927.3 ppm-min ÷ 240 min C = 230 ppm
8-h AEGL-3:	C = 166,927.3 ppm-min ÷ 480 min C = 130 ppm

APPENDIX C

CATEGORY GRAPH OF TOXICITY DATA AND AEGL VALUES FOR METHYL BROMIDE

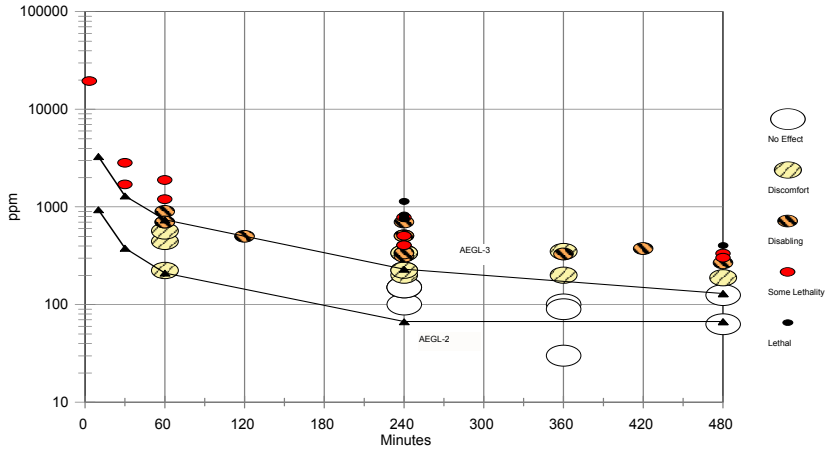

FIGURE C-1 Category graph of toxicity data on methyl bromide compared with AEGL values. All of the toxicity data pertain to laboratory animals; no clinical data were available.

APPENDIX D

ACUTE EXPOSURE GUIDELINE LEVELS FOR METHYL BROMIDE

Derivation Summary for Methyl Bromide

AEGL-1 VALUES

AEGL-1 values are not recommended because toxic effects might occur below the odor threshold for methyl bromide. Absence of AEGL-1 values does not imply that exposures below the AEGL-2 values are without adverse effects.

AEGL-2 VALUES

10 min	30 min	1 h	4 h	8 h
940 ppm	380 ppm	210 ppm	67 ppm	67 pm

Key references:
(1) Hurtt, M.E., D.A. Thomas, P.K. Working, T.M. Monticello, and K.T. Morgan. 1988. Degeneration and regeneration of the olfactory epithelium following inhalation exposure to methyl bromide: Pathology, cell kinetics, and olfactory function. Toxicol. Appl. Pharmacol. 94(2):311-328.
(2) Hastings, L. 1990. Sensory neurotoxicology: Use of the olfactory system in the assessment of toxicity. Neurotoxicol. Teratol. 12(5):455-459.
(3) Japanese Ministry of Labour. 1992. Toxicology and Carcinogenesis Studies of Methyl Bromide in F344 Rat and BDF Mice (Inhalation Studies). Industrial Safety and Health Association, Japanese Bioassay Laboratory, Tokyo (as cited in IPCS 1995).
(4) Newton, P.E. 1994a. An Up-and-Down Acute Inhalation Toxicity Study of Methyl Bromide in the Dog. Study No. 93-6067. Pharmaco LSR, East Millstone, NJ.

Test species/Strain/Number: (1) dog/beagle/1 (with support from higher and lower exposures; (2) rat/not specified/30; (3) rat/F-344/10 male and 10 female; and (4) rat/male F-344/15

Exposure route/Concentrations/Durations: Inhalation, (1) 233 ppm for 5 h; (2) 200 ppm for 4 h; (3) 225 ppm for 4 h; and (4) 200 ppm for 6 h

Effects:
(1) No toxic signs or brain lesions; (2) no clinical signs; (3) reversible metaplasia of the olfactory epithelium; and (4) no clinical signs, reversible olfactory epithelium degeneration.

End point/Concentration/Rationale: Threshold for neurotoxic signs is 200 ppm for 4 h. Neurotoxicity (e.g., ataxia) would inhibit the ability to escape. Olfactory lesions were considered specific to the rat.

(Continued)

AEGL-2 VALUES Continued

10 min	30 min	1 h	4 h	8 h
940 ppm	380 ppm	210 ppm	67 ppm	67 pm

Uncertainty factors/Rationale:
Total uncertainty factor: 3
Interspecies: 1, based on studies with methyl chloride, uptake is greater in rodents than in humans; GST concentrations are greater in rodents than humans, resulting in faster production of toxic metabolites.
Intraspecies: 3, differences in metabolism among humans are no greater than 3-fold (Nolan et al. 1985).

Modifying factor: Not applied

Animal-to-human dosimetric adjustment: Not applied

Time scaling: $C^{1.2} \times t = k$, based on lethality studies with the rat.

Data adequacy: Although there are no controlled clinical studies, the database of experimental animal studies is robust. Studies of dogs exposed to methyl bromide at 156 ppm (no clinical signs for first 2 days) or 268 ppm (severe signs during first day) indicate that 200 ppm for 4 h would be near the threshold for neurotoxicity in dogs (Newton 1994a).

AEGL-3 VALUES

10 min	30 min	1 h	4 h	8 h
3,300 ppm	1,300 ppm	740 ppm	230 ppm	130 ppm

Key Reference: Kato, N., S. Morinobu, and S. Ishizu. 1986. Subacute inhalation experiment for methyl bromide in rats. Ind. Health 24(2):87-103.

Test species/Strain/Number: Male rat/Sprague-Dawley/5 or 10

Exposure route/Concentrations/Durations: Inhalation at 502, 622, 667, 701, 767, 808, 832, or 896 ppm for 4 h

Effects: clinical signs were not described

502 ppm	no morality
622 ppm	no mortality
667 ppm	no mortality
701 ppm	no mortality
767 ppm	30% mortality
799 ppm	60% mortality
808 ppm	70% mortality
817 ppm	80% mortality
832 ppm	100% mortality
896 ppm	100% mortality

LC_{50} = 780 ppm (95% confidence limits of 760-810 ppm)
LC_{01} = 701 ppm
$BMCL_{05}$ = 701 ppm

(Continued)

AEGL-3 VALUES Continued

10 min	30 min	1 h	4 h	8 h
3,300 ppm	1,300 ppm	740 ppm	230 ppm	130 ppm

End point/Concentration/Rationale: BMCL05, 701 ppm, threshold for lethality

Uncertainty factors/Rationale:
Total uncertainty factor: 3
Interspecies: 1, based on studies with methyl chloride, uptake is greater in rodents than in humans; GST concentrations are greater in rodents than humans, resulting in faster production of toxic metabolites.
Intraspecies: 3, differences in metabolism among humans are no greater than 3-fold (Nolan et al. 1985). Use of an intraspecies uncertainty factor of 3 is supported by the steep dose-response curve for lethality which indicates that there might be little intraspecies variation (see Chapter 6). Furthermore, larger reduction of the AEGL-3 values would result in values near the AEGL-2 values.

Modifying factor: Not applied

Animal-to-human dosimetric adjustment: Not applied

Time scaling: $C^{1.2} \times t = k$, based on several lethality studies with the rat

Data adequacy: Although reliable human data are lacking, the database of animal studies is robust, consisting of acute, repeat-exposure, subchronic, chronic, neurotoxicity, genotoxicity, and carcinogenicity studies.

6

Methyl Chloride[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 are defined as follows:

AEGL-1 is the airborne concentration (expressed as parts per million or milligrams per cubic meter [ppm or 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 (Summitec Corporation), Julie M. Klotzbach (Syracuse Research Corporation), Chemical Manager George Rodgers (National Advisory Committee [NAC] on Acute Exposure Guideline Levels for Hazardous Substances), and Ernest V. Falke (U.S. Environmental Protection Agency). 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 guidelines reports (NRC 1993, 2001).

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 concentrations 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 AEGL values represent threshold concentrations 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

Methyl chloride is a substantially odorless, colorless gas with moderate flammability and explosiveness. Most methyl chloride produced today is used as a chemical intermediate in the production of silicones, agricultural chemicals, methyl cellulose, quaternary amines, butyl rubber, and tetraethyl lead. Previous use in refrigeration systems led to accidental exposures and, in some cases, deaths. In the late 1880s, methyl chloride had limited use as a general and local anesthetic. Data on toxicity to humans were available from accidental exposures, occupational exposures, and clinical studies. Animal studies, primarily with the rat and mouse, generally used a repeat-exposure scenario. Data were available on lethal and sublethal concentrations, neurotoxicity, developmental and reproductive effects, genotoxicity, and carcinogenicity. Metabolism is rapid. The human and animal studies document the central nervous system as the target of acute and chronic exposures. In animal studies, other organs such as the kidneys and testes have been affected by repeat exposures.

Clinical studies show that single exposures of healthy adults to methyl chloride at 200 ppm for 3 or 3.5 h (Putz-Anderson et al. 1981a,b) and a two-day repeat exposure of exercising adults exposed at 150 ppm for 7.5 h/day (Stewart et al. 1980) are without adverse neurotoxic effects. The subjects included both "fast" and "slow" metabolizers of methyl chloride. These exposures failed to elicit physiologic, neurologic, behavioral, or clinical symptoms. Furthermore, in

the absence of a clearly defined odor at these concentrations, the subjects were unable to differentiate between control and exposure days. None of the exposures produced mild, transient effects that define the AEGL-1 values. Because methyl chloride has no clearly defined odor or warning properties at concentrations that might be neurotoxic, an AEGL-1 is not recommended.

The AEGL-2 values were based on several studies with rats; a monitoring study was used as support. The basis for the AEGL-2 values was the absence of clinical signs in rats exposed at 1,500 ppm for 6 h/day for 1 day (Dodd et al. 1982) or 90 days (Mitchell et al. 1979). Because of the greater blood uptake of chemicals by rodents than humans (Landry et al. 1981, 1983; Nolan et al. 1985), an interspecies uncertainty factor of 1 was applied. Although humans differ in the rate at which they metabolize methyl chloride, the difference does not appear to be toxicologically significant (Nolan et al. 1985). Because of differences in uptake and metabolism among the human population, an intraspecies uncertainty factor of 3 was considered sufficient. Time scaling was performed using the equation $C^n \times t = k$, using the default values of n = 3 for shorter durations and n = 1 for longer durations. Because of the long exposure duration of the key study, the 10-min value was set equal to the 30-min value. In a monitoring study, accidental exposures at 1,000-2,000 ppm and repeated exposure at 2,000-4,000 ppm resulted in transient symptoms of blurred vision, dizziness, headache, and nausea in workers (MacDonald 1964). Exposure durations were not reported, but appeared to be throughout the workday. Application of an intraspecies uncertainty factor of 3 to 1,500 ppm, the mean concentration of methyl chloride in the occupational monitoring studies, results in 500 ppm, a value similar to the 4- and 8-h AEGL-2 values.

The only lethality data were 50% lethality (LC_{50}) values for the mouse, a particularly sensitive species. Two studies reported no deaths in rats during the first 4 days of exposures to methyl chloride at 5,000 ppm for 6 h/day (Morgan et al. 1982; Chellman et al. 1986a). A single 6-h exposure at 5,000 ppm was selected as the point-of-departure for the threshold for lethality. Interspecies and intraspecies uncertainty factors of 1 and 3, respectively, were applied as was done in the calculation for AEGL-2 values. Time scaling was performed using the equation $C^n \times t = k$, using n = 3 for shorter durations and n = 1 for longer durations. Because of the long exposure duration of the key study, the 10-min AEGL-3 was set equal to the 30-min value.

The AEGL values for methyl chloride are presented in the Table 6-1.

1. INTRODUCTION

Methyl chloride is a substantially odorless, colorless gas with moderate flammability and explosiveness. Additional chemical and physical properties are listed in Table 6-2. At “high concentrations” it has a mild ethereal odor and sweet taste. Methyl chloride is ubiquitous in the environment because it is produced by wood burning and is released by natural organic processes, such as

microbial fermentation. Most industrially-produced methyl chloride is used as a chemical intermediate. The primary use is in the manufacture of silicones (72%); other products in which it is used as an intermediate include agricultural chemicals, methyl cellulose, quaternary amines, butyl rubber, and tetraethyl lead. Previously, it was used as a refrigerant and as an agricultural pesticide or fumigant (ATSDR 1998; O'Neil et al. 2001; Reid 2001). It had limited use as a general and local anesthetic in the late 1800s. It comprised 16% of the anesthetic "Somnoform" (Henderson 1930). Skin contact with the liquid may cause frost-bite (DOT 1985).

Major production methods of methyl chloride involve the reaction of methanol and hydrogen chloride or the chlorination of methane (Holbrook 1992). Production in the United States was 920 million pounds in 1994 (CMR 1995). Methyl chloride (99.5-99.9% purity) is marketed as a liquefied gas under pressure (WHO 2000).

2. HUMAN TOXICITY DATA

The most important route of exposure to methyl chloride in humans is via the respiratory tract. Reported human exposures have primarily been the result of its use as a refrigerant gas and as a blowing agent for plastic foams. Early published reports of acute intoxications involved leaks in domestic refrigerators and overexposures of industrial workers. Uses as a refrigeration gas and as a blowing agent for plastic foams have been discontinued.

TABLE 6-1 Summary of AEGL Values for Methyl Chloride

Classification	10 min	30 min	1 h	4 h	8 h	End Point (Reference)
AEGL-1 (nondisabling)	NR[a]	NR[a]	NR[a]	NR[a]	NR[a]	
AEGL-2 (disabling)	1,100 ppm (2,277 mg/m^3)	1,100 ppm (2,277 mg/m^3)	910 ppm (1,884 mg/m^3)	570 ppm (1,180 mg/m^3)	380 ppm (787 mg/m^3)	NOAEL for clinical signs, tissue lesions in rats (Mitchell et al. 1979; Dodd et al. 1982)
AEGL-3 (lethal)	3,800 ppm (7,866 mg/m^3)	3,800 ppm (7,866 mg/m^3)	3,000 ppm (6,210 mg/m^3)	1,900 ppm (3,933 mg/m^3)	1,300 ppm (2,691 mg/m^3)	Threshold for lethality in rats (Morgan et al. 1982; Chellman et al. 1986a)

[a]AEGL-1 values are not recommended because methyl chloride has no odor or warning properties at concentrations that may be neurotoxic.
Abbreviations: NR, not recommended; NOAEL, no-observed-adverse-effect level.

The central nervous system (CNS) is the primary target of methyl chloride, with behavioral symptoms and neurologic effects resulting from both acute and chronic exposures. Overexposures can result in loss of equilibrium, dizziness, semiconsciousness, and delayed death. Case histories show that acute exposures at high concentrations and chronic exposures to moderately high concentrations result in degeneration of portions of the CNS. Symptoms include headache, confusion, ataxia, muscle weakness, and tremor. Gastrointestinal disturbances may also occur, but there is no effect on pulmonary function. Recovery may be protracted. Renal, hepatic, cardiovascular, gastrointestinal, and other complications also have been documented (Repko and Lasley 1979). Data on the toxicity of methyl chloride have been reviewed by the Agency for Toxic Substances and Disease Registry (ATSDR 1998), the International Agency for the Research on Cancer (IARC 1999), the World Health Organization (WHO 2000), and the Hazardous Substances Databank (HSDB 2005).

Although mortalities have been reported as a result of accidental overexposure to methyl chloride, no information was available on measured concentrations.

2.2. Nonlethal Toxicity

Methyl chloride is considered nonirritating to the eyes, nose, and throat; however, the liquid can cause frostbite (DOT 1985).

TABLE 6-2 Chemical and Physical Properties of Methyl Chloride

Parameter	Value	Reference
Synonyms	Chloromethane, monochloromethane	O'Neil et al. 2001
CAS registry no.	74-87-3	O'Neil et al. 2001
Chemical formula	CH_3Cl	O'Neil et al. 2001
Molecular weight	50.49	O'Neil et al. 2001
Physical state	Colorless gas	O'Neil et al. 2001
Melting point	-97.7°C	O'Neil et al. 2001
Boiling point	-23.7°C	O'Neil et al. 2001
Density		Holbrook 1992
Vapor	2.3 g/L at 0°C, 1 atm (air = 1)	
Liquid	0.9 g/mL at 20/4°C (water = 1)	
Solubility in water	4.8 g/L at 25°C	O'Neil et al. 2001
Vapor pressure	3670 mm Hg at 20°C	Holbrook 1992
Flammability limits	Flammable; 8.1-17.2%	DOT 1985
Conversion factors	1 ppm = 2.07 mg/m^3 1 mg/m^3 = 0.483 ppm	ACGIH 2003

2.1. Acute Lethality

2.2.1. Odor Threshold and Awareness

Data on the odor and irritation thresholds of methyl chloride are conflicting. The odor threshold has been reported at 10 ppm (Billings and Jonas 1981; Ruth 1986), and an irritation threshold was reported in a literature review as approximately 500 ppm (Ruth 1986). However, the specific source of the odor and irritation thresholds was not reported. In well-conducted clinical studies with male and female subjects, odor was not clearly perceived at concentrations of 150 ppm (Stewart et al. 1980) or 200 ppm (Putz-Anderson et al. 1981a). Several reviews, including one by Repko and Lasley (1979), state that methyl chloride is undetectable at concentrations that are dangerous to breathe. The odor is described as ethereal or sweet (Reid 2001).

2.2.2. Case Reports

Numerous case reports of exposure to methyl chloride as a result of refrigeration losses or industrial leaks have been reported. A few examples are cited here. Symptoms of fatigue, drowsiness, staggered walk, headache, blurred vision, mental confusion, vertigo, muscular cramping and rigidity, and tremor may be preceded by a latent period of 1-4 h. Depending on the severity of the exposure, symptoms may persist for several months, and personality changes, such as depression, may develop (ATSDR 1998).

MacDonald (1964) described nine case reports of employees at a synthetic-rubber plant where he was the medical supervisor. Where concentrations were noted in the work area, measurements were taken by gas chromatography or, in one case, by a Riken indicator, which gives immediate indication of the presence of methyl chloride at concentrations up to 10,000 ppm. In some cases exposures continued for several days before employees reported to the medical department. The cases were reported in short paragraphs, and no further details on exposure durations were reported. In the first case, an employee experienced vision disturbance, headache, dizziness, nausea, and staggering for several days prior to reporting to the medical department. Concentrations of methyl chloride in his work area was <25-1,600 ppm. Symptoms disappeared slowly, and he returned to work after 36 days. Two other employees working in the same area had similar symptoms, but medical examinations were normal. Apprehension and depression occurred for some time following the exposures.

A fourth employee neglected to wear a mask in an area where concentrations of methyl chloride were known to be 1,000-2,000 ppm. He experienced dizziness, blurred vision, headache, nausea, and vomiting. The exposure duration could not be quantified. The symptoms cleared quickly and he returned to work the next day. A second exposure one year later, although considered more moderate, resulted in more persistent symptoms. After a methyl chloride spill, a

fifth employee reported symptoms similar to those described above, and he appeared to be euphoric. He completed his work shift, but reported to the medical department the next day with persistent symptoms. He returned to work 2 weeks later. Over the next 10 years he experienced occasional periods of dizziness and headaches, which he attributed to mild overexposures. Concentrations in his work area rarely exceeded 100 ppm.

Following another accidental spill, an employee repeatedly entered and left an area that had methyl chloride in excess of 10,000 ppm (Riken indicator). Although he experienced symptoms of blurred vision, dizziness, and sight headache, he did not report to the medical department. At another time, he worked in an area with a leak that was not controlled for 13 days. Monitoring data showed concentrations of methyl chloride at 2,000-4,000 ppm. During the first week, the employee slept for long periods; the following week he experienced the typical symptoms described above. Although the symptoms lessened with time, the employee became irritable and depressed. This continued until his reassignment into another area of the plant. Another employee exposed at the same time, but not to the same degree, experienced milder symptoms.

An eighth employee was found unconscious lying in a cloud of escaping methyl chloride gas by other workmen. He was admitted to the hospital where he remained unconscious for several hours. Weakness and headaches were still present when he was discharged 10 days later. Follow-up examinations over the next 5 years revealed persistent symptoms, personality changes, and neurologic damage.

In 1963, 17 male crew members on an Icelandic fishing trawler were exposed for 2 days to methyl chloride from a leaking refrigerator located under their sleeping quarters (Gudmundsson 1977). No estimates of exposure concentrations were made. Fifteen of the crew members had signs of intoxication and abnormal neurologic symptoms. One survivor died within 24 h of exposure, two committed suicide 11 and 18 months later, and one died 10 years later. Six of 10 survivors (one survivor could not be located) still had neurologic deficits 20 months later. All survivors suffered from mild to permanent neurologic or psychiatric sequelae 13 years after the exposure occurred.

Lanham (1982) reported a case of a husband and wife who stored Styrofoam™ insulating boards in the basement of their new home prior to installation. The home was of tight, energy-efficient construction. Several days later they developed symptoms of blurred vision, fatigue, vertigo, tremor, and abnormal gait. Concentrations of methyl chloride measured by three different devices were above 200 ppm.

Battigelli and Perini (1955) described two workers exposed to methyl chloride while repairing a refrigeration system. On the basis of the room size and the amount of gas in the system, the exposure was estimated at >29,000 ppm (duration was not provided). The workers developed vertigo, tremors, dulled senses, nausea, vomiting, and abdominal pain 3-4 h after exposure. Symptoms disappeared 1 day after the exposure.

Four refrigeration-repair workers were exposed to methyl chloride at approximately 39,000, 50,000, 440,000, and 600,000 ppm (Jones 1942). Common symptoms were ataxia, staggering, headache, drowsiness, anorexia, blurred and double vision, convulsions, nausea, and vomiting. The exposure duration was not reported.

2.2.3. Occupational Exposures

Scharnweber et al. (1974) described six cases of prolonged worker exposure to "relatively low levels" of methyl chloride. Exposures were for 2-3 weeks, sometimes with 12- to 16-h workdays, before onset of symptoms. The analysis method was not reported. Two workers exposed at up to 300 ppm (8-h time-weighted average [TWA]) for several weeks were hospitalized with symptoms of confusion, blurry vision, difficulty in eating and swallowing, headache, and combativeness. Some symptoms, such as poor memory and headache, persisted for several months. Four workers exposed at 265 ppm (8-h TWA) for 2-3 weeks, with 12- to 16-h workdays, developed similar symptoms, including impaired memory, gait, and speech and slight elevation in blood pressure. Scharnweber et al. (1974) concluded that 8-h of exposure to methyl chloride at concentrations greater than 200 ppm is necessary for development of chronic methyl-chloride intoxication.

Continuous monitoring studies (for up to 4 months) during manufacturing operations at nine plants were conducted by the Dow Chemical Co. (personal communication, 1970, as cited in ACGIH 2003). Time-weighted average exposure concentrations were determined for 54 job classifications. The average TWA was 30 ppm with a range of 5-78 ppm; peaks as high as 400 ppm were recorded. Routine, periodic medical examinations did not identify any evidence of overexposure. Methyl chloride concentrations in relation to reported illnesses in Styrofoam^{TM}-manufacturing plants were summarized. On the basis of 100 sample points at 9 plants, illness was reported in plants where average concentrations of methyl chloride were 2-1,500 ppm; the range of average exposures was 195-475 ppm. Symptoms of illness included weakness, drowsiness, staggered gait, thickness of the tongue, and lapses of memory. At 141 plants (1,784 sample points) without reported illnesses, average concentrations at sample points were 2-500 ppm, and the range of average exposures was 15-195 ppm.

Repko et al. (1976) compared neurologic functions in a group of 122 healthy male and female workers exposed to methyl chloride in the manufacture of foam products with 49 workers also engaged in the manufacture of foam products but not exposed to methyl chloride. Average daily air concentrations were determined for each worker individually. Air concentrations were monitored by different methods in different plants and involved continuous and single-sampling techniques. For continuous monitoring with gas or infrared analyzers (five plants), the amount of time each employee spent in an area was used to

calculate TWA exposures. On testing days, carbon tubes were used to collect area samples. Results correlated "reasonably" with concentrations determined from conductivity and infrared analyzers. In the sixth plant, continuous monitoring was conducted with an automated gas chromatograph. Carbon tubes also were used during the battery of tests. The study was not blind; volunteers were paid and were told the objectives of the tests. Functional capacity was evaluated with a series of comprehensive neurologic, electroencephalogram (EEG), and behavioral test batteries.

Ambient concentrations of methyl chloride were 7.4-70 ppm, with an overall average of 33.6 ppm. There were no significant differences in results of neurologic tests or EEGs. Although the exposed group outperformed the control group on a few tasks, significant performance deficits were observed for most tasks. The concentration of methyl chloride was related to the decrease in performance deficits, primarily cognitive time sharing, and increased finger tremor. Methyl chloride concentration also was correlated with breath concentration, as well as urine pH and hematocrit. The authors concluded that daily exposure to methyl chloride below 100 ppm can cause significant, transitory changes in functional capacity. Because exposures before the study were higher and because questionable statistical methods were used, the study is of limited value (Torkelson and Rowe 1981).

The National Institute of Occupational Safety and Health (Cohen et al. 1980) conducted a survey of four U.S. chemical plants. Three of the plants produced methyl chloride and the fourth used methyl chloride as a blowing agent in the production of polystyrene foam. The personal 8-h TWA concentrations at the first three plants were 8.9-12.4 ppm, <0.2-7.5 ppm, and <0.1-12.7 ppm; personal exposures in the fourth plant were 3.0-21.4 ppm. In a Dutch methyl chloride plant, individual 8-h TWA area samples (which correlated closely with personal samples) were 30- 90 ppm during one working week (van Doorn et al. 1980). Symptoms, if present, were not reported in these studies.

2.2.4. Clinical Studies

Clinical studies of methyl chloride are summarized in Table 6-3. As part of a pharmacokinetic study of methyl chloride, six male volunteers were exposed at 10-50 ppm on separate days for 6 h (Nolan et al. 1985). Exposures took place in a 70-m^3 chamber. Atmospheres were measured continuously with an infrared spectrometer and at 15-min intervals with a gas chromatograph equipped with a flame ionization detector. There was no recognizable odor or irritation. No adverse effects were reported by the subjects or by the physicians conducting the post-exposure examinations.

Additional clinical studies are discussed below in the section on neurotoxicity (Stewart et al. 1980; Putz-Anderson et al. 1981a,b) or on metabolism (Lof et al. 2000).

TABLE 6-3 Summary of Clinical Studies of Methyl Chloride

Concentration (ppm)	Exposure Duration	Effect	Reference
10	2 h	No irritation or CNS effects.	Lof et al. 2000
10, 50	6 h	No recognizable odor or irritation.	Nolan et al. 1985
0, 20, 100, 150	1, 3, or 7.5 h, 2-5 d	No eye, nose, or throat irritation; no effect on physiologic, neurologic, behavioral, or clinical parameters; exercise incorporated into the protocol for male subjects.	Stewart et al. 1980
0, 100, 200	3 h	No odor perception; little to no effect on tests of alertness.	Putz-Anderson et al. 1981a
0, 200	3.5 h	No odor perception; no effect on tests of alertness.	Putz-Anderson et al. 1981b

2.3. Neurotoxicity

In a study using a controlled atmospheric chamber, nine male subjects (ages 19-34) were exposed to methyl chloride at 0, 20, 100, or 150 ppm for 1, 3, or 7.5 h, and nine female subjects were exposed at 0 or 100 ppm for identical periods of time (Hake et al. 1977; Stewart et al. 1980). Male subjects were exposed at 150 ppm on 2 consecutive days and male and female subjects were exposed at 100 ppm on 5 consecutive days. An additional exposure of male subjects involved fluctuating concentrations of 50, 100, and 150 ppm (TWA of 100 ppm) for 1, 3, or 7.5 h/day for 5 days. Groups were composed of 2-4 subjects. Groups were defined by exposure duration; for example, the four male subjects exposed for 7.5 h were exposed to methyl chloride at 0, 20, 100, fluctuating 50-150, and 150 ppm on different weeks. The entire testing period was 5 weeks. The male subjects were sedentary except for 11 min of exercise on a bicycle ergometer (6 min at 350 kpm and 5 min at 750 kpm) between the fifth and seventh hour h of exposure on the fourth day at all concentrations (day 2 for the male group exposed at 150 ppm). Concentrations were verified by gas chromatography and infrared analysis. Clinical symptoms and physiologic (EEG and visual evoked response patterns), clinical chemistry and hematology, neurologic, and behavioral effects were monitored; blood and alveolar breath samples were monitored for methyl chloride. Subjective responses were recorded immediately after entering the chamber, at the half hour, and hourly thereafter. The report form contained the descriptors headache; nausea; dizziness; abdominal pain; eye, nose, throat irritation; odor; and other, with modifiers of mild, moderate, and strong (only abnormalities reported). Neurologic studies consisted of a modified Romberg test, equilibrium test, spontaneous EEG, and visual evoked response. Cognitive testing, consisting of time estimation, eye-hand coordination, arithmetic, and number recognition, was performed after 2 and 3 h during

the 3- and 7.5-h exposures, respectively. The physiologic, neurologic, behavioral, clinical, and medical responses revealed no deleterious effects from methyl chloride (blood and breath analysis for methyl chloride are summarized in Section 4.1). The notation of a mild odor was reported as frequently for control exposure (0 ppm) as for test exposures.

Putz-Anderson et al. (1981a) assessed the behavioral effects of inhaled methyl chloride in groups of 8 or 12 healthy male and female subjects. Ages ranged from 18 to 32 years. Methyl chloride was administered at concentrations of 0, 100, or 200 ppm for 3 h. Three performance tests (visual vigilance, dual task, and time discrimination), designed to test attention or alertness, were administered before and during the treatment period. The net impairment resulting from exposure at 200 ppm was marginally significant (4.5%). The authors concluded that exposure at 200 ppm produced little or no behavioral impairment. In a second study (Putz-Anderson et al. 1981b), conducted in the same manner and using the same tests, groups of 12 healthy male and female subjects were exposed at 200 ppm for 3.5 h. The subjects did not experience any significant impairment on the tests. The authors note that the subjects were no more successful than chance in assessing whether they had been exposed to the control or chemical atmosphere.

2.4. Developmental/Reproductive Toxicity

No studies were found regarding reproductive or developmental effects in humans after inhalation of methyl chloride.

2.5. Genotoxicity

No studies were found regarding genotoxic effects in humans after inhalation exposure to methyl chloride. In an in vitro test, methyl chloride at 0.3-5% induced an increase in the frequency of sister chromatid exchanges in human lymphoblasts, but did not induce DNA damage (Fostel et al. 1985). Unscheduled DNA synthesis was induced in primary cultures of human hepatocytes of three individuals exposed at 1%, but not at 0.1-0.3% (Butterworth et al. 1989).

2.6. Carcinogenicity

Holmes et al. (1986) conducted a retrospective study of 852 workers exposed to methyl chloride in a butyl rubber manufacturing plant. Mortality from all causes was lower than expected compared with the U.S. male population. There was no statistical evidence that the death rate from cancer at any site was increased. No concentrations of methyl chloride were specified in this study.

Rafnsson and Gudmundsson (1997) conducted a long-term follow-up study of the survivors of the acute exposure described by Gudmundsson (1977)

in Section 2.2.2. The 24 crew members, which included individuals that had not been heavily exposed, were compared with a matched referent group. The authors found increased mortality from cardiovascular diseases and "vague signs" of cancer risk.

In reviewing the two studies described above for its Integrated Risk Information System (IRIS), EPA (2003) concluded that the studies failed to convincingly demonstrate an increased cancer mortality risk. The agency has not assigned a carcinogenicity classification to methyl chloride. The National Toxicology Program (NTP) has not classified methyl chloride with regard to carcinogenicity. IARC (1999) also concluded that evidence for carcinogenicity in humans is inadequate. IARC reviewed the study by Holmes et al. (1986), as well as a study by Ott et al. (1985). In the Ott et al. (1985) study, exposures were to mixtures of chemicals, including methyl chloride. The standard mortality ratio (SMR) for all cancers was 0.7. IARC (1999) also concluded that there was inadequate evidence for carcinogenicity in animals (see Section 3.6). The overall IARC evaluation is that methyl chloride is *"not classifiable as to its carcinogenicity to humans"* (Group 3). The American Conference of Governmental Industrial Hygienists (ACGIH 2003) considers methyl chloride *"not classifiable as a human carcinogen"* (A4). However, NIOSH (1984) has classified methyl chloride as a *"potential occupational carcinogen"* with no further categorization.

2.7. Summary

Acute and chronic exposures to methyl chloride primarily affect the CNS. Deaths have occurred from accidental exposure to methyl chloride, but there are no reliable data on concentrations and exposure durations. The odor is very faint and may not be noticed by individuals at concentrations that are life-threatening. Like many solvents, methyl chloride is nonirritating to the eyes and mucous membranes. Accidental workplace exposures have documented concentrations of 1,000-2,000, 2,000-4,000 (for up to a week), and 10,000 ppm, the latter concentration for short periods of time (MacDonald 1964). These exposures resulted in typical symptoms of blurred vision, dizziness, headache, nausea, and vomiting. In some cases symptoms were delayed for several hours. Daily, repeated exposures to methyl chloride at ≥265 ppm for 12- to 16-h workdays have resulted in similar symptoms (Scharnweber et al. 1974).

In a well-conducted clinical study, no toxicologically-significant effects were apparent when female and exercising male subjects were exposed to methyl chloride at 100 ppm for 7.5 h on 5 consecutive days, male subjects were exposed at 150 ppm for 7.5 h on two consecutive days, or male subjects exposed at 50-150 ppm (TWA of 100 ppm) for 7.5 h on 5 consecutive days (Stewart et al. 1980). In all cases male subjects exercised on a bicycle ergometer for 11 min per day. A concentration of 200 ppm was also a no-effect level for toxicologi-

cally significant neurobehavioral symptoms during separate 3- and 3.5-h exposures (Putz-Anderson et al. 1981a,b).

No studies on developmental or reproductive effects were found. In in vitro systems, methyl chloride is genotoxic only at very high concentrations. There is inadequate evidence for carcinogenicity from methyl chloride in humans.

3. ANIMAL TOXICITY DATA

Few studies of acute exposure to methyl chloride are available. Most studies address neurotoxicity and use repeat-exposure methods. These studies are summarized in Table 6-4.

3.1. Acute Lethality

In an early, comprehensive study (Smith and von Oettingen 1947a,b; Dunn and Smith 1947), several species were exposed to methyl chloride at 300, 500, 1,000, 2,000, 3,000, or 4,000 ppm for 6 h/day for up to several months. Concentrations were monitored by a titration method, from direct rotameter calibrations, and by weight loss from the methyl chloride tanks. Agreement among the measurement methods was fairly good. Animals were observed for frank toxicity, including ataxia and convulsions. Concentrations of 3,000 and 4,000 ppm were lethal to most species within a few days. For 5 adult monkeys, exposure at 2,000 ppm resulted in convulsive seizures and long periods of unconsciousness; no deaths were recorded during the first week. At 500 ppm, 3 of 4 dogs died after 4 weeks of exposure and both monkeys died after 16 weeks of exposure (with progressive debility and persisting unconsciousness). None of the monkeys, dogs, rats, mice, rabbits, or guinea pigs exposed at 300 ppm for up to 64 weeks died, and no tissue lesions were found. Symptoms developed later in young animals. Deaths were dependent on time and concentration. At high concentrations, mortality correlated with the product of exposure duration and concentration, but at about 500 ppm, mortality was more gradual. Details of this study were insufficient to estimate acute lethal concentrations for each species. Furthermore, although the values appear similar to those of more recent studies, the methods do not meet current standards. Therefore, this study and the following study are not presented in Table 6-4 with other mortality studies.

Additional studies were reported by this group. The average survival time of beagles exposed at 14,661 ppm was 5.9 h (von Oettingen et al. 1949). Death was preceded by an initial increase in heart rate and blood pressure, followed by reduced respiration, decreased heart rate, and progressive fall in blood pressure. This concentration had little narcotic action as measured by disappearance of corneal reflex, pupillary reflex, and muscular action before death. Groups of mice (strain and number not specified) were exposed to methyl chloride at various concentrations for 7 h (von Oettingen et al. 1949). Concentrations were es-

timated by the amount of chemical volatilized over the 7-h period and by a titration method. Mice exhibited increased activity soon after exposure began; this was followed by quiescence at 2 h. Death was preceded by clonic convulsions. Most deaths occurred during the first 8 h after exposure; none occurred after 16 h. The 7-h 50% lethality (LC_{50}) was approximately 3,100 ppm. No further details were provided.

3.1.1. Rats

In an unpublished study, groups of 40 male and 40 female Sprague-Dawley rats were exposed continuously to methyl chloride (99.5% purity) at 0, 200, 500, 1,000, or 2,000 ppm for 48 or 72 h (Burek et al. 1981). Twenty rats per sex were killed immediately after exposure and the remaining rats were observed for 12 days before they were also killed for necropsy. At 2,000 ppm, rats were lethargic, moribund, or dead at 48 h, and all were dead at 72 h. No male rats and 1 of 10 female rats exposed at 1,000 ppm for 48 h died during the 12-day post-exposure observation period; mortality in the rats exposed for 72 h was 6 of 10 males and 8 of 10 females. The authors attributed the deaths to kidney failure. No deaths occurred at 0, 200, or 500 ppm for up to 72 h of exposure, and no overt signs of toxicity were observed during the exposures. There were no gross or microscopic changes in organs of rats exposed at 200 ppm for 24 h; livers of female rats exposed at 200 ppm for 72 h showed reversible changes.

Groups of 10 male and 10 female F344 rats were exposed to methyl chloride at 0, 2,000, 3,500, or 5,000 ppm for 6 h/day for up to 12 days (Morgan et al. 1982). The chemical was 99.5% pure; analysis was by an infrared gas analyzer. At 5,000 ppm, clinical signs included diarrhea during the first days of exposure, incoordination of the forelimbs by day 3, hindlimb paralysis on day 5, and a moribund state (6 males and 5 females) on day 5. In the 3,500-ppm group, two females in a moribund state were killed after the fifth day of exposure. No other deaths were reported. Histopathologic changes included cerebellar degeneration in males and females exposed at 5,000 ppm, dose-related degeneration and necrosis of the renal proximal convoluted tubules in males exposed at 2,000 ppm and in both sexes at 3,500 and 5,000 ppm, hepatocellular degeneration in females exposed at 2,000 ppm and in both sexes at 3,500 and 5,000 ppm, testicular degeneration in males exposed at ≥2,000 ppm, and adrenal fatty degeneration in males and females exposed at 3,500 and 5,000 ppm. In a later study by the same group (Chellman et al. 1986a), exposure to methyl chloride at 5,000 ppm for 5 days resulted in weight loss and testicular, brain, liver, and adrenal lesions in male F344 rats (see Section 3.4 for reproductive effects). Tremors, ataxia, and forelimb and hindlimb paralysis were observed, but the day of onset was not specified. One rat of five rats died after the last day of exposure. Exposure to methyl chloride at 7,500 ppm for 6 h/day for 2 days resulted in 67% lethality (8/12 rats) during a 4-day post-exposure observation period.

TABLE 6-4 Summary of Acute, Repeat-Exposure, and Subchronic Inhalation Studies of Methyl Chloride in Laboratory Animals

Species	Concentration (ppm)	Exposure Duration	Effect[a]	Reference
Dog	0, 200, 500	23.5 h/d, 3 d	No clinical signs or microscopic lesions at 200 ppm; neurotoxic signs and brain-stem lesions at 500 ppm	McKenna et al. 1981a
Dog	0, 50, 150, 400	6 h/d, 5 d/wk, 13 wk	No clinical signs; no brain or spinal cord lesions.	McKenna et al. 1981b
Rat	0, 100, 500, 1,500	6 h	No clinical signs; no effect on organ weights (up to 18 h post-exposure); no microscopic examinations.	Dodd et al. 1982
Rat	0, 1,000, 3,000	6 h/d, 5 d	No deaths; initial weight loss of 3% and 20% in 1,000- and 3,000-ppm groups, respectively, followed by recovery.	Working et al. 1985a
Rat	0, 5,000	6 h/d, 5 d	Degeneration of cerebellar granular cells, lesions in liver and adrenal glands; death of 1 rat after fifth day of exposure	Chellman et al. 1986a
	0, 7,500	6 h/d, 2 d	67% lethality.	
Rat	0, 500, 1,000, 2,000, 3,500, 5,000	6 h/d, up to 12 d	No deaths during first 4 d; pathologic changes in cerebellum (5,000 ppm), kidneys (≥2,000 ppm), liver (≥2,000 ppm), adrenal glands (≥3,500 ppm), testes (≥2,000 ppm).	Morgan et al. 1982
Rat	200	48, 72 h	No deaths; no clinical signs.	Burek et al. 1981
	500	48, 72 h	No deaths; no clinical signs.	
	1,000	48 h	5% mortality.	
	2,000	48 h	Moribund state or death.	
Rat	400	6 h/d, 5 d/wk, 90 d	No clinical signs, no organ lesions.	McKenna et al. 1981b

(Continued)

TABLE 6-4 Continued

Species	Concentration (ppm)	Exposure Duration	Effect[a]	Reference
Rat	0, 375, 750, 1,500	6 h/d, 5 d/wk, 90 d	No clinical signs or tissue lesions; increased liver weight at 1,500 ppm.	Mitchell et al. 1979
Mouse	2,250 (males)	6 h	LC_{50}	White et al. 1982
	8,500 (females)	6 h	LC_{50}	
Mouse	2,200	6 h	LC_{50}	Chellman et al. 1986b
Mouse (3 strains)	0, 500, 1,000, 2,000	6 h/d, up to 12 d	2,000 ppm: death of one strain of male mice by day 2; ataxia, hematuria in females, death of males and females of other strains by day 5; pathologic changes in cerebellum (≥1,000 ppm), kidneys (≥1,000 ppm), liver (≥500 ppm).	Morgan et al. 1982
Mouse	1,500 ppm	6 h/d, 5 d/wk, 2 wk	2 of 10 mice died during first week, motor incoordination during second week; degenerative changes in cerebellum.	Jiang et al. 1985
Mouse	0, 15, 50, 100, 150, 200	22 h/d, 11 d	Cerebellar lesions at ≥100 ppm; 200 ppm lethal in 5 days.	Landry et al. 1985
	0, 150, 400, 800, 1,600, 2,400	5.5 h/d, 11 d	Cerebellar lesions at ≥400 ppm; moribund at 2,400 ppm by day 9.	
Mouse	0, 375, 750, 1,500	6 h/d, 5 d/wk, 90 d	Mild liver changes; no brain lesions.	Mitchell et al. 1979
Mouse	0, 50, 150, 400	6 h/d, 5 d/wk, 90 d	No clinical signs or organ lesions.	McKenna et al. 1981b
Guinea pig	0, 20,000	10 min/d, 6 d/wk, 61-70 d	No deaths, neurotoxic signs; lesions of cerebellar cortex.	Kolkmann and Volk 1975

3.1.2. Mice

The 6-h LC_{50} values for methyl chloride in male and female $B6C3F_1$ mice were 2,250 and 8,500 ppm, respectively (White et al. 1982). After exposure to methyl chloride at 2,500 ppm for 30 min, hepatic glutathione (GSH) was reduced to 9% of control values in both sexes. No further data were provided in this abstract. As reported in a later paper by the same authors (Chellman et al. 1986b), groups of five male $B6C3F_1$ mice were exposed for 6 h to methyl chloride at 500-ppm increments from nonlethal concentrations to a concentration that caused 100% mortality (exact concentrations not specified). Animals were killed 18 h after exposure. The LC_{50} was 2,200 ppm. Tremors, ataxia, and forelimb and hindlimb paralysis occurred prior to death.

Two strains of mice (C3H and C57Bl/6) and the cross between these two strains ($B6C3F_1$) were exposed to methyl chloride at 0, 500, 1,000, or 2,000 ppm for 6 h/day for up to 12 days (Morgan et al. 1982). Groups were composed of five mice of each sex. All male $B6C3F_1$ mice exposed at 2,000 ppm were moribund or dead by day 2, and all male and female mice in the remaining groups exposed at 2,000 ppm were moribund by day 5. The animals exhibited ataxia and had hematuria, the latter primarily in females. Histopathologic changes included: cerebellar degeneration in male and female C57Bl/6 mice exposed at 1,000 ppm and female $B6C3F_1$ mice exposed at 2,000 ppm (the cerebellums of C3H mice were unaffected at all concentrations); degeneration and necrosis of the renal proximal convoluted tubules in male C3H mice exposed at 1,000 ppm and in both sexes of all three strains exposed at 2,000 ppm; basophilic renal tubules in both sexes of all three stains (except female C57Bl/6 mice) exposed at 1,000 ppm, but not at 2,000 ppm; and hepatocellular degeneration at ≥500 ppm, with greatest severity in male mice exposed at 2,000 ppm. Jiang et al. (1985) also found degenerative changes in the cerebellum of female C57Bl/6 mice exposed to methyl chloride at 1,500 ppm for 6 h/day, 5 days/week for 2 weeks. Renal lesions were minimal or absent.

In a study of neurotoxicity, female C57Bl/6 mice were exposed either "continuously" for 11 days (22 h/day without interruption) to methyl chloride at 0, 15, 50, 100, 150, or 200 ppm or "intermittently" (5.5 h/day) at 0, 150, 400, 800, 1,600, or 2,400 ppm for 11 consecutive days (Landry et al. 1985). At the end of exposure, cerebellar lesions were observed in mice continuously exposed at ≥100 ppm and in mice intermittently exposed at ≥400 ppm. Thymus weights were reduced at the lower concentrations, but there were no histologic correlates. Continuous exposure at 200 ppm was lethal in 5 days, and intermittent exposure at 2,400 ppm resulted in moribund mice by day 9.

3.2. Nonlethal Toxicity

3.2.1. Dogs

Groups of three male beagle dogs were exposed to methyl chloride at 0, 200, or 500 ppm for approximately 23.5 h/day for 3 days (McKenna et al.

1981a). After 23.5 h of treatment, dogs exposed at 500 ppm appeared more tranquil, with one exhibiting intermittent tremor and slight excess salivation. After 72 h, the behavior of the control and 200-ppm dogs was comparable. Dogs exposed at 500 ppm for 72 h appeared weak and displayed signs of forelimb stiffness and incoordination, occasional slipping and falling, inability to sit up or walk, limb tremor, and excess salivation. Partial recovery was observed during the 27-day post-exposure period. Microscopic examination of tissues revealed no treatment-related abnormalities in the control dogs or dogs exposed at 200 ppm. All three dogs exposed at 500 ppm displayed lesions in the brain stem and spinal cord, consisting of vacuolization, swollen eosinophilic axons, axon loss, demyelinization, and microglial cells with phatocytosed debris. There were no lesions in the cerebrum, cerebellum, or peripheral nerves.

In a second study by the same investigators (McKenna et al. 1981b), there was no evidence of brain or spinal-cord lesions in dogs exposed to methyl chloride at 0, 50, 150, or 400 ppm for 6 h/day, 5 days/week for approximately 13 weeks. Groups of four young male beagles were exposed to each concentration for a total of 66 days. Chamber concentrations were monitored by infrared spectrometry. Dogs were monitored daily for clinical signs, and body weight and hematologic- and clinical-chemistry parameters were evaluated before and after exposure. The animals were killed at the end of the study, and tissues and organs were examined microscopically. No dogs died during the study. There were no clinical signs, weight gains were comparable to that of controls, and no organ or tissue lesions attributable to methyl chloride were found.

3.2.2. Rats

As part of a mechanism of toxicity study (see Section 4.3), groups of 20 male F344 rats were exposed to methyl chloride at 0, 100, 500, or 1,500 ppm for 6 h or at 500 ppm for 1, 2, or 4 h (Dodd et al. 1982). All rats appeared normal after the exposures. No differences were observed in lung, liver, or kidney weights between control and treated rats when measured 0, 2, 4, 8, or 18 h after exposure (groups of four animals). Organs were not examined microscopically.

No deaths occurred in male F344 rats exposed to methyl chloride at 1,000 or 3,000 ppm for 6 h/day for 5 days (Working et al. 1985a). Weight loss was 3% in the 1,000-ppm group, but it was recovered 3 weeks after exposure ended. In the 3,000-ppm group, animals experienced a 20% weight loss, with recovery 4 weeks later (see Section 3.4 for reproductive effects).

In a 90-day pilot study, groups of 10 male and 10 female F344 rats were exposed to methyl chloride at 0, 375, 750, or 1,500 ppm for 6 h/day, 5 days/week (Mitchell et al. 1979). Animals were observed for food consumption, body weight changes, and mortality. Hematology and clinical-chemistry tests were performed. Organs were weighed and all tissues and organs from animals in the control and high-concentration groups were examined microscopically. Effects on the liver were mild; no necrosis was observed. There were no expo-

sure-related histopathologic lesions of the brain and spinal cord and no effect on brain weight. In a similar 90-day study, the no-effect concentration for methyl chloride in Sprague-Dawley rats was 400 ppm (McKenna et al. 1981b). The exposure regimen was the same as that described for dogs (see earlier discussion). In addition, rats were evaluated weekly for motor and sensory response. The results of the evaluations were normal except for a slight decline in the performance of female rats (primarily those in the 400-ppm group) over time in the wire maneuver test. The significance of this effect is questionable, given the weight increase in female rats over 90 days and that the same tendency was present in the control group.

3.2.3. Mice

In a 90-day pilot study, male and female $B6C3F_1$ mice were exposed to methyl chloride at 0, 375, 750, or 1,500 ppm for 6 h/day, 5 days/week (Mitchell et al. 1979). Moderate changes in the liver included increased liver weight and cytoplasmic vacuolation in male and female mice exposed at 1,500 ppm, and to a lesser degree in mice exposed at 750 ppm. Female mice were more affected than male mice. There were no exposure-related histopathologic lesions of the brain or spinal cord and no effect on brain weight at any concentration. In a similar study, the no-effect level for clinical signs and organ lesions in CD-1 mice was 400 ppm (McKenna et al. 1981b). The protocol of this study is the same as that for dogs and rats (described earlier).

3.2.4. Guinea Pigs

Kolkmann and Volk (1975) exposed guinea pigs to methyl chloride at 20,000 ppm for 10 min/day for 21 days. Microscopic examination of the cerebellum revealed edema with necrosis of the granular cells.

3.3. Neurotoxicity

Neurotoxic effects were observed before death in many of the studies described earlier. In a study that addressed neurotoxicity at less than lethal concentrations, Landry et al. (1985) exposed female C57Bl/6 mice to various concentrations of methyl chloride either continuously (22 h/day) for 11 days or for 5.5 h/day for 11 days (see Table 6-4). The authors noted that female C57B1/6 mice are particularly sensitive to the neurotoxic effects of methyl chloride and do not have the complications of hepatic and renal toxicity. The mice were tested for their ability to maintain balance on an accelerating, rotating rod after 4, 8, and 11 days of exposure. Performance decrements on the rotating rod were observed after continuous exposure at 150 ppm for 4 and 8 days; mice were moribund or dead by the eleventh day. For intermittent exposures, slight decrements in per-

formance were observed at 800 and 1,600 ppm after 4 days, but not after 8 or 11 days. Cerebellar damage was observed at 400 ppm.

3.4. Developmental and Reproductive Toxicity

Methyl chloride has been shown to be a reproductive toxicant in a variety of animal studies. These studies are described below and in Table 6-5.

TABLE 6-5 Reproductive Toxicity of Methyl Chloride in Animal Models

Concentration (ppm)	Exposure Duration	Effects	Reference
Dog			
0, 200, 500	23.5 h/d for 3 d	No change in testes weight; no histopathologic lesions of testes.	McKenna et al. 1981a
Rat			
0, 150, 475, 1,500	6 h/d, 5 d/wk for 10 wk	No statistically significant effect on fertility of males exposed at 150 or 475 ppm for two generations (but dose-related trend of reduced fertility); 100% sterility in males at 1,500 ppm; no clear effect on fertility in females.	Hamm et al. 1985
200, 500, 1,000	48 or 72 h	No testicular lesions at 200 ppm; inflammation of the epididymides with testicular atrophy at 500 and 1,000 ppm.	Burek et al. 1981
3,500	6 h/d, 5 and 4 d, with 3-d break between exposures	Epididymal and testicular lesions; interference with neuroendocrine control of spermatogenesis; first observed at day 9.	Chapin et al. 1984
5,000, 7,500	6 h/d for 2 d	Testicular or epididymal granulomas; testicular lesions.	Chellman et al. 1986a; Working and Chellman 1989
3,000	6 h/d for 5 d	Epididymal inflammation and granulomas, sperm cytotoxicity, preimplantation loss in mated females.	Chellman et al. 1986c; 1987
0, 2,000, 3,500, 5,000	6 h/d for 5 and 4 d with 2-d break between exposures	Concentration-related testicular degeneration with reduced numbers of sperm.	Morgan et al. 1982
0, 1,000, 3,000	6 h/d for 5 d	At 1,000 ppm: no sperm granulomas, no effect on fertilization of females At 3,000 ppm: epididymal granulomas, preimplantation loss in mated females from fertilization failure (decrease in sperm quality), recovery by week 16.	Working et al. 1985a,b; Working and Bus 1986

The reproductive toxicity of methyl chloride in the male rat has been well characterized in studies performed at the Chemical Industry Institute of Toxicology (Burek et al. 1981; Morgan et al. 1982; Chapin et al. 1984; Working et al. 1985a,b; Chellman et al. 1986b,c, 1987; Working and Chellman 1989). Male F344 rats exposed to methyl chloride at concentrations of 1,000 ppm or greater developed testicular degeneration, epididymal inflammation, and sperm granulomas. Fertility might be decreased at 500 ppm. Recovery occurred 16 weeks after exposure. Unexposed females bred to treated males in a dominant lethal assay exhibited increased rates of postimplantation embryonic death during the first 2 weeks post-exposure and increased preimplantation embryonic loss during weeks 2 to 8 post-exposure. Studies on the mechanism of action suggest that preimplantation loss is from cytotoxic effects of methyl chloride on sperm in the testes (a significant decrease in the number of motile sperm of normal morphology 2 to 8 weeks post-exposure) and not to genotoxic effects on the sperm. Chellman et al. (1986a) showed that the effects of methyl chloride were virtually absent when male rats were pretreated with the anti-inflammatory agent 3-amino-1-[*m*(trifluoromethyl)phenyl]-2-pyrazoline (BW755C).

No changes in testes weights and no histopathologic lesions of the testes were observed in beagles after exposure to methyl chloride at 500 ppm almost continuously for 3 days (McKenna et al. 1981a; see Section 3.1.1 for study description).

A decrease in male fertility was also observed in a two-generation study with rats (Hamm et al. 1985). Groups of 40 male and 80 female F344 rats were exposed to methyl chloride at 0, 150, 475, or 1,500 ppm for 6 h/day, 5 days/week during a 10-week premating period. The only clinical sign during that time was a 10-20% reduced body weight gain in both sexes exposed at 1,500 ppm for 2 weeks and a 5-7% depression in body weight gain after day 57 in rats exposed at 475 ppm. During a 2-week mating period, both sexes were exposed for 7 days/week. Males were mated to two exposed females. Necropsies of 10 males in each group (at 12 weeks) showed severe bilateral testicular degeneration (10/10) and epididymal granulomas (3/10) in the 1,500-ppm group. Males exposed at 1,500 ppm were sterile when mated to either unexposed or exposed females. Fewer F_0 males exposed at 475 ppm and mated to unexposed females were fertile (12/28) than in the control (23/28) or 150-ppm (21/28) group. Also, fewer males exposed at 475 ppm and mated to exposed females were fertile (12/40) than in the control (18/40) or 150-ppm (20/39) group. However, there was no difference in fertility in the F_1 generation (the number of fertile males in the control, 150-ppm, and 475-ppm groups was 31/40, 26/40, and 14/23, respectively) although there was a trend toward decreased fertility. After a 9- or 18-week recovery period, fertility remained low in F_0 males previously exposed at 1,500 ppm (5 and 10%, respectively).

Several studies addressed developmental effects of methyl chloride (see Table 6-6). In the Hamm et al. (1985) study described earlier, no significant differences in litter size, sex ratio, pup viability, or pup growth were observed

TABLE 6-6 Developmental Effects of Methyl Chloride in Animal Models

Concentration (ppm)	Exposure Duration	Effects	Reference
Rat			
0, 150, 475, 1,500	6 h/d, 5 d/wk premating; 6 h/d, 7 d/wk from mating to postnatal day 28	No significant differences in litter size, sex ratio, pup viability, or pup growth.	Hamm et al. 1985
0, 100, 500, 1,500	6 h/d, gestation days 7-19	Maternal and fetal toxicity at 1,500 ppm; no external, skeletal, or visceral abnormalities in fetuses.	Wolkowski-Tyl et al. 1983a
Mouse			
0, 100, 500, 1,500	6 h/d, gestation days 6-17	Mortality in dams at 1,500 ppm; survival in other groups with no evidence of maternal or fetal toxicity; no external malformations; small, statistically significant increase in heart defects in litters of 500-ppm group.	Wolkowski-Tyl et al. 1983a
0, 250, 500, 750	6 h/d, gestation days 6-18	Decreased body weight gain in dams at 750 ppm; heart malformations in fetuses/litters at 500 and 750ppm.	Wolkowski-Tyl et al. 1983b
0, 250, 300	24 h, gestation days 11.5-12.5	No heart malformations	John-Green et al. 1985
1,000	12 h, gestation days 11.5-12	No heart malformations	

between control or treated (150, and 475 ppm) rats. Females were exposed for 6 h/day, 7 days/week from the start of mating to postnatal day 28, but were not exposed from gestation days 18 to postnatal day 4 and the pups were not exposed directly.

Results of other developmental toxicity tests with methyl chloride are conflicting. Wolkowski-Tyl et al. (1983a,b) found no external, skeletal, or visceral abnormalities in the fetuses of pregnant F344 rats exposed at 0, 100, 500, or 1,500 ppm on gestation days 7-19. Maternal and fetal toxicity were apparent at the highest concentration, as evidenced by reduced food consumption and decreased body weight and weight gain in dams and reduced fetal body weight. However, a small but statistically significant number of heart malformations were found in the $B6C3F_1$ fetuses of pregnant C57Bl/6 mice (bred to C3H males) exposed at 500 ppm on gestation days 6-17. The malformation involved the bicuspid and tricuspid valves, and primarily involved a reduced number of papillary muscles on the right side of the heart. No abnormalities were found in fetuses when dams were exposed at 100 ppm. Dams exposed at 1,500 ppm were

moribund and killed. In a subsequent study, the same malformations were observed in fetuses when mouse dams were exposed at 500 or 750 ppm. No other embryotoxicity, fetotoxicity, or malformations were observed at 250 ppm. In another study, no heart malformations were found in fetuses of C57Bl/6 mouse dams exposed at 250, 300, or 1,000 ppm during gestation days 11.5-12.5, considered the critical period for heart development by John-Green et al. (1985). Wolkowski-Tyl et al. (1983b) considered gestation day 14 the critical developmental period. John-Green et al. (1985) also noted the variability in the appearance of the papillary heart muscles of fetal mice and, owing to their small size, the difficulty in confirming their presence. When examinations of fetal hearts were conducted with technicians that were blind to the test conditions, the technicians failed to find either an absence of or a reduction in size of the papillary muscles of the tricuspid valve. Small numbers of animals were used in the John-Green et al. (1985) study, and the exposure was for 24 h at 250 or 300 ppm or for 12 h at 1,000 ppm.

3.5. Genotoxicity

Genotoxicity assays have been reviewed by ATSDR (1998) and EPA (2003). In in vitro assays, methyl chloride is a weak genotoxin at high concentrations. Methyl chloride has been shown to be mutagenic in several strains of *Salmonella typhimurium* and to induce unscheduled DNA synthesis in several types of cells at concentrations >3%, but not at 1%. It has not been shown to methylate the DNA of rat tissues. Exposures of male B6C3F_1 mice to methyl chloride at 1,000 ppm for 6 h/day for 4 days failed to induce DNA-protein crosslinks in the kidneys, and gave only minor evidence of single-strand DNA breaks (Jager et al. 1988).

In in vivo assays, cytotoxicity appears to dominate potential genotoxicity. At 15,000 ppm, but not at 3,500 ppm, methyl chloride was weakly positive for the induction of unscheduled DNA synthesis in rat liver. Concentrations of 2,000-3,000 ppm (but not 1,000 ppm) produced dominant lethal effects in several strains of rats. The authors of the individual studies, including Chellman et al. (1986c), stated that the effects appeared to be attributable to cytotoxic effects on sperm in the testes rather than to direct genotoxicity, and to the effects of genotoxic oxidative metabolites resulting from an induced inflammatory response in the epididymides.

3.6. Chronic Toxicity and Carcinogenicity

In a 2-year study, methyl chloride was tested for carcinogenicity in F344 rats (120 per sex) and B6C3F_1 mice (120 per sex) (Pavkov et al. 1981). Vapor concentrations were 0, 50, 225, or 1,000 ppm, and exposures were for 6 h/day. Mouse survival was affected at 1,000 ppm, whereas rat survival was unaffected. This outcome for mice might have been influenced by fighting for dominance

among males. Neurofunctional impairment was observed in mice exposed at 1,000 ppm beginning with the 18-month interim sacrifice. Histopathologic examinations of male and female mice at the 18-month sacrifice revealed cerebellar lesions and atrophy. These lesions were not observed at lower concentrations or in male or female rats. Beginning at 6 months, male rats exposed at 1,000 ppm developed bilateral atrophy of the testicular seminiferous tubules. However, this lesion, as a result of aging, was present in all male rats at the 24-month sacrifice. At 12 months, renal degenerative changes consisting of cortical tubular epithelial hypertrophy and hyperplasia and hepatocellular degeneration were observed in male mice exposed at 1,000 ppm. These lesions progressed in severity and prevalence throughout the study. At 24 months, the only evidence of carcinogenicity was a statistically significant increased incidence of benign and malignant renal tumors in male mice exposed at 1,000 ppm; two male mice in the 225-ppm group also had renal adenomas. EPA (2003) considered 250 ppm a no-observed-adverse-effect level (NOAEL) for any effect in rats. EPA did not designate a NOAEL for mice, but noted that the tumors observed at 225 ppm might be related to treatment.

3.7. Summary

Acute toxicity data on methyl chloride are sparse. The 6-h LC_{50} values for male and female $B6C3F_1$ mice were 2,200 and 8,500 ppm, respectively (White et al. 1982).

Studies with laboratory animals have shown adverse effects on the brain, kidneys, liver, testes, and spleen. These effects are generally seen at neurotoxic concentrations or after repeated exposures at lower concentrations. In laboratory animals, no-effect levels were 1,500 ppm for clinical signs in rats exposed for 6 h (Dodd et al. 1982), 400 ppm for brain and spinal cord lesions in dogs exposed for 6 h/day, 5 days/week for 13 weeks (McKenna et al. 1981b), 400 ppm for cerebellar lesions in female mice exposed for 5.5 h/day for 11 days (Landry et al. 1985), 1,500 ppm in rats and mice exposed for 6 h/day for 13 weeks (Mitchell et al. 1979), and 200 ppm for clinical signs and brain lesions in dogs and rats exposed continuously for 3 days (Burek et al. 1981; McKenna et al. 1981a).

In reproduction studies with rats, exposures to methyl chloride that did not cause inflammation of the epididymides did not affect reproduction. A nearly 72-h continuous exposure of dogs to methyl chloride at 500 ppm had no effect on testicular weight or function (McKenna et al. 1981a). In developmental and teratology studies, results were conflicting. In one study, exposures of mice to methyl chloride at ≥500 ppm on gestation days 6-18 caused a reduced number of papillary muscles of the tricuspid valve of the fetal heart (Wolkowski-Tyl et al. 1983b); whereas in a study performed in a slightly different manner, these malformations were not observed (John-Green et al. 1985). However, it is possible the critical day of heart development was not chosen in the latter study.

The cancer potential is low and species specific (Pavkov et al. 1981). Consistent with the low cancer potential demonstrated in laboratory animals, alkyla-

tion of DNA appears to be minimal. Mutagenicity is considered weak to moderate. Although positive in dominant lethal assays, the effect appears to be secondary to injury to a specific area of the epididymides.

4. SPECIAL CONSIDERATIONS

4.1. Metabolism and Disposition

Human and animal studies show that methyl chloride is rapidly absorbed from the lungs (Andersen et al. 1980; Stewart et al. 1980; Landry et al. 1981, 1983; Nolan et al. 1985; ATSDR 1998). In humans exposed at up to 200 ppm, steady state was reached during the first hour of exposure (Putz-Anderson et al. 1981a; Nolan et al. 1985; Lof et al. 2000). Methyl chloride is extensively distributed throughout the body, with greatest deposition in the liver, kidneys, and testes (Kornbrust et al. 1982; Landry et al. 1983). This deposition, however, might refer to metabolites. In the rat, methyl chloride is metabolized by conjugation with GSH to yield S-methylglutathione. Cleavage of the glutamic acid and glycine moieties of GSH yields S-methylcysteine, and transamination and decarboxylation yields the mercapturic acid, methylthioacetic acid (Kornbrust and Bus 1983). The latter sulfur-containing compounds can be excreted in the urine. Methylthioacetic acid might be further metabolized to methanethiol which might yield formaldehyde and formic acid via P-450 metabolism; the latter compounds can enter the one-carbon pool or be excreted as carbon dioxide. The reaction with GSH appears to be primarily enzyme catalyzed, probably by glutathione transferase. Formation of formaldehyde appears to be a minor pathway. Human kidneys lack detectable CYP2E1 protein which suggests that formaldehyde formation might not occur in this organ. In summary, methyl chloride is rapidly absorbed and metabolized (does not bioaccumulate) and is excreted as numerous metabolites indistinguishable from normal metabolites.

The metabolite S-methylcysteine has been identified in the urine of occupationally exposed humans (van Doorn et al. 1980), in humans during clinical exposures (Nolan et al. 1985), and exposed rats (Landry et al. 1983). Urinary S-methylcysteine excretion is extremely variable in human subjects and is not a good indicator of the concentrations to which subjects are exposed (Nolan et al. 1985). Methylmercapturic acid was not detectable in the urine of exposed workers (van Doorn et al. 1980). In the rat, the metabolite $^{14}CO_2$ may account for nearly 50% of inhaled ^{14}C-labeled methyl chloride (Kornbrust and Bus 1983). Unmetabolized methyl chloride is excreted via the lungs in human subjects (Stewart et al. 1980; Nolan et al. 1985; Lof et al. 2000).

4.2. Blood Concentrations and Pharmacokinetic Models

Blood and alveolar-air concentrations of methyl chloride are difficult to correlate with exposure (ATSDR 1998). Differences in uptake between individuals (Stewart et al. 1980; Nolan et al. 1985; Lof et al. 2000), as well as dif-

ferences in measurement methods (Nolan et al. 1985), might be responsible for the lack of correlation.

The pharmacokinetics of methyl chloride in six male subjects exposed at 10 or 50 ppm were studied by Nolan et al. (1985). Air concentrations of methyl chloride in the test chamber were compared with venous-blood and expired-air concentrations. Blood concentrations increased rapidly and reached a plateau during the first hour of exposure and were proportional to the exposure concentration. On the basis of blood and expired-air concentrations, the subjects could be divided into two distinct groups. Two of the subjects had blood concentrations three-fold greater than the other subjects. At 10 ppm, blood concentrations were approximately 30 and 8 ng/mL in the two groups and, at 50 ppm, blood concentrations were approximately 100 and 35 ng/mL (values read from graphs). Blood:air partition coefficients of 2.1 and 2.5 were calculated for the groups with the higher and lower blood values exposed at 10 ppm, respectively. At 50 ppm, blood:air partition coefficients of 1.7 and 1.8 were calculated for the respective groups. Following a single breath of methyl chloride at 500 ppm, held for 20 seconds, blood:air and serum:air partition coefficients were both 0.8 (Morgan et al. 1970). In the Nolan et al. (1985) study, expired-air concentrations exhibited the same temporal uptake; differences in expired-air concentrations were two-fold between the two groups, with higher concentrations in the group with the greater uptake. In slow and rapid metabolizers, expired air contained 30-40% or 70% of the concentration in inhaled air, respectively. Absorption rates of 1.4 µg/min/kg (slow metabolizers) and 3.7 µg/min/kg (rapid metabolizers) were calculated using a two-compartment model. Elimination was rapid in both groups after exposure ended. Elimination was more rapid in volunteers with the lower blood and expired-air concentrations. The authors explained the difference in the two groups by a two-fold difference in the rate at which they metabolized methyl chloride. They considered the difference of questionable toxicologic significance.

In a second controlled chamber study, breath and venous-blood samples were taken before exposure, immediately after exiting from the exposure chamber, and 15- and 30-min after exposure (Stewart et al. 1980). Subjects were exposed at 0, 20, 100, or 150 ppm for 1, 3, or 7.5 h (see study description details in Section 2.3). Blood and breath concentrations varied among individuals and, to a lesser degree, among days. For male subjects with low uptake (see Section 4.2), the mean breath concentrations measured 1 min after exposure to methyl chloride at 100 ppm for 1, 3, or 7.5 h were 33 (1 subject), 40, and 44 ppm, respectively. Two male subjects had considerably higher alveolar concentrations (up to 77 ppm). Weekly measurements of breath concentrations of methyl chloride in females with low uptake ranged from 33 to 47 ppm, with no correlation to exposure duration. Only one of the nine females was a "high-level responder" (up to 76 ppm). At 100 ppm for 7.5 h, blood concentrations pre-exit ranged from 0.1 to 1.3 ppm in low-responder males. Two males with higher alveolar concentrations (one each in the 1- and 3-h exposure groups) also had higher blood concentrations, 9.8 ppm after 1 h and 15.1 ppm after 3 h. Blood concentrations dropped

rapidly during the 15-min post-exposure period, and methyl chloride concentration in expired air dropped so rapidly as to be of little or no value in quantifying exposure. In four low-responder females exposed at 100 ppm for 7.5 h, the average pre-exit blood concentration was 6 ppm. Concentrations for the high-level female responder were 5 and 18 ppm on two different occasions.

The average venous-blood concentrations of methyl chloride in 24 healthy male and female subjects exposed at 100 or 200 ppm for 3 h were 36 and 63 ppm, respectively (Putz-Anderson et al. 1981a). Three of the individuals exposed at 200 ppm had breath concentrations greater than 100 ppm (50% of the exposure concentration). Blood concentrations for the respective exposures were 11.5 and 7.7 ppm. In a second study (Putz-Anderson et al. 1981b), the breath and blood concentrations of male and female subjects exposed at 200 ppm were 74 and 14.5 ppm. The number of high and low responders was not cited, but breath and blood standard deviations of the mean were 43 and 100%, respectively.

Blood concentrations also were studied in 24 subjects who differed in glutathione-*S*-transferase (GST) genotype (Lof et al. 2000). At a concentration of 10 ppm, blood concentrations were similar in individuals with high, intermediate, and low GSH activity (0.5-0.6 μmoL/L); however, uptake was greater and clearance was more rapid in the group with high GSH activity.

The average concentration of methyl chloride in the blood of five beagles exposed at 15,000 ppm over a 6-h period was 6.1 mg% (von Oettingen et al. 1949). Concentrations ranged from 5.1 mg% at 10 min to 7.3 mg% at 240 min. While exposed at 40,000 ppm for 4 h, blood concentrations rose from 12.0 mg% at 10 min to 18.9 mg% at 150 min.

Andersen et al. (1980) determined the kinetic constants for metabolism of inhaled methyl chloride in male F344 rats. Exposure concentrations were not specified (however, for the related chemical, methyl bromide, concentrations were 100, 100, 3,000, and 10,000 ppm); chamber depletion measurements were taken every 10 min for 180 min. Methyl chloride exhibited a mixed-form rate curve, possessing both a saturable and a first-order component. However, the overall uptake rate from first-order processes was negligible and all of the observed uptake was accounted for by the saturable term. Saturation dependence appeared to be associated with enzymatic metabolism. Data were transformed by modified Eadie-Hofstee plots to calculate the inhalational K_m (the ambient concentration at which uptake proceeds at half the maximum rate) and the inhalational V_{max} (the maximum rate of uptake). The authors developed a four-compartment, steady state, pharmacokinetic model to describe gas uptake in general. K_m was 640 ppm and V_{max} was 120 ppm.

In male F344 rats and beagles exposed at 50 or 1,000 ppm for 3 h, blood concentrations rapidly reached steady-state concentrations which were proportional to the exposure concentration (Landry et al. 1983). Blood concentrations were similar in the two species at each concentration (values were slightly higher for the rat than the dog). A linear two-compartment model with zero order uptake and first-order output described methyl chloride pharmacokinetics in

both species. Apparent steady-state blood values after exposure to methyl chloride at 50 or 1,000 ppm were 160 and 3,690 ng/g (dogs) and 194 and 3,930 ng/g (rats). The total areas under the blood-concentration curve (AUCs) were 70 and 3,930 μg/g/min for rats exposed at 50 and 1,000 ppm for 6 h, and 28 and 659 μg/g/min for dogs exposed at 50 and 1,000 ppm, respectively, for 3 h. The AUCs estimated for rats at 3 h are 35 and 710 μg/g/min, respectively. There was no indication of saturable metabolism based on blood concentrations. End-exposure blood concentrations for dogs were approximately 20-fold greater at 1,000 ppm compared with 50 ppm, reflecting the ratio of exposure concentrations. Metabolism ratios, normalized to body weight, were similar for the rat and dog.

Landry et al. (1983) and Nolan et al. (1985) reported that the relative steady-state blood concentrations of methyl chloride after a 3-h exposure was 194 ng/g in rats, 160 ng/g in dogs, 100 ng/g in rapidly metabolizing humans, and 35 ng/g in slowly metabolizing humans. The rat absorbed 10 μg/min/kg, and humans absorbed 3.7 μg/min/kg (rapid metabolizers) and 1.4 μg/min/kg (slow metabolizers).

4.3. Mechanism of Toxicity

Acute CNS effects might be from methyl chloride (this compound was once used as an anesthetic in combination with other anesthetics), but rapid metabolism probably limited its efficacy as an anesthetic.

Recent reviews state that the mechanism of neurotoxicity from methyl chloride is unclear, but most probably involves the metabolism of methyl chloride (i.e., the conjugation of methyl chloride with GSH). Acute exposures of rats and mice cause significant reductions in GSH concentrations in numerous organs, including the liver, kidneys, lungs, and brain (Dodd et al. 1982; Landry et al. 1983; Kornbrust and Bus 1984). A 6-h exposure of male F344 rats to methyl chloride at 1,500 ppm decreased the nonprotein sulfhydryl (primarily GSH) content of liver, kidney, and lungs to 17, 27, and 30% of control values, respectively (Dodd et al. 1982). At 500 ppm, values were 41, 59, and 55% of control values, respectively. At 1,500 ppm, recoveries to control concentrations occurred within 8 h. There were no changes in tissue GSH following a 6-h exposure to methyl chloride at 100 ppm. Blood concentrations of nonprotein sulfhydryl were not affected by the exposures. In a related study with male F344 rats, brain concentrations of GSH were reduced to a much lesser extent compared with the liver and kidney (Kornbrust and Bus 1984). The same tissues of male $B6C3F_1$ mice were affected to a much greater degree. In another study, tissue nonprotein sulfhydryl in the liver, kidney, testis, and epididymis of rats decreased in a concentration-dependent manner in rats exposed at 225, 600, or 1,000 ppm for 6 h (Landry et al. 1983). No significant decreases of GSH were observed in the brain or blood.

Inhibition of GSH conjugation decreases the toxicity of methyl chloride (White et al. 1982; Chellman et al. 1986b). Mice exposed to methyl chloride at 1,500 ppm for 6 h/day, 5 days a week for 2 weeks developed multiple degenerative, necrotic foci in the internal granule cell layer of the cerebellum. Tremors, ataxia, and forelimb and hindlimb paralysis were associated with the cerebellar damage. Pretreatment of mice with buthionine-S,R-sulfoxime, a GSH depleter, protected mice from the cerebellar degeneration. Methanethiol and formaldehyde have been suggested as the toxic metabolites. Treatment of mice with methanethiol produces the same CNS symptoms of tremors, convulsion, and coma, as seen in animals and humans acutely intoxicated with methyl chloride (Heck et al. 1982; Kornbrust and Bus 1983; Chellman et al. 1986b).

Methyl chloride induces dominant lethal effects in several strains of rats. The mechanism of reproductive toxicity appears attributable to cytotoxic effects on sperm in the testes and to the effects of genotoxic oxidative metabolites resulting from an induced inflammatory response in the epididymides (Working et al. 1985b; Chellman et al. 1986c). Using both male and female F344 rats and male and female $B6C3F_1$ mice exposed to methyl chloride at 1,000 ppm for 6 h/day for 4 days, Jager et al. (1988) found no biochemical sex differences in enzymatic transformation with respect to formaldehyde dehydrogenase or formaldehyde-induced genetic damage that would explain tumor formation. Mice, which have higher GSH activity in the kidneys than other species, appear to be more susceptible to methyl chloride.

Although the exact mechanism of action of methyl chloride is unclear, it appears to involve GSH depletion in target tissues and the production of toxic metabolites, such as formaldehyde or methanethiol, as well as lipid peroxidation. A lack of GSH might impair the ability of tissues to suppress lipid peroxidation reactions (Kornbrust and Bus 1984). The buildup of leukotrienes also has been suggested as a toxic mechanism of action. Under conditions of GSH depletion, 5-hydroperoxicoso-tetraenoic acid, the immediate precursor of leukotrienes, accumulates in the tissues. Leukotrienes are potent vasoconstrictors, and cause increased capillary permeability and tissue edema (AIHA 2003). Pretreatment of male F344 rats with the anti-inflammatory agent BW755C prevented inflammatory lesions of the brain, kidney, liver, and testis after exposure to methyl chloride at 5,000 ppm for 5 days (Chellman et al. 1986a).

A suggested mechanism of action for renal tumors in male mice is GSH depletion in the target tissue, increased lipid peroxidation, and formation of formaldehyde-induced DNA lesions (Bolt and Gansewendt 1993). Once GSH is depleted in the tissues, the alternate oxidative pathway via P-450 leads to formation of formaldehyde. Renal microsomes of male mice are more active than those of females in transforming methyl chloride to formaldehyde (Jager et al. 1988). Although Jager et al. (1988) did not observe increased formaldehyde concentrations in mouse liver or kidneys after a single, 8-h exposure at 1,000 ppm or increased DNA protein crosslinks after 4 days of exposure at 1,000 ppm, Ristau et al. (1989) observed an increase in DNA-protein crosslinks immediately

after an 8-h exposure. These crosslinks were attributed to the action of formaldehyde and were rapidly repaired.

4.4. Structure-Activity Relationships

von Oettingen et al. (1950) compared the toxicity of methyl chloride with that of dichloro-, trichloro-, and tetrachloro-methane in an unidentified strain of mice. The 7-h LC_{50} values were 3,080, 16,186, 5,761, and 9,528 ppm, respectively. The authors noted that methyl chloride had little narcotic action compared with the other chlorinated methanes. In the same study, dogs were found to be more susceptible to trichloromethane than methyl chloride, as measured by survival when exposed at 15,000 ppm.

For halogenated methanes, toxicity increases in the order methyl chloride, methyl bromide, and methyl iodide, but all produce similar toxic syndromes in man (Gosselin et al. 1984). However, few studies using comparable concentrations and exposure durations were available to make comparisons.

4.5. Other Relevant Information

4.5.1. Species Variability

There are differences in the rate at which species metabolize methyl chloride. The kinetics of methyl chloride in man are similar to those described for the rat and dog (Landry et al. 1983; Nolan et al. 1985). Blood concentrations reached a plateau during the first hour of exposure in all species and elimination was rapid once the exposures were terminated. Blood concentrations in slow human metabolizers reach a plateau at 60% of those found in the rat and 70% of those found in the dog. Post-exposure elimination was most rapid in the rat ($t_{1/2}$ = 15 min). The dog and rapid human metabolizers eliminated methyl chloride at the same rate ($t_{1/2}$ = 50 min), and the slow human metabolizers eliminated at the slowest rate ($t_{1/2}$ = 90 min). During exposures to methyl chloride at 50 ppm, the rat absorbed 10 µg/min/kg whereas the rapid and slow human metabolizers absorbed 3.7 and 1.4 µg/min/kg. According to Nolan et al. (1985), differences in the pharmacokinetics between these three species were adequately explained by the differences in respiratory minute-volume and basal-metabolic rates (rat > dog > man). Using measurements of liver and kidney cytosol, Thier et al. (1998) placed the metabolism of methyl chloride in the following order: $B6C3F_1$ female mouse > $B6C3F_1$ male mouse > F344 rat > hamster. Activity was two to seven times greater in liver cytosol than in kidney cytosol.

As noted in several laboratory studies, mice are more susceptible to the toxic effects of methyl chloride than rats. In both control and exposed animals (1,000 ppm, 6 h/day for 4 days), specific activity of GST was greater in the livers of male $B6C3F_1$ mice than in female mice (by a factor of 2) and greater in the livers of male mice than in F344 rats of either sex (by a factor of 3). GST

activity was similar in the kidneys of male and female mice. Formaldehyde dehydrogenase (FDH) activity was higher in the liver and kidneys of mice than in rats. There were no sex differences in activity of FDH. Exposure to methyl chloride at1,000 ppm for 1 or 4 days reduced the activity of GST in the kidneys of male mice by approximately 10%; activity levels in the livers of male mice and kidneys of female mice and male and female rats did not change (Jager et al. 1988). In in vitro assays, sex-, strain- and species-specific differences in bioactivation of methyl chloride by cytochrome P-450 was seen in the liver and kidneys of rats and mice (Dekant et al. 1995). In kidney microsomes, the rate of oxidation of methyl chloride in mice was significantly greater in males than in females, and in rats of both sexes than in mice. The rate of oxidation in kidney microsomes was faster in CD-1 mice and NMRI mice than in C3H/He or C57Bl/6J mice. Oxidative dechlorination of methyl chloride via the P-450 system might lead to the formation of formaldehyde.

The available data for the related chemical, methyl bromide, allowed comparisons between rats and mice. Where data were available for the same exposure durations, mice were more susceptible to the lethal effects of methyl bromide than rats (see Chapter 5). On the basis of 30-min and 1- and 4-h LC_{50} values, the mouse is two-fold more sensitive to methyl bromide than the rat. This increased sensitivity may be related to the higher concentrations of GST found in mouse tissues (Griem et al. 2002). Increased sensitivity might also be a reflection of the higher respiratory rate of mice compared with rats.

Erythrocyte cytoplasm of rats, mice, Rhesus monkeys, cows, pigs, and sheep does not metabolize monohalomethanes, whereas the erythrocyte cytoplasm of approximately 60% of humans does (Redford-Ellis and Gowenlock 1971; Peter et al. 1989). Lack of erythrocytic metabolism might explain the rapid equilibrium between the gas phase of methyl chloride and methyl bromide and concentrations in whole blood of rats observed in pharmacokinetic studies.

For the related chemical, methyl bromide, the available data indicate that rabbits are more sensitive than rats or guinea pigs. Guinea pigs and rats withstood 6-month exposures to methyl chloride at 66 ppm without demonstrable effects, but rabbits became paralyzed (Irish et al. 1940). A similar result was observed in a more recent study (Anger et al. 1981). Rabbits exposed at 65 ppm began to lose weight by the third week of exposure and eyeblink responses and nerve conduction velocity in rabbits were significantly reduced. Rats were unaffected under the same exposure regime. Maternal toxicity was high in pregnant rabbits exposed to methyl bromide at 70 ppm before mating and through gestation, whereas no maternal toxicity was evident in rats exposed under the same scenario (Sikov et al. 1981; Hardin et al. 1981).

Methyl bromide was specifically toxic to the olfactory epithelium of the rat, whereas the other nasal epithelia were unaffected (Hurtt et al. 1988). Histochemical techniques revealed degeneration of the sensory and sustentacular cells but not the basal cells from which the former cells are regenerated. This was a reversible effect as the basal cells were not affected. The olfactory region (dorsal meatus) of rats is highly exposed to chemicals because of the air-flow character-

istics in the nasal turbinates. In rodents, an inhaled vapor traverses a few millimeters of resistant respiratory epithelium before reaching sensitive olfactory tissue; whereas, in humans, an inhaled vapor has to traverse several centimeters and a much larger surface area of respiratory epithelium to reach the olfactory tissue. A mathematical model based on a combination of computational fluid dynamics and physiologically-based pharmacokinetics showed that the dorsal meatus region of the rat nose receives 12-20% of the inhaled air (Bush et al. 1998; Frederick et al. 1998). A comparison with airflow patterns in the human nose shows that the olfactory epithelium in the dorsal meatus region of the nasal cavity of the rat is exposed at two- to three-fold greater concentrations of chemicals. Therefore, compared with the rat, it is likely that higher concentrations of methyl bromide would be required to induce this lesion in humans.

Comparison of data from in vitro and in vivo studies indicates that GST enzymes are much lower in human tissues (liver and lung) than in mice or rats (Andersen et al. 1987; Reitz et al. 1989). The data are consistent with the hypothesis that the rate of activation of mono- and di-halomethanes to toxic metabolites by the GST pathway occurs much more slowly in humans than in rodents. Jager et al. (1988) investigated the concentration of GSH in rodent tissues. Activities of GSH were 2-3 times greater in the liver of male $B6C3F_1$ mice than in female mice or F344 rats of both sexes. Griem et al. (2002) compiled rodent-to-human ratios of GST activity in various tissues. Ratios of rat-to-human and mouse-to-human GST activity in liver are 3.95 and 7.64, respectively. On the other hand, nonprotein sulfhydral content (primarily GSH) is similar in human, monkey, and rat tissues on a μmol/mL of tissue basis (Frederick et al. 2002). This was true for major organs, but not nasal tissue. Nonprotein sulfhydral content was greater in the nasal tissues of rats than humans.

4.5.2. Susceptible Populations

Interindividual variation in the rate of metabolism of methyl chloride has been observed in humans. At least two distinct populations of humans with different rates of metabolism of methyl chloride have been identified (Nolan et al. 1985; ATSDR 1998; WHO 2000). Differences in metabolic rate are attributed to the genetic polymorphism of GST. Depending on the presence or absence of GST, humans may be "fast metabolizers" or "slow metabolizers" of methyl chloride. There might be a third phenotype, non-conjugators (Warholm et al. 1994; Lof et al. 2000). Fast metabolism might lead to the formation of toxic metabolites that can exert their action before they can be eliminated. Slow metabolizers would be expected to be less susceptible to the toxic effects of methyl chloride. Because the elimination of methyl chloride is rapid in both populations, the difference is of questionable toxicologic significance. In addition, Nolan et al. (1985) noted that an exposure to methyl chloride at 50 ppm for 6 h did not affect blood GST concentrations of either population.

Among Caucasians, the majority of individuals possess at least one copy of the GST gene; 10-25% are non-conjugators (Warholm et al. 1994; Nelson et al. 1995; Kempkes et al. 1996). Approximately 60% of Asians lack the gene (Nelson et al. 1995).

Some forms of GST are present in the human fetal liver and other forms are present at birth at a low rate and increase after birth (Cresteil 1998).

As noted in ATSDR (1998), populations that have kidney or liver disease, anemia, or neurologic deficits might be more susceptible to the toxic effects of methyl chloride. Persons with a deficiency of glucose-6-phosphate dehydrogenase might have reduced concentrations of GSH (Bloom and Brandt 2001). Additionally, accidental exposures suggest that infants are more susceptible than adults (Langard et al. 1996). However, in the latter case, death of an infant was from acute pneumonia resulting from vomiting and aspiration after methyl bromide inhalation.

4.5.3. Concentration-Exposure Duration Relationship

At constant concentrations of methyl chloride at ≤200 ppm, the highest concentration in clinical studies, blood concentrations reach steady state within 1 h (Putz-Anderson et al. 1981a). A similar relationship between air and blood values was observed in animal studies.

4.5.4. Concurrent Exposure Issues

Putz-Anderson et al. (1981a,b) assessed the behavioral effects of inhaled methyl chloride alone (200 ppm for up to 3.5 h) or in combination with 10 mg oral diazepam (a CNS depressant), alcohol (0.8 mL/kg), or caffeine (3 mg/kg). Subjects were groups of 8 or 12 healthy males and females, ages 18 to 32 years. Three performance tests (visual vigilance, dual task, and time discrimination), designed to test attention or alertness, were administered before and during the treatment period. Exposure to methyl chloride at 200 ppm produced little or no behavioral impairment, whereas diazepam and alcohol alone produced a 10% (statistically significant) impairment. Caffeine produced a small, but significant impairment on only one test. When the drugs were administered in combination with methyl chloride, the effect was equivalent to the sum of the separate effects (there were no behavioral interactions).

5. DATA ANALYSIS FOR AEGL-1

5.1. Summary of Human Data Relevant to AEGL-1

There are few data on acute exposures to methyl chloride. In well-conducted clinical studies, methyl chloride at 10 ppm for 2 h (Lof et al. 2000),

10 or 50 ppm for 6 h (Nolan et al. 1985), 100 ppm for 7.5 h/day for 5 days (Stewart et al. 1980), 150 ppm for 7.5 h/day for 2 days (Stewart et al. 1980), or 200 ppm for 3.5 h (Putz-Anderson et al. 1981b) produced no discernible irritant or neurotoxic effects in human subjects. Both male and female subjects were tested, exercise was incorporated into the protocol of the Stewart et al. (1980) study, and adequate numbers of subjects were tested.

In reports of accidental exposures, the threshold for neurotoxic symptoms such as blurred vision and sleepiness appeared to be 1,000-2,000 ppm (MacDonald 1964). In occupational monitoring studies, chronic exposures to ≤200 ppm TWA (15-195 ppm) with peaks up to 500 ppm did not produce neurotoxic symptoms, whereas prolonged exposures to a TWA ≥265 ppm for 12-16 h workdays for 2-3 weeks resulted in neurologic symptoms (Dow Chemical Co. 1970, personal communication, as cited in ACGIH 2003; Scharnweber et al. 1974).

5.2. Summary of Animal Data Relevant to AEGL-1

Most animal studies of methyl chloride involved a repeat-exposure scenario. In the only acute study, no clinical signs were observed in male F344 rats exposed to methyl chloride at 100, 500, or 1,500 ppm for 6 h (Dodd et al. 1982). No histopathologic examinations were performed in this study. No clinical signs or tissue or organ lesions were observed in F344 rats exposed to methyl chloride continuously at 200 ppm for 48 h (Burek et al. 1981). No microscopic lesions were observed in rats exposed at 1,000 ppm for 6 h/day for 12 days (Morgan et al. 1982). Mice were more sensitive than rats; some strains showed hepatocellular degeneration after exposure at 500 ppm for 6 h/day for 12 days (Morgan et al. 1982). Lesions were not observed in other tissues. The no-effect concentration in rats, mice, and dogs exposed to methyl chloride for 6 h/day, 5 days/week for 13 weeks was 400 ppm (McKenna et al. 1981b).

5.3. Derivation of AEGL-1 Values

No adverse neurotoxic effects were observed in a clinical study in which healthy adults were given a single exposure to methyl chloride at 200 ppm for 3 or 3.5 h (Putz-Anderson et al. 1981a,b) or in a 5-day repeated exposure study of exercising adults exposed at 150 ppm for 7.5 h/day (Stewart et al. 1980). The subjects included both “fast” and “slow” metabolizers. The exposures failed to elicit physiologic, neurologic, behavioral, or clinical symptoms. Furthermore, in the absence of a clearly defined odor at the concentrations tested, the subjects were unable to differentiate between control and exposure days. None of the exposures produced mild, transient effects on which to base the AEGL-1 values. Because methyl chloride has no clearly defined odor or warning properties at concentrations that might be neurotoxic, AEGL-1 values are not recommended.

6. DATA ANALYSIS FOR AEGL-2

6.1. Summary of Human Data Relevant to AEGL-2

No reliable human data on acute exposures to methyl chloride were available. Accidental exposures at chemical plants provide an indication of the threshold for neurotoxic symptoms. In these reports, only workplace TWA concentrations were provided; personal monitors were not utilized. Most of the cases involved repeat exposures. These data indicate that sleepiness, dizziness, and blurred vision might occur at concentrations of 1,000-2,000 ppm, although repeated exposure at 2,000-4,000 ppm induced similar symptoms; very short exposures at 10,000 ppm also were tolerated with similar symptoms (MacDonald 1964). Reports of accidental exposures at a chemical plant document neurotoxic symptoms following a short exposure to methyl chloride at 1,000-2,000 ppm, after work for several days in an area where the measured area concentration ranged from <25 to 1,600 ppm, during 13 days of exposure at 2,000-4,000 ppm, and after brief excursions into an area where the measured concentration was >10,000 ppm. Prolonged exposures to methyl chloride for 12-16 h over several weeks at concentrations as low as 300 ppm might cause symptoms in some workers (Scharnweber et al. 1974).

6.2. Summary of Animal Data Relevant to AEGL-2

Except for an acute exposure of F344 rats to methyl chloride at 1,500 ppm for 6 h, which resulted in no clinical signs (Dodd et al. 1982), all other animal studies involved repeat exposures. No clinical signs or tissue or organ lesions were observed in rats exposed at 1,500 ppm for 6 h/day, 5 days/week for 90 days (Mitchell et al. 1979). Slight decrements in performance on a rotating rod were observed in female mice exposed at 800 and 1,600 ppm for 5.5 h/day for 4 days, but not after 8 or 11 days of exposure (Landry et al. 1985). No brain or spinal-cord lesions were observed in dogs exposed at 400 ppm for 5 h/day for 13 weeks (McKenna et al. 1981b).

6.3. Derivation of AEGL-2 Values

The AEGL-2 values for methyl chloride were based on several rat studies, and a monitoring study was used as support. The basis for the AEGL-2 values was the absence of clinical signs in rats exposed to methyl chloride at 1,500 ppm for 6 h/day for 1 day (Dodd et al. 1982) or 90 days (Mitchell et al. 1979). Because of the greater blood uptake of methyl chloride by rodents compared with humans (Landry et al. 1983; Nolan et al. 1985), an interspecies uncertainty factor of 1 was applied. Although humans differ in the rate at which they metabolize methyl chloride, the difference does not appear to be toxicologically significant (Nolan et al. 1985). Furthermore, most humans are rapid metabolizers, the more susceptible group. Therefore, an uncertainty factor of 3 was considered

appropriate for intraspecies differences. In the absence of time-scaling information, the AEGL values were scaled using the equation $C^n \times t = k$, with default values of n = 3 for shorter durations and n = 1 for longer durations (NRC 2001). Because of the long exposure duration of the key study, the 10-min value was set equal to the 30-min value (see Table 6-7). Accidental exposures to methyl chloride at 1,000-2,000 ppm and repeated exposure at 2,000-4,000 ppm resulted in transient symptoms of blurred vision, dizziness, headache, and nausea in workers (MacDonald 1964). Exposure durations were not reported, but appeared to be throughout the workday. The application of an intraspecies uncertainty factor of 3 to the mean concentration of 1,500 ppm reported in this occupational monitoring report results in a value of 500 ppm, which is similar to the 4- and 8-h AEGL-2 values. Calculations are provided in Appendix A, and a category graph of the human and animal toxicity data in relation to the AEGL values are provided in Appendix B.

7. DATA ANALYSIS FOR AEGL-3

7.1. Summary of Human Data Relevant to AEGL-3

Although deaths have occurred from accidental exposures to methyl chloride, no reliable information on concentrations or exposure durations were available. Monitoring data indicate that healthy workers can withstand concentrations of methyl chloride at 2,000-4,000 ppm for several days and brief excursions at 10,000 ppm (MacDonald 1964).

7.2. Summary of Animal Data Relevant to AEGL-3

Only one group of researchers has published recent data on lethality (White et al. 1982; Chellman et al. 1986b). Older studies (before 1950) do not meet current testing standards, although results are similar to those reported by Chellman et al. (1986b). The LC_{50} values for methyl chloride in male and female $B6C3F_1$ mice are 2,250 and 8,500 ppm, respectively (White et al. 1982; Chellman et al. 1986b). Few acute lethality data were available for the rat. Repeat exposures of $B6C3F_1$ mice to methyl chloride at 1,000 ppm for 6 h/day for up to 12 days resulted in microscopic tissue lesions, but no deaths (Morgan et al. 1982). Similar results were observed when F344 rats were exposed to methyl chloride at 5,000 ppm for 5 days, although one rat died following the last exposure (Chellman et al. 1986a). The next highest concentration of 7,500 ppm for 6 h/day for 2 days resulted in 67% mortality during the 4-day post-exposure period (Chellman et al. 1986a).

TABLE 6-7 AEGL-2 Values for Methyl Chloride

10 min	30 min	1 h	4 h	8 h
1,100 ppm ($2,277 mg/m^3$)	1,100 ppm ($2,277 mg/m^3$)	910 ppm ($1,884 mg/m^3$)	570 ppm ($1,180 mg/m^3$)	380 ppm ($787 mg/m^3$)

7.3. Derivation of AEGL-3 Values

Data on lethality are limited to LC_{50} values for the mouse, a particularly sensitive species. Because of the higher respiratory rate in mice and the higher concentrations of GSH in mouse liver and kidney, particularly the male mouse, compared with human tissues, the mouse is not considered an appropriate surrogate for human response to methyl chloride. On the basis of data from a monitoring study in which humans withstood repeated exposures to methyl chloride at 1,000-4,000 ppm, the 6-h LC_{50} of 2,200 ppm in male mice is not realistic for application to humans.

Two studies reported no deaths in rats during the first 4 days of 5- and 12-day exposures to methyl chloride at 5,000 ppm for 6 h/day (Morgan et al. 1982; Chellman et al. 1986a). Chellman et al. (1986a) reported that 1/5 rats died following the fifth day of exposure. Morgan et al. (1982) reported that no deaths occurred in a 12-day exposure study, although 6/10 males and 5/10 females exposed at 5,000 ppm (6 h/day) and 2/10 females exposed to 3,500 ppm (6 h/day) were moribund and were killed after the fifth day of exposure. Thus, similar responses (death or near death) were observed in both studies. However, since a single 6-h exposure to methyl chloride at 5,000 ppm did not produce death in either study, this value was considered the point-of-departure for lethality. As was done for the AEGL-2 values, interspecies and intraspecies uncertainty factors of 1 and 3, respectively, were applied. Time-scaling was performed using the equation $C^n \times t = k$, with n = 1 for longer durations and n = 3 for shorter durations (NRC 2001). Because of the long exposure duration in the key studies, the 10-min value was set equal to the 30-min value (see Table 6-8). Calculations are provided in Appendix A, and a category graph of the toxicity data in relation to AEGL values are provided in Appendix B.

8.2. Comparison with Other Standards and Guidelines

Standards and guidelines for methyl chloride are summarized in Table 6-10. The American Industrial Hygiene Association did not derive a level-1 emergency response planning guideline (ERPG-1) for methyl chloride "because the odor threshold is greater than 200 ppm" (AIHA 2003). The 1-h ERPG-2 value of 400 ppm is one-half of the 1-h AEGL-2 value. The basis for the ERPG-2 value was several studies with laboratory animals; in particular, the basis was the no-observed effect level of 400 ppm for clinical signs and tissue lesions in rats, mice, and dogs exposed for 6 h/day, 5 days/week for 13 weeks (McKenna et al. 1981b). The AEGL-2 is based on a combination of human and animal data, but

TABLE 6-8 AEGL-3 Values for Methyl Chloride

10 min	30 min	1 h	4 h	8 h
3,800 ppm (7,866 mg/m^3)	3,800 ppm (7,866 mg/m^3)	3,000 ppm (6,210 mg/m^3)	1,900 ppm (3,933 mg/m^3)	1,300 ppm (2,691 mg/m^3)

particularly on the single and repeat exposures to methyl chloride at 1,500 ppm. The ERPG-3 was based on LC_{50} values for the mouse and rat which were apparently reduced by a factor of 2. The mouse and rat are more sensitive to methyl chloride than humans; therefore, although the AEGL-3 values were based on animal data, values were chosen that were supported by occupational exposure data.

8. SUMMARY OF AEGLS

8.1. AEGL Values and Toxicity Endpoints

AEGL values are listed in Table 6-9. A derivation summary is provided in Appendix C.

The NIOSH immediately dangerous to life and health (IDLH) value for methyl chloride is 2,000 ppm (NIOSH 1994). The IDLH is based on the report by MacDonald (1964) that a worker exposed to methyl chloride at 2,000 to 4,000 ppm for 13 days became sleepy and dizzy during the first week. A second worker exposed at 1,000-2,000 ppm during the workshift experienced dizziness, blurred vision, headache, nausea, and vomiting. The same study was used as support for the AEGL-3 values.

Chronic workplace standards for methyl chloride are lower than the AEGL values, and range from 25 ppm to 50 ppm. ACGIH (2003) noted that an exposure to methyl chloride at 100 ppm would be without health effects, but reduced the value to 50 ppm on the basis of a potentially limited margin of safety. ACGIH also recognized that repeated exposures might lead to cumulative effects. The German maximum workplace concentration (i.e., MAK) carries carcinogen, pregnancy, and skin absorption notations.

TABLE 6-9 Summary of AEGL Values for Methyl Chloride

Classification	10 min	30 min	1 h	4 h	8 h
AEGL-1 (nondisabling)	NR[a]	NR[a]	NR[a]	NR[a]	NR[a]
AEGL-2 (disabling)	1,100 ppm (2,277 mg/m^3)	1,100 ppm (2,277 mg/m^3)	910 ppm (1,884 mg/m^3)	570 ppm (1,180 mg/m^3)	380 ppm (787 mg/m^3)
AEGL-3 (lethal)	3,800 ppm (7,866 mg/m^3)	3,800 ppm (7,866 mg/m^3)	3,000 ppm (6,210 mg/m^3)	1,900 ppm (3,933 mg/m^3)	1,300 ppm (2,691 mg/m^3)

[a]AEGL-1 values are not recommended (NR) because methyl chloride has no odor or warning properties at concentrations that may be neurotoxic.

TABLE 6-10 Extant Standards and Guidelines for Methyl Chloride

Guideline	Exposure Duration				
	10 min	30 min	1 h	4 h	8 h
AEGL-1	NR[a]	NR[a]	NR[a]	NR[a]	NR[a]
AEGL-2	1,100 ppm	1,100 ppm	910 ppm	570 ppm	380 ppm
AEGL-3	3,800 ppm	3,800 ppm	3,000 ppm	1,900 ppm	1,300 ppm
ERPG-1 (AIHA)[b]			NA		
ERPG-2 (AIHA)			400 ppm		
ERPG-3 (AIHA)			1,000 ppm		
IDLH (NIOSH)[c]		2,000 ppm			
TLV-TWA (ACGIH)[d]					50 ppm (skin notation)[e]
REL-TWA (NIOSH)[f]					Lowest feasible concentration
PEL-TWA (OSHA)[g]					100 ppm
PEL-C (OSHA)[h]					200 ppm; 300 ppm (5-min maximum peak in any 3 h)
TLV-STEL (ACGIH)[i]	100 ppm				
MAK (Germany)[j]					50 ppm (skin notation)[e]
MAK Peak Limit (Germany)[k]		Excursions of 15 min duration at twice the MAK may take place 4 times/work shift at 1-h intervals			
MAC (The Netherlands)[l]					25 ppm

[a]AEGL-1 values are not recommended (NR) because methyl chloride has no odor or warning properties at concentrations that may be neurotoxic.

[b]ERPG (emergency response planning guidelines, American Industrial Hygiene Association (AIHA 2003).

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.
[c]IDLH (immediately dangerous to life or health, National Institute for Occupational Safety and Health) (NIOSH 1994) 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]Dermal absorption might contribute substantially to systemic intoxication.
[f]REL-TWA (recommended exposure limit - time weighted average, National Institute for Occupational Safety and Health) (NIOSH 2010) is defined analogous to the ACGIH TLV-TWA.
[g]PEL-TWA (permissible exposure limit – time weighted average, Occupational Safety and Health Administration) (NIOSH 2010) is defined analogous to the ACGIH TLV-TWA, but is for exposures of no more than 10 h/day, 40 h/week.
[h]PEL-C (permissible exposure limit-ceiling, Occupational Safety and Health Administration) (NIOSH 2010) is a value that must not be exceeded during any part of the workday.
[i]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 which should not be exceeded at any time during the workday even if the 8-h TWA is within the TLV-TWA. Exposures greater than the TLV-TWA and 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.
[j]MAK (maximale arbeitsplatzkonzentration [maximum workplace concentration]) [German Research Association (DFG 2003)] is defined analogous to the ACGIH TLV-TWA.
[k]MAK spitzenbegrenzung (peak limit) [German Research Association (DFG 2003)] constitutes the maximum average concentration to which workers can be exposed for a period up to 30 min with no more than two exposure periods per work shift; total exposure may not exceed 8-h MAK.
[l]MAC (maximaal aanvaarde concentratie [maximal accepted concentration]) (Dutch Expert Committee for Occupational Standards, The Netherlands) (MSZW 2004) is defined analogous to the ACGIH TLV-TWA.
Abbreviations: NA, not applicable; NR, not recommended.

8.3. Data Adequacy and Research Needs

The data from two well-conducted clinical studies define a no-effect concentration in healthy adults, and additional data were available from monitoring studies. Because there is inherent uncertainty in monitoring data, AEGL-2 and AEGL-3 values were based on both animal toxicity data and human monitoring studies. Most animal toxicity studies used a repeat-exposure scenario. Using

such studies for assess single exposures provides a margin of safety for AEGL-2 and AEGL-3 values. Although the neurotoxic mechanism of action of methyl chloride is not completely understood, metabolism is similar in various species and rate of metabolism appears related to body size and respiratory rate. Data were available on relative uptake and blood concentrations among species.

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APPENDIX A

DERIVATION OF AEGL VALUES FOR METHYL CHLORIDE

Derivation of AEGL-1 Values

AEGL-1 values are not recommended because methyl chloride has no odor or warning properties at concentrations that might be neurotoxic.

Derivation of AEGL-2 Values

Key studies:	Mitchell, R.I., K. Pavkov, R.M. Everett, and D.A. Holzworth. 1979. A Ninety Day Inhalation Toxicology Study in F-344 Albino Rats and B6C3F1 Mice Exposed to Atmospheric Methyl Chloride Gas. Battelle Columbus Laboratories for the Chemical Industry Institute of Toxicology, Research Triangle Park, NC. Microfiche No. OTS0205952. Dodd, D.E., J.S. Bus, and C.S. Barrow. 1982. Nonprotein sulfhydryl alterations in F-344 rats following acute methyl chloride inhalation. Toxicol. Appl. Pharmacol. 62(2):228-236.
Toxicity end point:	No clinical signs in rats exposed at 1,500 ppm for 6 h. No clinical signs in rats exposed at 1,500 ppm for 6 h/day, 5 days/week for 90 days.
Time scaling:	$C^n \times t = k$, default values of n = 3 for shorter exposure durations and n = 1 for longer exposure durations. $(1{,}500 \text{ ppm}/3)^3 \times 360 \text{ min} = 4.5 \times 10^{10} \text{ ppm}^3\text{-min}$ $(1{,}500 \text{ ppm}/3) \times 360 = 1.8 \times 10^5 \text{ ppm-min}$
Uncertainty factors:	1 for interspecies differences; uptake is greater in rats than in humans. 3 for intraspecies variability; differences in metabolism were not considered toxicologically significant.
Modifying factor:	Not applicable

Calculations:

10-min AEGL-2:	Set equal to the 30-min value because of the long exposure durations of the key studies.
30-min AEGL-2:	$C = (4.5 \times x\ 10^{10}\ ppm^3\text{-min} \div 30min)^{1/3}$ $C = 1{,}100$ ppm
1-h AEGL-2:	$C = (4.5 \times 10^{10}\ ppm^3\text{-min} \div 60\ min)^{1/3}$ $C = 910$ ppm
4-h AEGL-2:	$C = (4.5 \times 10^{10}\ ppm^3\text{-min} \div 240\ min)^{1/3}$ $C = 570$ ppm
8-h AEGL-2:	$C = (1.8 \times 10^5\ ppm\text{-min}) \div 480$ min $C = 380$ ppm

Derivation of AEGL-3 Values

Key studies:	Morgan, K.T., J.A. Swenberg, T.E. Hamm, Jr., R. Wolkowski-Tyl, and M. Phelps. 1982. Histopathology of acute toxic response in rats and mice exposed to methyl chloride by inhalation. Fundam. Appl. Toxicol. 2(6):293-299. Chellman, G.J., K.T. Morgan, J.S. Bus, and P.K. Working. 1986a. Inhibition of methyl chloride toxicity in male F-344 rats by the anti-inflammatory agent BW755C. Toxicol. Appl. Pharmacol. 85(3):367-379.
Toxicity end point:	5,000 ppm for 6 h/day for 12 days was nonlethal to rat for 5 days, one death following the fifth day of exposure. 5,000 ppm for 6 h/day for 5 days was nonlethal to rats.
Time scaling:	$C^n \times t = k$; default values of n = 3 for shorter exposure durations and n = 1 for longer exposure durations. $(5{,}000\ ppm \div 3)^3 \times 360\ min = 1.67 \times 10^{12}\ ppm^3\text{-min}$ $(5{,}000\ ppm \div 3) \times 360 = 6.0 \times 10^5\ ppm\text{-min}$

Uncertainty factors:	1 for interspecies difference; uptake is greater in rats than in humans 3 for intraspecies variability; differences in metabolism were not considered toxicologically significant.
Modifying factor:	Not applicable
Calculations:	
10-min AEGL-3:	Set equal to the 30-min value because of the long exposure durations of the key studies.
30-min AEGL-3:	$C = (1.67 \times 10^{12}\ ppm^3\text{-min} \div 30\ min)^{1/3}$ $C = 3{,}800$
1-h AEGL-3:	$C = (4.5 \times 10^{12}\ ppm^3\text{-min} \div 60\ min)^{1/3}$ $C = 3{,}000$ ppm
4-h AEGL-3:	$C = (1.67 \times 10^{12}\ ppm^3\text{-min} \div 240\ min)^{1/3}$ $C = 1{,}900$ ppm
8-h AEGL-3:	$C = (6.0 \times 10^{5}\ ppm^3\text{-min}) \div 480\ min$ $C = 1{,}300$ ppm

APPENDIX B

CATEGORY GRAPH OF HUMAN AND ANIMAL TOXICITY DATA AND AEGL VALUES FOR METHYL CHLORIDE

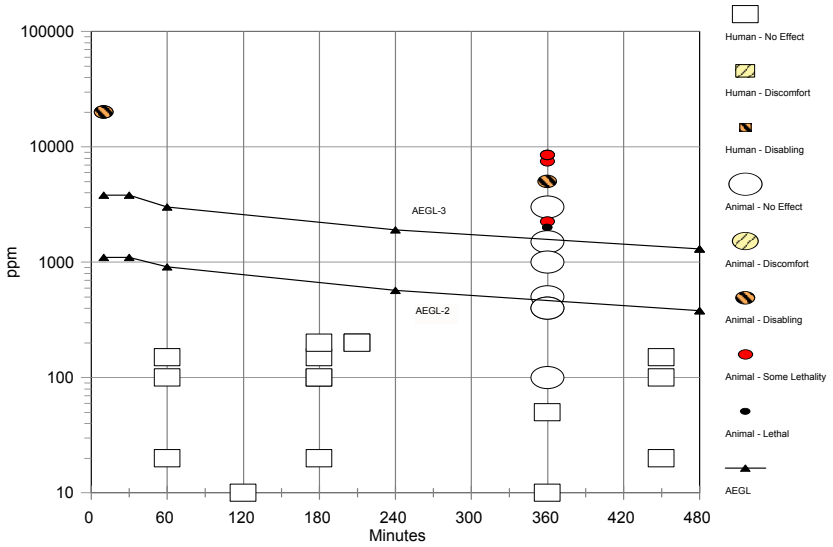

FIGURE B-1 Category graph of human and animal toxicity data in relation to AEGL values for methyl chloride. Some of the data points represent repeat exposures. The two lethal concentrations are for the mouse, a particularly sensitive species.

APPENDIX C

ACUTE EXPOSURE GUIDELINE LEVELS FOR METHYL CHLORIDE

Derivation Summary for Methyl Chloride

AEGL-1 VALUES

AEGL-1 values are not recommended because methyl chloride has no odor or warning properties at concentrations that might be neurotoxic.

AEGL-2 VALUES

10 min	30 min	1 h	4 h	8 h
1,100 ppm	1,100 ppm	910 ppm	570 ppm	380 ppm

Key references:
(1) Dodd, D.E., J.S. Bus, and C.S. Barrow. 1982. Nonprotein sulfhydryl alterations in F-344 rats following acute methyl chloride inhalation. Toxicol. Appl. Pharmacol. 62(2):228-236.
(2) Mitchell, R.I., K. Pavkov, R.M. Everett, and D.A. Holzworth. 1979. A Ninety Day Inhalation Toxicology Study in F-344 Albino Rats and B6C3F1 Mice Exposed to Atmospheric Methyl Chloride Gas. Battelle Columbus Laboratories. Microfiche No. OTS0205952.

Test species/Strain/Number: (1) groups of 20 male F344 rats; (2) 10 male and 10 female F344 rats/group

Exposure route/Concentrations/Durations: Inhalation, (1) 0, 100, 500, or 1,500 ppm for 6 h; (2) 0, 375, 750, or 1,500 ppm, 6 h/day, 5 days/week for 90 days.

Effects: (1) no clinical signs; (2) no clinical signs, no tissue lesions, increased liver weight at 1,500 ppm.

End point/Concentration/Rationale: NOAEL for clinical signs and tissue lesions.

Uncertainty factors/Rationale:
Total uncertainty factor: 3
Interspecies: 1, uptake is greater in rodents than in humans as measured by blood concentrations.
Intraspecies: 3, differences in uptake (by a factor of 2-3) and metabolism among humans are not considered toxicologically significant.

Modifying factor: Not applicable

Animal-to-human dosimetric adjustment: Not applied

Time scaling: $C^n \times t = k$; default values of n = 3 for durations shorter than 6 h and n = 1 for durations longer than 6 h. Because of the long exposure durations of the key studies, the 10-min value was set equal to the 30-min value.

(Continued)

AEGL-2 VALUES Continued

10 min	30 min	1 h	4 h	8 h
1,100 ppm	1,100 ppm	910 ppm	570 ppm	380 ppm

Data adequacy: The animal studies were well conducted. The 90-day exposure duration of one of the key studies ensures a true NOAEL after a single 6-h exposure. Animal tissues were examined microscopically after the 90-day exposure. The AEGL values are supported by a monitoring study in which accidental, repeat exposures to methyl chloride at 1,000-4,000 ppm (duration not known) resulted in transient neurotoxic symptoms (MacDonald 1964). The values are also supported by clinical studies in which no effects were observed after a 3.5-h exposure at 200 ppm (Putz-Anderson et al. 1981b) or after a 7.5-h exposure (with exercise) at 150 ppm (Stewart et al. 1980).

AEGL-3 VALUES

10 min	30 min	1 h	4 h	8 h
3,800 ppm	3,800 ppm	3,000 ppm	1,900 ppm	1,300 ppm

Key references:
(1) Morgan, K.T., J.A. Swenberg, T.E. Hamm, Jr., R. Wolkowski-Tyl, and M. Phelps. 1982. Histopathology of acute toxic response in rats and mice exposed to methyl chloride by inhalation. Fundam. Appl. Toxicol. 2(6):293-299.
(2) Chellman, G.J., K.T. Morgan, J.S. Bus, and P.K. Working. 1986a. Inhibition of methyl chloride toxicity in male F-344 rats by the anti-inflammatory agent BW755C. Toxicol. Appl. Pharmacol. 85(3):367-379.

Test species/Strain/Number: (1) Groups of 10 male and 10 female F344 rats; (2) groups of 5 male F344 rats

Exposure route/Concentrations/Durations: Inhalation, (1) 5,000 ppm for 6 h/day, 12 days; (2) 5,000 ppm for 6 h/day, 5 days.

Effects: (1) moribund state in 11/20 rats on day 5, tissue and organ lesions; (2) death of 1/5 on day 5.

End point/Concentration/Rationale: Threshold for lethality on day 1 at exposure concentration of 5,000 ppm.

Uncertainty factors/Rationale:
Total uncertainty factor: 3
Interspecies: 1, uptake is greater in rodents than in humans as measured by blood concentrations.
Intraspecies: 3, differences in uptake (by a factor of 2-3) and metabolism among humans are not considered toxicologically significant.

Modifying factor: Not applicable

Animal-to-human dosimetric adjustment: Not applied

Time scaling: $C^n \times t = k$; default values of n = 3 for durations shorter than 6 h and n = 1 for durations longer than 6 h. Because of the long exposure durations of the key studies, the 10-min value was set equal to the 30-min value.

(Continued)

AEGL-3 VALUES Continued

10 min	30 min	1 h	4 h	8 h
3,800 ppm	3,800 ppm	3,000 ppm	1,900 ppm	1,300 ppm

Data adequacy: Lethality data are sparse and, with the exception of the mouse, usually occurred after repeat exposures. The AEGL values are supported by a monitoring study in which accidental, repeat exposures to methyl chloride at 1,000-4,000 (durations not known) resulted in transient neurotoxic symptoms (MacDonald 1964).

7

Propane[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 are defined as follows:

AEGL-1 is the airborne concentration (expressed as parts per million or milligrams per cubic meter [ppm or 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 Peter Bos (RIVM, The Dutch National Institute of Public Health and the Environment), Julie M. Klotzbach (Syracuse Research Corporation), Chemical Managers Steven Barbee and Larry Gephart (National Advisory Committee [NAC] on Acute Exposure Guideline Levels for Hazardous Substances), and Ernest V. Falke (U.S. Environmental Protection Agency). 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 guidelines reports (NRC 1993, 2001).

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 concentrations 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 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

Propane is a colorless and odorless gas. It is poorly soluble in water. The lower explosive limit is 2.3%. Propane is an important constituent of liquefied petroleum gas and sometimes the main compound in liquefied petroleum gas used as (bus) fuel. It is a byproduct from various refinery processes. Its main use is in the synthesis of chemicals, such as ethylene and propylene. It is also used as an aerosol propellant and as a refrigerant. Because it is easily accessible, propane is often used in inhalant abuse and in suicide attempts.

The toxicity of propane is low, so very high concentrations can be assumed in propane abuse. The predominant effects observed in such cases are effects on the upper and lower airways of the respiratory tract and on the brain. Quantitative human data include an old study on the warning properties of propane and a study involving propane at low concentrations.

Toxicity and mortality data are sparse. Cardiac sensitization has been studied mainly in dogs and one study provides good quantitative data. Only an old study with guinea pigs focused on CNS depression. Propane was negative in the bacterial reverse mutation assay (Ames test). Carcinogenicity and reproductive toxicity studies are lacking.

The AEGL-1 values are based on a study of the warning properties of propane (Patty and Yant 1929). No effects were noted during a 10-min exposure to propane at 10,000 ppm, but distinct vertigo was reported by volunteers exposed at 100,000 ppm for 2 min. An intraspecies uncertainty factor of 1 was consid-

ered adequate because the concentration-response curve for CNS effects appears to be steep and, thus, interindividual variability will be relatively small. Further, 10,000 ppm appears to be a conservative starting point considering the effects reported at 100,000 ppm. The anesthetic potency of propane is estimated to be lower than that of butane (Drummond 1993). The AEGL-1 values for propane, therefore, should not be lower than those for butane, which are based on the same study by Patty and Yant (1929). For consistency, the AEGL-1 values for propane are derived similarly to those for butane, including the approach for time scaling. Data on butane suggest a relatively high value for n (Stoughton and Lamson 1936), so time extrapolation was performed with n = 3. Data on butane (Gill et al. 1991) and propane (Stewart et al. 1977) indicate that steady-state plasma concentrations for propane are reached within 30 min. By analogy with other substances that depress the CNS, the effects are assumed to be solely concentration dependent. Therefore, time extrapolation was performed from 10 min to 30-min and 60-min exposures. The calculated values for AEGL-1 are presented in Table 7-1. These values are considered protective of the irregular breathing observed in guinea pigs when exposed to propane at 20,000-29,000 ppm for up to 2 h (Nuckolls 1933). All of the AEGL-1 values are more than 10% of the lower explosive limit.

The AEGL-2 values are based on cardiac sensitization. In a well-performed cardiac sensitization test, beagle dogs were exposed to propane at 50,000, 100,000, or 200,000 ppm (Reinhardt et al. 1971). No cardiac sensitization occurred in six dogs exposed at 50,000 ppm, whereas it was observed in two of 12 dogs at 100,000 ppm. These findings were supported by a second study using the same protocol in which a median effective concentration (EC_{50}) of 180,000 ppm was reported (Clark and Tinston 1982). Cardiac sensitization in beagle dogs is relevant to human exposures because humans exposed at high concentrations to several substances might develop cardiac arrhythmia. The no-effect concentration of 50,000 ppm was chosen as the basis for the AEGL-2 values. The cardiac sensitization model with the dog is considered an appropriate model for humans and is highly sensitive because the response is optimized by the exogenous administration of epinephrine (Brock et al. 2003; ECETOC 2009). This protocol is designed conservatively with built-in safety factors and, thus, no additional safety factors are needed (ECETOC 2009). Accordingly, an interspecies uncertainty factor of 1 was applied. The information available indicates that cardiac sensitization is a concentration-related threshold effect and concentrations that do not produce a positive response in a short-term test also will not produce the effect when exposures are continued for longer durations. Although these considerations are mainly based on experiments with halocarbons (e.g., HFC-134a) reaching a steady-state plasma concentration in a very short timeframe, it also is considered to be true for a compound like propane because a steady-state plasma concentration is nearly reached within 30 min. Applying a total uncertainty factor of 3 to 50,000 ppm yields a (rounded) value of 17,000 ppm, which was assigned to all AEGL-2 durations. This concentration is greater than 50% of the lower explosive limit.

The same study used to derive the AEGL-2 values also was used as starting point for AEGL-3 values. Although a marked cardiac response occurred in two of 12 beagle dogs exposed to propane at 100,000 ppm, no deaths were occurred. One case of ventricular fibrillation and cardiac arrest occurred at 200,000 ppm. The concentration of 100,000 ppm was used as the basis for the AEGL-3 values. After applying a total uncertainty factor of 3, a (rounded) value of 33,000 ppm was assigned to all AEGL-3 time periods.

The AEGL values for propane are summarized below in Table 7-1.

1. INTRODUCTION

Propane is a byproduct of various refinery processes. It is often used to produce liquefied petroleum gas. Liquefied petroleum gas is generally a mixture of predominantly butane and propane in varying proportions, but sometimes propane is the main component liquefied petroleum gas used as (bus) fuel. Propane is also used in the manufacture of ethylene and propylene, as a basic material in chemical synthesis in a number of processes, as an aerosol propellant to replace the chlorofluorocarbons, as a refrigerant in chemical refining and gas processing operations, as a fuel in welding and cutting operations, and as a solvent and extractant in deasphalting and degreasing of crude oils (Low et al. 1987). Propane also has been reported to be used in cosmetic products like shaving creams (Moore 1982). Ethyl mercaptan is often added to propane to give it a pungent odor. Additional chemical and physical properties of propane are presented in Table 7-2.

2. HUMAN TOXICITY DATA

2.1. Acute Lethality

2.1.1. Case Reports

Several fatalities from propane have been reported. Most case reports deal with suicide attempts (Püschel 1979; Avis and Archibald 1994; Graefe et al. 1999; Fonseca et al. 2002) or inhalant abuse (Haq and Hameli 1980; Tsoukali et al. 1998). Also some autoerotic fatalities, considered to be accidental deaths involving inhalation or propane, have been reported (Püschel 1979; Rauschke and Harzer 1983; Pragst et al. 1991; McLennan et al. 1998). One accidental death from propane exposure in open space has been reported (Püschel 1979). There were 39 deaths in Virginia between 1987and 1996 likely as a direct consequence of exposure to an abused inhalant; five of the cases were associated with propane (Bowen et al. 1999). These reports do not provide quantitative dose-response data, so are not further described. In cases of propane abuse and with autoerotic fatalities, repeated exposure and regular abuse of other volatile organic solvents cannot be excluded. Data on intoxication by liquefied petroleum gas (mixture of predominantly propane and butane in varying proportions) are not considered.

TABLE 7-1 Summary of AEGL Values for Propane

Classification	10 min	30 min	1 h	4 h	8 h	End Point (Reference)
AEGL-1 (nondisabling)	10,000 ppm[a] (18,000 mg/m^3)	6,900 ppm[a] (12,000 mg/m^3)	5,500 ppm[a] (9,900 mg/m^3)	5,500 ppm[a] (9,900 mg/m^3)	5,500 ppm[a] (9,900 mg/m^3)	CNS depression (Patty and Yant 1929)
AEGL-2 (disabling)	See below[b]	See below[b]	See below[b]	See below[b]	See below[b]	Cardiac sensitization (Reinhardt et al. 1971)
AEGL-3 (lethal)	See below[c]	See below[c]	See below[c]	See below[c]	See below[c]	Cardiac sensitization (Reinhardt et al. 1971)

[a]The AEGL-1 value is greater than 10% of the lower explosive limit for propane in air of 23,000 ppm. Therefore, safety considerations against the hazard of explosion must be taken into account.
[b]The AEGL-2 values for all time periods is 17,000 ppm (31,000 mg/m^3), which is greater than 50% of the lower explosive limit for propane in air of 23,000 ppm. Therefore, extreme safety considerations against the hazard of explosion must be taken into account.
[c]The AEGL-3 values for all time periods is 33,000 ppm (59,000 mg/m^3), which is greater than the lower explosive limit for propane in air of 23,000 ppm. Therefore, extreme safety considerations against the hazard of explosion must be taken into account.

TABLE 7-2 Chemical and Physical Properties of Propane

Parameter	Value	Reference
Synonyms	Dimethylmethane, propylhydride	Lewis 1999
CAS registry no.	74-98-6	
Chemical formula	C_3H_8	
Molecular weight	44.11	Lide 1999
Physical state	Gas	Lewis 1999
Color	Colorless	Lewis 1999
Odor	Odorless when pure[a]	Lewis 1999
Melting point	-187.6°C -189.7°C	Lide 1999; Low et al. 1987
Boiling point	-42.1°C	Lide 1999
Solubility	65 mg/L in water	Low et al. 1987
Density		
Vapor	1.56 (air = 1)	Lewis 1999;
Liquid	0.585 g/cm^3 (-44.5°C) (water = 1)	Low et al. 1987
Vapor pressure	1.33 kPa (26.9°C)	Low et al. 1987
Flammability	Extremely flammable gas	ECB 2000
Explosive	Lower explosive limit = 2.3%	Lewis 1999
Conversion factors	1 mg/m^3 = 0.555 ppm 1 ppm = 1.80 mg/m^3	Low et al. 1987

[a]Although propane is considered to be odorless and ethyl mercaptan is often added as a warning agent, it has been reported that the odor of propane can be detected at 980-19,650 ppm (1,800-36,000 mg/m^3) (Ruth 1986).

Most deaths were from asphyxia induced by a combination of plastic bag suffocation and propane inhalation. Autopsy findings generally were very similar and included frothy material in the upper airways and oral cavity, petechial hemorrhages in the epicardium and pleural spaces, and cerebral and pulmonary congestion and edema. A few case reports of fatalities provide some quantitative information (Pragst et al. 1991; Graefe et al. 1999) or qualitative information (Haq and Hameli 1980) on propane concentrations in tissues. The highest concentrations of propane in tissues were reported by Graefe et al. (1999) as 1,100 µg/mL in the blood, 1,028 µg/g in the lungs, 820 µg/g in the brain, 572 µg/g in the liver, and 256 µg/g in the kidneys. Pragst et al. (1991) reported a blood concentration of 720 µg/mL, a lung concentration of 230 µg/mL, and a brain concentration of 120 µg/mL.

2.2. Nonlethal Toxicity

2.2.1. Case Reports

No data were available.

2.2.2. Experimental Studies

Caucasian volunteers (4-8 per group, males and females, 20-22 years of age) underwent single exposures to propane at 1,000 ppm for up to 10 min and at 250 or 500 ppm for up to 8 h (Stewart et al. 1977). In addition, some subjects were repeatedly exposed to propane at 1,000 ppm for 8 h/day for 9 days over 2 weeks. Exposure concentrations were continuously monitored. Clinical parameters (e.g., complete blood count, blood urea nitrogen, serum enzymes, urine analysis), adrenocortical function, neurological and neurobehavioral tests (a battery of cognitive tests, spontaneous electroencephalogram, and visual evoked response), pulmonary function (spirometry measurements), and cardiac responses (including electrocardiogram) were evaluated. No effects from propane on any of the parameters studied were found and no subjective responses were noted.

Patty and Yant (1929) studied the warning properties of several alkanes, including propane. In a continuous exposure test, subjects were exposed to propane at slowly increasing concentrations up to 50,000 ppm for an unknown duration (but at least 6 min). In an intermittent exposure test, subjects were exposed at fixed concentrations (10,000, 20,000, 50,000, and 100,000 ppm) for a few minutes. Exposure groups consisted of 3-6 people (males and females, 20-30 years of age). Propane was not detected in the continuous exposure test at concentrations up to 50,000 ppm, but was "readily perceptible" (mean score of 2) at 46,000 ppm in the intermittent exposure test. The odor-detection score was below moderate intensity at 100,000 ppm, with no signs of irritation reported. No symptoms were reported after 10 min of exposure at 10,000 ppm, but distinct vertigo was reported when volunteers were exposed at 100,000 ppm for 2 min. The subjects were fully capable of leaving the test chamber.

2.2.3. Human Experience

The data on human exposure to propane are very limited. Most data, especially the animal data, indicate that cardiac sensitization as an important effect. However, as with other alkanes, CNS depressing effects are also to be expected. The available data are not sufficient to determine which of the two effects occur at lower concentrations.

2.3. Summary

Fatal cases of propane intoxication (abuse, suicide attempts, autoerotic cases) have been reported. Death occurred as a result of asphyxia. Organs that were most often seriously affected in these cases were the brain and heart. These case reports do not provide adequate data for a quantitative evaluation of propane toxicity.

A single or repeated daily 8-h exposure to propane at up to 1,000 ppm had no effect on a number of clinical parameters, heart function, brain function, lung function, neurobehavioral parameters, or adrenocortical function (Stewart et al. 1977). No symptoms were noted following a 10-min exposure at 10,000 ppm, but "distinct vertigo" was reported after 2 min of exposure at 100,000 ppm. The exposed subjects were capable of leaving the exposure chamber unassisted. No complaints of irritation were reported at 100,000 ppm. Propane was "readily perceptible" at 46,000 ppm (Patty and Yant 1929).

3. ANIMAL TOXICITY DATA

3.1. Acute Lethality

3.1.1. Rats

Clark and Tinston (1982) exposed groups of six male or female Alderley Park rats to various concentrations of propane for 15 min. The 15-min LC_{50} (lethal concentration, 50% lethality) for propane was more than 800,000 ppm. At these high concentrations, oxygen was added to maintain an oxygen content of 20%. No further details were given.

3.2. Nonlethal Toxicity

3.2.1. Monkeys

Cardiac sensitization of propane was studied in groups of three anesthetized Rhesus monkeys in an open-chest preparation. Monkeys were artificially ventilated via an endotracheal cannula and several parameters of cardiac function (pulmonary arterial pressure, atrial pressure, aortic blood pressure, heart rate, and myocardial force) were studied. Monkeys were exposed to propane at 100,000 or 200,000 ppm propane for 5 min (Belej et al. 1974). No effects were

found on any parameter studied. In a similar series of experiments, exposure to propane at 200,000 ppm caused a decrease in respiratory volume, but the decrease was not statistically significant (Aviado and Smith 1975).

3.2.2. Dogs

Krantz et al. (1948) reported cardiac sensitization by propane (unspecified concentration) in unanesthetized dogs. Dogs were administered epinephrine hydrochloride (0.01 mg/kg) intravenously and were subsequently exposed to propane at concentrations between 150,000 to 900,000 ppm; an epinephrine challenge was injected after approximately 10 min of exposure.

Reinhardt et al. (1971) studied cardiac sensitization in unanesthetized, healthy, male, beagle dogs (13-26 months of age). Target propane exposure concentrations were 50,000 ppm (6 dogs), 100,000 ppm (12 dogs), and 200,000 ppm (12 dogs). Actual concentrations were measured, but were not reported. Marked responses to injected epinephrine (0.008 mg/kg), either defined as arrhythmias posing a serious threat to life (e.g., multiple consecutive ventricular beats) or which ended in cardiac arrest (e.g., ventricular fibrillation), were judged to represent significant cardiac sensitization. The incidences of marked responses were 0/6, 2/12, and 7/12 (one case of ventricular fibrillation and cardiac arrest) for the 50,000, 100,000, and 200,000 ppm exposure groups, respectively. No marked responses were observed in 13 unexposed dogs and challenged with epinephrine following the same procedure.

Clark and Tinston (1982; see Beck et al. [1973] for methodologic details) studied cardiac sensitization in unanesthetized beagle dogs exposed to propane for 5 min followed by an intravenous epinephrine injection. The procedure was similar to that of Reinhardt et al. (1971). Cardiac sensitization was judged to have occurred when ventricular tachycardia or ventricular fibrillation resulted from the challenge injection of epinephrine. An EC_{50} of 18% (180,000 ppm) was reported, with a 95% confidence interval of 12-26%.

Hemodynamic effects of propane were studied in groups of seven anesthetized adult mongrel dogs. Dogs were artificially ventilated via an endotracheal cannula and several parameters of cardiac function (pulmonary arterial pressure, atrial pressure, ventricular pressure, heart rate, and stroke volume) were studied (Zakhari 1977). Each dog was exposed to nominal concentrations of propane at 2.5, 5.0, 10.0, 15.0, and 20.0% (25,000, 50,000, 100,000, 150,000, and 200,000 ppm, respectively) via respirator for 5 min; each exposure immediately followed the preceding one. No further details were given on actual exposure concentrations. Myocardial contractility (the rate of rise in left ventricular pressure) showed a concentration-related decrease starting at 25,000 ppm. Other effects reported at higher concentrations included decrease in aortic pressure and stroke work, decrease in cardiac output, and increase in vascular resistance. The individual contribution of propane (as opposed to anesthesia) to produce these effects is unclear.

3.2.3. Guinea Pigs

Nuckolls (1933) exposed groups of three guinea pigs to propane at 22,000-29,000 ppm or 47,000-55,000 ppm for 5, 30, 60, or 120 min. The animals were observed during exposure and for 10 days after exposure. The concentrations were analyzed periodically and adjustments made to maintain the predetermined concentrations. Animals exposed at 22,000-29,000 ppm showed occasional chewing movements and irregular breathing, but these effects did not worsen as the exposure duration increased. Animals recovered quickly and appeared normal after exposure ended. Guinea pigs exposed at 47,000-55,000 ppm for 5 min showed occasional tremors and chewing movements. Continuation of exposure resulted in irregular breathing, occasional retching, and chewing movements, and the animals became somewhat stupid (as reported by study authors) but were able to walk. They sat huddled with their eyes partly shut. The description of the effects suggests that the effects did not increase in severity with continuation of exposure. One guinea pig exposed for 2 h at 47,000-55,000 ppm was examined histopathologically 7 days after exposure; no effects were found.

3.2.4. Rats

The 10-min EC_{50} for CNS depression (ataxia and loss of righting reflex) by propane was 28% (280,000 ppm; 95% confidence interval of 22-35%) (Clark and Tinston 1982). Oxygen was added at propane concentrations greater than 250,000 ppm to maintain an oxygen concentration of 20%. Groups of six male or female Alderley Park rats were exposed to various concentrations of propane. No further details were given.

3.2.5. Mice

Cardiac sensitization by propane was studied in groups of anesthetized Swiss male mice. Mice were exposed to propane at 10% (n = 3) or 20% (n = 5) for 6 min (Aviado and Belej 1974). At 20%, propane was balanced with oxygen in order to prevent asphyxia. Mice were exposed with and without a challenge dose of epinephrine hydrochloride (intravenous injection of 6 μg/kg) 2 min after the start of exposure. Electroencephalogram was continuously recorded during exposure. No effects were observed in unchallenged mice, but propane at both concentrations sensitized the heart to epinephrine.

3.3. Neurotoxicity

In an experimental study with humans, Patty and Yant (1929) reported no symptoms after 10 min of exposure to propane at 10,000 ppm, but distinct vertigo occurred after exposure for 2 min at 100,000 ppm. In rats, a 10-min EC_{50}

for CNS depression (ataxia and loss of righting reflex) of 28% (280,000 ppm) was reported for propane, with a 95% confidence interval of 22-35% (Clark and Tinston 1982). No other data were available.

3.4. Developmental and Reproductive Toxicity

No data were available.

3.5. Genotoxicity

Propane was negative for reverse mutations in the Ames test, with and without metabolic activation (citation of an unpublished report in Moore [1982]).

3.6. Carcinogenicity

No data were available.

3.7. Summary

Exposure to a mixture of propane at 800,000 ppm and oxygen at 20% for 15 min was not lethal to rats. Hemodynamic properties of propane and the potential for cardiac sensitization were studied in monkeys, dogs, and mice. Propane caused cardiac sensitization in these species. However, in most studies, the animals were tested under anesthesia, which make these studies unsuitable for a quantitative evaluation of the potency of propane for cardiac sensitization. The individual contribution of propane (as opposed to anesthesia) to produce the effects is unclear. In a well-performed study, cardiac sensitization was found in 2/12 unanesthetized dogs exposed to propane at 100,000 ppm and in 7/12 dogs at 200,000 ppm, of which one showed ventricular fibrillation and cardiac arrest (Reinhardt et al. 1971). Exposure to propane lasted for 10 min with a challenge injection of epinephrine after 5 min of exposure. No effects were observed at 50,000 ppm. These findings were supported by a second study using the same protocol in which an EC_{50} of 180,000 ppm was reported (Clark and Tinston 1982).

Slight effects on the respiratory rate were reported in guinea pigs exposed to propane at 22,000-29,000 ppm for up to 2 h. Guinea pigs exposed at 47,000-55,000 ppm for 2 h became somewhat stupid (as reported by study authors) but were able to walk. A 10-min EC_{50} for CNS depression of 280,000 ppm was reported; oxygen was added at propane concentrations greater than 250,000 ppm to maintain the oxygen concentration at 20%.

Propane was negative in the bacterial reverse mutation (Ames) test with and without metabolic activation.

4. SPECIAL CONSIDERATIONS

4.1. Metabolism and Disposition

Volunteers (Caucasian students, 20-22 years of age) underwent single exposures to propane at 250 ppm or 500 ppm for 1 h (1 male; 1 female), 2 h (1 male; 1 female), or 8 h (2 males, 2 females) (see Section 2.2.2 for more details) (Stewart et al. 1977). Post-exposure alveolar breath concentrations of propane decreased rapidly. Fifteen minutes after exposure, propane concentrations were less than 2 ppm and 10 ppm in subjects exposed at 250 and 500 ppm, respectively, for 2 h. Propane also was analyzed in blood sampled 15 min before the end of a 1-, 2-, and 8-h exposure at 250 or 500 ppm, and of an 8-h exposure at 1,000 ppm. The blood concentration of propane ranged from 0.04 to 0.09 μg/mL, and was somewhat greater in the 500-ppm group. Concentrations did not differ significantly for the different exposure durations. These results indicate that an equilibrium between propane in alveolar breath and in blood is rapidly reached. The propane concentration in blood at the end of an 8-h exposure was similar for subjects exposed at 500 and 1,000 ppm.

Tsukamoto et al. (1985) exposed male ICR mice (number of animals not specified) for 1 h to a mixture of propane, air, and oxygen (in the proportion of 2:1:1); animals were killed immediately after exposure. Besides propane, acetone and isopropanol were detected in blood and tissues as metabolites. Tissue concentrations of acetone ranged from 19 to 29 μg/g, with the highest concentrations found in the liver, followed by the blood, brain, and kidneys. Endogenous concentrations of acetone in unexposed mice were negligible. The isopropanol concentration in tissues ranged from 25 to 35 μg/g, with the highest concentrations in the blood.

4.2. Species Variability

No data were available.

5. DATA ANALYSIS FOR AEGL-1

5.1. Summary of Human Data Relevant to AEGL-1

No effects from a single or repeated daily 8-h exposure to propane at up to 1,000 ppm on a number of clinical parameters, heart function, brain function, lung function, neurobehavioral parameters, and adrenocortical function were found in 2 male and 2 female volunteers (Stewart et al. 1977). No symptoms were noted following a 10-min exposure to propane at 10,000 ppm, but "distinct vertigo" was reported after 2 min of exposure at 100,000 ppm. Exposure groups consisted of 3-6 volunteers (male and female). No complaints of irritation were reported at 100,000 ppm. Propane was "readily perceptible" (mean score of 2) at 46,000 ppm (Patty and Yant 1929).

5.2. Summary of Animal Data Relevant to AEGL-1

Nuckolls (1933) exposed groups of 3 guinea pigs to propane at low (22,000-29,000 ppm) or high concentrations (47,000-55,000 ppm) for 5, 30, 60, or 120 min. Guinea pigs exposed at 20,000-29,000 ppm showed occasional chewing movements and irregular breathing. In addition, guinea pigs exposed at 47,000-55,000 ppm showed irregular breathing, occasional retching, and chewing movements, and the animals became somewhat stupid (as reported by study authors) but were able to walk. They sat huddled up with their eyes partly shut. The description of the effects suggests that effects did not increase in severity with continuation of exposure. One guinea pig exposed for 2 h at the high concentrations was examined histopathologically 7 days after exposure; no effects were noted. Animals recovered quickly and appeared normal after exposure.

5.3. Derivation of AEGL-1

The human data presented by Patty and Yant (1929) form the basis for AEGL-1 derivation. No effects were noted during a 10-min exposure to propane at 10,000 ppm, but distinct vertigo was reported by volunteers when exposed at 100,000 ppm for 2 min. Although the study was performed with a small groups of volunteers (n = 3 or 6) of a relatively young age (20-30 years), an intraspecies uncertainty factor of 1 was considered adequate for the following reasons. First, the concentration-response curve for CNS effects appears to be very steep (analogous to butane, see Chapter 1); thus, interindividual variability will be relatively small. Second, compared with the effects reported for the next higher exposure concentration of 100,000 ppm (for 2 min), 10,000 ppm appears to be a conservative starting point. This is also supported by the relative ranking of several alkanes according their anesthetic potency by Drummond (1993). Drummond estimated anesthetic potency on the basis of lipid solubility and reported greater potency with increasing chain length. It was estimated that the anesthetic potency for propane was about 2- to 3-fold lower than for butane. Hence, because the AEGL-1 values for butane and propane are based on CNS depression, the AEGL-1 values for propane should not be lower than those for butane. The AEGL-1 values for butane also are based on the 10-min exposure study by Patty and Yant (1929). For consistency, the AEGL-1 values for propane were time-scaled in a manner similar to that for butane. The time scaling performed for butane is summarized below.

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 of $C^n \times t = k$, 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. Approxi-

mately 90% of the values of n ranged between 1 and 3. Consequently, these values were selected as the reasonable lower and upper bounds of n to use when data are not available to derive an empirical value for n. A value of n = 1 is used when extrapolating from shorter to longer time periods, because the extrapolated values are conservative and, therefore, reasonable in the absence of any data to the contrary. Conversely, a value of n = 3 is used when extrapolating from longer to shorter time periods because the extrapolated values are conservative and, therefore, reasonable in the absence of any data to the contrary. On the basis of CNS effects (complete anesthesia) observed in a study with mice exposed to butane or cyclopropane (Stoughton and Lamson 1936) it was concluded that n will be relatively high (3 or greater). Although the data cannot be used to provide an adequate estimate for n, it can be concluded that n will be relatively high and that the upper bound of n = 3 is an appropriate estimate for time scaling. This is consistent by analogy to other anesthetics, where the effects are assumed to be concentration dependent rather than time dependent.

No increase in the magnitude or severity of the response by duration is expected for concentration-dependent effects after reaching steady state. Although no appropriate kinetic data are available for to assess the time for propane to reach a steady state, it can be concluded from the pulmonary uptake data for butane from Gill et al. (1991) that a steady-state uptake, and hence, steady-state plasma values, will be reached within 30 min of exposure. In addition, it has been stated that gases which are relatively insoluble in blood increase rapidly toward equilibrium with the inhaled concentration and the less soluble in blood the faster the narcotic action of the gas (Drummond 1993). The increase to a quick equilibrium has been confirmed for propane. Concentrations of propane were approximately similar in blood samples taken 15 min before the end of 1-, 2-, and 8-h exposures to propane at 250 and 500 ppm (Stewart et al. 1977).

Because of the poor solubility of propane in water (65 mg/L), it is expected that exposure to propane will lead to a rapid equilibrium and that there will be no increase in the magnitude or severity of response at 2, 4, or 8 h. The other exposure duration-specific values were derived by time scaling according to the dose-response regression equation $C^n \times t = k$, using n = 3. Time extrapolation was performed from 10 min to 300 and 60-min durations, where the steady-state concentration was calculated. The resulting values for AEGL-1 are presented in Table 7-3; these values are considered to be protective of the irregular breathing observed in guinea pigs when exposed to propane at 20,000 to 29,000 ppm for up to 2 h.

6. DATA ANALYSIS FOR AEGL-2

6.1. Summary of Human Data Relevant to AEGL-2

No human data that address the level of effects defined by the AEGL-2 were found.

TABLE 7-3 AEGL-1 Values for Propane

10 min	30 min	1 h	4 h	8 h
10,000 ppm[a] (18,000 mg/m^3)	6,900 ppm[a] (12,000 mg/m^3)	5,500 ppm[a] (9,900 mg/m^3)	5,500 ppm[a] (9,900 mg/m^3)	5,500 ppm[a] (9,900 mg/m^3)

[a]The AEGL-1 value is greater than 10% of the lower explosive limit for propane of 23,000 ppm. Therefore, safety considerations against the hazard of explosion must be taken into account.

6.2. Summary of Animal Data Relevant to AEGL-2

The cardiac-sensitization potential of propane was evaluated in beagles exposed in a protocol developed by Reinhardt et al. (1971). Briefly, just before exposure, dogs received injections of epinephrine, and each group of 6-12 dogs was exposed to a different concentration of propane for 5 min, at which time a challenge injection of epinephrine was administered, and electrocardiography was used to evaluate the presence of cardiac sensitization. Under these exaggerated conditions, none of 6 dogs exposed at 50,000 ppm showed cardiac effects, but 2 of 12 dogs exposed at 100,000 ppm and 7 of 12 dogs exposed at 200,000 ppm showed effects. Those findings were confirmed in a similar study that reported an EC_{50} for cardiac sensitization of 180,000 ppm (95% confidence interval of 120,000-260,000 ppm) (Clark and Tinston 1982).

The relevant cardiac-sensitization study used an optimized conservative model in the beagle. The test involved the injection of epinephrine into the dog before and during exposure at very high concentrations. The administered epinephrine was given at a dose rate about 10 times greater than that calculated to occur in humans in times of stress (Brock et al. 2003). Although the model is very sensitive, it is relevant to humans because humans exposed to high concentrations of several substances might develop cardiac arrhythmias (ECETOC 2009).

6.3. Derivation of AEGL-2

Although it is an optimized, supersensitive model, cardiac sensitization in beagles is relevant to human exposures because humans exposed at high concentrations to several substances might develop cardiac arrhythmias. The no-effect concentration for propane of 50,000 ppm was selected as the basis for the AEGL-2 values. The cardiac sensitization model with the dog is considered an appropriate model for humans and is highly sensitive because the response is optimized by the exogenous administration of epinephrine (Brock et al. 2003; ECETOC 2009). This protocol is designed conservatively with built-in safety factors; thus, no additional uncertainty factors are needed (ECETOC 2009). Accordingly, an interspecies uncertainty factor of 1 was applied.

The information available indicates that cardiac sensitization is a concentration-related threshold effect (Reinhardt et al. 1971; Brock et al. 2003), and concentrations that do not produce a positive response in short-term tests will also not produce the effect when exposures are continued for longer periods. Although these considerations are mainly based on experiments with halocarbons (e.g., HFC-134a) reaching a steady-state plasma concentration in a very short time, they are also considered to be true for a compound as propane since a steady-state plasma concentration is considered to be nearly reached within 30 min (see Section 5.3). Starting with 50,000 ppm and applying a total uncertainty factor of 3, a (rounded) value of 17,000 ppm is assigned to all AEGL-2 time periods (see Table 7-4).

7. DATA ANALYSIS FOR AEGL-3

7.1 Summary of Human Data Relevant to AEGL-3

No human data that address the level of effects defined by the AEGL-3 were found. Case reports are inadequate to provide a basis for AEGL-3 values.

7.2. Summary of Animal Data Relevant to AEGL-3

Clark and Tinston (1982) exposed groups of 6 male or female Alderley Park rats to various concentrations of propane for 15 min. The 15-min LC_{50} for propane was reported to be more 800,000 ppm. Since oxygen was added at propane concentrations above 250,000 ppm to maintain the oxygen level at 20%, the only conclusion that can be drawn from this study is that the 15-min LC_{50} is greater than 250,000 ppm.

Propane has been reported to be a cardiac sensitizer in monkeys, dogs, and mice. Most of these studies used anesthetized animals, which makes them unsuitable for a quantitative evaluation for this end point. In a well-performed cardiac sensitization test conducted by Reinhardt et al. (1971), beagles were exposed to propane at 50,000, 100,000, or 200,000 ppm. At an exposure concentration of 100,000 ppm, 2 of 12 dogs showed cardiac sensitization, but no deaths occurred. At 200,000 ppm, 7 of 12 dogs showed cardiac sensitization, including one case of ventricular fibrillation and cardiac arrest. These findings were supported by a second study using the same protocol in which an EC_{50} of 180,000 ppm was reported (Clark and Tinston 1982).

TABLE 7-4 AEGL-2 Values for Propane

10 min	30 min	1 h	4 h	8 h
See below[a]	See below[a]	See below[a]	See below[a]	See below[a]

[a]The AEGL-2 values for all time periods is 17,000 ppm (31,000 mg/m^3), which is greater than 50% of the lower explosive limit for propane of 23,000 ppm. Therefore, extreme safety considerations against the hazard of explosion must be taken into account.

7.3. Derivation of AEGL-3

No human data or adequate mortality studies in animals were available. Although it is an optimized, supersensitive model, cardiac sensitization in beagles is relevant to human exposures because humans exposed to high concentrations of several substances might develop cardiac arrhythmias. Although a marked cardiac response occurred in 2 of 12 beagle dogs exposed at 100,000 ppm in the cardiac sensitization test, no deaths were occurred. One case of ventricular fibrillation and cardiac arrest occurred with propane at 200,000 ppm. The concentration of 100,000 ppm was used as the basis for the AEGL-3 values. The cardiac-sensitization model with the dog is considered an appropriate model for humans; therefore, an interspecies uncertainty factor of 1 was applied. Because the cardiac sensitization test is highly sensitive, as the response to epinephrine is optimized, an intraspecies uncertainty factor of 3 was applied to account for sensitive individuals.

The information available indicates that cardiac sensitization is a concentration-related threshold effect (Reinhardt et al. 1971; Brock et al. 2003), and concentrations that do not produce a positive response in short-term tests will also not produce the effect when exposures are continued for longer periods. Although these considerations are mainly based on experiments with halocarbons (e.g., HFC-134a), which reach a steady-state plasma level in a very short time, they are also considered to be true for a compound as propane since, a steady-state plasma concentration is considered to be nearly reached within 30 min (see Section 5.3). Starting with 100,000 ppm and applying a total uncertainty factor of 3, a (rounded) value of 33,000 ppm is assigned to all AEGL-3 time periods (see Table 7-5).

8. SUMMARY OF AEGLS

8.1 AEGL Values and Toxicity End Points

The AEGL values for propane are summarized in Table 7-6.

8.2. Comparison with Other Standards and Guidelines

Standards and guidelines for short-term exposures are presented in Table 7-7.

TABLE 7-5 AEGL-3 Values for Propane

10 min	30 min	1 h	4 h	8 h
See below[a]	See below[a]	See below[a]	See below[a]	See below[a]

[a]The AEGL-3 values for all time periods is 33,000 ppm (59,000 mg/m^3), which is greater than the lower explosive limit for propane in air of 23,000 ppm. Therefore, extreme safety considerations against the hazard of explosion must be taken into account.

TABLE 7-6 Summary of AEGL Values for Propane

Classification	10 min	30 min	1 h	4 h	8 h
AEGL-1 (nondisabling)	10,000 ppm[a] (18,000 mg/m^3)	6,900 ppm[a] (12,000 mg/m^3)	5,500 ppm[a] (9,900 mg/m^3)	5,500 ppm[a] (9,900 mg/m^3)	5,500 ppm[a] (9,900 mg/m^3)
AEGL-2 (disabling)	See below[b]	See below[b]	See below[b]	See below[b]	See below[b]
AEGL-3 (lethal)	See below[c]	See below[c]	See below[c]	See below[c]	See below[c]

[a]The AEGL-1 value is greater than 10% of the lower explosive limit for propane in air of 23,000 ppm. Therefore, safety considerations against the hazard of explosion must be taken into account.
[b]The AEGL-2 values for all time periods is 17,000 ppm (31,000 mg/m^3), which is greater than 50% of the lower explosive limit for propane in air of 23,000 ppm. Therefore, extreme safety considerations against the hazard of explosion must be taken into account.
[c]The AEGL-3 values for all time periods is 33,000 ppm (59,000 mg/m^3), which is greater than the lower explosive limit for propane in air of 23,000 ppm. Therefore, extreme safety considerations against the hazard of explosion must be taken into account.

TABLE 7-7 Extant Standards and Guidelines for Propane

Guideline	Exposure Duration				
	10 min	30 min	1 h	4 h	8 h
AEGL-1	10,000 ppm[a] (18,000 mg/m^3)	6,900 ppm[a] (12,000 mg/m^3)	5,500 ppm[a] (9,900 mg/m^3)	5,500 ppm[a] (9,900 mg/m^3)	5,500 ppm[a] (9,900 mg/m^3)
AEGL-2	See below[b]	See below[b]	See below[b]	See below[b]	See below[b]
AEGL-3	See below[c]	See below[c]	See below[c]	See below[c]	See below[c]
IDLH (NIOSH)[d]		2,100 ppm (10% of lower explosive limit)			
TLV-TWA (ACGIH)[e]					1,000 ppm; simple asphyxiant; oxygen content to be >18%
PEL-TWA (OSHA)[f]					1,000 ppm
REL-TWA (NIOSH)[g]					1,000 ppm
MAK (Germany)[h]					1,000 ppm
MAK Peak Limit (Germany)[i]			2,000 ppm		
MAC (The Netherlands)[j]					None, simple asphyxiant; oxygen content to be >18%

[a]The AEGL-1 value is greater than 10% of the lower explosive limit for propane in air of 23,000 ppm. Therefore, safety considerations against the hazard of explosion must be taken into account.

[b]The AEGL-2 values for all time periods is 17,000 ppm (31,000 mg/m^3), which is greater than 50% of the lower explosive limit for propane in air of 23,000 ppm. Therefore, extreme safety considerations against the hazard of explosion must be taken into account.
[c]The AEGL-3 values for all time periods is 33,000 ppm (59,000 mg/m^3), which is greater than the lower explosive limit for propane in air of 23,000 ppm. Therefore, extreme safety considerations against the hazard of explosion must be taken into account.
[d]IDLH (immediately dangerous to life or health, National Institute for Occupational Safety and Health) (NIOSH 1994) represents the maximum concentration from which one could escape within 30 min without any escape-impairing symptoms, or any irreversible health effects.
[e]TLV-TWA (threshold limit value - time weighted average, American Conference of Governmental Industrial Hygienists) (ACGIH 2004) 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.
[fe]PEL-TWA (permissible exposure limit - time weighted average, Occupational Safety and Health Administration) (29CFR 1910.1000[1990]) is defined analogous to the ACGIH TLV-TWA, but is for exposures of no more than 10 h/day, 40 h/week.
[g]REL-TWA (recommended exposure limit - time weighted average, National Institute for Occupational Safety and Health) (NIOSH 2010) is defined analogous to the ACGIH TLV-TWA.
[h]MAK (maximale arbeitsplatzkonzentration [maximum workplace concentration]) (Deutsche Forschungsgemeinschaft [German Research Association] DFG 2002) is defined analogous to the ACGIH TLV-TWA.
[i]MAK spitzenbegrenzung (peak limit) (German Research Association (DFG 2002) constitutes the maximum average concentration to which workers can be exposed for a period up to 60 min with no more than three exposure periods per work shift; total exposure may not exceed 8-h MAK.
[j]MAC (maximaal aanvaarde concentratie [maximal accepted concentration]) (Dutch Expert Committee for Occupational Standards, The Netherlands (MSZW 2004) is defined analogous to the ACGIH TLV-TWA.

8.3. Data Quality and Research Needs

The database for propane is very poor. Significant human data are absent or performed with propane concentrations that appear to be too low to be relevant for the derivation of AEGLs. The study with human volunteers (Patty and Yant 1929) is rather dated and focused on a limited number of parameters to examine the warning properties of propane. The available case reports are inadequate to be used for any quantitative concentration-response evaluation.

The only toxicity end point that has been properly studied for propane is cardiac sensitization. Besides a limited study with guinea pigs that dates back to 1933, no animal studies are available that properly addresses the anesthetic properties of propane. Mortality data with experimental animals are also lacking.

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APPENDIX A

DERIVATION OF AEGL VALUES FOR PROPANE

Derivation of AEGL-1 Values

Key study:	Patty, F.A., and W.P. Yant. 1929. Odor Intensity and Symptoms Produced by Commercial Propane, Butane, Pentane, Hexane, and Heptane Vapor. U.S. Bureau of Mines Report of Investigation No. 2979. Washington, DC: U.S. Department of Commerce, Bureau of Mines.
Toxicity end point:	10-min exposure to 10,000 ppm is no-observed-adverse-effect level CNS depression
Time scaling:	$C^3 \times t = k$ for extrapolation to 30 and 60 min, flatlining assumed for 60 min to 4- and 8-h exposure (based on 60-min steady-state concentration). $k = (10{,}000 \text{ ppm})^3 \times 10 \text{ min} = 10^{13} \text{ ppm}^3\text{-min}$
Uncertainty factors:	1 for interspecies variability 1 for interindividual variability Combined uncertainty factor of 1
Calculations:	
10-min AEGL-1:	10,000 ppm[a] (18,000 mg/m^3)
30-min AEGL-1:	$C^3 \times 30 \text{ min} = 10^{13} \text{ ppm}^3\text{-min}$ C = 6,900 ppm[a] (rounded) (12,000 mg/m^3)
1-h AEGL-1:	$C^3 \times 60 \text{ min} = 10^{13} \text{ ppm}^3\text{-min}$ C = 5,500 ppm[a] (rounded) (9,900 mg/m^3)
4-h AEGL-1:	Set equivalent to 1-h AEGL-1 of 5,500 ppm[a] (9,900 mg/m^3)
8-h AEGL-1:	Set equivalent to 1-h AEGL-1 of 5,500 ppm[a] (9,900 mg/m^3)

[a]The AEGL-1 value is greater than 10% of the lower explosive limit for propane in air of 23,000 ppm. Therefore, safety considerations against the hazard of explosion must be taken into account.

Derivation of AEGL-2 Values

Key study:	Reinhardt, C.F., A. Azar, M.E. Maxfield, P.E. Smith, Jr., and L.S. Mullin. 1971. Cardiac arrhythmias and aerosol "sniffing". Arch Environ. Health 22(2):265-279.
Toxicity end point:	Short-term exposure (10 min with epinephrine injection after 5 min) induced cardiac sensitization in dogs; no-observed-adverse-effect level was 50,000 ppm.
Time scaling:	Flatlining assumed for 10 and 30 min and for 1, 4, and 8 h.
Uncertainty factors:	1 for interspecies variability 3 for interindividual variability Combined uncertainty factor of 3
Calculations:	
10-min AEGL-2:	50,000 ppm ÷ 3 = 17,000 ppm[b] (rounded) 31,000 mg/m^3)
30-min AEGL-2:	Set equivalent to 10-min AEGL-2 of 17,000 ppm[b] (31,000 mg/m^3)
1-h AEGL-2:	Set equivalent to 10-min AEGL-2 of 17,000 ppm[b] (31,000 mg/m^3)
4-h AEGL-2:	Set equivalent to 10-min AEGL-2 of 17,000 ppm[b] (31,000 mg/m^3)
8-h AEGL-2:	Set equivalent to 10-min AEGL-2 of 17,000 ppm[b] (31,000 mg/m^3)

[b]The AEGL-2 value is greater than 50% of the lower explosive limit for propane in air of 23,000 ppm. Therefore, extreme safety considerations against the hazard of explosion must be taken into account.

Derivation of AEGL-3 Values

Key study:	Reinhardt, C.F., A. Azar, A., M.E, Maxfield, P.E. Smith Jr., and L.S. Mullin. 1971. Cardiac arrhythmias and aerosol "sniffing". Arch Environ. Health 22(2):265-279.

Toxicity end point:	Short-term exposure (10 min with epinephrine injection after 5 min) induced cardiac sensitization in dogs; no deaths occurred at 100,000 ppm.
Time scaling:	Flatlining assumed for 10 and 30 min and for 1, 4, and 8 h.
Uncertainty factors:	1 for interspecies variability 3 for interindividual variability Combined uncertainty factor of 3
Calculations:	
10-min AEGL-3:	100,000 ppm ÷ 3 = 33,000 ppm[c] (rounded) (59,000 mg/m^3)
30-min AEGL-3:	Set equal to 10-min AEGL-3 of 33,000 ppm[c] (59,000 mg/m^3)
1-h AEGL-3:	Set equal to 10-min AEGL-3 of 33,000 ppm[c] (59,000 mg/m^3)
4-h AEGL-3:	Set equal to 10-min AEGL-3 of 33,000 ppm[c] (59,000 mg/m^3)
8-h AEGL-3:	Set equal to 10-min AEGL-3 of 33,000 ppm[c] (59,000 mg/m^3)

[c]The AEGL-3 value is greater than the lower explosive limit for propane in air of 23,000 ppm). Therefore, extreme safety considerations against the hazard of explosion must be taken into account.

APPENDIX B

CATEGORY GRAPH FOR PROPANE

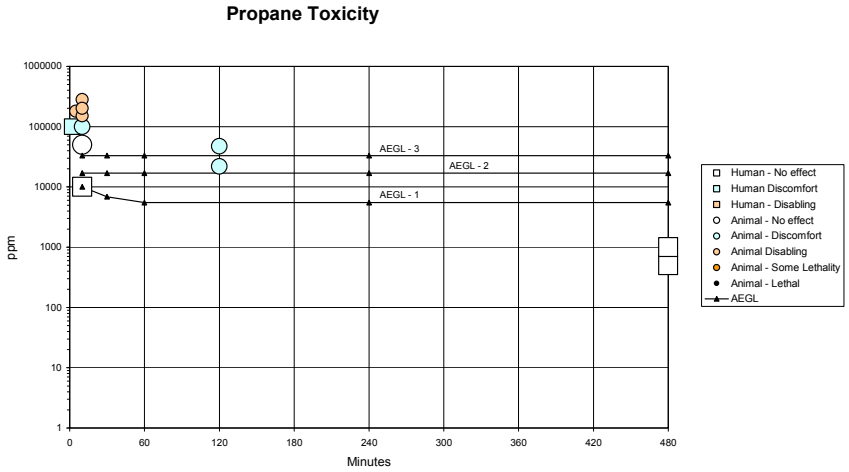

FIGURE B-1 Category graph of toxicity data and AEGLs values for propane.

APPENDIX C

ACUTE EXPOSURE GUIDELINE LEVELS FOR PROPANE

Derivation Summary for Propane

AEGL-1 VALUES

10 min	30 min	1 h	4 h	8 h
10,000 ppm[a] (18,000 mg/m^3)	6,900 ppm[a] (12,000 mg/m^3)	5,500 ppm[a] (9,900 mg/m^3)	5,500 ppm[a] (9,900 mg/m^3)	5,500 ppm[a] (9,900 mg/m^3)

Key reference: Patty, F.A., W.P. and Yant. 1929. Odor Intensity and Symptoms Produced by Commercial Propane, Butane, Pentane, Hexane, and Heptane Vapor. U.S. Bureau of Mines Report of Investigation. No. 2979. Washington, DC: U.S. Department of Commerce, Bureau of Mines.

Test species/Strain/Number: Groups of 3-6 human subjects (males and females, 20-30 years of age). The study was focused on the warning properties of several alkanes.

Exposure route/Concentrations/Durations: Subjects were exposed to slowly increasing concentrations up to 50,000 ppm (continuous exposure test, total exposure was at least 6 min), followed by exposure to fixed concentrations for a few minutes on the same day (intermittent exposure test). The fixed exposure concentrations were approximately 10,000, 20,000, 50,000, and 100,000 ppm.

Effects: No odor detection or irritation was reported during the continuous exposure test. No symptoms were reported after 10 min of exposure at 10,000 ppm, but distinct vertigo was reported when volunteers were exposed at 100,000 ppm for 2 min. No irritation was noticed at 100,000 ppm for 10 min. The subjects were capable of leaving the test chamber unassisted.

End point/Concentration/Rationale: No AEGL-1 effects at 10 min exposure to 10,000 ppm; consistent with butane.

Uncertainty factors/Rationale:
Total uncertainty factor: 1
Interspecies: 1, test subjects were humans
Intraspecies: 1, the concentration-response curve appears to be very steep indicating small interindividual variability; no irritation at 100,000 ppm for 10 min; a higher factor would result in unrealistically low values compared with occupational standards.

Modifying factor: Not applicable

Animal-to-human dosimetric adjustment: Not applicable

Time scaling: n = 3 for time scaling from 10 min to 60 min (animal data for cyclopropane and butane suggest high value of n); because steady state is reached within 30 min, the values for 4- and 8-h exposures are similar to the 60-min value.

Data adequacy: Database is poor.

[a]The AEGL-1 value is greater than 10% of the lower explosive limit for propane in air of 23,000 ppm. Therefore, safety considerations against the hazard of explosion must be taken into account.

AEGL-2 VALUES

10 min	30 min	1 h	4 h	8 h
See below[a]	See below[a]	See below[a]	See below[a]	See below[a]

Key reference: Reinhardt, C.F., A. Azar, M.E. Maxfield, P.E. Smith Jr., and L.S. Mullin. 1971. Cardiac arrhythmias and aerosol "sniffing". Arch Environ. Health 22(2):265-279.

Test species/Strain/Number: Male beagles, number of dogs exposed was 6 (low exposure group) or 12 (mid- and high-exposure group).

Exposure route/Concentrations/Durations: Inhalation exposure for 10 min at 50,000, 100,000, or 200,000 ppm.

Effects:

0 ppm	No effects
50,000 ppm	No effects
100,000 ppm	Marked response in 2/12 dogs
200,000 ppm	Marked response in 7/12 dogs, one of which had cardiac arrest

End point/Concentration/Rationale: No cardiac sensitization at 50,000 ppm

Uncertainty factors/Rationale:
Total uncertainty factor: 3
Interspecies: 1, canine cardiac sensitization assay appears to be a good model for the human heart.
Intraspecies: 3, the test is a conservative test for sensitive individuals because an excess of epinephrine is used.

Modifying factor: Not applicable

Animal-to-human dosimetric adjustment: Not applicable

Time scaling: Cardiac sensitization is a concentration-related threshold effect and concentrations that do not produce a positive response in short-term tests will also not produce the effect when exposures are continued for longer periods.

Data adequacy: Database is poor. Data on cardiac sensitization are sufficient but quantitative data are lacking on possible CNS effects so that a comparison of CNS effects and cardiotoxicity cannot be made.

[a]The AEGL-2 values for all time periods is 17,000 ppm (31,000 mg/m^3). The AEGL-2 value is greater than 50% of the lower explosive limit for propane in air of 23,000 ppm. Therefore, extreme safety considerations against the hazard of explosion must be taken into account.

AEGL-3 VALUES

10 min	30 min	1 h	4 h	8 h
See below[a]	See below[a]	See below[a]	See below[a]	See below[a]

Key reference: Reinhardt, C.F., A. Azar, M.E. Maxfield, P.E. Smith Jr., and L.S. Mullin. 1971. Cardiac arrhythmias and aerosol "sniffing". Arch Environ. Health 22(2):265-279.

Test species/Strain/Number: Male beagles, number of dogs exposed was 6 (low-exposure group) or 12 (mid- and high-exposure group).

(Continued)

AEGL-3 VALUES Continued

10 min	30 min	1 h	4 h	8 h
See below[a]	See below[a]	See below[a]	See below[a]	See below[a]

Exposure route/Concentrations/Durations: Inhalation exposure for 10 min at 50,000, 100,000, or 200,000 ppm.

Effects:

0 ppm	No effects
50,000 ppm	No effects
100,000 ppm	Marked response in 2/12 dogs
200,000 ppm	Marked response in 7/12 dogs, one of which had cardiac arrest

End point/Concentration/Rationale: No deaths from cardiac sensitization at 100,000 ppm.

Uncertainty factors/Rationale:
Total uncertainty factor: 3
Interspecies: 1, the canine cardiac sensitization assay appears to be a good model for the human heart.
Intraspecies: 3, the test is a conservative test for sensitive individuals because an excess of epinephrine is used.

Modifying factor: Not applicable

Animal-to-human dosimetric adjustment: Not applicable

Time scaling: Cardiac sensitization is a concentration-related threshold effect and concentrations that do not produce a positive response in short-term tests will also not produce the effect when exposures are continued for longer periods.

Data adequacy: Database is poor. Data on cardiac sensitization are sufficient but quantitative data are lacking on possible CNS effects so that a comparison on the potency for CNS effects and cardiotoxicity cannot be made.

[a]The AEGL-3 values for all time periods is 33,000 ppm (59,000 mg/m^3).The AEGL-3 value is greater than the lower explosive limit for propane in air of 23,000 ppm. Therefore, extreme safety considerations against the hazard of explosion must be taken into account.