Acute Exposure Guideline Levels for Selected Airborne Chemicals

VOLUME 14

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 fourteenth volume in that series. AEGL documents for BZ (2-quinuclidinyl benzilate), ethyl

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

phosphorodichloridate, hexane, methanesulfonyl chloride, nitric acid, propargyl alcohol, and vinyl acetate monomer 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 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 committee interim reports, which summarize the committee's conclusions and recommendations for improving NAC's AEGL documents for BZ (interim reports 19a, 20a, and 21a), ethyl phosphorodichloridate (interim reports 20a and 21a), hexane (interim reports 17 and 21a), methanesulfonyl chloride (interim reports 20a and 21a), nitric acid (interim reports 15, 18, and 21a), propargyl alcohol (interim reports 16 and 19a), and vinyl acetate monomer (interim reports 18 and 21a): Harvey Clewell (The Hamner Institutes for Health Sciences), Jeffrey Fisher (U.S. Food and Drug Administration), Sam Kacew (University of Ottawa), A. Wallace Hayes (Harvard School of Public Health), Rogene Henderson (Lovelace Respiratory Research Institute [retired]), James McDougal (Wright State University [retired], Charles Reinhardt (DuPont Haskell Laboratory [retired]), Andrew Salmon (California Environmental Protection Agency), 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 reports 15-21 was overseen by Robert Goyer (University of Western Ontario [retired]). Appointed by the NRC, he was responsible for making certain that an independent examination of the interim reports was

carried out in accordance with institutional procedures and that all review comments were carefully considered. Responsibility for the final content of this report rests entirely with the authoring committee and the institution.

The committee gratefully acknowledges the valuable assistance provided by Ernest Falke and Iris A. Camacho from EPA. 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 of 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

VOLUME 14

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

This report is the fourteenth 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 and 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 extrap-

olation 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 thirteen reports in the series *Acute Exposure Guideline Levels for Selected Airborne Chemicals* (NRC 2001b, 2002b, 2003, 2004, 2007b, 2008b, 2009, 2010a,b, 2011, 2012a,b,c). This report is the fourteenth volume in that series. AEGL documents for BZ (2-quinuclidinyl benzilate), ethyl phosphorodichloridate, hexane, methanesulfonyl chloride, nitric acid, propargyl alcohol, and vinyl acetate monomer 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

3

n-Hexane[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 effects. However, the effects are not disabling and are transient and reversible upon cessation of exposure.

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[1]This document was prepared by the AEGL Development Team composed of Peter Bos (Oak Ridge National Laboratory), Julie Klotzbach (SRC, Inc.), Chemical Manager Alfred Feldt (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).

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

n-Hexane is a colorless liquid with a slightly disagreeable, gasoline-like odor. It dissolves slightly in water. The lower explosive limit of *n*-hexane is 1.1%. *n*-Hexane is produced from natural gas and crude oil. Its main use in industry is in products known as solvents. The major uses for these solvents are in food processing to extract vegetable oils from crops, as cleaning agents in the printing, textile, furniture, and shoemaking industries (used in special glues), and in the manufacture of pharmaceuticals. Because of their easily accessibility, solvents and glues containing *n*-hexane are often used in inhalant abuse.

Human data on the acute toxicity of *n*-hexane are extremely limited and are insufficient for setting AEGL values. The data show that the acute toxicity of *n*-hexane is very low. No cases of lethality were reported after inhalation of *n*-hexane or *n*-hexane-containing mixtures, not even in solvent abuse. Furthermore, no severe clinical signs were reported in human volunteers after acute exposure to *n*-hexane both at rest and during physical exercise. Genotoxic and carcinogenic effects of the chemical have not been examined in humans. Chronic exposure to *n*-hexane frequently results in degenerative distal axonopathy in the peripheral nervous system, but this effect is not relevant for acute exposures.

Two LC_{50} (lethal concentration, 50% lethality) values for *n*-hexane have been reported for rats, but the original studies from which they were derived could not be obtained. Findings in toxicokinetic studies appear to have discrepancies with the LC_{50}s. Visible signs of acute toxicity from *n*-hexane are generally associated with effects on the nervous system, such as reduced respiration, ptosis, myoclonic seizures, ataxia, decreased motor activity, sedation, laying

down in a side position, and narcosis. Rats exposed to *n*-hexane exhibited acute effects on the brain and lungs and reversible lesions in the testis. The most significant effect in developmental and reproduction studies of *n*-hexane was a transient retardation in the growth of live pups; however, this effect is considered to be a result of repeated exposure. In general, *n*-hexane is not mutagenic in vitro although some positive results were obtained. *n*-hexane is not mutagenic in mice, but morphologic alterations in sperm, as well as chromatid breaks in bone marrow cells, were reported in rats. The limited information available on the carcinogenicity found hepatocellular neoplasms in mice and papillary tumors in the bronchiolar epithelium of rabbits exposed to *n*-hexane.

Because of insufficient human and animal data addressing the level of effects defined by AEGL-1, no AEGL-1 values are recommended for *n*-hexane.

Human and animal data indicate that central nervous system (CNS) depression is the most relevant adverse effect of acute exposure to *n*-hexane. However, adequate human data for evaluating concentration-response relationships for AEGL-2 effects are not available. Reporting insufficiencies in rat studies and confounding methodologic issues in studies of mice severely limit confidence in identifying no-effect levels for AEGL-2 effects. Although data are not available to define the concentration-response curve for *n*-hexane, a steep concentration-response relationship is observed for butane, a structural analog of *n*-hexane and central nervous system depressant (NRC 2012). On this basis, at steep concentration-response relationship is also expected for *n*-hexane. For chemicals with a steep concentration-response curve, AEGL-2 values may be derived by reducing AEGL-3 values by one-third (NRC 2001).

AEGL-3 values were based on a kinetic study of male Sprague-Dawley rats exposed to *n*-hexane at an actual concentration of 86,222 ± 1,330 ppm for 10, 15, 20, 25, or 30 min (Raje et al. 1984). Although the study focused on blood *n*-hexane concentrations, some toxicity data were provided; rats exposed for 25 or 30 min showed visible signs of toxicity (ataxia and decreased motor activity), but no deaths. From these results, a 30-min exposure at 86,222 ppm in rats was chosen as the point of departure for AEGL-3 values. Considering data on humans, rats, and mice, a total uncertainty factor of 10 appears to be sufficient for toxicokinetic and toxicodynamic differences between individuals and interspecies differences. The effects are attributed to *n*-hexane itself and no relevant differences in kinetics are assumed, so only small interindividual differences are expected. Steady-state blood concentrations for *n*-hexane will be reached in approximately 30 min. Thus, the 30-min AEGL-3 value was adopted as the 1-, 4-, and 8-h AEGL-3 values. The 10-min AEGL-3 value was derived from the 30-min value by time scaling using the equation $C^n \times t = k$, with $n = 3$. All of the AEGL-3 values are higher than 50% of the lower explosive limit for *n*-hexane and the 10-min value is higher than the lower explosive limit, so safety considerations against the hazard of explosion must be taken into account.

AEGL values are summarized in Table 3-1.

TABLE 3-1 AEGL Values for *n*-Hexane

Classification	10 min	30 min	1 h	4 h	8 h	End Point (Reference)
AEGL-1 (nondisabling)	NR	NR	NR	NR	NR	Insufficient data
AEGL-2 (disabling)	4,000 ppm[a] (14,000 mg/m^3)	2,900 ppm[a] (10,000 mg/m^3)	2,900 ppm[a] (10,000 mg/m^3)	2,900 ppm[a] (10,000 mg/m^3)	2,900 ppm[a] (10,000 mg/m^3)	One-third of AEGL-3 values
AEGL-3 (lethal)	See below[b]	See below[c]	See below[c]	See below[c]	See below[c]	No lethality in rats (Raje et al. 1984)

Abbreviations: NR, not recommended because of insufficient data.
[a]The AEGL-2 value is higher than 10% of the lower explosive limit of *n*-hexane in air of 1.1% (11,000 ppm). Therefore, safety considerations against the hazard of explosion must be taken into account.
[b]The 10-min AEGL-3 value of 12,000 ppm (42,000 mg/m^3) is higher than the lower explosive limit of *n*-hexane in air of 1.1% (11,000 ppm). Therefore, extreme safety considerations against the hazard of explosion must be taken into account.
[c]The AEGL-3 values for the 30-min, 1-h, 4-h, and 8-h durations are each 8,600 ppm (30,000 mg/m^3), which is higher than 50% of the lower explosive limit of *n*-hexane in air of 1.1% (11,000 ppm). Therefore, extreme safety considerations against the hazard of explosion must be taken into account.

1. INTRODUCTION

n-Hexane is a chemical isolated from natural gas and crude oil (WHO 1991; ATSDR 1999). Pure *n*-hexane is a colorless, volatile liquid with a slightly disagreeable, gasoline-like odor. It evaporates very easily in air and dissolves only slightly in water. *N*-Hexane is highly flammable, and its vapors can be explosive (ATSDR 1999).

Pure *n*-hexane is used in laboratories (WHO 1991; ATSDR 1999). Most of the *n*-hexane used in industry is mixed with similar chemicals in products known as solvents. Common names for some of these solvents are commercial hexane, mixed hexanes, petroleum ether, and petroleum naphtha (ATSDR 1999). Commercial hexane is mainly a mixture of hexane isomers and related 6-carbon compounds, and has an *n*-hexane content varying between 20 and 80% (WHO 1991). Several hundred million pounds of *n*-hexane are produced in the United States each year in the form of these solvents (WHO 1991; ATSDR 1999). The major use for these solvents is in food processing to extract vegetable oils from crops such as soybeans, flaxseed, peanuts, safflower seed, corn germ, and cottonseed. They are also used as cleaning agents in the printing, textile, furniture, and shoemaking industries. Certain kinds of special glues used in the roofing and shoe and leather industries also contain *n*-hexane. Several other products containing *n*-hexane are gasoline, low-temperature thermometers, adhesives, and lacquers. *n*-Hexane is also present in rubber cement. It is further used in the manufacture of pharmaceuticals. Selected physical and chemical properties of *n*-hexane are presented in Table 3-2.

TABLE 3-2 Chemical and Physical Data for *n*-Hexane

Parameter	Value	Reference
Synonyms	Hexane; hexyl hydride	ATSDR 1999
CAS registry no.	110-54-3	ACGIH 2001
Chemical formula	C_6H_{14}	Lide 1999
Molecular weight	86.18	Lide 1999
Physical state	Liquid	O'Neil et al. 2006
Color	Colorless	O'Neil et al. 2006
Odor	Faint, peculiar odor	O'Neil et al. 2006
Melting point	-95 to -100°C	O'Neil et al. 2006
Boiling point	69°C	O'Neil et al. 2006
Vapor density (air = 1)	2.97	WHO 1991
Liquid density (water = 1)	0.660	O'Neil et al. 2006
Solubility in water	Insoluble; 9.5 mg/L	ATSDR 1999; O'Neil et al. 2006
Vapor pressure	138 mm Hg @ 24°C; 150 mm Hg @ 25°C	WHO 1991; ATSDR 1999
Flammability	Highly flammable	ATSDR 1999
Explosive	Lower explosive limit = 1.1%	WHO 1991
Conversion factors	1 mg/m^3 = 0.284 ppm 1 ppm = 3.52 mg/m^3	WHO 1991

Commercial hexanes are manufactured by two-tower distillation of a suitable hydrocarbon feedstock (WHO 1991). The feedstock may be straight-run gasoline distilled from crude oil or natural gas. Hexanes are also obtained from the remains of catalytic reformates after the removal of aromatics. Very pure *n*-hexane can be produced from hexane mixtures by absorption on molecular sieves.

n-Hexane evaporates easily, so the greatest potential for exposure is through inhalation. Because gasoline contains *n*-hexane, almost everyone is exposed to small amounts of the chemical in the air. A concentration of 2 ppb in the air has been reported for *n*-hexane (ATSDR 1999). Foods, drinking water, and even cooking oils processed with solvents containing *n*-hexane do not generally contain *n*-hexane or contain only very small amounts. Exposure to *n*-hexane most frequently occurs among industrial workers in occupational settings (for example, refinery workers, shoe and footwear assembly workers, ball makers, laboratory technicians, and carpenters). Some people known as "sniffers" inhale volatile chemicals deliberately for their euphoric properties (reviews of Seppäläinen [1988] and Ritchie et al. [2001]). Exposure can also occur in the home if products containing *n*-hexane are used without proper ventilation.

Concentrations of *n*-hexane measured in extraction facilities were 0.9-97 ppm (olive extraction plants) and 4.4-13.2 ppm (soybean extraction facility) (WHO 1991). Concentrations measured for outside operators and transport drivers were 0.13 ± 0.17 ppm and 0.33 ± 0.25 ppm, respectively. Maximum time-weighted average (TWA) (8 h) concentrations of *n*-hexane at an extraction facility were found to be 26 ppm.

2. HUMAN TOXICITY DATA

Many studies of toxicologic effects of *n*-hexane in humans are available. However, most of these studies concerned industrial workers or substance abusers repeatedly exposed for long periods of time to commercial hexane. Commercial hexane generally contains 20-80% *n*-hexane, in addition to hexane isomers and small amounts of related carbon compounds (e.g., cyclopentane, cyclohexane, pentane, and heptane) and other chemicals (e.g., acetone, methyl ethyl ketone, and toluene). Often co-exposure to other solvents was also present. Since most of the studies concern repeated exposure to solvents with a low percentage of *n*-hexane (not pure), they were considered of little or no relevance for deriving AEGL values for *n*-hexane are not discussed in detail. However, it is noteworthy that no lethality was reported in humans abusively exposed to *n*-hexane or commercial hexane.

The main toxic effect reported for *n*-hexane in human studies is degenerative distal axonopathy in the peripheral nervous system, which is caused by the main toxic metabolite 2,5-hexanedione (2,5-HD). Peripheral neuropathy develops in workers who are occupationally exposed to rather high concentrations of *n*-hexane for months (ATSDR 1999). Therefore, this effect is a result of chronic exposure to *n*-hexane and is not relevant for deriving AEGL values.

2.1. Acute Lethality

2.1.1. Case Reports

No case reports of human lethality from acute exposure to *n*-hexane (pure or commercial) were found.

2.2. Nonlethal Toxicity

2.2.1. Case Reports

No relevant case reports of nonlethal toxicity from *n*-hexane were found.

2.2.2. Experimental Studies

Nelson et al. (1943) exposed human volunteers in a chamber (approximately 10 subjects; both sexes) to *n*-hexane at nominal concentrations of up to

500 ppm for 3-5 min. Exposure was well tolerated and there were no subjective complaints.

Four Caucasian male volunteers (22-52 years old; no occupational exposure to organic solvents) were exposed (whole body) for 2 h to *n*-hexane (purity 99%) at an actual concentration of 54.2 (±0.8) ppm during light physical exercise (50 W) on a bicycle ergometer (Shibata et al. 2002). Subjects rated the severity (somewhat, rather, quite, and very) of 10 main symptoms frequently associated with solvent exposure in a questionnaire. Symptoms in the questionnaire included: ocular discomfort, runny nose, discomfort in throat or airways, headache, fatigue, nausea, dizziness, feeling of being intoxicated, difficulty in breathing, and odor of solvents. Ratings for all symptoms except odor were below 10% of the whole scale and corresponded to verbal ratings of "not at all" and "hardly at all".

In addition, several toxicokinetic studies with volunteers were performed. Although the studies did not focus on adverse health effects, no mention of such effects was made. These studies are briefly described.

No adverse clinical effects or subjective complaints were reported in an absorption study with 10 volunteer students (healthy Japanese men and women; 18- to 25-years old) after a 4-h exposure (whole body) to *n*-hexane (purity not specified) at actual concentrations of 87-122 ppm (Nomiyama and Nomiyama 1974).

Veulemans et al. (1982) exposed healthy male subjects (25- to 35-years old) to *n*-hexane (purity unknown) at 100 and 200 ppm (360 and 720 mg/m^3, respectively) for 4 h at rest and at 100 ppm (360 mg/m^3) for 3 h under exertion (up to 100 W). No adverse clinical effects or subjective complaints were reported.

No adverse clinical effects or subjective complaints were also reported in healthy male volunteers (19- to 26-years old) exposed twice (nose only; in a sitting position) to *n*-hexane (purity 99%) at 60 ppm with a 4-h interval (van Engelen et al. 1997). Mean exposure durations were 15.5 min and 3.91 h, respectively.

2.2.3. Occupational and Epidemiological Studies

The group of Perbellini and Brugnone has published many reports on *n*-hexane in occupational settings. In one study, grasp samples of breathing zone air were collected from 20 workers (18 men and 2 women) employed in a shoe upper factory after 60, 165, 195, and 270 min. Average *n*-hexane concentrations were 99 ppm (349 mg/m^3), 150 ppm (531 mg/m^3), 167 ppm (589 mg/m^3), and 214 ppm (755 mg/m^3), respectively (Brugnone et al. 1978). No adverse clinical symptoms were reported. In a second study, the breathing zone air of workers in a shoe factory was collected at different time points (duration of sampling not specified). An average *n*-hexane concentration of 117 ppm (411 mg/m^3) was

reported (mean of 76 air samples, with a maximum concentration of 480 ppm [1,700 mg/m^3]) (Perbellini et al. 1980).

Ten healthy workers (18-30 years old) employed in a shoe factory were shown to be exposed to *n*-hexane at an 8-h TWA of 69 ppm (243 mg/m^3) (range 2-325 ppm [8-1,143 mg/m^3]) (Mutti et al. 1984). No adverse clinical signs were reported.

No adverse clinical effects were also reported in four healthy shoe factory workers (women, 41-54 years old) exposed during four working days to a mean concentration of *n*-hexane in the breathing zone of 1.9-31 ppm (6.7-108.7 mg/m^3). The exposure period was preceded by four days without exposure and followed by two exposure-free days (Ahonen and Schimberg 1988).

2.3. Neurotoxicity

No neurotoxicity studies of acute exposure to *n*-hexane in humans were found.

2.4. Developmental and Reproductive Toxicity

No studies on developmental and reproductive toxicity of *n*-hexane in humans are available. However, it has been reported that on the basis of data from experimental animals and according to the Nordic criteria, *n*-hexane has been classified into Group 1B: "The substance should be regarded as toxic to human reproduction" (Hansen 1992).

2.5. Genotoxicity

The genotoxicity of *n*-hexane has been evaluated by two organizations (WHO 1991; ATSDR 1999). Both evaluations reported only one in vitro test of *n*-hexane in human cells; no increase in unscheduled DNA synthesis was found in an assay using human lymphocytes. Genotoxic effects have not been examined in humans after *n*-hexane exposure.

2.6. Carcinogenicity

No epidemiological studies of occupational exposure to *n*-hexane and cancer were found, which was consistent with the reviews by WHO (1991) and ATSDR (1999).

2.7. Summary of Human Data

In humans, *n*-hexane is of low acute toxicity. No cases of lethality were reported after inhalation of *n*-hexane or commercial hexane. Furthermore, no

severe clinical signs were reported by volunteers after acute exposure at the highest *n*-hexane concentrations tested (200 ppm for 4 h at rest and 100 ppm for 1 h under physical exercise [20-100W]). The only symptom reported at more than 10% on a rating scale by volunteers exposed for 2 h to *n*-hexane at an actual concentration of 54.2 ppm while performing low physical exercise (50 W) was detection of the odor of *n*-hexane.

Corresponding to the evaluations of WHO (1991) and ATSDR (1999), no studies on genotoxicity in humans were located. Only a negative unscheduled DNA synthesis assay using human lymphocytes has been reported. Although genotoxic effects have not been examined in humans after *n*-hexane exposure, genotoxicity in humans cannot be excluded because some positive results were reported in limited animal studies (polyploidy, structural aberrations, and sister chromatid exchanges in in vitro tests with mammalian cells, and morphologic alterations in sperm and chromatid breaks in bone marrow cells in studies of rats) (see Section 3.5).

Consistent with the WHO (1991) and ATSDR (1999) reviews, no epidemiologic studies of occupational exposure to *n*-hexane and cancer in humans were found. However, carcinogenicity cannot be excluded because limited studies in experimental animals have reported hepatocellular neoplasms (adenoma and carcinoma) in mice and papillary tumors in the bronchiolar epithelium of rabbits.

3. ANIMAL TOXICITY DATA

3.1. Acute Lethality

3.1.1. Rats

Little data were available on acute lethality of *n*-hexane in rats. Two LC_{50} values were reported, but lacked details on experimental conditions. A 1-h LC_{50} of 76,900 ppm for rats was reported in a neurotoxicity study by Pryor et al. 1982 (see Section 3.2.1) and a 4-h LC_{50} of 48,000 ppm is mentioned in a review by Couri and Milks (1982) without any details or a reference.

Some studies on the toxicokinetics and metabolism (exposure durations of 10 min to 10 h) were available, in which high concentrations of *n*-hexane (10,000-86,222 ppm) were used (Böhlen et al. 1973; Baker and Rickert 1981; Bus et al. 1982; Raje et al. 1984; see Section 4.1 for details). No mortality was reported in these studies, not even at concentrations as high as 86,222 ppm for 30 min (male Sprague-Dawley rats; whole-body exposure) (Raje et al. 1984) or 48,280 ppm for 10 h (female albino rats; whole-body exposure; purity of *n*-hexane not specified) (Böhlen et al. 1973).

No mortality was reported in rats exposed to *n*-hexane at 48,000 ppm for 10 min, 6 times per day (at least 50 min between exposures), 5 days per week for 10 weeks, followed by an additional 8 weeks at an increased frequency of 12

exposures per day (10-min exposure, 20-min no exposure) and another 4 weeks at a frequency of 24 times per day (10-min exposure, 5-min no exposure). In addition, one repeated exposure study on metabolism was available in which high concentrations of *n*-hexane were used. In this study, male Fischer rats were exposed (whole body) for 12 weeks to *n*-hexane (purity 95%) at a concentration of either 48,000 ppm for 10 min every 30 min, 8 h/day, 5 days/week or 40,000 ppm for 10 min every 30 min with a background of *n*-hexane at 4,000 ppm continuously, 8 h/day, 5 days/week (Howd et al. 1982). No mortality was reported for either exposure regimen.

3.1.2. Mice

Fühner (1921) studied the narcotic action of *n*-hexane (pure, but percentage not specified) prepared from coal oil (initial concentrations approximately 34,800, 38,210, 41,620, 43,750, and 51,990 ppm [123, 134, 147, 154, and 183 g/m^3, respectively]) and *n*-hexane prepared from propyl iodide (initial concentrations approximately 37,640 and 40,060 ppm [132 and 141 g/m^3, respectively]) in white mice (sex and strain not specified). Animals were exposed whole-body in a so-called 'narcotic bottle' in which a watch glass was present for evaporation of required volumes of *n*-hexane. In this bottle, 1-2 mice could be exposed at the same time, but the number of animals exposed at each concentration was not specified. Animals were exposed for different durations (20-127 min) until they were removed or until they died. *n*-Hexane from coal oil induced mice to lay down in a side position without standing up after shaking the exposure chamber (narcotic action) after 34-90 min at the lowest concentration (34,800 ppm) and after 10 min at the highest concentration (51,990 ppm) (dose-dependent effect). Loss of reflexes occurred only at higher concentrations of approximately 38,210, 43,750, and 51,990 ppm after 75 min (1/1), 39 and 57 min (2/2), and 20 and 31 min (2/3), respectively. Animals losing reflexes died after exposure for 127 min (1/1), 73 and 119 min (2/2), and 51 min (1/2), respectively. At 51,990 ppm, one of three mice died very rapidly after 9 min with tetanic convulsions. The minimal fatal dose of *n*-hexane was approximately 38,210 ppm for 127 min. *n*-Hexane showed a marked depressant effect on respiration. Comparative results were obtained with *n*-hexane prepared from propyl iodide. Loss of reflexes occurred within 34 and 42 min (2/2) at 37,640 ppm and within 23 min at 40,060 ppm (dose-dependent effect). The minimal fatal concentration was 37,640 ppm; animals died after 39 and 45 min (2/2). No mortality was observed in mice exposed to *n*-hexane at 40,060 ppm for 26 min. This study could not be used for quantitative analysis, because mice were exposed in a closed system and *n*-hexane concentration as well as the oxygen concentration will have decreased during exposure while carbon dioxide will have increased; the number of animals exposed at each exposure concentration was unknown; and respiration rate steadily decreased during exposure.

These results appeared to be confirmed by Lazarew (1929) who determined a minimal narcotic concentration of *n*-hexane causing mice (sex and strain not specified) to lay down in a side position of approximately 28,000 ppm (100 g/m^3) and a minimal fatal concentration of 34,000-43,000 ppm (120-150 g/m^3). Exposures were for 2h, but the purity of the *n*-hexane and whether concentrations were actual or nominal concentrations were not specified. Reflexes in mice frequently persisted until death. These results showed a very small margin between narcotic and fatal concentration. Exposures were in a closed system, similar to the experiments by Fühner (1921).

Ten male NMRI mice were exposed to *n*-hexane under static conditions (Krämer et al. 1974); 3.2 mL of *n*-hexane was added in a 25-L glass box (equal to an initial concentration of about 24,000 ppm) and exposure was for at least 24 h. No mortality was reported under these conditions. The only adverse effect mentioned was that exposed mice suffered from body weight loss compared with controls.

Groups of four Swiss mice (sex not specified) were exposed head-only for 5 min to *n*-hexane (purity ≥99%) at nominal concentrations of 1,000, 2,000, 4,000, 8,000, 16,000, 32,000, and 64,000 ppm. No mention was made of whether concentrations were monitored during exposure (Swann et al. 1974). Test conditions were the same as those in the sensory irritation test (determination of the concentration that reduces the respiratory rate by 50% [RD_{50}]), and were considered to be screening tests. Mice were placed in individual plethysmographs to investigate effects on pulmonary physiology during exposure. Mice had light anesthesia at 16,000 ppm, whereas at 32,000 ppm they became directly anesthetized with occasional sporadic body movements. At 64,000 ppm, all mice had respiratory arrest within 4.5 min. During exposure at 64,000 ppm, respiration was highly irregular; excitation was followed by light anesthesia with body movements, irregular respiration, an increase in inspiratory effort, and a decrease in the expiratory effort. Respiratory arrest occurred at the end of inspiration. No convulsions were observed. No information on the methods used to assess anaesthesia was provided. Animals in this study were restrained, which could potentially affect assessments of *n*-hexane-induced anaesthesia, adding uncertainty to determination of effect levels for AEGL-2 value. A summary of relevant lethality data is presented in Table 3-3.

3.2. Nonlethal Toxicity

3.2.1. Rats

Three groups of 12 male Fischer rats (six or nine "intact animals" and six or three "surgically prepared animals" for neurologic testing) were intermittently exposed (whole body) to *n*-hexane (labeled; purity ≥95%) at target concentrations of 24,000 (one group) and 48,000 ppm (two groups) (Pryor et al. 1982).

TABLE 3-3 Summary of Acute Lethal Inhalation Data in Laboratory Animals

Species	Concentration (ppm)	Duration	Effect	Reference
Rat	76,900	1 h	LC_{50}	Reported in Pryor et al. (1982)
Rat	48,000	4 h	LC_{50}	Reported in Couri and Milks (1982)
Rat	48,280	2-10 h	No lethality	Böhlen et al. 1973 (kinetic study)
Rat	10,000	0.5-6 h	No lethality	Baker and Rickert 1981 (kinetic study)
Rat	10,000	6 h	No lethality	Bus et al. 1982 (kinetic study)
Rat	86,222	30 min	No lethality	Raje et al. 1984 (kinetic study)
Rat	48,000 40,000	10 min/30 min, 8 h/d, 5 d/wk for 12 wk 10 min/30 min, on a background of 4,000 ppm continuous, 8 h/day, 5 d/wk for 12 wk	No lethality No lethality	Howd et al. 1982
Mouse	38,210[a] 43,750[a] 51,990[a]	127 min 73-119 min 9-51 min	Lethality (1/1) Lethality (2/2) Lethality (2/3)	Fühner 1921
Mouse	37,640	39-45 min	Lethality (2/2)	Fühner 1921
Mouse	28,000, 34,000, and 43,000[a] (+ other unspecified concentrations)	2 h	Minimal fatal concentration 34,000-43,000 ppm[a]	Lazarew 1929
Mouse	32,000	5 min	No lethality	Swann et al. 1974
	64,000	<4.5 min	Lethality	

[a]Initial concentration in a closed system.

Concentrations were continuously monitored and were within 10% of the target concentrations. Rats were surgically prepared for recording the brainstem auditory-evoked response and peripheral nerve conduction velocity. Animals were exposed for 10 min, six times per day (at least 50 min between exposures), 5 days per week for at least 10 weeks. One of the 48,000 ppm exposure groups was exposed for an additional 8 weeks to *n*-hexane at 48,000 ppm at an in-

creased frequency of 12 exposures per day (10-min exposure, 20-min no exposure). After 18 weeks, surgically prepared rats were exposed 24 times per day (10-min exposure, 5-min no exposure) for an additional 4 weeks, and were then were allowed to recover. Actual concentrations were monitored daily throughout the experiment. No acute behavioral effects (grip strength, conditioned avoidance response, undifferentiated motor activity) were observed with 10-min exposures to *n*-hexane at 24,000 ppm or 48,000 ppm. Furthermore, the study reported (without further detail) that 48,000 ppm was approximately the highest concentration that did not cause myoclonic seizures in most of the rats during the repeated exposures. An additional group of 15 rats continuously exposed to *n*-hexane at 1,000 ppm for 11 weeks did not show neurobehavioral effects before 3 weeks of exposure.

Six male Wistar rats (trained to avoid shock; avoidance rate >80%) were exposed (whole body) for 4 h first to air (internal control), and then to *n*-hexane (purity >99%) at target concentrations of 50, 100, 200, 400, and 800 ppm (in ascending order on different days) (Ikeda et al. 1993). Concentrations were measured several times during every exposure. The interval between exposures to the different concentrations of *n*-hexane was 14 days. Sham exposure to air (internal control) was carried out every seventh day following exposure to *n*-hexane. Rats were tested on their shock avoidance response 1 h immediately before exposure, during the 4 h of exposure, and 1 h thereafter (6-h test period). As behavioral baseline, the level of performance of the 1-h pretest was used. At 50 ppm, *n*-hexane induced a transitory decrease in lever press and avoidance rates during part of the exposure. No behavioral changes could be detected in rats exposed to *n*-hexane at 100 and 400 ppm, while large, variable changes were observed at 200 ppm. Exposure at 800 ppm induced a considerable increase in the lever press rate during part of the exposure period, while no effect on avoidance rate was observed. Considering the wide variation and fluctuations in responses during exposure, no clear conclusions can be drawn from this experiment.

Three groups of four male albino rats (SD strain, Charles River Japan) were exposed (whole body) for 8 h to *n*-hexane (purity not specified) at nominal concentrations of 0 and 4,000 ppm (no monitoring of actual concentrations during exposure) (Honma et al. 1982). Immediately after exposure, brains were irradiated by microwave to inactivate brain enzymes. Midbrain tissue was homogenized and free amino acids (Tau, Asp, Glu, Gln, Gly, GABA, Thr, Ser, and Ala) were analyzed. In midbrain tissue, only glutamine and alanine were significantly increased by exposure to *n*-hexane. Glutamine is synthesized from glutamic acid by glutamine synthetase in the presence of ammonia. The authors felt that more detailed examination of the change in ammonia content and glutamine synthetase activity is needed for explanation of the increase in glutamine content. Also, the increase in alanine content needs further investigation.

Groups of four male albino rats (SD strain) were exposed (whole body) for 8 h to *n*-hexane gas (purity not specified) at actual concentrations of 0, 2,000, 4,000, and 8,000 ppm (Honma 1983). During exposure the concentration of

n-hexane was monitored (frequency not specified). Rats were killed immediately after exposure by decapitation and their brains were irradiated with microwave to prevent post-mortem changes. Acetylcholine (ACh) content, choline acetyltransferase (ChAT), and acetylcholine esterase (AChE) activities were measured in homogenized hippocampus. ACh was somewhat increased (approximately +20%) at 2,000 ppm, but was reduced extensively (approximately -25%) at 8,000 ppm. At all concentrations tested, *n*-hexane showed a weak trend in reducing ChAT activity (<15%). AChE activity was increased by *n*-hexane (significantly at 4,000 ppm, approximately +23%), but without a dose-relationship. Because ChAT synthesizes ACh from choline and acetyl CoA at nerve endings, the decrease in ACh at 8,000 ppm might be due to both the reduced ChAT activity and the elevated AChE activity. Rats exposed to *n*-hexane showed some symptoms including sedation, hypothermia, and ptosis. The incidence and severity of symptoms were dependent on exposure concentration. However, no further details were presented.

The functional implications of the biochemical effects reported by Honma et al. are difficult to assess considering the degree of neurologic effects observed in repeat-exposure studies with much higher concentrations of *n*-hexane (e.g., Pryor et al. 1982).

Two groups of three male Wistar rats were exposed (whole body) for 18 h to *n*-hexane (purity not specified) at target concentrations of 0 and 500 ppm on three different days (Edelfors and Ravn-Jonsen 1985). Concentrations were monitored during exposure (frequency of sampling not specified). At the end of the exposure period, rats were bled with heart puncture. Synaptosome preparations were obtained from the brain, except for the cerebellum, to measure calcium uptake at 0.5, 2, 4, and 8 min. No significant effects were found.

Male Wistar rats were exposed (whole body) to *n*-hexane (purity 96-99%) at nominal concentrations of 700 ppm for 8 h (3 rats) and 10,000 ppm for 4 h (2 rats) and 8 h (3 rats) (Schnoy et al. 1982). Concentrations were calculated from the consumption of *n*-hexane per hour (measured by gas chromatography) and total air volume (variation <±15%). Lungs were examined for histopathologic changes with special attention to the pneumocytes. Light microscopy did not reveal any pathologic finding. Electron microscopy showed direct toxic effects of *n*-hexane to pneumocytes (concentration and exposure duration not specified; changes observed within 24-48 h) with definite regressive alterations, such as fatty degeneration, changes in lamellar bodies of type II pneumocytes, and increased detachment of cells. In a later report of this study, the intrapulmonary nerve system in the hilus and central and peripheral lung segments was examined histopathologically. Ultrastructurally, no alterations of the intrapulmonary nerves were found after short-term exposure to *n*-hexane (Schmidt et al. 1984).

Groups of six male Wistar rats were exposed (whole body) for 5 h to *n*-hexane (purity not specified) at nominal concentrations of 0 and 4,260 ppm (Hadjiivanova et al. 1987). No monitoring of the actual concentration *n*-hexane during exposure was performed. Animals were killed one day after exposure,

and bronchoalveolar lavage fluid (BAL) was isolated and freed of whole cells and debris and the lungs were homogenized. Lipids were extracted from BAL and from lung tissue homogenate. *n*-Hexane caused a moderate increase in the phospholipids of BAL, which was mainly due to an increase in phosphatidylserine and sphingomyeline on day 1 after exposure. In lung tissue homogenate, the total amount of phospholipids was not affected, but the relative contribution of the individual phospholipids was changed. Phosphocholine (the main and physiologically most important phospholipid) and phosphatidylserine were found to be decreased with a concomitant increase in phosphatidylethanolamine. The increased surfactant phopholipids in the alveoli (BAL) was most probably a direct effect of *n*-hexane on type II cells, enhancing the release of phospholipids (mainly phosphocholine) from their secretory granules (lamellar bodies). Effects on pulmonary surfactants are in general the early events in lung toxic injury.

A group of 17 male Sprague-Dawley rats (Charles River, Italy) was exposed (whole body) for 24 h to *n*-hexane (99% purity) at a target concentration of 5,000 ppm (De Martino et al. 1987). The concentration was regulated at approximately 5,000 ppm. A control group (no details provided) was also used. At days 0, 2, 7, 14, and 30 after exposure, 6, 3, 4, 2, and 2 rats, respectively, were killed. Testes and epididymides were examined histopathologically. The proportion of animals with lesions in the testis was 50, 67, 75, 50, and 0% and in the epididymis 67, 33, 25, 0, and 0% at day 0, 2, 7, 14, and 30, respectively. At day 0, testicular lesions were characterized by focal degeneration of spermatocytes (cytoplasmic swelling, nuclear pyknosis, kariorhexis, and kariolysis) and by mild exfoliation of elongated spermatids. Affected epididymal tubules showed several degenerating germ cells mixed with normal spermatozoa in the lumen. At days 2 and 7, effects were more pronounced (e.g., vacuolization, nuclear swelling of Sertoli cells), and numerous inflammatory cells were seen in the epididimys. Recovery from the lesions in both the testis and epididimys was observed starting at 14 day, with complete recovery by day 30.

No histopathologic lesions of the lungs or testes were found in groups of 15 male and 15 female Fischer-344 rats (Cavender et al. 1984) or in groups of 10 male and 10 female B6C3F_1 mice (Dunnick et al. 1989) exposed to *n*-hexane concentrations of up to 10,000 ppm for 6 h/day, 5 days/week for 13 weeks. Because effects on the testes were induced following a 24-h exposure, the effects might not manifest until the exposure lasts for much longer than 6-8 h. Repeated 6-h exposures with 18-h periods of no exposure in between are not sufficient to induce such lesions. Therefore, the effects on the testes reported by De Martino et al. (1987) are not relevant for setting AEGL values. Effects on the lung could only be traced with electronic microscopy. Since no functional lung impairment was observed even after repeated exposure, the effects described by Schnoy et al. (1982) and Schmidt et al. (1984) were considered irrelevant for deriving AEGL values.

A few toxicokinetic studies have been performed with relatively high concentrations of *n*-hexane. These studies are described briefly below.

Five groups of four male Sprague-Dawley rats were exposed (whole body) to *n*-hexane (reagent grade or higher, not further specified) at an actual concentration of 86,222 ± 1,330 ppm for 10, 15, 20, 25, and 30 min (Raje et al. 1984). Animals were killed immediately after exposure. Although the study focused on blood *n*-hexane concentrations, it was mentioned that only the animals exposed for 25 and 30 min showed visible signs of toxicity (ataxia and decreased motor activity).

In a toxicokinetic study, groups of four to six female albino rats (strain not specified) were exposed (whole body) for 2-10 h to *n*-hexane (purity not specified) at a nominal concentration of 48,280 ppm (170 g/m^3). No mention was made of clinical signs of *n*-hexane toxicity (Böhlen et al. 1973). In a similar study, groups of three male Fischer-344 rats were exposed for 0.5, 1, 2, 3, 4, and 6 h (head only) or for 6 h (whole body) to *n*-hexane (purity 95.8%) at actual concentrations of 1,000, 3,000, and 10,000 ppm. No mention was made of clinical signs of toxicity (Baker and Rickert 1981).

In groups of three male Fischer-344 rats exposed for 6 h (whole body) to [1,2-^{14}C]-*n*-hexane (purity 98.5%; diluted to the required specific activities with *n*-hexane, purity 95.8%) at actual concentrations of 500, 1,000, 3,000, and 10,000 ppm, reduced respiration associated with narcosis was observed at the highest concentration (Bus et al. 1982).

3.2.2. Mice

Fühner (1921) studied the narcotic action of *n*-hexane (pure, but percentage not specified) prepared from coal oil (initial concentrations approximately 34,800, 38,210, 41,620, 43,750, and 51,990 ppm [123, 134, 147, 154, and 183 g/m^3, respectively]) and *n*-hexane prepared from propyl iodide (initial concentrations approximately 37,640 and 40,060 ppm [132 and 141 g/m^3, respectively]) in white mice (sex and strain not specified). Animals were exposed whole-body in a so-called 'narcotic bottle' in which a watch glass was present for evaporation of required volumes of *n*-hexane. In this bottle, 1-2 mice could be exposed at the same time, but the number of animals exposed at each concentration was not specified. Animals were exposed for different durations (20-127 min) until they were removed or until they died. *n*-Hexane from coal oil induced mice to lay down in a side position without standing up after shaking the exposure chamber (narcotic action) after 34-90 min at the lowest concentration (34,800 ppm) and 10 min at the highest concentration (51,990 ppm) (dose-dependent effect). Loss of reflexes occurred only at higher concentrations of approximately 38,210, 43,750, and 51,990 ppm after 75 min (1/1), 39 and 57 min (2/2), and 20 and 31 min (2/3), respectively. Animals losing reflexes died after exposure for 127 min (1/1), 73 and 119 min (2/2), and 51 min (1/2), respectively. At 51,990 ppm, one of three mice died very rapidly after 9 min with tetanic convulsions. The minimal fatal dose of *n*-hexane was approximately 38,210 ppm for 127 min. *n*-Hexane showed a marked depressant effect on respi-

ration. Comparative results were obtained with *n*-hexane prepared from propyl iodide. Loss of reflexes occurred within 34 and 42 min (2/2) at 37,640 ppm and within 23 min at 40,060 ppm (dose-dependent effect). The minimal fatal dose was 37,640 ppm. At this concentration, animals died after 39 and 45 min (2/2). Results of this study could not be used for quantitative analysis, because mice were exposed in a closed system and *n*-hexane concentration as well as the oxygen concentration will have decreased during exposure while carbon dioxide will have increased; the number of animals exposed at each exposure concentration was unknown; and respiration rate steadily decreased during exposure.

These results appeared to be confirmed by Lazarew (1929) who determined a minimal narcotic concentration of *n*-hexane causing mice (sex and strain not specified) to lay down in a side position of approximately 28,000 ppm (100 g/m^3) and a minimal fatal concentration of 34,000-43,000 ppm (120-150 g/m^3). Exposures were for 2h, but the purity of the *n*-hexane and whether concentrations were actual or nominal concentrations were not specified. Reflexes in mice frequently persisted until death. These results showed a very small margin between narcotic and fatal concentration. Exposures were in a closed system, similar to the experiments by Fühner (1921).

Groups of four Swiss mice (sex not specified) were exposed (head only) for 5 min to *n*-hexane (purity ≥99%) at target concentrations of 1,000, 2,000, 4,000, 8,000, 16,000, 32,000, and 64,000 ppm (no monitoring of actual concentrations during exposure) (Swann et al. 1974). Mice were placed in individual plethysmographs to investigate effects on pulmonary physiology during exposure. Light anesthesia occurred at 16,000 ppm. At 32,000 ppm, mice became directly anesthetized with occasional sporadic body movements. Anesthesia became deeper and irregular respirations occurred with increased expiratory effort. Apneic respirations (4-5 sec), an increase in rate and a decrease in amplitude followed this. There was a return to moderate to light anesthesia during early and late recovery, respectively. All animals survived. At 64,000 ppm, all mice had respiratory arrest within 4.5 min. During exposure, respiration was highly irregular; excitation was followed by light anesthesia with body movements, irregular respiration, an increase in inspiratory effort, and a decrease in the expiratory effort. Respiratory arrest occurred at the end of inspiration. No convulsions were observed.

The study by Swann et al. (1974) cannot be used to derive AEGL values. Mice in this study were restrained, which could potentially affect assessments of *n*-hexane-induced anaesthesia, adding uncertainty to determination of effect levels for AEGL-2 values. A summary of nonlethal toxicity data is provided in Table 3-4.

3.3. Neurotoxicity

According to ATSDR (1999), exposure to *n*-hexane produces toxicity to peripheral and sensory nerves. Neurotoxicity is due to the *n*-hexane metabolite

2,5-hexanedione, which interferes with neurofilament phosphorylation status and, thus, impairs axonal transport. *N*-Hexane-induced neurotoxicity is primarily associated with chronic exposure, so is not relevant to derivation of AEGL values. However, no data were identified to evaluate the potential for peripheral neuropathy to occur following exposure to a single high dose of *n*-hexane. Many studies reviewed in Section 3.2 (Animal Toxicity Data, Nonlethal Toxicity) included assessments of neurotoxicity outcomes associated with repeated exposure. See Section 3.2 for reviews of these studies.

3.4. Developmental and Reproductive Toxicity

Pregnant Wistar rats were exposed by inhalation (whole body) to air (control) or *n*-hexane (purity 99%) for 23 h/day, 7 days/week (Stoltenburg-Didinger et al. 1990). Exposure concentrations were 500 ppm (during gestation), 800 ppm (one group exposed during gestation and one group [both dams and pups] exposed during gestation and postnatally for 3 weeks), and 1,000 ppm (initial concentration of 1,500 ppm; one group exposed during gestation and one group [both dams and pups] exposed during gestation and postnatally for 30 days). *n*-Hexane concentrations were monitored continuously during exposure. Results were not clearly reported.

TABLE 3-4 Summary of Relevant Nonlethal Inhalation Effect of *n*-Hexane in Laboratory Animals

Species	Concentration (ppm)	Duration	Effect	Reference
Rat (n = 12)	24,000	6 times, 10 min/d, d/wk for 18 wk	No acute behavioral effects	Pryor et al. 1982
Rat (n = 12)	48,000	6 times, 10 min/d, 5 d/wk for 13 wk (n = 11) and 18 wk (n = 1)	No myoclonic seizures in most rats	
Rat (n = 4)	86,222	10, 15, and 20 min	No visible signs of toxicity	Raje et al. 1984
		25 and 30 min	Ataxia and decreased motor activity	
Rat (n = 3)	500, 1,000, and 3,000	6 h	No effects on respiration	Bus et al. 1982
Rat (n = 3)	10,000	6 h	Reduced respiration associated with narcosis	

Dams: Six of eight dams exposed to *n*-hexane at 800 ppm and four of eight dams exposed at 1,000 ppm carried pregnancy to full term compared with 100% of controls. In the dams that did not give birth, resorption of embryos or death during late fetal stage was confirmed by necropsy. No data on litter sizes were provided. Neurologic irregularities were not observed in dams exposed only during gestation, but at the two highest concentrations marked hindlimb weakness developed after giving birth. At the highest concentration, corresponding paranodal axonal swellings were recognizable on postpartum day 30.

Newborns: At all concentrations tested, exposure during gestation reduced body weight at a comparable litter size. This reduction was still present at postnatal day 25 after exposure to *n*-hexane at 500 ppm. In pups with only prenatal exposure, absolute brain weight was less reduced than body weight, resulting in an increased brain-weight to body-weight ratio. This effect was more pronounced in animals exposed during gestation and postnatally. Furthermore, a delay in the maturation of cerebellar cortex was observed (fissura prima of the vermis cerebella; delay in migration of the outer granular cells and a persistence of Purkinje cells) at all concentrations. Recovery was found on postnatal day 30 in animals exposed during gestation only. Other observations included retardation in growth and development, fur irregularities, and less activity. Recovery from these symptoms started about 2 weeks after exposure ended.

In a later publication, effects on brain biochemistry were discussed (Stoltenburg-Didinger 1991). The enzyme maturation pattern in the cerebellum of newborn animals was studied by histochemistry of succinic dehydrogenase (SDH) and NADH tetrazolium reductase (NADH-Tr). Enzyme activities were visualized by formazan deposition in the primary fissure of the cerebellar vermis (an early maturing region) on postnatal days 1, 9, and 21.

Newborns: In control animals, external and internal granular cells exhibited weak oxidative enzyme activity at all ages. Purkinje cells showed increasing oxidative enzyme activity from birth, reaching adult activity at the end of the fourth week. In all exposed animals, development of SDH and NADH-Tr activity paralleled that of normal rats with a delay. Exposure during gestation and postnatally resulted in a persisting apical cone and delayed formation of the apical dendritic tree of the Purkinje cells at day 9. Higher SDH and NADH-Tr activity was found in these cells, returning to normal levels at day 21. Also, a delay in migration of the outer granular cells was observed, even at 500 ppm administered only during gestation. No differences in SDH and NADH-Tr activity in external and internal granular cells were found.

In a well-performed study, pregnant Fischer-344 rats were exposed (whole body) for 6 h/day during various periods of gestation to *n*-hexane (purity 99%) at target concentrations of 0 and 1,000 ppm (Bus et al. 1979). Concentrations were monitored three times per hour (variation <5%). To investigate perinatal toxicity, three groups of pregnant rats were exposed at 1,000 ppm on gestation days 8-12 (n = 7), 12-16 (n = 9), and 8-16 (n = 8) and compared with control rats (n = 7, 6, and 3, respectively). On day 22, females were killed and fetuses examined. In a second experiment, pregnant rats (number not specified) were

exposed to *n*-hexane at 0 and 1,000 ppm on gestation days 8-16. Following delivery on day 23, litters (eight control litters, 14 treatment litters) were culled to six pups per litter and pups were examined weekly for up to 7 weeks. All litters were weaned 4 weeks after birth. After exposure on gestation days 8-12, 12-16, or 8-16 (first experiment), no significant alterations in fetal resorptions, fetal body weights, visible anomalies, or incidence of soft tissue or skeletal anomalies were observed in any of the treatment groups. These results indicated that *n*-hexane was not developmentally toxic at 1,000 ppm. After exposure on days 8-16 (second experiment), a significant reduction in the growth of the pups was found for up to 3 weeks after birth (no significant effect at birth, but 13.9% decrease in litter weight at postnatal day 21). Litter weights remained reduced between 4 (10.6%) and 6 (6.9%) weeks after birth, but returned to control values after 7 weeks (transient effect). No deaths or externally visible onset of neuropathy were observed.

Placental transfer of *n*-hexane may occur since *n*-hexane has been found in rat fetuses after maternal exposure (see Section 4.1). Comparison of the data reported by Bus et al. (1979) with those of Stoltenburg-Didinger et al. (1990) show that (near) continuous exposure to *n*-hexane is necessary to induce developmental toxicity. These effects are, therefore, not to be expected after exposure to *n*-hexane for up to 8 h. Bus et al. (1979) reported postnatal growth retardation following exposure to *n*-hexane during gestation day 8-16. However, in a review on the significance of developmental effects for acute exposures, it was concluded that this kind of effect is not expected to occur after a single exposure (van Raaij et al. 2003). Therefore, these effects are considered not relevant for deriving AEGL values for *n*-hexane.

3.5. Genotoxicity

The genotoxicity of *n*-hexane has been evaluated by several organizations (WHO 1991; ATSDR 1999). See ATSDR (1999) for more details and primary references.

Only limited mutagenicity testing of *n*-hexane has been conducted. In general, *n*-hexane appears to be negative in bacterial tester strains such as *Escherichia coli*, *Bacillus subtilis*, and *Salmonella typhimurium* both with and without metabolic activation. *n*-Hexane was not mutagenic in *S. typhimurium* strains TA98, TA100, TA1535, or TA1537 when tested with a pre-incubation protocol at doses up to 1,000 μg/plate with or without rat or hamster liver S9 fraction. *n*-Hexane was also negative in an in vitro test for induction of chromosome loss in *Saccharomyces cerevisiae*. Negative results for chromosomal aberrations, sister chromatid exchanges, and point mutation rate were generally also obtained in mammalian cells except for one observation of polyploidy in Chinese hamster lung (CHL) cells and polyploidy and structural aberrations in two studies on Chinese hamster ovary (CHO) cells exposed to undiluted *n*-hexane in the absence of S9. *n*-Hexane at concentrations up to 5,000 μg/mL in the presence or absence of rat liver S9 did not induce chromosomal aberrations in cultured CHO

cells. Sister chromatid exchanges were induced in CHO cells but only in the presence of S9 (no dose response).

In studies of in vivo genotoxicity (see Table 3-5), *n*-hexane was found to be negative in a dominant lethal test, micronucleus test, and sperm morphology test in mice. The highest ineffective dose in mice was 10,000 ppm (6 h/day, 5 days/week for 13 weeks). In contrast, morphologic alterations in sperm were noted in rats at 5,000 ppm (exposure duration not specified). This effect was reversible; no significant effects were found 5 weeks after exposure ended. Chromatid breaks were reported in rat bone marrow cells (concentration and exposure duration not specified).

3.6. Carcinogenicity

Little information was available on the carcinogenicity of *n*-hexane. Carcinogenicity was evaluated by WHO (1991) (a dermal study, not relevant for deriving AEGL values) and ATSDR (1999). ATSDR reported on an inhalation study with $B6C3F_1$ mice in which hepatocellular neoplasms (adenoma and carcinoma) were found. However, commercial hexane (51.5% purity) was used, so it was not clear what component of the *n*-hexane mixture caused the neoplasms. A parallel experiment carried out on rats showed no increase in incidence of neoplasms at any site. Papillary tumors have been reported in the bronchiolar epithelium of rabbits after a 24-week exposure to *n*-hexane at 3,000 ppm.

TABLE 3-5 Genetic Effects of *n*-Hexane in In Vivo Studies of Inhalation Exposure

Species	Tissue	Exposure (HID or LED)	End Point	Results
Mouse[a]		396 ppm[b]	Dominant lethal mutation	-
Swiss mouse		5,000 ppm; 20 h/d for 5 d	Dominant lethal mutation	-
$B6C3F_1$ mouse	Sperm	5,000 ppm; 20 h/d for 5 d	Sperm morphology	-
Mouse[a]	Peripheral blood	10,000 ppm; 6 h/day, 5 d/wk for 13 wk	Micronuclei formation	-
Mouse[a]	Peripheral blood	1,000 ppm; 22 h/d, 5 d/wk for 13 wk	Micronuclei formation	-
Rat[a]	Sperm	5,000 ppm[b]	Sperm morphology	+ (reversible)
Rat[a]	Bone marrow	Not specified	Chromatid breaks	+

Abbreviations: HID, highest ineffective dose; LED, lowest effective dose; +, positive results; -, negative results.
[a]Strain not specified.
[b]Exposure duration not specified.
Source: ATSDR 1999.

3.7. Summary of Animal Data

A summary of relevant lethality data is presented in Table 3-3. No original acute mortality studies could be retrieved. Reference has been made to two LC_{50} values in rats (a 1-h LC_{50} of 72,900 ppm and a 4-h LC_{50} of 48,000 ppm) without further details, but the original references could not be identified. Hence, these values cannot be evaluated properly. In addition, findings in toxicokinetic studies have discrepancies with these LC_{50} values. No lethality occurred in rats exposed to *n*-hexane at up to 48,280 ppm for 10 h or at 86,222 ppm for 30 min. Mice appeared to be more susceptible to *n*-hexane than rats, but the studies by Fühner (1921) and Lazarew (1929) could not be used for quantitative analysis because mice were exposed in a static system, the number of animals per exposure concentration was unknown, and respiration rate steadily decreased during exposure. Exposure for 5 min to *n*-hexane at 16,000 ppm caused light anesthesia in mice. Mice became directly anesthetized at 32,000 ppm, and exposure at 64,000 ppm caused respiratory arrest within 4.5 min (Swann et al. 1974).

A summary of nonlethal toxicity data is provided in Table 3-4. Visible signs of acute toxicity were associated with effects on the nervous system (e.g., reduced respiration, ptosis, myoclonic seizures, ataxia, decreased motor activity, sedation, laying down in a side position, and narcosis). In general, these effects were dose-related. Results showed a very small margin between concentrations causing narcotic effects (34,800 ppm for 90 min) and causing death (38,210 ppm for 127 min; 43,750 ppm for 96 min) in mice exposed in static systems. Clinical signs were found in rats exposed at 86,222 ppm for 25 min (Raje et al. 1984).

In rats, acute effects on the brain were found at concentrations of 2,000 ppm and higher for 8 h (increased glutamine and alanine content, effects on acetylcholine metabolism) but the functional implications are difficult to assess. Effects on the lung were seen after exposure to *n*-hexane at 4,260 ppm for 5 h (effects on the lung surfactant system followed by degenerative effects on the pneumocytes). Reversible lesions in the testis were found in rats exposed at 5,000 ppm for 24 h. However, no histopathologic changes in the lungs or testes were found in rats and mice exposed to *n*-hexane at 10,000 ppm for 6 h/day, 5 days/week for 13 weeks. Pulmonary effects were very minor and did not result in functional impairment after repeated exposure. An exposure duration longer than 6 h is necessary to induce the testes effects. Therefore, both types of effects are not relevant for AEGL-derivation.

Developmental and reproductive toxicity studies with *n*-hexane at up to 1,000 ppm showed no effects on dams exposed during gestation. A decreased number of litters were observed in dams exposed at 500 ppm for 23 h/day, 7 days/week throughout gestation (Stoltenburg-Didinger et al. 1990), but not in dams exposed at 1,000 ppm for 6 h/day on gestation days 8-16 (Bus et al. 1979). Therefore, a (near) continuous exposure regimen is necessary to induce these kind of effects. Postnatal growth retardation in offspring exposed during gestation was judged to be not relevant for setting AEGL values. Some transient ef-

fects on biochemical parameters and brain development were also reported by Stoltenburg-Didinger (1991).

Only limited mutagenicity testing has been conducted. In general, *n*-hexane was not mutagenic in vitro with some exceptions: polyploidy in hamster CHL cells, polyploidy and structural aberrations in CHO cells (undiluted *n*-hexane in the absence of S9), and sister chromatid exchanges in CHO cells (only in the presence of S9, no dose response). The available studies on in vivo genotoxicity are summarized in Table 3-5. *n*-Hexane was found to be not mutagenic in a dominant lethal test, micronucleus test, and sperm morphology test with mice. In rats, however, morphologic alterations in sperm and chromatid breaks in bone marrow cells were observed.

Little information was available on the carcinogenicity of *n*-hexane. Hepatocellular neoplasms (adenoma and carcinoma) were reported in mice and papillary tumors in the bronchiolar epithelium in rabbits.

4. SPECIAL CONSIDERATIONS

4.1. Metabolism and Disposition

Absorption

Ten volunteer students (healthy Japanese men and women, 18-25 years old) were exposed to *n*-hexane (purity not specified) at actual concentrations of 87-122 ppm for 4 h in an exposure room (Nomiyama and Nomiyama 1974). Concentrations of *n*-hexane in environmental and expired air were measured four times at a 1-h interval. Immediately after exposure, expired air was collected for 3 min following inhalation of fresh air. Respiratory retention decreased with exposure duration and reached a constant level after 2 h of exposure (saturation). Pulmonary retention was calculated to be 5.6 ± 5.7% (5.7 ± 7.1% for men, 5.4 ± 4.1% for women).

Brugnone and coworkers have published many reports on occupational exposure of shoe factory workers to *n*-hexane. In one study, grasp samples of breathing zone air and alveolar air samples were obtained from 20 workers (18 men and 2 women) employed in a shoe upper factory 60, 165, and 270 min after the start of a shift. Average *n*-hexane concentrations were 99 ppm (349 mg/m^3), 167 ppm (589 mg/m^3), and 214 ppm (755 mg/m^3), respectively (Brugnone et al. 1978). Alveolar retention of *n*-hexane was estimated to be equal to 14.9%, and the concentration in venous blood sampled after 270 min was 0.38 µg/mL.

Healthy males (25-35 years old) were exposed for 4 h to *n*-hexane (purity not specified) at 100 and 200 ppm (360 and 720 mg/m^3, respectively) at rest (four 50-min periods of exposure with 10-min non-exposed intervals) and at 100 ppm (360 mg/m^3) under increasing levels of physical exercise (peak exercise involved five consecutive 10-min periods of 20, 40, 60, 80, and 100 W or three consecutive 50-min periods with 10-min non-exposed intervals of 20, 40, and 60 W) on a bicycle ergometer (Veulemans et al. 1982). Exposure concentrations

were monitored. Experiments were conducted with at least 2-week intervals. *n*-Hexane concentrations were measured in inhaled and exhaled air. Physical exercise during exposure caused an important increase in lung clearance values from 2.30 L/min at rest to 5.36 L/min at 80 W of peak exercise. Retention decreased by more than 50% with increasing exercise from 24.1% at rest to 10.5% at 100 W of peak exercise. Uptake rate increased with exercise by more than two times the uptake at rest (from 0.84 mg/min at rest to 1.93 mg/min at 80 W of peak exercise). Steady-state concentrations of *n*-hexane in venous blood were reached within 15 min at 100 or 200 ppm at rest or under exertion. *n*-Hexane concentrations in peripheral venous blood (0.183 μg/mL) increased with increasing exercise levels, both after 10 min (0.290 μg/mL, 60 W) or 50 min of exercise (0.331 μg/mL, 100 W).

n-Hexane concentrations were measured in breathing zone air and in alveolar air of 10 young healthy workers (18-30 years old) employed in a shoe factory. The median 8-h TWA of *n*-hexane in the breathing zone was 69 ppm or 243 mg/m^3 (range 2-325 ppm or 8-1,143 mg/m^3). Alveolar retention was calculated to be about 25% (range 22.1-28.7%) of the inhaled *n*-hexane. Physical load while working was very slight (ventilation value 8 L/min). Urinary metabolites 2,5-dimethylfuran, 2-hexanol, 2,5-HD, and γ-valerolactone were found at low concentrations, but were related to *n*-hexane in the air. 2,5-HD was the main metabolite (Mutti et al. 1984).

Healthy male volunteers (19-26 years old) were exposed (nose only, in a sitting position) for 15.5 min to *n*-hexane (purity 99%) at concentration of about 60 ppm. Volunteers were exposed approximately 4 h later to *n*-hexane at 60 ppm for a mean exposure duration of 3.91 h (van Engelen et al. 1997). During and after exposure, the last part of the alveolar air was sampled after subjects held their breath for 30 seconds. During the first exposure, the mean inhaled concentration (C_i) of *n*-hexane was 64 ppm and the mean exhaled concentration (C_e) was 39 ppm, so alveolar retention was 39%. During the second exposure, the mean C_i was 63 ppm and the mean C_e was 41 ppm, so alveolar retention was 35%. Little variation in the toxicokinetics of *n*-hexane between the first and second exposure in the same subject was observed.

Distribution, Metabolism, and Excretion

Perbellini and Brugnone have published many reports on *n*-hexane metabolism, both in occupational workers and in animals. Two of the reports are discussed below. Solvents in the breathing air of workers in a shoe factory were found to contain *n*-hexane at 117 ppm (411 mg/m^3) (Perbellini et al. 1980). Metabolites 2-hexanol, 2,5-dimetylfuran, γ-valerolactone, and 2,5-HD were identified in the urine of workers sampled during the last 4 h of a work shift. 2,5-HD was the main metabolite. 2,5-Dimetylfuran and γ-valerolactone were also found in the urine of rats, but not in the urine of rabbits or monkeys (Perbellini et al. 1982). In this comparative study, male Sprague-Dawley rats, male New Zealand

rabbits, and one male monkey (*Macaca mulatta*) were subjected to a single, whole body exposure to *n*-hexane (purity 99%) at an actual concentration of 5,000 ppm for 6-24 h. Other metabolites present in the urine of rats, rabbits, and the monkey were 2-hexanol, 3-hexanol, methyl *n*-butyl ketone, and 2,5-HD. *n*-Hexane metabolites in rat blood were 2-hexanol, methyl-*n*-butyl ketone, 2,5-dimethylfuran, and 2,5-HD. Humans chronically exposed to a mixture of hexane isomers containing *n*-hexane at 10-40 ppm had urinary concentrations of 2,5-HD ranging from 0.4 to 21.7 mg/L, which is the same proportion as rats exposed once to *n*-hexane at 5,000 ppm for 6 h (1.7 mg/L) or for 12 h (12.8 mg/L).

Caucasian male volunteers (22-52 years old; no occupational exposure to organic solvents) were exposed (whole body) for 2 h to an actual concentration of *n*-hexane (purity 99%) of 54.2 ppm during light physical exercise (50 W) on an ergometer bicycle (Shibata et al. 2002). *n*-Hexane increased rapidly in arterialized capillary blood and reached a steady-state concentration of about 0.25 µg/mL (3 µmol/L) within 30-60 min of exposure. A rapid decline in the concentration of *n*-hexane occurred after exposure ended; *n*-hexane was not detected 2 h after exposure.

Urinary excretion of the *n*-hexane metabolite 2,5-HD was determined in four healthy shoe factory workers (women, 41-54 years old) during four working days (preceded by four exposure-free days and followed by two exposure-free days) (Ahonen and Schimberg 1988). Mean concentration of *n*-hexane in the breathing zone ranged from 1.9 ppm (6.7 mg/m^3) to 31 ppm (108.7 mg/m^3). Total absorption of *n*-hexane in the body was 22-352 mg, total excretion of 2,5-HD in the urine was 0.007-5.03 mg, and the relative excretion (excretion as percentage of absorption) of 2,5-HD was 2-100%, which increased as the exposure to *n*-hexane increased (100% reported for the highest exposed worker). 2,5-HD appeared to accumulate progressively in the body at the highest *n*-hexane concentration.

Female albino rats were exposed to *n*-hexane (purity not specified) at a target concentration of 48,280 ppm (170 g/m^3) for up to 10 h. *n*-Hexane concentration in blood, brain, adrenal glands, kidneys, and spleen increased until a saturation value was reached within 3-4 h (Böhlen et al. 1973). Blood concentrations of *n*-hexane reached a steady-state of about 0.15 mg/mL after 4 h, and a steady-state concentration in the brain of 0.39 mg/g was reached at the same time. Liver concentration of *n*-hexane increased linearly with inhalation duration, and did not reach saturation within 10 h. This was possibly caused by *n*-hexane-induced lipid accumulation (triglycerides) in the liver, and consequently the lower blood supply (lower access of *n*-hexane to fatty liver). Comparison of in vivo tissue/gas partition coefficients (calculated from *n*-hexane concentrations in saturated tissues) with total lipid content of these tissues showed a direct proportional relationship between *n*-hexane saturation concentration and total lipid content of brain, adrenal glands, kidneys, and spleen (saturation value 4 mg/g of lipid). Blood contained much more *n*-hexane in relation to its lipid content (saturation value 25 mg/g of lipid), which was considered to be possibly caused by protein binding. Calculated estimates of the *n*-hexane/lipid content ratio for the liver

essentially exceeded the saturation value of 4 mg/g of lipid. The exceedance was explained by the accumulation of triglycerides in the liver possessing a much higher solubility for organic solvents than other lipid fractions.

Blood from groups of four male Sprague-Dawley rats was collected immediately after whole-body exposure to *n*-hexane (purity at least reagent grade, presaturated air) for 10, 15, 20, 25, or 30 min (Raje et al. 1984). The concentration of *n*-hexane in the exposure chamber was 86,222 ppm (393 g/m^3) after introduction of the animals. Blood became saturated with *n*-hexane within 10 min, and saturation persisted through 30 min of exposure (mean saturation concentration 21 mg/mL).

Male Fischer rats were exposed to average 10-min concentrations of *n*-hexane (purity 95%) ranging from 4,800 to 21,000 ppm (Howd et al. 1982). For single exposures, animals were maintained under static conditions, and *n*-hexane concentrations reportedly decreased by 1% per min. For longer exposure durations, rats were exposed under dynamic conditions to regularly controlled concentrations of *n*-hexane. After 10-min of exposure to *n*-hexane at 4,800-21,000 ppm, *n*-hexane concentrations in blood and brain were found to be linearly related to the exposure concentration. Thereafter, *n*-hexane was rapidly eliminated ($t_{½}$ = 2.5 and 4 min in blood and brain, respectively). Blood concentration of *n*-hexane was approximately 10 µg/mL after 10 min of exposure at 21,000 ppm, and brain concentration was 60 µg/g. Lower concentrations were found in a second series of experiments with rats exposed at 24,500 or 46,100 ppm for 10 min. Blood concentrations of *n*-hexane immediately after exposure were approximately 4 and 8 µg/mL, respectively, and corresponding brain concentrations were less than 20 and approximately 40 µg/g. Despite rapid elimination of *n*-hexane, repeated 10-min exposures (10 min every half hour for 8 h/day) to a high concentration of *n*-hexane (48,000 ppm) resulted in an increase in 2,5-HD in blood (20 µg/mL from a 1-day exposure; 100 µg/mL from 3 or more days of exposure). The minimal sustained plasma 2,5-HD concentration resulting in neurotoxicity appeared to be 50 µg/mL.

Pregnant Fischer-344 rats (number not specified) were exposed (whole body) for 6 h to *n*-hexane (purity 99%) at target concentrations of 0 and 1,000 ppm on gestation day 12, day 20, or days 15-18 (Bus et al. 1979). Results from exposure on day 20 showed that *n*-hexane was rapidly and extensively metabolized to methyl *n*-butyl ketone (MBK) and 2,5-HD. Highest tissue concentrations were found immediately after exposure. Concentrations of *n*-hexane and the two metabolites in the fetus (µg/g wet wt) were approximately equal to those in maternal blood (0.45 µg/mL). Similar concentrations were also found immediately after exposure on day 12 and after exposure on days 15-18, indicating that the placenta was equally permeable during gestation. *n*-Hexane and MBK were rapidly eliminated (minimal to non-detectable concentrations 8 h after exposure) from all tissues examined (maternal blood, brain, kidneys, and liver and fetuses). In contrast, tissue concentrations of 2,5-HD increased between 0 and 4 h after exposure. Thereafter, 2,5-HD was eliminated significantly slower (non-detectable concentrations 24 h after exposure). The calculated half-life of 2,5-

HD in maternal blood (3.90 h) was significantly greater than for *n*-hexane (1.24 h) or MBK (0.99 h). Comparable half-lives for 2,5-HD and MBK were found for fetuses.

In male Fischer-344 rats exposed to *n*-hexane (purity 95.8%) at 500, 1,000, 3,000, and 10,000 ppm for 6 h, steady-state *n*-hexane concentrations were achieved within 30 min in blood and within 2 h in all other tissues examined (brain, liver, kidneys, lungs, testes, and sciatic nerve) (Baker and Rickert 1981). *n*-Hexane concentrations in air were regularly monitored. Steady-state *n*-hexane concentrations were lowest in blood (1, 2, 8, and 21 µg/mL, respectively) and highest in sciatic nerve (10-20 times the blood concentration). *n*-Hexane concentration in the brain was 54.2 µg/g after exposure at 10,000 ppm for 6 h. Steady-state concentrations in the other tissues were 2-5 times the blood concentration. Steady-state *n*-hexane concentrations in the blood and liver were shown to be directly proportional to exposure concentrations. The half-lives of *n*-hexane and MBK were approximately 1-2 h in all tissues except the kidneys ($t_{½}$ = 5-6 h). MBK was the first metabolite (<0.5 h) to appear in the blood and tissues. In blood, tissues, and urine, the following metabolites were demonstrated: 2,5-HD, MBK, 2,5-dimethylfuran (DMFU), 2-hexanol, and 1-hexanol. Concentrations of 2,5-HD were highest in the blood, kidneys, and sciatic nerve (in order of increasing concentrations) after exposure at 1,000 ppm. Tissue concentrations of 2,5-HD were not proportional to dose. The latter in combination with the finding that metabolism and elimination of *n*-hexane were dependent on exposure concentration indicated that severity of neuropathy might not be directly correlated to *n*-hexane exposure concentration.

Lam et al. (1990) demonstrated that 94% of the blood concentration of *n*-hexane was present in erythrocytes of Sprague-Dawley rats. Groups of four male rats were exposed to an actual *n*-hexane (purity not specified) concentration of 515 (± 25) ppm for 2 h. *n*-Hexane concentration immediately after exposure was 0.06 µg/mL in plasma and 0.86 µg/mL in erythrocytes. Very similar results were obtained with rat blood in vitro. In vitro studies of human blood showed that 66% of *n*-hexane in blood was present in erythrocytes, indicating that erythrocytes from humans and rats exhibit substantial differences in affinity for *n*-hexane. Proteins, chiefly hemoglobin, were demonstrated to be the major carriers of *n*-hexane.

In male Fischer-344 rats, the disposition of radioactivity after exposure to [1,2-^{14}C]-*n*-hexane (purity 98.5%; diluted to the required specific activities with *n*-hexane, purity 95.8%) at 500, 1,000, 3,000, and 10,000 ppm for 6 h was studied (Bus et al. 1982). A dose-dependent deposition of radioactivity was found. This finding was unlikely to be attributable to saturation of renal excretion of *n*-hexane metabolites since the estimated half-lives for excretion of urinary metabolites were similar for the groups exposed at 1,000-10,000 ppm. Inhibition of *n*-hexane metabolism at high concentrations (the metabolism of *n*-hexane to $^{14}CO_2$ and urinary metabolites was less in the 10,000-ppm than in the 3,000-ppm group) was suggested as a possible mechanism that would account for the dose-dependent disposition of *n*-hexane. The mechanism of the inhibition is un-

known. The total amount of radioactivity recovered did not increase linearly between the 3,000- and 10,000-ppm groups. This observation might be due to the reduced respiration associated with narcosis observed in the 10,000-ppm group.

Ten male NMRI mice were exposed to *n*-hexane (purity not specified) under static conditions (Krämer et al. 1974). Exposure conditions were very poorly characterized. In general, 3.2 mL of *n*-hexane was added to a 25-L glass box (initial concentration of about 24,000 ppm) and exposure was for at least 24 h. Evidence was obtained that the high turnover rate of *n*-hexane might be correlated with an inducing effect on the monooxygenase system in the liver. The following results supported this hypothesis: (1) increased microsomal protein/g liver ratio mainly caused by an increase in the proteins cytochrome P450, cytochrome b_5, and NADPH-DCPIP reductase (NADPH reductase activity specifically measured by reduction of dichlorophenolindophenol); (2) enhanced microsomal hydroxylation activity caused by an enhanced specific activity of cyclohexane hydroxylation and cyclohexane binding difference spectrum; (3) increase in the total cytochrome P450 content per body weight, liver weight, and total amount of microsomal protein; and (4) a qualitative alteration in the cytochrome P450 species.

The following metabolites were found in the urine of male Wistar rats exposed (whole body) to *n*-hexane (purity not specified) at an actual concentration of 997 ± 23 ppm for 8 h: 1-hexanol, 2-hexanol, 3-hexanol, 2-hexanone, 2,5-HD, 2,5-dimethyltetrahydrofuran, 2,5-dimethyl-2,3-dihydrofuran, and γ-valerolactone (Fedtke and Bolt 1986). Analysis of the urine of male Wistar rats exposed (whole body) to *n*-hexane (purity 99.5%) at an actual concentration of 2,096 ± 124 ppm for 8 h revealed the formation of 4,5-dihydroxy-2-hexanone via 5-hydroxy-2-hexanone or 2,5-HD (Fedtke and Bolt 1987a,b). 4,5-Dihydroxy-2-hexanone is converted to 2,5-dimethylfuran or 2,5-HD, depending on the conditions of urine treatment. Detection of 2,5-dimethylfuran after analysis of urine from workers exposed to *n*-hexane suggested that 4,5-dihydroxy-2-hexanone was formed as a precursor of 2,5-dimethylfuran in humans, too. In urine of male Wistar rats exposed (whole body) for 8 h to *n*-hexane (purity 99.5%) at mean actual concentrations of 50-3,074 ppm, the occurrence of the metabolites 1-hexanol, 2-hexanol, 3-hexanol, 2-hexanone, 2,5-HD, and 4,5-dihydroxy-2-hexanone was demonstrated (Fedtke and Bolt 1987a,b). Excretion of metabolites was linearly dependent on the exposure concentration at up to about 300 ppm; above 300 ppm, saturation kinetics occurred.

The amount of 4,5-dihydroxy-2-hexanone excreted in urine was approximately 10 times higher than that of 2,5-HD. 4,5-Dihydroxy-2-hexanone could also be demonstrated in the urine of a male volunteer (28 years old) exposed by breathing mask to *n*-hexane (purity 99.5%) at a mean actual concentration of 217 ppm for 4 h. The urinary concentration of 4,5-dihydroxy-2-hexanone 22 h after exposure was about four times higher than the concentration of 2,5-HD. Formation of 4,5-dihydroxy-2-hexanone was viewed as a route of detoxification in both rat and man.

Summary

Alveolar retention of *n*-hexane in humans is relatively low. In experimental settings, retention was 25-35%, and a lower retention of about 10% was found with exercise. Pulmonary retention of 5% reported by Nomiyama and Nomiyama (1974) appears to be very low. For men and women, comparable results were obtained. Alveolar retention in workers ranged from 15 to 25%. Blood *n*-hexane concentrations were highly correlated with environmental and alveolar concentrations. Post-exposure, alveolar excretion of *n*-hexane was about 10% of the total uptake. Steady-state *n*-hexane concentrations in blood are rapidly reached in approximately 30 min. Physical exercise resulted in an increase in lung clearance values, a decrease in alveolar retention, and an increase in blood *n*-hexane concentration. In general, a rapid decline in *n*-hexane concentrations was observed after exposure ended, with no detection after 2 h. Metabolite concentrations in urine were related to *n*-hexane concentrations in air.

In experimental animals, steady-state levels (saturation) were generally reached very rapidly in blood and other organs. Most studies indicate a steady-state concentration of *n*-hexane in blood within 30 min. Only Böhlen et al. (1973) reported that a steady-state concentration was reached in female albino rats after 4-5 h of exposure at 48,280 ppm (170 g/m^3). A steady-state blood concentration of about 0.15 mg/mL was also rather high when compared with other studies. Concentrations of *n*-hexane in blood vary to a large extent. In rats, exposure to *n*-hexane at 10,000 ppm for 6 h resulted in blood *n*-hexane concentrations of up to 21 µg/mL, whereas a blood concentration of 150 µg/mL was reported within 5 h of exposure at 48,280 ppm and of 22 mg/mL after a 10-min exposure at 86,222 ppm. The latter value reported by Raje et al. (1984) is probably not correct; it may be that the concentrations should read µg/mL instead of mg/mL. The difference between the 150 µg/mL reported by Böhlen et al. (1973) and the blood concentrations in other studies is difficult to explain. It might be that by direct injection of a blood sample into a gas chromatograph (as done by Böhlen et al. 1973), the *n*-hexane fraction bound to hemoglobin is more precisely estimated. By injecting headspace samples or extraction solvents, as was the method of choice in the other studies, the fraction bound to hemoglobin (which is about 95% in rats, see below) might be underestimated.

n-Hexane saturation concentrations were shown to be directly proportional to the total lipid content in tissues like brain, adrenal glands, kidneys, and spleen. Blood contained much more *n*-hexane in relation to its lipid content, caused by binding to erythrocytes. In rats, about 94% of the *n*-hexane in blood appeared to be noncovalently bound to hemoglobin, whereas in humans the percentage was 66%. In addition, relatively high concentrations of *n*-hexane were found in liver, which may be explained by accumulation of triglycerides in the liver that possess a much higher solubility for *n*-hexane than other lipid fractions. High concentrations of *n*-hexane were also found in sciatic nerve tissue.

n-Hexane is rapidly eliminated by an inducing effect on the monooxygenase system in the liver in animals and is extensively metabolized to MBK and

2,5-HD, the main neurotoxic metabolite. Maximum blood concentrations of 2,5-HD are reached at 4 h post-exposure. For *n*-hexane and MKB, high turn-over rates ($t_{½}$ = 1-2 h) were found in all tissues except the kidneys ($t_{½}$ = 5-6 h). Both compounds were rapidly eliminated to minimal or not detectable concentrations at 8 h post-exposure. In contrast, 2,5-HD increased between 0-4 h after exposure and was more slowly eliminated ($t_{½}$ = about 4 h) to minimal concentrations at 24 h post-exposure. Exposure of pregnant rats showed comparable kinetics of *n*-hexane and its metabolites in maternal and fetal tissues.

The main urinary metabolite of *n*-hexane was 4,5-dihydroxy-2-hexanone, which was about 4 or 10 times higher than that of excreted 2,5-HD in humans and rats, respectively. Additionally, many metabolites were identified in the blood and urine of humans, rats, mice, rabbits, and monkeys. 2,5-Dimethylfuran and γ-valerolactone could be demonstrated in the urine of humans and rats, but not in the urine of rabbits or monkeys. Excretion of metabolites was linearly dependent on the exposure concentration of *n*-hexane to about 300 ppm; above 300 ppm, saturation kinetics occurred.

4.2. Mechanism of Toxicity

The role of kinetics in the acute inhalation toxicity *n*-hexane was analyzed with a physiologically based pharmacokinetic model for the rat (De Jongh et al. 1998). Model compartments included: (1) localized fat tissue; (2) a lumped compartment representing all slowly perfused tissues except fat tissue; (3) liver tissue; (4) a lumped compartment representing all rapidly perfused tissues except liver and brain tissue; and (5) brain (central nervous system [CNS]) tissue. Two CNS subcompartments were defined, representing aqueous and lipid brain components. A common form of inhalation toxicity from nonreactive, volatile organic compounds in mammals, including humans, is general anesthesia possibly followed by death. Application of an internal dose surrogate C_{bl} (concentration in the brain's lipid constituents) instead of the traditional external exposure parameter LC_{50}-t (77,000 ppm × 1 h) resulted in a more than 10-fold reduction in the toxic range of 15 nonreactive, volatile organic compounds (including *n*-hexane) (simulated dose surrogate: 70 ± 31 mM for all volatile organic compounds). These observations support the presumption that nonspecific, acute narcotic lethality is directly related to the extent of *n*-hexane distribution in the phospholipid bilayer of nerve cell membranes.

4.3. Other Relevant Information

4.3.1. Species Variability

In lethality studies (see Section 3.1 and Table 3-3), mice seemed to be more susceptible to *n*-hexane toxicity than rats. Minimal fatal doses of 37,640 ppm for 39-45 min (Fühner 1921) and 64,000 ppm for 4.5 min (Lazarew 1929)

were found for mice. However, these studies were carried out in closed exposure chambers and were, therefore, not suitable for quantitative analysis (see Section 3.1 for explanation).

Differences in the amount of the main urinary metabolite 4,5-dihydroxy-2-hexanone were demonstrated in humans and rats (see Section 4.1). Concentrations of 4,5-dihydroxy-2-hexanone were about four times higher than that of excreted 2,5-HD in humans, whereas it was about 10 times greater than 2,5-HD in the rat (Fedtke and Bolt 1987a,b). Additionally, both quantitative and qualitative differences in the metabolites present in urine and blood of humans, rats, mice, rabbits, and monkeys were observed. Animals were exposed to *n*-hexane at 5,000 ppm for up to 72 h, while humans were exposed occupationally at 10-140 ppm during work shifts. 2,5-Dimetylfuran and γ-valerolactone could be demonstrated in the urine of humans and rats, but not in the urine of rabbits or monkeys (Perbellini et al. 1982). 3-Hexanol, a major metabolite in animals, could not be detected in human urine. The significance of these metabolic differences in the acute toxicity of *n*-hexane is unclear.

4.3.2. Irritation and Sensitization

Skin and Ocular Irritation

Little information was available on skin and ocular irritation in humans and laboratory animals after acute exposure to *n*-hexane vapor. No clinical signs (rubbing, scratching, redness, lacrimation) of ocular or skin irritation were reported in acute inhalation studies (see Sections 2.2 and 3.2). In a study by Shibata et al. (2002), in which Caucasian male volunteers were asked to rate ocular discomfort (burning, irritation), the rating was below 10% of the whole scale and corresponded verbally to ratings between ‘not at all’ and ‘hardly at all’ (see Section 2.2.2 for details).

In an evaluation by WHO (1991), only one study in humans was reported in which volunteers were exposed to *n*-hexane vapor at a concentration of 500 ppm (1,760 mg/m^3) for 3-5 min. No signs of ocular irritation were noted. No skin sensitization has been reported in exposed workers and no skin sensitization was noted in a maximization test with *n*-hexane solution. However, operators at a soybean hexane-extraction facility had a higher incidence of dry or irritated skin than maintenance workers (65% vs. 20%). It could not be deduced from the WHO (1991) report whether the operators were exposed to *n*-hexane vapor or solutions of *n*-hexane.

Respiratory Tract Irritation

Several effects on the lungs of laboratory animals have been reported following acute exposure to *n*-hexane. These studies are described in detail in Section 3.2 (Nonlethal Toxicity). In summary, Schnoy et al. (1982) reported effects

on pneumocytes (fatty degeneration, changes in lamellar bodies of type II pneumocytes, and increased detachment of cells) in rats exposed to *n*-hexane at 700 ppm for 8 h, 10,000 ppm for 4 h, and at 10,000 ppm for 8 h. Hadjiivanova et al. (1987) demonstrated effects on pulmonary surfactant in rats exposed to *n*-hexane at 4,260 ppm for 5 h, which are in general the early events in lung toxicity. However, no histopathologic lesions in the respiratory tract were found in groups of 15 male and 15 female Fischer-344 rats (Cavender et al. 1984) or in groups of 10 male and 10 female $B6C3F_1$ mice (Dunnick et al. 1989) exposed to *n*-hexane concentrations of up to 10,000 ppm for 6 h/day, 5 days/week for 13 weeks. Swann et al. (1974) reported sensory irritation of the respiratory system of mice exposed to *n*-hexane at 1,000-64,000 ppm for 5 min. Irregular respiration was observed at 32,000 ppm and 64,000 ppm. This effect was accompanied by increased expiratory effort, apneic respirations, an increase in respiration rate, and a decrease in amplitude at 32,000 ppm, and an increase in inspiratory effort and a decrease in the expiratory effort at 64,000 ppm. Respiratory arrest occurred at the end of inspiration at 64,000 ppm.

Caucasian male volunteers exposed to *n*-hexane at 54.2 ppm for 2 h under physical exertion were asked to rate a running nose and discomfort in throat or airways. Their ratings were below 10% of the whole scale (Shibata et al. 2002). This corresponded verbally to ratings between ‘not at all’ and ‘hardly at all’ (see Section 2.2.2 for details).

5. DATA ANALYSIS FOR AEGL-1

5.1. Human Data Relevant to AEGL-1

Human data relevant to deriving AEGL-1 values for *n*-hexane are limited. Nelson et al. (1943) did not report symptoms of irritation in volunteers exposed to the chemical at nominal concentration of 500 ppm for 5 min. Male volunteers exposed to *n*-hexane at 54 ppm for 2 h during light physical exercise did not experience any signs of discomfort. Toxicokinetic studies of *n*-hexane with volunteers included exposures up to 200 ppm for 4 h at rest and up to 100 ppm for 3 h under increasing levels of exercise (Veulemans et al. 1982). No adverse effects were described, but it was unclear whether subjects were questioned about such effects. No clinical signs were reported by workers exposed to *n*-hexane at 8-h TWA concentrations of up to 325 ppm (Mutti et al. 1984).

5.2. Animal Data Relevant to AEGL-1

Data on *n*-hexane are predominantly from studies of mice and rats. Most studies with mice are rather old and were performed in static systems (Fühner 1921; Lazarew 1929). In such systems, *n*-hexane concentrations would have decreased during testing, and oxygen concentrations would also have decreased

and the carbon dioxide concentrations would have increased making the studies difficult to interpret. Swann et al. (1974) observed light anesthesia in mice exposed to *n*-hexane at 16,000 ppm for 5 min but no CNS-effects were present at 8,000 ppm. However, no information on the methods used to assess anaesthesia was provided. In addition, animals in this study were restrained, potentially adding uncertainty regarding observations that would be indicative of *n*-hexane-induced anaesthesia. Therefore, data from the Swann et al. (1974) study were not suitable for deriving AEGL-1 values.

No clear effects on shock-avoidance rate were observed in rats exposed to *n*-hexane at 800 ppm for 4 h (Ikeda et al. 1993). Continuous exposure to *n*-hexane at 1,000 ppm did not cause changes in grip strength, conditioned avoidance response, and undifferentiated motor activity before 3 weeks of exposure. These effects were also absent in rats repeatedly exposed to *n*-hexane at 48,000 ppm (10-min exposures followed 5-min intervals without exposure, 24 times per day) for several weeks (Pryor et al. 1982). However, the latter study reported that "48,000 ppm was about the highest concentration that did not cause myoclonic seizures in most of the rats during exposure". When such effects occurred was not reported but this statement might indicate that 48,000 ppm can cause myoclonic seizures in rats. A kinetic study reported that no visible signs of toxicity were observed in rats exposed to *n*-hexane at 86,222 ppm for up to 20 min, but that ataxia and decreased motor activity was observed in rats exposed for 25 or 30 min (Raje et al. 1984). Reduced respiration associated with narcosis was reported in a kinetic study of rats exposed to *n*-hexane at 10,000 ppm for 6 h, but not in rats exposed at 3,000 ppm (Bus et al. 1982).

5.3. Derivation of AEGL-1 Values

As with other alkanes, the predominant effect in acute exposure to *n*-hexane is CNS depression. Human data do not provide adequate information to characterize a concentration-response relationship for *n*-hexane. Data obtained from studies of mice do not provide a suitable point of departure for AEGL-1 values. Experiments were performed under static conditions or under stress, conditions that can easily induce CNS-like effects in mice. Rat data on *n*-hexane also do not adequately address the level of effects defined by the AEGL-1. Therefore, no AEGL-1 values are recommended because of insufficient data.

6. DATA ANALYSIS FOR AEGL-2

6.1. Human Data Relevant to AEGL-2

No adequate human data that address the level of effects defined by AEGL-2 are available.

6.2. Animal Data Relevant to AEGL-2

Data on *n*-hexane relevant to AEGL-2 values are predominantly from studies of mice and rats. However, most studies with mice are rather old (1920s) and were performed in static systems (initial exposure concentrations were up to 51,990 ppm) (Fühner 1921; Lazarew 1929) or under conditions of restraint, with poorly reported methods regarding clinical observations (Swann et al. 1974). Therefore, these studies are not suitable for AEGL-2 derivation.

Clear CNS depression was absent in rats exposed to *n*-hexane at 48,000 ppm 24 times per day for several weeks (exposure for 10 min followed by 5 min of no exposure). However, it was reported that "48,000 ppm was about the highest concentration that did not cause myoclonic seizures in most of the rats during exposure" (Pryor et al. 1982). When these effects occurred was not specified but this statement might indicate that 48,000 ppm can cause myoclonic seizures in rats. A kinetic study reported that no visible signs of toxicity were seen in rats exposed to *n*-hexane at 86,222 ppm for up to 20 min; ataxia and decreased motor activity was reported in rats exposed for 25 or 30 min (Raje et al. 1984). Reduced respiration associated with narcosis (an AEGL-2 level effect) was reported in rats exposed to *n*-hexane at 10,000 ppm for 6 h, but not in rats exposed at 3,000 ppm (Bus et al. 1982). However, the Bus et al. (1979) study is a toxicokinetic study that was not designed to assess toxicity; the methods and results sections of the study report did not include any information on how toxicity (including narcosis) was assessed or specific observations related to toxicity. The observation of narcosis associated with a 6-h exposure to *n*-hexane at 10,000 ppm was a single statement in the discussion section of the publication. In addition, no information was reported regarding toxicity in rats exposed to *n*-hexane at 3,000 ppm. Because of these reporting insufficiencies, there is considerable uncertainty regarding the no-effect level for narcosis in this study, so the data are not appropriate as the basis of AEGL-2 values.

Honma (1983) reported that some symptoms of sedation, hypothermia, and ptosis were observed in rats exposed to *n*-hexane at 2,000, 4,000, or 8,000 ppm for 8 h in a dose-dependent manner (Honma 1983). However, no details were provided and the severity of effects could not be related to the exposure concentrations. Results of kinetics studies, which were not designed to assess toxicity, do not provide adequate information to define a no-effect level for AEGL-2 effects.

Acute effects on the biochemistry in the brain were in rats exposed to *n*-hexane at concentrations of 2,000 to 8,000 ppm for 8 h (Honma et al. 1982; Honma 1983), but the functional implications of these changes are difficult to assess. Effects on rat lung tissue (effects on the lung surfactant system and degenerative effects on the pneumocytes) were seen at *n*-hexane concentrations of up to 10,000 ppm for 8 h (Schnoy et al. 1982; Hadjivanova et al. 1987). Reversible lesions in the testis were found in rats exposed to *n*-hexane at 5,000 ppm for 24 h (De Martino et al. 1987). However, no histopathologic changes in

these organs were found in rats (Cavender et al. 1984) or mice (Dunnick et al. 1989) exposed to *n*-hexane at 10,000 ppm for 6 h/day, 5 days/week for 13 weeks, and the animals did not appear to show any functional impairment related to these effects. Therefore, these effects were not considered relevant for setting AEGL-2 values.

Developmental and reproductive toxicity studies of *n*-hexane showed no effects on dams exposed during gestation at concentrations up to 1,000 ppm. A decreased number of litters was observed in dams exposed to *n*-hexane at 500 ppm for 23 h/day, 7 days per week during the entire gestation period (Stoltenburg-Didinger et al. 1990), but not in dams exposed at 1,000 ppm for 6 h/day on days 8-16 of gestation (Bus et al. 1979). On the basis of these studies, the possible effects of acute exposure to *n*-hexane on the fetus were considered not relevant for setting AEGL-2 values.

6.3. Derivation of AEGL-2 Values

CNS depression is the most relevant adverse effect from acute exposure to *n*-hexane. Adequate human data addressing the level of effects defined by the AEGL-2 were not available. Because of reporting insufficiencies in studies with rats and confounding methologic issues in studies with mice, there is considerable uncertainty regarding the no-effect level for AEGL-2 level effects. Although data are not available to define the concentration-response curve for *n*-hexane, a steep concentration-response relationship is observed for butane, a structural analog of *n*-hexane and CNS depressant (NRC 2012). Thus, a steep concentration-response relationship is also expected for *n*-hexane. For chemicals with steep concentration-response curves, AEGL-2 values may be derived by reducing AEGL-3 values by one-third (NRC 2001). AEGL-2 values for *n*-hexane are presented in Table 3-6.

7. DATA ANALYSIS FOR AEGL-3

7.1. Human Data Relevant to AEGL-3

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

TABLE 3-6 AEGL-2 Values for *n*-Hexane

10 min	30 min	1 h	4 h	8 h
4,000 ppm[a] (14,000 mg/m^3)	2,900 ppm[a] (10,000 mg/m^3)	2,900 ppm[a] (10,000 mg/m^3)	2,900 ppm[a] (10,000 mg/m^3)	2,900 ppm[a] (10,000 mg/m^3)

[a]The AEGL-2 value is higher than 10% of the lower explosive limit of *n*-hexane in air of 1.1% (11,000 ppm). Therefore, safety considerations against the hazard of explosion must be taken into account.

7.2. Summary of Animal Data Relevant to AEGL-3

Only limited animal data on mortality following acute exposure to *n*-hexane are available. Two LC_{50} values have been reported for rats: a 1-h LC_{50} of 76,900 ppm (Pryor et al. 1982) and a 4-h LC_{50} of 48,000 ppm (Couri and Milks 1982). However, because the study details are not available, the data cannot be judged on their merits. The latter value appears to be inconsistent with results of the kinetic study by Böhlen et al. (1973), in which no mortality was reported in rats were exposed to *n*-hexane at 48,280 ppm for up to 10 h. In addition, repeated daily exposure to *n*-hexane at 48,000 ppm (10-min exposures followed 5-min intervals without exposure, 24 times) for 4 weeks caused no mortality (Pryor et al. 1982). In addition, no mortality was found in rats exposed to *n*-hexane at 5,000 ppm for 24 h (De Martino et al. 1987) or in rats exposed at up to 10,000 ppm for up to 8 h (Bus et al. 1982; Schnoy et al. 1982; Honma 1983). The 1-h LC_{50} of 76,900 ppm is also inconsistent with the finding of no mortality in rats exposed to *n*-hexane at 86,222 ppm for 30 min (Raje et al. 1984).

Studies with mice are rather old (1920s) and performed in static systems (initial exposure concentrations were up to 51,990 ppm) (Fühner 1921; Lazarew 1929) or under restraint, with poorly reported methods and results (Swann et al. 1974), making these studies unsuitable for deriving AEGL-3 values.

7.3. Derivation of AEGL-3 Values

Adequate human data addressing the level of effects defined by the AEGL-3 are not available. Mouse studies are not suitable because of study flaws, including use of static exposure systems (Fühner 1921; Lazarew 1929) or exposure under restraint, with poorly reported methods and results (Swann et al. 1974). Therefore, rat data were considered to be the most reliable and well founded for AEGL-3 values. The two LC_{50} values for rats (Couri and Milks 1982; Pryor et al. 1982) could not be judged properly because of inadequate documentation, and appear to be inconsistent with other reports, as discussed above. Therefore, the study by Raje et al. (1984) was chosen to determine the best point of departure for AEGL-3 values. In that study, no deaths occurred in rats exposed to *n*-hexane at 86,222 ppm for 30 min. A total uncertainty factor of 10 was applied; a factor of 3 for interspecies differences and a factor for 3 for intraspecies variability. These factors were judged to be adequate because the effects of *n*-hexane are attributed to itself and no relevant differences in kinetics are assumed, so only small interindividual differences are expected. In addition, mortality from *n*-hexane is preceded by CNS depression. Variation in susceptibility for CNS-depressing effects is not very great in the human population. The 30-min AEGL-3 value was calculated to be approximately 8,600 ppm. The 10-min AEGL-3 value was derived from 30-min AEGL-3 value. Time scaling was performing using the equation $C^n \times t = k$, using a default value of $n = 3$.

As with other alkanes, the anesthetic effects of *n*-hexane are considered to be predominantly concentration dependent. No increase of effect-size by dura-

tion is expected for concentration-dependent effects after reaching a steady state. The majority of human data (Veulemans et al. 1982; Shibata et al. 2002) and animal data (e.g., Baker and Rickert 1981; Raje et al. 1984) indicate a rapid steady-state concentration in blood and brain, with steady-state blood concentrations reached in approximately 30 min. In addition, gases that are relatively insoluble in blood rise quickly toward equilibrium with the inhaled concentration, and the less soluble in blood the faster the narcotic action of the gas (Drummond 1993). Quick equilibrium of such gases has been confirmed for other alkanes like propane and butane as well. Hence, no increase of effect-size by exposure duration is expected from 30 min to 8 h. Therefore, AEGL-3 values for the 1-h, 4-h, and 8-h durations were set equal to the 30-min AEGL-3 value. The 8-h AEGL-3 value of 8,600 ppm appears to be conservative and protective for lethality when considered in context with the result of the study by Böhlen et al. (1973), which found no mortality in rats exposed to *n*-hexane 48,280 ppm for 10 h.

AEGL-3 values for n-hexane are presented in Table 3-7.

8. SUMMARY OF AEGL VALUES

8.1. AEGL Values and Toxicity End Points

AEGL values for *n*-hexane are presented in Table 3-8. AEGL-1values are not recommended because of insufficient data. AEGL-2 values were set at one-third of the AEGL-3 values, and the AEGL-3 values were based on a study in which no lethality was observed in rats.

8.2. Comparison with Other Standards and Guidelines

Standards and guidelines for workplace and community exposures to *n*-hexane are presented in Table 3-9. No ERPG (emergency response planning guidelines) values have been set for *n*-hexane. The 1-h IDLH (immediately dangerous to life or health) of 1,100 ppm is set at 10% of the lower explosive limit of 1.1%. AEGL values for *n*-hexane cannot be compared with these general guidelines because those values are meant for long-term exposure. For long-term exposure, the most important effect is the degenerative distal axonopathy caused by the metabolite 2,5-HD. This effect is relevant for acute exposures.

8.3. Data Quality and Research Needs

An adequate acute toxicity study on *n*-hexane is lacking, and the available data do not provide a strong basis for AEGL values. An acute toxicity study with emphasis on anesthesia, narcosis, and mortality would be very helpful. Two LC_{50} values were reported but neither the original reports nor the underlying data could be identified. These values appear to be inconsistent with other reported studies. One of the values, the 4-h LC_{50} of 48,000 ppm, appears to have

been based on a study with rats exposed to *n*-hexane concentrations ranging from 1,000 to 64,000 ppm. This could be a valuable study but the original data are not available.

TABLE 3-7 AEGL-3 Values for *n*-Hexane

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-3 value of 12,000 ppm (42,240 mg/m^3) is higher than the lower explosive limit of *n*-hexane in air of 1.1% (11,000 ppm). Therefore, extreme safety considerations against the hazard of explosion must be taken into account.
[b]The AEGL-3 values for the 30-min, 1-h, 4-h, and 8-h durations are each 8,600 ppm (30,000 mg/m^3), which is higher than 50% of the lower explosive limit of *n*-hexane in air of 1.1% (11,000 ppm). Therefore, extreme safety considerations against the hazard of explosion must be taken into account.

TABLE 3-8 AEGL Values for *n*-Hexane

Classification	10 min	30 min	1 h	4 h	8 h
AEGL-1 (nondisabling)	NR	NR	NR	NR	NR
AEGL-2 (disabling)	4,000 ppm[a] (14,000 mg/m^3)	2,900 ppm[a] (10,000 mg/m^3)	2,900 ppm[a] (10,000 mg/m^3)	2,900 ppm[a] (10,000 mg/m^3)	2,900 ppm[a] (10,000 mg/m^3)
AEGL-3 (lethal)	See below[b]	See below[c]	See below[c]	See below[c]	See below[c]

Abbreviations: NR, not recommended because of insufficient data.
[a]The AEGL-2 value is higher than 10% of the lower explosive limit of *n*-hexane in air of 1.1% (11,000 ppm). Therefore, safety considerations against the hazard of explosion must be taken into account.
[b]The 10-min AEGL-3 value of 12,000 ppm (42,000 mg/m^3) is higher than the lower explosive limit of *n*-hexane in air of 1.1% (11,000 ppm). Therefore, extreme safety considerations against the hazard of explosion must be taken into account.
[c]The AEGL-3 values for the 30-min, 1-h, 4-h, and 8-h durations are each 8,600 ppm (30,000 mg/m^3), which is higher than 50% of the lower explosive limit of *n*-hexane in air of 1.1% (11,000 ppm). Therefore, extreme safety considerations against the hazard of explosion must be taken into account.

TABLE 3-9 Standards and Guidelines for *n*-Hexane

	Exposure Duration				
Guideline	10 min	30 min	1 h	4 h	8 h
AEGL-1	NR	NR	NR	NR	NR
AEGL-2	4,000 ppm[a] (14,000 mg/m^3)	2,900 ppm[a] (10,000 mg/m^3)	2,900 ppm[a] (10,000 mg/m^3)	2,900 ppm[a] (10,000 mg/m^3)	2,900 ppm[a] (10,000 mg/m^3)
AEGL-3	See below[b]	See below[c]	See below[c]	See below[c]	See below[c]

(Continued)

TABLE 3-9 Continued

	Exposure Duration				
Guideline	10 min	30 min	1 h	4 h	8 h
IDLH (NIOSH)[d]		1,100 ppm (3,880 mg/m^3)			
TLV-TWA (ACGIH)[e]					50 ppm (180 mg/m^3)
REL-TWA (NIOSH)[f]					50 ppm (180 mg/m^3)
PEL-TWA (OSHA)[g]					500 ppm (1,800 mg/m^3)
REL-STEL (NIOSH)[h]	510 ppm (1,800 mg/m^3)				
MAK (Germany)[i]					50 ppm (180 mg/m^3)
MAK Peak Limit (Germany)[j]					180 ppm (630 mg/m^3)
MAC (The Netherlands)[k]					25 ppm (90 mg/m^3)

Abbreviations: NR, not recommended.

[a]The AEGL-2 value is higher than 10% of the lower explosive limit of *n*-hexane in air of 1.1% (11,000 ppm). Therefore, safety considerations against the hazard of explosion must be taken into account.

[b]The 10-min AEGL-3 value of 12,000 ppm (42,000 mg/m^3) is higher than the lower explosive limit of *n*-hexane in air of 1.1% (11,000 ppm). Therefore, extreme safety considerations against the hazard of explosion must be taken into account.

[c]The AEGL-3 values for the 30-min, 1-h, 4-h, and 8-h durations are each 8,600 ppm (30,000 mg/m^3), which is higher than 50% of the lower explosive limit of *n*-hexane in air of 1.1% (11,000 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 2001, 2012) is the time-weighted average concentration for a normal 8-h workday and a 40-h workweek, to which nearly all workers may be repeatedly exposed, day after day, without adverse effect.

[f]REL-TWA (recommended exposure limit - time weighted average, National Institute for Occupational Safety and Health) (NIOSH 2011) is defined analogous to the ACGIH TLV-TWA.

[g]PEL-TWA (permissible exposure limit - 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.

[h]REL-STEL (recommended exposure limit - short term exposure limit, National Institute for Occupational Safety and Health) (NIOSH 1977) is defined as a 15-min TWA exposure that should not be exceeded at any time during the workday.

[i]MAK (maximale arbeitsplatzkonzentration [maximum workplace concentration], Deutsche Forschungsgemeinschaft [German Research Association]) (DFG 2005) is defined analogous to the ACGIH TLV-TWA.
[j]MAK Spitzenbegrenzung (peak limit) (German Research Association (DFG2003) 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.
[k]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 OF AEGL VALUES FOR *n*-HEXANE

Derivation of AEGL-1 Values

Data were insufficient for deriving AGEL-1 values for *n*-hexane, so no values are recommended.

Derivation of AEGL-2 Values

In the absence of data for deriving AEGL-2 values for *n*-hexane and because *n*-hexane has a steep concentration-response curve, AEGL-2 values were calculating by dividing the AEGL-3 values by 3 (NRC 2001).

10-min AEGL-2:	12,000 ppm ÷ 3 = 4,000 ppm
30-min AEGL-2:	8,600 ppm ÷ 3 = 2,900 ppm
1-h AEGL-2:	8,600 ppm ÷ 3 = 2,900 ppm
4-h AEGL-2:	8,600 ppm ÷ 3 = 2,900 ppm
8-h AEGL-2:	8,600 ppm ÷ 3 = 2,900 ppm

Derivation of AEGL-3 Values

Key study:	Raje, R.R., M. Greening, and M.T. Fine. 1984. Blood n hexane concentration following acute inhalation exposure in rats. Res. Commun. Chem. Pathol. Pharmacol. 46(2):297-300.
Toxicity end point:	No mortality in rats exposed to *n*-hexane at 86,222 ppm for 30 min.
Time scaling:	The 10-min value was time-scaled using the equation $C^n \times t = k$, with n = 3. $(8{,}600\ \text{ppm})^3 \times 30\ \text{min} = k$ $k = 19.08 \times 10^{12}$ ppm-min Because a steady-state blood concentration will be reached within 30 min, no increase in effect-size by exposure duration is expected from 30 min to 8 h.

	Therefore, AEGL-2 values for the 1-h, 4-h, and 8-h durations were set equal to the 30-min AEGL-2 value.
Uncertainty factors:	3 for interspecies differences 3 for intraspecies variability
10-min AEGL-3:	$C^3 \times 10$ min = 19.08×10^{12} ppm-min C ≈ 12,000 ppm (42,000 mg/m^3)
30-min AEGL-3:	86,222 ppm ÷ 10 ≈ 8,600 ppm (30,000 mg/m^3) (point of departure)
1-h AEGL-3:	Set equal to 30-min AEGL-3 of 8,600 ppm (30,000 mg/m^3)
4-h AEGL-3:	Set equal to 30-min AEGL-3 of 8,600 ppm (30,000 mg/m^3)
8-h AEGL-3:	Set equal to 30-min AEGL-3 of 8,600 ppm (30,000 mg/m^3)

APPENDIX B

CATEGORY PLOT FOR *n*-HEXANE

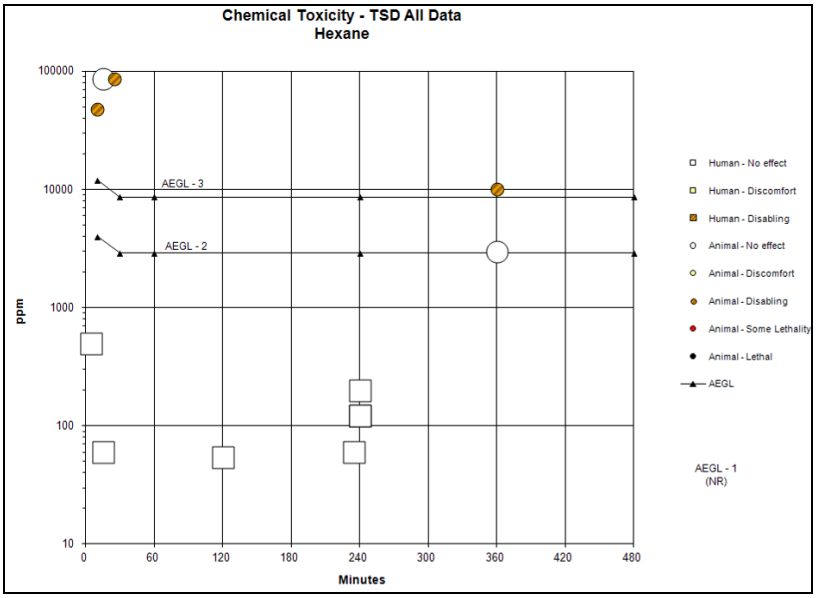

FIGURE B-1 Category plot of toxicity data on *n*-hexane compared with AEGL values. Lethal concentrations in animals were not plotted, because the available data were not reliable. Studies reporting lethality in animals used static exposure conditions or animals were exposed under restraint, and had poor descriptions of methods and results (see Section 7.2 for discussion).

TABLE B-1 Data Used in Category Plot

Source	Species	Sex	No. Exposures	ppm	Minutes	Category
NAC/AEGL-1				NR	10	AEGL
NAC/AEGL-1				NR	30	AEGL
NAC/AEGL-1				NR	60	AEGL
NAC/AEGL-1				NR	240	AEGL
NAC/AEGL-1				NR	480	AEGL
NAC/AEGL-2				4,000	10	AEGL
NAC/AEGL-2				2,900	30	AEGL
NAC/AEGL-2				2,900	60	AEGL
NAC/AEGL-2				2,900	240	AEGL

(Continued)

TABLE B-1 Continued

Source	Species	Sex	No. Exposures	ppm	Minutes	Category
NAC/AEGL-2				2,900	480	AEGL
NAC/AEGL-3				12,000	10	AEGL
NAC/AEGL-3				8,600	30	AEGL
NAC/AEGL-3				8,600	60	AEGL
NAC/AEGL-3				8,600	240	AEGL
NAC/AEGL-3				8,600	480	AEGL
Nelson et al. 1943	Human	Both	1	500	5	0
Shibata et al. 2002	Human	Male	1	54.2	120	0
Nomiyama and Nomiyama 1974	Human	Both	1	122	240	0
Nomiyama and Nomiyama 1974	Human	Both	1	122	240	0
Veulemans et al. 1982	Human	Male	1	200	240	0
van Engelen et al. 1997	Human	Male	1	60	15.5	0
	Human	Male	1	60	234.6	0
Bus et al. 1982	Rat		1	3,000	360	0
	Rat		1	10,000	360	2
Raje et al. 1984	Rat		1	86,222	15	0
	Rat		1	86,222	25	2
Pryor et al. 1982	Rat		1	48,000	10	2

For category: 0 = no effect, 1 = discomfort, 2 = disabling, 3 = lethal; L = lethality.

APPENDIX C

ACUTE EXPOSURE GUIDELINE LEVELS FOR *n*-HEXANE

Derivation Summary

AEGL -1 VALUES

Data were insufficient for deriving AGEL-1 values for *n*-hexane, so no values are recommended.

AEGL-2 VALUES

10 min	30 min	1 h	4 h	8 h
4,000 ppm[a] (14,000 mg/m^3)	2,900 ppm[a] (10,000 mg/m^3)	2,900 ppm[a] (10,000 mg/m^3)	2,900 ppm[a] (10,000 mg/m^3)	2,900 ppm[a] (10,000 mg/m^3)

Data adequacy: Data are not available to define the concentration-response curve for *n*-hexane. A steep concentration-response relationship is observed for butane, a structural analog of *n*-hexane and CNS depressant (NRC 2012), so a similar relationship is expected for *n*-hexane. For chemicals with a steep concentration-response curve, AEGL-2 values may be derived by reducing AEGL-3 values by one-third (NRC 2001). Therefore, AEGL-2 for values *n*-hexane were calculated by dividing the AEGL-3 values by 3.

[a]The AEGL-2 value is higher than 10% of the lower explosive limit of *n*-hexane in air of 1.1% (11,000 ppm). Therefore, safety considerations against the hazard of explosion must be taken into account.

AEGL-3 VALUES

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

Key reference: Raje, R.R., M. Greening, and M.T. Fine. 1984. Blood n hexane concentration following acute inhalation exposure in rats. Res. Commun. Chem. Pathol. Pharmacol. 46(2):297-300.

Test species/Strain/Number: Rat, Sprague-Dawely, groups of 4 male

Exposure route/Concentrations/Durations: Inhalation, 86,222 ppm for 10, 15, 20, 25, or 30 min.

Effects:

Duration (min)	Effects
10	No effects
15	No effects
20	No effects
25	Ataxia, no deaths
30	Ataxia, no deaths

(Continued)

AEGL-3 VALUES Continued

End point/Concentration/Rationale: Absence of mortality.

Uncertainty factors/Rationale:
Total uncertainty factor: 10
Interspecies: 3
Intraspecies: 3
A total uncertainty factor of 10 was considered sufficient because the effects are attributed to *n*-hexane itself and no relevant differences in kinetics are assumed. Mortality from *n*-hexane exposure is preceded by CNS depression, and variation in susceptibility for CNS-depressing effects is not very great in the human population.

Modifying factor: None

Animal-to-human dosimetric adjustment: Not applicable

Time scaling: Because a steady-state blood concentration will be reached within 30 min of exposure, no increase in effect-size by exposure duration is expected from 30 min to 8 h. Therefore, the AEGL-3 values for the 1-h, 4-h, and 8-h durations are set equal to the 30-min AEGL-3 value. The 10-min AEGL-3 value was derived from the 30-min AEGL-3 value by time-scaling using the equation $C^n \times t = k$, with n = 3.

Data adequacy: The database is very poor. Available data for derivation of AEGL-3 values were predominantly from toxicokinetics studies. Adequate toxicity studies are lacking.

[a]The 10-min AEGL-3 value of 12,000 ppm (42,240 mg/m^3) is higher than the lower explosive limit of *n*-hexane in air of 1.1% (11,000 ppm). Therefore, extreme safety considerations against the hazard of explosion must be taken into account.

[b]The AEGL-3 values for the 30-min, 1-h, 4-h, and 8-h durations are each 8,600 ppm (30,000 mg/m^3), which is higher than 50% of the lower explosive limit of *n*-hexane in air of 1.1% (11,000 ppm). Therefore, extreme safety considerations against the hazard of explosion must be taken into account.