Spacecraft Maximum Allowable Concentrations for Selected Airborne Contamnants: Volume 5

ISBN: 0-309-12845-5, pages, , ()

Spacecraft Maximum Allowable Concentrations for Selected Airborne Contaminants

Volume 5

Committee on Spacecraft Exposure Guidelines

Committee on Toxicology

Board on Environmental Studies and Toxicology

Division on Earth and Life Studies

NATIONAL RESEARCH COUNCIL
OF THE NATIONAL ACADEMIES

THE NATIONAL ACADEMIES PRESS
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This project was supported by Grant No. NNX07AP75G between the National Academy of Sciences and the National Aeronautics and Space Administration. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the organizations or agencies that provided support for this project.

Library of Congress Catalog Card Number 95-73151
International Standard Book Number-13: 978-0-309-12844-5
International Standard Book Number-10: 0-309-12844-7

Additional copies of this report are available from:

The National Academies Press
500 Fifth Street, NW
Box 285
Washington, DC 20055

800-624-6242
202-334-3313 (in the Washington metropolitan area)
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Printed in the United States of America.

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[1]This study was planned, overseen, and supported by the Board on Environmental Studies and Toxicology.

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Preface

The National Aeronautics and Space Administration (NASA) is aware of the potential toxicologic hazards to crew that might be associated with prolonged spacecraft missions. Despite major engineering advances in controlling the atmosphere within spacecraft, some contamination of the air appears inevitable. NASA has measured numerous airborne contaminants during space missions. As the missions increase in duration and complexity, ensuring the health and well-being of astronauts traveling and working in this unique environment becomes increasingly difficult. As part of its efforts to promote safe conditions aboard spacecraft, NASA requested the National Research Council (NRC) to develop guidelines for establishing spacecraft maximum allowable concentrations (SMACs) for contaminants and to review SMACs for various spacecraft contaminants to determine whether NASA's recommended exposure limits are consistent with the guidelines recommended by the committee. In response to this request, the NRC first developed criteria and methods for preparing SMACs for airborne contaminants, published in its 1992 report *Guidelines for Developing Spacecraft Maximum Allowable Concentrations for Space Station Contaminants*. Since then, the NRC's Committee on Spacecraft Exposure Guidelines has been reviewing NASA's documentation of chemical-specific SMACs. This report is the fifth volume in the series *Spacecraft Maximum Allowable Concentrations for Selected Airborne Contaminants*. The first volume was published in 1994, the second and third in 1996, and the fourth in 2000. This report presents SMACs for acrolein, C3 to C8 aliphatic saturated aldehydes, C2 to C9 alkanes, ammonia, benzene, carbon dioxide, carbon monoxide, 1,2-dichloroethane, dimethylhydrazine, ethanol, formaldehyde, limonene, methanol, methylene dichloride, *n*-butanol, propylene glycol, toluene, trimethylsilanol, and xylenes.

The committee's review of the SMAC 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 SMAC documents based on the advice in the interim reports and presented them for re-examination by the committee as many times as necessary until the committee was satisfied that the SMACs were scientifi-

cally justified and consistent with the 1992 NRC guideline report. Once these determinations are made for a SMAC document, it is ready to be published as an appendix in a volume like this one.

The committee's interim reports were reviewed in draft form by individuals chosen 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 manuscripts remain confidential to protect the integrity of the deliberative process. We wish to thank the following individuals for their review of one or more of the interim reports listed above:

Lawrence S. Betts, Eastern Virginia Medical School
H. Tim Borges, Oak Ridge National Laboratory
Barbara G. Callahan, University Research Engineers and Associates
Janice E. Chambers, Mississippi State University
David Dankovic, National Institute for Occupational Safety and Health
Donald E. Gardner, Inhalation Toxicology Associates, Inc.
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Rogene Henderson, Lovelace Respiratory Research Institute
Samuel Kacew, University of Ottawa
Gary Krieger, NewFields
Loren D. Koller, Loren Koller & Associates, LLC
John L. O'Donoghue, University of Rochester, School of Medicine and Dentistry
George M. Rusch, Honeywell, Inc.

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 the interim report or this volume before their release.

The review of each interim report was overseen by a review coordinator, and we thank the following individuals for serving in this capacity for one or more of the interim reports listed above:

James V. Bruckner, University of Georgia
Samuel Kacew, University of Ottawa
David P. Kelly, DuPont
George M. Rusch, Honeywell, Inc.
Robert Snyder, Rutgers, The State University of New Jersey

Appointed by the National Research Council, the coordinators were responsible for making certain that an independent examination of these 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.

Special thanks are extended to John James, Noreen Khan-Mayberry, and Torin McCoy (NASA) and Hector Garcia, Shannon Langford, Chiu Wing Lam, and Raghupathy Ramanathan (Wyle Laboratories) for preparing and revising the SMAC documents. We also thank members of the committee who contributed to the development of this document, including A. John Bailer, Miami University; Darol Dodd, the Hamner Institute for Health Sciences; Kevin Driscoll, Procter and Gamble Pharmaceuticals; David Gaylor, Gaylor and Associates; Jack Harkema, Michigan State University; David Kaufman, University of North Carolina; Kenneth Rosenman, Michigan State University; Kenneth Thummel, University of Washington; Joyce Tsuji, Exponent Environmental Group; Rochelle Tyl, RTI International; and Judith Zelikoff, New York University School of Medicine.

We are grateful for the assistance of the NRC staff in supporting this project and preparing the report. James J. Reisa, director of the Board on Environmental Studies and Toxicology, contributed to this effort. We especially wish to recognize the contributions of Eileen Abt, program director; Jennifer Saunders, project director (through December 2007); Heidi Murray-Smith, research associate; Ruth Crossgrove, senior editor; Mirsada Karalic-Loncarevic, manager of the Technical Information Center; Radiah Rose, editorial projects manager; Tamara Dawson, program associate; and Panola Golson, senior program assistant.

Garold S. Yost, Ph.D., *Chair*,
Committee on Spacecraft
Exposure Guidelines

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Volume 5

Introduction

Construction of the International Space Station (ISS)—a multinational effort—began in 1999. In its present configuration, it is expected to carry a crew of three to six astronauts for up to 180 days (d). Because the ISS is a closed and complex environment, some contamination of its internal atmosphere is unavoidable. Several hundred chemical contaminants may be found in its closed-loop atmosphere, most at very low concentrations.

Important sources of atmospheric contaminants include off-gassing of cabin materials, operation of equipment, and metabolic waste products of crew. Other potential sources of contamination are releases of toxic chemicals from experiments and from manufacturing activities performed on the ISS as well as accidental spills and fires. The water recycling system also produces chemical contaminants that can enter the cabin air. Astronauts potentially can be chronically exposed to low concentrations of airborne contaminants and, in the event of an accident—such as a leak, spill, or fire—to high concentrations of contaminants.

The National Aeronautics and Space Administration (NASA) seeks to ensure the health and safety of astronauts and to prevent their exposure to toxic amounts of spacecraft contaminants. Consequently, exposure limits need to be established for continuous exposure of astronauts to spacecraft contaminants for up to 180 d (for normal spacecraft operations) and for short-term (1 to 24 h) emergency exposures to high concentrations of contaminants.

To protect space crews from air contaminants, NASA requested that the National Research Council (NRC) provide guidance for developing spacecraft maximum allowable concentrations (SMACs) and review NASA's development of exposure guidelines for specific chemicals. The NRC convened the Committee on Spacecraft Exposure Guidelines to address this task. The committee published *Guidelines for Developing Spacecraft Maximum Allowable Concentra-*

tions for Space Station Contaminants (NRC 1992). A second report, *Spacecraft Maximum Allowable Concentrations for Selected Airborne Contaminants, Volume 1* (NRC 1994), addressed 11 chemicals: acetaldehyde, ammonia, carbon monoxide, formaldehyde, freon 113, hydrogen, methane, methanol, octamethyltrisiloxane, trimethylsilanol, and vinyl chloride. Volume 2 of the report (NRC 1996a) presented SMACs for acrolein, benzene, carbon dioxide, 2-ethoxyethanol, hydrazine, indole, mercury, methylene chloride, methyl ethyl ketone, nitromethane, 2-propanol, and toluene. Volume 3 (NRC 1996b) addressed bromotrifluoromethane (halon 1301), 1-butanol, *tert*-butanol, diacetone alcohol, dichloroacetylene, 1,2-dichloroethane, ethanol, ethylbenzene, ethylene glycol, glutaraldehyde, trichloroethylene, and xylene. Volume 4 (NRC 2000a) reviewed acetone, C3 to C8 aliphatic saturated aldehydes, hydrogen chloride, isoprene, methylhyrazine, perfluoropropane and other aliphatic perfluoroalkanes, polydimethylcyclosiloxanes, dichlorofluoromethane (freon 21), chlorodifluoromethane (freon 22), trichlorofluoromethane (freon 11), dichlorodifluoromethane (freon 12), 4-methyl-2-pentanone, chloroform, furan, and hydrogen cyanide.

This report (Volume 5) presents SMACs for acrolein, C3-C8 aliphatic saturated aldehydes, ammonia, benzene, *n*-butanol, C2-C9 alkanes, carbon dioxide, carbon monoxide, 1,2-dichlorethane, dimethylhydrazine, ethanol, formaldehyde, limonene, methanol, methylene chloride, propylene glycol, toluene, trimethylsilanol, xylenes. Most of these chemicals were reviewed in previous SMAC volumes, with the exception of C2-C9 alkanes, methylene chloride, dimethylhydrazine, limonene, and propylene glycol.

The reason for the review of chemicals in Volume 5 is that many of them have not been examined for more than 10 years, and new research necessitates examining the documents to ensure that they reflect current knowledge. New knowledge can be in the form of toxicologic data or in the application of new approaches for analysis of available data. In addition, because NASA anticipates longer space missions beyond low Earth orbit, SMACs for 1,000-d exposures have also been developed.

SMACs are defined as the maximum concentrations of airborne substances that will not produce adverse health effects, cause significant discomfort, or degrade crew performance. SMACs are classified into 1- and 24-hour (h) emergency SMACs and 7-, 30-, and 180-d continuous SMACs. The SMACs for 1,000-d exposures are intended for longer space missions. The 1- and 24-h SMACs are to be used in emergency situations, such as accidental spills or fires. Temporary discomfort (such as mild skin or eye irritation) might occur, but if the 1- and 24-h SMACs are not exceeded, there should be no marked effect on judgment, performance, or the ability to respond to emergencies. The 7-, 30-, and 180-d SMACs are guidance concentrations intended to prevent adverse health effects, either immediate or delayed (over the course of a lifetime), and to avoid impairing crew performance after continuous exposures (which can last as long as 180 d) to contaminants in the ISS environment. These values are for normal operations of the ISS.

SUMMARY OF REPORT ON METHODS FOR DEVELOPING SMACS

In developing SMACs, several types of data should be evaluated, including (1) the physical and chemical characteristics of the contaminant, (2) in vitro toxicity studies, (3) toxicokinetic studies, (4) mechanistic studies, (5) animal toxicity studies conducted over a range of exposure durations, (6) genotoxicity studies, (7) carcinogenicity bioassays, and (8) human clinical and epidemiology studies. All observed toxic effects should be considered, including mortality, morbidity, functional impairment, specific organ system toxicities (such as renal, hepatic, and endocrine), neurotoxicity, immunotoxicity, reproductive toxicity, genotoxicity, and carcinogenicity. Toxicity data from human studies are most applicable and are used when available in preference to data from animal studies and in vitro studies. The toxicity data from animal species most representative of humans in terms of pharmacodynamic and pharmacokinetic properties are used for determining SMACs. Toxicity data from inhalation exposures are most useful for setting SMACs for airborne contaminants because inhalation is the most likely route of exposure.

There are several important determinants for deriving a SMAC, including identifying the most sensitive target organ or body system affected, the nature of the effect on the target tissue, the exposure duration in relation to the SMAC being developed, the dose-response relationship for the target tissue, the rate of recovery, the nature and severity of the injury, cumulative effects, pharmacokinetic data, interactions with other chemicals, and effects of microgravity. Derivation of the SMACs in this report is informed by NRC (1992, 2000b) guidelines.

Risk Assessment

Several risk assessment methods can be used to derive SMACs. Risk assessments for exposure to noncarcinogenic substances traditionally have been based on the premise that an adverse health effect will not occur below a specific threshold exposure. Given this assumption, an exposure guidance level can be established by dividing the no-observed-adverse-effect level (NOAEL) or the lowest-observed-adverse-effect level (LOAEL) by an appropriate set of uncertainty factors. This method requires making judgments about the critical toxicity end point relevant to a human in space, the appropriate study for selecting a NOAEL or LOAEL, and the magnitudes of the uncertainty factors used in the process.

For carcinogenic effects known to result from direct mutagenic events, no threshold dose would be assumed. However, when carcinogenesis results from nongenotoxic mechanisms, a threshold dose can be considered. Estimating carcinogenic risk involves fitting mathematical models to experimental data and extrapolating to predict risks at doses that are usually well below the experimen-

tal range. A linearized form of the multistage model has historically been used in cancer risk assessment. According to multistage theory, a malignant cancer cell develops from a single stem cell as a result of several biologic events (for example, mutations) that must occur in a specific order. Other models, such as two-stage clonal expansion models, have been used in cancer risk assessment. EPA's *Guidelines for Carcinogen Risk Assessment* (EPA 2005) also introduce modifications to the assessment process.

An alternative to the traditional NOAEL and LOAEL risk assessment methods that are used to set carcinogenic and noncarcinogenic concentrations is the benchmark dose (BMD) approach. The BMD is the dose associated with a specified low level of excess health risk, generally in the risk range of 1% to 10%, that can be estimated from modeled data with little or no extrapolation outside the experimental dose range. $BMDL_{01}$ and $BMDL_{10}$ are defined as the statistical lower confidence limits of doses that correspond to excess risks of 1% and 10% above background concentrations, respectively, and these are often used as a point of departure for estimating doses thought to be of negligible risks. Use of the lower confidence limit provides a suitable method to account for sampling variability. However, the use of a point estimate of the BMD, with incorporation of an additional uncertainty factor to account for experimental variation, may be more appropriate for certain types of data. There are many ways to apply BMD models. Ideally, mechanistic information about a compound's toxic action can guide the choice of a model. In the absence of this insight, model averaging approaches can be used to estimate points of departure. Like the NOAEL and LOAEL, $BMDL_{01}$ and $BMDL_{10}$ are points of departure for establishing exposure guidelines and should be modified by appropriate exposure conversions and uncertainty factors.

Scientific judgment is often a critical, overriding factor in applying the methods described above. It is recommended that, when sufficient dose-response data are available, the BMD approach be used to calculate exposure guidelines. However, in the absence of sufficient data, or when special circumstances dictate, the other, more traditional approaches should be used.

Special Considerations for NASA

When deriving SMACs, by either the NOAEL/LOAEL or the BMD approach, it is necessary to use exposure conversions and uncertainty factors to adjust for weaknesses or uncertainties about the data. When adequate data are available, exposure conversions that NASA should use include those to adjust for target tissue dose, differences in exposure duration, species differences, and differences in routes of exposure. Uncertainty factors should also be used to extrapolate animal exposure data to humans, when human exposure data are unavailable or inadequate; to extrapolate data from subchronic studies to chronic exposure; to account for using $BMDL_{10}$ instead of $BMDL_{01}$ (or a LOAEL instead of a NOAEL); to account for experimental variation; and to adjust for

spaceflight factors that could alter the toxicity of contaminants. The latter factors are used to account for uncertainties associated with microgravity, radiation, and stress. Some of the ways astronauts can be physically, physiologically, and psychologically compromised include decreased muscle mass, decreased bone mass, decreased red blood cell mass, depressed immune systems, altered nutritional requirements, behavioral changes, shift of body fluids, altered blood flow, altered hormonal status, altered enzyme concentrations, increased sensitization to cardiac arrhythmias, and altered drug metabolism. There is generally little information to permit a quantitative conversion that would reflect altered toxicity resulting from spaceflight environmental factors. Thus, spaceflight uncertainty factors should be used when available information on a substance indicates that it could affect one or more aspects of an astronaut's condition that might already be compromised in space.

Another commonly used uncertainty factor is one that accounts for variable susceptibilities in the human population. That uncertainty factor is used to protect sensitive members of the general population, including young children, pregnant women, and the immune compromised. Because the astronaut population is typically composed of healthy nonpregnant adults, the committee considers that an uncertainty factor for intraspecies differences should be used only if there is evidence that some individuals could be especially susceptible to the contaminant. These differences could be observed among astronauts who have genetic polymorphisms for well-established genes.

Exposure Guidelines Set by Other Organizations

Several regulatory agencies have established exposure guidance levels for some of the contaminants of concern to NASA. Those guidance levels should be reviewed before SMACs are established. The purpose of this comparison would not be simply to mimic the regulatory guidelines set elsewhere but to determine how and why other exposure guidelines might differ from NASA's guidelines and to assess whether those differences are reasonable in light of NASA's special needs.

REVIEW OF SMAC REPORTS

NASA is responsible for selecting the contaminants for which SMACs will be established and for developing documentation on how SMAC values were determined. As described above, the procedure for developing SMACs involves identifying toxicity effects relevant to astronauts and calculating exposure concentrations on the basis of those end points. The lowest concentration is selected as the SMAC, because the lowest value would be expected to protect astronauts from manifesting other effects.

To ensure that the SMACs are developed in accordance with the NRC guidelines (NRC 1992), NASA requested that the NRC committee independ-

ently review NASA's draft SMAC documents. NASA's draft documents summarize data relevant to assessing risk from exposure to individual contaminants in air only; they are not comprehensive reviews of the available literature on specific contaminants. Furthermore, although the committee is mindful that contaminants will be present as mixtures and the potential exists for interactions, it was asked to consider each chemical on an individual basis. The committee reviews NASA's SMAC documents and provides comments and recommendations in a series of interim reports (see NRC 2004a,b, 2005a,b, 2006a,b, 2007a,b, 2008). The committee reviews NASA's documents as many times as necessary until it is satisfied that the SMACs are scientifically justified.

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

This report is the fifth volume in the series *Spacecraft Maximum Allowable Concentrations for Selected Airborne Contaminants*. The SMACs presented here supersede values presented in earlier volumes; however, the older volumes often contain a more complete review of the literature. Volume 5 supplements the earlier volumes by describing new data relevant to setting SMACs and by showing how new approaches can be applied to both older and newer data. SMAC reports for acrolein, C3-C8 aliphatic saturated aldehydes, ammonia, benzene, *n*-butanol, C2-C9 alkanes, carbon dioxide, carbon monoxide, 1,2-dichlorethane, dimethylhydrazine, ethanol, formaldehyde, limonene, methanol, methylene chloride, propylene glycol, toluene, trimethylsilanol, xylenes are included in the appendix of this report. The committee concludes that the SMACs developed in those documents are scientifically valid based on data reviewed by NASA and are consistent with the guideline reports (NRC 1992, 2000).

REFERENCES

EPA (U.S. Environmental Protection Agency). 2005b. Guidelines for Carcinogen Risk Assessment. EPA/630/P-03/001F. Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, DC. March 2005 [online]. Available: http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=116283 [accessed August 1, 2008].

NRC (National Research Council). 1992. Guidelines for Developing Spacecraft Maximum Allowable Concentrations for Space Station Contaminants. Washington, D.C.: National Academy Press.

NRC (National Research Council). 1994. Spacecraft Maximum Allowable Concentrations for Selected Airborne Contaminants, Vol. 1. Washington, D.C.: National Academy Press.

NRC (National Research Council). 1996a. Spacecraft Maximum Allowable Concentrations for Selected Airborne Contaminants, Vol. 2. Washington, D.C.: National Academy Press.

NRC (National Research Council). 1996b. Spacecraft Maximum Allowable Concentrations for Selected Airborne Contaminants, Vol. 3. Washington, D.C.: National Academy Press.

NRC (National Research Council). 2000a. Spacecraft Maximum Allowable Concentrations for Selected Airborne Contaminants, Vol. 4. Washington, DC: National Academy Press.

NRC (National Research Council). 2000b. Methods for Developing Spacecraft Water Exposure Guidelines. Washington, DC: National Academy Press.

NRC (National Research Council). 2004. Interim Report 9 on Spacecraft Exposure Guidelines. Washington, DC: National Academy Press.

NRC (National Research Council). 2005a. Interim Report 10 on Spacecraft Exposure Guidelines. Washington, DC: National Academy Press.

NRC (National Research Council). 2005b. Interim Report 11 on Spacecraft Exposure Guidelines. Washington, DC: National Academy Press.

NRC (National Research Council). 2006a. Interim Report 12 on Spacecraft Exposure Guidelines. Washington, DC: National Academy Press.

NRC (National Research Council). 2006b. Interim Report 13 on Spacecraft Exposure Guidelines. Washington, DC: National Academy Press.

NRC (National Research Council). 2007a. Interim Report 14 on Spacecraft Exposure Guidelines. Washington, DC: National Academy Press.

NRC (National Research Council). 2007b. Interim Report 15 on Spacecraft Exposure Guidelines. Washington, DC: National Academy Press.

NRC (National Research Council). 2008. Interim Report 16 on Spacecraft Exposure Guidelines. Washington, DC: National Academy Press.

Appendix

1

Acrolein

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BACKGROUND

Spacecraft maximum allowable concentrations (SMACs) for acrolein were documented in Volume 2 of *Spacecraft Maximum Allowable Concentrations for Selected Airborne Contaminants* (Wong 1996). They were established for 1 and 24 h and for 7, 30, and 180 d. These time points were based on expected nominal spacecraft mission timelines and potential exposure durations (nominal or emergency/contingency) of that era.

Acrolein (2-propanal, molecular weight 56.06) is a volatile liquid with an extremely irritating vapor. As Wong (1996) pointed out, it is not a normal component used in the United States or International Partner spacecraft design or production, nor is it intentionally included in a spacecraft before launch. However, this compound has been reported in off-gassing tests from Spacelab missions (rates of 0.007 mg/d) (Geiger 1984).

A limited number of studies pertaining to acrolein toxicity have been reported since the first SMACs were adopted for this compound. Key pertinent reports, including comprehensive examinations of acrolein toxicology by the U.S. Environmental Protection Agency (EPA) (EPA 2003) and the National Research Council (NRC) (NRC 2007) are examined. The information from reports since the initial release of acrolein SMACs is used here to confirm the existing SMAC guidelines and to establish the new 1,000-d value. This report is intended as a companion document to complement and update the existing acrolein SMAC document. This document is organized as follows:

- The approach taken in developing existing acrolein SMACs is summarized,
- Recent data that may affect existing SMACs are examined and the 1,000-d acrolein SMAC is established, and

- A rationale for setting the 1,000-d acrolein SMAC and revising existing SMACs is provided.

REVIEW OF EXISTING ACROLEIN SMACs

NASA established the existing SMACs for acrolein in 1996 (Wong 1996). After the toxicologic information in the literature was assessed, one toxicologic end point was identified as being critical (mucosal irritation) and acceptable concentrations (ACs) were developed for this end point. Table 1-1 summarizes the SMACs established for acrolein using mucosal irritation as the toxicologic end point.

SUMMARY OF ORIGINAL APPROACH USED TO SET SMACS

Acrolein exposure produces mucosal irritation at concentrations lower than those that produce histopathologic changes in the respiratory tract (Lyon et al. 1970, Weber-Tschopp et al. 1977, Feron et al. 1978, Steinhagen and Barrow 1984). In addition, the eye is more susceptible than the nose to irritation produced by acrolein (Weber-Tschopp et al. 1977). Therefore, both the 1- and 24-h SMACs were established based on minimizing irritation to the eye and nose and to preclude irreversible injury and significant crew performance decrements resulting from exposure.

The 1-h acrolein SMAC was based on the report of Weber-Tschopp et al. (1977) describing moderate eye irritation in humans after 1 h of exposure to acrolein at 0.3 part per million (ppm) (0.68 milligram per cubic meter [mg/m^3]). Further evidence from the studies of Weber-Tschopp et al. indicates no effect on the eyes after exposure to acrolein at 0.15 ppm (0.34 mg/m^3) for 1.5 min. In addition, Darley et al. (1960) reported decreased eye irritation at progressively lower acrolein exposure concentrations. They observed a reduction in reported eye irritancy (by half a grade, from moderate-to-severe to moderate) when acrolein concentration decreased by 35% to 40%. Weber-Tschopp et al. (1977) observed a reduction in reported eye irritation (from mildly irritating to no effect) when the acrolein exposure concentration declined by a factor of 4 (from 0.6 to 0.15 ppm).

Wong (1996) asserted that the sensitivity of the eyes and nose observed in humans toward irritation caused by acrolein depends on exposure concentration and duration. In longer exposures (1 h), irritation to the eyes prevails over irritation to the nose, whereas short exposures show more irritation to the nose (exposures ≤ 0.3 ppm for 40-60 min) (Weber-Tschopp et al. 1977, Wong 1996). Additionally, 1.5-min exposures to 0.6 ppm of acrolein caused similar irritation in the eyes and nose of human subjects, but 1.5-min exposures to 0.15 ppm of acrolein caused only slight nose irritation and no reported eye irritation (Weber-Tschopp et al. 1977). Wong (1996) proposed that acrolein exposure concentration is more influential than exposure duration in causing acute irritation in humans.

TABLE 1-1 SMACs for Acrolein, 1996

Duration	ppm	mg/m^3	Toxic End Point to Avoid
1 h	0.075	0.17	Mucosal irritation
24 h	0.035	0.08	Mucosal irritation
7 d	0.015	0.034	Mucosal irritation
30 d	0.015	0.034	Mucosal irritation
180 d	0.015	0.034	Mucosal irritation

Abbreviations: mg/m^3, milligrams per cubic meter; ppm, part per million.

As supporting evidence, a study by Sim and Pattle (1957) was cited, which reported that lacrimation was induced in 20 s in human subjects exposed to 0.8 ppm of acrolein but 1.2 ppm of acrolein caused the same response in 5 s. The report of Sim and Pattle (1957) suggests that irritation from acrolein in this study does not adhere to Haber's rule. Wong (1996) applied a safety factor of 4 to the LOAEL observed by Weber-Tschopp, reasoning that a 4-fold reduction in acrolein concentration should provide a dose only mildly irritating to the eyes (an acceptable effect for short-term SMACs).

Hence, the 1-h SMAC was established at 0.075 ppm (0.17 mg/m^3), a concentration expected to cause only mild eye irritation to spacecraft crew (Equation 1).

1-h SMAC based on eye irritation

$$\text{1-h AC}_{(\text{mucosal irritation})} = 0.3 \text{ ppm}_{(\text{LOAEL})} \times 1/4_{(\text{safety factor})} = 0.075 \text{ ppm} \quad (1)$$

The 24-h acrolein SMAC was set at 0.035 ppm (0.08 mg/m^3) (Equation 2). This value was established by further reducing the 1-h SMAC by a factor of 2. The rationale for this more stringent AC was based partly on the observations of Weber-Tschopp et al. (1977) showing a concomitant 2-fold lowering of reported eye irritation when exposure concentration decreased by half. Wong (1996) reasoned that the 24-h acrolein SMAC could therefore be set at the same value as the 1-h SMAC but thought it was prudent to reduce the 24-h SMAC to lessen the degree of mucosal irritation that astronauts could experience. This factor was chosen based on data of Weber-Tschopp et al. (1977), leading Wong (1996) to surmise that reducing the mildly irritating 1-h SMAC by a factor of 2 would result in an acrolein AC that would cause only minimal eye irritation. Thus, the 24-h SMAC was set to decrease possible adverse effects to crew over a longer duration (24 h) resulting from exposure to acrolein (Equation 2).

24-h SMAC based on eye irritation

$$\text{24-h AC}_{(\text{mucosal irritation})} = 0.3 \text{ ppm}_{(\text{LOAEL})} \times 1/4_{(\text{safety factor})} \times 1/2_{(\text{safety factor})} = 0.035 \text{ ppm} \quad (2)$$

The acrolein SMACs for 7, 30, and 180 d (0.015 ppm, 0.034 mg/m^3) were set based on extending the nonirritating 1-h acrolein SMAC adjusted for LOAEL to NOAEL extrapolation (factor of 4) and an additional safety factor of $10/\sqrt{n}$ for potential differences among individuals in a human population; adjustment for small size of the study sample is applied to account for potential uncertainty relative to the results from a small number of study subjects (Equation 3). The rationale for this AC estimate was predicated on the fact that mucosal irritation is a surface-contact phenomenon that does not depend on exposure duration. The work of Lyon et al. was cited at the time as supportive of this exposure-duration independence. Specifically, these investigators reported diminished evidence of mucosal irritation in dogs after 1 wk of continuous or repeated exposure to acrolein (Lyon et al. 1970).

7- , 30- , and 180-d SMACs based on eye irritation

$$\text{7, 30, and 180-d AC}_{(\text{mucosal irritation})} = 0.075 \text{ ppm}_{(\text{1-h AC})} \times 1/4_{(\text{LOAEL to NOAEL})} \times \sqrt{53/100}_{(\text{small n})} = 0.015 \text{ ppm} \quad (3)$$

SUMMARY OF NEW RELEVANT DATA FROM LITERATURE

A review of recent scientific literature (1996 to present) regarding acrolein exposures suggests that mucosal irritation remains the toxicologic end point of concern for acute acrolein exposures (up to and including 30 d). Available human studies, which were previously examined to establish the existing SMACs in 1996, are limited. These studies indicate that exposure to acrolein at concentrations below 1 ppm can produce ocular and nasal irritation (moderate eye and nose irritation in humans after 1 h of exposure at 0.3 ppm) and can cause a decrease in respiratory rate (approximately 25% decrease in respiratory rate in mice exposed for 10 min at 0.22 ppm) (Sim and Pattle 1957, Weber-Tschopp et al. 1977, Steinhagen and Barrow 1984). Most relevant studies pertaining to acrolein inhalation exposure were examined during initial SMAC development (Wong 1996). Some new data reporting reduced respiratory rate and alterations to the nasal epithelium of rats have become available since 1996.

In work reported by Cassee et al. (1996), male Wistar rats, with five or six animals per exposure group, underwent nose-only exposure to acrolein or chemical mixtures including combinations of formaldehyde, acrolein, and acetaldehyde. Animals were exposed to acrolein concentrations of 0, 0.25, 0.67, or 1.40 ppm for 6 h/d for three consecutive days. Slight histopathologic changes, including disarrangement and thickening of the respiratory epithelium, were reported at the lowest exposure concentration (0.25 ppm). Morris and coworkers exposed male and female C57Bl/6J mice to 0.3, 1.6, and 3.9 ppm of acrolein and measured changes in breathing frequency (Morris et al. 2003). C57Bl/6J mice without ovalbumin-induced allergic airway disease that were exposed to the lowest acrolein concentration of the study (0.3 ppm) demonstrated a 10% reduction in respiratory frequency compared with that observed

during preexposure baseline measurements (data reported as group mean ± standard deviation [SD] with three to six animals per group). The LOAEL reported in the Cassee et al. study (0.25 ppm) as well as the respiratory rate depression reported by Morris et al. at the lowest acrolein concentration tested (0.3 ppm) is complementary to the LOAEL of 0.3 ppm reported by Weber-Tschopp et al. in 1977 using human-derived data.

The EPA completed a review of acrolein toxicity in 2003 in support of their information on the Integrated Risk Information System (EPA 2003). In this review, the EPA set an inhalation reference concentration (RfC) using the base study of Feron et al. (1978). The work of Feron et al. was also considered in setting the 1996 SMACs. A whole-body exposure system was used to expose sex- and weight-matched groups of hamsters, rats, and rabbits to 0, 0.4, 1.4, and 4.9 ppm of acrolein vapor for 6 h/d, 5 d/wk for 13 wk. The authors examined mortality, growth, food consumption, hematologic changes, blood chemistry, urinalyses, and organ weight and pathology in these animals. Of the species examined, rats were the most sensitive to acrolein exposure, exhibiting slightly depressed growth and histopathologic changes of the nasal cavity at the lowest concentration (Feron et al. 1978). A LOAEL of 0.4 ppm (0.9 mg/m^3) was derived from the data obtained in this study. The EPA considered three studies (Kutzman 1981, Kutzman et al. 1985, Costa et al. 1986) as supportive of the findings of Feron et al. (1978). The EPA modified the LOAEL reported by Feron et al. to obtain a LOAEL human equivalent concentration ($LOAEL_{HEC}$) of 0.02 mg/m^3. Uncertainty factors totaling 1,000 were then applied to the $LOAEL_{HEC}$: 3 for animals to humans, 10 for intrahuman variability, 10 for extrapolation from subchronic to chronic effects, and 3 for extrapolation using a minimal LOAEL. Thus, the EPA set the RfC at 0.00001 ppm (2×10^{-5} mg/m^3).

The NRC, in conjunction with the U.S. Navy, proposed 1-h, 24-h, and 90-d exposure guidelines for acrolein in 2007 (NRC 2007). The NRC Committee on Toxicology recommended a 1- and 24-h emergency exposure guideline (EEGL) for submariners at 0.1 ppm (0.23 mg/m^3) based on the work of Weber-Tschopp et al. The 90-d continuous exposure guidance level (CEGL) used as its basis the 90-d continuous exposure study of Lyon et al (1970), which found evidence of emphysema in dogs exposed to 0.22 ppm of acrolein for 24 h/d for 90 d. The NRC applied an interspecies uncertainty factor of 3 and a factor of 3 for extrapolation from a LOAEL to a NOAEL. This committee proposed a 90-d CEGL of 0.02 ppm (0.045 mg/m^3).

The Agency for Toxic Substances and Disease Registry (ATSDR) recently updated the toxicologic profile for acrolein, which has been submitted for public comment (ATSDR 2005). The ATSDR proposes both acute (≤14 d) and intermediate (15 to 364 d) inhalation minimal risk levels (MRLs) for humans. The acute MRL of 0.003 ppm (0.007 mg/m^3) was based on the LOAEL reported by Weber-Tschopp et al. (1977) (0.3 ppm for 60-min exposure resulting in irritation of nasal passages and throat and decreased respiratory rate). The ATSDR ap-

plied uncertainty factors to account for extrapolation from a LOAEL (factor of 10) and for intrahuman variability (factor of 10). The ATSDR proposed an intermediate MRL of 0.00004 ppm (0.00009 mg/m^3). Uncertainty factors adjusting for using a LOAEL (factor of 10), for extrapolation from animals to humans (factor of 3), and to account for intrahuman variability (factor of 10) were applied to a duration-adjusted, human equivalent LOAEL of 0.012 (derived from the LOAEL of 0.4 ppm reported by Feron et al. 1978). The ATSDR cited as the toxicologic end point was nasal epithelial metaplasia in rats, as reported by Feron et al. The ATSDR remarked that no human exposure data were available that could be used in setting their proposed intermediate MRL. Furthermore, the ATSDR found studies on chronic-duration exposure to acrolein inadequate for deriving a chronic MRL for this substance.

Several studies (one originally reviewed by Wong [1996] and others published in the intervening years) have examined the impact of acrolein exposure on antibacterial defenses of the lung. Jakab (1977) reported a significant increase in surviving bacteria (*Staphylococcus aureus and Proteus mirabilis*) in mice exposed to 1-2 ppm of acrolein for 24 h. A follow-on study demonstrated a significant increase in survival of *S. aureus* in mouse lungs after 8 h of exposure to acrolein at 3 ppm or greater (3, 6.2, 7.5, and 9 ppm) (Astry and Jakab 1983). Aranyi et al (1986) conducted a study in which mice exposed to 0.1 ppm of acrolein 3 h/d for 5 d showed a significant decrease in bacteriacidal activity (to *Klebsiella pneumoniae*). A more recent study by Jakab (1993) which used coexposure of acrolein and carbon black showed less straightforward results with increased bacteriacidal activity toward *P. mirabilis* and decreased bacteriacidal activity toward *Listeria monocytogenes*. Bacteriacidal activity toward *S. aureus* at first decreased 1 d after acrolein exposure but returned to control concentrations 7 d postexposure (Jakab 1993). The effects reported by Jakab (1977, 1993), Astry and Jakab (1983), and Aranyi et al. (1986) may represent potential secondary effects of the direct irritation effects of acrolein. However, the significance of altered bacteriacidal activity was not clear to the U.S. Navy and the ATSDR (NRC 2007, ATSDR 2005) or was categorized as “other effects” by the EPA (EPA 2003). Although these groups acknowledge pulmonary defense effects due to acrolein exposure, they do not apply a correction factor to account for these possible effects in deriving their respective acrolein ACs. The uncertainty on the part of the Navy, the ATSDR, the EPA, and NASA about the relevance or significance of the bacterial killing and clearance data leads us to choose not to modify our AC derivation based on these findings. Furthermore, protection against irritation, for which the ACs derived in the current review are set, presumably would address issues of altered bacteriacidal activity as well, specifically for longer-term exposures. The longer-term ACs presented here are well below concentrations associated with bacteriacidal effects in the literature and therefore should address effects on the defense mechanisms of the lungs as well.

ADDITIONAL CONSIDERATION OF NONTOXIC ODOR THRESHOLD

The possibility of detrimental effects (nontoxic) on job performance caused by aversion to noxious chemical smells such as that exhibited by acrolein should be considered. Acrolein has a noxious, choking odor with a reported odor threshold of 0.16 ppm (0.4 mg/m^3) (Amoore and Hautala 1983). This odor thresholds is about 10 to 20 times higher than the 7-, 30-, 180-, and 1,000-d acrolein SMACs proposed in this review and about 2 times higher than the short-term 1-h SMAC. Thus, it is assumed that the lower SMACs (designed to protect against adverse health effects) will prevent spacecraft crew discomfort due to noxious odor. A footnote will be included with the revised acrolein SMAC table describing the concentrations at which the odor of the compound may become a concern.

RATIONALE FOR 1,000-DAY SMAC AND REVISION OF 180-DAY SMAC

The existing 7-, 30-, and 180-d acrolein SMACs (0.015 ppm, 0.034 mg/m^3) used a departure concentration of 0.3 ppm (Weber-Tschopp et al. 1977, Wong 1996) modified by a safety factor of 4 (for extrapolation from a LOAEL to NOAEL) and an additional safety factor of $10/\sqrt{n}$. The additional safety factor was applied to adjust for uncertainties resulting from a study with fewer than 100 subjects (Wong 1996). Examination of relevant data pertaining to the potential for chronic acrolein exposure to produce pathologic changes to the lungs warrants reevaluation of the long-term 180-d SMAC.

The original rationale for adopting the same SMAC for 30- and 180-d exposures was based on data that indicated the toxic end point of mucosal irritation did not appear to depend on exposure duration. When the 180-d acrolein SMAC was established, the work of Lyon et al. was cited as supportive of this exposure-duration independence. They reported diminished signs of respiratory tract irritation (difficulty breathing and nasal discharge) in dogs after 1 wk of continuous (1.0 ± 0.2 ppm for 24 h/d, 90 d) or repeated (discontinuous at 3.7 ± 0.8 ppm for 8 h/d, 5 d/wk for six consecutive weeks) acrolein exposure, suggesting reduced sensitivity to acrolein with increasing exposure duration (Lyon et al. 1970). However, these authors also reported evidence of emphysematous pulmonary changes in laboratory dogs who were continuously exposed for 90 d as well as acute congestion and vacuolization of bronchial epithelial cells, troubling pathologic findings. The authors characterized the pathologic findings reported in the 90-d continuous acrolein (1.0 ± 0.2 ppm) exposures as "moderate" (Lyon et al. 1970).

The Navy recognized the applicability and appropriateness of the 90-d continuous exposure study and considered the findings of emphysematous pulmonary changes in laboratory dogs as key in setting their LOAEL at 0.22 ppm

(point of departure for the Navy's 90-d CEGL, NRC 2007). The study of Lyon et al. has significant limitations that preclude it from being used as the basis for setting long-term exposure guidelines. Most notable among these limitations are the small number of animals per exposure group as well as the lack of reported controls, which complicates interpretation of the findings. Emphysematous changes occurred in the lungs of two dogs exposed to 0.22 ppm of acrolein but not in the lungs of dogs exposed to higher concentrations. However, in consideration of the emphysematous changes reported by Lyon et al., we propose that the existing 180-d acrolein SMAC be set lower.

Using a LOAEL of 0.4 ppm (Feron et al. 1978) as a point of departure, we propose a revised 180-d acrolein SMAC of 0.008 ppm (0.0183 mg/m^3). Exposure times used by Feron et al. were discontinuous with animals exposed 6 h/d, 5 d/wk for 13 wk (91 d). Extrapolation factors of 0.25 and 0.71 are proposed to adjust for continuous exposure conditions. Like the Navy, we propose that an uncertainty factor of 3 be applied to extrapolate from LOAEL to NOAEL. We agree with the NRC and the Navy's previous assessment of an interspecies extrapolation factor of 3 and choose to adopt that value as well in our long-term SMAC derivations. Adoption of the interspecies correction factor of 3 is based on similarities in irritancy and a steep concentration-response relationship between species (rodents and humans). In addition, the resulting AC is below the reported effect level in human studies. Based on extension of the original argument for the exposure-duration independent nature of acrolein-induced mucosal irritation (Lyon et al. 1970, Wong 1996), no additional factor (Haber's rule) was deemed necessary to adjust for exposure duration (from 91 to 180 d) (Equation 4).

Revised 180-d SMAC

$$\text{180-d AC}_{(\text{mucosal irritation})} = 0.4 \text{ ppm}_{(\text{LOAEL})} \times [6/24 \times 5/7]_{(\text{time extrapolation})} \times 1/3_{(\text{LOAEL to NOAEL})} \times 1/3_{(\text{interspecies})} = 0.008 \text{ ppm} \quad (4)$$

By comparison, if the 0.22-ppm LOAEL from the study of Lyon et al. (1970) is modified by the same uncertainty factors of 3 (for the LOAEL to NOAEL and the interspecies factors) and application of the same adjustments for exposure duration, a 180-d SMAC of 0.004 ppm can be obtained. This value is similar to the 180-d SAMC of 0.008 ppm proposed here.

The proposed 1,000-d SMAC uses the base study of Feron et al. (1978) and is derived similarly to the revised 180-d AC. Beginning with the LOAEL of 0.4 ppm, we propose that the same exposure extrapolation factors, LOAEL to NOAEL uncertainty factor, and interspecies extrapolation factor be applied. The risk due to low-level exposure to aldehydes, including acrolein, is not well understood. Acrolein is an extremely reactive aldehyde (electrophilic) that reacts readily with sulfhydryl and thiol-containing compounds and can result in depletion of certain species such as reduced glutathione (McNulty et al. 1984, Lam et al. 1985, Grafstrom et al. 1990). Heck and co-workers (1986) reported that respiratory and nasal mucosal nonprotein sulfhydryls, but not protein sulfhydryls,

were significantly depleted after 3 h of exposure to acrolein at 0.1 to 2.4 ppm. Overt toxicity to lung tissues may be attenuated by the reaction of acrolein with protein and nonprotein sulfhydryls, especially reduced glutathione. Although in situ defense mechanisms may afford protection for relatively acute exposures, it is unclear whether these mechanisms would remain effective under conditions of low-level continuous exposure to acrolein for more than 1,000 d. Application of Haber's rule would result in an additional factor of 0.091 (extrapolating from 91 to 1,000 d of exposure). In light of the discussion above and the argument that acrolein-induced irritation is exposure-duration independent, it is likely that the application of Haber's rule would result in an overly conservative AC. Therefore, no additional factor was applied to adjust for exposure duration (from 91 to 1,000 d). The proposed 1,000-d acrolein SMAC is 0.008 ppm (0.02 mg/m^3) (Equation 5).

$$\begin{aligned}\text{Proposed 1000-d SMAC 1,000-d AC}_{(\text{mucosal irritation})} &= 0.4 \text{ ppm}_{(\text{LOAEL})} \\ &\times [6/24 \times 5/7]_{(\text{time extrapolation})} \times 1/3_{(\text{LOAEL to NOAEL})} \times 1/3_{(\text{interspecies})} \\ &= 0.008 \text{ ppm} \end{aligned} \quad (5)$$

There is no evidence suggesting that the microgravity environment of planetary orbit or interplanetary travel will modify mechanisms associated with acrolein toxicity. Similarly, no foreseeable change in toxicity is expected as a result of space crews living in moon and Mars gravity conditions (accelerations of 1/6 and 1/3 gravity, respectively). Thus, no additional safety factors are warranted to compensate for possible gravity-induced physiological changes. Table 1-2 presents the proposed SMACs for acrolein for 2008.

COMPARISON OF APPROACH OF ORIGINAL AND CURRENT NRC COMMITTEE ON TOXICOLOGY

The current trend for risk assessment among many regulatory bodies, including the NRC Committee on Toxicology, is to apply a benchmark dose method (BMD) to set acceptable human exposure guidelines and limits. In particular, this approach is recommended by the NRC for setting spacecraft water exposure guidelines—and by inference SMACs—when sufficient and appropriate dose-response data are available (NRC 2000). Furthermore, the NOAEL-based method is recommended by the NRC in the absence of sufficient dose-response data or when special considerations are warranted. In the case of acrolein, few studies of either acute or chronic exposure are available. The U.S. Navy, the EPA, and the ATSDR have performed recent comprehensive examination of the relevant acrolein inhalation toxicologic studies. On the basis of the available studies, these organizations chose to establish exposure guidelines predicated on reference concentrations. The same methodology was used in establishing the existing (1996) acrolein SMACs. We have chosen to adhere to a

TABLE 1-2 SMACs for Acrolein, 2008

Duration	ppm	mg/m^3	Toxic End Point to Avoid	Change from 1996 SMAC
1 h	0.075	0.17	Mucosal irritation	No change
24 h	0.035	0.08	Mucosal irritation	No change
7 d	0.015	0.034	Mucosal irritation	No change
30 d	0.015	0.034	Mucosal irritation	No change
180 d	0.008	0.02	Mucosal irritation	~1.9-fold reduction
1,000 d	0.008	0.02	Mucosal irritation	New SMAC

Note: The average odor threshold concentration is 0.16 ppm (Amoore and Hautala 1983). Although acrolein exhibits a noxious odor, the odor detection threshold is about 2 times higher than the 1-h SMAC.

LOAEL-NOAEL methodology in confirming existing acrolein SMACs and for revising and establishing new SMACs. The following section presents an additional explanation of our rationale for using each base and supporting study and provides BMD analysis of these studies when applicable.

Short-Term SMACs

Weber-Tschopp et al. 1977

The study of Weber-Tschopp et al. (1977) is considered the base study for establishing the original acrolein 1-h, 24-h, 7-d, and 30-d SMACs. The ATSDR also uses this study's corresponding LOAEL of 0.3 ppm—based on nasal and throat irritation and reduction in respiratory rate in human subjects—as the point of departure for setting the ATSDR acrolein MRL. We chose this study in setting a point of departure for our SMACs, as was chosen in setting the guidelines of the ATSDR, because it reports human-derived data. Using human- as opposed to animal-derived data eliminates the need to include factors to adjust for uncertainties due to species differences in RfC derivations. The LOAEL observed by Weber-Tschopp et al. (1977) of 0.26 to 0.3 ppm is corroborated by the animal data presented by Cassee et al. (1996), who report a LOAEL of 0.25 ppm. Although the study reported by Weber-Tschopp et al. (1977) used an appreciable number of both male and female human subjects, it nevertheless relies on subjective self-reporting of effects by the study subjects. The results presented by Weber-Tschopp et al. (1977) are presented as average scores for response categories. The unavailability of the raw data precludes modeling the findings of Weber-Tschopp et al. via BMD methodology for this review.

Morris et al. 2003

The work of Morris et al. (2003) helps to validate the point of departure used to derive our acrolein SMACs based on NOAEL-LOAEL methodology.

This study is considered supportive of the base study of Weber-Tschopp et al. (1977) used in setting the original 1-h, 24-h, 7-d, 30-d, and 180-d acrolein SMACs. This study is also considered supportive in setting the revised 180-d and the new 1,000-d acrolein SMACs. Morris and co-workers exposed male and female mice to acrolein as part of their study to compare responses in healthy mice and in mice with allergic airway disease. Of concern to this review, these authors observed a 10% reduction from baseline in the respiratory rate of normal mice (C57Bl/6J mice without ovalbumin-induced allergic airway disease) at the lowest acrolein concentration of 0.3 ppm. As mentioned, the respiratory rate change noted in this study is considered supportive of the point of departure value and subsequent NOAEL-LOAEL extrapolations. Although applying BMD methodology to this respiratory rate data is problematic, it is nevertheless presented here for comparison.

Morris et al. give only a range of animals in each exposure group—four to six animals in control exposures (0 ppm of acrolein) and three to six animals exposed to 0.3 and 1.3 ppm of acrolein. For the BMD analysis presented here, the number of animals was estimated at five for controls and 4.5 for higher-dose groups. The SD for the low acrolein dose (0.3 ppm) was not reported numerically in the text but had to be estimated from the report's graphic presentation (estimated at ±five breaths/min based on interpretation of Figure 4 of Morris et al. [2003]). A polynomial model with the dose-response data procedure for continuous data in the EPA Benchmark Dose Software (BMDS) program was used to analyze the end point of interest (respiratory rate of control and acrolein-exposed mice). For normally distributed data, a shift of the mean from the baseline of 1 SD can be assumed to result in an excess risk of abnormal levels of about 10% (Crump 1995). Because the SD in the case of the breathing frequency data reported by Morris and co-workers is less than 10% of the baseline values, the SD in the benchmark concentration (BMC) calculation using these continuous data will approximate an increased risk of 10%. The BMC and lower 95% confidence limit of the benchmark concentration (BMCL) associated with an excess risk of 10% (e.g., a 10% incidence above background) were estimated to be 0.015 and 0.011 ppm, respectively. Using the BMD lower confidence limit (BMDL) as the point of departure, correction factors of 0.25 (interspecies variability) and 0.17 (extrapolation from 10- to 60-min exposure) were applied, yielding an AC of 0.0005 ppm.

Intermediate SMACs

Cassee et al. 1996

The study of Cassee et al. (1996) used relatively high resolution analysis designed to elucidate the severity of effects from exposure to mixtures or single chemical irritants. Only the data from this study pertaining to histopathologic nasal changes and nasal epithelium biotransformation enzyme activities are pre-

sented by the authors in enough detail to allow BMD analysis. In one part of the study of interest here, rats were exposed for 6 h/d for three consecutive days. For histopathologic analysis, 19 rats were used as controls (0 ppm of acrolein), 5 rats were exposed to 0.25 ppm of acrolein, and 6 rats were exposed to 0.67 ppm of acrolein. The data are reported as three levels of incidence (slight, moderate, and severe) for each histologic end point. One end point, "disarrangement, necrosis, thickening and desquamation of respiratory/transitional epithelium" exhibited a 100% incidence at both doses tested (above 0 ppm) and therefore was not modeled. The other end points measured did not exhibit a significant biological response at either dose tested.

The end point "basal cell hyperplasia and/or increased number of mitotic figures in respiratory/transitional epithelium" from Cassee et al. (1996) showed a dose-dependent change amenable to BMD analysis. A multistage model for dichotomous data (EPA BMDS) was used to estimate the dose-response relationship. For the purpose of this analysis, the three levels of incidence were summed to obtain a total incidence per end point per exposure. The BMC and lower BMCL associated with an excess risk of 10% were 0.08 and 0.012 ppm, respectively. The BMCL was then used as a point of departure for estimating a maximum allowable concentration for 24-h and 7-d exposures. A factor of 0.25, the same correction factor for interspecies variability as that used to derive the 24-h SMAC, was applied to this point of departure. No additional uncertainty factors for intraspecies variability or a potential risk at the point of departure (NOAEL or BMCL) were applied. The maximum AC for 24 h and 7 d was derived as 0.003 ppm.

For nasal epithelium biotransformation enzyme activity determination, only three rats each were included in control (0 ppm of acrolein), medium-exposure (0.67 ppm of acrolein), and high-exposure (1.4 ppm of acrolein) groups. A polynomial model with the dose-response data procedure for a continuous data (EPA BMDS) program was used to analyze the enzyme activity data. SDs for the dose-dependent nasal epithelium biotransformation enzyme activity of the Cassee et al. (1996) study were all less than 10% of their respective baseline values. Therefore, using the SDs in the BMC calculations will approximate an increased risk of 10% (Crump 1995). The most sensitive end points for severity were glutathione *S*-transferase and aldehyde dehydrogenase activities, both having BMC and lower BMCL associated with an excess risk of 10% of 0.045 and 0.028 ppm, respectively. Using the BMCL as the point of departure and applying the same correction factors as above for the histopathology end point, a maximum AC of 0.007 ppm was derived for the 24-h and 7-d exposures. The study of Cassee et al. (1996) is not ideal to derive a BMC as there are no "no-effect" levels reported and a limited number of exposure concentrations were tested. Importantly, there are a small number of animals in each exposure group (six animals per group for the histopathology assessment and three animals per group for the biotransforming enzyme assays), which can lead to derivation of wide confidence limits.

Long-Term SMACs

Feron et al. 1978

The work of Feron et al. is selected as our base study for determining a point of departure for the revised 180-d SMAC as well as our new 1,000-d SMAC. The EPA in 2003 also chose to use this study as their base study. This study characterized several toxicologic end points by examining responses in three groups in three species as well as in both sexes. Of primary interest in the Feron et al. (1978) study is the extended exposure duration of 5 d/wk for 13 wk. Although not continuous, this exposure duration more closely approximates conditions that could be expected on long-duration space voyages and stays. Despite the positive aspects of this study, it has limitations with respect to BMD analysis. As with the report of Weber-Tschopp et al. (1977), results reported by Feron et al. for treatment-related effects (other than for body weight and organ weight ratios) are presented as summary scores averaged for all animals in each exposure group. The unavailability of the raw data precludes the BMD modeling of these histopathologic findings. BMD analysis was nevertheless applied to the body weight and organ weight ratio (g per 100 g of body weight) data reported by Feron et al. to estimate maximum allowable concentrations that could then be compared with the proposed SMACs, particularly the 180- and 1,000-d SMACs.

Rats were the most sensitive of the species examined by Feron et al., exhibiting treatment-related effects at the lowest dose (0.4 ppm). Four groups of 12 rats each (divided by body weight and sex) were exposed to 0, 0.4, 1.4, and 4.9 ppm of acrolein for 6 h/d, 5 d/wk for 13 wk (91 d discontinuously). Body weight was measured as well as the organ weight ratios of multiple organs including heart, kidneys, liver, spleen, brain, testicles, ovaries, thymus, adrenals, and lungs. Statistically significant dose-related changes were reported for body weight and heart, lung, kidney, and adrenal organ weight ratios in rats. A polynomial model with the dose-response data procedure for a continuous data program (EPA BMDS) was applied to each of these data sets**.** The SDs reported by Feron et al. for body weight and organ weight changes were all either less than or in two midlevel doses of acrolein in the female rat adrenals, or equal to 10% of their respective baseline values. Therefore, using the SDs in the BMC calculations will approximate an increased risk of 10% (Crump 1995). The BMC and BMCL associated with an excess risk of 10% were calculated for each end point of concern. The BMCL for each end point was used as a point of departure for estimating a maximum allowable concentration. Estimates of BMC and BMCL for 13-wk exposures reported by Feron et al. (1978) with estimated 180- and 1,000-d ACs are listed in Table 1-3.

The most sensitive end points, lowest BMCL = 0.04 ppm, occurred for male rat body weight gain, which was decreased, and male rat adrenal weight ratio, which was also decreased. To derive an AC for 180-d exposures, factors of 0.25 and 0.71 were used to adjust for continuous exposure conditions. An inter-

TABLE 1-3 Estimates of BMC and BMCL for 13-wk Exposures in Rats, Reported by Feron et al. (1978), with 180- and 1,000-d ACs

End Point	BMD, ppm	BMDL, ppm	AC (180 d), ppm	AC (1,000 d), ppm
Body weight				
Male	0.050	0.040	0.002	0.002
Female	0.080	0.070	0.004	0.004
Lung weight ratio[a]				
Male	0.060	0.050	0.003	0.003
Female	0.012	0.098	0.006	0.006
Heart weight ratio				
Male	0.130	0.100	0.006	0.006
Female	0.180	0.140	0.008	0.008
Kidney weight ratio				
Male	0.290	0.210	0.012	0.012
Female	0.130	0.110	0.007	0.007
Adrenal weight ratio				
Male	0.040	0.040	0.002	0.002
Female	0.100	0.090	0.005	0.005

[a]Organ weight ratio = g per 100 g of body weight.
Abbreviations: AC, acceptable concentration; BMD, benchmark dose concentration; BMDL, lower 95% confidence limit of the benchmark concentration.

species extrapolation factor of 3 was also applied. No uncertainty factors for intraspecies variability of a potential risk at the point of departure (NOAEL or BMCL) were applied. The resulting estimated 180-d AC is 0.002 ppm.

To derive an AC for 1,000-d exposures, factors of 0.25 and 0.71 were used to adjust for continuous exposure conditions. An interspecies extrapolation factor of 3 was also applied. No uncertainty factors for intraspecies variability or for a potential risk at the point of departure (NOAEL or BMCL) were applied. The resulting estimated 1,000-d AC is 0.002 ppm.

Lyon et al. 1970

The emphysematous changes in laboratory dogs reported by Lyon et al. (1970) were carefully considered in setting the current revised 180-d and the new 1,000-d acrolein SMAC. However, limitations in the reporting detail and experimental design of this study preclude its choice as a base study and prohibit applying BMD methods to the data. As mentioned previously, this study used continuous exposure conditions (24 h/d) for 90 d. This exposure protocol closely approximates the longer-duration SMAC intervals (especially 180 d). However, this study used small numbers of animals per exposure group and, most significantly, lacked reporting of control data; both factors exclude the

reported LOAEL as a valid point of departure and preclude application of BMD modeling.

BMD analysis results for the base studies and supporting studies discussed above are summarized in Table 1-4. The ACs derived from BMDLs estimated from these studies fall below the current and proposed SMACs, which are based on LOAEL-NOAEL methods. BMD analysis of data from Morris et al. (2003) and the AC derived from this analysis falls well below (approximately 150 times below) the proposed 1-h SMAC. Because of the lack of available raw data and the need to estimate the number of animals in each exposure group, confidence in the derived 1-h AC resulting from this BMD analysis is low. We choose to rely solely on the NOAEL-LOAEL-derived 1-h SMAC based on Weber-Tschopp et al. (1977) and use the LOAEL of 0.3 ppm reported by Morris et al. (2003) as supportive data.

BMD analysis of data presented by Cassee et al. (1996), although resulting in 24-h and 7-d ACs slightly below the current and proposed SMACs, are considered supportive of the values already established. Confidence in the BMD analysis and derivation of subsequent ACs is medium. It is important to point out that the study design involved in producing the data used for the BMD analysis used a limited number of exposure concentrations—control, low, and medium acrolein for the "basal cell hyperplasia" end point and control, medium, and high acrolein for the biotransformation enzyme activity end point. Also, the study for biotransformation enzyme activity involved only three animals per exposure group. Because of these limitations, we have chosen to use the base study of Weber-Tschopp et al. and consider the LOAEL of 0.3 ppm as the point-of-departure concentration for determining the intermediate 7- and 30-d SMACs.

COMPARISON WITH OTHER AIR QUALITY LIMITS

Exposure guidelines for acrolein exist with various public health and occupational health entities as well as with industry and government advisory bodies. Table 1-5 presents a selected list of some of these guidelines and regulatory standards for comparison with the current and proposed NASA acrolein SMACs. The proposed NASA 180- and 1,000-d acrolein SMACs are more conservative than the 90-d CEGL proposed by the Navy for submariners in 2007 (Table 1-5). These differences result from use of a different LOAEL point of departure (LOAEL of 0.4 ppm for NASA based on Feron et al. (1978) versus LOAEL of 0.22 ppm for the Navy based on Lyon et al. (1970) and the application of additional correction factors to account for differences between discontinuous and continuous exposure conditions. In contrast, both the revised 180-d and the proposed 1,000-d SMACs are approximately 900 times higher than the RfC set by the EPA. Explanation of this difference in ACs can be found in the inherent nature of the expected exposure conditions for which each guideline was established.

TABLE 1-4 Summation of Benchmark Dose Analysis Results

Study	End Point	BMD, ppm	BMDL, ppm	AC, ppm	BMD-Specific Limitations	Comments
Weber-Tschopp et al. 1977	Nasal and throat irritation, reduction in respiratory rate	ND	ND	ND	BMD analysis not performed	Base study, LOAEL used as point of departure
Morris et al. 2003	Respiratory rate	0.015	0.011	0.0005 (1 h)	Required estimation of data, lack of reported raw data. Required estimation of n	150 times lower than current or proposed SMAC
Cassee et al. 1996	"Basal cell hyperplasia and/or increased number of mitotic figures in respiratory/transitional epithelium."	0.08	0.012	0.003 (24 h)	No "no-effect" level reported, limited number of exposure concentrations tested, small n in each exposure group	12 times lower than current or proposed SMAC
Cassee et al. 1996	"Basal cell hyperplasia and/or increased number of mitotic figures in respiratory/transitional epithelium."	0.08	0.012	0.003 (7 d)	No "no-effect" level reported, limited number of exposure concentrations tested, small n in each exposure group	12 times lower than current or proposed SMAC
Cassee et al. 1996	Nasal epithelium biotransformation enzyme activity	0.045	0.028	0.007 (24 h)	No "no-effect" level reported, limited number of exposure concentrations tested, small n in each exposure group	5 times lower than current or proposed SMAC

(Continued)

TABLE 1-4 Continued

Study	End Point	BMD, ppm	BMDL, ppm	AC, ppm	BMD-Specific Limitations	Comments
Cassee et al. 1996	Nasal epithelium biotransformation enzyme activity	0.045	0.028	0.007 (7 d)	No "no-effect" level reported, limited number of exposure concentrations tested, small n in each exposure group	2 times lower than current or proposed SMAC
Feron et al. 1978	Body weight, adrenal weight ratio	0.05	0.04	0.002 (180 d)	No BMD-specific limitations	4 times lower than current or proposed SMAC
Feron et al. 1978	Body weight, adrenal weight ratio	0.05	0.04	0.002 (1,000 d)	No BMD-specific limitations	4 times lower than proposed SMAC
Lyon et al. 1970	Emphysematous changes	ND	ND	ND	BMD analysis not performed	Supporting study

Abbreviation: ND, not done.

TABLE 1-5 Selected Inhalation Exposure Levels for Acrolein from Various Agencies

Organization	Exposure Level, ppm	Reference
OSHA PEL TWA	0.1	29 CFR 1910.1000 [1993]
ACGIH TLV	0.1	ACGIH 2001
NIOSH REL TWA	0.1	NIOSH 2005
ATSDR		ATSDR 2005
acute inhalation MRL (≤14 d)	0.003	
intermediate inhalation MRL (15-364 d)	0.00004	
NAC/NRC AEGL-1[a]		EPA 2005
1 h	0.03	
8 h	0.03	
NRC/Navy (submariner)		NRC 2007
EEGL 1 h	0.1	
EEGL 24 h	0.1	
CEGL 90 d	0.02	
EPA RfC	0.0000088	EPA 2003
NRC/NASA SMAC		
1 h	0.075	Wong 1996
24 h	0.035	Wong 1996
7 d	0.015	Wong 1996
30 d	0.015	Wong 1996
180 d	0.008	Revised in current document
1,000 d	0.008	Proposed in current document

[a]AEGL-1 is the airborne concentration of a substance above which it is predicted that the general population, including susceptible individuals, could experience notable discomfort, irritation, or certain asymptomatic nonsensory effects. However, the effects are not disabling and are transient and reversible upon cessation of exposure.

Abbreviations: ACGIH, American Conference of Governmental Industrial Hygienists; AEGL, acute exposure guideline level; ATSDR, Agency for Toxic Substances and Disease Registry; CEGL, Continuous Exposure Guideline Level; EEGL, Emergency Exposure Guideline Level; EPA, U.S. Environmental Protection Agency; MRL, minimal risk level; NAC, National Advisory Council; NASA, National Aeronautics and Space Administration; NIOSH, National Institute for Occupational Safety and Health; NRC, National Research Council; OSHA, Occupational Safety and Health Administration; PEL, permissible exposure limit; TLV, threshold limit value; TWA, time-weighted average; REL, recommended exposure limit; RfC, reference concentration; SMAC, Spacecraft Maximum Allowable Concentration.

SMACs and the Navy's analogous acute exposure guideline levels, EEGLs, and CEGLs differ from the usual public health and occupational health standards of exposure. Public and occupational health standards are aimed at

protecting sensitive subpopulations, including children, the elderly, and those with underlying chronic health conditions. SMACs are geared to protecting a healthy and relatively homogeneous population of adults. Occupational exposure standards are set for conditions of repeated exposure to the toxic agent throughout a worker's lifetime (exposed 8 h/d × 5 d/wk × 52 wk/year × approximately 30 years ≈ 62,400 h). Whereas crew engaged in long-term space exploration as well as submariners potentially could be exposed to a toxicant such as acrolein for 24 h/d for up to 90 d (submariners) or 1,000 d (spacecraft crew), these resulting exposure durations are far shorter than those considered by occupational health standards (24 h/d × 90 d = 2,160 h for submariners or 24 h/d × 1,000 d = 24,000 h for space crews). Potential exposure conditions aboard spacecraft would preclude "recovery periods" associated with nonworking days that workers normally experience in a traditional workplace. If acrolein were present in confined living spaces such as those found in long-duration spaceflights and aboard modern submarines conducting extended submerged operations, circumstances of potentially similar acrolein exposure conditions could result.

RECOMMENDATIONS FOR ADDITIONAL RESEARCH

Establishing exposure limits for toxicants that cause sensory irritation is inherently difficult. In the case of acrolein, reports of ocular and respiratory tract irritation experienced by human subjects are subjective. The results of controlled human exposures to acrolein use descriptors such as "mild" and "mild to moderate." Furthermore, sensory irritation thresholds for acrolein and related materials can be highly variable from person to person. Current and proposed NASA acrolein SMACs are derived through use of a RfC "threshold dose" method with applicable safety factors applied. The supporting studies from which reference dosages were obtained are based in large part on subjective responses. Additional studies that rely on less subjective subject responses about the effects of acrolein exposure are needed to better define exposure guidance levels.

REFFERENCES

ACGIH (American Conference of Governmental Industrial Hygienists). 2001. Acrolein in Documentation of Threshold Limit Values and Biological Exposure Indices, 7th Ed. American Conference of Government Industrial Hygienists, Cincinnati, OH.

Amoore, J.E., and E. Hautala. 1983. Odor as an aid to chemical safety: Odor thresholds compared with threshold limit values and volatilities for 214 industrial chemicals in air water dilution. J. Appl. Toxicol. 3(6):272-290.

Aranyi, C., W.J. O'Shea, J.A. Graham, and F.J. Miller. 1986. The effects of inhalation of organic chemical air contaminants on murine lung host defenses. Fundam. Appl. Toxicol. 6(4):713-720.

Astry, C.L., and G.J. Jakab. 1983. The effects of acrolein exposure on pulmonary antibacterial defenses. Toxicol. Appl. Pharmacol. 67(1):49-54.

ATSDR (Agency for Toxic Substances and Disease Registry). 2005. Toxicological Profile for Acrolein (Draft for Public Comment). U.S. Department of Health and Human Services, Public Health Service, Atlanta, GA.

Cassee, F.R., J.P. Groten, and V.J. Feron. 1996. Changes in the nasal epithelium of rats exposed by inhalation to mixtures of formaldehyde, acetaldehyde, and acrolein. Fundam. Appl. Toxicol. 29(2):208-218.

Costa, D.L., R.S. Kutzman, J.R. Lehmann, and R.T. Drew. 1986. Altered lung function and structure in the rat after subchronic exposure to acrolein. Am. Rev. Respir. Dis. 133(2):286-291.

Crump, K.S. 1995. Calculation of benchmark doses from continuous data. Risk Anal. 15(1):79-89.

Darley, E.F., J.T. Middleton, and M.J. Garber. 1960. Plant damage and eye irritation from ozone-hydrocarbon reactions. J. Agr. Food Chem. 8(6):484-485.

EPA (U.S. Environmental Protection Agency). 2003. Toxicological Review of Acrolein (CAS No. 107-02-8) In Support of Summary Information on the Integrated Risk Information System (IRIS). EPA/635/R-03/003. U.S. Environmental Protection Agency, Washington, DC [online]. Available: http://www.epa.gov/iris/toxreviews/0364-tr.pdf [accessed April 1, 2008].

EPA (U.S. Environmental Protection Agency). 2005. Acrolein (CAS RN 107-02-8). Interim Acute Exposure Guidelines Levels (AEGLs). Prepared for NAS/COT Subcommittee for AEGLs. AEGLS Program, U.S. Environmental Protection Agency. August/September 2005 [online]. Available: http://www.epa.gov/oppt/aegl/pubs/tsd303.pdf [accessed July 16, 2008].

Feron, V.J., A. Kruysse, H.P. Til, and H.R. Immel. 1978. Repeated exposure to acrolein vapor: Subacute studies in hamsters, rats, and rabbits. Toxicology 9(1-2):47-57.

Geiger, T. 1984. P. 11 in Spacelab Mission 3 Aggregate Trace Contaminant Assessment. Publ. No. EP45(84-148). NASA, Marshall Space Flight Center, Huntsville, AL.

Graftstrom, R.C. 1990. In vitro studies of aldehyde effects related to human respiratory carcinogenesis. Mutat. Res. 238(3):175-184.

Heck, H., M. Casanova, M.J. McNulty, and C.W. Lam. 1986. Mechanisms of nasal toxicity induced by formaldehyde and acrolein. Pp. 235-247 in Toxicology of the Nasal Passages, C.S. Barrow, ed. Washington, DC: Hemisphere Publishing.

Jakab, G.J. 1977. Adverse effect of a cigarette smoke component, acrolein, on pulmonary antibacterial defenses and on viral-bacterial interactions in the lung. Am. Rev. Respir. Dis. 115(1):33-38.

Jakab, G.J. 1993. The toxicologic interactions resulting from inhalation of carbon black and acrolein on pulmonary antibacterial and antiviral defenses. Toxicol. Appl. Pharmacol. 121(2):167-175.

Kutzman, R.S. 1981. A Subchronic Inhalation Study of Fisher 344 Rats Exposed to 0, 0.4, 1.4, or 4.0 ppm Acrolein. Conducted for the National Toxicology Program: Interagency Agreement No. 222-Y01-ES-9-0043. Brookhaven National Laboratory, Upton, NY.

Kutzman, R.S., E.A. Popenoe, M. Schmaleler, and R.T. Drew. 1985. Changes in rat lung structure and composition as a result of subchronic exposure to acrolein. Toxicology 34(2):139-151.

Lam, C.W., M. Casanova, H.D. Heck. 1985. Depletion of nasal mucosal glutathione by acrolein and enhancement of formaldehyde-induced DNA-protein cross-linking by simultaneous exposure to acrolein. Arch. Toxicol. 58(2):67-71.

Lyon, J.P., L.J. Jenkins, Jr., R.A. Jones, R.A. Coon, and J. Siegel. 1970. Repeated and continuous exposure of laboratory animals to acrolein. Toxicol. Appl. Pharmacol. 17(3):726-732.

McNulty, M.J., H.D. Heck, and M. Casanova-Schmitz. 1984. Depletion of glutathione in rat respiratory mucosa by inhaled acrolein. Fed. Proc. 43(3):575 [Abstr.1695].

Morris, J.B., P.T. Symanowicz, J.E. Olsen, R.S. Thrall, M.M. Cloutier, and A.K. Hubbard. 2003. Immediate sensory nerve-mediated respiratory responses to irritants in healthy and allergic airway-diseased mice. J. Appl. Physiol. 94(4):1563-1571.

NIOSH (National Institute for Occupational Safety and Health). 2005. NIOSH Pocket Guide to Chemical Hazards. DHHS (NIOSH) No. 2005-151. National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention, U.S. Department of Health and Human Services, Cincinnati, OH.

NRC (National Research Council). 2000. Methods for Developing Spacecraft Water Exposure Guidelines. Washington, DC: National Academy Press.

NRC (National Research Council). 2007. Emergency and Continuous Exposure Guidance Levels for Selected Submarine Contaminants, Vol. 1. Washington, DC: The National Academies Press.

Sim, V.M., and R.E. Pattle. 1957. Effect of possible smog irritants on human subjects. J. Am. Med. Assoc. 165(15):1908-1913.

Steinhagen, W.H., and C.S. Barrow. 1984. Sensory irritation structure-activity study of inhaled aldehydes in B6C3F1 and Swiss-Webster mice. Toxicol. Appl. Pharmacol. 72(3):495-503.

Weber-Tschopp, A., T. Fisher, R. Geier, and E. Grandjean. 1977. Experimentally induced irritating effects of acrolein on men [in German]. Int. Arch. Occup. Environ. Health 40(2):117-130.

Wong, K.L. 1996. Acrolein. Pp. 19-38 in Spacecraft Maximum Allowable Concentrations for Selected Airborne Contaminants, Vol. 2. Washington, DC: National Academy Press.

2

C3 to C8 Aliphatic Saturated Aldehydes

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BACKGROUND

Spacecraft maximum allowable concentrations (SMACs) of C3 to C8 straight-chain, aliphatic aldehydes have been established and were documented in Volume 4 of *Spacecraft Maximum Allowable Concentrations for Selected Airborne Contaminants* (James 2000). These aldehydes, shown in Table 2-1 with their associated physical properties, can enter habitable compartments of spacecraft and contaminate breathing air by several routes, including incomplete oxidation of alcohols in the environmental control and life support system air revitalization subsystem, as a by-product of human metabolism, through materials off-gassing, and during food preparation. These aldehydes have been detected in the atmosphere of manned space vehicles in the past. The National Aeronautics and Space Administration (NASA) analyzed air samples from the crew cabin of the Russian Mir Space Station and found that C3 to C8 aldehyde concentrations peaked at approximately 0.1 milligram per cubic meter (mg/m^3) (James 2000, unpublished NASA technical data from 1995).

NASA has reviewed most existing reports pertaining to aliphatic aldehyde toxicity in support of establishing the SMACs published in 2000. This report is intended to be a companion document to complement and update James (2000) on C3 to C8 saturated aliphatic aldehyde SMACs. This update summarizes the approach taken in developing the existing SMACs, identifies recent data that may affect existing SMAC values, and establishes and provides rationale for a new 1,000-day SMAC.

REVIEW OF EXISTING SMACs AND SUMMARY OF ORIGINAL APPROACH

The initial review in 2000 resulted in establishment of 1- and 24-h as well

as 7-, 30-, and 180-day SMACs for the C3 to C8 straight-chain, aliphatic aldehydes. Table 2-2 presents the SMACs NASA established for these compounds. Respiratory irritation potential threshold data from rats and mice indicate similar properties (sensory irritation) within this group of compounds and the closely related acetaldehydes (Sim and Pattle 1957; Steinhagen and Barrow 1984; Babiuk et al. 1985). Because the C3 to C8 aldehydes exhibited similar toxicities for this particular end point, the Committee on Spacecraft Exposure Guidelines chose to establish SMACs for these compounds as a group instead of setting a separate SMAC for each compound. The toxicological end points of concern identified previously include mucosal irritation, nasal-cavity injury, nausea and vomiting, and liver damage. SMACs for each exposure time were selected based on the most conservative acceptable concentration (AC) for each toxicological end point.

Protection Against Mucosal Irritation

An early study reported that exposure to propanal at 134 parts per million (ppm) for 30 min was mildly irritating to mucosal surfaces in humans (Sim and Pattle 1957). The same study found that exposure to 230 ppm butanal and 207 ppm isobutanal for 30 min was not irritating to human subjects. Human data were available only for the three aldehydes propanal, butanal, and isobutanal. However, animal data available at the time indicated the possibility that other aldehydes in the group may be two to three times more irritating than propanal (Salem and Cullumbine 1960; Abdo et al. 1998). Thus, ACs for the 1- and 24-h SMACs based on mucosal irritation were set at 50 ppm (Equation 1).

TABLE 2-1 Physical Properties of C3 to C8 Straight-Chain Aliphatic Aldehydes

Name	Propanal	Butanal	Pentanal	Hexanal	Heptanal	Octanal
$CH_3(CH_2)_nCHO$:	n = 1	n = 2	n = 3	n = 4	n = 5	n = 6
CAS no.:	123-38-6	123-72-8	110-62-3	66-25-1	111-71-7	124-13-0
Molecular weight:	58.1	72.1	86.1	100.2	114.2	128.2
Boiling point (°C):	49	76	103	128	154	171
Melting point (°C):	–81	–99	–92	–56	–45	N/A
Vapor pressure (mmHg):	687	92	50	10	3	N/A
(At °C):	45	20	25	20	25	N/A
Conversion factors[a]						
1 ppm =	2.3 mg/m^3	2.9	3.5	4.1	4.6	5.2
1 mg/m^3 =	0.422 ppm	0.340	0.284	0.245	0.215	0.191

[a] 1 ppm converted to mg/m^3, and 1 mg/m^3 converted to ppm.
Abbreviations: CAS, Chemical Abstracts Service; N/A, not available; ppm, parts per million; mg/m^3, milligrams per cubic meter.

TABLE 2-2 SMACs for C3 to C8 Aliphatic Saturated Aldehydes from James (2000)

Duration	Ppm	mg/m^3	Toxic End Point to Avoid
1 h	50	125-250[a]	Mucosal irritation
24 h	50	125-250	Mucosal irritation
7 d	6	15-30	Liver injury, mucosal irritation
30 d	1.5	4-8	Liver injury
180 d	1.5	4-8	Liver injury

[a]Value depends on molecular weight of the aldehyde.
Source: James 2000, P. 52.

$$134\ ppm_{(LOAEL)} \text{ reduced downward by a factor of 2 to 3}$$
$$1\text{- and 24-h } AC_{(mucosal\ irritation)} = 50\ ppm \quad (1)$$

where LOAEL is lowest-observed-adverse-effect level.

Although short-term ACs were established conservatively to protect against mucosal irritation, some risk of this toxicological end point is allowed. However, for exposure durations exceeding 24 h, mucosal irritation should be precluded. Therefore, the NASA 7-, 30-, and 180-d SMACs based on mucosal irritation were established by dividing the human-derived mildly irritating concentration of 134 ppm for propanal (Sim and Pattle 1957) by 10, yielding an AC of 13 ppm (James 2000) (Equation 2).

$$\text{7-, 30-, and 180-day } AC_{(mucosal\ irritation)} = 134\ ppm_{(LOAEL)} \times 1/10_{(LOAEL\ to\ NOAEL)} = 13\ ppm \quad (2)$$

Protection Against Nasal-Cavity Injury

Long-term studies of isobutanal exposure in rats and mice were used to estimate ACs protective for injury to the nasal cavity (squamous metaplasia and olfactory epithelial degeneration in the nose) (Abdo et al. 1998). In the first study of this report, an isobutanal vapor cumulative exposure time of 390 h (6 h/d, 5 d/wk for up to 13 wk) resulted in a no-observed-adverse-effect level (NOAEL) of 500 ppm in rats and mice. Similarly, 500 ppm was reported as the LOAEL in female rats. In the second study from this report, the cumulative exposure time to isobutanal vapor was 3,120 h (6 h/d, 5 d/wk for 2 y). NASA ACs protective of nasal-cavity injury based on the NOAEL and LOAEL values from Abdo et al. (1998) are shown in Equations 3, 4, 5:

$$\text{7-day } AC_{(nasal\text{-}cavity\ injury)} = 500\ ppm_{(NOAEL)} \times 1/10_{(species\ factor)} = 50\ ppm \quad (3)$$

$$\text{30-day } AC_{(nasal\text{-}cavity\ injury)} = 500\ ppm_{(NOAEL)} \times 1/10_{(species\ factor)} \times 390\ h/720\ h_{(time\ extrapolation)} = 27\ ppm \quad (4)$$

$$\text{180-day AC}_{(\text{nasal-cavity injury})} = 500 \text{ ppm}_{(\text{LOAEL})} \times 1/3_{(\text{LOAEL to NOAEL})} \times 1/10_{(\text{species factor})} \times 3{,}120 \text{ h}/4{,}320 \text{ h}_{(\text{time extrapolation})} = 12 \text{ ppm} \quad (5)$$

Protection Against Liver Injury

ACs protective against possible liver injury from accumulation of organic acids from aldehyde metabolism were conservatively set at 6.4 ppm (7-d AC) and 1.5 ppm (30- and 180-d AC) (James 2000). The choice of liver injury as a presumptive toxicological end point was based on the observation of vacuoles within hepatocytes of rats exposed six times to 1,300 ppm propanal for 6 h each exposure (Gage 1970). To derive the ACs, NASA used, as a point of departure, the 90-ppm exposure level reported by Gage (20 exposures of 6 h each) to yield no observable liver changes (cumulative exposure of 120 h). It was assumed that harmful metabolites would not accumulate in liver cells below a threshold exposure concentration. Extrapolations to adjust for exposure duration based on application of Haber's rule would correct the AC to a level below this threshold concentration. The resulting NASA ACs protective for liver injury are shown in Equations 6 and 7:

$$\text{7-day AC}_{(\text{liver injury})} = 90 \text{ ppm}_{(\text{NOAEL})} \times 1/10_{(\text{species factor})} \times 120 \text{ h}/168 \text{ h}_{(\text{time extrapolation})} = 6.4 \text{ ppm} \quad (6)$$

$$\text{30- and 180-d AC}_{(\text{liver injury})} = 90 \text{ ppm}_{(\text{NOAEL})} \times 1/10_{(\text{species factor})} \times 120 \text{ h}/720 \text{ h}_{(\text{time extrapolation})} = 1.5 \text{ ppm} \quad (7)$$

As reviewed previously, the toxicities of the C3 to C8 aliphatic saturated aldehydes appear to be similar (James 2000). Upon review of the AC established for each toxicological end point, group SMACs were established for toxic effects by selecting the acceptable concentration for the most active compound for that end point. Table 2-3 presents the individual ACs for each toxicological end point of concern.

SUMMARY OF NEW RELEVANT DATA FROM LITERATURE

No toxicity studies (including those examining relevant routes of pulmonary exposure) since 2000 with a bearing on C3 to C8 aliphatic saturated aldehydes SMACs were located during this assessment.

ADDITIONAL CONSIDERATION OF NONTOXIC ODOR THRESHOLD

The group of C3 to C8 aliphatic saturated aldehydes have different odors, described as agreeably fruity to choking and suffocating (Furia and Bellanca 1975; Furia 1980; U.S. Coast Guard 1985; NFPA 1986; NIOSH 1994). The re-

ported odor threshold for one aldehyde in this group, pentanal (the only member of the group for which an odor threshold value is available), is 0.028 ppm (Amoore and Hautala 1983). Table 2-4 summarizes reported odor characteristics for the C3 to C8 aliphatic saturated aldehydes.

Odor thresholds—the lowest concentration of a chemical in the air that people can smell—are imprecise measurements. Humans exhibit a wide sensitivity to odors, which can be further affected by factors such as illness. Because odor threshold detection can vary, the concentrations are often reported as ranges. Odor threshold values are not absolute points but rather an average of the sampled populations' response. In addition, "fruity," "choking," and "suffocating" are descriptions of smells individuals have reported. In the case of many chemicals, continued exposure to a chemical odor can also affect detection of the odor. Olfactory adaptation is a very common phenomenon that results from continued exposure to an odor and is characterized by a reduction or loss in smell sensitivity to a particular chemical (Pryor et al. 1970).

The lowest concentration of a chemical causing acute stinging, burning sensations, or tear generation in the nose and eyes is reported as the irritation threshold value. These values are distinctly different from odor thresholds and usually require higher ambient chemical concentrations to elicit an irritation response compared with detection of odor (Amoore and Hautala 1983). Acetaldehyde, although not included in the group of C3 to C8 aldehydes, exhibits human irritancy similar to that of pentanal and propanal. As with propanal, human subjects exposed for 30 min to 134 ppm acetaldehyde reportedly experienced slight irritation (Sim and Pattle 1957). Acetaldehyde has a pungent suffocating odor with an odor threshold of 0.05 ppm and a threshold limit value of 25 ppm, very similar to values for propanal and pentanal (Amoore and Hautala 1983; EPA 1987; ACGIH 1999). Amoore and Hautala (1983) reported an irritation threshold of 2,200 ppm for the nose and an ocular level of 11,000 ppm for acet-

TABLE 2-3 Acceptable Concentrations for Identified Toxicological End Points, 2000

	Uncertainty Factors				Acceptable Concentration (ppm)				
End Point	NOAEL	Time	Species	Space flight	1 h	24 h	7 d	30 d	180 d
Mucosal Irritation	2-3	1	1	1	50	50	—	—	—
	10	1	1	1	—	—	13	13	13
Nasal-cavity injury	1	HR	10	1	—	—	50	27	—
	3	HR	10	1	—	—	—	—	12
Liver injury	1	$HR_{threshold}$	10	1	—	—	6	1.5	1.5
SMACs[a]					*50*	*50*	*6*	*1.5*	*1.5*

[a]SMACs for each exposure time are selected based on the most conservative AC for each toxicological end point.
Abbreviation: HR, Haber's rule.

TABLE 2-4 Selected Odor Characteristics of C3 to C8 Aliphatic Saturated Aldehydes

Compound	Odor	Odor Threshold	Reference
Propanal	Suffocating, fruity, similar to acetaldehyde, pungent, unpleasant, choking	Not available	Furia and Bellanca 1975; Furia 1980; USCG 1985; NFPA 1986
Butanal	Pungent, aldehyde	Not available	Lewis 1997
Pentanal	Powerful, acrid, pungent, strong	0.028 ppm	Amoore and Hautala 1983; NIOSH 1994
Hexanal	Fruity, strong green grass, sharp aldehyde	Not available	Furia and Bellanca 1975; Furia 1980; Lewis 1997
Heptanal	Fatty pungent, penetrating fruity	Not available	Furia 1980; Budavari 1989
Octanal	Sharp fatty, fruity	Not available	Furia and Bellanca 1975

Abbreviations: NFPA, National Fire Protection Association; NIOSH, National Institute for Occupational Safety and Health; USCG, U.S. Coast Guard.

aldehyde—approximately 44,000 times higher (nose) than the odor threshold value for this compound. Amoore and Hautala (1983) classified both acetaldehyde and pentanal as "Class A" substances, which can serve as bellwether indicators because their odor threshold values are much lower than their threshold limit values.

The low odor threshold of pentanal (and, by inference, the other C3 to C8 aldehydes) could serve as a means to alert spacecraft crew to the presence of a substance at levels far lower than would be expected to cause toxicological effects. Granted, crew could experience smell aversion as a result of exposure to noxious chemical smells. Although such aversion could impede crew performance, it should not be categorized as a toxic effect. Therefore, odor threshold values are not used here as a toxicological end point. The odor threshold for pentanal (0.028 ppm) is several times higher than the lowest SMAC values for the C3 to C8 aldehydes (about 143 times higher). Therefore, it is understood that the SMAC levels, which are designed to protect against adverse health effects, will not necessarily prevent spacecraft crew from experiencing smell aversion due to noxious odors.

REVISION OF EXISTING SMACs AND ESTABLISHMENT OF 1,000-DAY SMAC

After review of the studies considered in setting the original SMACs in 2000, the Committee on Spacecraft Exposure Guidelines decided to revise all the ACs for the C3 to C8 aldehydes.

The uncertainty factor of 2 to 3 originally applied to the acute 1- and 24-h SMAC in 2000 will be revised to a factor of 3. A factor of 3 is considered the most conservative for this group of aldehydes and reflects animal data suggesting that some members of this group of compounds are two to three times more irritating than the base compound—propanal (Salem and Cullumbine 1960; Abdo et al. 1998). The 1- and 24-h ACs remain based on the point of departure of 134 ppm (mildly irritating to mucosal surfaces after 30 min of exposure in humans) (Sim and Pattle 1957). Therefore, the revised 1- and 24-h C3 to C8 aldehyde SMACs based on mucosal irritation are set at 45 ppm (Equation 8).

$$\text{1- and 24-h AC}_{(\text{mucosal irritation})} = 134 \text{ ppm}_{(\text{LOAEL})} \times 1/3_{(\text{safety factor})} = 45 \text{ ppm} \quad (8)$$

The 2006 Committee revisited the original rationale used in setting the 7- through 180-d SMACs in 2000. In 2000, the long-term SMACs were predicated on protecting against liver pathology, which was based on acute (5-d) exposure data from rats (Gage 1970). The study reported by Gage used discontinuous exposures to propanal. The 2000 SMACs then used a factor to correct for continuous versus discontinuous exposure conditions. No additional factors were applied to account for differences in the duration of exposure (5 d to 7, 30, or 180 d) based on the assumption that a threshold dose, below which no liver pathology would occur, had been established. However, upon reevaluation, the acute exposure protocol and the now questioned relationship between cellular vacuoles and liver pathology eliminated use of the NOAEL reported by Gage as a point of departure for the longer-term SMACs. The Committee chose instead to select the study of Abdo et al. (1998). This study design more closely corresponds to exposure durations bounded by the longer-term SMACs (7 through 1,000 d). In addition, the end point (squamous metaplasia of respiratory epithelium) was believed to be more toxicologically appropriate and defensible.

Abdo et al. used exposure of rats and mice to select concentrations of isobutanal vapor for 6 h/d, 5 d/wk, for up to 13 wk or 2 y. The 13-wk study revealed a NOAEL of 500 ppm in both rats and mice, whereas the 2-y study revealed a LOAEL of 500 ppm in female rats. The LOAEL of 500 ppm reported for female rats exposed for 2 y was selected as the point of departure for the 7- through 1,000-day SMACs. A factor of 10 was applied to extrapolate from a LOAEL to a NOAEL. A correction factor of 3 was applied to account for interspecies differences in response. Finally, a factor of 3 was applied, as was the case for the new 1- and 24-h ACs (discussed previously), to reflect animal data suggesting differences in irritating potential for these aldehydes (Salem and Cullumbine 1960; Abdo et al. 1998). The revised 7-, 30-, and 180-d and the new 1,000-d SMACs based on nasal-cavity injury are set at 5 ppm (Equation 9).

$$\text{7- through 1,000-d AC}_{(\text{nasal-cavity injury})} = 500 \text{ ppm}_{(\text{LOAEL})} \times 1/10_{(\text{LOAEL to NOAEL})} \times 1/3_{(\text{species factor})} \times 1/3_{(\text{safety factor})} = 5 \text{ ppm} \quad (9)$$

The 2008 SMACs for C3 to C8 aliphatic saturated aldehydes are presented in Table 2-5.

DIFFERENCES BETWEEN ORIGINAL AND CURRENT APPROACH OF THE NATIONAL RESEARCH COUNCIL COMMITTEE ON TOXICOLOGY

The National Research Council (NRC) Committee on Toxicology, as well as other regulatory bodies, is primarily interested in using benchmark dose modeling (BMD) to interpret toxicological data, as BMD modeling is favored over traditional threshold dose (NOAEL, LOAEL) methods, since it allows for greater use of the available data. The NRC recommends that BMD methods be used when sufficient and appropriate dose-response data are available (NRC 2000). However, the NOAEL/LOAEL-based method is recommended by the NRC in the absence of sufficient data or when special considerations are warranted.

The original SMACs for C3 to C8 straight-chain, aliphatic aldehydes were established based on a LOAEL/NOAEL and uncertainty factor method. BMD methodology was applied to data from the long-term SMAC (7- through 1,000-d) study of Abdo et al. (1998). The BMD analysis is summarized below.

Background for BMD Analysis of Long-Term Exposure Data

Isobutyraldehyde was administered to male and female F344/N rats and to B6C3F$_1$ mice by inhalation (6 h/d, 5 d/wk) for up to 13 wk or 2 y (Abdo et al. 1998). These results were used to the calculate benchmark concentration (BMC) for various toxic effects. Uncertainty factors were applied to the lower 95% confidence limit of the benchmark concentration (BMCL) to arrive at maximum allowable concentrations. These values are compared with the proposed current SMACs.

TABLE 2-5 SMACs for C3 to C8 Aliphatic Saturated Aldehydes, 2008

Duration	ppm	mg/m^3	Toxic End Point to Avoid
1 h	45	113	Mucosal irritation
24 h	45	113	Mucosal irritation
7 d	5	11.8	Nasal-cavity injury
30 d	5	11.8	Nasal-cavity injury
180 d	5	11.8	Nasal-cavity injury
1,000 d	5	11.8	Nasal-cavity injury

Note: A representative average odor threshold concentration for the C3 to C8 aliphatic saturated aldehydes is 0.028 ppm (pentanal) (Amoore and Hautala 1983). Some aldehydes in this group exhibit strong noxious odors detectable by humans at levels well below SMAC levels for these compounds.

13-Week Exposures Reported by Abdo et al. 1998

Ten animals per group were exposed to 0, 500, 1,000, 2,000, 4,000, and 8,000 ppm of isobutyraldehyde. All rats died at 8,000 ppm and three male and six female rats died at 4,000 ppm. All mice died at 8,000 ppm, and all except one mouse, a male, died at 4,000 ppm. Body weight gains were reduced at 4,000 ppm in both sexes of rats and at 1,000 ppm in both sexes of mice. Several end points could not be ascertained at 8,000 ppm. Hence, data from the 8,000-ppm group were not used for estimating low-dose BMCs. Incidence rates for the most sensitive end point for each sex of rats and mice are listed in Table 2-6.

Because the multistage model can describe a wide variety of dose-response shapes, it was used to estimate the dose-response relationships. BMCs and BMCLs associated with an excess risk of 10% (BMC_{10} and $BMCL_{10}$) are listed in Table 2-6. Using the $BMCL_{10}$ as a point of departure for establishing a maximum allowable concentration is generally more conservative (stringent) than using the NOAEL.

Consistent with the calculation of SMACs, an uncertainty factor of 10 was used for interspecies extrapolation; no uncertainty factor was used for intraspecies variability or a potential risk at the point of departure (NOAEL or BMCL). Further, adjustment for the duration of exposure used Haber's rule, which assumes equal toxic effects for equal cumulative exposures. Hence, experimental exposures of 6 h/d for 5 d/wk are assumed to be equivalent to continuous exposures of $(6/24) \times (5 / 7) = 0.18$ times the exposure administered over the $(13 \times 7) = 91$ d.

The most sensitive end point, lowest $BMCL_{10} = 340$ ppm, occurred for serous exudate in male mice (Table 2-6). This results in a 30-d SMAC of

$$\text{30-d SMAC} = 340_{(\text{BMCL10})} \times 1/10_{(\text{interspecies})} \times [6 / 24 \times 5/7]_{(\text{discontin. to contin.})} \times 91/30_{(\text{time extrapolation})} = 18.4 \text{ ppm}$$

and a 180-d SMAC of

$$\text{180-d SMAC} = 340_{(\text{BMCL10})} \times 1/10_{(\text{interspecies})} \times [6/24 \times 5/7]_{(\text{discontin. to contin.})} \times 91/180_{(\text{time extrapolation})} = 3.1 \text{ ppm}$$

These results are complementary to the proposed SMAC of 5 ppm, which is based on a LOAEL/NOAEL method.

Further, average severity scores were examined by using a polynomial model with the dose-response data procedure for continuous data in the EPA Benchmark Dose Software program. The most sensitive end point for severity was for olfactory epithelium degeneration in male rats. The benchmark response corresponding to an average severity grade of 1 (minimal effect) produced a BMCL of 1,110 ppm, which exceeds the minimum BMCL of 340 ppm obtained for the incidences of effects.

TABLE 2-6 Incidence of Effects in the Most Sensitive End Point in Each Sex of Rats and Mice (10 Animals per Dose Group) and Estimates of BMC_{10} and BMCL10 for 13-wk Exposures

	Exposure (ppm)					BMC	BMCL
Species/Sex End Point	0	500	1,000	2,000	4,000	(ppm)	(ppm)
Rat (male) Olfactory epithelium degeneration	0	0	0	10	10	880	680
Rat (female) Suppurative inflammation	2	6	2	0	10	2,030	1,000
Mice (male) Serous exudate	0	2	0	4	10	1,000	340
Mice (female) Serous exudate	0	0	0	3	10	1,480	1,020

Two-Year Exposures Reported by Abdo et al. 1998

Initially, 50 animals per group were exposed to 0, 500, 1,000, and 2,000 ppm of isobutyraldehyde. Incidence rates for the most sensitive end point for each sex of rats and mice are listed in Table 2-7. Because the multistage model can describe a wide variety of dose-response shapes, it was used to estimate the dose-response relationships. BMCs and BMCLs associated with an excess risk of 5% (BMC_5 and $BMCL_5$) are listed in Table 2-7. With 50 animals per group, at least 5 animals (10%) with an effect are required to achieve a statistically significant increase ($P \leq 0.05$) above a background of 0 of 50 animals with the effect. However, using the $BMCL_5$ rather than the $BMCL_{10}$ as a point of departure for establishing a maximum allowable concentration is generally more conservative (stringent) than using the NOAEL.

Consistent with the calculation of SMACs, an uncertainty factor of 10 was used for interspecies extrapolation; no uncertainty factor was used for intraspecies variability or a potential risk at the point of departure (NOAEL or BMCL). Further, adjustment for the duration of exposure used Haber's rule, which assumes equal toxic effects for equal cumulative exposures. Hence, experimental exposures of 6 h/d for 5 d/wk are adjusted by the factor (6/24) × (5/7) for equivalency to continuous exposure.

The most sensitive end point (lowest $BMCL_5$ = 150 ppm) occurred for respiratory epithelium squamous metaplasia in female rats (Table 2-7). Presumably, a 2-y lifetime exposure in rodents would be adequate to provide protection for a 1,000-d exposure to humans. The resulting 1,000-d SMAC is

$$\text{1,000-d SMAC} = 150_{\text{(BMCL5)}} \times 1/10_{\text{(interspecies)}} \times [6/24 \times 5/7]_{\text{(discontin. to contin.)}} = 2.7 \text{ ppm}$$

TABLE 2-7 Incidence of Effects and Estimates of BMC_5 and $BMCL_5$ for 2-y Exposures

	Exposure (ppm)				BMC	BMCL
Species/Sex End Point	0	500	1,000	2,000	(ppm)	(ppm)
Respiratory epithelium squamous metaplasia						
Rat (male)	1/50[a]	1/49	10/49	44/50	590	450
Rat (female)	1/49	11/50	9/49	44/50	270	150
Olfactory epithelium degeneration						
Mice (male)	0/50	0/50	11/50	45/50	580	480
Mice (female)	1/50	1/50	27/50	49/50	440	320

[a]Observed/total.

These results again are indistinguishable from (within a factor of 2) the proposed SMAC of 5 ppm, which is based on a LOAEL/NOAEL method.

Further, average severity scores were examined by using a polynomial model with the dose-response data procedure for continuous data in the EPA Benchmark Dose Software program. The most sensitive end point for severity was for olfactory epithelium degeneration in female mice. The benchmark response corresponding to an average severity grade of 1 (minimal effect) produced a BMCL of 1,420 ppm, which exceeds the minimum BMCL of 150 ppm obtained for the incidences of effects. Therefore, the lower BMCL value (for incidence rather than severity) will be used to develop a proposed SMAC.

Summary of Conclusions from BMD Analyses

Results from the 13-wk exposures to isobutyraldehyde were used to calculate 30- and 180-d SMACs. The most sensitive end point was the incidence of serous exudate in male mice, leading to 30- and 180-d SMACs of 18.4 and 3.1 ppm, respectively. The most sensitive end point from the 2-y exposures was the incidence of respiratory epithelium squamous metaplasia in female rats producing a 1,000-d SMAC of 2.7 ppm. The proposed 7- to 1,000-d SMAC of 5 ppm, derived via application of a LOAEL/NOAEL method should provide protection for the effects observed in the study by Abdo et al. (1998).

COMPARISON WITH OTHER AIR-QUALITY LIMITS

Exposure guidelines for a limited subset of C3 to C8 aliphatic saturated aldehydes exist with various public health and occupational health entities as well as with industry and government advisory bodies. Table 2-8 lists of some of these guidelines and regulatory standards for comparison with the current and proposed NASA SMACs.

TABLE 2-8 Selected Inhalation Exposure Levels for Selected C3 to C8 Aliphatic Saturated Aldehydes

Compound	Organization/ Reference	Exposure Guideline	Exposure Level
Propanal	ACGIH 2004	TLV (8 h TWA)	20 ppm
Pentanal	ACGIH 2005	TLV (8 h TWA)	50 ppm
Pentanal	NIOSH 2005	REL (10 h TWA)	50 ppm
Butanal	AIHA 2003	WEEL (8 h TWA)	25 ppm
C3 to C8 aliphatic aldehydes	NASA[a]	SMAC (1 h and 24 h)	45 ppm

[a]Only 1- and 24-h SMACs are listed here for comparison with similar exposure duration guidelines from other organizations.
Abbreviations: ACGIH, American Conference of Governmental Industrial Hygienists; AIHA, American Industrial Hygiene Association; NIOSH, National Institute for Occupational Safety and Health; REL, recommended exposure limit; TLV, threshold limit value; TWA, time-weighted average; WEEL, workplace environmental exposure level.

The current NASA 1- and 24-h SMACs are very similar to exposure levels from other organizations at comparable exposure durations. Exposure limits and guidelines for pentanal (for which values are available for comparison) have remained stable for several years. No guidelines are available for long-term exposure durations to compare with the 7-, 30-, 180-, and 1,000-d SMACs.

RECOMMENDATIONS FOR ADDITIONAL RESEARCH

Shortcomings in the toxicity database pertaining to C3 to C8 aliphatic saturated aldehydes persist. Lack of data on the effects of acute (humans) and chronic (humans and animals) exposures as well as lack of data elucidating the nonlethal exposure effects to animals confounds attempts to establish exposure guidelines. Recommendations for additional research pertaining to toxicity of this group of aldehydes are unchanged from those proposed by James (2000). Increasing the number of exposure concentrations used as well as expanding the end point measurements examined for all aldehydes in this group would be most beneficial. The long-term exposure guidelines established here are designed to protect against nasal epithelial squamous metaplasia. Long-term pulmonary studies would be beneficial in confirming and extending the work of Gage (1970) and validating the protective assumptions made in establishing our intermediate and long-term SMACs.

REFERENCES

Abdo, K.M., J.K. Haseman, and A. Nyska. 1998. Isobutyraldehyde administered by inhalation (whole body exposure) for up to 13 weeks or 2 years was a respiratory tract

toxicant but was not carcinogenic in F344/N rats and B6C3F$_1$ mice. Toxicol. Sci. 42(2):136-151.

ACGIH (American Conference of Governmental Industrial Hygienists). 1999. TLVs and BEIs. Threshold Limit Values for Chemical Substances and Physical Agents, Biological Exposure Indices. Cincinnati, OH: American Conference of Governmental Industrial Hygienists.

ACGIH (American Conference of Governmental Industrial Hygienists). 2004. TLVs and BEIs: Based on the Documentation of the Threshold Limit Values for Chemical Substances and Physical Agents & Biological Exposure Indices. Cincinnati, OH: American Conference of Governmental Industrial Hygienists.

ACGIH (American Conference of Governmental Industrial Hygienists). 2005. TLVs and BEIs: Based on the Documentation of the Threshold Limit Values for Chemical Substances and Physical Agents & Biological Exposure Indices. Cincinnati, OH: American Conference of Governmental Industrial Hygienists.

AIHA (American Industrial Hygiene Association). 2003. The AIHA Emergency Response Planning Guidelines and Workplace Environmental Exposure Levels Guide Handbook. Fairfax, VA: American Industrial Hygiene Association.

Amoore, J.E., and E. Hautala. 1983. Odor as an aid to chemical safety: Odor thresholds compared with threshold limit values and volatilities for 214 industrial chemicals in air water dilution. J. Appl. Toxicol. 3(6):272-290.

Babiuk, C., W.H. Steinhagen, and C.S. Barrow. 1985. Sensory irritation response to inhaled aldehydes after formaldehyde pretreatment. Toxicol. Appl. Pharmacol. 79(1):143-149.

Budavari, S., ed. 1989. The Merck Index—Encyclopedia of Chemicals, Drugs and Biologicals. Rahway, NJ: Merck and Co., Inc.

EPA (U.S. Environmental Protection Agency). 1987. Health Assessment Document for Acetaldehyde. EPA/600/8-86-015A. Environmental Criteria and Assessment Office, Office of Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC.

Furia, T.E., ed. 1980. CRC Handbook of Food Additives, Vol. 2, 2nd Ed. Boca Raton, FL: CRC Press.

Furia, T.E., and N. Bellanca. 1975. Fenaroli's Handbook of Flavor Ingredients, Vol. 2, 2nd Ed. Cleveland, OH: The Chemical Rubber Co.

Gage, J.C. 1970. The subacute inhalation toxicity of 109 industrial chemicals. Br. J. Ind. Med. 27(1):1-18.

James, T.J. 2000. C3 to C8 aliphatic saturated aldehydes. Pp. 42-59 in Spacecraft Maximum Allowable Concentrations for Selected Airborne Contaminants, Vol. 4. Washington, DC: National Academy Press.

Lewis, R.J., Sr., ed. 1997. Hawley's Condensed Chemical Dictionary, 13th Ed. New York, NY: John Wiley & Sons.

NFPA (National Fire Protection Association). 1986. Fire Protection Guide on Hazardous Materials, 9th Ed. Boston, MA: National Fire Protection Association.

NIOSH (National Institute for Occupational Safety and Health). 1994. NIOSH Pocket Guide to Chemical Hazards. NIOSH Publication No. 94-116. Washington, DC: U.S. Government Printing Office.

NIOSH (National Institute for Occupational Safety and Health). 2005. NIOSH Pocket Guide to Chemical Hazards. DHHS (NIOSH) No. 2005-151. Cincinnati, OH: National Institute for Occupational Safety and Health, Center for Disease Control and Prevention, U.S. Department of Health and Human Services.

NRC (National Research Council). 2000. Methods for Developing Spacecraft Water Exposure Guidelines. Washington, DC: National Academy Press.

Pryor, G.T., G. Steinmetz, and H. Stone. 1970. Changes in absolute detection threshold and in subjective intensity of supra-threshold stimuli during olfactory adaptation and recovery. Percept. Psychophys. 8:331-335.

Salem, H., and H. Cullumbine. 1960. Inhalation toxicities of some aldehydes. Toxicol. Appl. Pharmacol. 2:183-187.

Sim, V.M., and R.E. Pattle. 1957. Effect of possible smog irritants on human subjects. J. Am. Med. Assoc. 165(15):1908-1913.

Steinhagen, W.H., and C.S. Barrow. 1984. Sensory irritation structure-activity study of inhaled aldehydes in B6C3F1 and Swiss-Webster mice. Toxicol. Appl. Pharmacol. 72(3):495-503.

USCG (U.S. Coast Guard). 1985. CHRIS—Hazardous Chemical Data, Vol. II. Washington, DC: U.S. Government Printing Office.

3

Ammonia

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Spacecraft maximum allowable concentrations (SMACs) for ammonia were published in Volume 1 of this series, *Spacecraft Maximum Allowable Concentrations for Selected Airborne Contaminants*, for exposure durations of 1 h, 24 h, 7 d, 30 d, and 180 d (Wong 1994). In anticipation of longer-duration exploration missions, this document establishes a SMAC for ammonia for an extended exposure duration of 1,000 d and revisits the SMACs for 1 h, 24 h, 7 d, 30 d, and 180 d.

OCCURRENCE AND USE

Ammonia vapor (NH_3) is found naturally in air at reported background concentrations of 0.001 to 0.005 ppm (ATSDR 2004), but typical concentrations of ammonia in urban and nonurban areas are on the order of 0.029 and 0.007 part per million (ppm), respectively (Ontario Ministry of the Environment 2001). Values of 5 to 20 ppm have been reported for the odor threshold of ammonia (ATSDR 2006; OSHA 2008). Ammonia, which is highly water soluble, is produced in humans and animals as a by-product of amino acid metabolism. It is required or produced by most living organisms (ATSDR 2004). Humans produce an estimated 17 grams (g) of ammonia per day, of which about 4 g is produced in the gut by intestinal bacteria (ATSDR 2004). Ammonia is produced in the environment by the breakdown of manure and dead plants and animals. It is used in fertilizer; in the manufacture of synthetic fibers, plastics, and explosives; and in household cleaning agents, floor waxes, and smelling salts. Anhydrous ammonia is used in large quantities in the U.S. Space Shuttle and in the International Space Station (ISS) as a refrigerant (several hundred kilograms) in external coolant loops. Ammonia concentrations in the ISS atmosphere have been measured by the Russians and nominal concentrations are reported to range

from 1.0 to 1.5 ppm, but the Russians reported that they had little confidence in the values produced by the "GANK" measurement system they were using. Measurements taken in recent months with a new measurement system (Draeger CMS) have indicated that ammonia concentrations were below the detection limit of 2 ppm for the new system.

SUMMARY OF ORIGINAL APPROACH

The SMACs for exposure durations of 1 h to 180 d were set by King-Lit Wong in 1994 (Table 3-1) and were based on mucosal irritation, which is the most sensitive toxic end point for exposures at low to moderate concentrations of ammonia. To establish the SMACs, Wong used data from subjects who were not inured to ammonia because, in adapted workers, mild inflammation (conjunctival erythema) was reported even in those who did not complain of discomfort at exposures to 20 ppm (Vigliani and Zurlo 1955; Wong 1994). The 1- and 24-h SMACs were set at 30 and 20 ppm, respectively, to permit no more than slight mucosal irritation during emergency situations, whereas the SMACs for 7, 30, and 180 d were set at 10 ppm, the estimated maximum nonirritating concentration.

The 1-h SMAC was based on a report (MacEwen et al. 1970) that exposure of volunteers to 30 ppm of ammonia for 10 min was not perceptible in three of five noninured subjects and was barely perceptible in the other two. Thus, a 1-h exposure to 30 ppm of ammonia is expected to produce no more than mild irritation, which is acceptable for emergency situations.

For the 24-h SMAC, a lower concentration was desired so as to reduce the degree of discomfort that astronauts would have to endure during a longer emergency situation. A concentration of 20 ppm was selected, because it produced only eye and respiratory discomfort in workers (Vigliani and Zurlo 1955; Furguson et al. 1977).

No data were available on the maximum concentration that would be nonirritating for longer-term exposures to ammonia. Therefore, the 7-, 30-, and 180-d SMACS were based on a comparison of dose-response data from occupational and laboratory studies in humans (Vigliani and Zurlo 1955; Verberk 1977; MacEwen et al. 1970). A lowest-observed-adverse-effect level (LOAEL) of 20 ppm was used to set the 24-h SMAC, but, rather than apply the traditional safety factor of 10 for extrapolation from the LOAEL to a no-observed-adverse-effect level (NOAEL), dose-response data from several studies in the literature were used to decrease the safety factor from 10 to 2. Verberk (1977) reported that, for 1-h exposures, a reduction in the ammonia concentration from 120 to 80 ppm decreased the reported degree of eye irritation from "nuisance" to between "just perceptible" and "distinctly perceptible," whereas a reduction from 80 to 50 ppm decreased the irritation ratings from "just perceptible" and "distinctively noticeable" to "just noticeable." MacEwen et al. (1970) reported that, for 10-min ex-

TABLE 3-1 SMACs for Ammonia Vapors, 1994

Duration	ppm	mg/m^3	Target Toxicity
1 h	30	20	Irritation
24 h	20	14	Irritation
7 d	10	7	Irritation
30 d	10	7	Irritation
180 d	10	7	Irritation

Source: Wong 1994

posures, reducing the ammonia concentration from 50 to 30 ppm decreased the reported degree of irritation from "moderate" to "just perceptible." Because of the dose responses in these two studies, Wong concluded that a 50% reduction in the LOAEL of 20 ppm should yield a concentration that does not produce irritation or discomfort. Thus, the 7-, 30-, and 180-d SMACs were set at 10 ppm, assuming that, because of adaptation, a concentration that would be nonirritating for 7 d would remain nonirritating for longer exposures. Conversion factors of 0.69 milligram per cubic meter (mg/m^3) per ppm or 1.44 ppm per mg/m^3 were used.

CHANGES IN FUNDAMENTAL APPROACHES RECOMMENDED BY THE NATIONAL RESEARCH COUNCIL

The original SMACs for ammonia, set in 1994, were calculated using safety factors applied to a LOAEL. More recently, the National Research Council has recommended the use of a benchmark dose (BMD) analysis (preferred) or ten Berge's generalization ($C^N \times T = K$) of Haber's rule when the data permit.

NEW DATA SINCE 1994

A single case report (Brautbar et al. 2003) was found of a patient with long-term repetitive occupational exposure to ammonia at concentrations at or above odor recognition (0.043 to 53 ppm, with a geometric mean of 17 ppm) (Ontario Ministry of the Environment 2001) who developed interstitial lung disease. Little weight can be given to a single case report, because causation cannot reasonably be established. No other reports were found describing interstitial lung disease associated with ammonia exposures in humans or animals.

Swedish researchers (Sundblad et al. 2004) reported the respiratory effects on 12 volunteers of controlled exposures (18 to 20 air changes per h) in a chamber (one to four persons per session) to sham or ammonia vapors at 5 and 25 ppm (randomly) on three occasions, with each 3-h session consisting of a total of 1.5 h of resting plus 1.5 h of exercising (50 watts on a bicycle ergometer), changing activity every 30 min. Exposures were separated by at least 1 wk. Par-

ticipants rated the perceived discomfort on a questionnaire with 10 symptom descriptions, each having a 0- to 100-mm visual analog scale (VAS) (0 = no symptoms, 100 = almost unbearable), immediately before, during (3, 28, 58, 88, 118, 148, and 178 min from the start of exposure), and after (270 min from the start of) the exposure. Bronchial responsiveness to methacholine, lung function, and exhaled nitric oxide (NO) were measured 1 wk before and 7 h after the start of exposures. Nasal lavage was performed and peripheral blood samples were drawn 0.5 h before and 7 h after the start of exposures. All 10 perceived discomfort ratings increased significantly during the exposure to 25 ppm of ammonia compared with the control exposure. However, no differences were observed in lung function or bronchial responsiveness when the exposure to fresh air (sham) was compared with the exposure to 5 and 25 ppm of ammonia. Ammonia exposure did not cause detectable upper-airway inflammation and did not significantly affect the levels of exhaled NO, total cell or interleukin 8 concentration in nasal lavage fluid, or complement factor 3b in plasma. At 5 ppm, the authors reported statistically significant increases in eye discomfort, solvent smell, headache, dizziness, and a feeling of intoxication, but not for the other five subjective measures (nose discomfort, throat or airway discomfort, breathing difficulty, fatigue, and nausea).

No odor adaptation was apparent at 25 ppm, but Sundblad reported a tendency toward adaptation at 5 ppm (Sundblad et al. 2004), a concentration that has been reported as the lower end of the range of odor thresholds for ammonia (ATSDR 2006; OSHA 2008). Examination of the responses for the individuals exposed to 5 ppm of ammonia reveals that, although 5 of 12 subjects reported decreasing irritation over time, 4 of 12 reported relatively constant irritation over the 3-h exposure and 3 of 12 reported increasing irritation that appeared to plateau after 1 to 2 h of exposure. This result suggests that a significant proportion of the population may not show signs of "adapting" to the effects of ammonia before the end of a 3-h exposure to 5 ppm. NASA's experience is that some newly arriving crew members note a "gym locker" smell when first entering the ISS, which might be due to the 1- to 1.5-ppm measured concentrations of ammonia, but is more likely due to some organic amines such as those that have been detected in humidity condensate. No measurements have been made, however, of amine concentrations in the ISS atmosphere. In every case, the U.S. crew members have adapted to the smell, so that they no longer notice it after a short while.

Belgian and French researchers measured histologic changes (by light and scanning electron microscopy) in tissues from the respiratory tracts of pigs (four or five pigs per group) exposed continuously for 6 d to ammonia at 5 (baseline "control"), 25, 50, or 100 ppm (Urbain et al. 1996). Quantitative histologic analysis of the nasal and tracheal mucosa revealed considerable "mucosal injuries" (epithelial hyperplasia and increased numbers of neutrophils in the epithelial layer and in the lamina propria) compared with the 5-ppm "controls." Except for the lamina propria, all these changes were significant at ammonia concentrations as low as 25 ppm in the turbinates but not in the trachea, although func-

tional disturbances of the tracheal smooth muscle contractions were found at concentrations as low as 25 ppm (Urbain et al. 1996). Ammonia induced a dose-related increase in the efficacy (peak contraction strength) but not the potency (dose/response) of carbechol in measurements of contractile strength in vitro of strips of tracheal smooth muscle as indicated by hyperresponsiveness to acetylcholine. Ammonia did not influence the response of isolated strips of tracheal smooth muscle to isoproterenol. A nonsignificant decrease was observed in the area of ciliated surface of the turbinate mucosa with increasing ammonia concentration (Urbain et al. 1996). Because all effects were measured at only one exposure duration (6 d), no conclusions can be drawn from these results about the time course of the observed effects. In addition, this study could not achieve an exposure to 0 ppm of ammonia. The reported average "background" concentration of 5 ppm of ammonia was achieved by washing out the pig manure from the chambers twice a day (99% of total particles were <5 micrometers at 0.40 ± 0.05 mg/m^3 with respirable concentrations of 0.05 ± 0.01 mg/m^3).

The results of the human study of Sundblad et al. and the pig study of Urbain et al. are summarized in Table 3-2.

NEW RISK ASSESSMENT APPROACHES

Neither the data available when the original SMACs were set in 1994 nor the data currently available for ammonia toxicity are amenable to application of the ten Berge approach to data analysis. Use of the ten Berge equation ($C^N \times T = K$) requires chemical-specific information on the relationship between concentration, duration, and effects in order to determine the value of N for a given effect level. Such data are not available for ammonia. In a recent review (Shusterman et al. 2006) of how well the published data on various sensory irritants follow Haber's rule (a specific case of the ten Berge equation in which N = 1), Shusterman et al. noted that usable published studies of ammonia show a stronger effect of concentration than of time on the intensity of sensory irritation. For ammonia, diminution of the time effect (plateauing) was apparent within the first 10 s of exposure. They concluded that "The studies reviewed here for ammonia … suggest that extrapolation of effects utilizing the formulation c × t = k ("Haber's Law") may overestimate risk of sensory irritation (if extrapolating from short to long durations) or underestimate risk (if extrapolating from long to short durations)."

A BMD analysis was performed on the raw data from the Sundblad study (obtained from the first author). The VAS scores at seven exposure durations ranging from 3 to 178 min for each subject for ammonia concentrations of 0, 5, and 25 ppm for each measured effect were entered into the BMDS version 1.4.1 software of the U.S. Environmental Protection Agency (EPA) using the polynomial model for continuous data with nonhomogeneous variance. Results obtained using the linear and power models for continuous data were almost identical to the results obtained from the polynomial model.

TABLE 3-2 Human and Pig Responses to Ammonia Vapors

Reference	Species	Time	[NH_3], ppm	Effects
Sundblad et al. 2004	Human	3 hour exposure, 3 separate occasions	0	None
Sundblad et al. 2004	Human	3 hour exposure, 3 separate occasions	5	Significant odor, very minor ("hardly at all") discomfort
Sundblad et al. 2004	Human	3 hour exposure, 3 separate occasions	25	Significant increase in mild discomfort
Urbain et al. 1996	Pig	24 h/d, 6 d	5[a]	Baseline "control"
Urbain et al. 1996	Pig	24 h/d, 6 d	25	Epithelial hyperplasia and increase in neutrophils in nasal turbinates

[a]An atmospheric concentration of 5 ppm of ammonia was the mean background concentration for the "control" pigs. It was achieved by twice daily washing out the manure from the floor under the grating of the pig exposure chambers. No ammonia vapors were added to achieve this concentration.

Figure 3-1 presents an example of the dose-response curve for eye irritation output by the BMDS computer program. Benchmark response values of 2.26, 5.6, and 2.48 standard deviations for eye irritation, solvent smell, and headache, respectively, were selected to correspond to a score of 6 ("hardly at all") on the VAS used in the Sundblad study. The average VAS score (average for all participants at multiple exposure time points) for each symptom was calculated for each exposure concentration (see Table 3-3). For each ammonia concentration, the VAS scores obtained at 3, 28, 58, 88, 118, 148, and 178 minutes from the beginning of an exposure were averaged for each symptom rated by each participant. The average for all participants of their average score for each symptom was used in calculating BMDs. For the purposes of establishing SMACs, VAS scores of ≤6 are considered to be nonadverse and acceptable effects. The calculated BMD and BMDL (lower confidence limit on BMD) values for the symptoms (eye discomfort, solvent smell, headache) that showed significant increases over background and whose average VAS scores at the highest tested concentration (25ppm) were >6.0 are shown in Table 3-4. The SMACs determined as described in the Rationale section below are listed in Table 3-5

RATIONALE FOR A 1,000-D SMAC AND REVISED 7-, 30-, AND 180-D SMACS

Acceptable concentrations (ACs) were determined following the guidelines of the National Research Council's committee on Spacecraft Exposure Guidelines (NRC 2000).

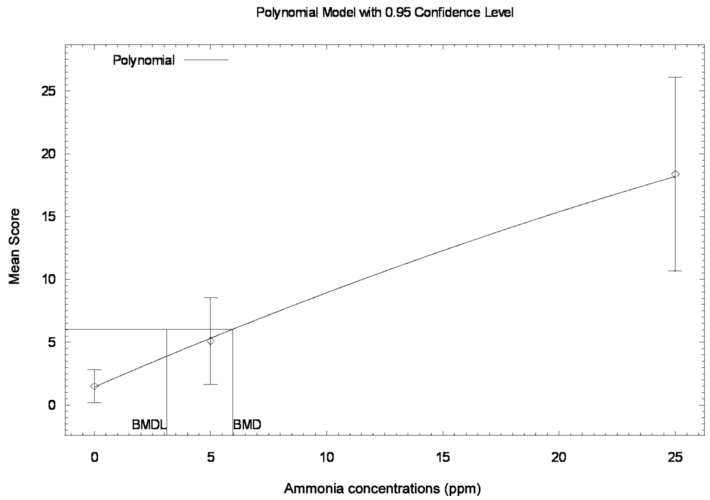

FIGURE 3-1 BMDS graphic representation of results for data on eye irritation. Source: Calculated from raw data provided by B.M. Sundblad.

TABLE 3-3 VAS Severity Score Averages for Exposures of 3 to 178 min

	Average VAS Scores		
Symptom	0 ppm	5 ppm	25 ppm
Eye irritation	1.5	5.1	18
Nose discomfort	4.9	8.6	25
Throat discomfort	5.6	8.1	20
Breathing difficulty	2.5	2.5	14
Solvent smell	0.73	39	66
Headache	1.6	3.2	9
Fatigue	6.6	8	16
Nausea	1.2	1.7	3.7
Dizziness	1	2.4	5.8
Feeling of intoxication	0.86	2.6	5.6

Source: Calculated from raw data provided by B.M. Sundblad.

TABLE 3-4 Results from BMD Analysis of Sundblad et al. 2004 Data

Symptom	BMD, ppm	BMDL, ppm
Eye discomfort: burning, irritated, or running eyes	6	3
Solvent smell	1	0.5
Headache	15	8

Source: Calculated from raw data provided by B.M. Sundblad using the continuous, polynomial model of the BMD software distributed by EPA.

TABLE 3-5 SMACs for Ammonia Vapors, 2008

SMAC Duration	ppm	mg/m^3	Target Toxicity
1 h	30	20	Eye irritation
24 h	20	14	Eye irritation
7 d	3	2	Eye irritation
30 d	3	2	Eye irritation
180 d	3	2	Eye irritation
1,000 d	3	2	Eye irritation

The original SMACs for 1- and 24-h exposure durations will not be changed by the new data. These short-term SMACs are set to allow minor effects to crew members who are working to clean up a release of ammonia.

Two major studies of the toxicity of ammonia vapors have been published since the original SMACs for ammonia were established in 1996. The Sundblad et al. study in human volunteers was well done but was limited to exposures durations of ≤3 h. The study of Urbain et al. in pigs was also well done but was limited to a single exposure duration of 6 d and did not include an exposure to 0 ppm of ammonia. Because human data are preferable to animal data for setting human exposure limits, the data of Sundblad et al. rather than Urbain et al.'s pig data are used to calculate ACs. Although generally test data for a 3-h exposure would not be used to set exposure limits for chronic exposures, in the case of ammonia such an extrapolation is justified because ammonia at low concentrations is not known to produce any adverse effects that increase in severity or are cumulative with prolonged exposures.

Sundblad et al. reported that 5 of the 10 measured effects (see Table 3-6) significantly increased at 5 ppm of ammonia compared with controls: eye discomfort and irritation, solvent smell, headache, dizziness, and a feeling of intoxication. Nevertheless, statistical significance does not imply that low-severity effects should be considered adverse. For the case of "solvent smell", although some subjects in the Sundblad study rated the severity of odor as "quite" even after 278 min of exposure to 5 ppm of ammonia, NASA's experience has shown that, although some ISS crew members have reported an odor like ammonia ("locker room smell") upon first entry, in all cases, they quickly adapted so that they no longer noticed the odor. Note also that the "locker room smell" is presumed to be partly due to ammonia, but other organic amine compounds have been detected (but not quantitated) in ISS air samples that could account for some or all of the reported smell upon first entry into ISS. Nevertheless, based on NASA's experience showing adaptation to low ambient concentrations of ammonia and organic amines, long-term SMACs will not be set to protect against smell.

TABLE 3-6 Time-Averaged Scores for Each Subject and Measured Effect

	Eye Irritation			Solvent Smell			Headache		
	Average Score (3 to 178 min)			Average Score (3 to 178 min)			Average Score (3 to 178 min)		
Subject	0 ppm	5 ppm	25 ppm	0 ppm	5 ppm	25 ppm	0 ppm	5 ppm	25 ppm
2	0	0.9	10.4	0	55	49	0.14	0	3.9
3	1.5	19.6	20.4	1.5	23	71	2.5	2.6	2.4
4	4	7.3	38.4	3.1	4	48	3	4.4	11
5	6.9	7.6	23.1	0	64	76	5.9	4.1	20
6	0.4	5.4	43.7	0.43	67	82	0.29	1.7	8.1
8	1.7	1.9	20.4	1.1	23	79	1.1	0.86	2.3
9	1.4	1.9	5.0	0.57	49	67	3.6	4.6	9.1
10	0	0.0	14.8	0	36	72	0	5.1	20
11	0	1.0	13.1	0	26	51	0.14	0.43	1
12	0.1	3.1	17.3	0	19	79	0	2.1	23
13	2.1	9.1	8.4	2	74	34	2	10	3.4
14	0	3.4	5.6	0	27	82	0.14	2.9	3.9
Average	1.51	5.1	18.38	0.73	38.9	65.83	1.57	3.23	9.008
Standard deviation	2.09	5.44	12.13	1.01	22.3	16.16	1.88	2.72	7.855

Each score in the table is the average of the scores reported by an individual subject at exposure durations of 3, 28, 58, 88, 118, 148, and 178 min. Source: Sundblad et al. 2004.

Although Sundblad et al. reported that the scores at 5 ppm for symptoms eye irritation, solvent smell, headache, dizziness, and feeling of intoxication (refer to Table 3-3) are statistically significant, NASA does not consider the severity scores for dizziness and feeling of intoxication (≤6 or "hardly at all") to be adverse. Because mild, transient effects in astronauts are acceptable for exposure durations of 1 and 24 h, no factor will be applied to protect sensitive individuals when calculating ACs for 1 and 24 h. The chronic (12.2-year, occupational) NOAEL of 9.2 ppm time-weighted average (TWA) reported by Holness et al. (1989) and described below supports the conclusion that chronic exposure to low concentrations of ammonia does not produce cumulative injury to the respiratory tract. Thus, a lack of adverse effects observed during a 3-h exposure should remain a lack of adverse effects for all longer durations for both sensory irritation and injury to the respiratory tract. Because workers have been reported to adapt to both the smell and the eye irritant effects of ammonia vapors in the workplace, the use of a score of "hardly at all" as a nonadverse effect is considered conservative. ACs were calculated for eye irritation and headache by setting them equal to the BMDL values (see Table 3-4) for those symptoms.

Table 3-7 presents exposure limits for ammonia vapors set by other organizations, while Table 3-8 presents ACs and SMACs developed in this document.

OTHER STANDARDS FOR AMMONIA IN AIR

Holness et al. (1989) investigated production workers exposed to ammonia in a soda ash facility. No statistical difference in the prevalence of reporting symptoms (eye, skin, and respiratory symptoms) between exposed and control groups was found for TWA ammonia exposures to 9.2 and 0.3 ppm, respectively, for 8.4 h/d, 5 d/wk (Holness et al. 1989; Ontario Ministry of the Environment 2001), although workers reported that exposure at the plant had aggravated specific problems including coughing, wheezing, nasal complaints, eye irritation, throat discomfort, and skin problems. Based on the lack of subjective symptoms and changes in spirometry, the EPA established a TWA NOAEL of 9.2 ppm (6.4 mg/m^3). An uncertainty factor of 10 was applied to protect sensitive individuals and an additional factor of 3 was applied for deficiencies in the database, including the lack of chronic exposure data, the proximity of the LOAEL to the NOAEL, and the lack of reproductive and developmental studies. RfC values are meant to be protective of the entire population, including the elderly, children, and unusually sensitive individuals.

SPACEFLIGHT EFFECTS

None of the reported adverse effects of ammonia exposures are known to be affected by spaceflight other than transient nausea (spaceflight motion sickness) that lasts no more than 2 to 3 d.

TABLE 3-7 Air Standards for Ammonia Set by Other Organizations

Organization, Standard	Value	Reference
ACGIH		ACGIH 2001
TLV TWA	25 ppm (17 mg/m^3)	ACGIH 2001
STEL	35 ppm (27 mg/m^3)	
OSHA		29 CFR 1910.1000
PEL TWA	50 ppm (35 mg/m^3)	
NIOSH		NIOSH 2005
REL TWA	25 ppm (17 mg/m^3)	NIOSH 2005
REL STEL	35 ppm (27 mg/m^3)	NIOSH 1996
IDLH	300 ppm	
EPA		EPA 1991
RfC[a]	0.144 ppm (0.1 mg/m^3)	EPA 1991
NOAEL TWA	9.2 ppm (6.4 mg/m^3)	EPA 1991
NOAEL ADJ	3.3 ppm (2.3 mg/m^3)	EPA 1991
LOAEL HEC	2.8 ppm (1.9 mg/m^3)	

[a]RfC is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily inhalation exposure of the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. The basis for this calculation is explained in the EPA (1991) reference cited in Table 3-7.

Abbreviations: ACGIH, American Conference of Governmental Industrial Hygienists; ADJ, adjusted; EPA, U.S. Environmental Protection Agency; HEC, human equivalent concentration; IDLH, immediately dangerous to life or health; LOAEL, lowest-observed-adverse-effect-level; NOAEL, no-observed-adverse-effect level; NIOSH, National Institute for Occupational Safety and Health; OSHA, Occupational Safety and Health Administration; PEL, permissible exposure limit; REL, recommended exposure limit; RfC, reference concentration; STEL, short-term exposure limit; TLV, threshold limit value; TWA, time-weighted average.

RECOMMENDATIONS FOR ADDITIONAL RESEARCH

An extension of the study by Sundblad et al. (with a larger number of subjects and for continuous exposure durations of ≥24 h at ammonia concentrations between 5 and 25 ppm) is needed to test the assumption that ammonia concentrations that are NOAELs at short exposure durations remain NOAELs at longer exposure durations. Because mucosal injury has been reported to occur without subjective complaints of irritation, the study should incorporate objective measures of mucosal injury.

TABLE 3-8 ACs for Ammonia

			Uncertainty Factors				Acceptable Concentrations, ppm					
Effect	Exposure Data	Species and Reference	NOAEL	Species	Time	Space flight	1 h	24 h	7 d	30 d	180 d	1,000 d
Eye irritation	30 ppm, 10 min	Human (MacEwen et al. 1970)	LOAEL	1	1	1	30	NC	NC	NC	NC	NC
Eye irritation	20 ppm, occupational	Human (Fergeson et al. 1977) (Vigliani and Zurlo 1955)	LOAEL	1	1	1	NC	20	NC	NC	NC	NC
Eye irritation	20 ppm, occupational	Human (Fergeson et al. 1977) (Vigliani and Zurlo 1955)	2	1	1	1	NC	NC	10	10	10	10
Eye irritation	0, 5, 25 ppm, 3 h	Human (Sundblad et al. 2004)	BMDL	1	1	1	NS[a]	NS[a]	3	3	3	3
Headache	0, 5, 25 ppm, 3 h	Human (Sundblad et al. 2004)	BMDL	1	1	1	NS[a]	NS[a]	8	8	8	8
SMAC							*30*	*20*	*3*	*3*	*3*	*3*

[a]AC values for 1 and 24 h were previously set by King Lit Wong in 1994 and were not changed because these short-term limits, unlike the limits for exposures of >1 d, are set to allow minor adverse effects, thereby permitting the crew to attempt to clean up or contain minor releases if they can do so within about 24 h.

Abbreviations: NC, not calculated; NS, not set in this document.

REFERENCES

ACGIH (American Conference of Governmental Industrial Hygienists). 2001. Ammonia. Documentation of the Threshold Limit Values and Biological Exposure Indices, 7th Ed. American Conference of Governmental Industrial Hygienists, Cincinnati, OH.

ATSDR (Agency for Toxic Substances and Disease Registry). 2004. Toxicological Profile for Ammonia. U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry, Atlanta, GA. September 2004 [online]. Available: http://www.atsdr.cdc.gov/toxprofiles/tp126.html [April 21, 2008].

ATSDR (Agency for Toxic Substances and Disease Registry). 2006. Medical Management Guidelines for Ammonia(NH_3). U.S. Department of Health and Human Services, Agency for Toxic Substances and Disease Registry [online]. Available: http://www.atsdr.cdc.gov/MHMI/mmg126.html [accessed Feb. 22, 2007].

Brautbar, N., M.P. Wu, and E.D. Reichter. 2003. Chronic ammonia inhalation and interstitial pulmonary fibrosis: A case report and review of the literature. Arch. Environ. Health 58(9): 592-596.

EPA (U.S. Environmental Protection Agency). 1991. Ammonia. Integrated Risk Information System, U.S. Environmental Protection Agency [online]. Available: http://www.epa.gov/iris/subst/0422.htm [accessed Apr. 22, 2008].

Furguson, W.S., W.C. Koch, L.B. Webster, and J.R. Gould. 1977. Human physiological response and adaptation to ammonia. J. Occup. Med. 19(5): 319-326.

Holness, D.L., J.T. Purdham, and J.R. Nethercott. 1989. Acute and chronic respiratory effects of occupational exposure to ammonia. Am. Ind. Hyg. Assoc. J. 50(12): 646-650.

MacEwen, J.D., J. Theodore, and E.H. Vernot. 1970. Human exposure to EEL concentrations of monomethylhydrazine. Pp. 355-363 in Proceedings of the 1st Annual Conference Environmental Toxicology, September 9-11, 1970, Wright-Patterson Air Force Base, OH. AMRL-TR-70-102, Paper No 23. Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, OH.

NIOSH (National Institute for Occupational Safety and Health). 2005. NIOSH Pocket Guide to Chemical Hazards. DHHS (NIOSH) Publication No. 2005-149. National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention, U.S. Department of Health and Human Services, Cincinnati, OH [online]. Available: http://www.cdc.gov/niosh/npg/ [accessed Apr. 22, 2008].

NIOSH (National Institute for Occupational Safety and Health). 1996. Ammonia. In Documentation for Immediately Dangerous to Life or Health Concentrations (IDLH): NIOSH Chemical Listing and Documentation of Revised IDLH Values (as of 3/1/95). National Institute for Occupational Safety and Health [online]. Available: http://www.cdc.gov/niosh/idlh/7664417.html [accessed July 11, 2008].

NRC (National Research Council). 2000. Methods for Developing Spacecraft Water Exposure Guidelines. Washington, DC: National Academy Press.

Ontario Ministry of the Environment. 2001. Ontario Air Standards for Ammonia. Standards Development Branch, Ontario Ministry of the Environment. March 2001 [online]. Available: http://www.ene.gov.on.ca/envision/env_reg/er/documents/2001/airstandards/pa00e0003.pdf [accessed Apr. 21, 2008].

OSHA (Occupational Safety and Health Administration). 2008. Ammonia Refrigeration. Safety and Health Topics. U.S. Department of Labor, Occupational Safety and

Health Administration [online]. Available: http://www.osha.gov/SLTC/ammoniarefrigeration/index.html [accessed April 21, 2008].

Shusterman, D., E. Matovinovic, and A. Salmon. 2006. Does Haber's Law apply to human sensory irritation? Inhal. Toxicol. 18(7): 457-471.

Sundblad, B.M., B.M. Larsson, F. Acevedo, L. Ernstgard, G. Johanson, K. Larsson, and L. Palmberg. 2004. Acute respiratory effects of exposure to ammonia on health persons. Scand. J. Work Environ. Health 30(4): 313-321.

Urbain, B., P. Gustin, G. Charlier, F. Coignoul, J.L. Lambotte, G. Grignon, B. Foliguet, B. Videc, D. Beerens, J.F. Prouvost, and M. Ansay. 1996. A morphometric and functional study of the toxicity of atmospheric ammonia in the extrathoracic airways in pigs. Vet. Res. Commun. 20(4): 381-399.

Verberk, M.M. 1977. Effects of ammonia in volunteers. Int. Arch. Occup. Environ. Health 39(2): 73-81.

Vigliani, E.C., and N. Zurlo. 1955. Experiences of occupational clinics with some maximum concentrations of poisons of industry at the place of work [in German]. Arch. Gewerbepathol. Gewerbehyg. 13: 528-534.

Wong, K.L. 1994. Ammonia. Pp. 39-59 in Spacecraft Maximum Allowable Concentrations for Selected Airborne Contaminants, Vol. 1. Washington, DC: National Academy Press.

4

Benzene

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Spacecraft maximum allowable concentrations (SMACs) for benzene were initially published in Volume 2 of *Spacecraft Maximum Allowable Concentrations* for 1-h, 24-h, 7-d, 30-d, and 180-d exposure durations (James and Kaplan 1996). As NASA will be conducting longer exploration missions, longer-duration SMACs are required. This document establishes a benzene SMAC for 1,000-d extended duration exposure. It also demonstrates that a review of published research since the original publication supports the original SMACs for 1-h, 24-h, 7-d, 30-d, and 180-d exposures.

OCCURRENCE AND USE

Benzene is a clear liquid with a sweet odor (see Hazardous Substance Data Bank (HSDB 2005). This aromatic hydrocarbon is used as a solvent; however, this use has decreased in many countries because of concerns about carcinogenicity. Benzene occurs naturally but is primarily produced from petroleum products. It is a constituent of gasoline, in which it is used to enhance octane rating and as an antiknock agent (Krewski et al. 2000). Uses for benzene are numerous including acting as the intermediate in the manufacture of several chemicals, such as ethylbenzene, cumene, cyclohexane, and nitrobenzene. Benzene is a precursor in the manufacture of urethanes, chlorobenzene, and maleic anhydride (HSDB 2005).

Benzene can enter the environment during any of the stages involved in its production, storage, use, and transport (Krewski et al. 2000). Vehicular emissions constitute the main source of benzene in the environment. Benzene has been detected in approximately 10% of recent air samples in the space-shuttle cabin and in Spacelab at concentrations of 0.01 to 0.1 milligrams per cubic meter (mg/m^3) (James and Kaplan 1996).

In September 2006, overheating of the oxygen generator in the Russian segment of the International Space Station resulted in high concentrations of several aromatic compounds. Samples taken several hours after the incident showed a concentration of benzene in the U.S. segment of 0.5 mg/m^3.

TOXIC MECHANISM OF ACTION

Acute benzene toxicity causes gastrointestinal problems and neurotoxicity (HSDB 2005). Chronic benzene toxicity can lead to hematotoxicity. In the body, benzene is metabolized by a hepatic enzyme (CYP2E1) to benzene oxide, which spontaneously forms phenol. Phenol is further metabolized to hydroquinone by the same hepatic enzyme. Hydroquinone and related hydroxy metabolites are converted to benzoquinones by myeloperoxidase in the bone marrow. Benzoquinones are hematotoxic, genotoxic compounds that can be transformed to less toxic hydroxyl metabolites by NAD(P)H: quinone oxidoreductase 1 (Rothman et al. 1997).

SUMMARY OF ORIGINAL APPROACH

James and Kaplan (1996), along with the National Research Council (NRC) Subcommittee on Toxicology, analyzed the acute and chronic toxicity of benzene by assessing available research. They set SMACs based on four categories of benzene toxic effects; nervous system effects, hematologic effects, immunologic effects, and risk of leukemia. The lowest values were selected as the final SMACs, all of which were set to protect the immune system, with the exception of the 180-d SMAC, which was also set to be protective against leukemia. Their analysis of toxic effects followed the guidelines provided to NASA by the NRC Committee on Toxicology, with a few notable exceptions (NRC 1992).

Deviations from defaults require an explanation. Some key deviations from past practices include the following: (1) using a species factor of 3 instead of 10 for effects caused by metabolites of benzene, (2) applying a spaceflight factor to an immunotoxicant because of the immune-modulating effects of spaceflight, (3) applying a radiation uncertainty factor because of benzene's leukemogenic properties and the relatively high radiation exposure of astronauts, and (4) deviating their analysis from the NRC-recommended linearized multistage model because of uncertainty about the human epidemiology database and variations in low-dose extrapolation methods used by investigators (James and Kaplan 1996). Explanations are provided for specific acceptable concentrations (ACs) below.

1- and 24-h SMACs, 1996

The 1- and 24-h SMACs were set at 10 and 3 parts per million (ppm), re-

spectively. These short-term values are meant to be protective against a decrease in the number of peripheral lymphocytes. The Dempster et al. (1984) study was used to calculate this value; it found that five 6-h exposures to benzene at 100 ppm induced a 30% reduction in circulating lymphocytes in mice. No significant change was noted after a single 6-h exposure; therefore, the no-observed-adverse-effect level (NOAEL) was determined to be 100 ppm for 6 h. The short-term ACs were calculated as follows:

$$\text{1-h AC} = 100 \text{ ppm}_{(\text{NOAEL})} \times 1/3_{(\text{species factor})} \times 1/3_{(\text{spaceflight factor})} = 11 \text{ ppm}$$

$$\text{24-h AC} = 100 \text{ ppm}_{(\text{NOAEL})} \times 1/3_{(\text{species factor})} \times 1/3_{(\text{spaceflight factor})} \times 6/24_{(\text{time extrapolation})} = 3 \text{ ppm}$$

James and Kaplan (1996) noted that because immunologic effects, which are similar or greater in mice than in humans, were presumably induced by benzene's toxic metabolites such as phenol, catechol, and hydroquinone, as opposed to benzene itself, the species factor should be 3. A spaceflight factor of 3 was deemed appropriate because of numerous reports on spaceflight effects on immune function in rats and to a lesser extent in astronauts (Taylor 1993).

7-, 30-, and 180-d SMACs, 1996

The 7- and 30-d ACs were set based on the data of Rosenthal and Snyder (1985), which showed that 12, 6-h exposures (72 h total) of mice to benzene at 10 ppm did not increase their susceptibility to infection by *Listeria monocytogenes*. The ACs were calculated as follows:

$$\text{7-d AC} = 10 \text{ ppm}_{(\text{NOAEL})} \times 1/3_{(\text{species factor})} \times 1/3_{(\text{spaceflight factor})} \times 72/168_{(\text{time extrapolation})} = 0.5 \text{ ppm}$$

$$\text{30-d AC} = 10 \text{ ppm}_{(\text{NOAEL})} \times 1/3_{(\text{species factor})} \times 1/3_{(\text{spaceflight factor})} \times 72/720_{(\text{time extrapolation})} = 0.1 \text{ ppm}$$

These values were the lowest of the immunotoxicity ACs calculated by James and Kaplan (1996) and were also the lowest for any toxic effect known to be caused by benzene (see Table 4-1). No long-term exposure data were available on the immunotoxicologic effects of benzene exposure. Haber's rule was used to extrapolate a 180-d AC of 0.07 ppm from the 30-d AC of 0.4 ppm (calculated from Green et al. 1981a, as cited by James and Kaplan 1996), which was set to be protective against leukemia. The 30-d AC used a spaceflight factor of 3 to be protective against radiation effects.

$$\text{180-d AC} = 0.4 \text{ ppm} \times 30/180_{(\text{time extrapolation})} = 0.07 \text{ ppm}$$

TABLE 4-1 Benzene End Points and Acceptable Concentrations, 1996

			Uncertainty Factor			Acceptable Concentration, ppm				
End Point	Exposure Data	Genus and Reference	Time	Species	Space-flight	1 h	24 h	7 d	30 d	180 d
Nervous system toxicity, loss of hind-limb grip strength	NOAEL at 300 ppm, 10 × 6 h	*Mus* (Dempster et al. 1984)	1	10	1	30	30	30	30	30
Hematotoxicity										
Anemia	NOAEL at 300 ppm, 2 × 6 h	*Mus* (Dempster et al. 1984)	1	3	3	33	16	—[a]	—	—
Hemotoxic effects	NOAEL at 10 ppm, 50 × 6h	*Mus* (Green et al. 1981b)	1 or HR	3	3	—	—	1.1	0.5	—
	NOAEL at 10 ppm, 8 wk continuous	*Mus* (Toft et al. 1982)	HR	3	3	—	—	—	—	0.3
Immunotoxicity										
Decrease in peripheral lymphocytes	NOAEL at 100 ppm, 6 h	*Mus* (Dempster et al. 1984)	1 or HR	3	3	11	3	—	—	—
Resistance to bacterial infection and reduced splenic lymphocyte count	NOAEL at 10 ppm, 12 × 6 h	*Mus* (Rosenthal and Snyder 1986)	1 or HR	3	3	—	—	0.5	0.1	—
Decrease in peripheral lymphocytes	NOAEL at 9.6 ppm, 50 × 6 h	*Mus* (Green et al. 1981b)	1 or HR	3	3	—	—	1.1	0.4	0.07
Leukemia	Lowest of 0.01% risk estimates at 0.2 ppm, 180 d continuous	Varieties	—	—	3 rad	—	12	1.7	0.4	0.07
SMAC						*10*	*3*	*0.5*	*0.1*	*0.07*

[a]Extrapolation to these exposure durations produces unacceptable uncertainty in the values.
Abbreviation: HR, Haber's rule; rad, radiation.
Source: James and Kaplan 1996.

NEW DATA SINCE 1996

Short-Term AC Data

Acute exposure to benzene results in central nervous system (CNS) depression such as dizziness, ataxia, and confusion. These effects are believed to be caused by benzene and not its metabolites, because the onset of CNS effects at extremely high doses is too rapid for metabolism to have occurred. Fatality due to acute benzene exposure has been attributed to asphyxiation, respiratory arrest, CNS depression, or cardiac dysrhythmia. Pathologic results in fatal cases have noted respiratory tract inflammation, lung hemorrhage, kidney congestion, and cerebral edema (ATSDR 1992). There were no new data supporting a change in short-term (1 or 24 h) benzene ACs.

Long-Term AC Data

A review of the long-term exposure data since the original SMAC publication primarily focused on identifying biomarkers of benzene exposure in urine. No data were identified that would support changing the 7-, 30-, or 180-d benzene ACs. There are extensive case study data on long-term occupational (5 years) exposure to benzene. Yin et al. (1987) investigated workers in 28 provinces of China between 1979 and 1981. This group of industrial workers (painting, paint production, shoe manufacturing, organic synthesis, insulation varnish, printing, rubber and petroleum refineries) was exposed to benzene or benzene mixtures. Yin et al. (1987) concluded that the prevalence of aplastic anemia in these individuals was 5.8 times the rate in the general population. The same group of investigators claimed that chronic occupational exposure to low levels (<1 ppm) of benzene caused increased risk of hematotoxicity (Lan et al. 2004, Vermeulen et al. 2004). This group previously reported (Qu et al. 2002) red blood cell, white blood cell (WBC), and neutrophil changes in the lowest benzene exposure group (at or below 0.25 ppm).

In the studies of Qu et al. (2002), Vermeulen et al. (2004), and Lan et al. (2004), 250 exposed workers from the shoe industry along with 140 age- and sex-matched controls from the clothing industry near Tianjin, China, were compared for exposure and toxic effects from benzene. The workforce used had at least 5 years of exposure, with little or no shoe-making task rotation. The workers were classified on the basis of their tasks and exposure to glue containing benzene and toluene (dominant exposures) along with exposures to 18 other hydrocarbons. Expected benzene exposure concentrations were distinguished on the basis of the work task. Individual benzene and toluene exposure was monitored repeatedly with organic vapor monitors, which were attached to the worker's lapel for the full shift (total of 2,783 measurements); home (personal) exposure measurements were taken on up to three different occasions (a total of 595 measurements).

The Vermeulen et al. (2004) study focused on determining the broad range of benzene exposures in the two shoe manufacturing facilities. Although the work exposures were to several compounds, the authors did a good job of estimating the predicted benzene exposure by calculating the personal exposures from the benzene monitors by a gas chromatography flame ionization detector and showing a high correlation between the logged air levels of benzene and the monitors analyzed in China and at a commercial laboratory in the United States. The workers wore personal benzene monitors for 16 months.

The same group provided postshift urine samples for the Lan et al. (2004) study. These samples were collected from each subject at the end of the 16-month monitoring study (Vermeulen et al. 2004). Subjects in the Lan et al. (2004) research study were categorized into four groups by mean level of benzene during the month before phlebotomy (controls, <1 ppm, 1 to <10 ppm, and ≥10 ppm). More than 100 of the exposed workers had exposures below 1 ppm. In the <1 ppm, 1 to <10 ppm, and ≥10 ppm exposure groups, WBC counts decreased. The authors contend that these data show evidence of hematotoxic effects at <1 ppm, but the WBC, granulocyte, lymphocyte, CD4 and CD8 T-cell, B-cell, NK-cell, monocyte, and platelet counts for the <1-ppm and 1 to <10-ppm groups are still in a range that would be considered normal. NASA contends that the 1-ppm threshold appears to be at worst a marginal lowest-observed-adverse-effect level (LOAEL) for hematotoxicity (Lan et al. 2004). The amount of hemoglobin (grams/deciliter) remained unchanged in these two exposure groups as well.

This observation is bolstered by Lamm and Grunwald (2006), who published a response to the Lan et. al (2004) study in which they agreed with hematotoxicity data at >10 ppm, but their data do not show consistent evidence of hematotoxicity at lower concentrations. Lamm and Grunwald (2006) specifically stated that the Lan et al. (2004) study showed a monotonically increasing effect only for platelets and B cells but not for the measured cell lines that might be expected to lead to myeloid leukemic lines; WBC counts and granulocyte counts that showed a reduction in cell number at <1 ppm did not show a further reduction among workers with exposures up to 10 ppm. Lamm and Grunwald (2006) presented a figure adapted from data Lan et al. (2004) supplied to them. The data were requested because Lamm and Grunwald (2006) noted that the Lan et al. (2004) article did not separate progenitor cell colony data below 10 ppm and could not demonstrate an effect below 1 ppm. Lamm and Grunwald stated that their figure shows a monotonically increasing trend only for granulocyte-macrophage colony formation, which appears at >1 ppm in the absence of erythropoietin and at <1 ppm in the presence of erythropoietin, and that neither reduction is statistically significant until the >10-ppm exposure group is considered, concluding that the Lan et al. (2004) data do not support hematotoxicity at concentrations below 10 ppm.

In response to Lamm and Grunwald (2006), Lan et al. (2006) remarked that a spline regression analysis of benzene exposure and WBC counts, which used the total study population from Lan et al. (2004), demonstrates an inverse

relationship with a slope that was significantly less than zero for every point between 0.2 and 15 ppm and finds no apparent threshold within this occupational range. Lan et al. (2006) showed a figure of this spline analysis, which displays solely the regression line and confidence limits without the actual data points. The figure presented by Lan et al. (2006) does not provide enough data to identify potential thresholds or breakpoints in the relationship.

Also in response to the assertions of Lamm and Grunwald (2006), Kim et al. (2006) published metabolite production data (also from the Qu, Lan, and Vermeulen China study) in which 13 groups of 30 workers were distinguished by their benzene exposures (median concentrations about 1.2 ppm). Kim et al. (2006) found that the urine concentration of each measured metabolite (phenol, *E*,*E*-muconic acid, hydroquinone, and catechol as well as the minor metabolite *S*-phenylmercapturic acid) was elevated at or above air concentrations of 0.2 ppm for *E*,*E*-muconic acid and *S*-phenylmercapturic acid, 0.5 ppm for phenol and hydroquinone, and 2 ppm for catechol. They concluded that at benzene concentrations less than 1 ppm, metabolism favors production of the toxic metabolites hydroquinone and *E*,*E*-muconic acid.

A study by Shen et al. (2006) reported an association between total counts of WBCs, granulocytes, lymphocytes, B cells, and platelets (hematotoxicity) and the cohort's benzene exposure that occurred in the preceding month (mean about 5 ppm). These investigators performed a follow-up study on the cohort used by Lan et al. (2004). Their study population included the same 250 workers who were exposed to benzene in two shoe manufacturing factories and 140 unexposed controls from comparable populations who worked in three Chinese clothing manufacturing factories. The exposed group had a mean benzene air concentration of 5 ppm in the month before phlebotomy. Total WBC counts were lower in the exposed group than in the unexposed controls. An association was reported between total counts of WBCs, granulocytes, lymphocytes, B cells, and platelets (hematotoxicity) and the cohort's benzene exposure that occurred in the preceding month. Four single nucleotide polymorphisms were associated with decreased WBCs in the benzene-exposed workers. The NRC Committee on Emergency and Continuous Exposure Guidance Levels for Selected Submarine Contaminants (NRC 2008) recently selected the point of departure from the Shen et al. (2006) study for derivation of the 90-d continuous exposure guidance level—specifically, 0.2 ppm. The mean value of about 5 ppm reported by Shen et al. (2006) was used as a LOAEL for hematologic effects relevant for submariners.

The original 180-d SMAC was set based on the following logic (James and Kaplan 1996). A survey of nine estimates of the leukemogenic potency of benzene in humans was summarized in a single table with calculations for 6 months of continuous exposure (180 d). The predictions ranged from 0.2 to 2.2 ppm for the various risk estimate methods. The lowest estimate was based on the work of Infante and White (1985) from an epidemiologic study of benzene-exposed workers. We selected this lowest value as our point of departure. We noted that radiation exposure in space is typically elevated, so we placed a factor

of 3 on this to compensate for the likelihood that the radiation and benzene are known leukemogens. Thus, the 180-d SMAC was determined to be 0.2/3 = 0.07 ppm.

PROPOSED 1,000-d AC

NASA will base the 1,000-d AC calculations on the Shen et al. (2006) study of WBC reductions and polymorphisms in the same occupational group studied by Lan et al (2004). Shen et al. (2006) showed a LOAEL of 5 ppm for hematologic effects. Therefore, NASA will calculate the 1,000-d AC using 5 ppm as a LOAEL, with the following adjustment factors: 10 for LOAEL to NOAEL, 3 for variability among humans as reported by Shen et al. (2006), and 3 for spaceflight risk for anemia. These adjustments result in a 1,000-d AC of 0.06 ppm.

$$\text{1,000-d AC} = 5\ \text{ppm}_{\text{(LOAEL)}} \times 1/10_{\text{(LOAEL to NOAEL)}} \times 1/3_{\text{(interindividual variability)}} \times 1/3_{\text{(spaceflight anemia)}} = 0.06\ \text{ppm}$$

NASA will base the 1,000-d SMAC on an extrapolation of the 180-d SMAC set to protect against the risk of leukemia. The 180-d AC will be time adjusted to 1,000 d, resulting in an AC of 0.013 ppm.

$$\text{1,000-d SMAC} = 0.07\ \text{ppm}_{\text{(180-d SMAC)}} \times 180\text{d}/1{,}000\ \text{d}_{\text{(time adjustment)}} = 0.013\ \text{ppm}\ (0.04\ \text{mg/m}^3)$$

CONCLUSIONS

The NASA AC is conservative compared with the benzene exposure recommendations set by other organizations (see Table 4-2). We are confident, after a review of the literature since our original AC publication, that NASA's SMACs are fully protective of astronaut crews during missions of short- and long-term duration.

TABLE 4-2 Exposure Limits Set by Other Organizations

Organization, Standard	Recommended Exposure Level, ppm[a]	Reference
OSHA		NIOSH 2005
PEL, 8-h TWA	1	
ATSDR		ATSDR 1992
PEL, STEL	5	
Action level TWA	0.5	

(Continued)

TABLE 4-2 Continued

Organization, Standard	Recommended Exposure Level, ppm[a]	Reference
ACGIH		ATSDR 1992
TWA, 8 h	0.5	
STEL, 15-min ceiling	2.5	
NIOSH		NIOSH 2005
10-h TWA (advisory)	0.1	
STEL, 15-min ceiling	1	
IDLH	500	
NRC		NRC 2008
EEGL, 1 h	40	
EEGL, 24 h	3	
CEGL, 90 d	0.2	
NASA		
SMAC, 1 h	10	James and Kaplan 1996
SMAC, 24 h	3	
SMAC, 7 d	0.5	
SMAC, 30 d	0.1	
SMAC, 180 d	0.07	
SMAC, 1,000 d (proposed)	0.013	

[a]Conversion factors at 25°C, 1 atm: 1 ppm = 3.26 mg/m^3; 1 mg/m^3 = 0.31 ppm.

Abbreviations: ACGIH, American Conference of Governmental Industrial Hygienists; ATSDR, Agency for Toxic Substances and Disease Registry; CEGL, continuous exposure guidance level; EEGL, emergency exposure guidance level; IDLH, immediately dangerous to life and health; NASA, National Aeronautics and Space Administration; NIOSH, National Institute for Occupational Safety and Health; NRC, National Research Council; OSHA, Occupational Safety and Health Administration; PEL, permissible exposure limit; STEL, short-term exposure limit; TWA, time-weighted average; SMAC, Spacecraft Maximum Allowable Concentration.

REFERENCES

ATSDR (Agency for Toxic Substances and Disease Registry). 1992. Benzene Toxicity Standards and Regulations. Case Studies in Environmental Medicine (CSEM). Course: SS3039. U.S. Department of Health and Human Services, Agency for Toxic Substances and Disease Registry [online]. Available: http://www.atsdr.cdc.gov/HEC/CSEM/benzene/standards_regulations.html [accessed April 11, 2008].

Dempster, A.M., H.L. Evans, and C.A. Snyder. 1984. The temporal relationship between behavioral and hematological effects of inhaled benzene. Toxicol. Appl. Pharmacol. 76(1):195-203 (as cited in James and Kaplan 1996).

Green, J.D., C.A. Snyder, J. LoBue, B.D. Goldstein, and R.E. Albert. 1981a. Acute and chronic dose/response effects of inhaled benzene on multipotential hematopoietic stem (CFU-S) and granulocyte/macrophage progenitor (GM-CFU-C) cells in CD-1 mice. Toxicol. Appl. Pharmacol. 58(3):492-503 (as cited in James and Kaplan 1996).

Green, J.D., C.A. Snyder, J. LoBue, B.D. Goldstein, and R.E. Albert. 1981b. Acute and chronic dose/response effect of benzene inhalation on the peripheral blood, bone

marrow, and spleen cells of CD-1 male mice. Toxicol. Appl. Pharmacol. 59(2):204-214 (as cited in James and Kaplan 1996).

HSDB (Hazardous Substance Data Bank). 2005. Benzene (CASRN: 71-43-2). TOXNET, Specialized Information Services, U.S. National Library of Medicine, Bethesda, MD [online]. Available: http://toxnet.nlm.nih.gov/cgi-bin/sis/search [accessed Nov. 2005].

Infante, P.F., and M.C. White. 1985. Projections of leukemia risk associated with occupational exposure to benzene. Am. J. Ind. Med. 7(5-6):403-413.

James, J.T., and H.L. Kaplan. 1996. Benzene. Pp. 39-103 in Spacecraft Maximum Allowable Concentrations for Selected Airborne Contaminants, Vol. 2. Washington, DC: National Academy Press.

Kim, S., R. Vermeulen, S. Waidyanatha, B.A. Johnson, Q. Lan, N. Rothman, M.T. Smith, L. Zhang, G. Li, M. Shen, S. Yin, and S.M. Rappaport. 2006. Using urinary biomarkers to elucidate dose-related patterns of human benzene metabolism. Carcinogenesis 27(4):772-781.

Krewski, D., R. Snyder, P. Beatty, G. Granville, B. Meek, and B. Sonawane. 2000. Assessing the health risks of benzene: A report on the benzene state-of-the-science workshop. J. Toxicol. Environ. Health A. 61(5-6):307-338.

Lamm, S.H., and H.W. Grunwald. 2006. Benzene exposure and hematotoxicity. Science 312(5776):998.

Lan, Q., L. Zhang, G. Li, R. Vermeulen, R.S. Weinberg, M. Dosemeci, S.M. Rappaport, M. Shen, B.P. Alter, Y. Wu, W. Kopp, S. Waidyanatha, C. Rabkin, W. Guo, S. Chanock, R.B. Hayes, M. Linet, S. Kim, S. Yin, N. Rothman, and M.T. Smith. 2004. Hematotoxicity in workers exposed to low levels of benzene. Science 306(5702):1774-1776.

Lan, Q., R.S. Vermeulen, L. Zhang, G. Li, P.S. Rosenberg, B.P. Alter, M. Shen, S.M. Rappaport, R.S. Weinberg, S. Chanock, S. Waidyanatha, C. Rabkin, R.B. Hayes, M. Linet, S. Kim, S. Yin, N. Rothman, and M.T. Smith. 2006. Response to benzene exposure and hematotoxicity. Science 312(5776):998-999.

NIOSH (National Institute for Occupational Safety and Health). 2005. NIOSH Pocket Guide to Chemical Hazards. DHHS (NIOSH) Publication No. 2005-149. National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention, U.S. Department of Health and Human Services, Cincinnati, OH [online]. Available: http://www.cdc.gov/niosh/npg/ [accessed April 11, 2008].

NRC (National Research Council). 1992. Guidelines for Developing Spacecraft Maximum Allowable Concentrations for Space Station Contaminants. Washington, DC: National Academy Press.

NRC (National Research Council), 2008. Emergency and Continuous Exposure Guidance Levels for Selected Submarine Contaminants, Vol. 2. Washington, DC: The National Academies Press.

Qu, Q., R. Shore, G. Li, X. Jin, L.C. Chen, B. Cohen, A.A. Melikian, D. Eastmond, S.M. Rappapport, S. Yin, H. Li, S. Waidyanatha, Y. Li, R. Mu, X. Zhang, and K. Li. 2002. Hematological changes among Chinese workers with a broad range of benzene exposures. Am. J. Ind. Med. 42(4):275-285.

Rosenthal, G.J., and C.A. Snyder. 1985. Modulation of the immune response to Listeria monocytogenes by benzene inhalation. Toxicol. Appl. Pharmacol. 80(3):502-510.

Rosenthal, G.J., and C.A. Snyder. 1986. Altered T-cell responses in C57B1 mice following sub-chronic benzene inhalation. Toxicologist 61:68 (as cited in James and Kaplan 1996).

Rothman, N., M.T. Smith, R.B. Hayes, R.D. Traver, B. Hoener, S. Camplemen, G.L. Li, M. Dosemeci, M. Linet, L. Zhang, L. Xi, S. Wacholder, W. Lu, K.B. Meyer, N. Titenko-Holland, J.T. Stewart, S. Yin, and D. Ross. 1997. Benzene poisoning, a risk factor for hematological malignancy, is associated with the NQO1 $^{609}C \rightarrow T$ mutation and rapid fractional excretion of chlorzoxazone. Cancer Res. 57(14):2839-2842.

Shen, M., Q. Lan, L. Zhang, S. Chanock, G. Li, R. Vermeulen, S.M. Rappaport, W. Guo, R.B. Hayes, M. Linet, S. Yin, M. Yeager, R. Welch, M.S. Forrest, N. Rothman, and M.T. Smith. 2006. Polymorphisms in genes involved in DNA double-strand break repair pathway and susceptibility to benzene-induced hematotoxicity. Carcinogenesis 27(10):2083-2089.

Taylor, G.R. 1993. Immune changes during short-duration missions. J. Leukoc. Biol. 54(3):202-208 (as cited in James and Kaplan 1996).

Toft, K., T. Olofsson, A. Tunek, and M. Berlin. 1982. Toxic effects on mouse marrow caused by inhalation of benzene. Arch. Toxicol. 51:295-302 (as cited in James and Kaplan 1996).

Vermeulen, R., G. Li, Q. Lan, M. Dosemeci, S.M. Rappaport, X. Bohong, M.T. Smith, L. Zhang, R.B. Hayes, M. Linet, R. Mu, L. Wang, J. Xu, S. Yin, and N. Rothman. 2004. Detailed exposure assessment for a molecular epidemiology study of benzene in two shoe factories in China. Ann. Occup. Hyg. 48(2):105-116.

Yin, S.N., Q. Li, Y. Liu, F. Tian, C. Du, and C. Jin. 1987. Occupational exposure to benzene in China. Br. J. Ind. Med. 44(3):192-195.

5

n-Butanol

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BACKGROUND AND SUMMARY OF ORIGINAL APPROACH

The alcohol *n*-butanol is consistently found in International Space Station (ISS) atmospheric samples at concentrations below 0.1 part per million (ppm). The source is presumed to be off-gassing from materials present inside the vehicle. Since the original document on spacecraft maximum allowable concentrations (SMACs) was written (James 1996), one remarkable event involving *n*-butanol pollution of the ISS has occurred. It involved the thermal desorption of charcoal filters that accidentally had been positioned to adsorb atmospheric contaminants over a period of 6 months. The effluent compounds from that desorption included copious amounts of *n*-butanol, which produced concentrations several-fold above odor thresholds for that compound, and caused an emergency situation in the U.S. segment of the station. The crew took refuge for 30 h in the Russian segment of the ISS while the *n*-butanol and other pollutants were scrubbed from the atmosphere of the U.S. segment (James et al. 2003).

NASA set the original SMACs for *n*-butanol in the mid-1990s based primarily on its irritant properties (James 1996). The intensity and effect levels for irritation were gleaned from three human studies published in the 1940s. From these studies, the threshold for eye irritation was estimated to be between 50 and 100 ppm; thus, for a 1-h exposure, a concentration of 50 ppm was deemed acceptable. Given some evidence of increasing irritation sensitivity during the work week, the 24-h acceptable concentration (AC) was set at 25 ppm. Because adaptation to the presence of *n*-butanol would be expected, the long-term AC was set at 25 ppm (80 milligrams per cubic meter [mg/m^3]) to protect against eye irritation.

The potential central nervous system (CNS) effects of *n*-butanol were considered in two ways. First, its potency in inducing CNS deficits has been noted to be 5-10 times greater than that of ethanol. Performance decrements have not

been reported for ethanol concentrations below 50 mg per liter (L) of blood; thus, blood concentrations of *n*-butanol below 5 mg/L would be acceptable. A stable blood concentration of *n*-butanol is attained after 30 min of inhalation at 100 ppm. That blood concentration is reported to be 3 mg/L (Astrand et al. 1976); therefore, an exposure to 100 ppm of *n*-butanol would not be expected to induce any CNS effects. This is consistent with a second approach that depends on epidemiologic evidence that CNS effects do not occur unless concentrations are well above 100 ppm (Tabershaw et al. 1944).

Long-term inhalation exposures were not found, so we relied on a 90-d oral study in rats in which there were no pathology findings at a dose of 500 mg per kilogram of body weight per day (mg/kg/d) (TRL 1986). Using body surface modeling, a 40% uptake in the respiratory system, and a nominal human inhalation rate, this equates to humans breathing a concentration of 250 ppm. Applying a 10-fold species factor gave an AC of 25 ppm for avoiding systemic effects (80 mg/m^3) for exposures up to 90 d. The 180-d AC for such effects was set at half this value (90 d/180 d) to give 12 ppm as the 180-d AC. This was the lowest of the ACs, so the 180-d SMAC was set at 12 ppm or 40 mg/m^3. The 7- and 30-d SMACs were set at 25 ppm. The SMACs are presented in Table 5-1.

In summary, the original approach relied on irritation reports in humans, comparison of the relative CNS-depression potency and blood concentrations of ethanol and *n*-butanol, and rather weak evidence that long-term ingestion of *n*-butanol does not cause observable pathology in rats.

CHANGES IN FUNDAMENTAL APPROACHES RECOMMENDED BY THE NATIONAL RESEARCH COUNCIL

The toxicity database on *n*-butanol was, and still is, sparse and not suitable for any of the approaches sanctioned by the National Research Council such as benchmark dose analyses or the "ten Berge" approach for time-dose extrapolations. For example, the human exposure data on irritancy come from three human studies published in the 1940s, and they give only a general idea of the exposure level at which most people would cease to experience irritation. As far as CNS effects are concerned, the data consist of blood concentrations of *n*-butanol that are deduced to be below the threshold for CNS effects by analogy with ethanol blood concentrations. Fortunately, these are reasonably consistent with an epidemiologic report that CNS effects are not observed at exposures below 100 ppm. For ototoxicity (a new end point), the data on rats show no effect at any exposure concentration (Crofton et al. 1994). Comparative data on *n*-butyl acetate (*n*-BA) are used to discount the relevance of putative hematologic and immunotoxicity effects of *n*-butanol. There are simply no data left on which to apply approaches that require discrete, quantitative end points.

NASA has considered whether genetic differences in alcohol dehydrogenase (ADH) could affect the ability of certain individuals to deal with exposures to *n*-butanol. Because of the multiplicity of human ADH isoforms, which

have the capacity to metabolize small-chain alcohols such as ethanol and *n*-butanol (Ehrig et al. 1988), and because cytochrome P450 enzymes can also catalyze ethanol and *n*-butanol oxidation, interindividual differences in clearance of these alcohols is relatively invariant. Variations among ethnic populations are not large, as shown in a study of ethanol metabolism in African-Americans with genetic polymorphisms at the *ADH2* locus (Thomasson et al. 1995). By analogy, this extends to metabolism of *n*-butanol; thus, we have not used a factor for interindividual variability in setting new SMACs for *n*-butanol.

RELEVANT DATA SINCE 1993

Data from Samples of ISS Air

The ISS has been operating for several years since the original SMACs for *n*-butanol were set. During this time, the nominal range of *n*-butanol concentrations has been from 0.02 to 0.08 ppm (0.05 to 0.25 mg/m^3), with an occasional excursion to 0.3 ppm. After the attempted regeneration of the Metox canisters (used to purify air during extravehicular activity) on February 20, 2002, the concentration of *n*-butanol reached 2.5 ppm (7.5 mg/m^3), and the crew reported a noxious smell (other pollutants undoubtedly contributed to the odor). The average odor threshold for this compound is reported to be 0.8 ppm (2.5 mg/m^3) (Amoore and Hautala 1983); thus, this compound probably contributed to the smell the crew reported. Within 33 h, the trace contaminant control system of the U.S. laboratory module had reduced the concentration of *n*-butanol to 0.1 ppm, and the crew reported only a very faint odor when they reentered. Because this "noxious odor" event forced the crew to take refuge in the Russian segment of the ISS for 30 h, it suggests that we must rethink the importance of odor for space operations.

TABLE 5-1 SMACs Set in Volume 3 for *n*-Butanol

	Safe Concentration		
Exposure Time	mg/m^3	ppm	Effect to Avoid
1 h	150	50	More than mild eye irritation
24 h	80	25	More than mild eye irritation
7 d	80	25	Eye irritation, systemic injury
30 d	80	25	Eye irritation, systemic injury
180 d	40	12	Systemic injury, eye irritation

Source: James 1996.

New Toxicity Data

New toxicologic data relevant to *n*-butanol inhalation risk assessment consist primarily of physiologically based pharmacokinetic studies of *n*-butanol and *n*-BA (Barton et al. 2000, Teeguarden et al. 2005), a subchronic inhalation study of *n*-butanol (Korsak et al. 1994), an evaluation of the ototoxicity of several solvents in rats (Crofton et al. 1994), and a subchronic inhalation study looking at the general toxicity (David et al. 2001) and neurotoxicity (David et al. 1998) of *n*-BA. The first of these studies allows us to link *n*-butanol and *n*-BA exposures and also to link human and rat exposures to *n*-butanol. The *n*-BA study will allow us to discount the apparent hematologic and immune effects suggested by the Korsak et al. (1994) report by comparing its findings with those reported in other studies. Finally, the ototoxicity study will enable us to evaluate old epidemiologic reports that *n*-butanol could cause ototoxicity.

Putative Hematology and Immune Effects

Korsak et al. (1994) exposed groups of 12 rats for 6 h/d, 5 d/wk to 50 or 100 ppm of *n*-butanol for 3 months. They evaluated some biochemical parameters, body and organ weights, rotorod performance (a measure of neuromuscular performance), and hematology. Their findings revealed primarily an effect on blood cells in samples from the tail vein as shown in Table 5-2. These inhalation results are compared with results from a gavage study of equal length done earlier in another laboratory (TRL 1986). However, a direct comparison is confounded by the bolus nature of the gavage dose and the first-pass effects on the gavaged alcohol. Nonetheless, the findings appear to be difficult to reconcile, so given these data alone, we would be compelled to begin with the more relevant data from Korsak et al. (1994).

Hematology data from David et al. (2001) provide compelling evidence that the apparent decrease in hematologic parameters reported by Korsak et al. (1994) are not real. The former investigators exposed male and female Sprague Dawley (SD) rats to *n*-BA at 0, 500, 1,500, and 3,000 ppm 6 h/d, 5 d/wk for 13 wk. They reported slight increases in hematologic parameters in the 3,000-ppm group (see Table 5-2 for male data). Because the hydrolysis of *n*-BA to *n*-butanol is 99% complete in 2.7 min in rats, we can establish an inhalation equivalent of *n*-butanol based on the relative absorption of the compounds. The acetate is 100% absorbed, whereas the alcohol is absorbed at 40% to 50% in the respiratory system (Barton et al. 2000). According to the physiologically based pharmacokinetic modeling of Barton et al. (2000), an inhalation exposure to rats of 500 ppm of *n*-butanol is equivalent to an exposure to 820 ppm of *n*-BA, both giving 26 μM *n*-butanol as a steady-state blood concentration and identical areas under the curve (0.16 μmol × h/mL). These exposures were taken to be no-observed-adverse-effect levels (NOAELs) by these authors.

TABLE 5-2 Comparison of Blood Parameters in Male Rats after 3 Months of Exposure to *n*-Butanol or *n*-BA

	n-Butanol (Korsak et al. 1994)			*n*-Butanol Daily Gavage Dosing (TRL 1986)				*n*-BA (David et al. 2001)			
Parameter	0 ppm	50 ppm	100 ppm	0 mg/kg/d	30 mg/kg/d	125 mg/kg/d	500 mg/kg/d	0 ppm	500 ppm	1,500 ppm	3,000 ppm
Hct, %	40.0	38.6	38.5	44.8	43.5	44.0	43.3	42.3	42.1	42.0	45.1[a]
HgB, g/dL	15.9	14.2[b]	14.1[b]	14.9	14.7	14.6	14.4	14.2	14.1	14.1	15.1[a]
RBC, $\times 10^6$/mm^3	9.97	9.45	8.35[b]	7.93	7.89	7.84	7.74	8.1	8.2	8.2	8.6[a]
WBC, $\times 10^3$/mm^3	10.5	13.1	16.5[b]	9.6	11.5	11.0	9.5	10.0	10.3	8.8	8.0

[a] $P < 0.05$ statistically significant difference compared with controls.
[b] $P < 0.01$ statistically significant difference compared with controls.
Abbreviations: Hct, hematocrit; HgB, hemoglobin; RBC, red blood cells; WBC, white blood cells.

Similarly, Teeguarden et al. (2005) found in their modeling that in rats inhaling 100 ppm, the steady-state concentration of *n*-butanol was 5.5 µM if the administered compound was *n*-butanol, whereas if the administered compound was *n*-BA, the steady-state blood concentration was 7.4 µM. Thus, we must conclude that inhalation of a specific concentration of *n*-BA results in higher blood concentrations of *n*-butanol than inhalation of the same concentration of *n*-butanol. Thus, the data of David et al. (2001) showing no hematologic effects from exposure to *n*-BA suggests that the hematologic results on *n*-butanol reported by Korsak et al. (1994) may be misleading.

Another approach to understanding the Korsak et al. (1994) report is to ask whether other small-chain alcohols exhibit similar hematotoxicity by inhalation. No subchronic studies of either 1-propanol or 1-pentanol could be identified; however, two subchronic inhalation studies were found on 2-propanol. In the first study (Nakaseko et al. 1991), male rats were exposed 4 h/d, 5 d/wk for 3 months to 2-propanol concentrations of 0, 400, 1,000, 4,000, or 8,000 ppm. The authors reported a significant lowering of the red blood cell count after 12 wk of exposure to 2-propanol at 4,000 ppm and more rapid effects in the 8,000-ppm group; however, 4 wk after the exposure ended there were no effects (perhaps also 1 wk after the end of exposure, but the figure is too small to answer definitively).

In another study (Burleigh-Flayer et al. 1994), male and female rats and mice were exposed 6 h/d, 5d/wk for 13 wk to 2-propanol vapor at concentrations of 0, 100, 500, 1,500, or 5,000 ppm. A slight anemia was observed at 6 wk in the highest-concentration group of male and female rats, but it was not observed at 14 wk. From this result, it was concluded that 2-propanol induces hematologic effects only at concentrations 40 to 50 times higher than those *n*-butanol concentrations that Korsak et al. (1994) reported to be hematotoxic. Furthermore, the effects of 2-propanol on red cell parameters are transient.

One further interesting argument against using the Korsak et al. (1994) finding centers on reports in the same paper that *m*-xylene also induces hematotoxicity with about the same potency as *n*-butanol. Both compounds apparently have mild effects at 50 ppm and more pronounced effects at 100 ppm (e.g., about a 20% decrease in red blood cell count). This must be considered in light of a report from the same laboratory 2 years earlier in which rats of the same strain were exposed 6 h/d, 5d/wk for 3 months to 1,000 ppm of *m*-xylene (Korsak et al. 1992). Twenty-four h after exposure terminated, no statistically significant exposure-related changes were found for hematologic parameters except for differential white blood cell counts (EPA 2003). The paper published in 1994 makes no reference to the 1992 paper.

In summary, *n*-butanol will not be treated as a hematotoxicant for three reasons: (1) *n*-BA inhalation exposures equivalent to much higher concentrations of *n*-butanol do not show an effect (David et al. 2001), (2) subchronic inhalation studies of other small-chain alcohols show transient effects only at 40 to 50 times the concentration of *n*-butanol that supposedly induced hematotoxicity,

and (3) the hematologic results on xylene from the same laboratory are inconsistent (Korsak et al. 1992, 1994).

Neurotoxicity

The key new data for this effect come from a study by David et al. (1998) in which rats exposed for 6 h/d, 5 d/wk for 14 wk to *n*-BA at concentrations of 0, 500, 1,500, and 3,000 ppm were subjected to a battery of functional neurologic tests to detect neuropathology. The study noted that "minimal to minor" reduced activity levels were observed at the two highest doses during exposures; however, the severity of the reduced activity did not increase as the exposures progressed to 14 wk. No effects on activity were noted in the controls or in the 500-ppm group. The authors concluded that 3,000 ppm was a NOAEL for cumulative neurotoxicity; however, we cannot accept reduced activity as a no-effect end point because it might be associated with a reduced capability to perform complex tasks. We will take 500 ppm *n*-BA as the neurotoxicity NOAEL from this study, and this is equivalent to 820 ppm of *n*-butanol.

Ototoxicity

This adverse effect was not considered in the original SMAC document; however, for completeness it is noted here that an old report of ototoxicity that some groups had used to set human exposure limits has been superseded by more recent findings (Velazquez et al. 1969). Crofton et al. (1994) exposed rats for 6 to 8 h/d, 5 d/wk for 5 d to various solvents at concentrations ranging from 1,600 to 4,000 ppm. They conducted auditory testing 5 to 8 wk after exposure using reflex modification audiometry to define thresholds for frequencies from 0.5 to 40 kilohertz. Hearing deficits were found in the midfrequency range for all solvents except *n*-butanol, which caused no hearing loss, even at 4,000 ppm. This is the ototoxicity NOAEL for *n*-butanol regardless of time of exposure.

NEW RISK ASSESSMENT APPROACHES

Our approach to dealing with compounds that have a significant odor at concentrations well below those that could cause adverse health effects will be to continue to set SMACs based on potential physical harm but include a footnote in the table describing the concentrations where the presence of the compound may create an unpleasant odor. One issue with this approach is that odor sensitivities appear to change during spaceflight; therefore, even if there were ground-based data describing odor properties in detail, their applicability to the spaceflight situation would be questionable. Other issues center on adaptation to odors with time. There are few specific data describing this phenomenon.

We point out that the situation for air is different than for water. Crews will be forced to breathe air with an unpleasant smell when their supply of fresh

air or their respirators are expended, even though the air is not harmful. They will adapt to the presence of a continuous odor. However, they can choose not to ingest water that tastes or smells bad, even if it is not harmful. They will not adapt to this because they experience the odor only when trying to drink water. Thus, our concerns with health risks from reduced water consumption caused by poor aesthetic properties of the water do not have a parallel concern for breathing air.

RATIONALE FOR REVISIONS TO THE PREVIOUS SMACS

Odor Thresholds and Noxious Odors

The odor thresholds reported for *n*-butanol have been summarized from 29 original sources (Amoore and Hautala 1983) dating from 1892. The thresholds ranged from 0.05 to 60 ppm (0.15 to 190 mg/m^3), more than 3 orders of magnitude. This provides no more than a rough guide to which concentrations might represent the threshold of detection.

Hematotoxicity and Immunotoxicity

Based on the information presented in Table 5-2, we will not do a risk assessment on the apparent hematologic and immune effects suggested by the inhalation study of Korsak et al. (1994).

Neurotoxicity

As pointed out previously, exposures equivalent to 820 ppm of *n*-butanol (500 ppm *n*-BA) elicited no detectable effect in rats given a subchronic inhalation exposure. The AC is as follows:

$$AC_{(\text{neurotoxicity})} = 820 \text{ ppm}_{(\text{NOAEL})} \times 1/10_{(\text{species})} = 80 \text{ ppm}$$

This result is consistent with the earlier neurotoxicity estimate based on analogies to ethanol and from epidemiologic evidence. Because there is no cumulative neurotoxicity (David et al. 1998), this AC will be taken as applicable to all exposure times.

Ototoxicity

The data from Crofton et al. (1994) revealed that styrene, xylenes, toluene, and 1,1,2-trichloroethylene at 1,600 to 3,500 ppm caused midfrequency hearing loss in rats exposed for 5 d, but 4,000 ppm of *n*-butanol did not. This is sufficient evidence that *n*-butanol is not a significant ototoxicant; therefore, we will

take 4,000 ppm to be a NOAEL regardless of time of exposure. The AC for ototoxicity is as follows:

$$AC_{(ototoxicity)} = 4000 \text{ ppm}_{(NOAEL)} \times 1/10_{(species)} = 400 \text{ ppm}$$

This applies to indefinite exposure times. Note that we will not consider nasal injury from the *n*-BA exposures as relevant to *n*-butanol exposures because the effect is probably mediated through hydrolysis of the acetate to the alcohol and acetic acid, a process that does not occur when the exposure is only to an alcohol.

RATIONALE FOR THE 1,000-DAY SMAC

Unfortunately, there are no long-term data on *n*-butanol by any route of administration; however, neither *n*-butanol nor its metabolites (organic aldehydes and acids) are expected to accumulate at exposures below 50 ppm beyond that achieved from short-term *n*-butanol exposures, so we do not expect an increased risk of adverse effects with prolonged exposure times. This conclusion is supported by the observation of David et al. (1998) that cumulative neurotoxicity was not observed using a battery of end points during a subchronic study of *n*-BA. Accordingly, the 1,000-d SMAC was set at the same value, 12 ppm, as the 180-d SMAC.

COMPARISON OF SMACS WITH OTHER AIR QUALITY LIMITS

The current threshold limit value (TLV) for *n*-butanol is 20 ppm (60 mg/m^3) to protect against irritation. The Occupational Safety and Health Administration (OSHA) permissible exposure limit (PEL) is 100 ppm (300 mg/m^3), and the National Institute for Occupational Safety and Health (NIOSH) ceiling limit is 50 ppm (150 mg/m^3).

Our long-term exposure limits (Table 5-3) of 12 and 25 ppm (40 or 80 mg/m^3) straddle the TLV of 20 ppm and are well below the PEL of 100 ppm. The 1-h SMAC and the NIOSH ceiling limit are the same.

RECOMMENDATIONS FOR ADDITIONAL RESEARCH

A detailed review of the odor threshold work, which is beyond the scope of this review, might suggest a more focused statement about odor thresholds and noxious concentrations; however, one is plagued by anecdotal reports from crew that odor perceptions in space can be quite different than on the ground.

TABLE 5-3 ACs for *n*-Butanol Toxicity[a,b] and Proposed SMACs

			Uncertainty Factors			Acceptable Concentrations, mg/m^3					
Effect	Reference	Species	Species	Time	Small *n*	1-h	24-h	7-d	30-d	180-d	1,000-d
Mild irritation at 150 mg/m^3	3 studies (see text)	Human, n >100	1	1	1	150	80	80	80	80	80
CNS epidemiology NOAEL	Tabershaw et al. (1944)	Human	1	1	1	300	300	300	300	300	300
CNS ethanol comparison	Several references (see text)	Human	1	1	1	300	300	300	300	300	300
Systemic injury, oral NOAEL (92 d)	TRL (1986)	Rat	10	1 or Haber's rule	1	n/a	n/a	80	80	40	40
SMACs						*150*	*80*	*80*	*80*	*40*	*40*

[a]The only value that has changed from the original SMACs is the addition of the 1,000-d value.
[b]The odor threshold and noxious odor concentrations are uncertain. These concentrations may not preclude odor detection by the crew.
Abbreviation: n/a, not applicable.

REFERENCES

Amoore, J.E., and E. Hautala. 1983. Odor as an aid to chemical safety: Odor thresholds compared with threshold limit values and volatilities for 214 industrial chemicals in air and water diulution. J. Appl. Toxicol. 3(6):272-290.

Astrand, I., P. Ovrum, T. Lindqvist, and M. Hultengren. 1976. Exposure to butyl alcohol: Uptake and distribution in man. Scand. J. Work Environ. Health 2(3):165-175.

Barton, H.A., P.J. Deisinger, J.C. English, J.N. Gearhart, W.D. Faber, T.R. Tyler, M.I. Banton, J. Teeguarden, and M.E. Andersen. 2000. Family approach for estimating reference concentrations/doses for series of related organic chemicals. Toxicol. Sci. 54(1):251-261.

Burleigh-Flayer, H.D., M.W. Gill, D.E. Strother, L.W. Masten, R.H. McKee, T.R. Tyler, and T. Gardiner. 1994. Isopropanol 13-week vapor inhalation study in rats and mice with neurotoxicity evaluation in rats. Fundam. Appl. Toxicol. 23(3):421-428.

Crofton, K.M., T.L. Lassiter, and C.S. Rebert. 1994. Solvent-induced ototoxicity in rats: An atypical selective mid-frequency hearing deficit. Hear.Res. 80(1):25-30.

David, R.M., T.R. Tyler, R. Ouellette, W.D. Faber, M.I. Banton, R.H. Garman, M.W. Gill, and J.L. O'Donoghue. 1998. Evaluation of subchronic neurotoxicity of n-butyl acetate vapor. Neurotoxicology 19(6):809-822.

David, R.M., T.R. Tyler, R. Ouellette, W.D. Faber, and M.I. Banton. 2001. Evaluation of subchronic toxicity of n-butyl acetate vapor. Food Chem. Toxicol. 39(8):877-886.

Ehrig, T., K.M. Bohren, B. Wermuth, and J.P. von Wartburg. 1988. Degradation of aliphatic alcohols by human liver alcohol dehydrogenase: Effect of ethanol and pharmacokinetic implications. Alcohol. Clin. Exp. Res. 12(6):789-794.

EPA (U.S. Environmental Protection Agency). 2003. Xylene (CASRN 1330-20-7) Part I.B.2 Principal and Supporting Studies (Inhalation RfC), Integrated Risk Information System, U.S. Environmental Protection Agency [online]. Available: http://www.epa.gov/NCEA/iris/subst/0270.htm [accessed Apr. 3, 2008].

James, J.T. 1996. 1-Butanol. Pp. 53-77 in Spacecraft Maximum Allowable Concentrations for Selected Airborne Contaminants, Vol. 3. Washington, DC: National Academy Press.

James, J.T. 2003. Toxicological Assessment of the International Space Station Atmosphere with Emphasis on Metox Canister Regeneration. Paper No. 2003-01-2647. Presentation at the International Conference on Environmental Systems, July 2003, Vancouver, BC, Canada.

Korsak, Z., J.A. Sokal, and R. Gorny. 1992. Toxic effects of combined exposure to toluene and m-xylene in animals. III. Subchronic inhalation study. Pol. J. Occup. Med. Environ. Health 5(1):27-33.

Korsak, Z., J. Wisniewska-Knypl, and R. Swiercz. 1994. Toxic effects of subchronic combined exposure to n-butyl alcohol and m-xylene in rats. Int. J. Occup. Med. Environ. Health 7(2):155-166.

Nakaseko, H., K. Teramoto, S. Horiguchi, F. Wakitani, T. Yamamoto, M. Adachi, H. Tanaka, and S. Hozu. 1991. Toxicity of isopropyl alcohol. Part 2: Repeated inhalation exposure in rats [in Japanese]. Sangyo Igaku 33(3):200-201.

Tabershaw, I.R., J.P. Fahy, and J.B. Skinner. 1944. Industrial exposure to butanol. J. Ind. Hyg. Toxicol. 26(10):328-330.

Teeguarden, J.G., P.J. Deisinger, T.S. Poet, J.C. English, W.D. Faber, H.A. Barton, R.A. Corley, and H.J. Clewell III. 2005. Derivation of a human equivalent concentration

for n-butanol using a physiologically based pharmacokinetic model for n-butyl acetate and metabolites n-butanol and n-butyric acid. Toxicol. Sci. 85(1):429-446.

Thomasson, H.R., J.D. Beard, and T.K. Li. 1995. ADH2 gene polymorphisms are determinants of alcohol pharmacokinetics. Alcohol. Clin. Exp. Res. 19(6):1494-1499.

TRL (Toxicology Research Laboratories). 1986. Rat Oral Subchronic Toxicity Study of Normal Butanol. TRL Study No. 032-006. Toxicology Research Laboratories, Muskegon, MI.

Velazquez, J., R. Escobar, and A. Almaraz. 1969. Audiologic impairment due to n-bytil alcohol exposition. Pp. 231-234 in Proceedings of the 16th International Congress on Occupational Health. Tokyo: Excerpta Medica Foundation.

6

C2-C9 Alkanes[1]

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SUMMARY OF APPROACH

A number of hydrocarbons fall under the category of C2-C9 aliphatic saturated alkanes. Rather than address each compound individually, this document initially sought to establish a group spacecraft maximum allowable concentration (SMAC) for the C2-C9 alkanes in accordance with their similarities in toxic action and their physical and chemical properties. A SMAC for methane (C1) has already been established based on its lower limit of explosivity rather than on its chemical toxicity.

Estimating health-protective environmental limits for groups of chemicals can be a useful and practical risk-assessment approach, as evidenced by its success in establishing guidelines for total petroleum hydrocarbon fractions (MA DEP 1994). Group SMACs can be an efficient way to evaluate spacecraft air concentrations, while helping to limit redundancy in developing individual SMACs. However, group SMACs lose some of their usefulness when there are broad variations in toxicity among group members or when the toxic effects produced by one member are poorly relevant to the remainder of the group. Some variation in toxicity among a group is unavoidable, and group SMACs often serve a screening function in the sense that the developed SMACs may be driven by one or two group representatives that exhibit the greatest toxicologic response for an end point that is relevant to the entire group.

In this evaluation, we determined that it was neither realistic nor productive to establish group SMACs for the C2-C9 alkane range. We based this conclusion on two critical observations. First, for ethane (C2), propane (C4), and butane (C4), there is clearly limited potential for toxicity associated with environmentally relevant exposures to these compounds in air. Consistent with the

[1]Not including *n*-hexane.

approach taken in SMAC development for methane, individual SMACs for these compounds were based on 10% of the lower limits of explosivity for each of these gases. By maintaining concentrations below each chemical's limit, health concerns (including the potential for them to act as simple asphyxiants) are minimized. The C5-C9 alkanes are treated as a group, with the exception of *n*-hexane. Significant research and epidemiologic findings have established that *n*-hexane differs from the other alkanes (and from the other hexane isomers) in its potential to cause peripheral neuropathy (Spencer et al. 1980, Takeuchi et al. 1980, Frontali et al. 1981, Filser et al. 1996, ACGIH 2008). Because consideration of this specific effect for *n*-hexane would likely result in a group SMAC toxicologically irrelevant for most of members of the group, *n*-hexane is reserved for separate SMAC development and is not fully addressed in this document from a toxicologic standpoint.

PHYSICAL AND CHEMICAL PROPERTIES

Physical and chemical properties for the C2-C9 alkanes are presented in Table 6-1. Ethane (C2) through butane (C4) exist as gases at standard temperature and pressure, whereas pentane (C5) to nonane (C9) are liquids. Various branched isomers exist for many of the *n*-alkanes, and the physical and chemical properties for these isomers may differ from those presented in Table 6-1, which are specific to the *n*-alkanes.

OCCURRENCE AND USE

The group of compounds that comprise the C2-C9 saturated aliphatic alkanes (the linear and branched alkanes from ethane through nonane) are present in earth gases and crude oil and have a variety of commercial and industrial applications. The gaseous alkanes within this group (ethane, propane, and butane) are principal components of natural gas and are used widely as fuels and propellants (Sandmeyer 1981). They are also greenhouse gases and contribute to the formation of ground-level ozone (Katzenstein et al. 2003). The liquid alkanes (pentane, hexane, heptane, octane, and nonane) also may be found at low concentrations in natural gas but are known more for their applications as solvents and as important components of crude oil, diesel, and gasoline. *n*-Hexane is a widely used industrial solvent, as are pentane and heptane (Finkel 1983). Octane is used extensively as a preignition additive for high-compression engine fuels; other alkanes are either found in or are added to gasoline and other fuels. They are also formed and released after combustion of the fuels in automobiles, boilers, and other machinery (Sandmeyer 1981). The human body produces some of the more volatile alkanes (e.g., ethane and pentane) endogenously as a result of the breakdown of polyunsaturated fatty acids (Galvin and Marashi 1999); they can be measured at low concentrations in human breath (Frank et al. 1980).

TABLE 6-1 Physical and Chemical Properties of C2-C9 *n*-Alkanes

	C2	C3	C4	C5	C6	C7	C8	C9
Formula	C_2H_6	C_3H_8	C_4H_{10}	C_5H_{12}	C_6H_{14}	C_7H_{16}	C_8H_{18}	C_9H_{20}
Name	Ethane	Propane	Butane	Pentane	Hexane	Heptane	Octane	Nonane
CAS registry no.	78-84-0	74-98-6	106-97-8	109-66-0	110-54-3	142-82-5	111-65-9	111-84-2
Molecular weight	30.1	44.1	58.1	72.1	86.1	100.2	114.2	128.3
Boiling point (°C)	−88.6	−42.1	−0.5	36.1	69	98.4	125.7	150.8
Lower explosive limit	3.2%	2.3%	1.9%	1.4%	1.2%	1.2%	1.0%	0.9%
Upper explosive limit	12.5%	9.5%	8.4%	7.8%	7.8%	6.7%	4.7%	2.9%
Vapor pressure (mm Hg)	Gas	Gas	Gas	400 (18°C)	100 (20°C)	40 (25°C)	10 (19°C)	10 (38°C)
Conversion factor (ppm to mg/m^3) 25°C	1.23	1.80	2.38	2.95	3.52	4.10	4.67	5.25
Isomers	1	1	2	3	5	9	18	33

Abbreviations: mg/m^3, milligrams per cubic meter; ppm, parts per million.
Source: Sandmeyer 1981, ACGIH 1991.

Given their widespread uses, these saturated hydrocarbons are commonly encountered in ambient air. For example, Hawas et al. (2001) found that C5-C9 aliphatic alkanes composed more than 80% of the total concentration of volatile organic compounds measured in ambient air from a light industrial area of Brisbane, Australia. Individual alkanes were measured in ambient air at concentrations as high as 200 parts per billion (ppb) (*n*-pentane), with the highest three averages reported for *n*-pentane (70 ppb), 2,3-dimethylbutane (60 ppb), and *n*-hexane (19 ppb). The C2-C4 gases have been studied because of their importance in the formation of ground-level ozone and were recently measured in the near-surface atmosphere of Texas at 34 ppb (ethane), 20 ppb (propane), and 13 ppb (butane) (Katzenstein et al. 2003).

C2-C9 alkanes may be present in certain spacecraft payloads and are found in small amounts in certain flight hardware (e.g., detectors). Some members of this group have been measured in air samples collected onboard the International Space Station (ISS), although they typically occur at very low concentrations. For example, Russian analysis of AK-1M tubes collected before the return of 6 Soyuz (Nov. 2003) reported that most C5-C9 alkanes were present at concentrations below 0.1 ppm. In 2003, Russian analysts reported much higher concentrations of alkanes in an air sample collected from the Progress resupply vehicle (total hydrocarbon concentrations of 34 milligrams per cubic meter [mg/m^3], with pentane isomers as the primary components), although their source remains unclear. The C2-C9 alkanes either are not reported or are found in very low concentrations (e.g., 0.1-0.2 mg/m^3) in U.S. analyses of ISS air samples.

Toxicokinetics

Given that a group SMAC is being developed for C2-C9 alkanes, the toxicokinetics of these compounds is discussed as it applies to the class of compounds. Thus, the focus is on presenting an accurate general toxicokinetic picture for this group rather than detailing the process for each member of the group. Because of the commercial importance of *n*-pentane and *n*-hexane, more specific information exists about their toxicokinetics than for some of the other alkanes. Because information on these two alkanes cannot necessarily be used to make general statements for the entire group, an attempt was made to describe how variations in carbon chain length or structure (e.g., branched isomers) may affect each area of toxicokinetics.

Absorption

There is some variation in respiratory absorption among the aliphatic C2-C9 saturated alkanes, but they are generally not as well absorbed as the unsaturated alkanes (Zahlsen et al. 1990). Among the C2-C9 saturated alkanes, absorption into the bloodstream after inhalation exposures will generally be greater for

the linear members of this group and for those with higher molecular weights (Galvin and Marashi 1999). Perbellini et al. (1985) conducted in vivo studies of human tissues and blood and found that alkane solubility in blood typically increased with increasing molecular weight, as evidenced by blood:air partitioning coefficients of 0.4, 0.8, and 1.9 for *n*-pentane, *n*-hexane, and *n*-heptane, respectively. *n*-Hexane reached steady-state blood concentrations within 100 min during a limited 4-h inhalation study with human volunteers (Veulemans et al. 1982). In a longer study, Zahlsen et al. (1990) observed that peak blood concentrations of *n*-nonane occurred within the first day (12 h) of exposure during a 14-d inhalation test with rats.

Distribution

Once dissolved in the bloodstream, the saturated alkanes can be distributed to various organ systems. In general, in accordance with the lipid solubility of the alkanes, most can easily cross biologic membranes, and the group has an affinity for lipid-soluble tissues (Perbellini et al. 1985, Robinson 2000). Perbellini et al. (1985) found that *n*-pentane was distributed to a significant degree to adipose, brain, liver, and kidney tissue. The higher molecular weight alkanes would be expected to move from blood into these organs even more readily because of their greater solubility in lipids. That was observed in a study of *n*-nonane by Zahlsen et al. (1990) where, for example, *n*-nonane accumulated in the brain at concentrations twice as high as trimethylbenzene, although *n*-nonane was present in the blood at only a third of the concentration of trimethylbenzene. Peak brain tissue concentrations of *n*-nonane were reached after 12 h of inhalation exposure. This transfer from the blood occurs in spite of the possibility of size restriction, which can slow the absorption of high molecular weight compounds across the blood-brain barrier (Hau et al. 2002).

Metabolism and Excretion

Frank et al. (1980) studied the rate of elimination of *n*-pentane in rats after inhalation and reported an inhalation half-life of 2.3 h. They found that the addition of peroxidation inhibitors (dithiocarb or ethanol) significantly increased the half-life. Consistent with similar observations reported by Allerheilingen et al. (1987), who observed that clearance decreased in the presence of carbon tetrachloride (which destroys cytochrome P-450), it appears that metabolism by the mixed-function oxygenase system plays a significant role in the elimination of *n*-pentane and other saturated alkanes. A closed-chamber test with radiolabeled *n*-pentane revealed that 50% of the radioactivity was excreted as CO_2 after 8 h, with 7.6% metabolized and released in urine (Daugherty et al. 1988). The authors assumed that most of the remainder (about 40%) was primarily excreted unchanged in the breath. This percentage released in the breath is probably an overestimate for the alkanes with lower volatility and increased blood solubility.

For example, no more than 20% was estimated to be excreted unchanged in the breath for *n*-hexane (ATSDR 1999).

With respect to the fraction of inhaled alkanes absorbed into the bloodstream and metabolized, there are important metabolic distinctions among the individual C2-C9 alkanes. To a significant degree, it is thought that a major elimination pathway for the *n*-alkanes is chain breakdown and elimination of the carbon as expired CO_2 (Daugherty et al. 1988). Dahl (1989) exposed rats to radiolabeled *n*-octane and found that to be the case. However, Dahl's studies of a branched alkane isomer (isooctane) produced very different results. Instead of being metabolized to CO_2, the branched structure prevented normal chain breakdown and resulted in significant excretion as urinary metabolites (e.g., alcohols).

Instead of being released as CO_2, alkanes can be oxidized by microsomal P-450 activity and undergo aliphatic hydroxylation to form an alcohol (Sandmeyer 1981). Studies with *n*-pentane (Frommer et al. 1970) have shown that 2-pentanol is the major metabolite (89%) formed by rat liver microsomes, with 3-pentanol as a minor metabolite (11%), consistent with the aliphatic hydroxylation process. However, as is observed with *n*-hexane, the alcohol can also be further oxidized to form ketones, diketones, diols, and carboxylic acids (ATSDR 1999). These intermediates can be released in urine or exhaled (EEMA 1995). Some of these metabolites have exhibited specific toxicity that is not relevant to other metabolites of the same parent hydrocarbon. In particular, exposure to *n*-hexane has been associated with the development of neuropathy that is mediated by the formation of a γ-diketone (2,5-hexanedione) following metabolism of the parent hydrocarbon (Schaumburg and Spencer 1976). The same effect is not observed with 2,4- or 3,5-diketones, and it is thought that the neurotoxicity of the γ-diketone is due to its high water solubility and ability to form stable conjugated Schiff bases (Graham and Abou-Donia 1980).

TOXICITY SUMMARY

Available data suggest that a distinction can be made between the gaseous (C2-C4) and liquid (C5-C9) saturated alkanes in terms of their toxicity. Because of the widespread harmless exposures associated with their presence as components of natural gas, ethane, propane, and butane are generally viewed as having extremely low chemical toxicity (Sandmeyer 1981; Finkel 1983). One of the main hazards with these gases is their potential to cause asphyxiation after their release in an enclosed environment. These light gases can dilute available concentrations of oxygen, thus decreasing oxygen uptake by the lung (Galvin and Marashi 1999). That is not to say that there is no toxicity associated with these alkanes. For example, in summarizing the toxicity of these gases, the European Agency for the Evaluation of Medicinal Products reported the following effects: (1) propane concentrations of 100,000 parts per million (ppm) (10%) caused respiratory depression and bronchospasm in exposed mice; (2) isobutane resulted in increased pulmonary resistance and depressed minute volume in rats

exposed at 50,000 ppm; (3) in humans, 100,000 ppm of *n*-butane caused vertigo after 2 min of exposure; (4) 350,000 ppm of isobutane had an anaesthetic effect in mice after 25 min; and (5) 520,000 ppm of isobutane was a 2-h LC_{50} (dose lethal to 50% of subjects) for mice (EEMA 1995). These concentrations are not environmentally relevant and confirm only that there is very limited potential for these gases to be toxic. However, these gases do have significant potential to explode if allowed to accumulate to sufficient concentrations (lower explosive limits of 1.9% to 3.2%), and consideration of these hazards appears to be more than adequately protective of any potential toxicity (Sandmeyer 1981).

This conclusion does not extend to the C5-C9 saturated alkanes because a number of toxicologic effects at more realistic concentrations of exposure have been reported in the scientific literature (although there is still variability in the toxic potential among members of this group). Those acute, subchronic, and chronic effects of particular relevance to SMAC development are more fully described in the following sections (refer to Table 6-2 for study details). When possible, general trends in toxicologic effect are noted among the different members of the series of C5-C9 saturated alkanes. Knowledge of these general trends is useful, especially when scanty toxicity data are available for a specific alkane (Robinson 2000).

Acute Exposures

As might be predicted based on their physical and chemical properties, the central nervous system (CNS) is a main target for the C5-C9 saturated alkanes (Hau et al. 2002). Adverse CNS effects may include narcosis and anesthetic effects, and have been reported in humans in the form of headaches, exhilaration, nausea, a loss of fine motor skills, difficulty concentrating, confusion, and loss of appetite (Sandmeyer 1981, Finkel 1983). Swann et al. (1974) noted that an increase in the length of the carbon chain among the C5-C9 alkanes typically results in a greater potential for narcotic effects, although exceptions have been cited (Nilsen et al. 1988). For example, in mice exposed to *n*-pentane, concentrations of at least 32,000 ppm were necessary to produce light anesthesia, although 8,000 ppm of *n*-heptane was capable of producing the same effect (Swann et al. 1974). Branched alkanes may not have the same potential for anesthetic effects as their linear alkane counterparts (Sandmeyer 1981) because isooctane did not result in anesthesia in this study, even at lethal concentrations of 32,000 ppm.

A number of studies have evaluated the acute toxicity of *n*-pentane (comprehensive review by Galvin and Marashi 1999). It is clear from reviewing the results of these studies that *n*-pentane has very low acute toxicity, because many studies failed to find any adverse effects, and those that did typically required extremely high concentrations (32,000-100,000 ppm). Carpenter et al. (1978) evaluated the acute toxicity of *n*-nonane vapors in rats. The LC_{50} (after 14 d) for rats subjected to inhalation exposures to *n*-nonane for 4 h was 3,200 ppm.

TABLE 6-2 Toxicity Summary for C5-C9 Saturated Alkanes (Excluding *n*-Hexane)

Effect Concentration (ppm)	Exposure Duration	Species	Effects	Reference
n-Pentane				
3,000	16 wk (12 h/d)	Wistar rats	No neurotoxic effects.	Takeuchi et al. 1980
3,000	30 wk (9 h/d, 5 d/wk)	Sprague-Dawley rats	No neurotoxic effects.	Frontali et al. 1981
4,500 (50:50, pentane: butane)	13 wk (6 h/d, 5 d/wk)	Fischer 344 rats	No reported kidney effects.	Aranyi et al. 1986
7,000	90 d (6 h/d, 5 d/wk)	Sprague-Dawley rats	No effects (including survival, body weight changes, organ weight, tissue pathology [including reproductive tissue], blood chemistry).	McKee et al. 1998
10,000	2 wk (6 h/d, 5 d/wk)	Charles River rats	No effects besides reversible and slight clinical pathology changes.	Stadler et al. 2001
10,000	Gestation day 6-15	Pregnant Charles River rats	No developmental toxicity.	Hurtt and Kennedy 1999
32,000	5 min	Swiss mice	Light anesthetic effects.	Swann et al. 1974
128,000	5 min	Swiss mice	Respiratory arrest (1/4 mice).	Swann et al. 1974
2-Methylpentane				
1,500	14 wk (9 h/d, 5 d/wk)	Sprague-Dawley rats	No neurotoxic effects.	Frontali et al. 1981
3-Methylpentane				
1,500	14 wk (9 h/d, 5 d/wk)	Sprague-Dawley rats	No neurotoxic effects.	Frontali et al. 1981
n-Heptane				
800	28 d (6 h/d)	Long Evans rats	Ototoxicity NOAEL.	Simonsen and Lund 1995

1,500	30 wk (9 h/d, 5 d/wk)	Sprague-Dawley rats	No neurotoxic effects.	Frontali et al. 1981
3,000	16 wk (12 h/d)	Wistar rats	No neurotoxic effects.	Takeuchi et al. 1980
4,000	28 d (6 h/d)	Long Evans rats	Increased auditory threshold, ototoxicity.	Simonsen and Lund 1995
6,700	10 min	CF-1 mice	10% reduced respiratory rate (RD_{10} [d]) calculated from concentration-response relationship.	Kristiansen and Nielsen 1988
8,000	5 min	Swiss mice	Anesthetic effects.	Swann et al. 1974
17,400	10 min	CF-1 mice	50% reduced respiratory rate (RD_{50} [b]) calculated from developed concentration-response relationship.	Kristiansen and Nielsen 1988
48,000	5 min	Swiss mice	Respiratory arrest (3/4 mice)	Swann et al. 1974
Isooctane				
16,000	5 min	Swiss mice	Respiratory arrest (1/4 mice).	Swann et al. 1974
32,000	5 min	Swiss mice	Respiratory arrest (4/4 mice).	Swann et al. 1974
n-Octane				
3,800	10 min	CF-1 mice	10% reduced respiratory rate (RD_{10} [d]), calculated from concentration-response relationship.	Kristiansen and Nielsen 1988

(Continued)

TABLE 6-2 Continued

Effect Concentration (ppm)	Exposure Duration	Species	Effects	Reference
n-Nonane				
590	13 wk (6 h/d, 5 d/wk)	Wistar rats	NOAEL for reduced weight gain; also, no shorter term effects that were noted in the 7-d study at 1,500 ppm.	Carpenter et al. 1978
1,100	10 min	CF-1 mice	10% reduced respiratory rate (RD_{10} [a]), calculated from concentration-response relationship.	Kristiansen and Nielsen 1988
1,500	7 d (6 h/d)	Wistar rats	Mild tremors, coordination loss, irritation.	Carpenter et al. 1978
1,600	13 wk (6 h/d, 5 d/wk)	Wistar rats	Mild tremors, coordination loss, salivation, and significantly reduced weight gain over the exposure duration relative to controls	Carpenter et al. 1978
2,414	8 h (14 d observed	Sprague-Dawley rats	NOAEL for CNS effects.	Nilsen et al. 1988
3,200	4 h (14 d observed)	Wistar rats	LC_{50}	Carpenter et al. 1978
3,560	8 h (14 d observed)	Sprague-Dawley rats	LOAEL, gross ataxia, spasms, loss of Purkinje cells.	Nilsen et al. 1988

[a] RD_{10} is the concentration expected to result in a 10% decrease in respiratory rate relative to controls.
[b] RD_{50} is the concentration expected to result in a 50% decrease in respiratory rate relative to controls.
Abbreviations: LOAEL, lowest-observed-adverse-effect level; NOAEL, no-observed-adverse-effect level.

Rats exposed to *n*-nonane at 1,500 ppm for 6 h/d for 7 d exhibited mild tremors, loss of coordination, and slight irritation. The same effects were exhibited in a longer term experiment with *n*-nonane at 1,600 ppm, which was part of the Carpenter et al. study, but the effects were not observed with exposures to *n*-nonane at 590 ppm.

Nilsen et al. (1988) evaluated the toxicity of *n*-nonane and other higher alkanes in association with inhalation exposures of 8 h followed by a 14-d observation period in Sprague-Dawley mice. They focused on evaluating CNS and respiratory system effects. They evaluated four exposure groups, with concentrations ranging from 2,414 to 5,289 ppm, and found a good dose-response relationship with tremors, ataxia, spasms, and limb paralysis after 2-4 h of exposure. An 8-h LC_{50} of 4,500 ppm was estimated for *n*-nonane, with death occurring as a result of cardiopulmonary insufficiency (the authors were not able to distinguish whether it was due to CNS depression or to direct action on the heart or lungs).

One interesting finding was the report of neurotoxicity in mice surviving exposure to *n*-nonane at 4,438 ppm. On autopsy (14-d postexposure), surviving rats (6/10) were found to have a significant loss of Purkinje cells and a marked increase in damaged cerebellar neurons compared with controls. No neuropathic changes were found in any of the mice dying before the 14-d observation period, nor was it observed with C10-C13 alkanes tested. The authors suggested that this effect is likely specific to *n*-nonane and is indicative of its neurotoxicity. However, they also cautioned that the effect could be attributable to general hypoxia. As described later in this document, the neuropathy reported by Nilsen et al. (1988) is inconsistent with the current weight of evidence for the C5-C9 alkanes, which points only to *n*-hexane as having specific neurotoxicologic properties that distinguish it from the other members of this group.

In the same study, anesthetic effects were not noted, even at the highest tested concentrations for *n*-nonane. This observation can be partially explained by other studies that have noted slower absorption into the brain for the larger alkanes despite their lipophilicity (Hau et al. 2002). However, as other significant CNS effects were noted in this 8-h study, more research is needed to fully understand the factors controlling the anesthetic properties of these alkanes.

Swann et al. (1974) also observed that mice commonly exhibited respiratory irregularities progressing to arrest after inhalation exposures to saturated alkanes. One in four mice experienced respiratory arrest in association with 5 min of exposure to *n*-pentane at 128,000 ppm, although only 16,000 ppm produced the same effect with isooctane. All four mice went into arrest within 3 min at 32,000 ppm. The authors suggested that these observed respiratory effects may not simply be due to direct alkane action on CNS depression and noted that the observed respiratory changes corresponded to irritation (determined by assessing body movements). This irritation increased in severity and sensitivity with increasing carbon chain length of the tested alkanes.

Other authors have studied specific respiratory changes in laboratory animals in response to exposure to saturated alkanes in the context of deter-

mining the potential for these compounds to act as sensory irritants (Kristiansen and Nielsen 1988). Alarie (1973, 1981) suggested that the trigeminal nerve endings in the nasal mucosa would respond to sensory irritants (resulting in a burning sensation in the mucous membranes of the eyes, nose, and throat) and that this response could be measured by a decrease in respiratory rate. This type of sensory irritation would affect the upper respiratory tract, as distinguished from pulmonary irritants, which act on the lower respiratory tract (Alarie 1973). To study these effects, Kristiansen and Nielsen (1988) used an inhalation test chamber to expose groups of four CF-1 mice for 30 min to a range of C7-C11 alkane concentrations (Table 6-2). Certain groups of mice were exposed through a trachea cannula, thus bypassing the trigeminal nerve to assess nonsensory irritation. The authors measured the response at each exposure by evaluating the peak percentage change in the average respiratory rate of each group of four mice relative to their average preexposure level (as measured during a 10-min control period). On the basis of this testing, they determined a relationship between exposure concentration and the reduction in respiratory rate relative to controls for each alkane (Figure 6-1). They applied the regression equation for this relationship to predict RD_0, RD_{10} (the concentration expected to result in a 10% decrease in respiratory rate relative to controls), and RD_{50} (the concentration expected to result in a 50% decrease in respiratory rate relative to controls) for each alkane. Pulmonary irritation seemed not to be a significant factor with the alkanes. This study affirmed previous observations (Swann et al. 1974, Sandmeyer 1981) about the relationship between carbon chain length and the potential for irritation, with *n*-nonane resulting in respiratory rate reduction at the lowest concentration of the alkanes tested that were relevant to this group SMAC. Table 6-3 presents the RD_0, RD_{10}, and RD_{50} values for the C7-C9 alkanes (with data for C10 and C11 alkanes also provided for comparison) based on results observed within the first 10 min of the test (when effects could be most clearly attributed to sensory irritation).

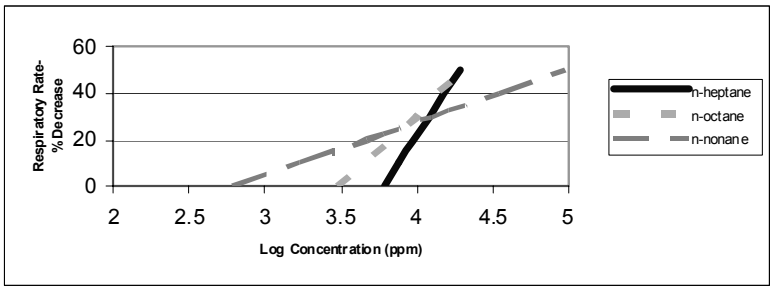

FIGURE 6-1 Concentration-response slopes for decreases in respiratory rate after exposures to *n*-heptane, *n*-octane, and *n*-nonane (Kristiansen and Nielsen 1988). The slopes extend to an RD_{50}, but only *n*-heptane exhibited an RD_{50} response at the concentrations tested.

TABLE 6-3 Predicted RD_0, RD_{10}, and RD_{50} Values for C7-C11 Alkanes from Sensory Irritation Investigations

Compound	RD_0 (ppm)	RD_{10} (ppm)	RD_{50} (ppm)
n-Heptane	6,000	6,700	17,400
n-Octane	3,000	3,800	18,100
n-Nonane	620	1,100	62,200
n-Decane	110	420	$>1 \times 10^6$
Undecane	Not predicted because of lack of significance of the regression	Not predicted because of lack of significance of the regression	Not predicted because of lack of significance of the regression

Source: Kristiansen and Nielsen 1988. Reprinted with permission; copyright 1988, *Archives of Toxicology*.

An important finding is that, among the alkanes and concentrations tested, only *n*-heptane exhibited enough of a sensory response to reduce respiration by 50% relative to controls. The authors postulated that this response may be because of stabilization of the receptor-substance complex or because the greater water solubility of the shorter chain alkanes may allow them more contact with receptors deep within the nasal mucosa. Thus, these findings suggest that exposure to *n*-nonane may cause sensory irritation at the lowest air concentrations (among the C5-C9 alkanes), although the severity of the response above that threshold may not be drastic (see Figure 6-1).

Subchronic and Chronic Exposures

Several longer term studies and reviews of *n*-pentane toxicity described in the literature consistently have shown *n*-pentane to have very low toxicity. McKee et al. (1998) conducted a 90-d inhalation study in which Sprague-Dawley rats were exposed to *n*-pentane at concentrations of 0, 1,700, 3,500, and 7,000 ppm. Various end points were examined, including weight gain, changes in various organ weights, and organ morphology. No treatment-related effects were observed at any concentration. Stadler et al. (2001) also exposed rats through inhalation to *n*-pentane for 2 wk to concentrations as high as 10,000 ppm. They observed subtle changes in clinical pathology at the 3,000- and 10,000-ppm dosing concentrations (changes in serum calcium and phosphorous concentrations), but these changes were slight, reversible, and within the range of variation seen in historical controls. They observed no outward signs of toxicity in any of the rats, and histopathologic evaluation of organs and the assessment of body weight showed no adverse effects after the *n*-pentane exposure. Taken as a whole, the scientific evidence suggests that *n*-pentane has little potential for adverse health effects, especially in association with exposures to environmentally relevant concentrations (Galvin and Marashi 1999).

Subchronic and chronic exposures to *n*-hexane have been shown to result in a specific polyneuropathy in human epidemiologic studies as well as in studies with laboratory animals (Yamada 1967, Takeuchi et al. 1980, Frontali et al. 1981, Filser et al. 1996). This has been found to be attributable to the neurotoxic potential of a specific metabolite of *n*-hexane—2,5-hexanedione, which is a γ-diketone (DiVincenzo et al. 1980). Similar metabolites (for example, 2,4-hexanedione) do not exhibit the same neurotoxicity (Graham and Abou-Donia 1980). For many of the epidemiologic cohorts, it was not possible to rule out the presence of other alkanes besides *n*-hexane, and there has been some discussion about whether other alkanes could be metabolized to form sufficient quantities of neurotoxic γ-diketones (Truhaut et al. 1973, 54 Fed. Regist. 2424[1989]). However, convincing work (Takeuchi et al. 1980, Frontali et al. 1981, Filser et al. 1996) suggests that the formation of necessary γ-diketones is favored by *n*-hexane metabolism, whereas it is not favored for other closely related alkanes. Therefore, the weight of evidence suggests that *n*-hexane has significantly greater potential to cause neurotoxicity than the other C5-C9 alkanes. For this reason, *n*-hexane is not included with the other C5-C9 alkanes in the development of a group SMAC; a separate SMAC will be developed in another document.

Hydrocarbon-induced nephropathy in rodents has been widely addressed in the scientific literature, especially for the C5-C9 hydrocarbons that are present in gasoline vapors (Aranyi et al. 1986, Halder et al. 1986). This nephrotoxicity (observed in male rats only) includes progressive renal tubular disease and primary renal neoplasms (Dahl 1989); several C5-C9 saturated alkanes have been shown to exhibit this type of nephrotoxicity (e.g., isooctane, 2,3,4-trimethylpentane). However, it has been accepted that this effect is likely mediated by binding to α2μ-globulin (Short et al. 1986, Loury et al. 1987, Dahl 1989), a protein unique to male rats and therefore irrelevant to SMAC development.

Simonsen and Lund (1995) conducted a study to evaluate the potential for exposure to aliphatic saturated alkanes (*n*-heptane) to result in ototoxicity. Previous work (Pryor et al. 1984, Rebert et al. 1991) showed auditory impairments in rats after exposure to other organic solvents (e.g., toluene, trichloroethylene). Simonsen and Lund tested whether *n*-heptane inhalation could reduce auditory thresholds in mice. They used implanted electrodes and needle electrodes to measure auditory brain stem responses elicited by an instrument that emitted sounds at frequencies of 3, 8, 16, and 32 kilohertz (kHz) and eight levels of sound intensity ranging from 25 to 95 decibels (dB). The rats were exposed through inhalation to *n*-heptane at 0, 800, and 4,000 ppm 6 h/d for 28 d. For the 4,000-ppm exposure group, effects were observed on the auditory brain stem response, which was most marked at the higher sound intensities. These effects translated into an increase in the auditory threshold by approximately 10 dB for the rats in this exposure group (although the effect was observed at all frequencies, it was statistically significant for controls only at 8 and 16 kHz). Rats in the 800-ppm exposure group showed no effects that were significantly different

from controls, and this concentration was proposed as a no-observed-adverse-effect level (NOAEL) for the study. Although this study was not designed to elucidate the mechanism of the observed ototoxicity, other studies have suggested that the effects may be related to a loss of outer hair cells in the cochlea—cells that aid in frequency selectivity (Pryor et al. 1984, Yano et al. 1992).

With respect to studies involving longer term inhalation exposures to alkanes, Carpenter et al. (1978) exposed rats to *n*-nonane vapors 6 h/d, 5 d/wk for 13 wk at concentrations of 0, 590, and 1,600 ppm. They observed excess salivation and lacrimation throughout the study for the rats in the highest exposure group. These rats also experienced a significant decrease in body weight gain relative to controls. These effects were not observed in the 590-ppm exposure group, and microscopic histopathology revealed no lesions or other effects attributable to the *n*-nonane exposure. Thus, 590 ppm was suggested as a NOAEL for these effects with *n*-nonane.

Genotoxicity and Mutagenicity

Limited information is available on the genotoxicity or mutagenicity of the C5-C9 alkanes in the scientific literature. Some information is available on effects due to *n*-hexane, especially its 2,5-hexanedione metabolite, but evaluation of this information is not relevant to this document. For *n*-pentane, no evidence of mutagenicity was observed in five species of *Salmonella* in the in vitro Ames assay (Kirwin et al. 1980). *n*-Pentane was also not mutagenic in a dominant lethal study when it was injected into the peritoneum of male mice (Epstein et al. 1972). A chromosome aberration test in Chinese hamster ovary cells conducted by McKee et al. (1998) produced equivocal results, especially given the high concentrations used (>10 millimolar) in the testing. Given these results, McKee et al. (1998) also conducted an in vivo bone marrow micronucleus test by examining the bone marrow from the rats exposed to *n*-pentane at up to 7,000 ppm for 90 d (another experiment reported in the same paper). They reported no increase in micronuclei formation and no evidence of cytotoxicity in the bone marrow.

Brooks et al. (1988) evaluated the potential for genetic toxicity with *n*-heptane by means of a bacterial mutation assay, a yeast assay for mitotic gene conversion, and cultured mammalian cells (rat liver and Chinese hamster ovary). *n*-Heptane did not produce a positive mutagenic response in any of the assays.

Developmental and Reproductive Toxicity

With respect to developmental toxicity, Hurtt and Kennedy (1999) exposed groups of eight pregnant female rats to *n*-pentane at 0, 1,000, 3,000, and 10,000 ppm by inhalation for 6 h/d from gestational days 6 to 15. They observed no maternal toxicity or adverse developmental effects on the fetuses. McKee et al. (1998) examined the reproductive tissues in the rats exposed to *n*-pentane as part of their 90-d inhalation study (exposures as high as 7,000 ppm) and found

no differences in organ weight or pathology relative to controls. Although relevant reproductive and developmental studies for the other alkanes were not found (*n*-hexane was not included in this SMAC evaluation), the lack of adverse effects in *n*-pentane, the negative findings from mutagenicity and genotoxicity assays, and the rapid clearance of the C5-C9 alkanes from the body suggest that these are not likely to be critical toxicologic end points for these compounds.

Interactions with Other Chemicals

No specific data were found indicating that the C5-C9 saturated alkanes would interact with other compounds to modify their toxic effect.

RATIONALE FOR ACCEPTABLE CONCENTRATIONS

Table 6-4 presents occupational exposure limits set by other organizations for the C2-C9 saturated alkanes. In general, occupational exposure limits decrease with increasing length of the carbon chain for this class of compounds. *n*-Hexane is a specific exception because lower limits are provided to address the potential for exposure to result in neurotoxicity. For the American Council of Governmental Industrial Hygienists (ACGIH) and the Occupational Safety and Health Administration (OSHA), the limits for the remaining C2-C9 saturated alkanes are set primarily to minimize their potential for irritation or narcosis. However, the National Institute for Occupational Safety and Health (NIOSH) takes a different approach and does not view *n*-hexane as the sole member of this chemical class with the potential to cause neuropathy. Russian PDK (Russian acronym representing their long-term air quality guideline) values are also provided for comparison purposes.

Table 6-5 includes the SMACs for the C2-C9 saturated alkanes established by the National Aeronautics and Space Administration through the evaluation presented in this document. For the C2-C4 alkane gases (ethane, propane, and butane), 10% of each chemical's lower explosive limit was established as the SMAC. As described earlier in this document, it was determined that these gases were not likely to result in toxicity in association with environmentally relevant exposures, and ensuring that levels of explosivity are not approached would be adequately protective of any direct toxicity associated with these compounds. The C2-C4 alkanes were not grouped with the C5-C9 alkanes for that reason.

A literature review identified three general categories of effects potentially relevant to the C5-C9 alkanes: sensory irritation, CNS effects, and auditory toxicity. These effects were further considered for acceptable concentration (AC) development and are discussed later in this document (for a summary of ACs, see Table 6-6). Because of the lack of individual studies for each C5-C9 alkane, data were not available for each effect. Accordingly, an attempt was made to use available data for conservative representatives for the C5-C9 group that could adequately represent C5-C9 alkane toxicity for each effect without underesti-

mating health risks. For example, studies suggest that sensory irritation may be a concern with the C5-C9 alkanes. Although sensory irritation data on every alkane of interest were not available, the data that were available suggested that *n*-nonane was a conservative representative for the group because it was predicted to have the lowest threshold for sensory irritant effects.

TABLE 6-4 Exposure Limits Set by Other Organizations

	ACGIH-TLV[a,d] ppm	OSHA PEL[b,d] ppm	NIOSH REL[b, d] ppm	Russian PDK[c] (360 d), ppm
Ethane	1,000	—	—	—
Propane	1,000	1,000	1,000	—
Butane	1,000	—	800	—
Pentane	600	1,000	120	3
n-Hexane	50	500	50	1
Other hexane isomers	500 1,000 STEL/CEIL	— —	100 510 STEL/CEIL	1 —
Heptane	400 500 STEL/CEIL	500 —	85 440 STEL/CEIL	2 —
Octane	300	500	75	—
Nonane	200	—	200	—

[a]Source: ACGIH 2008.
[b]Source: NIOSH 2005.
[c]International Space Station Medical Operations Requirements Document (MORD), Johnson Space Center 50620, National Aeronautics and Space Administration.
[d]Value in column is a time-weighted average (TWA) unless otherwise specified.
Note: Limits apply to all isomers unless otherwise specified.
Abbreviations: —, not applicable; PEL, permissible exposure limit; REL, recommended exposure limit; STEL/CEIL, short-term exposure limit/ceiling; TLV, threshold limit value.
Source: ACGIH 2008. Reprinted with permission; copyright 2008, American Conference of Industrial Hygienists.

TABLE 6-5 Spacecraft Maximum Allowable Concentrations C2-C9 Alkanes (ppm)

	1 h	24 h	7 d	30 d	180 d
Ethane[a]	3,200	3,200	3,200	3,200	3,200
Propane[a]	2,300	2,300	2,300	2,300	2,300
Butane[a]	1,900	1,900	1,900	1,900	1,900
C5-C9[b] (target toxicity)	150 (irritation, CNS effects)	80 (irritation, CNS effects)	60 (CNS effects)	20 (ototoxicity[c], CNS effects)	3 (ototoxicity[c])

[a]10% of the LEL for this compound is set as the SMAC.
[b]Includes all isomers and members except *n*-hexane, which has its own SMAC.
[c]Overall ototoxicity risk will depend on noise levels in the specific environment, because hearing damage may be caused by both mechanical and chemical injury.
Abbreviation: LEL, lower explosive limit.

TABLE 6-6 Acceptable Concentrations

			Uncertainty Factors				Acceptable Concentration (ppm)				
End Point	Data and Reference	Species	NOAEL	Time	Species	Space-flight	1 h	24 h	7 d	30 d	180 d
Sensory irritation	*n*-Nonane as surrogate; 95% lower confidence limit on the RD_{10}; 440 ppm, 0-10 min; Kristiansen and Nielsen 1988	CF-1 mice	1	1	3	1	150	150	—	—	—
CNS effects	*n*-Nonane; 2,414 ppm (NOAEL), 8 h; Nilsen et al. 1988	Sprague-Dawley rats	1	1 (1 h) 8/24 h (24 h)	10	1	240	80	—	—	—
	n-Nonane; 590 ppm (NOAEL), 6 h/d, daily evaluation over 13-wk study; Carpenter et al. 1978	Wistar rats	1	1	10	1	—	—	60	60	60
Auditory toxicity	*n*-Heptane; 800 ppm NOAEL, 6 h/d, 28 d; Simonsen and Lund 1995	Long Evans rats	1	6/24 h 28/180 d	10	1	—	—	—	20	3
SMACs							*150*	*80*	*60*	*20*	*3*

Efforts were made to eliminate differences that might limit the usefulness and applicability of a group SMAC. Thus, *n*-hexane has its own SMAC in accordance with the understanding that its neurotoxicity is not relevant to the other C5-C9 alkanes. Even with these efforts, however, it is clear that the remaining alkanes are not equal in terms of their potential for adverse health effects. Accordingly, the measurement of an exceedance of the C5-C9 SMAC over a particular time frame should prompt a closer examination of the specific alkane to ensure that any actions taken are commensurate with the actual risk that compound poses. For example, if the group SMAC is being applied to evaluate *n*-pentane measurements, slight exceedances of the SMAC would be viewed with less health significance than if *n*-nonane measurements were being evaluated.

Although there are limitations inherent in establishing group SMACs, it can often be a useful and practical approach to risk assessment when reliable data are scanty for all group members but there is a reasonable basis and ability to predict toxicologic response within a grouping (Robinson 2000). For example, establishing a reference compound that can represent a broader group of chemicals plays a central role in some advocated approaches to risk assessment for total petroleum hydrocarbonfractions. The Massachusetts Department of Environmental Protection has recommended grouping C9-C17 alkanes and identifying a reasonably conservative reference compound (in this case, *n*-nonane) for the purposes of establishing a reference dose (MA DEP 1994).

Benchmark dose analysis was considered but was determined to be inappropriate for the end points and studies evaluated. Specifically, the approach taken in developing sensory irritation ACs utilized a statistical procedure that precluded benchmark dose analysis. For other end points, critical studies did not include sufficient dosing groups to allow meaningful modeling of the dose-response relationship.

Sensory Irritation

The 1-h and 24-h ACs for sensory irritation were calculated for C5-C9 alkanes based on the work of Kristiansen and Nielsen (1988). These sensory irritation effects are evidenced as a burning sensation in the eyes, nose, or throat that is mediated by receptors on the trigeminal nerve endings within the nasal mucosa (Alarie 1981). The effect is measured by evaluating exposure concentrations and corresponding reductions in respiratory rate in exposed mice. Predicted concentration-response relationships were calculated for *n*-heptane, *n*-octane, and *n*-nonane and were expressed in regression equations developed for each alkane. To use these study results in SMAC development, it was necessary to identify a specific degree of sensory irritation that is consistent with the level of irritation allowable for varying exposure durations. With guidance from the National Research Council committee, we used the 95% lower confidence limit on the RD_{10} (lower confidence limit on the concentration that corresponds to a 10% reduction in the respiratory rate relative to controls). We found this level of

response to be appropriately conservative for use in evaluating short-term exposures and generally consistent with the guidelines used in establishing a point of departure for benchmark dose analysis (that is, a lower confidence limit on a specific response rate is identified).

Among the alkanes evaluated, *n*-nonane produced an irritant response at the lowest concentration. Thus, we used *n*-nonane as a conservative representative compound in setting ACs for sensory irritation for the alkanes. To better reflect sampling variability inherent in the RD_{10} estimate, we used a bootstrap analysis to generate a distribution of possible RD_{10} values for *n*-nonane, with 5th, 50th, and 95th percentile estimates of the RD_{10} of 440 ppm, 1,095 ppm, and 1,700 ppm, respectively (90% confidence interval of 440-1,700 ppm) (Efron and Tibshirani 1993). We applied the lower 95% confidence limit on the RD_{10} (440 ppm) in establishing the AC for this end point.

Although these effects were measured in mice, the few human observations available seem to correlate reasonably well with these predictions (Alarie 1981, Kristiansen and Nielsen 1988), and mice are not thought to underpredict the human health risks for sensory irritants (Buckley et al. 1984, Schaper 1993). Therefore, we applied an uncertainty factor (UF) of only 3 to account for necessary species extrapolation. It was not necessary to further adjust the RD_{10} estimate when determining a 24-h AC, because any sensory irritant effects are likely to be exhibited during the first few minutes of exposure and are not expected to occur at progressively lower concentrations in conjunction with longer term exposures. Also, because *n*-nonane is being used as a basis for the AC, any short-term irritation would be extremely mild and would also be acceptable for 24 h of exposure.

Sensory irritation:

1- and 24-h $_{(\text{AC sensory irritation})}$ = 440 ppm $_{(\text{lower 95\% confidence limit on the RD10})}$
× 1/3 $_{(\text{species factor})}$ = 147 ppm, rounded to 150 ppm

CNS Effects

Available studies addressing CNS effects related to the C5-C9 alkanes are relevant to the 1-h, 24-h, 7-d, 30-d, and 180-d ACs.

With respect to the 1-h and 24-h ACs, Nilsen et al. (1988) reported CNS effects including ataxia, focal seizure, and spasms in rats exposed to 3,560 ppm, 4,438 ppm, and 5,280 ppm of *n*-nonane for 8 h. Effects were observed within 2-4 h of exposure, and they proposed a NOAEL of 2,414 ppm. In calculating the ACs, a UF of 10 was applied to account for necessary species extrapolations. Although CNS effects are generally more dependent on the attainment of critical blood concentrations than on exposure duration, a time adjustment was applied for the 24-h AC to account for the possibility that enough time may not have passed during the 8-h study for the CNS effects to be fully realized (Zahlsen et al. 1990).

CNS:

1-h AC $_{(CNS)}$ = 2,414 ppm $_{(NOAEL)}$ × 1/10 $_{(species\ factor)}$ = 241 ppm, rounded to 240 ppm

24-h AC $_{(CNS)}$ = 2,414 ppm $_{(NOAEL)}$ × 1/10 $_{(species\ factor)}$ × 8 h/24 h $_{(time\ extrapolation)}$ = 80 ppm

The CNS effects reported by Carpenter et al. (1978) after inhalation exposures to *n*-nonane were used as the basis for the 7-d, 30-d, and 180-d ACs. Rats exposed to 1,500 ppm of *n*-nonane (6 h/d) were observed to exhibit mild tremors and coordination loss. However, in a separate experiment described in the same paper, no such effects were reported in rats exposed to *n*-nonane at 590 ppm and evaluated daily over 13 wk (6 h/d, 5 d/wk), although rats in the 1,600-ppm exposure group did show such effects. Thus, 590 ppm was applied as a NOAEL for these CNS effects in developing the 7-d, 30-d, and 180-d ACs. A UF of 10 was applied to account for species extrapolation. Time adjustments were not considered to be necessary for these longer term ACs because CNS effects are generally expected to be more dependent on the attainment of critical blood concentrations. This is supported by the observations of Carpenter et al. (1978) that CNS effects occurred within the first few days of exposure and that these effects did not appear to worsen with longer exposures. This is further corroborated by modeling predictions (Robinson 2000) and measured data on rat inhalation of *n*-nonane (Zahlsen et al. 1990). It was observed that peak brain concentrations of *n*-nonane, which are thought to be proportional to the potential for hydrocarbon-induced CNS effects (Baker et al. 1985), are likely attained within the first day of exposure.

CNS:

7-d AC $_{(CNS)}$ = 590 ppm $_{(NOAEL)}$ × 1/10 $_{(species\ factor)}$ = 59 ppm, rounded to 60 ppm

30-d AC $_{(CNS)}$ = 590 ppm $_{(NOAEL)}$ × 1/10 $_{(species\ factor)}$ = 59 ppm, rounded to 60 ppm

180-d AC $_{(CNS)}$ =590 ppm $_{(NOAEL)}$ × 1/10 $_{(species\ factor)}$ = 59 ppm, rounded to 60 ppm

Ototoxicity

In a study of inhalation exposures of rats to *n*-heptane, Simonsen and Lund (1995) observed ototoxicity in rats as measured through changes in audi-

tory brain stem responses. They exposed groups of rats to *n*-heptane at 0 ppm, 800 ppm, and 4,000 ppm for 6 h/d over 28 d. Rats in the 4,000-ppm exposure group experienced changes in auditory brain stem responses that corresponded to an approximate 10 decibel (dB) increase of the auditory threshold. Rats in the 800-ppm group did not experience significant differences relative to controls. Thus, 800 ppm was taken as a NOAEL for this effect, and 30-d and 180-d ACs were calculated. A UF of 10 was applied to account for species extrapolation. In addition, a time adjustment was made to account for the less-than-continuous exposure regimen (6 h/d). In extending the 28-d findings to calculate a 180-d AC, an additional time adjustment (180 d/28 d) was also applied to account for the shorter exposure. There is some uncertainty in terms of the need for this adjustment for exposure duration. Simonsen and Lund (1995) noted that *n*-heptane is a relatively weak ototoxic agent that would likely act similarly to trichloroethylene and toluene (Pryor et al. 1984, Rebert et al. 1991), which generally exhibit a threshold in animal testing below which even long-term exposures do not result in ototoxicity. However, scanty data are available on the role of exposure duration in the ototoxicity of the saturated alkanes. Also, epidemiologic data from occupational cohorts suggest that length of exposure can be a contributing factor to ototoxicity for certain solvents (Morata et al. 2002, Sliwinska-Kowalska et al. 2004), although confounding factors make it difficult to assess fully the relevancy of these findings in interpreting animal study results.

Overall risk of hearing damage will depend on noise levels in the actual space environment because damage may be caused by both mechanical and chemical injury. Noise is an important aeromedical factor for both ISS and Shuttle operations, and flight rules limit the amount of noise that can be tolerated for 24 h (65 dB for ISS and 74 dB for Shuttle). The more stringent noise requirement for ISS is partially because of the longer period of crew habitation than for Shuttle. Measurements above these limits prompt mitigation efforts to reduce noise as well as precautionary actions such as the use of hearing protection. Shorter term noise limits have also been established to guide decision making (e.g., Shuttle flight rules state that noise shall not exceed 86 dBA for any time period). Although not specifically intended to address cumulative effects due to noise and chemical exposures, limiting noise levels plays an important role in minimizing the potential for auditory damage.

Ototoxicity:

$$\text{30-d AC}_{(\text{ototoxicity})} = 800\ \text{ppm}_{(\text{NOAEL})} \times 1/10_{(\text{species factor})} \times 6\ \text{h}/24\ \text{h}_{(\text{time extrapolation})} = 20\ \text{ppm}.$$

$$\text{180-day AC}_{(\text{ototoxicity})} = 800\ \text{ppm}_{(\text{NOAEL})} \times 1/10_{(\text{species factor})} \times [6\ \text{h}/24\ \text{h} \times 28\ \text{d}/180\ \text{d}]_{(\text{time extrapolation})} = 3\ \text{ppm}.$$

BOOTSTRAPPING THE SENSORY IRRITATION DATA

This section describes the nonparametric bootstrap method used to estimate confidence intervals for the regression on the sensory irritation data for *n*-nonane reported by Kristiansen and Nielsen (1988). Bootstrapping represents a statistical analysis that allows for better characterization of the sampling variability inherent in estimating an RD_{10} concentration (Efron and Tibshirani 1993).

From the authors:

n-Nonane concentration (ppm)	% decrease relative to controls (0-10 min)
1,159	8.77
1,521	17.18
2,873	21.97
3,443	21.25
4,439	20.54
4,862	12.71
5,433	27.70
6,182	29.95
6,358	34.37

Starting with the linear model: $y = b_0 + b_1 \times \log_{10}(x)$.

$\log (RD_{10}) = \log_{10}(x) = (y - b_0)/b1 = (10 - b_0)/b_1$.

The goal of the bootstrap method is to generate bootstrap sample data [x, y] from the original data, (x_i, y_i), i = 1, 2, ..., 9. Specifically, bootstrap sample data are data sets of the same size (n = 9) sampled from the original data set with replacement. An example of a bootstrap resample of the data could be as follows:

1 (x_3, y_3)
2 (x_1, y_1)
3 (x_4, y_4)
4 (x_7, y_7)
5 (x_7, y_7)
6 (x_2, y_2)
7 (x_3, y_3)
8 (x_2, y_2)
9 (x_5, y_5)

For each bootstrap sample data set, the linear model is refit to obtain b_0, b_1; hence, $\log_{10}(x10)$. From 1,000 bootstrap samples, we acquire 10,000 corresponding estimates of $\log_{10}(x\ 10)$. The quantiles of this empirical distribution for *n*-nonane are as follows:

	5%	50%	95%
$\log_{10}(x10)$	2.6	3.0	3.2
$x10$	440.5	1095.9	1723.5

The 90% bootstrap confidence interval of log $RD_{10}(x10)$ is [2.6, 3.2] and the 90% confidence interval of the RD_{10} is [440, 1723].

REFERENCES

ACGIH (American Council of Governmental Industrial Hygienists). 1991. Documentation of the Threshold Limit Values and Biological Exposure Indices, 6th Ed. American Council of Governmental Industrial Hygienists, Cincinnati, OH.

ACGIH (American Council of Governmental Industrial Hygienists) 2008. Guide to Occupational Exposure Values. American Council of Governmental Industrial Hygienists, Cincinnati, OH.

Alarie, Y. 1973. Sensory irritation by airborne chemicals. CRC Crit. Rev. Toxicol. 2(3):299-363.

Alarie, Y. 1981. Dose-response analysis in animal studies: Prediction of human responses. Environ. Health Perspect. 42:9-13.

Allerheiligen, S.R., T.M. Ludden, and R.F. Burk. 1987. The pharmacokinetics of pentane, a by-product of lipid peroxidation. Drug Metab. Dispos. 15(6):794-800.

Aranyi, C., W.J. O'Shea, C.A. Halder, C.E. Holdsworth, and B.Y. Cockrell. 1986. Absence of hydrocarbon-induced nephropathy in rats exposed subchronically to volatile hydrocarbon mixtures pertinent to gasoline. Toxicol. Ind. Health 2(1):85-98.

ATSDR (Agency for Toxic Substances and Disease Registry). 1999. Toxicological Profile for Hexane. Agency for Toxic Substances and Disease Registry, Atlanta, GA.

Baker, E.L., T.J. Smith, and P.J. Landrigan. 1985. The neurotoxicity of industrial solvents: A review of the literature. Am. J. Ind. Med. 8(3):207-217.

Brooks, T.M., A.L. Meyer, and D.H. Hutson. 1988. The genetic toxicology of some hydrocarbon and oxygenated solvents. Mutagenesis 3(3):227-232.

Buckley, L.A., X.Z. Jiang, R.A. James, K.T. Morgan, and C.S. Barrow. 1984. Respiratory tract lesions induced by sensory irritants at the RD50 concentration. Toxicol. Appl. Pharmacol. 74(3):417-429.

Carpenter, C.P., D.L. Geary, R.C. Myers, D.J. Nachreiner, L.J. Sullivan, and J.M. King. 1978. Petroleum hydrocarbon toxicity studies: XVII. Animal response to n-nonane vapor. Toxicol. Appl. Pharmacol. 44(1):53-61.

Dahl, A.R. 1989. The fate of inhaled octane and the neprotoxicant, isooctane in rats. Toxicol. Appl. Pharmacol. 100(2):334-341.

Daugherty, M.S., T.M. Ludden, and R.F. Burk. 1988. Metabolism of ethane and pentane to carbon dioxide by the rat. Drug Metab. Dispos. 16(5):666-671.

DiVincenzo, G.D., W.J. Krasavage, and J.L. O'Donoghue. 1980. Role of metabolism in hexacarbon neuropathy. Dev. Toxicol. Environ. Sci. 6:183-200.

EEMA (European Agency for the Evaluation of Medicinal Products). 1995. Summary Report: Propane, n-butane, Isobutene. Committee for Veterinary Medicinal Products, European Agency for the Evaluation of Medicinal Products, London.

Efron, B, and R.J. Tibshirani. 1993. An Introduction to the Bootstrap. New York: Chapman & Hall.

Epstein, S.S., E. Arnold, J. Andrea, W. Bass, and Y. Bishop. 1972. Detection of chemical mutagens by dominant lethal assay in the mouse. Toxicol. Appl. Pharmacol. 23(2):288-325.

Filser, J.G., G.Y. Csanady, W. Dietz, W. Kessler, P.E. Kreuzer, M. Richter, and A. Stormer. 1996. Comparative estimation of the neurotoxic risks of n-hexane and n-heptane in rats and humans based on the formation of the metabolites 2,5-hexanedione and 2,5-heptanedione. Pp. 1002-1023 in Biological Reactive Intermediates V: Basic Mechanistic Research in Toxicology and Human Risk Assessment, R. Snyder, J.J. Kocsis, I.G. Sipes, G.F. Kalf, D.J. Jollow, H. Greim, T.J. Monks, and C.M. Witmer, eds. New York: Plenum Press.

Finkel, A. 1983. Aliphatic hydrocarbons. Pp. 245-255 in Hamilton and Hardy's Industrial Toxicology, 4th Ed. London: John Wright PSG.

Frank, H., T. Hintze, D. Bimboes, and H. Remmer. 1980. Monitoring lipid peroxidation by breath analysis: Endogenous hydrocarbons and their metabolic elimination. Toxicol. Appl. Pharmacol. 56(3):337-344.

Frommer, U., V. Ullrich, and H. Staudinger. 1970. Hydroxylation of aliphatic compounds by liver microsomes. I. The distribution pattern of isomeric alcohols. Hoppe Seylers Z. Physiol. Chem. 351(8):903-912.

Frontali, N., M.C. Amantini, A. Spagnolo, A.M. Guarcini, M.C. Saltari, F. Brugnone, and L. Perbellini. 1981. Experimental neurotoxicity and urinary metabolites of the C5-C7 aliphatic hydrocarbons used as glue solvent in shoe manufacturing. Clin. Toxicol. 18(12):1357-1367.

Galvin, J.B., and F. Marashi. 1999. n-Pentane. CAS No. 109-66-0. J. Toxicol. Environ. Health A 58(1-2):35-56.

Graham, D.G., and M.B. Abou-Donia. 1980. Studies of the molecular pathogenesis of hexane neuropathy. 1. Evaluation of the inhibition of glyceraldehydes-3-phosphate dehydrogenase by 2,5-hexanedione. J. Toxicol. Environ. Health 6(3):621-631.

Halder, C.A., G.S. Van Gorp, N.S. Hatoum, and T.M. Warne. 1986. Gasoline vapor exposures. Part II. Evaluation of the nephrotoxicity of the major C4/C5 hydrocarbon components. Am. Ind. Hyg. Assoc. J. 47(3):173-175.

Hau, K.M., D.W. Connell, and B.J. Richardson. 2002. A study of the biological partitioning behavior of n-alkanes and n-alkanols in causing anesthetic effects. Regul. Toxicol. Pharmacol. 35(2 Pt. 1):273-279.

Hawas, O., D. Hawker, A. Chan, D. Cohen, E. Christensen, G. Golding, and P. Voweles. 2001. Characterization and identification of sources of VOCs in an industrial area of Brisbane. Australian Nuclear Science and Technology Organization Publication.

Hurtt, M.E., and G.L. Kennedy. 1999. A limited developmental toxicity study of pentane by inhalation in the rat. Food Chem. Toxicol. 37(5):565-567.

Katzenstein, A.S., L.A. Doezema, I.J. Simpson, D.R. Blake, and F.S. Rowland. 2003. Extensive regional atmospheric hydrocarbon pollution in the southwestern United States. Proc. Natl. Acad. Sci. 100(21):11975-11979.

Kirwin, C., W. Thomas, and V. Simmon. 1980. In vitro microbiological mutagenicity studies of hydrocarbon propellants. J. Soc. Cosmet. Chem. 31:367-370.

Kristiansen, U., and G.D. Nielsen. 1988. Activation of the sensory irritant receptor by C70- C11 n-alkanes. Arch. Toxicol. 61(6):419-425.

Loury, D.J., T. Smith-Oliver, and B.E. Butterworth. 1987. Assessment of the covalent binding potential of 2,2,4-trimethylpentane to rat alpha2u-globulin. Toxicol. Appl. Pharmacol. 88(1):44-56.

MA DEP (Massachusetts Department of Environmental Protection). 1994. Interim Final Petroleum Policy: Development of Health-Based Alterative to the Total Petroleum Hydrocarbon Parameter. Massachusetts Department of Environmental Protection, Boston, MA.

McKee, R., E. Frank, J. Heath, D. Owen, R. Przygoda, G. Trimmer, and F. Whitman. 1998. Toxicology of n-pentane (CAS No. 109-66-0). J. Appl. Toxicol. 18(6):431-442.

Morata, T.C., A.C. Johnson, P. Nylen, E.B. Svensson, J. Cheng, E.F. Krieg, A.C. Lindblad, L. Ernstgard, and J. Franks. 2002. Audiometric findings in workers exposed to low levels of styrene and noise. J. Occup. Environ. Med. 44(9):806-814.

Nilsen, O.G., O.A. Haugen, K. Zahlsen, J. Halgunset, A. Helseth, H. Aarset, and I. Eide. 1988. Toxicity of n-C9 to n-C13 alkanes in the rat on short-term inhalation. Pharmacol. Toxicol. 62(5):259-266.

NIOSH (National Institute for Occupational Safety and Health). 2005. NIOSH Pocket Guide to Chemical Hazards: Carbon dioxide. NIOSH Publication No. 2005-149. National Institute for Occupational Safety and Health [online]. Available: http://www.cdc.gov/niosh/npg/npgd0103.html [accessed August 26, 2008].

Perbellini, L., F. Brugnone, D. Caretta, and G. Maranelli. 1985. Partition coefficients of some industrial aliphatic hydrocarbons (C5-C7) in blood and human tissues. Br. J. Ind. Med. 42(3):162-167.

Pryor, G.T., C.S. Rebert, J. Dickinson, and E.M. Feeney. 1984. Factors affecting toluene-induced ototoxicity in rats. Neurobehav. Toxicol. Teratol. 6(3):223-238.

Rebert, C.S., V.L. Day, M. Matteucci, and G.T. Pryor. 1991. Sensory-evoked potentials in rats chronically exposed to trichloroethylene: Predominant auditory dysfunction. Neurotoxicol. Teratol. 13(1):83-90.

Robinson, P. 2000. Pharmacokinetic Modeling of JP-8 Jet Fuel Components. I. Nonane and C9-C12 Aliphatic Components. AFRL-HE-WP-TR-2000-0046. U.S. Air Force Research Laboratory, Wright Patterson AFB OH.

Sandmeyer, E.E. 1981. Aliphatic hydrocarbons. Pp. 3175-3220 in Patty's Industrial Hygiene and Toxicology, Volume II B, 3rd Rev. Ed., G.D. Clayton, and F.E. Clayton, eds. New York: John Wiley & Sohn.

Schaper, M. 1993. Development of a database for sensory irritants and its use in establishing occupational exposure limits. Am. Ind. Hyg. Assoc. J. 54(9):488-544.

Schaumburg, H.H., and P.S. Spencer. 1976. Degeneration in central and peripheral nervous systems produced by pure n-hexane: An experimental study. Brain 99(2):183-192.

Short, B.G., V.L. Burnett, and J.A. Swenberg. 1986. Histopathology and cell proliferation induced by 2, 2, 4-trimethylpentane in the male rat kidney. Toxicol. Pathol. 14(2):194-203.

Simonsen, L., and S.P. Lund. 1995. Four week inhalation exposure to n-hexane causes loss of auditory sensitivity in rats. Pharmacol. Toxicol. 76(1):41-46.

Sliwinska-Kowalska, M., E. Zamyslowska-Szmytke, W. Szymczak, P. Kotylo, M. Fiszer, W. Wesolowski, M. Pawlaczyk-Luszczynska, M. Bak, and A Gajda-Szadkowska. 2004. Effects of coexposure to noise and mixture of organic solvents on hearing in dockyard workers. J. Occup. Environ. Med. 46(1):30-38.

Spencer, P.S., H.H. Schaumberg, M.I. Sabri, and B. Veronesi. 1980. The enlarging view of hexacarbon neurotoxicity. Crit. Rev. Toxicol. 7(4):279-356.

Stadler, J.C., A.J. O'Neill, G.S. Elliot, and G.L. Kennedy. 2001. Repeated exposure inhalation study of pentane in rats. Drug Chem. Toxicol. 24(2):75-86.

Swann, H.E., B.K. Kwon, G.K. Hogan, and W.M. Snellings. 1974. Acute inhalation toxicology of volatile hydrocarbons. Am. Ind. Hyg. Assoc. J. 35(9):511-518.

Takeuchi, Y., Y. Ono, N. Hisanaga, J. Kitoh, and Y. Sugiura. 1980. A comparative study of the neurotoxicity of n-pentane, n-hexane, and n-heptane in the rat. Br. J. Ind. Med. 37(3):241-247.

Truhaut, R., P. Laget, G. Piat, P.L. Nguyen, H. Dutertre-Catella, V.N. Huyen, F. Frédéric, and E. Shechter 1973. Preliminary electrophysiologic results following experimental poisoning with technical hexane and heptane in white rats [in French]. Arch. Mal. Prof. Med. Trav. Secur. Soc. 34(7-8):417-426.

Veulemans, H., E. Van Vlem, H. Janssens, R. Masschelein, and A. Leplat. 1982. Experimental human exposure to n-hexane. Study of the respiratory uptake and elimination, and of n-hexane concentrations in peripheral venous blood. Int. Arch. Occup. Environ. Health 49(3-4):251-263.

Yamada, S. 1967. Polyneuritis in workers exposed to n-hexane, its causes and symptoms [in Japanese]. Jpn. J. Ind. Health 9:651-659.

Yano, B.L., D.A. Dittenber, R.R. Albee, and J.L. Mattsson. 1992. Abnormal auditory brainstem responses and cochlear pathology in rats induced by an exaggerated styrene exposure regimen. Toxicol. Pathol. 20(1):1-6.

Zahlsen, K, A.M. Nilsen, I. Eide, and O.G. Nilsen. 1990. Accumulation and distribution of aliphatic (n-nonane) aromatic (1,2,4-trimethylbenzene) and naphthenic (1,2,4, trimethylcyclohexane) hydrocarbons in the rat after repeated inhalation. Pharmacol. Toxicol. 67(5):436-440.

7

Carbon Dioxide

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OCCURRENCE AND USE

Carbon dioxide is the major expired by-product of human metabolism; if not effectively controlled, it can rapidly accumulate to dangerous concentrations in spacecraft atmospheres. On earth, the outdoor CO_2 concentration is typically about 0.03%, and average indoor air contains CO_2 in the range of 0.08% to 0.1% (IEQ 2006). In nominal spacecraft operations, the CO_2 concentration is typically about 0.5%, but the concentration approached 2% during the troubled Apollo 13 mission (Michel et al. 1975). Carbon dioxide can also enter the atmosphere of a space habitat from accidental combustion of materials, from operation of payloads that use CO_2 as an intravehicular propellant, and from use of the fire extinguisher, which, on the U.S. segment of the International Space Station (ISS), is CO_2.

SUMMARY OF ORIGINAL APPROACH

The original spacecraft maximum allowable concentrations (SMACs) for CO_2 were set by Wong (1996) and the National Research Council (NRC) SMACs Subcommittee, with substantial input and cooperation from National Aeronautics and Space Administration (NASA) environmental control engineers and the ISS Program Office. The following end points were evaluated: neurological (visual impairment, tremor, central nervous system [CNS] depression), headache, dyspnea and intercostal pain, increases in airway resistance, intolerance to hyperventilation, exercise impairment, and testicular injury. For all end points except testicular injury, the data came from human studies; testicular injury data came from exposed guinea pigs and rats. The "small n factor" was often used to compensate for the fact that many results were from human studies

in which a no-effect level was identified for the specific end point, but there was not an effect (or low) response level. Under these conditions, the no-observed-adverse-effect level (NOAEL) was multiplied by the factor $\sqrt{(n)}/10$, where n was the number of subjects in the study. The original summary table is presented in Appendix A. The end points were considered as described in the following sections (Wong 1996).

Neurological

This end point was evaluated from the observation that 3% CO_2 was a NOAEL for CNS effects in two studies; one was a 5-d exposure involving 7 subjects and the other was a 2-wk exposure involving 12 subjects (Glatte et al. 1967; Storm and Giannetta 1974). The calculation was as follows:

$$AC_{(CNS)} = 3\%_{(NOAEL)} \times \sqrt{(19)}/10_{(\text{small n factor})} = 1.3\%$$

where AC is acceptable concentration.

This AC was applied to all exposure times because CNS effects would not be acceptable even for brief periods; however, there is no basis for supposing that prolonged exposures would result in an accumulation of CO_2 that could have CNS effects.

Headaches

Evidence was presented that CO_2-induced headaches are transient and that they were rare in a 30-d study of six humans exposed to 2% CO_2 and exercising periodically during their exposure (Radziszewski et al. 1988). On the basis of this observation, 2% was assigned as a NOAEL for CO_2-induced headaches (Wong 1996).

Dyspnea and Intercostal Pain

Wong used two studies to determine the AC for this adverse effect. Menn et al. (1970) found that exposure to 2.8% CO_2 for 0.5 h did not elicit intercostal pain or dyspnea in eight subjects. Likewise, Sinclair et al. (1971) found none of his four subjects experienced dyspnea or intercostal pain when exposed to 2.8% CO_2 for 1 h or 15-20 d. From these data, Wong derived a short-term AC to protect against this end point. He did not use the small n factor, because some risk of minor effects is tolerated for short-term exposures. The 1- and 24-h ACs to protect against dyspnea and intercostal pain were as follows:

$$\text{1- and 24-h } AC_{(\text{dyspnea, intercostal pain})} = 2.8\%.$$

For the longer-term SMACs, these effects would not be tolerated, so the small n factor was used with data from Sinclair et al. (1971) (n = 4) and Radziszewski et al. (1988) (n = 6) as follows:

$$\text{7-, 30-, and 180-d AC}_{\text{(dyspnea, intercostal pain)}} = 2.8\%_{\text{(NOAEL)}} \times \sqrt{(10)}/10_{\text{(small n factor)}} = 0.9\ \%$$

Hyperventilation and Exercise Ability

For short durations (1 or 24 h), hyperventilation was viewed as a physiological adaptation and SMACs were not set for contingencies for this effect. For long-term exposures, hyperventilation would not be acceptable, so Wong used the results of three studies to set this limit (Sinclair et al. 1969; Guillerm and Radziszewski 1979; Radziszewski et al. 1988). He noted that one can conclude from those three studies (n=14) that prolonged exposure to 2% CO_2 does not cause noticeable hyperventilation. The calculation was as follows:

$$\text{7-, 30-, and 180-d AC}_{\text{(hyperventilation)}} = 2\%_{\text{(NOAEL)}} \times \sqrt{(14)}/10_{\text{(small n factor)}} = 0.7\%$$

For long-term exposures, Wong also considered whether the crew's ability to exercise would be impaired. Looking at three studies (Glatte et al. 1967; Sinclair et al. 1971; Radziszewski et al. 1988) with a total of 16 subjects, Wong (1996) concluded that prolonged exposures to 2% CO_2 did not limit the ability to exercise. The calculation was as follows:

$$\text{7-, 30-, 180-d AC}_{\text{(exercise ability)}} = 2\%_{\text{(NOAEL)}} \times \sqrt{(16)}/10_{\text{(small n factor)}} = 0.8\%$$

Increases in Airway Resistance

On the basis of a report by Glatte et al. (1967) using seven subjects, Wong (1996) deduced that an exposure to 3% CO_2 for 5 d was a NOAEL for increased airway resistance. This level was not adjusted for the small n factor in setting ACs for 1- and 24-h exposures, because the safety factor is not needed for contingency situations. Thus,

$$\text{1- and 24-h AC}_{\text{(incr. airway resistance)}} = 3\%$$

Wong (1996) pointed out that the increased resistance is thought to be due to direct, local hypercapnia effects on the larynx; hence, severity would not be expected to increase with time of exposure. The long-term ACs to prevent increased airway resistance were calculated by using the small n factor as follows:

7-, 30-, and 180-d AC $_{\text{(incr. airway resistance)}}$ = 3% $_{\text{(NOAEL)}}$
× √(7)/10 $_{\text{(small n factor)}}$ = 0.8%

Testicular Effects

One of the more challenging findings Wong (1996) encountered was the report that rats exposed to CO_2 concentrations as low as 2.5% showed sloughing of mature spermatids and Sertoli cells after only 4 h of exposure (Vandemark et al. 1972). Since the changes in the testis were reversible 36 h after an 8-h exposure, this observation was not used to set a short-term AC for testicular effects.

To set the long-term standard in humans, Wong noted that a study in which guinea pigs and rats were exposed to 3% CO_2 for 42 d turned up no evidence of testicular effects in either species (Schaefer et al. 1971). The NRC SMAC Subcommittee advised that the toxicity is due to acidosis, and therefore the sensitivities of rodent and human testes to this effect should not differ. Thus,

7-, 30-, and 180-d AC $_{\text{(testicular effects)}}$ = 3% $_{\text{(NOAEL in rodents)}}$

CHANGES IN FUNDAMENTAL NRC-RECOMMENDED APPROACHES

The primary new tool for interpreting toxicity data is the benchmark dose modeling provided by the U.S. Environmental Protection Agency. This tool is regarded as an improvement over the NOAEL and lowest-observed-adverse-effect level (LOAEL) approach because it uses the entire dose-response curve to predict behavior of the dose-response relationship at concentrations below the lowest tested dose.

RELEVANT DATA SINCE 1995

Relevant data have emerged since the original SMAC was written in 1995. Two human studies were published shortly after that date, but they have serious limitations for risk assessment. Sun et al. (1996) exposed three subjects to 2.5% CO_2 for about ½ h (the time is unclear) and found that their depth perception (stereoacuity) decreased. In a related experiment, Yang et al. (1997) found that the ability of the three subjects to detect motion decreased with exposure to 2.5% CO_2. Both effects disappeared once the CO_2 exposure ended. These are interesting findings, but they are not suitable for human risk assessment because of the small value of n and because the relevance to crew performance during a contingency is unclear. The original acceptable concentration for preventing visual impairment was 1.3 %, which seems consistent with these reports (Wong 1996). Certainly, if CO_2 impairs visual ability in operationally significant ways, that must be considered in setting limits. Further experiments are required to determine the significance of these preliminary findings.

In a study of four human subjects (males in their 20s), exposures to 0.7% and 1.2% CO_2 for 20 d were found to increase the velocity of cerebral blood flow for the first 1-3 days of exposure; however, the flow gradually returned to preexposure values over the next 20 d (Sliwka et al. 1998). Only the subjects exposed to the higher concentration of CO_2 reported headaches, and that occurred only during the first days of exposure before adaptation. The authors concluded that autoregulation of cerebral vascular blood flow was preserved during chronic (20-d) exposure to these low levels of CO_2.

In what appears to be a companion paper to the one of Sliwka et al. (1998)., Manzey and Lorenz (1998) investigated the mental performance of four subjects continuously exposed to 0.7% and 1.2% CO_2 in a confined space for 26 d. They used four standardized performance tests: grammatical reasoning, memory search, unstable tracking, and dual task (doing unstable tracking and memory search together). Subjects were tested three times before CO_2 was introduced, 12 times during the exposures, and once after the exposures ended. They concluded that concentrations up to 0.7% in the ambient atmosphere do not cause any detrimental effects on human subjective mood or performance. A slight decrement was noted in the tracking task during exposures to 1.2% CO_2, but, in the judgment of the investigators, the magnitude of the effect was much smaller than those caused by other space flight stressors. These findings are consistent with the SMACs set by Wong (1996).

Evidence from Russian Mir Space Station had suggested that sleep quality changed in space, so this was investigated in a manner similar to the two investigations described above (Gundel et al. 1998). Four males exposed to 0.7% or 1.2% CO_2 were evaluated with "sleep polygraphs," which involved monitoring seven channels that included electroencephalogram and electrocardiogram parameters. The authors found that neither level of CO_2 altered sleep quality over the 26 d of the test.

Horn et al. (2003) reported on the incidence of minor health complaints in 122 submarine crew members exposed to an average CO_2 concentration of 0.49% during a 101-d mission. The only minor health problem that might reasonably be associated with CO_2 exposure is trouble sleeping and no apparent change was noted when the premission, first-half mission, and last-half mission were compared (not a statistically based conclusion). There was no control group, and the end points were poorly defined, so the results of this study add little to our understanding of the potential long-term effects of exposure to CO_2. There are a few studies of 5-7 d duration that evaluate crew survival during disabled-submarine tests. Typically, these studies focus on gross parameters of survival as opposed to detailed visual, memory, and neuromuscular testing, and they involve extreme cold along with high concentrations of CO_2. These studies were judged not to be useful for setting SMACs for CO_2 exposure.

The NRC (2007) set exposure limits for Navy submarine operations based largely on the SMAC document (Wong 1996). The only new studies identified in that report were those described above (Sun et al. 1996; Yang et al. 1997).

NEW RISK ASSESSMENT APPROACHES

Table 7-1 summarizes the opportunities that may exist to apply new risk assessment tools to the key studies supporting the original SMAC document.

RATIONALE FOR REVISIONS TO PREVIOUS SMACS

A large body of information exists on the physiological effects of excess CO_2 inhalation in human volunteers and in submariners chronically exposed to concentrations often exceeding 1%. No single study or small subset of studies gives definitive guidance for each potential adverse effect of long-term exposure to CO_2. Wong (1996) pieced together a rational picture of the potential adverse effects of exposure to CO_2 and derived a defensible set of SMACs, with which the NRC SMAC Subcommittee agreed. None of the studies meet current-day standards and many were never published in the peer-reviewed literature. Since Wong's effort, there have been no new studies to suggest that the original long-term SMACs need to be revised. On the basis of the visual-effects data of Sun et al. (1996) and Yang et al. (1997), the NRC Continuous Exposure Guidance Level (CEGL) Submarine Subcommittee (2007) determined a 90-d CEGL as follows:

$$\text{90-d CEGL} = 2.5\%_{\text{(LOAEL, visual effects)}} \times 1/3_{\text{(limited data factor)}} = 0.8\%$$

The factor of 3 was applied for "limited data." Indeed, the two studies on which this is based involved only three subjects given acute exposures of uncertain duration.

RATIONALE FOR THE 1,000-DAY SMAC

No studies are available that qualify as authentic chronic studies. The longest human exposures to elevated concentrations of CO_2 have occurred on submarines, and they seldom lasted longer than 100 d. Physiologically, the SMAC set for 180 d of exposure elicits a mild, subclinical adaptation to CO_2, and there is no reason to suppose such an adaptation could not persist for 1,000 d.

However, three factors present unique problems for chronic exposures to CO_2 in space: (1) crews on exploration missions *will* receive sustained exposure to elevated CO_2 levels and will not have the option to "fly up" more CO_2 scrubbing capability if the CO_2 levels become intolerable, (2) the repair and rescue options for missions in deep space are much more limited than low-earth-orbit missions, and (3) anecdotal data from ISS crews suggest that a few individuals may have a risk of mild headaches at concentrations near or above 0.6% CO_2 (Carr 2006). The 1,000-d SMAC is set conservatively at 0.5% to compensate for

TABLE 7-1 Applicability of a Benchmark Dose Modeling Approach

Study	Description	Applicability of the BMD Approach
Glatte et al. (1967)	Humans exposed to 3% (n = 7) or 4% (n = 4) CO_2 for 5 d. Findings were not reported as dose-response data. For example, at 3% subjects could exercise for 1 h, but at 4% they struggled to do 10 min. No psychomotor changes were found. Blood pH changes were compensated.	Unsuited for BMD due to lack of dose-response data and small n value.
Menn et al. (1970)	Humans (n = 7-8) exercised for 30 min, exposed to 1%, 2%, 2.8%, and 4% CO_2. The highest two levels caused dyspnea and intercostal pain, and the highest level caused headache. The only quantitative data were physiology data, not toxicity data.	Data unsuited for BMD because any apparent adverse effects were not given in quantitative dose-response terms.
Radziszewki et al. (1988)	Human experiments in days (and %) as follows: 1 d (4.3%), 9 d (3.8%), 8 d (2.9%), 30 d (1.9%, 1%, and 0.5%); n = 5 or 6. A number of physiological changes and exercise limitations were noted at the higher concentrations, but they were not indicative of adverse effects, nor were they quantitatively expressed.	No suitable toxicity data.
Schaefer et al. (1971)	The data consist of transient adaptations, changes in organ weights, changes in enzymes, and descriptive histological effects in rats and guinea pigs exposed to concentrations from 1% to 30% CO_2 for various times. The emphasis was on exposures to 3% or 15% CO_2. Large differences were noted in the susceptibility of rats and guinea pigs.	Only a small portion of the data appear to be quantitative, and they do not yield a dose-response relationship.
Sinclair et al. (1969)	From 4 to 8 subjects were exposed to 1% to 4% CO_2 and exercised for 30 min. Subjects (n = 3 or 4) were exposed for 5 or 11 d to 4% CO_2; blood and cerebrospinal fluid CO_2, bicarbonate, pH, and ventilation parameters were measured. Nine preventricular complexes were noted in the group that exercised.	Data in original report are illegible and provide no basis for a dose-response curve.
Sinclair et al. (1971)	Four subjects exposed to 2.8% CO_2 for 1 h or 15-20 d. CO_2 retention increased during work. The level was well tolerated at rest and with exercise. Some physiological changes.	No adverse effects were identified, one exposure level.
Storm and Giannetta (1974)	Four groups (n = 6) were exposed to air or 4% CO_2 for 2 wk in an active or a bed-rested state. They showed no decrement in psychomotor performance.	No adverse effects on parameters measured.

Abbreviation: BMD, benchmark dose modeling.

these realities and to force a somewhat more robust air revitalization design that provides a larger safety margin during exploration-class missions.

RATIONALE FOR AN INCREASE IN THE 1-HOUR SMAC

The original SMAC for 1 h was based on extrapolation of observed NOAELs in 19 subjects exposed to 3 % CO_2 for 5 to 14 d (Wong 1996). In our view, that approach is too conservative for a 1-h emergency exposure. The aggregate of data in human studies suggests that, during a 1-h exposure to 2% CO_2, physiological adaptation, mild headache, and hyperventilation may occur, but performance decrements will be insignificant. The short-term SMACs are set for emergencies, and some risk of mild effects on the crew is acceptable. According to current flight rules, the flight surgeon would not allow the crew to exercise if the CO_2 level were anywhere near 2%. Also, we expect that the crew will have adapted somewhat to relatively high CO_2 (about 0.5%) levels during the space flight, so a "sudden" increase above the running levels would be better tolerated than if no preexposure had occurred. Mild headache and hyperventilation would be easily tolerated for 1 h, thus the 1-h SMAC is increased to 2%.

COMPARISON OF SMACS WITH OTHER AIR QUALITY LIMITS

The 1-h SMAC is close to the Navy CEGL, but the 24-h SMAC is about half the 24-h CEGL. This seems reasonable because the space crew is likely to have fewer resources to deal with an emergency situation if CO_2 becomes elevated above nominal.

For example, the space crew might have to repair the CO_2 scrubber (a sophisticated task requiring at least several hours), whereas the submarine crew should be able to engage additional scrubbers as needed to stabilize the level of CO_2.

Table 7-2 compares SMACs with other air quality limits. The long-term SMACs (0.7%) are between the Navy CEGL (0.8%) and the industrial-worker exposure limits (0.5%). This is reasonable; industrial workers may need a lower limit because they never get a chance to physiologically adapt to workplace CO_2 exposures, whereas physiological adaptation occurs in long-term continuous exposures and astronauts are unaffected by CO_2 exposures confined to less than 0.7%. The rationale for a lower 1,000-d SMAC of 0.5% has already been stated.

RECOMMENDATIONS FOR ADDITIONAL RESEARCH

The most useful practical experiment would be to repeat the work of Sun et al. (1996) and Yang et al. (1997) in which they found evidence of visual disturbances. Before such an experiment begins, the user community must agree on how much of a "visual" deficit can be accepted given the methods available to

TABLE 7-2 Comparison of Exposure Standards

Source (Year Set)	Time	Limit (%)	Reference
NRC			NRC 2007
EEGL	1 h	2.5	
EEGL	24 h	2.5	
CEGL	90 d	0.8	
OSHA			NIOSH 2005
PEL	Working lifetime	0.5	
NIOSH			NIOSH 2005
REL	Working lifetime	0.5	
IDLH	Brief	4.0	
ACGIH			ACGIH 2004
TLV	Working lifetime	0.5	
STEL	15 min	3.0	
NASA SMAC	1 h	2.0[a]	Updated in current document
	24 h	1.3	Wong 1996
	7-180 d	0.7	Wong 1996
	1,000 d	0.5[b]	Updated in current document

[a]New value replaces 1.3%; mild headache and hyperventilation acceptable for 1 h.
[b]New value based on avoiding any risk of mild headache; no previous value set.
Abbreviations: ACGIH, American Conference of Governmental Industrial Hygienists; CEGL, continuous exposure guidance level; EEGL, emergency exposure guidance level; IDLH, immediately dangerous to life and health; NIOSH, National Institute for Occupational Safety and Health; NRC, National Research Council; OSHA, Occupational Safety and Health Administration; PEL, permissible exposure limit; REL, recommended exposure limit; SMAC, Spacecraft Maximum Allowable Concentration; STEL, short -term exposure limit; TLV, threshold limit value.

measure such a deficit. The experiment must be done with several visual end points and with at least 10 subjects, and it must use at least three exposures. In addition, it would be useful to develop a dose-response curve for the testicular lesions Vandemark et al. (1972) reported, because their findings appear to be inconsistent with those of other studies. The mechanism(s) of these effects needs to be understood in order to determine whether the effects are due to acidosis.

REFERENCES

ACGIH (American Conference of Governmental Industrial Hygienists). 2004. Threshold Limit Values. Cincinnati, OH: American Conference of Governmental Industrial Hygienists.

Carr, C. 2006. NASA White Paper. Houston, TX: National Aeronautics and Space Administration.

Glatte, H., B.O. Hartman, and B.E. Welch. 1967. Nonpathologic hypercapnia in man. Pp. 110-129 in Lectures in Aerospace Medicine. Report SAM-TR-68-116. Brooks Air Force Base, TX: USAF Aerospace Medical Division, USAF School of Medicine.

Guillerm, R., and E. Radziszewski. 1979. Effects on man of 30-day exposure to PI_{CO2} of 14 torr (2%): Application to exposure limits. Undersea Biomed. Res. (Suppl. 6): S91-S114.

Gundel, A., R.A. Parisi, R. Strobel, and M.R. Weihrauch. 1998. Characterization of sleep under ambient CO_2 levels of 0.7 % and 1.2%. Aviat. Space Environ. Med. 69(5):491-495.

Horn, W.G., T.L. Thomas, K. Marino, and T.I. Hooper. 2003. Health experience of 122 submarine crewmembers during a 101-day submergence. Aviat. Space Environ. Med. 74(8):858-862.

IEQ. 2006. Carbon Dioxide. Fact Sheets. IEQ Corporation [online]. Available: http://www.ieqcorp.com/carbon_dioxide.htm [accessed May 14, 2007].

Manzey, D., and B. Lorenz. 1998. Effects of chronically elevated CO_2 on mental performance during 26 days of confinement. Aviat. Space Environ. Med. 69(5):506-514.

Menn, S.J., R.D. Sinclair, and B.E. Welch. 1970. Effect of inspired P_{CO_2} up to 30 mmHg on response of normal man to exercise. J. Appl. Physiol. 28(5):663-671.

Michel, E.L., J.M. Waligora, D.J. Horrigan, and W.H. Schumate. 1975. Environmental factors. Section 2, Chapter 5 in Biomedical Results of Apollo, R.S. Johnston, L.F. Dietlein, and C.A. Berry, eds. NASA-SP 368. Washington, DC: National Aeronautics and Space Administration.

NIOSH (National Institute for Occupational Safety and Health). 2005. NIOSH Pocket Guide to Chemical Hazards: Carbon dioxide. NIOSH Publication No. 2005-149. National Institute for Occupational Safety and Health [online]. Available: http://www.cdc.gov/niosh/npg/npgd0103.html [accessed March 27, 2007].

NRC (National Research Council). 2007. Carbon dioxide. Pp. 46-66 in Emergency and Continuous Exposure Guidance Levels for Selected Submarine Contaminants, Vol. 1. Washington, DC: The National Academies Press.

Radziszewski, E., L. Giacomoni, and R. Guillerm. 1988. Physiological effects in man as a result of long duration confinement in an atmosphere enriched with carbon dioxide. Pp. 19-23 in Proceedings of the Colloquium on Space and Sea, November 24-27, 1987, Marseille, France, T.D. Guyenne, ed [in French]. Paris: European Space Agency. [Translated to English by N. Timacheff].

Schaefer, K.E., H. Niemoeller, A. Messier, E. Heyder, and J. Spencer. 1971. Chronic CO_2 Toxicity: Species Differences in Physiological and Histopathological Effects. Report 656. Groton, CT: Naval Submarine Medical Research Laboratory.

Sinclair, R.D., J.M. Clark, and B.E. Welch. 1969. Carbon dioxide tolerance levels for space cabins. Proceedings of the Fifth Annual Conference on Atmospheric Contamination in Confined Spaces, Sept. 16-18, Wright-Patterson Air Force Base, Dayton, Ohio, R.D. O'Donnell, H.A. Leon, A. Azar, C.H. Wang, R.L. Patrick, W. Mautner, M.E. Umstead, and ML. Taylor, eds. Air Force Aerospace Medical Research Lab Wright-Patterson AFB, OH.

Sinclair, R.D., J.M. Clark, and B.E. Welch. 1971. Comparison of physiological responses of normal man to exercise in air and in acute and chronic hypercapnia. Pp. 409-417 in Underwater Physiology, C.J. Lambertsen, ed. New York, NY: Academic Press.

Sliwka, U., J.A. Kransney, S.G. Simon, P. Schmidt, and J. North. 1998. Effects of sustained low-level elevations of carbon dioxide on cerebral blood flow and autoregu-

lation of the intracerebral arteries in humans. Aviat. Space Environ. Med. 69(3):299-306.

Storm, W.F., and C.L. Giannetta. 1974. Effects of hypercapnia and bedrest on psychomotor performance. Aerospace Med. 45(4):431-433.

Sun, M., C. Sun, and Y. Yang. 1996. Effect of low-concentration CO_2 on stereoacuity and energy expenditure. Aviat. Space Environ. Med. 67(1):34-39.

Vandemark, N.L., B.D. Schanbacher, and W.R. Gomes. 1972. Alterations in testes of rats exposed to elevated atmospheric carbon dioxide. J. Reprod. Fertil. 28(3):457-459.

Wong, K.L. 1996. Carbon dioxide. Pp. 105-187 in Spacecraft Maximum Allowable Concentrations for Selected Airborne Contaminants, Vol. 2. Washington, DC: National Academy Press.

Yang, Y., C. Sun, and M. Sun. 1997. The effect of moderately increased CO_2 concentration on perception of coherent motion. Aviat. Space Environ. Med. 68(3):187-191.

APPENDIX A

Table 7-3 summarizes the original SMACs set by Wong (1996). The current SMACs established by this committee are summarized in Table 7-2.

TABLE 7-3 End Points and Acceptable Concentrations (Wong 1996)

			Uncertainty Factors		Acceptable Concentrations				
End Point	Exposure Data	Species and Reference	Species	Small n	1 h	24 h	7 d	30 d	180 d
Visual impairment, tremor, CNS depression	NOAEL at 3%, 24 h/d, 5 d or 2 wk	Human (n = 7, 12) (Glatte et al. 1967; Storm and Giannetta 1974)	—	$10/(19)^{1/2}$	1.3	1.3	1.3	1.3	1.3
Headache	NOAEL at 2%, 24 h/d, 30 d	Human (n = 6) (Radziszewski et al. 1988; Guillerm and Radziszewski 1979)	—	—	2	2	2	2	2
Dyspnea, intercostal pain	NOAEL at 2.8%, 0.5 or 1 h	Human (n = 8, 4) (Menn et al. 1970; Sinclair et al. 1971)	—	—	2.8	2.8	—	—	—
	NOAEL at 2.8%, 15 or 20 d	Human (n = 4, 6) (Sinclair et al. 1971; Radziszewski et al. 1988)	—	$10/(10)^{1/2}$	—	—	0.9	0.9	0.9
Airway resistance increases	NOAEL at 3%, 24 h/d, 5 d	Human (n = 7) (Glatte et al. 1967)	—	—	3	3	—	—	—
	NOAEL at 3%, 24 h/d, 5 d	Human (n = 7) (Glatte et al. 1967)	—	$10/(10)^{1/2}$	—	—	0.8	0.8	0.8

(Continued)

TABLE 7-3 Continued

End Point	Exposure Data	Species and Reference	Uncertainty Factors		Acceptable Concentrations				
			Species	Small n	1 h	24 h	7 d	30 d	180 d
Hyperventilation									
Tolerability	NOAEL at 2%, 24 h/d, 11 or 30 d	Human (n = 4, 4, 6) (Sinclair et al. 1969; Radziszewski et al. 1988; Guillerm and Radziszewski 1979)	—	$10/(14)^{½}$	—	—	0.7	0.7	0.7
Exercise impairment	NOAEL at 2%, 24 h/d, 5, 15, or 30 d	Human (n = 6, 4, 6) (Glatte et al. 1967; Sinclair et al. 1971; Radziszewski et al. 1988)	—	$10/(16)^{½}$	—	—	0.8	0.8	0.8
Testicular injury	NOAEL at 3%, 24 h/d, 42 d	Rat and guinea pig (Schaefer et al. 1971)	1	—	—	—	3	3	3
SMAC 1996					*1.3*	*1.3*	*0.7*	*0.7*	*0.7*

Source: Wong 1996.

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Carbon Monoxide

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Spacecraft maximum allowable concentrations (SMACs) for carbon monoxide (CO) were initially published in Volume 1 of *Spacecraft Maximum Allowable Concentrations* for 1-h, 24-h, 7-d, 30-d, and 180-d exposures (Wong 1994). As NASA will be conducting longer missions, extended duration SMACs are required. This document will establish a CO SMAC value for 1,000-d extended duration exposure; it will also reassess original SMACs and propose new values for 1-h, 24-h, 7-d, 30-d, and 180-d exposures.

OCCURRENCE AND USE

CO is a colorless, odorless, and tasteless gas that is produced by incomplete combustion of carbon-containing materials (Penney 2000a). It is produced in the human biological system through hemoglobin (Hb) metabolism at a rate of 0.4 milliliter (mL)/h, which results in a carboxyhemoglobin (COHb) concentration of 0.4% (Coburn et al. 1965). However, Doherty reported in 2000, that CO is produced endogenously during heme metabolism, accounting for a normal COHb concentration of approximately 1%. Doherty also reported that smoke, especially from the tips of cigarettes, is a source of CO that may lead to COHb concentrations as high as 10% to 15% in heavy smokers.

In a study with 80 Boston police officers working in city street conditions, McFarland reported in 1973 that almost all the nonsmokers had COHb concentrations below 4%; for most of the smokers, it was below 6%, indicating much higher endogenous COHb concentrations in outdoor workers.

CO interferes with the oxygenation of blood and the delivery of oxygen to tissues because it has approximately 245 times greater affinity for Hb than oxygen (Roughton 1970, as cited by NRC 2007). COHb reduces the oxygen-carrying capacity of blood thereby shifting the oxygen dissociation curve and

reducing oxygen delivery to tissues. Hypoxemia and resulting tissue hypoxia are the best understood mechanisms of CO toxicity. A log-log plot of estimated percentage of COHb saturation and exposure duration for different CO concentrations computed from the Coburn-Foster-Kane (CFK) equation is shown in Figure 8-1 (Peterson and Stewart 1975, Penney 1999). Details of the CFK equation are presented in Appendix A.

The central nervous system (CNS) and cardiovascular (CV) system are the primary targets of CO toxicity. Adverse effects resulting from CO exposure vary widely from subtle vascular and neurologic changes to loss of consciousness and death (NRC 2007). There is no known use of CO in spacecraft; however, it is predicted to be an off-gas product (Leban and Wagner 1989, as cited by Wong 1994). CO is also a by-product of a fire event occurring aboard spacecraft. Such an event occurred aboard the Mir Spacestation since publication of the 1994 CO SMAC (Wong 1994). CO and COHb concentrations as well as resulting health effects were documented and provide direct evidence of CO toxicity to spacecraft crews (James and Garcia 1994, James 2008).

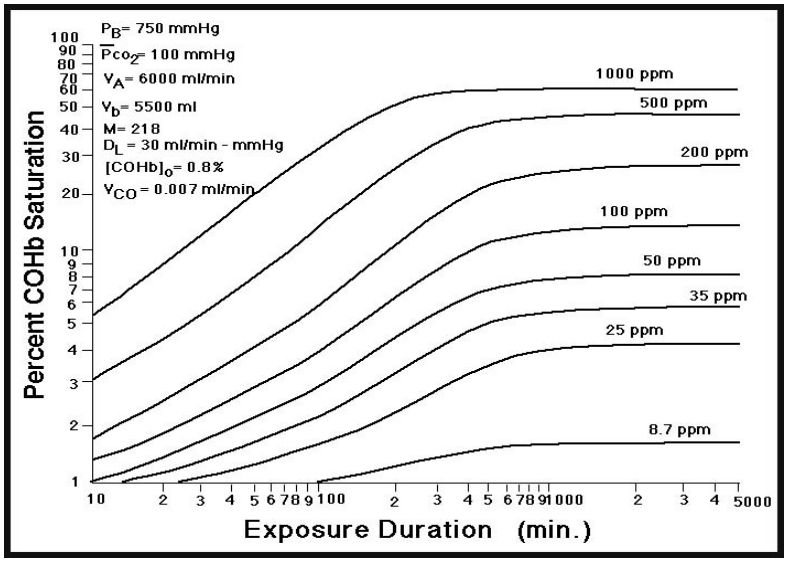

FIGURE 8-1 Prediction of CO uptake and COHb saturation using CFK equation. Log-log plot of CO uptake by humans from very low ambient CO concentrations as computed from the CFK equation. Abbreviations: D_L, diffusing capacity of lungs, $[COHb]_0$, value before CO exposure; M, equilibrium constant; P_B, barometric pressure; $\overline{P}_{CO2}$, mean partial pressure of O_2 in lung capillaries; ppm, parts per million; V_A, alveolar ventilation rate; V_b, blood volume; V_{CO}, rate of endogenous CO production. Source: Peterson and Stewart 1975, as cited by Penney 1999. Reprinted with permission; copyright 1975, *Applied Physiology*.

SUMMARY OF ORIGINAL APPROACH

The SMAC values that were set in 1994 were targeted to protect crew from CNS and CV toxicity. Wong used the CFK equation as tested by Peterson and Stewart (1975) to calculate COHb concentrations (see Figure 8-1). The CFK equation is a prediction based on a fitted model and has been used by all regulatory agencies throughout the United States for setting CO exposure limits. These values are based on National Ambient Air Quality Standards (NAAQS), with an additional 2% safety margin. The longer-term SMACs—7, 30, and 180 d—were set at concentrations lower than the typical COHb levels of 1.6% in smokers (Wong 1994).

1-h SMAC, 1994

The 1-h SMAC used the studies of Ramsey (1972) and Putz et al. (1979), in which 5% COHb was found to increase reaction time and impair hand-eye coordination (Wong 1994). Even though there were several conflicting reports on COHb concentrations higher than 5% affecting reaction time and hand-eye coordination, the lowest concentration was selected, because the author believed these impairments would interfere with the crew's ability to deal with a contingency event. Nevertheless, Benignus et al. (1987) (Table 8-1) repeated the Putz et al. (1976, 1979) studies and found no statistically significant change in reaction time at a COHb concentration of 8.24%. Benignus and others went on to show no effect on visual detection at 17% COHb (Hudnell and Benignus 1989) and no decrease in human vigilance (numerical monitoring) (Benignus et al. 1977) at 12.62% COHb.

Wong (1994) selected 3% COHb as the target concentration for the 1-h SMAC to account for a 2% safety margin against the NAAQS value at that time. It also protected against cardiotoxicity, because 4% COHb for more than 1 h failed to increase the frequency of ventricular premature depolarization in cardiac patients (Sheps et al. 1990, as cited by Wong 1994). A minute volume of 20 L/min, corresponding to light activity of an adult (NRC 1992), was used to calculate the CO concentration. In addition, the COHb concentration of 0.6% and the in-flight Hb concentration measured in Skylab were used (Kimzey 1977, as cited by Wong 1994), which resulted in 55 parts per million (ppm) yielding 3% COHb an hour. The 1-h SMAC was set at 55 ppm.

24-h SMAC, 1994

The 24-h SMAC was based on research conducted by Putz et al. (1979). A COHb concentration of 5% impaired hand-eye coordination in 4 h (Putz et al. 1979, as cited by Wong 1994) and was assumed also to impair hand-eye coordination in 24 h. The same target of 3% COHb was then used to set the SMAC.

TABLE 8-1 COHb Effect Level (2% to 24%)

COHb, %	Time Period (CO Concentration)	Effects	Type of Individual	*n*	Reference
3-24	1 h (intermittent)	Increase in muscle sympathetic nerve activity. No increase in heart rate or ventilation.	Adult males	15	Hanada et al. (2003)
5, 10, 15, and 20	1-2 h (27, 55, 83, and 100 ppm, respectively)	No effect on upper and lower submaximal exercise, no overt CV injury.	Adult males	16	Kizakevich et al. (2000)
>15-20	2.5 h (1,000 ppm continuous to peak concentration)	CO intoxication evidenced by severe headache and delayed response, EEG and clinical chemistry normal.	Adult males	2	Stewart et al. (1970)
17	2 h (continuous)	No effect (visual detection).	Adult males	11	Hudnell and Benignus (1989)
17	1+ h (700 ppm CO continuous)	Minimal effect on dark adaptation (driving skills). Effect on peripheral light psychomotor response.	Adult males	27	McFarland (1973)
8	24 h (50 ppm continuous)	No effect on performance (time estimation, reaction time, manual dexterity, steadiness, EEG, and evoked response in driving simulator).	Adult males	3	Stewart et al. (1970)
11-13	8 h (100 ppm continuous)	No effect on performance (time estimation, reaction time, manual dexterity, steadiness, EEG, and evoked response in driving simulator).	Adult males	24	Stewart et al. (1970)
16	4 h (200 ppm continuous)	Mild headache in 3 of 3, subsided in 30 min for 2 subjects and in 2 h for 1 subject. No effect on performance (time estimation, reaction time, manual dexterity, steadiness, EEG, and evoked response in driving simulator).	Adult males	3	Stewart et al. (1970)
12.62	2 h (200 ppm)	No effect on vigilance (numerical monitoring).	Adult males	19	Benignus et al. (1977)

11.22	45 min (950 ppm)	No effect on depth perception visual discrimination for brightness and flicker fusion discrimination. Decrease in reaction time to visual stimulus, but improvement in reaction time in 5 of 20 subjects.	Adult males	20	Ramsey (1973)
11	1+ h (700 ppm CO continuous)	Minimal effect on dark adaptation (driving skills), slight effect on peripheral light psychomotor response.	Adult males	27	McFarland (1973)
8.3	1 h (1,000 ppm for 30 min + 100 ppm for 30 min continuous)	Effects of CO on muscle sympathetic nerve activity, forearm blood flow, blood pressure, heart rate, minute ventilation, and forearm vascular resistance not statistically significant.	Adult males	12	Hausberg and Somers (1997)
8.24	4 h (100 ppm continuous)	Effect noted not statistically significant (event monitoring and visual tracking of light).	Adult females Adult males (1 adult per chamber)	22	Benignus et al. (1987) (repeat of Putz et al. 1976, 1979)
7.61	45 min (650 ppm)	No effect on depth perception visual discrimination for brightness, flicker fusion discrimination. Decrease in reaction time to visual stimulus.	Adult males	20	Ramsey (1973)
7	8 d (50 ppm continuous)	P-wave changes in 6 of 15 subjects.	Adult males	9	Davies and Smith (1980)
5	4 h (70 ppm continuous)	Decreased ability to keep cathode ray tube on a moving spot while simultaneously detecting bright light flashes interspersed with dimmer flashes.	Adult males Adult females (2 subjects in same chamber)	6	Putz et al. (1976, 1979)
2.4	8 d (15 ppm continuous)	P-wave changes in 3 of 16 subjects.	Adult males	9	Davies and Smith (1980)

Abbreviation: EEG, electroencephalogram.

With a breathing rate of 20 cubic meters (m^3)/d used by the National Research Council (NRC) in 1992 and in-flight Hb concentrations from Skylab, 20 ppm was calculated to yield 3% COHb in 24 h. The 24-h SMAC was set at 20 ppm.

7-d SMAC, 1994

Only one study was found in humans for continuous exposure lasting 7 d or longer (Davies and Smith 1980, as cited by Wong 1994). In this study, P-wave changes were detected at 15 ppm (2.4% COHb) in 3 of 16 subjects and at 50 ppm (7.1% COHb) in 6 of 15 subjects during an 8-d exposure. Wong (1994) targeted a COHb below 2.4% and set the 7-d SMAC based on the U.S. Environmental Protection Agency's NAAQS of 9 ppm for 8 h, yielding 1.6% COHb in an exercising individual. Using the CFK equation and a minute volume of 20 m^3/d and in-flight levels in Skylab, a 7-d SMAC was set at 10 ppm.

30-d and 180-d SMAC, 1994

The 7-d SMAC target of 1.6% was used for the 30- and 180-d SMACs. The same rationale for the 7-d exposure was applied to the 30- and 180-d SMACs (Wong 1994); with 1.6% being lower than the COHb concentrations commonly detected in smokers, it was thought that this value would be protective against neurologic and CV effects in a continuous 30- or 180-d exposure. SMACs for 30 and 180 d were set at 10 ppm.

The NAAQS value, selected to protect the most sensitive individuals in the population, was used as a basis for setting NASA SMACs in 1994. (The NAAQS values are presented in Table 8-2, along with recommended exposure levels from other organizations.) NASA accepts a much higher risk for astronaut crews, because they are in top physical condition; therefore, the ultraconservative values for sensitive individuals are not necessarily appropriate for setting SMACs.

CARDIOVASCULAR RISKS OF SPACEFLIGHT

NASA has reviewed its position on the CV risks of spaceflight (Convertino and Cooke 2005) and has determined that, based on data from astronauts with spaceflight experience, there is no conclusive experimental evidence of cardiac dysrhythmias, manifestation of asymptomatic CV disease, or reduction in myocardial contractile function. The primary CV risks of spaceflight are compromised hemodynamic responses to central hypovolemia resulting in reduced orthostatic tolerance and exercise capacity. NASA performs a rigorous health screening process and selects astronauts who are in excellent physical condition. The NASA process includes health screening for anemia and CV disease.

TABLE 8-2 Other Organizations' Recommendations for CO Exposure

Organization, Standard	Recommended Exposure, ppm	Reference
OSHA		29 CFR 1910.1000
PEL 8 h TWA	50	
ACGIH		ACGIH 2002
TLV TWA	25	
NIOSH		NIOSH 2004
REL TWA	35	
Ceiling	200	
IDLH	1,200	
AIHA		AIHA 1999
ERPG-1	200	
ERPG-2	350	
ERPG-3	500	
IPCS WHO, CO in ambient air		WHO 1999
15 min	87	
30 min	52	
1 h	26	
8 h	9	
NAAQS, CO in ambient air		EPA 2008
1 h	37	
8 h	9	
NAC, general public		EPA 2001
AEGL-2, 8 h (proposed)	27	
AEGL-3, 8 h (proposed)	130	
NRC, Submarine		NRC 2007
EEGL 1 h	180	
EEGL 24 h	45	
CEGL 90 d	9	
NRC, Navy SEAL		NRC 2002
SEAL-1 (10 d)	125	
SEAL-2 (24 h)	150	

Abbreviations: ACGIH, American Conference of Governmental Industrial Hygienists; AEGL, acute exposure guideline level; AIHA, American Industrial Hygiene Association; CEGL, continuous exposure guidance level; EEGL, emergency exposure guidance level; EPA, U.S. Environmental Protection Agency; ERPG, emergency response planning guidelines; IDLH, immediately dangerous to life and health; IPCS, International Programme on Chemical Safety; NAAQS, National Ambient Air Quality Standard; NIOSH, NAC, National Advisory Committee; National Institute for Occupational Safety and Health; ; NRC, National Research Council; OSHA, Occupational Safety and Health Administration; PEL, permissible exposure limit; REL, recommended exposure limit; SEAL, submarine escape action level; TLV, threshold limit value; TWA, time weighted average; WHO, World Health Organization.

NASA IN-FLIGHT EXPOSURE DATA SINCE ORIGINAL SMAC PUBLICATION: PREVIOUS EXPOSURE OF ASTRONAUT CREWS TO ELEVATED CO DURING SPACEFLIGHT

In 1994, a fire occurred aboard the Mir Spacestation (see Figure 8-2 and Table 8-3). This event resulted in direct increased CO and COHb exposures to the crew. NASA recorded CO concentrations ranging from 570 ppm (15% COHb) at 1 h (see Table 8-3) post-fire event (pfe) to 4 ppm (9% COHb) at 90 h pfe; the CO measured decreased continuously over this time period. The sustained exposure to CO resulted in a peak COHb of 37% at 5 h pfe, which produced the most severe toxic effects noted—headache and nausea, as reported by only one crew member. At 1-h pfe (15% COHb) at 570 ppm CO, there were no reported effects (James and Garcia 1994, James 2008).

NEW RESEARCH DATA SINCE 1994

Hausberg and Somers (1997) studied the contribution of CO to the acute CV effects of smoking. This protocol examined the effects of CO on sympathetic and hemodynamic measurements in healthy humans. The test pool consisted of 10 healthy normotensive subjects (8 men and 2 women) aged 27 ± 5 years. Only one subject smoked (10 cigarettes per week). Subjects were exposed to either room air (control) or 1,000 ppm of CO for 30 min followed by the continuation of room air inhalation or 100 ppm of CO for 30 min. During the exposure to either CO or control (vehicle), measurements were obtained for 5 of every 10 min, and COHb was measured every 10 min. While COHb concentrations did not change in control subjects, baseline COHb concentrations of 0.2% ± 0.1% increased to 8.3% ± 0.5% after 30 min of inhalation of 1,000 ppm of CO ($P < 0.05$) and were maintained at about this concentration during the 30 min of inhalation of 100-ppm of CO. Baseline minute ventilation, end-tidal partial pressure of O_2 in lung capillaries, muscle sympathetic nerve activity (MSNA), forearm blood flow (FBF), blood pressure (BP), and heart rate (HR) did not change in CO-exposed or control subjects. Forearm vascular resistance (FVR) increased slightly during control inhalation exposure but did not change during CO inhalation. The authors concluded that CO is not a contributing factor to the reduction in central sympathetic outflow or to other hemodynamic changes observed with smoking in humans (Hausberg and Somers 1997). They further postulated that modest increases in COHb concentrations, equivalent to that resulting from cigarette smoking, do not have appreciable acute effects on MSNA, BP, HR, or FBF and thus are unlikely to contribute to the acute sympathetic and hemodynamic effects of smoking in healthy humans. The unchanged FVR with CO as opposed to a slight increase in FVR with vehicle may indicate a peripheral vasodilator action of even modest CO concentrations (Hausberg and Somers 1997).

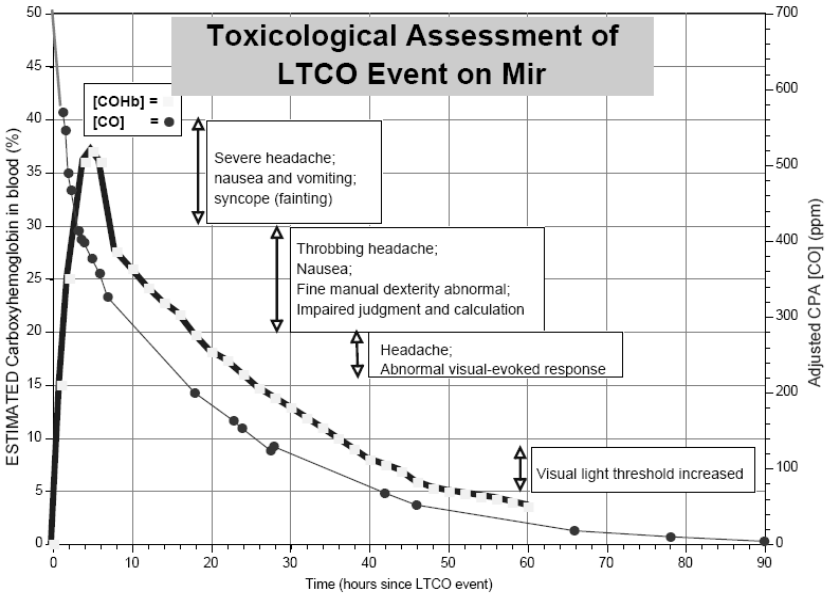

FIGURE 8-2 CO and COHb concentrations and toxic health effects observed on spacestation. (Expected effects are noted in boxes on graph and do not reflect actual observed effects in astronauts.) Abbreviations: CPA, Combustion Products Analyzer; LTCO, Low Temperature Catalytic Oxidizer. Source: James and Garcia 1994.

In 2000, Kizakevich et al. exposed healthy young men to 1-2 h of CO at COHb concentrations of up to 20%. Sixteen healthy nonsmoking men ranging in age from 18 to 29 years were the test subjects. Kizakevich et al. (2000) used a combination of exposures to CO by breathing from a bag or in an environmental chamber. Test subjects performed a randomized sequence of 5-min multilevel treadmill and hand-crank exercises on different days at less than 2% COHb and after attaining target levels of 5%, 10%, 15%, and 20% COHb. The team measured cardiac output, stroke volume, HR, cardiac contractility, and time to peak ejection time. They assessed myocardial irritability and ischemia and changes in cardiac rhythm. Their results established that the CV system compensated for the reduced O_2-carrying capacity of the blood by augmenting HR, cardiac contractility, and cardiac output for upper-body and lower-body exercise. They concluded that young, apparently healthy men can perform submaximal upper- and lower-body exercise without overt impairment of CV function after CO exposures attaining 20% COHb. They demonstrated that these subjects can perform submaximal upper- and lower-body exercise without blatant CV injury at up to 20% COHb (Kizakevich et al. 2000).

TABLE 8-3 Calculated COHb and Recorded CO Aboard Mir Spacestation Post-Fire Event

Time, h	CoHb, %
1	15
2	25
4	36
5[a]	37
6	36
8	27.52
10	25.88
12	24.16
14	22.72
16	21.61
18	19.68
20	18.07
22	17.24
24	15.96
26	14.64
28	13.74
30	12.82
32	11.87
34	10.91
36	9.93
38	8.93
40	7.9
42	7.38
44	6.85
46	5.77
48	5.22
50	4.89
52	4.67
54	4.45
56	4.22
58	3.89
60	3.54

Time, h	CO, ppm
1.17	570
1.5	546
1.83	489
2.2	467
3.13	413
3.48	402
3.83	398
4.83	377
5.83	357
6.83	326
17.83	200
22.75	163
23.83	153
27.42	124
27.83	129
41.83	68
45.85	52
65.83	18
78.00	10
89.83	4

[a]Peak COHb at 37%; one of three crew members reported headache and nausea symptoms (James and Garcia 1994).

Hanada et al. (2003) measured the role of arterial free oxygen partial pressure (P_{a,O_2}) on increases in MSNA, HR, ventilation, and leg hemodynamics at rest and during rhythmic handgrip exercise. Twenty healthy male subjects aged 26 ± 1 years were studied in the supine position. CO was used to mimic the effect of systemic hypoxia on arterial oxyhemoglobin (about 20% lower arterial oxyhemoglobin), while normalizing or increasing P_{a,O_2} (40-620 mmHg). Four experimental conditions were used: (1) normoxia, ~110 mmHg P_{a,O_2} and ~2% COHb; (2) hypoxia, ~40 mmHg P_{a,O_2} and ~2% COHb; (3) CO + normoxia, ~110 mmHg P_{a,O_2} and ~23% COHb; (4) CO + hyperoxia, ~620 mmHg P_{a,O_2} and ~24% COHb. Conditions (3) and (4) caused an increase in MSNA compared with condition (1) but did not increase HR or ventilation. In spite of the 4-fold elevation in MSNA in conditions (3) and (4), with hypoxemia and exercise no change was noted in O_2 uptake, resting leg blood flow, and vascular conductance. This research also noted that, despite normal or elevated P_{a,O_2} , conditions (3) and (4) increased MSNA at rest and during exercise similarly to acute systemic hypoxia (Hanada et al. 2003). This study is the first to provide direct evidence for a CO-induced increase in MSNA. It also demonstrated that COHb concentrations of 24% do not increase HR and ventilation during normoxic and hyperoxic conditions.

PROPOSED SMAC VALUES 2006

A review of available guidance levels, including NASA's original CO SMACs, has found that most levels set by various organizations do not align with NASA's mission objectives for protection of crew health. The bulk of these values are set to protect the most sensitive individuals. NASA accepts a much higher risk for spaceflight crews, who are expected to be in prime physical condition. The revised SMACs being proposed by NASA are presented in Table 8-4.

TABLE 8-4 Spacecraft Maximum Allowable Concentrations

Duration	ppm	mg/m^3	Target COHb, %	Target toxicity
1 h	425	485	15	CNS/CV
24 h	100	114	15	CNS/CV
7 d	55	63	8	CNS/CV
30 d	15	17	2	CNS/CV
180 d	15	17	2	CNS/CV
1,000 d	15	17	2	CNS/CV

Conversion factor: 1 ppm = 1.14 mg/m^3.

For 1- and 24-h SMACs, NASA defines acceptable risk as a concentration of a substance in air that may be acceptable for the performance of specific tasks during emergency conditions lasting for less than 1 h or less than 24 h (NRC 1992). The 1- and 24-h SMACs could include reversible effects that do not impair judgment and do not interfere with proper responses to the emergency, such as shutting a valve, closing a hatch, removing a source of heat or ignition, or using a fire extinguisher (NRC 1992). Exposure at the 1- and 24-h levels may produce effects such as increased respiratory rate from increased CO_2, headache or mild CNS effects from CO, and respiratory tract and eye irritation from ammonia or sulfur dioxide (NRC 1992). SMACs for up to 180 d are concentrations designed to avoid adverse health effects, immediate or delayed, as well as to avoid degradation in performance of crew after a continuous exposure.

In contrast to 1- and 24-h SMACs, which are intended to guide exposures during emergencies, SMACs lasting up to 180 d, and now 1,000 d, are intended to provide guidance for operations during those time periods (180 d in an environment like the Spacestation and 1,000 d for extended stays on the lunar, Martian, or other planetary surfaces). Accounting for accumulation, detoxification, excretion, and repair of toxic injuries is important in determining long-term SMACs (NRC 1992). Whether a material has a cumulative effect must be taken into account for long-duration SMACs. Neuropathologic regeneration or repair of toxic injuries occurs more readily in intermittent than in continuous exposures, making repair important in setting long-term SMACs (NRC 1992).

The emergency exposure guidance level (EEGL) values set by the NRC in 2007 for submariner protection most closely resemble the closed environment experienced during spaceflight, with the exceptions of the lack of microgravity and the allowance of smoking aboard their vessels. Research conducted after original publication of the CO SMAC, along with a review of older research, provides evidence for raising the current SMACs. Anecdotal support is also provided by observed effects in crewmembers on NASA's Spacestation fire event. SMACs proposed here are all below a COHb threshold of 15%. In all SMAC calculations, the use of Peterson and Stewart's (1975) CFK calculation with a minute volume of 20 L/min, corresponding to light activity of an adult (NRC 1992), was used to calculate CO concentration. In addition, the initial COHb concentration of 1.0% was used based on a report from Doherty (2000) and the in-flight Hb concentration measured in Skylab was used (Kimzey 1977, as cited by Wong 1994).

1-h SMAC

In 2007, the NRC proposed a 1-h EEGL to remain below a 20% COHb threshold based on the research of Kizakevich et al. (2000), in which healthy young men were exposed to 1-2 h of CO at COHb concentrations of up to 20% and identified no decrement to the CV system during submaximal and lower-body exercise. NRC began with a value of 200 ppm, which is a 5% COHb con-

centration on the basis of the calculations by Peterson and Stewart (1975) in Figure 8-1. The EEGL was adjusted to 180 ppm to be protective against severe headaches and was adjusted for low oxygen atmosphere to account for the differences between smokers and nonsmokers, making it tolerable to both groups of individuals and causing no neurobehavioral performance impairments (based on guidance from Stewart et al. 1970, as cited by NRC 2002). No additional factors were applied because the NRC considered this a no-observed-adverse-effect level (NOAEL). For CV protection, NASA proposes a 1-h SMAC that remains below the 20% COHb threshold.

Mayr et al. (2005) exposed healthy young individuals (mean age 25 years) to 500 ppm of CO for 1 h via inhalation (full face masks), yielding a peak of 7% COHb. The exposures did not have a significant effect on vital parameters, leukocyte and neutrophil counts, and cytokine levels. While the focus of this study was to determine whether CO had an anti-inflammatory effect on humans at 250 ppm as had been reported in rodents, it did show a NOAEL of 500 ppm at 1 h (Mayr et al. 2005).

NASA selects a value of 15% COHb to be protective against CNS and CV effects. The Committee on Spacecraft Exposure Guidelines (SEGs) recommended 15% COHb. This recommendation was based on the Kizakevich et al. (2000) study and the Mir Spacestation pfe. The NOAEL of 20% COHb was reported for CV effects in the Kizakevich et al. (2000) study. While NASA's in-flight experience is not a conclusive scientific study, NASA has previously observed this COHb level (15%) as a no-reported-effect level in three crewmembers at 1 h pfe on Mir Spacestation. The NASA information from the pfe on the Mir Spacestation was recommended by the SEGs committee because the report of no effects at 15% COHb in crewmembers was assumed to cover all effects (CNS and CV). NASA selects a SMAC of 425 ppm for a 1-h exposure, which should result in a COHb value of 15% on the basis of the calculations of Peterson and Stewart (1975) as indicated in Figure 8-1. No additional safety factors are applied, because this value is considered a NOAEL by Kizakevich et al. (2000).

NASA's 1-h SMAC is set at 425 ppm.

24-h SMAC

Wong cited the only 24-h human exposure study (Stewart et al. 1970), which showed a NOAEL for CNS effects of 50 ppm, yielding 8% COHb. NASA's experience during the Mir Spacestation fire showed no adverse responses in all three crewmembers at 24 h at 15% COHb. While Wong did not base the 24-h SMAC on this study, the NRC (2004) did use this research value. NRC reduced the value to 45 ppm for a low-oxygen environment and applied no further safety factors. The NRC (2002) proposed the Navy submarine escape action level 2 (SEAL-2) for a 24-h exposure, to be 150 ppm. This would not result in a COHb concentration higher than 20% COHb. They expect some

submariners to experience slight headache and some cognitive function decrement; however, it would not impair the crew from escaping a disabled submarine. Their value was also supported by research of Theodore et al. (1971) as cited by Wong (1994), in which monkeys were exposed continuously to 380 ppm for 99 d, yielding 31% COHb; no adverse health effects were observed.

NASA selects a 24-h SMAC value of 100 ppm, yielding a COHb of no more than 15%. The calculation of a 24-h exposure to 100 ppm results in a COHb concentration of 13.55%. No additional safety factors are applied because this is a NOAEL.

NASA's 24-h SMAC is set at 100 ppm.

7-d SMAC

The Navy SEAL-1 for 10-d exposure to CO in submarines is proposed to be 125 ppm, which would yield a COHb value of 15%, based on the studies by Stewart et al. (1973) and Hudnell and Benignus (1989) (as cited by NRC 2002). This SEAL-1 value showed no perceptual function or cognitive effects in healthy individuals. It is noted that this value was for oxygen values of 20.95% and the SEAL should be lowered if O_2 concentrations fall below this value, which is the case with NASA, who maintains a 20% O_2 atmospheric value on its spacecraft. Davies and Smith (1980) noted that a determination of the end point of effect (P-wave changes) was to be evaluated as to whether it was an adverse effect. If these p-wave changes are not considered adverse, the Davies and Smith (1980) paper supports a 7-d SMAC for CO of 55 ppm, 7% COHb. NASA considers the target of 7% COHb as appropriate for the 7-d SMAC, because it is expected to be a NOAEL.

NASA's 7-d SMAC is set at 55 ppm.

30-d and 180-d SMAC

The NRC proposed a 10-ppm SMAC for CO in 1994 based on a study by DeBias et al. (1973) (as cited by NRC 2007). This study exposed normal and infarcted monkeys to CO at 100 ppm for 23 h/d for 24 wk. The test subjects experienced a higher incidence of T-wave inversions. Both normal and infarcted monkeys presented higher P-wave changes after 2 d of exposure. The 100-ppm value was thought to be a lowest-observed-adverse-effect level (LOAEL). They adjusted the value for a low-oxygen atmosphere to 90 ppm, applied an interspecies factor, and applied a LOAEL to NOAEL factor to set a value of 10 ppm.

A 99-d continuous exposure to CO at 380 ppm (31% COHb) caused no reduction in operant behavior in monkeys (Theodore et al. 1971, as cited by Wong 1994). However, Wong did not apply an interspecies factor and he did not use this study as a basis for setting the 30- and 180-d SMACs. Instead, he targeted 1.6% COHb to calculate a value of 10 ppm, which is lower than the background concentrations of COHb typically detected in smokers. Wong also noted

that this concentration would not be expected to cause tissue degradation based on the studies of Eckardt et al. (1972) (2 years of continuous exposure to 3.4% COHb) and Theodore et al. (1971) (32% to 33% COHb for 168 d), which produced no histological morphology changes (as cited by Wong 1994).

NASA selects a threshold of 2% COHb for 30- and 180-d SMACs, which is calculated to be 15 ppm. Hanada et al. (2003) used this amount as the control COHb value in normoxic and hypoxic exposure. This amount of COHb showed no decrement to CNS activities. This concentration is also below the threshold limit value time weighted average for working lifetime exposure of occupational workers, currently set at 25 ppm (ACGIH 2002).

NASA's 30- and 180-d SMAC is set at 15 ppm.

1,000-d SMAC

Because there are no long-term (1,000 d) human exposure studies on CO exposure, it may be considered prudent to set a SMAC that is close to the ambient CO concentrations that are experienced on Earth, because humans do not appear to experience any adverse health effects at these concentrations. However, no conclusive research has been conducted on long-term health effects resulting from exposure to ambient CO concentrations, which change over the years. Background concentrations on submarines have been reported to average 5 ppm and range from 0 to 14 ppm (NRC 2007). Ambient air CO levels in large European cities generally average 17 ppm, with peaks at 53 ppm (WHO 1999). NAAQS accepts an 8-h average of 9 ppm, which is often exceeded throughout the United States (HSDB 2005, EPA 2008). NASA will therefore set its 1,000-d SMAC at the same level of 2% COHb as the 30- and 180-d SMACs.

NASA's 1,000-d SMAC is set at 15 ppm.

RECOMMENDATIONS AND CONCLUSIONS

Further research is needed for long-term exposures to CO. We are confident in the continued use of Peterson and Stewart's (1975) CFK calculations to set CO SMACs. Whereas the formula provides point estimates, our calculations include spaceflight variables from actual COHb concentrations attained during long-term (6 month) missions, which preclude the use of any additional spaceflight factors.

REFERENCES

ACGIH (American Conference of Governmental Industrial Hygienists). 2002. Carbon Monoxide. Pp. 20 in Threshold Limit Values (TLVs) for Chemical Substances and Physical Agents and Biological Exposure Indices (BEIs) for 2002. American Conference of Governmental Industrial Hygienists, Cincinnati, OH.

AIHA (American Industrial Hygiene Association). 1999. P. 25 in The AIHA 1999 Emergency Response Planning Guidelines and Workplace Environmental Exposure Level Guides Handbook. American Industrial Hygiene Association. Fairfax, VA.

Benignus, V.A., D.A. Otto, J.D. Prah, and G. Benignus. 1977. Lack of effects of carbon monoxide on human vigilance. Percept. Mot. Skills 45(3 Pt 1):1007-1014.

Benignus, V.A. K.E. Muller, C.N. Barton, and J.D. Prah. 1987. Effect of low level carbon monoxide on compensatory tracking and event monitoring. Neurotoxicol. Teratol. 9(3):227-234.

Coburn, R.F., R.E. Forster, and P.B. Kane. 1965. Considerations of the physiological variables that determine the blood carboxyhemoglobin concentration in man. J. Clin. Invest. 44(11):1899-1910.

Convertino, V.A., and W.H. Cooke. 2005. Evaluation of cardiovascular risks of spaceflight does not support the NASA bioastronautics critical path roadmap. Aviat. Space Environ. Med. 76(9): 869-876.

Davies, D.M., and D.J. Smith. 1980. Electrocardiographic changes in healthy men during continuous low-level carbon monoxide exposure. Environ. Res. 21(1):197-206.

DeBias, D.A., C.M. Banerjee, N.C. Birkhead, W.V. Harrer, and L.A. Kazal. 1973. Carbon monoxide inhalation effects following myocardial infarction in monkeys. Arch. Environ. Health 27(3):161-167 (as cited in NRC 2007).

Doherty, S. 2000. History, pathophysiology, clinical presentation and role of hyperbaric oxygen in acute carbon monoxide poisoning. Emerg. Med. 12(1):55-61.

Eckhart, R.E., H.N. MacFarland, Y.C. Alarie, and W.M. Busey. 1972. The biologic effect from long-term exposure of primates to carbon monoxide. Arch. Environ. Health 25(6):381-387(as cited in Wong 1994).

EPA (U.S. Environmental Protection Agency). 2001. Carbon Monoxide Results. AEGL Program [online]. Available: http://www.epa.gov/oppt/aegl/results50.htm [accessed Nov. 2005].

EPA (U.S. Environmental Protection Agency). 2008. National Ambient Air Quality Standards (NAAQS). Office of Air and Radiation, U.S. Environmental Protection Agency [online]. Available: http://epa.gov/air/criteria.html [accessed Apr. 3, 2008].

Hanada, A., M. Sander, and J. Gonzalez-Alonso. 2003. Human skeletal muscle sympathetic nerve activity, heart rate and limb haemodynamics with reduced blood oxygenation and exercise. J. Physiol. 551(2):635-647.

Hausberg, M., and V.K. Somers. 1997. Neural circulatory responses to carbon monoxide in healthy humans. Hypertension 29(5):1114-1118.

HSDB (Hazardous Substance Data Bank). 2005. Carbon Monoxide (CASRN 630-08-0). TOXNET, Specialized Information Services, U.S. National Library of Medicine, Bethesda, MD [online]. Available: http://toxnet.nlm.nih.gov/cgi-bin/sis/search [accessed Nov. 2005].

Hudnell, H.K., and V.A. Benignus. 1989. Carbon monoxide exposure and human visual detection thresholds. Neurotoxicol. Teratol. 11(4):363-371.

James, J.T. 2008. Health effects of atmospheric contamination. Chapter 21 in Principles of Clinical Medicine for Spaceflight, M.R. Barratt, and S.L. Pool, eds. New York: Springer.

James, J.T. and H. Garcia. 1994. Space Station Fire—Report on Toxicological Event. National Aeronautics and Space Administration, Johnson Space Center, Houston, TX.

Kimzey, S.L. 1977. Hematology and immunology studies. P. 249-282 in Biomedical Results from Skylab, R.S. Johnson, and L.F. Dietlein, eds. NASA SP-377. Wash-

ington, DC: National Aeronautics and Space Administration [online]. Available: http://lsda.jsc.nasa.gov/books/skylab/Ch28.htm [accessed Apr. 2, 2008].

Kizakevich, P.N., M.L. McCartney, M.J. Hazucha, L.H. Sleet, W.J. Jochem, A.C. Hackney, and K. Bolick. 2000. Noninvasive ambulatory assessment of cardiac function in healthy men exposed to carbon monoxide during upper and lower body exercise. Eur. J. Appl. Physiol. 83(1):7-16.

Leban, M.I., and P.A. Wagner. 1989. Space Station Freedom Gaseous Trace Contaminant Load Model Development. SAE PAPER 891513. Society of Automotive Engineers, Warrendale, PA (as cited in Wong 1994).

Mayr, F.B., A. Spiel, J. Leitner, C. Marsik, P. Germann, R. Ullrich, O. Wagner, and B. Jilma. 2005. Effects of carbon monoxide inhalation during experimental endotoxemia in humans. Am. J. Respir. Crit. Care Med. 171(4):354-360.

McFarland, R.A. 1973. Low level exposure to carbon monoxide and driving performance. Arch. Environ. Health. 27(6):355-359.

NIOSH (National Institute for Occupational Safety and Health). 2004. NIOSH Pocket Guide to Chemical Hazards. DHHS (NIOSH) 2004-103. National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention, U.S. Department of Health and Human Services, Cincinnati, OH.

NRC (National Research Council). 1992. Guidelines for Developing Spacecraft Maximum Allowable Concentrations for Space Station Contaminants. Washington, DC: National Academy Press.

NRC (National Research Council). 2002. Carbon monoxide. Pp. 69-96 in Review of Submarine Escape Action Levels for Selected Chemicals. Washington, DC: National Academy Press.

NRC (National Research Council). 2007. Carbon monoxide. Pp. 67-102 in Emergency and Continuous Exposure Guidance Levels for Selected Submarine Contaminants, Vol. 1. Washington, DC: The National Academies Press.

Penney, D.G. 1999. Carbon Monoxide Poisoning. Carbon Monoxide Headquarters – COHQ [online]. Available: http://www.coheadquarters.com/figco08.htm [accessed April 1, 2008].

Penney, D.G. 2000a. Carbon Monoxide Toxicity. Boca Raton: CRC Press.

Penney, D.G. 2000b. Carbon Monoxide. Coburn-Forster-Kane Equation. Carbon Monoxide Headquarters-COHQ. [online]. Available: http://www.coheadquarters.com/CFKEqu1.htm [accessed April1, 2008].

Peterson, J.E., R.D. Stewart. 1975. Predicting the carboxyhemoglobin levels resulting from carbon monoxide exposures. J. Appl. Physiol. 39(4):633-638.

Putz, V.R., B.L. Johnson, and J.V. Setzer. 1976. Effects of CO on Vigilance Performance: Effects of Low-Level Carbon Monoxide on Divided Attention, Pitch Discrimination, and the Auditory Evoked Potential. DHEW (NIOSH) 77-124. Cincinnati, OH: U.S. Department of Health, Education, and Welfare, National Institute of Occupational Safety and Health.

Putz, V.R., B.L. Johnson, and J.V. Setzer. 1979. A comparative study of the effects of carbon monoxide and methylene chloride on human performance. J. Environ. Pathol. Toxicol. 2(5):97-112.

Ramsey, J.M. 1972. Carbon monoxide, tissue hypoxia, and sensory psychomotor response in hypoxaemic subjects. Clin. Sci. 42(5):619-625.

Ramsey, J.M. 1973. Effects of single exposures of carbon monoxide on sensory and psychomotor response. Am. Ind. Hyg. Assoc. J. 34(5):212-216.

Roughton, F.J. 1970. The equilibrium of carbon monoxide with human hemoglobin in whole blood. Ann. N.Y. Acad. Sci. 174(1):177-188.

Sheps, D.S., M.C. Herbst, A.L. Hinderliter, K.F. Adams, L.G. Ekelund, J.J. O'Neil, G.M. Goldstein, P.A. Bromberg, J.L. Dalton, and M.N. Ballenger. 1990. Production of arrhythmias by elevated carboxyhemoglobin in patients with coronary artery disease. Ann. Intern. Med. 113(5):343-351.

Stewart, R.D., J.E. Peterson, E.D. Baretta, R.T. Bachand, M.J. Hosko, and A.A. Hermann. 1970. Experimental human exposure to carbon monoxide. Arch. Environ. Health 21(2):154-164.

Stewart, R.D., P.E. Newton, M.J. Hosko, and J.E. Peterson. 1973. Effect of carbon monoxide on time perception. Arch. Environ. Health 27(3):155-160.

Theodore, J., R.D. O'Donnell, and K.C. Back. 1971. Toxicological evaluation of carbon monoxide in humans and other mammalian species. J. Occup. Med. 13(5):242-255.

WHO (World Health Organization). 1999. Carbon Monoxide, 2nd Ed. Environmental Health Criteria 213. Geneva: World Health Organization [online]. Available: http://www.inchem.org/documents/ehc/ehc/ehc213.htm [accessed Apr. 2, 2008].

Wong, K.L. 1994. Carbon Monoxide Pp. 61-90 in Spacecraft Maximum Allowable Concentrations for Selected Airborne Contaminants, Vol. 1. Washington, DC: National Academy Press.

APPENDIX A

Coburn-Forster-Kane (CFK) Equation

The CFK equation is the most sophisticated approach available for modeling CO uptake by humans and other animals. Disadvantages of its use are the large number of variables and the fact that the value of many variables must be obtained from other equations (Penney 2000b).

$$\{A[HbCO]_t - (BV_{CO} + PI_{CO})\}/\{A[HbCO]_0 - (BV_{CO} + PI_{CO})\} = e^{-tAV_bB}$$

Terms of Equation

$A = \overline{P}_{CO2}/M\ [HbO_2]$
$[HbCO]_t$, see below
$B = 1/DL_{CO} + PL/VA$
V_{CO}, see below
PI_{CO}, see below
$[HbCO]_0$, see below
e, see below
t, see below
V_b, see below

where
$\overline{P}_{CO2}$ is the average partial pressure of O_2 in lung capillaries (mmHg), at sea level PI_{O_2} (159) – 49 = 110,
$PI_{O_2} = 148.304 - 0.0208\ PI_{CO}$,

M is the ratio of the affinity of blood for CO to that for O_2, approximately 218, $[HbO_2]$ is mL of O_2 per mL of blood, or = 0.22 – $[HbCO]_t$, $[HbCO]_t$ is mL of CO per mL of blood at time *t*, or = $[COHb\%]_t \cdot 0.0022$ (term to be solved for), DL_{CO} is diffusivity of the lung for CO (mL/min/mmHg), or = $35VO_2\ e^{0.33}$, VO_2 = RMV/22.274 – 0.0309, RMV is respiratory minute volume (L/min), PL is barometric pressure minus vapor pressure of water (49) at body temperature (mmHg), V_{CO} is rate of endogenous CO production (mL/min); approximately 0.007 mL/min, PI_{CO} is partial pressure of CO in inhaled air (mmHg), V_A is alveolar ventilation rate (mL/min), or = 0.933 V_E – 132 *f*, V_E is ventilation volume (mL/min), *f* is ventilation frequency, $[HbCO]_0$ is mL of CO per mL of blood at beginning of exposure (approximately 0.8% COHb, or 0.0176 mL of CO per mL of blood for a nonsmoker), e is the base of the natural logarithm (2.7182), *t* is exposure duration (min), and V_b is blood volume (mL); assume a body weight of 74 mL/kg. Data were obtained from Penney 2000b.

9

1,2 - Dichloroethane

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BACKGROUND

1,2-dichloroethane (EDC; ethylene dichloride) is a colorless liquid with an odor characteristic of a chlorinated hydrocarbon. It has molecular structure CH_2Cl-CH_2Cl and CAS number 107-06-2. It has a vapor pressure of 87 mmHg at 25°C. The conversion factors are 1 part per million (ppm) = 4.05 milligrams per cubic meter (mg/m^3) and 1 mg/m^3 = 0.25 ppm. NASA reviewed the toxicologic properties of this compound with respect to various exposure durations at different atmospheric concentrations. Spacecraft maximum allowable concentrations (SMACs) were derived by NASA, reviewed by the National Research Council (NRC) Committee on Toxicology, and published by the National Academy Press in 1996 in Volume 3, Appendix B6, of *Spacecraft Maximum Allowable Concentrations for Selected Airborne Contaminants* (Wong 1996). That document listed SMACs for 1 h, 24 h, 7 d, 30 d, and 180 d. With NASA now focusing on exploration missions going beyond low Earth orbit and targeted at the Moon and Mars, there is a need to reevaluate existing SMACs to derive acceptable concentrations (ACs) for long-duration missions, such as 1,000 d.

The effort presented in this chapter consisted of identifying new (since 1996) toxicology literature on EDC and evaluating data most appropriate for deriving a 1,000-d SMAC as well as identifying a need to update previously derived SMACs or approaches taken.

OCCURRENCE AND USE

EDC is used on Earth to manufacture vinyl chloride and as a solvent, degreaser, and fumigant. This compound is present in the air in the International Space Station and the Space Shuttle as a result of outgassing from experimental and system hardware. Because the materials designed for exploration missions

remain under development, future sources of such solvents in the spacecraft cannot be determined.

SUMMARY OF ORIGINAL APPROACH

The literature indicated that EDC can be absorbed from various exposure routes such as inhalation, oral, and dermal (IARC 1999). Exposure can lead to adverse effects, such as central nervous system (CNS) depression, corneal opacity, gastrointestinal (GI) irritation, hepatic necrosis, renal tubular necrosis, and neurotoxicity, and can result in death. The severity and incidence of these effects depend on exposure route, concentration, and duration. Acute inhalation exposure studies showed that EDC, in addition to causing neurotoxic, nephrotoxic, and hepatotoxic effects, also caused respiratory distress, congestion of the lungs, pulmonary edema, cardiac arrhythmia, nausea, and vomiting. In rodents, EDC impaired immune defense mechanisms and produced carcinogenic effects, especially by the oral route.

No adequate data were available to evaluate the carcinogenicity of EDC in humans. Coexposure to other solvents may have confounded such evaluations (see NTP 2005).

EDC has been reported to be a weak mutagen in the standard bacterial mutation assay (McCann et al. 1975), but it was mutagenic when bacteria were incubated with the liver S-9 fraction and glutathione and it was found to bind to DNA and proteins (Rannug et al. 1978, Banerjee et al. 1980, Guengerich et al. 1980). EDC has also been shown to be genotoxic in vivo (Reitz et al. 1982, Storer et al. 1984). Notably, although DNA lesions were observed in mice 4 h after exposure to EDC by intraperitoneal injection or given as gavage (Storer et al. 1984), no evidence of DNA damage was seen after a 4-h inhalation exposure of mice to up to 500 ppm.

EDC is metabolized by a cytochrome P-450-dependent microsomal oxidation system as well as by the cytosolic enzyme glutathione *S*-transferase, which conjugates EDC with glutathione, leading to the formation of *S*-(2-chloroethyl)glutathione (Reitz et al. 1982). These conjugates are excreted in the urine. D'Souza et al. (1988) and Reitz et al. (1982) proposed that conjugation with glutathione produced an alkylating agent—an episulfonium ion that is primarily responsible for EDC genotoxicity—but no later papers were found to confirm the supposition. Nevertheless, glutathione conjugation has been shown to result in genotoxic metabolites for other chlorinated alkanes.

In the 1996 SMAC derivations, Wong (1996) derived ACs for critical effects including CNS effects, GI symptoms, liver toxicity, and impaired immune defenses. He reviewed the data from a Russian paper (Kozik 1957) describing the effects of EDC exposure in an occupational setting in the Russian aviation industry. The original article was in Russian and, according to a translation of the essential parts of the paper, the study provided data collected from workers in the aviation industry during 1951 to 1955. The author reported that the num-

ber of workers who complained of GI disorders and in whom CNS effects were measured (increased errors in hand-eye coordination tests) and who reported they were sick depended on the number of years they worked in the industry. The time-weighted average (TWA) workplace concentration associated with such effects was found to be 15 ppm (NIOSH 1976). Using 15 ppm as the lowest-observed-adverse-effect level (LOAEL) for CNS effects, Wong (1996) calculated the 1-d AC using CNS effects as the adverse end point. Even though this study is not an acute exposure study, because exposure continued over several months and perhaps years, Wong (1996) concluded that this level of EDC can be used to evaluate CNS effects for all exposure durations. The rationale was based on the pharmacokinetic behavior of inhaled EDC—specifically, the finding that blood concentrations reach steady-state within 2 h of exposure at or above 150 ppm (Reitz et al. 1982), continuous exposures are not cumulative in blood, and the fact that EDC in blood affects the CNS. This also implies that CNS effects did not need to have a time adjustment factor for continuous exposures.

After a factor of 10 was applied for extrapolating from the LOAEL to no-observed-adverse-effect level (NOAEL), the 1-d AC was derived as 1.5 ppm.

Wong (1996) also used the GI effects data from the Russian occupational exposure study to derive a 180-d AC, which he adopted for 1 h, 24 h, 7 d, and 30 d for GI symptoms. Wong used the NOAEL adjusted concentrations for continuous exposure, taking into account 40 h/wk as the number of work hours. The calculation is as follows:

$$\text{7-, 30-, and 180-d AC}_{(\text{GI symptoms})} = 15 \text{ ppm}_{(\text{LOAEL})} \times 1/10_{(\text{LOAEL to NOAEL})} \times 40 \text{ h/wk}_{(\text{EDC duration exposure})} \div [24 \text{ h/d} \times 7 \text{ d/wk}]_{(\text{discontinuous to continuous})} = 0.36 \text{ ppm, rounded to } 0.4 \text{ ppm}$$

The GI effects are supported by Byers' report (1943) that U.S. workers exposed to about 100 ppm of EDC for 7.5 h/d developed nausea, vomiting, and abdominal pain within a few hours after they left work each day. The concentration of 15 ppm from the Kozik study is much lower.

As the Kozik data involved only one LOAEL (TWA of 15 ppm) concentration and the reports of illnesses could not be quantitative, the data were not amenable to benchmark dose (BMD) modeling. Because the publication is quite old and the documentation and references are in a foreign language, NASA did not analyze these data further. Several animal studies evaluated for acute toxicity effects were considered unsuitable because they used single high doses and had very high mortalities (Heppel et al. 1946; Spencer et al. 1951).

NASA reviewed the literature for toxicology data available since 1995 that can be used to derive 7-, 30-, and 180-d ACs, and no data were found. In addition to CNS and GI effects, another toxicologic end point that Wong (1996) used for deriving 1-h, 24-h, 7-d, 30-d, and 180-d ACs was hepatotoxicity reported in studies by Spencer et al. (1951) and Heppel et al. (1946) on monkeys, rats, and guinea pigs. Exposing monkeys to 400 or 200 ppm of EDC leads to fatty liver. One of these studies also noted degeneration of renal tubules. Spencer et al.

(1951) reported that monkeys exposed to 100 ppm of EDC for 7 h/d, 5 d/wk, for 29 wk showed no adverse effects on behavior, gross and microscopic tissue morphology, and hematologic parameters. They also studied the effects of a series of concentrations of EDC on rats and guinea pigs.

Wong (1996) determined ACs for various durations using the NOAEL dose of 100 ppm for hepatotoxicity for rats and guinea pigs reported by Spencer et al. (1951).

As NOAELs were found to be 100 ppm for 15 and 30 wk EDC exposures, Wong (1996) calculated the 24 h and 7-d ACs conservatively without adjusting for discontinuous-to-continuous exposure. Thus, the 24-h and 7-d ACs for liver toxicity were derived as follows:

$$\text{24-h and 7-d AC}_{\text{(liver toxicity)}} = 100\ \text{ppm}_{\text{(NOAEL)}} \times 1/10_{\text{(species factor)}} = 10\ \text{ppm}$$

Using the 15 wk (105 d) NOAEL of 100 ppm Wong (1996) calculated the 30-d AC for liver toxicity as follows after applying a factor for discontinuous-continuous exposure.

$$\text{30-d AC}_{\text{(liver toxicity)}} = 100\ \text{ppm}_{\text{(NOAEL)}} \times 1/10_{\text{(species factor)}} \times [7\ \text{h}/24\ \text{h} \times 5\ \text{d}/7\text{d}]\ (_{\text{discontin. to cont.)}} \times 105\ \text{d}/30\ \text{d}_{\text{(time extrapolation)}} = 7.3\ \text{ppm}$$

Using a NOAEL of 100 ppm based on exposure of rats and guinea pigs for 7h/d, 5 d/wk, for 30 wk (210 d) a 180-d AC for hepatotoxicity was calculated:

$$\text{180-d AC}_{\text{(liver toxicity)}} = 100\ \text{ppm}_{\text{(NOAEL)}} \times 1/10_{\text{(species factor)}} \times [7\ \text{h}/24\ \text{h} \times 5\ \text{d}/7\ \text{d}]\ (_{\text{discontin. to cont.)}} \times 210\ \text{d}/180\ \text{d}_{\text{(time extrapolation)}} = 2.4\ \text{ppm}$$

NASA evaluated the hepatotoxicity data used for these durations to determine whether BMD modeling could be used. Although Spencer et al. (1951) used various concentrations of EDC (100, 200, and 400 ppm for repetitive exposures) in guinea pigs and rats and stated that they had measured several parameters to assess toxicity, the only quantitative data they presented were changes in body weight and tissue weight. In general, the NRC SMAC and spacecraft water exposure guideline committees do not consider body weight changes or organ weight changes to be robust variables for calculating ACs (NRC 1992, 2000). Thus, because of the absence of quantitative dose-response data, BMD modeling could not be carried out on the results from this study.

Wong (1996) also used impaired immune defense as an adverse end point and derived 1-h, 24-h, 7-d, 30-d, and 180-d ACs from the results of Sherwood et al. (1987), who reported that a single 3-h exposure of young mice to 5-11 ppm of EDC by inhalation increased mortality after the mice were infected (challenge) with *Streptococcus zooepidemicus*. However, at 2.5 ppm this effect was not seen (NOAEL is 2.5 ppm for 3 h). Sherwood et al. also pointed out that the immunotoxic response differed among species; young male rats exposed to 200

ppm of EDC for 5 h or to 100 ppm for 5 h/d for 12 days were unaffected when challenged via inhalation with *Klebsiella pneumoniae*, whereas young mice showed infection in response to this organism even with just 11 ppm of EDC for 3 h. The most sensitive response was used to derive the AC very conservatively for humans.

For all durations up to 180 d (1 h, 24 h, 7 d, 30 d, and 180 d), Wong (1996) used the impaired host defense end point described above. He also used a space flight factor of 3 to protect against microgravity-induced impairment of the cell-mediated immune response (Taylor 1993).

The AC for the immunologic end point for all durations was calculated as follows:

$$\text{1- and 24-h AC}_{\text{(impaired host defense)}} = 2.5 \text{ ppm}_{\text{(NOAEL)}} \times 1/3_{\text{(spaceflight factor)}} = 0.8 \text{ ppm}$$

Thus, an AC of 0.8 ppm for impaired host defense was derived for all durations up to 180 d.

Wong (1996) also derived a 180-d AC for carcinogenic effects. He used the report of Ward (1980), who presented a National Cancer Institute (NCI) study (NCI 1978) showing that EDC produced tumors in multiple organs of Osborne-Mendel rats (50/dose/sex) and in B6C3F1 mice (50/dose/sex) that were administered EDC as a daily gavage in corn oil, 5 d/wk, for 78 wk. The two estimated TWA doses were 47 and 97 mg per kg of body weight for male and female rats, 97 and 195 mg/kg for male mice, and 149 and 299 mg/kg for female mice. Observed tumors included squamous cell carcinomas of the forestomach, hemangiosarcomas of the circulatory system, and fibrosarcomas of the subcutaneous tissue. Tumors were also found at other organ sites. Hepatocellular carcinomas and alveolar/bronchiolar adenomas were seen in male and female mice. In female rats and mice, mammary carcinomas were also noted. A summary of these results is presented in Table 9-1.

TABLE 9-1 Tumors Found in NCI Bioassay of EDC[a]

Species/Sex	Adverse Effect	Site
Rat/male	Squamous cell carcinoma	Forestomach
	Hemangiosarcoma	Circulatory system
	Fibroma	Subcutaneous tissue
Rat/female	Adenocarcinoma	Mammary gland
Mouse/female	Adenocarcinoma	Mammary gland
Mouse/female	Stromal polyp	Endometrium
	Stromal sarcoma	Endometrium
Mouse/male & female	Adenoma	Alveoli and bronchioli

[a]The observed incidence rate of tumors in exposed animals is statistically different from that of controls.
Source: NCI 1978.

Although these serious tumorigenic responses were found in the gavage study, Maltoni et al. (1980) found no evidence of carcinogenicity in a lifetime EDC inhalation exposure study in which Sprague-Dawley rats and Swiss mice were exposed to EDC at 5 to 150 ppm (20 to 600 mg/m^3). Another study by Cheever et al. (1990), in which groups of 50 male and 50 female Sprague-Dawley rats were exposed by inhalation to 50 ppm EDC for 7 h/d, 5 d/wk, for 2 y, showed no significant increase in incidence of any tumor type in male and female rats. However, the study included only one dose and one species.

Reitz et al. (1982) compared the pharmacokinetics of EDC in rats after oral and inhalation routes of administration. The oral dose selected (150 mg/kg in corn oil) was the same as that of the National Toxicology Program's (NTP 1991) EDC gavage study. The concentration and duration selected for inhalation exposure (150 ppm for 6 h) was the same as that of Maltoni et al.'s EDC inhalation study (1980), except Maltoni et al. exposed rats for 7 h/d. The peak blood concentrations were about 5-fold higher in the EDC-gavaged animals than in rats exposed by inhalation. The amounts of EDC metabolites binding to liver, spleen, kidney, and stomach DNA were 2 to 5 times greater in the gavaged animals than in those exposed by inhalation; however, the overall extent of DNA alkylation was low.

In spite of this notable difference in cancer incidence between the gavage studies and inhalation exposure studies, and although no adequate data were available to evaluate the carcinogenicity of EDC in humans, both the NCI and the International Agency for Research on Cancer (IARC) (NCI 1978, IARC 1999) declared that EDC is reasonably anticipated to be a human carcinogen based on sufficient evidence of carcinogenicity in experimental animals. IARC (1999) classified EDC as Group 2B (the agent [mixture] is possibly carcinogenic to humans). The U.S. Environmental Protection Agency (EPA) classified EDC as B2, a probable human carcinogen. They extrapolated the gavage data to an inhalation exposure scenario and calculated an inhalation unit risk (unit risk = upper-bound excess lifetime cancer risk estimated to result from continuous exposure to an agent at a concentration of 1 microgram [μg]/m^3 in air) using the linearized multistage procedure extrapolation method. One hundred percent absorption from inhalation and metabolism at low dose were assumed when oral data were used to calculate inhalation unit risk.

Wong (1996) used the tumor data from the NCI gavage study to derive an AC for inhalation exposure, from which U.S. EPA estimated that 4 μg/m^3 would yield an excess tumor risk of less than 1 in 10,000 for continuous lifetime exposure of humans. Following the approach recommended by the NRC (1992), which assumes the earliest age of exposure is 30 y and the average life span of an astronaut is 70 y, an adjustment factor of 146.7 (see NRC 1992, pp. 88-89) was calculated and used to compress the EPA estimated value of 4 mg/m^3 into a much shorter continuous exposure of 180 d that would yield the same tumor risk.

With these adjustment factors, the concentration of EDC for a risk of 1×10^{-4} was calculated to be 0.2 ppm (0.8 mg/m^3).

RATIONALE

The original SMACs were set in 1996, before the current NRC approaches to data analysis such as BMD modeling came to be used by many regulatory organizations in risk assessment. Thus, the 1996 calculations of ACs and SMACs were based on the conventional NOAEL-LOAEL approach. In derivation of the 1,000-d AC, the dose-response data were evaluated for applicability to dose-response modeling.

Exposure limits and recommended amounts set by other organizations are shown in Table 9-2. The EPA did not derive an oral reference dose or an inhalation reference concentration for non-caricogenic effects, but did calculate an oral slope factor for the carcinogenic potency of EDC based on the oral carcinogenicity data from the NCI (1978) study in rats described elsewhere in this document. The EPA (1991) arrived at an oral slope factor (an upper-bound estimate of the human cancer risk per mg of agent/kg body weight/d) of 0.091 $(mg/kg/d)^{-1}$ by using a linearized multistage procedure. This corresponds to a drinking water unit risk of 2.6×10^{-6} µg/liter. An inhalation unit risk of 2.6×10^{-5} µg/m^3 was derived from the oral data assuming 100% absorption from inhalation. This equals a risk of 4 µg/m^3 (EPA 1991) using a nominal adult body weight of 70 kg and a daily respiratory volume of 20 m^3. Inhalation unit risk represents the potential excess cancer risk for a person exposed for a lifetime to EDC at 1 µg/m^3 and is at most 22 in 1,000,000. The EPA's estimated inhalation carcinogenic risks and associated EDC air concentrations, summarized in IRIS (1991), are shown in Table 9-3.

TABLE 9-2 A Summary of Exposure Standards or Recommended Levels by Other Organizations for EDC Vapors

Organization, Standard	Exposure Limit (ppm)	References
ACGIH		ACGIH 1996
TLV-TWA	10	
OSHA		NIOSH 2005
PEL TWA	50	
STEL ceiling	100	
NIOSH REL		NIOSH 2005
TWA	1	
ceiling	2	
NIOSH IDLH		NIOSH 1996
Original[a]	1,000	
Revised[b]	50	
NIOSH STEL	2	NIOSH 2005

Conversion: 1 ppm = 4.05 mg/m^3.
[a]Original IDLH was based on rat data of Spencer et al. (1951).

(Continued)

TABLE 9-2 Continued

[b]Basis for revised IDLH: acute inhalation toxicity data from Polish agricultural workers (Brzozowski et al. 1954).
Abbreviations: ACGIH, American Conference of Governmental Industrial Hygienists; IDLH, immediately dangerous to life and health; NIOSH, National Institute for Occupational Safety and Health; OSHA, Occupational Safety and Health Administration; PEL, permissible exposure limit; ; REL, recommended exposure limit; STEL, short-term exposure limit; TLV, threshold limit value; TWA, time-weighted average.

TABLE 9-3 Air Concentration and Specified Carcinogenic Risk Levels

Carcinogenic Risk	EDC Concentration ($\mu g/m^3$)
1 in 10,000	4
1 in 100,000	0.4
1 in 1,000,000	0.04

Source: EPA 1991.

Minimal Risk Levels from Agency for Toxic Substances and Disease Registry

An acute-duration inhalation minimal risk level (MRL) for EDC has not been derived by the Agency for Toxic Substances and Disease Registry (ATSDR). An intermediate-duration MRL (15-364 days) adopted the chronic-duration MRL (ATSDR 2001). An MRL of 0.6 ppm (2.4 mg/m^3) was derived for chronic-duration inhalation exposure to EDC, which will be protective for intermediate-duration inhalation exposure to EDC. This was derived by using the NOAEL for liver histopathology data from the study of Cheever et al. (1990), which exposed rats to EDC at 50 ppm for 7 h/d, 5 d/wk, for 2 y. The details of the Cheever et al. (1990) study are described elsewhere in this document. ATSDR used an uncertainty factor of 90 (3 for species extrapolation, 10 for human variability, and 3 as a modifying factor for database deficiency including lack of dose-response data). Using liver as the target organ for EDC, toxicity was justified because several studies have shown hepatotoxicity after exposure to EDC. In deriving this MRL, ATSDR did not use a conversion factor for adjusting the intermittent exposure to a continuous exposure and did not derive a carcinogen potency factor even though the literature on cancer from exposures to EDC was discussed in the ATSDR toxicology profile for EDC (ATSDR 2001).

Changes to Previously Established SMACs for EDC

As mentioned previously, none of the AC values calculated in the previous EDC SMAC document could be recalculated by using newer methods such as

BMD modeling. There has been no study since 1996 that is suitable to rederive the ACs for these durations. Therefore, it was decided to leave the values as they are. Initially, Wong (1996) used CNS depression as an end point to derive 1- and 24-h ACs. This approach resulted in a value of 1.5 ppm for both 1- and 24-h durations. However, Wong (1996) also considered GI effects to derive the 1- and 24-h ACs, because GI effects had been the critical end point in deriving the 7-, 30-, and 180-d ACs. This approach resulted in all ACs (1 h to 180 d) having the same value of 0.4 ppm. The 180-d AC was lowered to 0.2 ppm when carcinogenesis was used as the critical end point for long-term derivation. Considerable concern has been raised about the validity and strength of the rationale behind using the oral data to compute the inhalation risk factor for cancer while experiments using inhalation exposure for a sufficiently long time did not provide convincing evidence that EDC could be carcinogenic to humans via inhalation. NASA decided to reassess this approach, as shown below under derivation of the 1,000-d AC. As a result, NASA decided to withdraw the 180-d AC value of 0.2 ppm derived for a carcinogenic end point, which was the driver for the 180-d SMAC.

Relevant Data Since 1996

NASA reviewed the literature to find a long-term EDC inhalation exposure study to use for deriving a 1,000-d AC (and a SMAC) for both a noncarcinogenic toxicity end point and, if applicable, a carcinogenic risk factor for 1 in 10,000. NASA did not find a study that could be used since 1996, when the previous SMACs for durations were derived.

Derivation of 1,000-Day ACs for EDC

First, data from the human occupational exposure study published by a Russian investigator were considered for 1,000-d AC. Kozik (1957) studied workers in the aircraft industry in Russia who applied glue containing EDC as a solvent to large rubber sheets. On the basis of Kozik's data, the National Institute for Occupational Safety and Health estimated that, in the first half of the shift, the TWA exposure concentrations of EDC were 28 ppm (113 mg/m^3) during glue application and 16 ppm (65 mg/m^3) during the time the glue dried (NIOSH 1976). In the second half of the shift, the TWA exposure concentration of EDC was 11 ppm (44.6 mg/m^3). Therefore, the EDC TWA exposure concentration for the entire shift was 15 ppm (60.8 mg/m^3) (NIOSH 1976). Effects reported by Kozik (1957) were likely caused by exposures to much higher (up to about 50 ppm) repeated short-term exposures for certain job categories rather than to the TWA exposure. Thus, the TWA of 15 ppm represents a very protective concentration to use as a LOAEL and may actually be a NOAEL for short-term exposures, although the data presented by this study are insufficient to determine that. Comparing the morbidity data of the gluers and the machinists,

who were not exposed to EDC, Kozik (1957) reported that the EDC exposure increased both the number of cases of acute GI disorders per 100 workers and the number of workdays lost to acute GI disorders per 100 workers. Kozik also measured the hand-eye coordination speed of the gluers and machinists at the start and end of the workday for 14 d in 17 gluers and 10 machinists (as controls). The speed did not differ among the groups. However, the EDC-exposed gluers made more errors in the test than did the nonexposed machinists (error rates of 30% for the gluers and 10% for the machinists). A number of factors were considered in the study even though the concentration versus effect was not clearly discernable because of the uncertainty in the exposure concentration.

NASA and the NRC Committee on Spacecraft Exposure Guidelines (SEGs) reevaluated the Kozik (1957) study for results on neurotoxicity and GI disturbances and concurred that the occupational exposure data from the Kozik (1957) study can be used for the 1,000-d AC. Kozik (1957) also included a laboratory study of changes in conditioned reflex activity in rats exposed to very low concentrations of EDC (2.5 ppm). However, because of a considerable lack of detail about the experimental design (e.g., sex, strain, age of rats, number of rats per group, presence of a control group, and details of EDC analysis and exposure data) and results (e.g., no data tables), it was concluded that the rat study should not be considered for AC derivation for any duration.

The 1,000-d AC for GI disturbances and neurotoxicity can be calculated based on a LOAEL of 15 ppm as follows. AC is derived for a prolonged continuous duration of 1,000 d, and for GI effects and neurological effects the concentration was adjusted for discontinuous-to-continuous exposures.

$$\text{LOAEL}_{(\text{adjusted})} = 15 \text{ ppm}_{(\text{LOAEL})} \times [8 \text{ h}/24 \text{ h} \times 5 \text{ d}/7 \text{ d}]_{(\text{discontin. to contin.})} = 3.57 \text{ ppm}$$

$$1{,}000\text{-d AC}_{(\text{GI disturbances and neurotoxicity})} = 3.57 \text{ ppm}_{(\text{LOAEL adjusted})} \times 1/10_{(\text{LOAEL to NOAEL})} = 0.357, \text{ rounded to } 0.4 \text{ ppm}$$

The 7-y exposure data are used for the shorter duration of only 1,000 d. Because of this margin of safety, 0.357 was rounded to 0.4 ppm. No time extrapolation is needed.

Some rodent studies were also considered for 1,000-d AC derivation. It was decided to use the absence of abnormal liver histopathology data reported in the Cheever study for noncarcinogenic effects at 1,000 d. In the Cheever et al. (1990) study, groups of male and female Sprague-Dawley rats (50/sex/dose) were exposed to EDC by inhalation of 50 ppm for 7 h/d, 5 d/wk, for 2 y. Body weights, survival rates, and absolute and relative liver weights of animals were not affected. Gross pathology and histopathologic lesions were evaluated for incidence of intrahepatic bile duct cholangiomas in liver, mammary, and testicular tissues; incidence of subcutaneous fibromas, neoplastic nodules, and interstitial cell tumors in the testes; and incidence of mammary adenocarcinomas. No significant increase in the number of any tumor type was observed in rats ex-

posed to EDC. There were also no histological lesions of the respiratory tract. The limitations of this study are that no clinical biochemical variables were measured and no dose-response data were collected. Only a NOAEL has been identified, without a LOAEL; thus, only limited evaluations of toxicity could be derived. There may be some uncertainty about the NOAEL. As large numbers of both sexes of animals were used, it may not be a serious issue. No new methodology could be used to derive a 1,000-d AC.

Using 50 ppm as a NOAEL for the absence of abnormal liver or lung histopathology, a 1,000-d AC can be calculated after using an appropriate adjustment factor (Adj) for exposure duration. For calculation of the NOAEL (Adj), the NRC Committee on Spacecraft Exposure Guidelines recommends modifying the previous approach so that when data are extrapolated from a chronic-duration animal study (especially a 2-y study) to 1,000 d for human exposure an additional time factor of 728 d (2 y) to 1,000 d is not necessary, because 2 y is a greater fraction of a rat's lifetime than 1,000 d is of a human's lifetime.

The 1,000-d AC for histopathology and general toxicity can be calculated as follows:

$$\text{NOAEL}_{(\text{adjusted})} = 50 \text{ ppm}_{(\text{NOAEL})} \times [7 \text{ h}/24 \text{ h} \times 5 \text{ d}/7 \text{ d}]_{(\text{discontin. to contin.})} = 10.40 \text{ ppm}$$

$$1{,}000\text{-d AC}_{(\text{histopathology and general toxicity})} = 10.40 \text{ ppm}_{(\text{NOAEL adjusted})} \times 1/10_{(\text{species factor})} = 1.04 \text{ ppm, rounded to } 1.00 \text{ ppm}$$

Thus, the 1,000-d AC for hepatotoxicity is 1.00 ppm.

Another chronic exposure inhalation study by Spreafico et al. (1980) that showed both liver and kidney toxicity were used to derive a 1,000-d AC for a noncarcinogenesis end point. In this study, rats (8-10/sex/dose) were exposed to EDC at 0, 5, 10, 50, and 150-250 ppm for 7 h/d, 5 d/wk, for up to 18 mo. The authors did not state how many animals were exposed to 150 ppm after some deaths at 250 ppm and also did not say clearly how long after the deaths occurred the dose was reduced to 150 ppm. Serum chemistries were measured at 3, 6, 12, and 18 mo. Animals were exposed starting at 3 mo of age. In this study, some older animals (14 mo old) were also exposed to EDC but only for 12 mo. Serum chemistries were unremarkable up to and including the 50-ppm group. However, rats exposed to higher amounts of EDC for 12 mo had increased serum alanine transaminase activity at the two highest exposure concentrations, indicative of chronic liver damage. Changes in lactate dehydrogenase and aspartate transaminase concentrations did not appear to be dose related. Increased blood urea nitrogen concentrations in the 150-ppm group and increased uric acid levels at the two highest exposure groups indicated renal toxicity. This study

identified an 18-mo NOAEL of 50 ppm. As 18 mo is a significant fraction of a rat's lifetime compared with 1,000 d in a human lifetime, an additional time extrapolation factor for 18 mo to 1,000 d was not used.

A 1,000-d AC for hepatotoxicity and nephrotoxicity as a critical adverse end point can be calculated after adjusting the NOAEL for going from a discontinuous to a continuous exposure dose.

$$NOAEL_{(adjusted)} = 50 \text{ ppm}_{(NOAEL)} \times [7 \text{ h}/24 \text{ h} \times 5 \text{ d}/7 \text{ d}]_{(discontin.\ to\ contin.)} = 10.40 \text{ ppm}$$

$$1{,}000\text{-d } AC_{(hepatotoxicity\ and\ nephrotoxicity)} = 10.40 \text{ ppm}_{(NOAEL\ adjusted)} \times 1/10_{(species\ factor)} = 1.04 \text{ ppm, rounded to } 1.00 \text{ ppm}$$

Thus, a 1,000-d AC for hepatotoxicity and nephrotoxicity was calculated as 1.00 ppm.

The subchronic study of Spencer et al. (1951) was also evaluated for the 1,000-d AC derivation. In this study, male and female rats, guinea pigs, rabbits, and monkeys were exposed to EDC by inhalation at various concentrations. Rats were exposed to 400 and 100 ppm for 7 h/d, 5 d/wk, for 6 mo, and some additional rats were exposed to 200 ppm for 30 wk. Male and female guinea pigs were exposed to 200 ppm for 36 wk. In all the animal species tested, no adverse effects were observed in groups exposed to 100 ppm or less. Although no adverse effects were found in rats exposed to 200 ppm for 30 wk, mild hepatotoxic effects were noted in the guinea pigs (such as parenchymatous degeneration with some vacuolization). Severe effects, including hepatotoxicity and death, were observed in rats and guinea pigs exposed at 400 ppm. As the data indicated a clear NOAEL of 100 ppm for hepatotoxicity, this concentration was chosen for deriving a 1,000-d AC.

A 1,000-d AC for hepatotoxicity as the critical effect can be calculated with data from the study by Spencer et al. (1951), as shown below. Concentration adjustments for intermittent to continuous exposures and factors for time extrapolation from 210 d to 1,000 d are used.

$$NOAEL_{(adjusted)} = 100 \text{ ppm}_{(NOAEL)} \times [7 \text{ h}/24 \text{ h} \times 5 \text{ d}/7 \text{ d}]_{(discontin.\ to\ contin.)} \times 210 \text{ d}/1{,}000 \text{ d}_{(time\ extrapolation)} = 4.375 \text{ ppm}$$

$$1{,}000 \text{ d } AC_{(hepatotoxicity)} = 4.375 \text{ ppm}_{(NOAEL\ adjusted)} \times 1/10_{(species\ factor)} = 0.4375 \text{ ppm, rounded to } 0.45 \text{ ppm}$$

A summary of ACs derived for 1,000 d for various end points is shown in Table 9-4. The 1,000-d SMAC is 0.4 ppm (1.6 mg/m^3), based on the lowest 1,000-d AC for all end points.

TABLE 9-4 Summary of 1,000-d ACs for Vapors to EDC by Inhalation

1,000-d AC as ppm	1,000-d AC as mg/m^3	Critical Adverse Effect	Principal Study
0.40	1.6	GI effects and neurological effects	Kozik 1957
1.00	4.0	Tissue histopathology	Cheever et al. 1990
1.00	4.0	Hepatotoxicity	Spreafico et al. 1980
0.45	1.8	Hepatotoxicity	Spencer et al. 1951

Evaluation of Studies for Deriving a 1,000-Day AC for Carcinogenic Risk Level

This discussion also applies to the derivation of a 180-d AC by Wong (1996) based on the NTP 2-y EDC oral carcinogenicity bioassay. NASA has decided to withdraw use of the oral carcinogenesis bioassay data for calculating an inhalation cancer risk factor for EDC for 1,000 d.

The strength of the NCI cancer bioassay, which showed tumors in multiple sites, is that the bioassay was conducted in both sexes and in two species and with at least two doses of EDC. Nevertheless, one needs to provide a strong justification to resort to a "route-to-route extrapolation" method for extrapolation of results from this ingestion study to inhalation exposures, especially when sufficient data are already available from some long-term EDC inhalation exposure studies in which no tumors were found. Adequate understanding of the explanations offered for the striking differences in the tumor-induction response between these two routes of exposures is limited. However, NASA took several factors into account in its decision to not use the NTP cancer bioassay data to derive an inhalation cancer risk factor. Differences in the internal dose and pharmacokinetics between these two routes of exposure, the confounding factor of pharmacokinetic changes due to the use of corn oil for the gavage, the metabolic saturation behavior and potential consequences, and the levels of DNA alkylations, DNA damage, and in vitro and in vivo genotoxicity are the factors NASA considered in its decision.

Supporting evidence includes pharmacokinetic data and comparative toxicity studies of bolus dosing of solvents in corn oil versus administration in drinking water (more similar to dosing associated with inhalation). One can make certain inferences based on results from some existing EDC pharmacokinetic data from oral and inhalation studies (Reitz et al. 1982). When Reitz et al. (1982) administered EDC at dose rates approximately comparable to those used by NCI (1978) and Maltoni et al. (1980), the peak blood concentration of EDC from a one-time oral bolus was about 5 times higher than that from an inhalation exposure for 6 h, even though the EDC gavage dose of 150 mg/kg was only 1.3 times higher than the dose associated with the 150-ppm, 6-h EDC inhalation protocol (delivered dose estimated to be 113 mg/kg). When blood concentra-

tions are high, saturation of the primary oxidation pathway via the microsomal mixed function oxidase system (followed by detoxification) will shunt the EDC to form reactive species via a direct glutathione binding pathway. In spite of a significant difference in EDC pharmacokinetics between ingestion and inhalation exposures, and even though the amounts of DNA alkylations measured in various target and nontarget tissues (as a surrogate parameter for genetic damage and potency for cancer induction) were 2- to 5-fold higher in rats dosed with EDC by gavage than in those exposed by inhalation, the levels of DNA alkylations in these tissues were found to be on the order of only 2 to 20 per 10^6 nucleotides (Reitz et al. 1982).

According to Reitz et al. (1982), it has been argued that the differences lie mostly in the metabolic saturation kinetics and behavior of the two pathways that are known to be involved with the biotransformation of EDC in the body. They estimated that the metabolic pathway for EDC would be saturated after an animal received an EDC dose of 25 mg/kg by gavage or 150 ppm by inhalation. In short, Reitz et al. (1982) proposed that EDC exposure by inhalation did not result in high enough EDC concentrations in blood to saturate its oxidation and detoxification pathway. This limited the amount of EDC available for the formation of *S*-(2-chloroethyl)glutathione, a reactive intermediate formed by direct conjugation of EDC to glutathione, which can bind to cellular macromolecules including DNA (Reitz et al. 1982). This difference in the metabolic saturation can partly explain why no significant increase in tumors was found in the inhalation experiments. The data on metabolic saturation are consistent with the observation by Spreafico et al. (1980) that saturation occurs at inhalation concentrations around 150 to 250 ppm, based on the finding in rats that when the EDC concentration was increased from 50 to 250 ppm (5-fold) the blood EDC concentration increased 22-fold. Some studies reported in the literature on single versus continuous boluses indirectly support the conclusions from the Reitz et al. acute exposure pharmacokinetic study (see gavage versus drinking-water studies described below).

The NCI cancer bioassay protocol involved gavage administration of EDC in corn oil. Whether the positive results for tumor incidence in the NTP oral ingestion study might be due to the vehicle used for administration has been questioned. NASA reviewed studies in which a few volatile organics were administered by gavage in corn oil that resulted in a large amount in the system in a short period of time, and in drinking water, which delivered the chemical in small doses over an extended time. A subchronic study of EDC compared the two different types of administration. For example, a 13-wk NTP drinking water and gavage toxicity study was conducted to investigate the potential differences in strain susceptibility to EDC toxicity in F344/N rats, Sprague-Dawley rats, and Osborne-Mendel rats. The study concluded that administration of EDC in drinking water resulted in less toxicity than administration of similar doses by gavage (in corn oil) (NTP 1991). Similar results were reported in studies using other haloalkanes. For example, Larson et al. (1994) showed that chloroform increased the incidence of liver tumors in B6C3F1 mice when administered in

corn oil by gavage but not when similar daily doses were given ad libitum in drinking water. Similarly, Geter et al. (2004) reported that two other haloalkanes, bromodichloromethane and tribromomethane, which produced neoplasia in the large intestine when given as a corn oil gavage, failed to elicit such effects in male rats when given via drinking water for 26 wk.

EDC has been shown to cause mutagenesis in *Salmonella typhimurium* TA 1535 and TA 100 and in several short-term genotoxicity assays (see WHO 1996). Although EDC tested positive in the in vitro micronucleus test in isolated human lymphocytes, a clear dose-dependent mutagenic activity was not found (Tafazoli et al. 1998). More importantly, Storer et al. (1984) studied the in vivo genotoxicity of EDC in the livers of male mice (B6C3F1) after single oral, intraperitoneal, or inhalation exposures of 150 and 500 ppm for 4 h by measuring single-strand breaks in liver DNA (alkali-labile lesions). Although genotoxic effects were found after intraperitoneal and oral administration in corn oil (even at the lowest dose of 100 mg/kg), EDC failed to produce any evidence of a genotoxic effect (hepatic DNA damage) in mice exposed to it for 4 h at 500 ppm, a dose that all animals survived. In the gavage-dosed animals, the DNA damage persisted even at 24 h (Storer et al. 1984). When the genetic activity of EDC was tested in the germ cells and somatic tissue of *Drosophila melanogaster* after inhalation treatment with EDC, the induction of interchromosomal recombination was very low (Ballerring et al. 1993).

The most important consideration is that the studies that primarily aimed to assess the carcinogenic potential of EDC by the inhalation route did not show carcinogenic activity even at maximal tolerated doses. One might argue that, in one study (Cheever et al. 1990), only one dose (50 ppm) and one species (rats, both sexes) were used. However, in the other study (Maltoni et al. 1980), doses close to the maximum tolerated doses, two species of animals (rats and mice), and both sexes were used, with 90 animals in each treatment group, and the animals were exposed for a fairly long time (78 wk), which is most of their life span.

Thus, extrapolating oral ingestion study data to inhalation data to derive a carcinogen risk factor for inhalation exposure cannot be fully justified.

NASA is interested in setting a SMAC for 1,000 d, which is only a fraction of a human lifetime. It is unlikely that a space crew would be chronically exposed to concentrations of EDC used in the inhalation studies. The absence of a tumorigenic response from the results of inhalation studies provides sufficient confidence to believe that there will not be a carcinogenic risk to the crew on a 1,000-d mission.

If there is almost no probability of carcinogenic risk for 1,000 d, there is a larger margin of safety for a 180-d AC for this effect. Thus, the 180-d AC value for a carcinogenic end point will be withdrawn and will not be the basis for the 180-d SMAC.

A comprehensive summary of SMACs for all durations from 1 h to 1,000 d is presented in Table 9-5.

TABLE 9-5 A Summary of SMACs for EDC for Various Durations

Duration	Concentration (ppm)	Concentration (mg/m^3)	Adverse Endpoint	Principal Study
1 h	0.40	1.6	GI symptoms	Kozik 1957
24 h	0.40	1.6	GI symptoms	Kozik 1957
7 d	0.40	1.6	GI symptoms	Kozik 1957
30 d	0.40	1.6	GI symptoms	Kozik 1957
180 d	0.40	1.6	GI effects	Kozik 1957
1,000 d	0.40	1.6	Hepatotoxicity and GI effects	Spencer et al. 1951 and Kozik 1957

REFERENCES

ACGIH (American Conference of Governmental Industrial Hygienists). 1996. Ethylene Dichloride. Documentation of the Threshold Limit Values and Biological Exposure Indices, 6th Ed. American Conference of Governmental Industrial Hygienists, Cincinnati, OH.

ATSDR (Agency for Toxic Substances and Disease Registry). 2001. Toxicological Profile for 1,2-Dichloroethane (Update). U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry, Washington, DC [online]. Available: http://www.atsdr.cdc.gov/toxprofiles/tp38.html [accessed Oct. 3, 2007].

Ballering, L.A., M.J. Nivard, and E.W. Vogel. 1993. Characterization of the genotoxic action of three structurally related 1,2-dihaloalkanes in *Drosophila melanogaster*. Mutat. Res. 285(2):209-217.

Banerjee, S., B.L. Van Duuren, and F.I. Oruambo. 1980. Microsome-mediated covalent binding of 1,2-dichloroethane to lung microsomal protein and salmon sperm DNA. Cancer Res. 40(7): 2170-2173.

Brzozowski, J., J. Czajka, T. Dutkiewicz, I. Kesy, and J. Wojcik. 1954. Hygiene and the health condition of workers employed in eradication of potato beetle *Leptinotarsa decemlineata* with hexachlorocyclohexane and with dichloroethane [in Polish]. Med. Pr. 5(2): 89-98.

Byers, D.H. 1943. Chlorinated solvents in common wartime use. Ind. Med. 12(7):440-443.

Cheever, K.L., J.M. Cholakis, A.M. el-Hawari, R.M. Kovatch, and E.K. Weisburger. 1990. Ethylene dichloride: The influence of disulfiram or ethanol on oncogenicity, metabolism, and DNA covalent binding in rats. Fundam. Appl. Toxicol. 14(2):243-261.

D'Souza, R.W., W.R. Francis, and M.E. Andersen. 1988. Physiological model for tissue glutathione depletion and increased resynthesis after ethylene dichloride exposure. J. Pharmacol. Exp. Ther. 245(2):563-568.

EPA (U.S. Environmental Protection Agency). 1991. 1, 2-Dichloroethane (CASRN 107-06-2). Quantitative Estimate of Carcinogenic Risk from Inhalation Exposure. Integrated Risk Information System, U. S. Environmental Protection Agency [online]. Available: http://www.epa.gov/iris/subst/0149.htm [accessed Oct. 3, 2007].

Geter, D.R., M.H. George, T.M. Moore, S.R. Kilburn, G. Huggins-Clark, and A.B. DeAngelo. 2004. Vehicle and mode of administration effects on the induction of

aberrant crypt foci in the colons of male F344/N rats exposed to bromodichloromethane. J. Toxicol. Environ. Health A. 67(1):23-29.

Guengerich, F.P., W.M. Crawford, Jr., J.Y. Domoradzki, T.L. Macdonald, and P.G. Watanabe. 1980. In vitro activation of 1,2-dichloroethane by microsomal and cytosolic enzymes. Toxicol. Appl. Pharmacol. 55(2):303-317.

Heppel, L.A. P.A. Neal, T.L. Perrin, K.M. Endicott, and V.T. Porterfield. 1946. The toxicology of 1,2-dichloroethane (ethylene dichloride). V. The effects of daily inhalations. J. Ind. Hyg. Toxicol. 28(4):113-120.

IARC (International Agency for Research on Cancer). 1999. 1, 2-Dichloroethane. Pp. 501-529 in Re-evaluation of Some Organic Chemicals, Hydrazine and Hydrogen Peroxide. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans Vol. 71, Part 2. Lyon: IARC.

Kozik, I. 1957. Problems of occupational hygiene in the use of dichloroethane in the aviation industry [in Russian]. Gig. Tr. Prof. Zabol. 1:31-38 [as cited in Wong 1996].

Larson, J.L., D.C. Wolf, and B.E. Butterworth. 1994. Induced cytotoxicity and cell proliferation in the hepatocarcinogenicity of chloroform in female B6C3F1 mice: Comparison of administration by gavage in corn oil vs ad libitum in drinking water. Fundam. Appl. Toxicol. 22(1):90-102.

Maltoni, C., L. Valgimigli, and C. Scarnato. 1980. Long-term carcinogenic bioassays on ethylene dichloride administered by inhalation to rats and mice. Pp. 3-33 in Ethylene Dichloride: A Potential Health Risk? B. Ames, P. Infante, and R. Reitz, eds. Banbury Report 5. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

McCann, J., V. Simmon, D. Streitwieser, and B.N. Ames. 1975. Mutagenicity of chloroacetaldehyde, a possible metabolic product of 1,2-dichloroethane (ethylene dichloride), chloroethanol (ethylene chlorohydrin), vinyl chloride, and cyclophosphamide. Proc. Natl. Acad. Sci. USA 72(8):3190-3193.

NCI (National Cancer Institute). 1978. Bioassay of 1,2-Dichloroethane for Possible Carcinogenicity (CAS No. 107-06-2). Technical Report No. 55. DHEW (NIH) 78-1361. U.S. Department of Health, Education and Welfare, Public Health Service, National Institute of Health, Bethesda, MD.

NIOSH (National Institute for Occupational Safety and Health). 1976. Criteria for a Recommended Standard: Occupational Exposure to Ethylene Dichloride (1,2-Dichloroethane). NIOSH 76-139. U.S. Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, National Institute of Occupational Safety and Health, Washington, DC. March 1976.

NIOSH (National Institute for Occupational Safety and Health). 1996. Ethylene dichloride. In Documentation for Immediately Dangerous to Life or Health Concentrations (IDLH): NIOSH Chemical Listing and Documentation of Revised IDLH Values (as of 3/1/95). National Institute for Occupational Safety and Health [online]. Available: http://www.cdc.gov/niosh/idlh/107062.html [accessed July 11, 2008].

NIOSH (National Institute for Occupational Safety and Health). 2005. NIOSH Pocket Guide to Chemical Hazards. DHHS (NIOSH) Publication No. 2005-149. National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention, U.S. Department of Health and Human Services, Cincinnati, OH [online]. Available: http://www.cdc.gov/niosh/npg/ [accessed Apr. 22, 2008].

NRC (National Research Council). 1992. Pp. 88-89 in Guidelines for Developing Spacecraft Maximum Allowable Concentrations for Space Station Contaminants. Washington, DC: National Academy Press.

NRC (National Research Council). 2000. Methods for Developing Spacecraft Water Exposure Guidelines. Washington, DC: National Academy Press.

NTP (National Toxicology Program). 1991. Toxicity Studies of 1,2-Dichloroethane (Ethylene Dichloride) (CAS No. 107-06-2) in F344/N Rats, Sprague Dawley Rats, Osborne-Mendel Rats, and B6C3F1 Mice (Drinking Water and Gavage Studies). NTP TOX 4. NIH Publication No. 91-3123. National Toxicology Program, U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC.

NTP (National Toxicology Program). 2005. 1,2-Dichloroethane (Ethylene Dichloride) CAS No. 107-06-2. In Report on Carcinogens, 11th Ed. U.S. Department of Health and Human Services, Public Health Service, National Toxicology Program [online]. Available: http://ntp.niehs.nih.gov/ntp/roc/eleventh/profiles/s065dich.pdf [accessed Oct. 4, 2007].

Reitz, R.H., T.R. Fox, J.C. Ramsey, J.F. Quast, P.W. Langvardt, and P.G. Watanabe. 1982. Pharmacokinetic and macromolecular interactions of ethylene dichloride in rats after inhalation or gavage. Toxicol. Appl. Pharmacol. 62(2):190-204.

Rannug, U., A. Sundvall, and C. Ramel. 1978. The mutagenic effect of 1,2-dichloroethane on *Salmonella typhimurium* I. Activation through conjugation with glutathion in vitro. Chem. Biol. Interact. 20(1):1-16.

Sherwood, R.L., W. O'Shea, P.T. Thomas, H.V. Ratajczak, C. Aranyi, and J.A. Graham. 1987. Effects of inhalation of ethylene dichloride on pulmonary defenses of mice and rats. Toxicol. Appl. Pharmacol. (3):491-496.

Spencer, H.C., V.K. Rowe, E.M. Adams, D.D. McCollister, and D.D. Irish. 1951. Vapor toxicity of ethylene dichloride determined by experiments on laboratory animals. Ind. Hyg. Occup. Med. 4(5):482-493.

Spreafico, F., E. Zuccato, F. Marcucci, M. Sironi, S. Paglialunga, M. Madonna, and E. Mussini. 1980. Pharmacokinetics of ethylene dichloride in rats treated by different routes and its long-term inhalatory toxicity. Pp. 107-133 in Ethylene Dichloride: A Potential Health Risk? B. Ames, P. Infante, and R. Reitz, eds. Banbury Report 5. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Storer, R.D., N.M. Jackson, and R.B. Conolly. 1984. In vivo genotoxicity and acute hepatotoxicity of 1,2-dichloroethane in mice: Comparison of oral, intraperitoneal, and inhalation routes of exposure. Cancer Res**.** 44(10):4267-4271.

Tafazoli, M., A. Baeten, P. Geerlings, and M. Kirsch-Volders. 1998. In vitro mutagenicity and genotoxicity study of a number of short-chain chlorinated hydrocarbons using the micronucleus test and the alkaline single cell gel electrophoresis technique (Comet assay) in human lymphocytes: A structure-activity relationship (QSAR) analysis of the genotoxic and cytotoxic potential. Mutagenesis 13(2):115-126.

Taylor, G.R. 1993. Immune changes during short-duration missions. J. Leukoc. Biol. 54(3):202-208.

Ward, J.M. 1980. The carcinogenicity of ethylene dichloride in Osborne-Mendel rats and B6C3F1 mice. Pp. 35-53 in Ethylene Dichloride: A Potential Health Risk? B. Ames, P. Infante, and R. Reitz, eds. Banbury Report 5. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

WHO (World Health Organization). 1996. Guidelines for Drinking-Water Quality Criteria, 2nd Ed., Vol. 2. Health Criteria and Other Supporting Information. Geneva, Switzerland: World Health Organization.

Wong, K.L. 1996. 1,2-Dichloroethane. Pp.135-170 in Spacecraft Maximum Allowable Concentrations for Selected Airborne Contaminants, Vol. 3. Washington DC: National Academy Press.

10

Dimethylhydrazine

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PHYSICAL AND CHEMICAL PROPERTIES

1,1-Dimethylhydrazine (UDMH) is a highly corrosive, clear, colorless, flammable, hygroscopic fuming liquid that gradually turns yellow upon contact with air. It is miscible with water, ethanol, ether, dimethylformamide, and hydrocarbons (EPA 1984). It has an amine-like, ammonia, or fish-like odor, which is characteristic of aliphatic hydrazines (O'Neil et al. 2001). UDMH is highly corrosive and irritating to skin, eyes, and mucous membranes (HSDB 2005). The vapor is flammable in air at concentrations ranging from 2.5% to 95% (Wade 2003), and it may ignite spontaneously when in contact with heat, flame, or oxidizers. Table 10-1 describes UDMH's physical and chemical properties.

OCCURRENCE AND USE

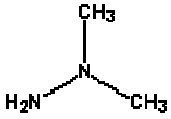
Dimethylhydrazine occurs as symmetrical (1,2-dimethylhydrazine) and unsymmetrical (1,1-dimethylhydrazine [UDMH]) isomers. UDMH occurs naturally and is synthesized for use in a variety of applications. It can be produced commercially by nitrosation of dimethylamine, followed by reduction of the intermediary to UDMH and ensuing purification (Wade 2003). UDMH has also been used for chemical synthesis and as an intermediate in the manufacture of aminimides; it is still used as an intermediate for organic chemical synthesis.

In plants, UDMH occurs naturally in small amounts (ATDSR 2007). It has been used to control vegetation, flowers, and fruit crops and as an absorbent for acid gases (IARC 1974, EPA 1984). Small amounts of UDMH, up to 147 nanograms per gram (ng/g), have been detected in tobacco products, leading to exposure of persons who chew tobacco, smoke cigarettes, or are exposed to

cigarette smoke (NTP 2000). UDMH is also used in the manufacture of *N*-dimethylaminosuccinamic acid, a plant growth regulator.

UDMH is used primarily in military applications, as a storable, high-energy propellant for liquid-fueled rockets, in fuel for thrusters, and in small electrical power-generating units. Development of UDMH started in the Soviet Union in 1949 (Wade 2003) and became the storable liquid fuel of choice by the mid-1950s. It is used, in effect, in all storable liquid rocket engines, with the exception of some orbital maneuvering engines made in the United States, in which monomethylhydrazine is preferred because of its higher density and performance (Wade 2003). UDMH has been widely used as the fuel source for a number of Russian, European, and Chinese rockets (Zelnick et al. 2003).

TABLE 10-1 Physical and Chemical Properties of UDMH

Formula	$C_2H_8N_2$
Chemical name	Dimethylhydrazine
Synonyms	Unsymmetrical dimethylhydrazine U-Dimethylhydrazine UDMH DMH Dimethylhydrazine AS-Dimethylhydrazine Asymmetric dimethylhydrazine ASYM dimethylhydrazine Dimazin Dimazine *N*,*N*-Dimethylhydrazine Dimethylhydrazine unsymmetrical
	
CAS number	57-14-7
Physical description	Liquid (HSDB 2005)
Molecular weight	60.10 (HSDB 2005)
Boiling point	63.9°C (HSDB 2005)
Freezing/melting point	–58°C (HSDB 2005)
Flash point (closed cup)	–15°C (HSDB 2005)
Liquid density at 25°C	0.782 (HSDB 2005)
Vapor density	1.94 (HSDB 2005)
Vapor pressure	156.8 mm Hg at 25°C
Solubility	Soluble in water and ethanol; miscible with dimethylformamide, hydrocarbons, alcohol, and ether
Specific gravity	0.782 at 25°C
Odor threshold	0.3 ppm (Rumsey and Cesta 1970) 1.7 ppm (Amoore and Hautala 1983) 6-14 ppm (Jacobson et al. 1955)
Conversion factor at 25°C, 1 atm	1 ppm = 2.5 mg/m^3 (HSDB, 2005)

UDMH is used on the functional cargo block (Russian *Funkcionalnij Gruzovoj Blck* [FGB]), service module, Soyuz and Progress thrusters, and reboost engines (Wikipedia, 2008). UDMH should not be present within the spacecraft cabin atmosphere unless it is introduced via contamination of a spacesuit as a consequence of contact during extravehicular activity. The amount of contaminant that may be introduced by this route is difficult to establish; however, because of the procedural safeguards currently used, theoretically, the amount of UDMH contamination should be less than a few grams (Garcia and James 1996).

TOXICOKINETICS

Toxicokinetic data are available on the uptake, metabolism, and elimination of UDMH via intraperitoneal (i.p.) and inhalation exposure routes in several species. The weight of evidence (Back et al. 1963) suggests that UDMH is nonselectively distributed throughout the body and metabolized through pathways that have not been fully defined.

Absorption

Weeks et al. (1963) researched the retention of inhaled UDMH in six anesthetized dogs (mongrel) by using an endotracheal tube or an oronasal mask. They exposed each animal to a known concentration for about 1 h and determined the percent retention of the inhaled dose. They did not describe in detail the method used to do this. Respiratory volumes and electrocardiograms were recorded during the exposure. The reported exposure concentrations ranged from 3 to 20 milligrams per liter (mg/L) (1,200 to 8,000 parts per million [ppm]) and the percent retention ranged from 71% to 93%.

Skin absorption of liquid UDMH was studied in anesthetized dogs given 5-30 millimoles of UDMH per kilogram of body weight (mmol/kg) applied to a shaved area of the chest (Smith and Clark 1971). The lower doses were absorbed more rapidly, so that the blood concentrations after the lowest dose peaked 60 min after application, whereas, at a dose of 30 mmol/kg, the peak blood concentration occurred 200 min after application. No studies were found that reported dermal absorption of UDMH vapor.

Distribution

The distribution of UDMH was reported in a limited research study. Back et al. (1963) conducted a study on albino rabbits (1.7 to 4.4 kg), injecting [^{14}C]UDMH intravenously (i.v.) at 50 mg/kg and removing tissue from two rabbits at each point of sacrifice (eight rabbits). The rabbits were euthanized 2, 4, 18, and 24 h postinjection to determine tissue distribution. They reported that the maximum difference between UDMH concentration in brain tissue, kidney,

spleen, and liver was approximately 2-fold after a 24-h period. The concentrations reported were the average of what was identified in each pair of rabbits. The tissues were not rendered bloodless, and that could account for a portion of the UDMH identified and reported. Within 24 h 10% to 28% of the injected dose was accounted for. UDMH did not preferentially concentrate in any of the vital organs studied. The authors suggested that the bulk of body weight, including skeletal muscle, bone, and adipose and cutaneous tissues, which were not examined, may represent a reservoir for a good percentage of UDMH not identified in the other tissues. On the other hand, much of the UDMH may have been eliminated by urinary excretion or through metabolism and CO_2 exhalation (see Excretion section).

Metabolism

The metabolism of UDMH has been partially characterized through i.p. and i.v. studies on a variety of species and also by in vitro studies using tissues, cells, or subcellular components. These studies have not led to a complete picture of UDMH metabolism.

Dost et al. (1966) administered [^{14}C]UDMH by i.p. injection to rats at doses from 0.013 to 1.33 mmol/kg and found that 30% of the lower doses were converted to CO_2, whereas at the highest doses only 13% were converted to CO_2 over a much longer time (10 h versus 20 h). At least half the administered radioactivity appeared in the urine during the 2 d after injection. Based on the results reported by Mitz et al. (1962) from rats given UDMH at 40 mg/kg, the urinary metabolites were the glucose hydrazone of UDMH and another hydrazone of UDMH. Clearly, demethylation of UDMH occurs in vivo.

Godoy et al. (1983), using rat liver microsomes and S9 fractions under aerobic conditions, identified a nonenzymatic and an enzymatic component involved in UDMH metabolism to formaldehyde. The metabolism led to covalent binding to proteins, with the process dominated by nonenzymatic processes; however, UDMH did not produce metabolites that bound covalently to nucleic acids. The reactive metabolites produced by UDMH may be free radical intermediates. Albano et al. (1989) studied activation of UDMH in isolated hepatocytes and liver microsomes of male Wistar rats. Hepatocytes incubated with 2 millimolar (mM) UDMH resulted in the formation of free radical metabolites (measured by spin trapping techniques). The authors noted that the oxidative metabolism of UDMH was largely mediated by FAD-containing monooxygenase; this study observed that methimazole, a competitive inhibitor of monooxygenase, decreased the free radical activation of UDMH. They also noted that the detection of free radicals suggested that these reactive species could be responsible for the toxic and carcinogenic effects of UDMH and other methylhydrazines (Albano et al. 1989). Using a series of selective inhibitors and copper ions, Tomasi et al. (1987) showed that one nonenzymatic mechanism and two enzymatic pathways were involved in UDMH metabolism.

Excretion

Back et al. (1963) reported that 30% to 50% of UDMH is excreted in urine in its original form in dogs and cats, respectively, within 5 h. Both i.p. and i.v. doses of UDMH ranging from 10 to 50 mg/kg were injected into the test animals. In some experiments in this study, they observed urinary concentrations of considerable magnitude as early as 3 min after administration of the compound, regardless of the route of administration. Back et al. (1963) also noted that urinary UDMH concentration is the most sensitive biomarker of exposure, because urine concentrations can be found at doses that do not produce detectable concentrations in blood. However, when urine is used as evidence of exposure, concentrations would not be considered an absolute indicator of the dose received because an unknown amount of drug administered would be lost to various transformation pathways within the body.

TOXICITY SUMMARY

Information on acute exposures to UDMH in humans is generally limited to case reports of accidental exposures. Most animal toxicity research on UDMH was conducted in military laboratories from the 1950s to the 1970s. These studies were published in peer-reviewed journals or in technical reports, in some cases without peer review. Furthermore, they were conducted before good laboratory practices became widely accepted in toxicity studies, so they typically lack the detail and organization of more recent toxicity studies. Toxicity data of different degrees of completeness are available for numerous species of laboratory animals, including rhesus monkeys, dogs, rats, mice, and hamsters (Weeks et al. 1963).

Acute Toxicity (≤1 d)

Lethality in Humans

Review of research indicates that lethal exposures of UDMH have caused death by respiratory arrest, and postmortem examinations reveal pulmonary edema. Authoritative data on these lethal exposures, including concentration and duration of exposure, were not available for these incidents (NRC 2000).

Lethality in Animals

Several studies characterized the lethal concentrations of UDMH in a variety of species. These studies reported consistent signs of convulsive activity, other central nervous system (CNS) effects, and respiratory effects before death, regardless of species. Dogs exposed to UDMH at 24, 52, or 111 ppm for 4 h had mortality rates of 0/3, 1/3, and 3/3 for the three exposure groups, respectively

(Jacobson et al. 1955). The authors noted that all deaths or terminations occurred within the first day of exposure. All three dogs in the highest exposure group displayed vomiting, panting, and convulsions before death. The sole dog that expired in the 52-ppm exposure group also exhibited the aforementioned signs before death, while the remaining two dogs exhibited nausea, panting, or lack of coordination. One dog in the low-exposure (24 ppm) group also exhibited vomiting and convulsions but did not die. No hematologic changes were observed in any of the surviving dogs. The surviving dogs were sacrificed for examination after 14 d. Necropsy revealed pulmonary edema and patchy hemorrhage in animals that had convulsions; according to the authors, these observations possibly resulted as a secondary consequence from seizures as opposed to being a direct result of UDMH exposure.

Weeks et al. (1963) exposed male rats (100 to 120 g), 10 per group, to UMDH at concentrations ranging from 252 to 24,500 ppm for 5, 15, 30, 60, and 240 min. They were observed for signs of toxicity 7 days postexposure. The authors conducted histopathologic studies on rats sacrificed immediately and 1, 3, and 7 d after exposure. Rats exposed to UDMH demonstrated signs of irritation, including sneezing, eye closure, and restlessness. Deaths occurred within 24 h postexposure and were preceded by alternating episodes of tonic-clonic convulsions and depressed activity. The median lethal concentration (LC_{50}) values for this study are given in Table 10-2. The purity of UDMH and recovery in the chamber were not reported. Several physiological tests were done on exposed animals, but the extent of any pathologic study was unclear.

TABLE 10-2 LC_{50} Values for UDMH (95% Confidence Interval)

Concentration, ppm	Exposure Duration	Species, Sex, Strain	Reference
52[a]	4 h	Dog, N/A, mongrel	Weeks et al. 1963
172 (150-194)	4 h	Mouse, N/A, N/A	Jacobsen et al. 1955
252 (219-290)	4 h	Rat, male, N/A	Weeks et al. 1963, Jacobsen et al. 1955
392 (376-413)	4 h	Hamster, N/A	Jacobsen et al. 1955
981 (862-1,120)	1 h	Dog, N/A, mongrel	Weeks et al. 1963
1,410 (1,300-1,530)	1 h	Rat, male, N/A	Weeks et al. 1963
3,580 (2,330-5,500)	15 min	Dog, N/A, mongrel	Weeks et al. 1963
4,010 (3,730-4,310)	30 min	Rat, male, N/A	Weeks et al. 1963
8,230 (6,930-9,780)	15 min	Rat, male, N/A	Weeks et al. 1963
18,315	12 min	Rat, N/A	Chevrier and Pfister 1974
22,300 (22,000-22,600)	5 min	Dog, N/A, mongrel	Weeks et al. 1963
24,500 (23,400-25,500)	5 min	Rat, male N/A	Weeks et al. 1963

[a]Approximate lethal concentration = lowest concentration producing mortality.

CNS Effects

Most studies have demonstrated that exposures to elevated concentrations of UDMH produce signs and symptoms originating in the CNS. CNS effects are expected to be the greatest acute hazard to crews.

The mechanism of initiation of CNS effects is uncertain. In rats, the i.p. median lethal dose (LD_{50}), which is the dose where the highest frequency of convulsions occurs, can be increased approximately 2-fold by i.p. injection of pyridoxine at 0.15 mmol/kg, 5 min after injection of the neurotoxicant (O'Brien et al. 1964). A study of brain, plasma, and erythrocyte cholinesterase concentrations in rats injected i.p. with UDMH showed that cholinesterase inhibition is not a factor in causing convulsions (Cornish et al. 1965). In the same study, a comparison of latency times to convulsions between i.p. and intracerebral injection suggested that metabolism outside the brain may not be important.

Back and Thomas (1962) studied the convulsive threshold of UDMH, noting that they did not discover the cause of the latency between dosage (i.p.) and ensuing CNS effects. Latency periods varied by species and dosage; mice showed CNS effects 50 to 120 min postdosing (131 mg/kg, i.p.), dogs ranged from 30 to 120 min (100 mg/kg, i.p. and i.v.) and 2 to 4 h (50 mg/kg, i.p. and i.v.), and *Macaca iris* monkeys had effects within 2 to 4 h (30 mg/kg, threshold dose, i.p.).Weeks et al. (1963) conducted a study similar to that of Back and Thomas (1962) in which they observed subdued behavior, muscle fasciculations, tremors, and convulsions in exposed dogs. All dogs in the Weeks et al. (1963) study survived the low dose (50 mg/kg) and symptoms occurred at the same time regardless of the route of administration (i.p. or i.v.). The *M. iris* monkey test subjects, in the same study, had no more than two convulsive episodes. UDMH did not affect the appetites of these animals, and the authors noted that some ate within 5 min after experiencing a convulsion (Weeks et al. 1963).

Accidental human exposures to UDMH caused dizziness, lack of coordination, headaches, and convulsions (ACGIH 1991). Dhennin et al. (1988, as cited in HSDB 2005) reported a case history of a 31-year-old man with extensive UDMH burns whose symptoms were predominantly neurological. Although the relative susceptibilities of various species are unknown, CNS symptoms caused by UDMH exposure appear qualitatively consistent across the species tested.

Respiratory Effects

Shook and Cowart (1957) as summarized by the National Research Council (NRC) (2000) reported that two people exposed to an undetermined concentration of UDMH first exhibited choking and breathing difficulty. The researchers suggested that some of these respiratory consequences may be an indirect result of CNS toxicity such as tonic-clonic convulsions leading to respiratory arrest (Back and Thomas 1962). However, evidence that the respiratory toxicity

is only a secondary manifestation has not been demonstrated. An acute toxicity study of groups of 20 mice, utilizing 4-h UDMH exposure assays at doses of 24, 52, and 111 ppm, was conducted by Jacobson et al. (1955). For the exposure period of 4 h, the mice displayed restlessness, dyspnea, convulsions, clonic but in some cases tonic-clonic, and exophthalmos at 52 and 111 ppm. Postmortem examination revealed no significant histopathologic findings with the exception of pulmonary edema and occasional localized pulmonary hemorrhage; the authors did not specify the concentrations at which these findings were noted. Hemorrhage was noted only in animals that had convulsions but was not noted in every animal that convulsed.

Short-Term Toxicity (2 to 10 d)

We did not find any studies using exposures in the above range.

Subchronic Toxicity (11 to 100 d)

Back (1963) reported no visible signs of toxicity or body weight changes in surviving ICR Swiss mice, Sprague-Dawley rats, and rhesus monkeys after inhalation exposure to 0.56 ppm of UDMH continuously for 90 d. One monkey died on day 41, three rats died between days 59 and 82, and six mice died between days 3 and 41. Back noted some long-term exposure effects, which included degenerative lesions in the liver of rhesus monkeys, and the cytoplasm in central zones appeared swollen and reticulated. The authors note that the heart was dilated in all monkeys except one; calcium deposits were noted in the myocardium of two monkeys, including the one that died on day 41, which also had necrosis of muscle fibers. Calcification was indicated in the adrenal gland of one monkey. Mite infestation of the lung was identified in six monkeys, including the one that died on day 41. The infestation does not appear to be attributable to the UDMH exposure. Hemosiderin deposits in the Kuppfer and liver cells of mice and lesions in rat kidney (vacuolization of renal tubular epithelium) and heart (cardiac fibrosis in one and cardiac necrosis in six others) were also noted. It is difficult to attribute the concentration of 0.56 ppm as a true effect level based on the findings noted. We also note that the report was not peer reviewed.

Weeks et al. (1963) exposed groups of dogs to UDMH at 50, 200, or 600 ppm for 60, 15, or 5 min, respectively, twice weekly for 6 wk. The authors expected the dogs not to produce any signs of toxicity based on results previously obtained from acute exposures of three groups of four mixed-breed dogs to the same concentrations and exposure sessions twice weekly for 6 wk (50, 200, and 600 ppm for 60, 15, and 5 min, respectively). All these animals were observed for general health, characteristic behavioral signs, coordination, and reflex reactions for 2 months before exposures. Baseline values for white blood cells, reticulocyte counts, hematocrit, nonprotein nitrogen, glucose, bilirubin, and cholinesterase levels were also determined. Two dogs from each group were trained

to perform a conditioned avoidance test. During the exposure period, each trained dog's behavior was recorded. The trials were recorded on each nonexposure day, except weekends and 15 min before and after exposure. No effects attributable to the exposures were found at these concentrations.

After 6 wk of exposure the three groups of dogs were scheduled to be exposed twice weekly for 2 wk at twice the previous concentrations (100, 400, and 1,200 ppm for 60, 15, and 5 min, respectively). Responses in the conditioned avoidance test remained unchanged, and the clinical laboratory parameters were unchanged (except possibly red blood cell (RBC) count and hematocrit); however, severe toxic signs developed even during the first exposures. Exposures were discontinued during the second week because of the continued appearance of severe toxic signs, including convulsion, tremors, vomiting, depressed behavior, salivating, apprehensive behavior, and death.

Chronic Toxicity (>101 d)

Chronic inhalation exposures to UDMH using conventional protocols in use today have not been published; however, one peer-reviewed study in dogs was published some time ago. Rinehart et al. (1960) set out to expose three dogs per group to UDMH concentrations of 5 and 25 ppm for 26 wk (6 h/d, 5 d/w). A control group was not used; however, they conducted preexposure hematologic assessments on dogs exposed to UDMH. Results from the exposed dogs were compared with preexposure values. At the higher concentration, one dog died on the third day, but the other two were exposed for a total of 13 wk. During this time, signs of depression, salivation, emesis, diarrhea, ataxia, convulsions, conjunctival injection, bradycardia, and fever were noted. No severe signs were noted in the lower concentration group. Blood was drawn weekly or monthly from the dogs to determine hematologic parameters.

The two dogs surviving the 13-wk exposure to UDMH at 25 ppm showed a large decrease in RBC count (9.8 to 5.9 $\times$ $10^6/mm^2$) and in hematocrit (50.8% to 45.0 %). The three dogs exposed to 5 ppm showed a decreased RBC count (7.2 to 6.0 $\times$ $10^6/mm^2$), decreased hemoglobin (15.4% to 11.4 gm%), and decreased hematocrit (52.9% to 46.0 %). Serum bilirubin was also measured in these animals and was found to increase from 0.2 mg% before the study to 0.7 mg% after 26 wk. Pathology for this group revealed only hemosiderosis of the spleen. These data may reflect a hemolytic process giving rise to the modest anemia observed at 13 wk. Other organs examined and found to be devoid of lesions included the brain, heart, kidney, stomach, intestines, pancreas, trachea, adrenals, testes, bladder, and trachea. The protocol for this examination was not clearly defined; however, a microscopic examination of stained sections from each of these organs was likely performed, as reporting of tissue section examinations included detail such as pigment in lymph nodes, bone marrow, and Kupffer cells.

Carcinogenicity

Cancer in Humans

No information is available regarding carcinogenicity to humans after inhalation exposure to UDMH (NRC 2000).

Cancer in Animals

UDMH was reported to be carcinogenic in mice after lifetime drinking water exposures (Toth 1973, NRC 2000). A concentration of 1,000 mg/L was linked to elevated incidences of angiosarcomas, pulmonary adenomas, malignant lymphoma, kidney adenomas, and hepatomas. Toth (1977) conducted a similar study in hamsters that identified excessive numbers of tumors in the cecum and blood vessels. Mice administered 0.5 mg of UDMH (dosage in mg/kg not given) daily for 40 wk exhibited a marginal increase in lung tumors. Rats and mice developed liver tumors after a 2-year exposure to UDMH in drinking water (Toth 1977; NRC 2000).

U.S. Air Force inhalation studies reported an increase in hemangiosarcomas and Kupffer cell sarcomas in mice exposed to UDMH at 5 ppm for 6 h/d, 5 d/wk for 6 months (Haun et al. 1979, 1984). Similarly, rat exposures to UDMH at 5 ppm caused an increase in the incidence of squamous cell carcinomas of the lung and hepatocellular carcinomas. The study used relevant test species and an adequate number of animals (total number of animals: 400 C571B1/6 mice, 200 Fischer rats, 200 golden Syrian hamsters, and 8 beagle dogs). The UDMH used in these studies contained 0.12% or 6 parts per billion of dimethylnitrosamine (DMNA). However, Haun et al. (1984) tested the purified UDMH along with the DMNA-contaminated UDMH and concluded that the UDMH caused the oncogenic tumors. The results of the Haun et al. (1984) 6-month study demonstrated that UDMH is tumorigenic in rats and mice, mainly at the highest concentration (5 ppm) studied. Tumors were not produced in hamsters and dogs through inhalation. Lesions in hamsters were not hepatotoxic, and in no case were lesions that were identified statistically different from those in control animals.

The liver was the primary target of UDMH-induced neoplastic and preneoplastic changes in rats and mice. An indication of hepatotoxicity in dogs was demonstrated by transitory elevations of serum enzyme levels and liver function values in test subjects exposed to 5 ppm of UDMH. Six- to 12-month exposures resulted in a significantly increased oncogenic response to purified UDMH; this proved particularly true for lung adenomas (rarely seen) and for nasal tumors and liver adenomas not previously reported in dogs (Haun et al. 1984). These findings confirm the authors' contention that the toxicity of UDMH is not intensified by the 0.12% DMNA contained in the UDMH studied. However, in certain cases (6- to 12-month studies) purified UDMH had greater toxicity (increased oncogenic response) than the DMNA-contaminated UDMH.

We examined the technical report of Haun et al. (1984) to determine whether a defensible cancer risk can be gleaned from the information presented. The authors reported a dose-related increase in tumors in female mice for the following tumors: hemangiosarcomas, thyroid carcinomas, and Kupffer cell sarcomas. However, our inspection of the data does not support this conclusion (Table 10-3). A statistical trend analysis was not done on the data.

Although several of the exposed-group incidences are statistically higher than control incidences, there is not a clear dose response for any of the cancers. The situation is no different for male rats exposed to DMNA-contaminated UDMH for 6 months.

The results from female mice exposed to purified UDMH for 6 months do not clarify the situation. This study used only a single dose (5 ppm) and a control group. Thyroid carcinomas and Kupffer cell sarcomas were not reported as increased in the exposed group. The incidence of hemangiosarcomas was 0.053 in exposed mice, and in controls it was 0.021. Although the incidence in the exposed group is a good match to the previous result (0.053 versus 0.047), the incidence in controls is much higher than in the earlier experiment (0.021 versus 0.007). We conclude that there are no dose-response data suitable for a cancer risk assessment. If cancers are caused by UDMH, then it is only at the highest dose of 5 ppm.

Haun et al. (1984) reported an array of nonmalignant tumors in the noses of mice exposed to purified UDMH. These lesions were not reported in mice from the DMNA-contaminated exposures, presumably because they were not looked for. The incidences of papillomas and adenomatous polyps were statistically higher than in controls; however, the rodent nares are well known to be a poor model of the human nasal cavities. This background, along with the fact that the purified UDMH study involved a single dose, precludes a defensible cancer risk assessment, even if one were to consider the nonmalignant lesions as cancer harbingers. Furthermore, none of the data has been peer reviewed, and the study was completed before good laboratory practices were the norm for inhalation toxicology studies.

TABLE 10-3 Incidence of Cancers in Female Mice Exposed 6 Months to DMNA-Contaminated UDMH

Lesion/Exposure Group	Control	0.05 ppm	0.5 ppm	5.0 ppm
Hemangiosarcomas	0.007 (5/701)	0.024[a] (9/374)	0.008 (3/368)	0.047[a] (17/360)
Thyroid carcinomas	0.004 (2/551)	0.003 (1/311)	0.029[a] (8/278)	0.017[a] (5/286)
Kupffer cell sarcomas	0.001 (1/701)	0.010[a] (4/374)	0.000 (0/368)	0.022[a] (8/360)

[a]Incidence statistically different from control group at $P = 0.01$.
Source: Haun et al. 1984

Peripheral Neurocarcinogenicity

UDMH's toxicity in the peripheral nervous system was observed in a study by Ernst et al. (1987). Peripheral nerve sheath tumors (PNST) were induced in European hamsters (EH) after lifetime exposure to weekly subcutaneous administration of UDMH. The increase in PNST was up to 43% in EH. Fifteen males and 15 females were given 1/10th of the LD_{50} of UDMH (373 mg/kg for males and 325 mg/kg for females) dissolved in 1 mL 0.9% saline once weekly for life. No sex differences were discovered with regard to the number of tumors, the incidence, and the type. The overall neoplastic response was elevated, which showed a broad spectrum of malignant neoplasms, especially in the female EH, where, along with other tumors, malignant dermal melanomas, hepatocellular carcinomas, and adenocarcinomas of the stomach were detected. The first observed PNST caused death in a male as early as week 17. Histologic examination of the malignant PNST showed neurofibrosarcomas and melanotic and unpigmented schwannomas (Ernst et al. 1987).This study is not useful for risk assessment because it used a less relevant route of exposure (noninhalation), a bolus weekly dosing routine, a single dose concentration, a near-lethal dose, and an insufficient number of test subjects for cancer study.

Genotoxicity

In a review of UDMH genotoxicity, the Hazardous Substance Data Bank (2005) noted that it is genotoxic in test animals, inducing DNA damage, mutations, sister chromatid exchanges, and oncogenic transformation in vitro.

Rogers and Back (1981) studied the mutagenicity of UDMH in L5178Y mouse lymphoma cells. They examined the ability of UDMH to induce forward mutations of ouabain and excess thymidine, thioguanine, and cytosine arabinoside. Doses of 0.1, 1, 2.5, and 5 mM UDMH were tested in triplicate using a population of 10^7 cells at each dose. A survival rate of at least 40% was deemed the lowest acceptable rate for mutation experiments. UDMH induced forward mutations at the thymidine kinase locus and was negative in all other assays. It also induced mutations in a dose-response relationship. This study was well conducted, L5178Y cells were maintained by routine methods, and the soft agar cloning technique of Cole and Artlett was employed using McCoy's 5A medium instead of Fisher's medium. Appropriate procedures were used to routinely screen cell lines for pleuropneumonia-like organism (PPLO) contamination. The UDMH obtained was redistilled to contain less than 0.001% DMNA.

Reproductive Toxicity

The reproductive toxicity of UDMH has been researched in rodents. Toxic effects included testicular abnormalities and fetotoxicity at doses near the LD_{50}; however, this was not noted at lower doses. This result suggests that reproduc-

tive impairment is not a primary concern when considering low-level exposure to UDMH.

Male mice given five i.p. injections of UDMH at 10 to 100 mg/kg demonstrated no sperm abnormalities (Trochimowicz 1994). The lack of damage was established in an assay in which mice were examined 35 days after the same dose regime. Doses nearing the LD_{50} produced a transient change in the count of abnormal sperm without affecting sperm numbers, testicular histology, or testis weight (Trochimowicz 1994). We were unable to locate reproductive toxicity studies with female test animals, including pregnant females and fetuses.

Developmental Toxicity

Developmental toxicity of UDMH was reported in Fischer-344 rats following parental administration of maternally toxic doses (NRC 2000). Rats were given i.p. injections of UDMH at 10, 30, or 60 mg/kg on gestation days 6 through 15. No effects were observed in maternal or fetal rats exposed to UDMH at 10 or 30 mg/kg. Those animals exposed to 60 mg/kg exhibited a reduction in maternal body weight gain and a reduction in fetal weight. The number of fetal implants and viable fetuses also decreased at this dose. The incidence of malformations (rib abnormalities, delayed vertebral ossification) also increased (Keller et al. 1984). The authors noted that these abnormalities were not statistically significant. They also noted that the signs of UDMH embryotoxicity may be related to maternal toxicity instead of embryotoxic effects of UDMH (Keller et al. 1984). The study was well designed. The UDMH obtained was redistilled to remove any DMNA contaminant.

Immunotoxicity

Immunotoxicty of UDMH was demonstrated in two in vivo and in vitro studies. The first group (Frazier et al. 1992) had previously demonstrated that UDMH decreased interleukin 2 (IL-2) production both in vivo and in vitro. Bauer et al. (1990) also reported that UDMH interferes with IL-2 regulatory action.

Tarr et al. (1982) studied the in vivo and in vitro effects of UDMH on selected immune functions after short-term (six groups of 10 mice each injected i.p. with UDMH at 10, 25, 50, 100, or 150 mg/kg) and long-term (five groups of 10 mice each received i.p. injections of UDMH at 25, 50, 100, or 150 mg/kg 3 d/wk for 14 wk) exposure in Swiss mice. Spleen cells were examined postexposure to determine immune function. These exposures resulted in an increase in Jerne plaque-forming cells (an assay for identifying antibody-forming spleen cells) in long-term exposure groups exposed to UDMH at 10 and 50 mg/kg. In both short- and long-term groups, a trend toward decreased induction of suppressor cell activity by concanavalin A (ConA) was found; none was statistically

significant, but the 50-, 100-, and 150-mg/kg dose groups changed the most in both experiments.

The in vitro experiments of Tarr et al. (1982) exposed spleen cells harvested from 24 normal mice to UDMH at 5, 10, 25, 50, 75, 100, and 150 micrograms (μg)/mL. UDMH suppressed the lymphocyte blast transformation response of normal cells to ConA at concentrations from 25 to 150 μg/mL. An enhanced response of lipopolysaccharide was observed at lower doses (10 and 25 μg/mL) and was depressed at higher doses (100 to 150 μg/mL). Both in vivo and in vitro experiments suggest that UDMH inhibits suppressor cell function, which is important when considering autoimmune diseases such as Graves' disease and multiple sclerosis, which are associated with decreased suppressor cell function (Tarr et al. 1982). The research of Tarr et al. (1982) shows that low doses of UDMH enhance immune function, which could possibly offset some of the immune changes associated with the stress of spaceflight. These low levels may or may not be seen in long-term space exploration and are not useful in deducing a long-term acceptable concentration (AC) for immune effects.

In 1988, the same laboratory (Tarr et al. 1988) noted an enhanced murine mixed lymphocyte response (MLR) by UDMH. Mice (Balb/C and C57B1/6) were injected i.p. with UMDH at 5, 10, 25, 50, 75, or 100 mg/kg/d for 7 d. Splenocytes were then pooled from these subjects to examine responder and stimulator cells. The indications, while varied in magnitude and pattern of response, demonstrate that UDMH at all doses significantly enhances MLR. In vivo UDMH exposure also showed enhanced MLR. However, the authors' noted that the effect was not dose dependent and that which cell population (responder or stimulator) was most affected could not be determined. That is why they exposed mice splenocytes in vitro (Tarr et al. 1988). This set of experiments expands the understanding of the immunomodulatory effects of UDMH using the MLR response assay. The authors stated that a possible target cell subpopulation for the immunoenhancing effect of UDMH consists of macrophages and B cells (Tarr et al. 1988). Tarr et al. (1988) proposed that inhibition of synthesis of prostaglandin E_2 is a possible mechanism of action for this effect. Coupled with the results of their previous study (Tarr et al. 1982) they inferred that UDMH may heighten humoral (Jerne plaque) and MLR immune responses.

Bauer et al. (1990) conducted a cell suspension exposure and reported that UDMH interferes with IL-2 regulatory action. They used CTLL-20 cells at concentrations of 10 to 100 μg/mL. They also found that DNA synthesis in murine splenocytes stimulated by ConA was inhibited at subtoxic UDMH concentrations of 10 to 50 μg/mL and that UDMH suppressed IL-2 production stimulated by phorbol myristic acetate in EL-4 cells (Bauer et al. 1990). They proposed that UDMH has the potential to modify immune function through its interference with IL-2 production and lymphoproliferative response to IL-2 (Bauer et al. 1990).

Frazier et al. (1992) studied altered immune responsiveness in mice promulgated by UDMH exposure (Frazier et al. 1992). The mice were sacrificed by

cervical dislocation for extractions of cell suspensions. The relative membrane potential of murine splenocytes was determined by cytofluorometry. Splenocytes were cultured alone, with ConA at 2 μg/mL, or with ConA at 2 μg/mL and UDMH at 10, 25, or 100 μg/mL for 24 and 48 h at 37°C in a 5% dehumidified incubator.

UDMH induced hyperpolarization of cellular membranes compared with controls at all doses except 50 μg/mL, where slight depolarization was induced. Frazier et al. (1992) concluded that hyperpolarization of UDMH may alter normal ionic fluctuation and may explain the reduced mitogenic potential of spleen cells, because hyperpolarization can inhibit lymphoproliferation. The specific action of UDMH on ionic regulation was not clear because no dose-response relationship was observed. Intracellular free Ca^{2+} was significantly increased with UMDH at 50 μg/mL in murine splenocytes. A significant increase in intracellular Ca^{2+} occurred at all concentrations except 25 μg/mL in thymocytes. The possibility that UDMH affects the abilities of different lymphocyte subpopulations to regulate intracellular ion concentrations for normal immune function is suggested as a possible conclusion of the research, requiring further investigation as suggested by the authors (Frazier et al. 1992).

Interaction with Other Chemicals

Reports of pertinent toxicologic interactions with other chemicals were not found.

Inhalation Toxicity Summary

Table 10-4, provides a data summary of UDMH inhalation toxicity studies.

EXPOSURE LIMITS

Table 10-5 presents exposure limits for UDMH set by other organizations.

SMACs were derived in accordance with guidelines developed by the SMAC subcommittee of the Committee on Toxicology (NRC 1992). Table 10-6 presents SMACs set by choosing the lowest values among the ACs (see Table 10-7).

RATIONALE FOR ACS

Although the mechanism of its toxicity is uncertain, UDMH has been tested by inhalation on a variety of species, at a variety of concentrations, and for exposure times from a few minutes to 26 wk. The database is sufficient to set human exposure guidelines with a moderate degree of confidence. Depending on the time of exposure, the toxic effects can include CNS effects, anemia, and hepatotoxicity.

TABLE 10-4 Inhalation Toxicity Summary

Concentration, ppm	Exposure Duration	Species, Sex, Strain	Effects	Reference
24,500	5 min	Rat, M, N/A	Sneezing, eye closure, restlessness, tonicoclonic convulsions, and depressed activity	Weeks et al. 1963
8,230	15 min	Rat, M, N/A	Sneezing, eye closure, restlessness, tonicoclonic convulsions, and depressed activity	Weeks et al. 1963
4,010	30 min	Rat, M, N/A	Sneezing, eye closure, restlessness, tonicoclonic convulsions, and depressed activity	Weeks et al. 1963
1,410	60 min	Rat, M, N/A	Sneezing, eye closure, restlessness, tonicoclonic convulsions, and depressed activity	Weeks et al. 1963
252	4 h	Rat, M, N/A	Sneezing, eye closure, restlessness, tonicoclonic convulsions, and depressed activity	Weeks et al. 1963
1,200	5 min	Dog, N/A, mongrel	Convulsions, vomiting, tremors, and death	Weeks et al. 1963
400	15 min	Dog, N/A, mongrel	Convulsions, tremors, vomiting, death	Weeks et al. 1963
100	60 min	Dog, N/A, mongrel	Convulsions, tremors, vomiting, death, muscle fasciculations, depressed, salivation, apprehensive	Weeks et al. 1963
600	5 min	Dog, N/A, mongrel	No signs of toxicity	Weeks et al. 1963
200	15 min	Dog, N/A, mongrel	No signs of toxicity	Weeks et al. 1963
50	60 min	Dog, N/A, mongrel	No signs of toxicity	Weeks et al. 1963
0.43	90 d, continuous	Mouse, N/A	1 d no observable effect level	House 1964

(Continued)

TABLE 10-4 Continued

Concentration, ppm	Exposure Duration	Species, Sex, Strain	Effects	Reference
172	4 h	Mouse, N/A	LC_{50}	Jacobsen et al. 1955
140	4 h	Mouse, N/A	LC_{20}	Jacobsen et al. 1955
52	4 h	Dog, N/A	1 of 3 dogs expired	Jacobsen et al. 1955
24	4 h	Mouse, N/A	No toxicity in 2; vomiting & convulsions & full recovery in 1	Jacobsen et al. 1955
5	6 h/d, 5 d/wk, 26 wk	Dog, N/A, beagle	Some hemolytic anemia, slight bilirubinemia, some lethargy	Rinehart et al. 1960
25	6 h/d, 5 d/wk, 13 wk	Dog, N/A, beagle	Depression, salivation, emesis, diarrhea, ataxia (hind quarters) tonicoclonic convulsive seizures, bradycardia, fever in 2 dogs—1 expired, 3rd dog depression and salivation only; hemolytic anemia	Rinehart et al. 1960
75	6 h/d, 5 d/wk, 6 wk	Mice, F, CF-1	8 of 20 mice died (tonicoclonic convulsions in all fatalities)	Rinehart et al. 1960
140	6 h/d, 5 d/wk, 7 wk	Mice, F, CF-1	29 of 30 mice died (tonicoclonic convulsions in all fatalities)	Rinehart et al. 1960
75	6 h/d, 5 d/w, 6 w	Rats, M, Wistar	Periods of dyspnea & lethargy	Rinehart et al. 1960
140	6 h/d, 5 d/w, 7 w	Rats, M, Wistar	1 of 20 rats died (tonicoclonic convulsions in fatality)	Rinehart et al. 1960
0.56	90 d, continuous	Mouse, N/A, ICR Swiss	Hemosiderin deposit on Kupffer & liver cells; cysts in hearts of 2; 6 mice died between days 3 and 41	Back et al. 1963
0.56	90 d, continuous	Rat, N/A, Sprague-Dawley	Vacuolization of renal tubular epithelium; necrosis of heart; 3 rats died between days 58 and 82	Back et al. 1963

0.56	90 d, continuous	Monkey, M, rhesus	One fatality at day 41; degenerative lesions in liver; heart dilation; calcium deposits in myocardium of 2; necrosis of muscle fibers in fatality; calcification of adrenal in 1; mite infestation of lung in 6	Back et al. 1963
5	8.5 wk, daily	Hamster, N/A Golden Syrian	NOAEL	MacEwen and Vernot 1970
5[a]	6 h/d, 5 d/wk, 6 mo	Mice, N/A	Increase in tumor response, hemangiosarcomas, Kupffer cell sarcomas	Haun et al. 1979
5[a]	6 h/d, 5 d/wk, 6 mo	Rat, N/A	Increase in squamous cell carcinomas, lung tumors, and hepatocellular carcinomas	Haun et al. 1979
0.5[a]	6 h/d, 5 d/wk, 6 mo	Rat, N/A	Islet cell adenomas of pancreas, slight increase in fibrous histiocytomas	Haun et al. 1979
5	6 h/d, 5 d/wk, 6 mo	Dog, M & F, beagle	1 fatality 15 mo postexposure, neoplastic lesions in heart & lung; reticulum cell sarcoma; increased SGPT levels in all dogs; BSP levels high	Haun et al. 1984
0.5	6 h/d, 5 d/wk, 6 mo	Dog, M & F, beagle	No toxic effects	Haun et al. 1984
0.05	6 h/d, 5 d/wk, 6 mo	Dog, M & F, beagle	No toxic effects	Haun et al. 1984
5	6 h/d, 5 d/wk, 6 mo	Mice, F, C57BL	Increase in tumor response, hemangiosarcomas, Kupffer cell sarcomas, thyroid sarcomas; increased uterine cysts	Haun et al. 1984
0.5	6 h/d, 5 d/wk, 6 mo	Mice, F, C57BL	Increased uterine cysts	Haun et al. 1984
0.05	6 h/d, 5 d/wk, 6 mo	Mice, F, C57BL	Increased uterine cysts	Haun et al. 1984

(Continued)

TABLE 10-4 Continued

Concentration, ppm	Exposure Duration	Species, Sex, Strain	Effects	Reference
5	6 h/d, 5 d/wk, 6 mo	Rat, M, CDF	Bronchiolar adenomas; pituitary chromophobe adenomas	Haun et al. 1984
0.5	6 h/d, 5 d/wk, 6 mo	Rat, M, CDF	Pancreatic islet cell adenomas; pituitary chromophobe adenomas	Haun et al. 1984
0.05	6 h/d, 5 d/wk, 6 mo	Rat, M, CDF	Hepatocellular adenomas; pituitary chromophobe adenomas	Haun et al. 1984
5	6 h/d, 5 d/wk, 6 mo	Hamster, M, Golden Syrian	No toxic effects	Haun et al. 1984
0.5	6 h/d, 5 d/wk, 6 mo	Hamster, M, Golden Syrian	No toxic effects	Haun et al. 1984
0.05	6 h/d, 5 d/wk, 6 mo	Hamster, M, Golden Syrian	No toxic effects	Haun et al. 1984

[a]UDMH contained 0.12% of the known carcinogen DMNA.
Abbreviations: F, female; M, male; N/A, not applicable.

TABLE 10-5 Exposure Limits Set by Other Organizations

Organization, Standard	Exposure Limit	Reference
ACGIH		HSDB 2005
TLV-TWA, skin	0.01 ppm	
OSHA		HSDB 2005
PEL, skin	0.5 ppm	
NIOSH		HSDB 2005
REL, 2-h ceiling	0.06 ppm	
IDLH	50 ppm	
NRC		NRC 2000
AEGL-2, 1 h	3 ppm	
AEGL-2, 8 h	0.38 ppm	
AEGL-3, 1 h	11 ppm	
AEGL-3, 8 h	1.4 ppm	
ATSDR		ATSDR 2007
MRL	0.0002 ppm	

Abbreviations: ACGIH, American Conference of Governmental Industrial Hygienists; AEGL-2, acute exposure guideline level (disabling); AEGL-3, acute exposure guideline level (life-threatening); Agency for Toxic Substances & Diseases Registry minimum risk level (inhalation); ATSDR MRL; IDLH, immediately dangerous to life or health concentration; NIOSH, National Institute of Occupational Safety and Health; OSHA, Occupational Safety and Health Administration; PEL, permissible exposure limit; REL, recommended exposure limit; TLV, threshold limit value; TWA, time-weighted average.

TABLE 10-6 Spacecraft Maximum Allowable Concentrations

Duration	Concentration, ppm	Concentration, mg/m^3	Target Toxicity
1 h	3	7.5	CNS effects
24 h	0.12	0.3	CNS effects
7 d	0.03	0.075	Anemia
30 d	0.017	0.0425	Anemia
180 d	0.003	0.0075	Hepatotoxicity

To set SMACs for UDMH, ACs were calculated for the induction of each adverse effect (CNS effects, anemia, or hepatotoxicity) using the guidelines established by the NRC (1992). Five key inhalation studies were identified. These studies show fairly good consistency over a wide range of exposure times and species. For every putative astronaut exposure time (1 h, 24 h, 7 d, 30 d, and 180 d), the lowest AC was selected as the SMAC value (Table 10-7).

TABLE 10-7 End Point and Acceptable Concentrations

End Point	Selected End Point Data	Species, Reference Test Group Size	Uncertainty Factor				Acceptable Concentration, ppm				
			NOAEL	Time	Species	Space-flight	1 h	24 h	7 d	30 d	180 d
Lethality	860 ppm = LL LC_{50} 1 h exposure	Dogs, n = 3 (Weeks et al. 1963)	30	1	10	1	3	--	--	--	--
CNS	5 ppm NOAEL 6 h/d, 5 d/wk, 6 wk	Dogs, n = 3 (Rinehart et al. 1960)	1	6	10	1	--	0.12	--	--	--
Anemia	5 ppm LOAEL 6 h/d, 5 d/wk, 6 wk	Dogs, n = 3 (Rinehart et al. 1960)	5	1	10	3	--	--	0.03	--	--
Anemia	5 ppm LOAEL 6 h/d, 5 d/wk, 24 wk	Dogs, n = 3 (Rinehart et al. 1960)	10	1	10	3	--	--	--	0.017	--
Hepatotoxicity	5 ppm LOAEL 6 h/d, 5 d/wk, 26 wk	Dogs, n = 8 (Haun et al. 1984)	10	1	10	1	--	--	--	0.05	--
Hepatotoxicity	0.5 ppm NOAEL 6 h/d, 5 d/wk, 26 wk	Dogs, n = 8 (Haun et al. 1984)	1	6	10	1	--	--	--	--	0.01
Anemia	5 ppm LOAEL 6 h/d, 5 d/wk, 24 wk	Dogs, n = 3 (Rinehart et al. 1960)	10	6	10	3	--	--	--	--	0.003
SMAC							*3*	*0.12*	*0.03*	*0.017*	*0.003*

Abbreviation: --, not calculated.

1-h AC

A 1-h AC can be estimated from the inhalation study by Weeks et al. (1963). They exposed rats (n = 10 rats per group) and mongrel dogs (n = 3 dogs per group) to UDMH vapor in a dynamic flow gassing chamber for 1 h or less. Dogs were more sensitive than rats. From another study, it is clear that, at least for 4-h exposures, dogs are more sensitive than mice (Jacobsen et al. 1955); therefore, we use dog data even though the number of test animals was small. From the Weeks et al. study, the lower limit (LL) of the 95% confidence interval of the 1-h LC_{50} value in dogs was found to be 860 ppm. One can roughly estimate a safe concentration as follows:

$$AC_{(\text{lethality})} = 860 \text{ ppm}_{(\text{LL of LC50})} \times 1/3_{(\text{to LOAEL})} \times 1/10_{(\text{LOAEL to NOAEL})} \times 1/10_{(\text{species})} = 3 \text{ ppm}$$

where the factor 1/3 is used for the LC_{50} to LOAEL, which is reasonable to apply based on Table 10-5 of Weeks et al. (1963) showing that a 1-h exposure under added stress to dogs yields an LC_{50} of 300 to 350 ppm and a LOAEL of 80 to 120 ppm; a default factor of 1/10 is applied for LOAEL to no-observed-adverse-effect level (NOAEL). An additional default species extrapolation factor of 1/10 is applied. Benchmark estimation was not attempted because of the small number of animals in each group.

Alternatively, one can estimate an AC for CNS effects from the observation of Weeks et al. (1963) in nine stressed dogs exposed for 1 h to a concentration of 80 to 120 ppm. One of the nine dogs showed slight tremors from which it recovered in 1 h. The calculation is as follows:

$$AC_{(\text{CNS effects})} = 100 \text{ ppm}_{(\text{average LOAEL})} \times 1/3_{(\text{LOAEL to NOAEL})} \times 1/10_{(\text{species})} = 3 \text{ ppm}$$

The reduced factor for extrapolation for a LOAEL to a NOAEL was due to the fact that the dogs were already stressed before their exposure.

Additionally, we note that the odor threshold may be exceeded by concentrations in this range (3 ppm); therefore, an odor may be experienced or noted by crewmembers exposed to 3 ppm.

24-h AC

A 24-h AC can be estimated from the inhalation study by Rinehart et al. (1960) This peer-reviewed study exposed three dogs to UDMH at 5 and 25 ppm for 6 h/d, 5 d/wk for 26 wk. The authors observed some hemolytic anemia, slight bilirubinemia, and some lethargy, but the effects were very minimal. The minimal anemia effects at this level would not be expected to enhance spaceflight anemia and are not a concern for the 24-h short-term contingency SMAC.

None of the CNS effects noted at the high dose (25 ppm) was observed at the low dose (5 ppm). This is considered a NOAEL for CNS effects. The AC can be calculated as follows:

$$AC_{(CNS\ effects)} = 5\ ppm_{(NOAEL)} \times 1/10_{(species)} = 0.5\ ppm$$

Assuming exposure is not cumulative = 0.5 ppm × 6 h/24 h = 0.12 ppm.

7-d AC

For the longer-term ACs we also used the studies by Rinehart et al. (1960). They exposed dogs, rats, and mice 6 h/d, 5 d/wk to UDMH concentrations ranging from 5 to 140 ppm, and for times ranging from 6 to 26 wk. In three dogs exposed to UDMH at 5 ppm for 26 wk, they observed weight loss, mild anemia, splenic hemosiderosis, and an increase in serum bilirubin. However, 6 wk into the study there were minimal, if any, changes to the blood and there was no evidence of hepatotoxicity (see Table III of the paper).

The 7-d AC to protect against anemia was estimated as follows:

$$AC_{(anemia)} = 5\ ppm_{(LOAEL)} \times 1/10_{(species)} \times 1/3_{(spflt\ anemia)} \times 1/3_{(LOAEL\ to\ NOAEL)} = 0.05\ ppm$$

where the factors were 10 for species extrapolation, 3 for anemia of spaceflight, and 3 to extrapolate to a true NOAEL because the degree of anemia was marginal at most. By marginal, we mean that at the 6-wk point the RBC count had dropped from 7.2 to 7.0 × $10^6/mm^3$, the hemoglobin was unchanged, and the hematocrit decreased from 53% to 48%.

30-d AC

The 30-d AC for hematologic effects can be estimated from the data on dogs used for the 7-d AC; however, we turn to the data on the dogs after 24 wk of exposure. At this time, the RBC count had dropped from 7.2 to 6.0 × $10^6/mm^3$, the hemoglobin dropped from 15.4 to 11.4 gram %, and the hematocrit decreased from 53% to 43%. We take 5 ppm for 24 wk of exposure for 6 h/d 5 d/wk (30 d cumulative) to be a LOAEL. Using the same calculation as in the equation above, with a LOAEL to NOAEL factor of 10 instead of 3, gives the following:

$$AC_{(anemia)} = 5\ ppm_{(LOAEL)} \times 1/10_{(species)} \times 1/3_{(spflt\ anemia)} \times 1/10_{(LOAEL\ to\ NOAEL)} = 0.017\ ppm$$

Note that 24 wk of intermittent exposure at a rate of 30 h/wk is the same cumulative time as 30 d of continuous exposure for 24 h/d. This is not an ideal approach to converting intermittent exposures to continuous ones, but it is commonly used.

The 30-d AC for hepatotoxicity can be estimated from the study of Haun et al. (1984). They found significant transitory hepatotoxic effects of inhaled UDMH at 5 ppm for 6 h/d, 5 d/wk for 6 months in dogs, mice, rats, and hamsters. The dogs showed elevated serum glutamic pyruvic transaminase (SGPT) by week 4 of exposure. At 6 wk, the average SGPT value was 3 times the level in the control group. Through the remaining 20 wk of exposure, SGPT values stabilized at 3 to 4 times that of the control group. Recovery was noted to be 50% at 2 wk postexposure; however, there was no further reduction at 4, 8, and 11 wk postexposure. Sampling at weeks 27 and 47 showed a return to normal values. Bromsulphalein (BSP) concentrations were used to measure for liver function. BSP concentrations in blood after a 10-mg/kg injection showed significant retention at exposure termination 4 and 8 wk postexposure. The BSP measurements for liver function returned to normal at 11 wk postexposure. With UDMH at 0.5 ppm, Haun et al. (1984) found no observable adverse effects.

The AC for hepatotoxicity was calculated from the NOAEL of 0.5 ppm using a factor of 10 for species extrapolation:

$$AC_{(\text{hepatotoxicity})} = 0.5 \text{ ppm}_{(\text{LOAEL})} \times 1/10_{(\text{species})} = 0.05 \text{ ppm}$$

180-d AC

The 180-d ACs for anemia and hepatotoxicity were estimated by multiplying the 30-d ACs by the time default extrapolation factor of 30 d/180 d which is Haber's rule. Thus, the ACs were as follows:

$$AC_{(\text{anemia})} = 0.017 \text{ ppm}_{(\text{30-d AC, anemia})} \times 30 \text{ d}/180 \text{ d}_{(\text{time extrapolation})} = 0.003 \text{ ppm}$$

$$AC_{(\text{hepatotoxicity})} = 0.05 \text{ ppm}_{(\text{30-d AC, hepatotoxicity})} \times 30 \text{ d}/180 \text{ d}_{(\text{time extrapolation})} = 0.01 \text{ ppm}$$

COMPARISON WITH OTHER LIMITS

The SMACs compare reasonably well with other exposure guidelines (see Table 10-5) where comparisons are possible. The 1-h SMAC of 3 ppm matches the NRC acute exposure guideline level "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-2) (NRC 2000). The 24-h SMAC of 0.12 ppm is almost 3-fold lower than the NRC AEGL-2 for 8 h of exposure at 0.38 ppm. The AEGL-2

was derived from the data of Weeks et al. (1963) starting with the exposure of 360 ppm for 15 min, which caused reversible effects (behavioral changes and mild muscular fasciculations) in dogs. This was divided by a factor of 30 (3 for interspecies variability and 10 for intraspecies variability) to give a 15-min AEGL-2 of 12 ppm. Using Haber's rule ($C^n \times t = k$) where n = 1, the AEGL-2 for a 1-h exposure was estimated at 3 ppm; likewise, the AEGL-2 for 8 h was estimated at 0.38 ppm. From the Weeks et al. (1963) study, we elected to start with 100 ppm, a mild lowest-observed-adverse-effect level (LOAEL) for exposure to dogs that were stressed before exposure; one of nine dogs displayed slight tremors and experienced full recovery within 1 h, which calculates to a 1-h AC of 3 ppm.

The long-term SMACs of 0.017 and 0.003 ppm for 30 and 180 d of continuous exposure compare favorably with the threshold limit value of 0.010 ppm set by the American Conference of Governmental Industrial Hygienists. The 1-h SMAC of 3 ppm is substantially higher than the National Institute of Occupational Safety and Health (NIOSH) 2-h ceiling value of 0.06 ppm; however, the Occupational Safety and Health Administration permissible exposure limit of 0.5 ppm for long-term worker protection seems inconsistent with the NIOSH value. The SMACs are somewhere in the middle of the array of values set by other organizations.

RECOMMENDATIONS

We are confident that the values used to set ACs for astronaut crew health are moderately sound, but current peer-reviewed studies are needed to update inhalation exposure data on UDMH. Most descriptive toxicity studies were conducted from the late 1950s to the 1970s. Several of the published reports were not peer reviewed. A limited number of inhalation studies were identified in the 1980s. An updated study on the carcinogenic effects of UDMH inhalation and dermal exposure would aid in determining the potential increased risk of carcinogenesis.

REFERENCES

ACGIH (American Conference of Governmental Industrial Hygienists). 1991. Documentation of the Threshold Limit Values and Biological Exposure Indices, 6th Ed. American Conference of Governmental Industrial Hygienists, Cincinnati, OH.

Albano, E., A. Tomasi, L. Goria-Gatti, and A. Iannone. 1989. Free radical activation of monomethyl and dimethyl hydrazines in isolated hepatocytes and liver microsomes. Free Radic. Biol. Med. 6(1):3-8.

Amoore, J.E., and E. Hautala. 1983. Odor as an aid to chemical safety: Odor thresholds compared with threshold limit values and volatiles for 214 industrial chemicals in air and water dilution. J. Appl. Toxicol. 3(6):272-290.

ATSDR (Agency for Toxic Substances and Diseases Registry). 2007. Minimal Risk Levels (MRLs) for Hazardous Substances [online]. Available: http://www.atsdr.cdc.gov/mrls.html#bookmark02 [accessed April 15, 2008].

Back, K.C., and A.A. Thomas. 1962. Pharmacology and Toxicology of 1,1-Dimethylhydrazine (UDMH). Technical Report No. AMRL-TDR-62-118. Wright-Patterson Air Force Base, OH. October 1962.

Back, K.C., M.K. Pinkerton, A.B. Cooper, and A.A. Thomas. 1963. Absorption, distribution and excretion of 1, 1-dimethylhydrazine (UDMH). Toxicol. Appl. Pharmacol. 5:401-413.

Bauer, R.M., M.J. Tarr, and R.G. Olsen. 1990. Effect of 1,1-dimethylhydrazine on lymphoproliferation and interleukin 2 immunoregulatory function. Arch. Environ. Contam. Toxicol. 19(1):148-153.

Chevrier, J.P., and A. Pfister. 1974. The toxicity of 1,1-dimethylhydrazine in animals. II. Chronic poisoning. J. Eur. Toxicol. 7(4):242-246.

Cornish, H.H., C.L. Geake, and M.L. Barth. 1965. Biological action of 1,1-dimethylhydrazine. Biochem. Pharmacol. 14(12):1901-1904.

Dhennin, C., L. Vesin, and J. Feauveaux. 1988. Burns and the toxic effects of a derivative of hydrazine. Burns Incl. Therm. Inj. 14(2):130-134.

Dost, F.N., D.J. Reed, and C.H. Wang. 1966. The metabolic fate of monomethylhydrazine and unsymmetrical dimethylhydrazine. Biochem. Pharmacol. 15(9):1325-1332.

EPA (U.S. Environmental Protection Agency). 1984. Health and Environmental Effects Profile for 1,1-Dimethylhydrazine. EPA/600/x-84/134. Environmental Criteria and Assessment Office, Office of Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, OH.

Ernst, H., S. Rittinghausen, U. Wahnschaffe, and U. Mohr. 1987. Induction of malignant peripheral nerve sheath tumors in European hamsters with 1,1-dimethylhydrazine (UDMH). Cancer Lett. 35(3):303-311.

Frazier, D.E., Jr., M.J. Tarr, and R.G. Olsen. 1992. Evaluation of murine lymphocyte membrane potential, intracellular free calcium, and interleukin-2 receptor expression upon exposure to 1,1-dimethylhydrazine. Toxicol. Lett. 61(1):27-37.

Garcia, H.D., and J.T. James. 1996. Hydrazine. Pp. 213-233 in Spacecraft Maximum Allowable Concentrations for Selected Airborne Contaminants, Vol. 2. Washington, DC: National Academy Press.

Godoy, H.M., M.I. Diaz Gomez, and J.A. Castro. 1983. Metabolisms and activation of 1,1-dimethylhydrazine and methylhydrazine, two products of nitrosodimethylamine reductive biotransformation, in rats. J. Natl. Cancer Instit. 71(5):1047-1051.

Haun, C.C., A. Hall, R.L. Amster, G.B. Baskin, J.T. Young, R.L. Eason, R.E. Schmidt, W.F. MacKenzie, and K.M. Ayers. 1979. A six-month chronic inhalation exposure of animals to UDMH to determine its oncogenic potential. Pp. 141-153 in Proceedings of the Ninth Conference of Environmental Toxicology, 28-30 March 1979, Irvine, CA. Report No. AMRL-TR-79-68. Aerospace Medical Research Laboratory,Wright-Patterson Air Force Base, OH. August 1979.

Haun, C.C., E.R. Kinkead, E.H. Vernot, C.L. Gaworski, J.D. MacEwen, A. Hall, III, R.L. Amster, and R.H. Bruneer. 1984. Chronic Inhalation Toxicity of Unsymmetrical Dimethylhydrazine: Oncogenic Effects. AFAMRL-TR-85-020. ADA152208. Air Force Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, OH. October 1984.

House, W.B. 1964. Tolerance Criteria for Continuous Inhalation Exposure to Toxic Materials: III. Effects on Animals of 90-Day Exposure to Hydrazine, Unsymmetrical

Dimethylhydrazine(UDMH), Decaborane, and Nitrogen Dioxide. ASD-TR-61-519 (III). Wright- Patterson Air Force Base, Dayton, OH.

HSDB (Hazardous Substances Data Bank). 2005. 1,1-Dimethylhydrazine (CASRN:57-14-7). Specialized Information Service, U.S. National Library of Medicine, Bethesda, MD [online]. Available: http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB [accessed Apr. 15, 2008].

IARC (International Agency for Research on Cancer). 1974. 1,1- dimethylhydrazine. Pp. 137-143 in Some Aromatic Amines, Hydrazine and Related Substances, *N*-Nitroso Compounds and Miscellaneous Alkylating Agents. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Vol.4. Lyon, France: International Agency for Research on Cancer.

Jacobson, K.H., J.H. Clem, H.J. Wheelwright Jr., and N. Mayes. 1955. The acute toxicity of the vapors of some methylated hydrazine derivatives. AMA Arch. Ind. Health 12(6):609-616.

Keller, W.C., C.T. Olson, K.C. Back, and C.L. Gaworski. 1984. Teratogenic assessment of three methylated hydrazine derivatives in the rat. J. Toxicol. Environ. Health 13(1):125-131.

MacEwen, J.D., and E.H. Vernot. 1970. Toxic Hazards Research Unit Annual Technical Report: 1970. Report No. AMRL-TR-70-77. AD0714694. Aerospace Medical Research Laboratory,Wright-Patterson Air Force Base, OH. August 1970.

Mitz, M.A., F.L. Aldrich, and B.M. Vasta. 1962. Study of Intermediary Metabolic Pathways of 1,1-Dimethylhydrazine (UDMH). Report No. AMRL-TDR-62–110. AD290590. Aerospace Medical Research Laboratories, Wright-Patterson Air Force Base, OH. September 1962.

NRC (National Research Council). 1992. Guidelines for Developing Spacecraft Maximum Allowable Concentrations for Space Station Contaminants. Washington, DC: National Academy Press.

NRC (National Research Council). 2000. Acute Exposure Guideline Levels for Selected Airborne Chemicals, Vol. 1. Washington, DC: National Academy Press.

NTP (National Toxicology Program). 2000. Report on Carcinogens, 9th Ed. U.S. Department of Health and Human Services, Public Health Service, National Toxicology Program, Research Triangle Park, NC.

O'Brien, R.D., M. Kirkpatrick, and P.S. Miller. 1964. Poisoning of the rat by hydrazine and alkyhydrazines. Toxicol. Appl. Phamacol. 6:371-377.

O'Neil, M.J., A.Smith, P.E. Heckelman, and S. Budavari, eds. 2001. P. 571 in The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals, 13th Ed. Whitehouse Station, NJ: Merck and Co., Inc.

Rinehart, W.E., E. Donati, and E.A. Greene. 1960. The sub-acute and chronic toxicity of 1,1-dimethylhydrazine vapor. Am. Ind. Hyg. J. 21:207-210.

Rogers, A.M., and K.C. Back. 1981. Comparative mutagenicity of hydrazine and 3 methylated derivatives in L5178Y mouse lymphoma cells. Mutat. Res. 89(4):321-328.

Rumsey, D.W., and R.P. Cesta. 1970. Odor threshold levels for UDMH and NO_2. Am. Ind. Hyg. Assoc. J. 31(3):339-342.

Shook, B.S., and O.H. Cowart. 1957. Health hazards associated with unsymmetrical dimethylhydrazine. Ind. Med. Surg. 26(7):333-336.

Smith, E.B., and D.A. Clark. 1971. Absorption of unsymmetrical dimethylhydrazine (UDMH) through canine skin. Toxicol. Appl. Pharmacol. 18(3):649-659.

Tarr, M.J., R.G. Olsen, and D.L. Jacobs. 1982. In vivo and in vitro effects of 1,1-dimethylhydrazine on selected immune functions. Immunopharmacology 4(2): 139-148.

Tarr, M.J., B.J. McKown, and R.G. Olsen. 1988. Enhancement of murine mixed lymphocyte response by 1,1-dimethylhydrazine: Characterization and possible mechanism. Cancer Detect. Prev. 12(1-6):573-581.

Tomasi, A., E. Albano, B. Botti, and V. Vannini. 1987. Detection of free radical intermediates in the oxidative metabolism of carcinogenic hydrazine derivatives. Toxicol. Pathol. 15(2):178-183.

Toth, B. 1973. 1,1-dimethylhydrazine (unsymmetrical) carcinogenesis in mice. Light microscopic and ultrastructural studies on neoplastic blood vessels. J. Natl. Cancer Instit. 50(1):181-194.

Toth, B. 1977. The large bowel carcinogenic effects of hydrazines and related compounds occurring in nature and in the environment. Cancer 40(Suppl. 5):2427-2431.

Trochimowicz, H.J. 1994. Heterocyclic and miscellaneous nitrogen compounds. Pp. 3442-3451 in Patty's Industrial Hygiene and Toxicology, 4th Ed, G.D. Clayton, and F.E. Clayton, eds. New York: John Wiley & Sons.

Wade, M. 2003. Encyclopedia Astronautica. N_2O_4/UDMH-Rocket engine propellants and the engines that use them. Encyclopedia Astronautica [online]. Available: http://www.astronautix.com/props/n2o4udmh.htm [accessed Mar. 21, 2008].

Weeks, M.H., G.C. Maxey, M.E. Sicks, and E.A. Greene. 1963. Vapor toxicity of UDMH in rats and dogs from short exposures. Am. Ind. Hyg. Assoc. J. 24:137-143.

Wikipedia, 2008. TKS Spacecraft [online]. Available: http://en.wikipedia.org/wiki/TKS_Spacecraft.

Zelnick, S.D., D.R. Mattie, and P.C. Stepaniak. 2003. Occupational exposure to hydrazines: Treatment of acute central nervous system toxicity. Aviat Space Environ Med. 74(12):1285-1291.

11

Ethanol

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INTRODUCTION

This document presents the results of an update and reassessment of the toxicity of ethanol as it relates to the establishment of appropriate spacecraft maximum allowable concentrations (SMACs). This reassessment refers to a chapter on ethanol published in Volume 3 of *Spacecraft Maximum Allowable Concentrations for Selected Airborne Contaminants* (James 1996). Since publication of Volume 3, a number of research articles on ethanol have been published in the scientific literature. However, a large quantity of them focus on the health consequences of oral consumption and abuse of ethanol and have limited relevance to inhalation exposures in a spacecraft environment. As documented in Volume 3, the overall lack of information on inhalation exposures seems to be driven by the presumption that exposures to ethanol vapors are not widely applicable and that ethanol vapors have low toxicity and are a minimal health risk.

A fairly recent development that somewhat catalyzed a renewed interest in the health consequences of exposure to ethanol by inhalation is the emergence of ethanol as a significant additive or replacement for motor vehicle fuels. For example, ethanol is often introduced into gasoline as an oxygenate (5-10%) to help limit emissions of carbon monoxide, ozone, and various volatile organic compounds (Ahmed 2001). Additionally, certain vehicles are equipped to use E85 fuels (85% ethanol blended with 15% unleaded gasoline). Although exposure assumptions for these routes can differ significantly from spacecraft applications, some useful data have been generated and were considered in this reassessment (Winebrake et al. 2001, Nadeau et al. 2003, Chu et al. 2005).

This reassessment evaluates new data to determine whether the SMACs established in Volume 3 can still be supported as the most appropriate spacecraft exposure limits for ethanol. The toxicologic end points previously evaluated for which acceptable concentrations (ACs) were calculated in Volume 3 include neurotoxicity, irritation, hepatotoxicity, and flushing. New studies applicable to

these end points were reviewed and are incorporated in this reassessment. Any other relevant end points for which data emerged since publication of Volume 3 were addressed as they were identified. Another important aspect of this review is the need to set 1,000-d SMACs for longer-term crew exposures. Longer exposures would be of interest in assessing possible lengthy missions on the International Space Station (ISS) or in future lunar or Martian exploration efforts.

The intent of this reassessment is not to revisit all the studies that were considered in the original Volume 3 chapter. Much of the foundation for this review (e.g., discussion on metabolism, rationale for not addressing fetal risks) was provided in Volume 3; thus, these two write-ups should be viewed as complementary.

ISS MONITORING DATA

Volume 3 was written before NASA's full involvement with ISS and thus does not contain information on the relevance of ethanol in that environment or on monitored concentrations of ethanol in the ISS atmosphere. As a compound potentially used in a variety of spacecraft applications, ethanol can be introduced to the ISS atmosphere through many sources, including cleaning products such as alcohol wipes, payloads, substances in medical kits, and crew hygiene products. Other possible contributors to consider are the small amounts of ethanol that can be formed and released endogenously by humans. Ethanol is generally monitored (near-instantaneous readings) around 5-8 milligrams per cubic meter (mg/m^3) in the ISS atmosphere, although measurements have approached 20 mg/m^3 on occasion.

Because of its extreme solubility in water, a main concern with ethanol in the ISS atmosphere is its potential to affect the processing of humidity condensate by the Russian water processing system. Recycled humidity condensate provides a significant percentage of potable water on ISS (50%+). Ethanol is a common organic component of ISS condensate, being measured at concentrations as high as 156 mg/liter (L), with average ethanol concentrations around 50-55 mg/L. For reasons that are not fully understood, U.S. Laboratory condensate frequently contains higher ethanol concentrations than condensate from the Russian Service Module (Figure 11-1).

As these ethanol concentrations can double the system design limit for the Russian processing equipment (80 mg/L), significant efforts have been made to identify and limit releases of ethanol and other volatiles to the ISS atmosphere. The presence of excess volatiles in humidity condensate can affect the processing system in several ways. As the Russian system includes an oxidizing reactor and downstream multifiltration beds, system resources may be spent in oxidizing and removing relatively low-toxicity liquid components (e.g., ethanol). This has operational impacts for these limited life items and can impair performance when the system is also challenged with more toxic organic compounds.

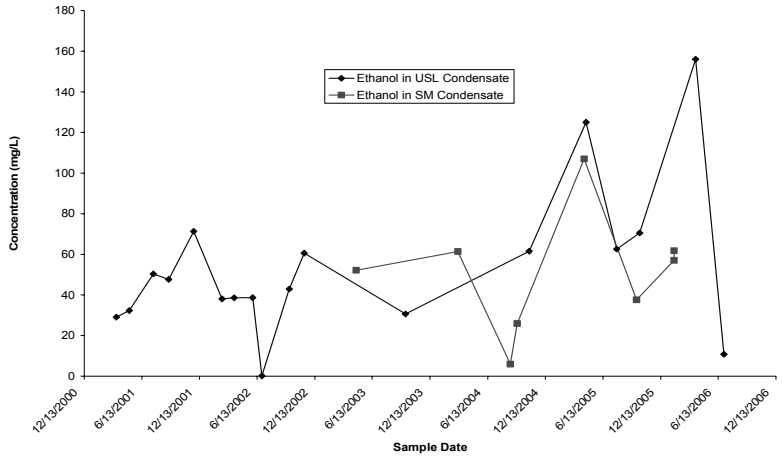

FIGURE 11-1 Ethanol concentrations (mg/L) measured in U.S. Lab Condensate (USL) and Russian Service Module (SM) condensate on ISS. Source: Data generated by NASA Johnson Space Center, Water and Food Analytical Laboratory.

REVIEW OF ACS FOR ETHANOL IN VOLUME 3 (James 1996)

In Volume 3, available toxicologic data on ethanol were presented and discussed in terms of specific end points. The review did not discuss the full range of toxicologic data on ethanol, as much of the data were not relevant to spacecraft applications or were deemed to be an issue only in association with chronic abuse (e.g., effects on skeletal or vascular smooth muscle). The document discussed adverse reproductive and developmental effects resulting from ethanol intake in some detail, but developing an AC was not deemed necessary given the lack of evidence that non-narcotic exposures could cause these effects (James 1996). Ultimately, ACs were developed for neurotoxicity, irritation, "flush response" (elevated skin temperature, pulse rate, and observable facial responses), and hepatotoxicity (Table 11-1). Most ACs were consistent across exposure time frames, as peak blood ethanol concentrations (BECs) are generally achieved in the first few hours of exposure (Pastino et al. 1997), and effects were not expected to be time dependent. For the irritation and flush response ACs, the 7- to 180-d ACs were set lower than the 1- and 24-h ACs not because of exposure time considerations but because a small margin of discomfort is allowable for the shorter-term ACs.

CONSIDERATION OF NEW DATA

The subsequent sections discuss new data available on the potential effects of inhalation exposure to ethanol. This includes studies not reviewed in Volume 3, and new studies published since 1996, as summarized in Table 11-2.

TABLE 11-1 Acceptable Concentrations for Ethanol End Points in Volume 3

	Acceptable Concentration (mg/m^3)				
End Point	1 h	24 h	7 d	30 d	180 d
Neurotoxicity	7,000	7,000	7,000	7,000	7,000
Mucosal irritation (eye, nose)	10,000	10,000	2,000	2,000	2,000
Flush response	4,000	4,000	2,000	2,000	2,000
Hepatotoxicity	N/A[a]	N/A[a]	2,000	2,000	2,000

[a]For hepatotoxicity, it was determined that non-narcotic exposures could not cause the relevant effects during these exposure time frames.
Source: James 1996.

TABLE 11-2 Toxicity Summary (For New Studies or Those Not Reviewed in Volume 3 SMAC Document)

Concentration (mg/m^3)	Exposure Duration	Species	Results	Reference[a]
Inhalation 0, 500, 1,000, 2,000 (NOAEL)	6 h	Human (n=5)	No observable adverse neuromotor effects. BEC reached only 0.44 mg/dL (0.0004%).	Nadeau et al. 2003
Inhalation 12,000	4 wk (6 h/d, 5 d/wk)	SD rats (15 male, 15 female)	Measured levels of certain neurochemicals (mediodorsal thalamus 5-hydroxyindoleacetic acid and hippocampal 5-hydroxytryptamine) were significantly reduced relative to controls in female but not in male rats.	Chu et al. 2005
Inhalation 2,500 (NOAEL), 3,100, 3,900, 4,900, 6,100, and 7,600	Nasal lateralization evaluated with 1- to 10-s pulses.	Human (n=6)	None of the 6 volunteers could reliably lateralize ethanol at 2,500 mg/m^3, but 4/6 (66%) could lateralize 3,100 mg/m^3.	Wise et al. 2006
Inhalation 19,000 (LOAEL)	Nasal lateralization evaluated with 1- to 3-s pulses.	Human anosmics (n=3)	Identified nasal irritation threshold. Questions exist about exposure duration.	Cometto-Muñiz and Cain 1990
Inhalation 90,000 (LOAEL)	Nasal lateralization evaluated with 1- to 3-s pulses.	Human (n=10)	Identified eye irritation threshold. Questions exist about exposure duration and test method.	Cometto-Muñiz and Cain 1996
Inhalation constant exposure 1,500 (NOAEL); Variable exposure 3,600 (NOAEL)	Both conditions were 4-h exposures, but the variable exposure regime is not clearly defined.	Human constant exposure (n=24); Variable exposure (n=16)	Volunteers were asked to assess irritation of the eye, nose, throat, or skin and to rate it on a scale of 0 (not at all) to 5 (strong). For ethanol, the mean response at 1,500 mg/m^3 remained about the same as the response for clean air. The mean response with variable exposures up to 3,600 mg/m^3 was also consistent with clean air, but the authors do not adequately describe the regime.	Seeber et al. 2002

[a]None of these studies were used to set ACs or SMACs.

Abbreviation: SD, Sprague-Dawley, LOAEL, lowest-observed-adverse-effect level; NOAEL, no-observed-adverse-effect level.

Neurotoxicity

The existing neurotoxicity AC (7,000 mg/m^3 for all time frames) was based on observations (Lester and Greenberg 1951) that three individuals exposed to ethanol in air at 15,000 mg/m^3 over 3-6 h did not report neurologic symptoms. Although lack of reported effects does not confirm the lack of performance detriments, it was noted that the BEC in this group reached only 10 mg/dL (0.01%), well below the level at which performance detriments have been observed in other studies reported in the scientific literature. Volume 3 noted one particularly applicable example in the work of Kennedy et al. (1993), which focused on the development of testing procedures that could be used to predict operational performance of air and space flight crew. The authors evaluated individual responses to a computer-based battery of performance tests (represented by the Armed Services Vocational Aptitude Battery) at different oral ethanol exposures (BECs of 150, 125, 100, 75, and 50 mg/dL). At each evaluated BEC except for 50 mg/dL (0.05%), the mean test score was below baseline performance, indicating various degrees of performance impairment. For example, the mean score at a BEC of 150 mg/dL was 17% lower than the baseline mean score.

New Data

Evaluation of the scientific literature since publication of Volume 3 found relatively few studies that specifically evaluated adverse neurologic impairment associated with inhalation exposures to ethanol. Nadeau et al. (2003) evaluated neuromotor effects in association with relatively low-level exposures to ethanol vapors. They exposed five volunteers to ethanol at 0, about 500, 1,000, and 2,000 mg/m^3 for 6 h. Reaction time, body sway, hand tremor, and rapid alternating movements were evaluated before and after the exposures. Subjects were exposed for five consecutive days, although the exposures were separated by 24 h. The authors reported that exposures up to 2,000 mg/m^3 did not result in significant neuromotor changes. Consistent with this conclusion, the BECs for test subjects exposed to ethanol at 500 and 1,000 mg/m^3 were below detection limits, whereas exposure to ethanol at 2,000 mg/m^3 resulted in a BEC of only 0.44 mg/dL (0.0004%).

Chu et al. (2005) exposed 15 male and 15 female rats to ethanol at 12,000 mg/m^3 for 4 wk (6 h/d, 5 d/wk) by inhalation. For the female rats, the authors noted that concentrations of certain neurochemicals (mediodorsal thalamus 5-hydroxyindoleacetic acid and hippocampal 5-hydroxytryptamine) were significantly reduced relative to controls. The biological significance of these results for humans in spaceflight is somewhat unclear, but it is worth noting that these compounds do relate to moods. However, the results do not outweigh the human data showing no adverse performance effects at similar ethanol concentrations.

Pastino et al. (1997) evaluated the pharmacokinetics of inhaled ethanol in B6C3F1 mice and F344 rats to construct a physiologically based pharmacokinetic (PBPK) model of ethanol inhalation in humans. They exposed rats and mice to ethanol by inhalation of 100, 400, and 1,200 mg/m^3 over 6 h and measured peak BECs. They developed a PBPK model to allow predictions of peak BECs in humans. The authors reported that the model accurately predicted the pharmacokinetics of ethanol in their inhalation study in mice and rats and was consistent with observed peak BECs for human males reported in the literature (primarily Lester and Greenberg 1951). The PBPK model predicted that the peak BEC in human males would reach a maximum of 293 μM (1.4 mg/dL or 0.001%) after inhalation of 1,200 mg/m^3. The authors noted that this BEC was significantly lower than concentrations at which diminished fine motor skills and impaired judgment might begin to occur and was 1-2 orders of magnitude below legal blood ethanol limits.

These studies suggest that the general range of BECs associated with mild performance impairment is 50-150 mg/dL (0.05-0.15%) (Nadeau et al. 2003). Consistent with this range, many states in the United States set their legal driving limit at 100 mg/dL (0.1%) or less.

AC Development

Ideally, the ACs would be based on an inhalation study that specifically evaluated the neurotoxicity of ethanol, as in the Nadeau et al. (2003) study. However, as the highest concentration used in that study (2,000 mg/m^3) resulted in a BEC of only 0.44 mg/dL, it would be an inappropriately low no-observed-adverse-effect level (NOAEL) to use. Accordingly, the approach taken in this reassessment was to use the same Kennedy et al. (1993) and Lester and Greenberg (1951) data that served as the basis for neurotoxicity ACs in Volume 3 but to evaluate them in a slightly different manner. Kennedy et al. (1993) reported a neurotoxicity NOAEL of 50 mg/dL. Applying an adjustment from this target BEC to account for the sample size ($\sqrt{27/10}$) provides a target BEC of 26 mg/dL. Lester and Greenberg (1951) demonstrated (n = 3) that inhalation of ethanol at 15,000 mg/m^3 resulted in a BEC of only 10 mg/dL, less than half the target BEC cited above. Given this margin, further adjustments to account for the small sample size in the Lester and Greenberg (1951) study were not deemed to be necessary. Further, as recent data from the Nadeau et al. (2003) study (n = 5) observed a BEC of only 0.44 mg/dL after inhalation of ethanol at about 2,000 mg/m^3, it appears that the Lester and Greenberg (1951) results provide a reasonably conservative BEC estimate. Accordingly, an AC of 15,000 mg/m^3 was established for all time frames as peak BECs are expected to occur within the first few hours of exposure, and because adverse neurologic health effects are not acceptable for any exposure period. Although benchmark dose modeling was initially considered for this end point, the small difference between the

NOAEL (50 mg/dL) and the lowest-observed-adverse-effect level (LOAEL) (75 mg/dl) suggests that it would not significantly improve the risk estimate.

Target BEC = 50 mg/dL $_{\text{(from Kennedy et al. 1993)}}$
$\times (\sqrt{27}/10)_{\text{(small n factor)}} = 26$ mg/dL

Lester and Greenberg (1951) BEC after ethanol exposure at 15,000 mg/m^3 = 10 mg/dL (2.6 times lower than target BEC)

1- and 24-h, 7-, 30-, 180-, and 1,000-d AC = 15,000 mg/m^3

Hepatotoxicity

The Volume 3 SMACs were based on the work of Di Luzio and Stege (1979). The authors evaluated hepatotoxicity in Sprague-Dawley rats after 26 d of continuous exposure to ethanol at 20,000 mg/m^3. The study observed transient changes in liver triglyceride concentrations and glutamic-pyruvic transaminase activity on days 3, 6, and 9 of testing. However, these changes were considered adaptive, and the same parameters did not differ from controls by the end of the 26-d study. With 20,000 mg/m^3 used as a NOAEL for hepatotoxicity, ACs were set for the 7-, 30-, and 180-d time frames (2,000 mg/m^3 after application of an uncertainty factor of 10 for animal-to-human extrapolation). No exposure time adjustments were considered to be necessary given that BECs and liver triglyceride levels were reached quickly in the testing and declined sharply by the end of the exposure period. Additionally, no short-term ACs were established, as hepatotoxicity over these time frames was not deemed to be credible without narcotic exposures.

New Data

No new studies were identified that specifically evaluated the hepatotoxicity of ethanol after inhalation exposures.

AC Development

Given that no new studies were identified, the Volume 3 ACs based on the Di Luzio and Stege (1979) findings were retained. Consistent with the rationale in Volume 3 for the lack of necessity for exposure time adjustments, a 1,000-d AC for hepatotoxicity was also established (2,000 mg/m^3). Di Luzio and Stege (1979) noted that inhalation exposure concentrations would have to be increasing on a stepwise basis for BECs to be sustained to a point where sustained hepatic effects might be observed. The authors concluded that "the ethanol vapor inhalation technique does not induce the classical hepatic alterations associated with ethanol liquid formula diets or following the acute oral administration of

ethanol." Given that only one dose was used in the critical study, benchmark dose modeling was not pursued for this end point as part of this reevaluation.

Mucosal Irritation

The Volume 3 ACs for mucosal irritation were based on the findings of Lester and Greenberg (1951). In their study, the five volunteers reported only mild irritation (coughing and smarting of the eyes and nose) when exposed to ethanol at 10,000 mg/m^3, and the effects dissipated so that no irritation was noted after 5-10 min of exposure. In contrast, there was continuous lacrimation at 30,000 mg/m^3, and 40,000 mg/m^3 was reported as intolerable. On the basis of these results, short-term ACs were set at 10,000 mg/m^3, with long-term ACs set at 2,000 mg/m^3 based on incorporation of an adjustment factor to account for the small sample size.

New Data

Several studies evaluated the nasal pungency associated with ethanol through nasal lateralization techniques (Wise et al. 2006, Cometto-Muñiz and Cain 1990). In these studies, both ethanol and clean air were introduced into a subject's nostrils, and the concentration at which the subject could consistently identify which nostril received the ethanol exposure (through interaction with nerve endings and reports of burning, stinging, or other signs of irritation) was recorded. A limitation of these studies for SMAC purposes is that the developed detection thresholds may correspond to a relatively minor degree of irritation, and it is difficult to evaluate the impact of exposure duration. The exposure duration may have not been long enough to elicit a maximum response; conversely, any observed nasal sensation may dissipate with time. Using these techniques, Wise et al. (2006) found that none of the six volunteers could reliably lateralize ethanol at concentrations of 2,500 mg/m^3, whereas four of six (66%) could lateralize about 3,000 mg/m^3. These results support the long-term AC of 2,000 mg/m^3 for ethanol set in Volume 3. Using similar lateralization techniques, Cometto-Muñiz and Cain (1990) reported that the threshold for nasal pungency (irritation) was about 19,000 mg/m^3 in a group of three anosmics. These results are consistent with the upper end of the 10,000-20,000 mg/m^3 proposed as an irritation threshold range by Lester and Greenberg (1951).

With regard to eye irritation, Cometto-Muñiz and Cain (1996) observed that irritation did not occur until about 90,000 mg/m^3, although the degree to which the test method (a specialized bottle cap) influenced the study findings is not clear. Seeber et al. (2002) also evaluated sensory irritation associated with inhalation exposures to ethanol. They exposed volunteers to ethanol under two test conditions: (1) 4 h of constant exposure to 0, 150, 760, and 1,500 mg/m^3, and (2) 4 h of variable concentration exposure at 0, 200, 2,000, and 3,600 mg/m^3. The volunteers (n = 24 for the constant exposure group, n = 16 for the

variable exposure group) were asked to assess irritation of the eye, nose, throat, or skin and to rate it on a scale between 0 (not at all) and 5 (strong). With constant exposures, the mean evaluated response to ethanol at 1,500 mg/m^3 remained approximately the same as the response reported for clean air. For variable exposures, the mean response at 3,600 mg/m^3 was also consistent with the clean air response, although the authors did not adequately describe how the variable exposure regime was implemented.

AC Development

Available study results support the existing Volume 3 short- and long-term ACs for ethanol, which are based on the Lester and Greenberg (1951) observations. Benchmark dose analysis was not pursued for this end point because of the qualitative nature of the response measurement with irritation (presence or absence of irritant effect). With regard to setting a 1,000-d AC for irritation, the same long-term AC (2,000 mg/m^3) can be applied, as solvent irritation is expected to peak within an hour or less (Hempel-Jorgensen et al. 1999) and not to demonstrate time dependency beyond that point.

1- and 24-h ACs $_{\text{(mucosal irritation)}}$ = 10,000 mg/m^3

7-, 30-, 180-, and 1,000-d ACs $_{\text{(mucosal irritation)}}$ = 10,000 mg/m^3 $_{\text{(1- and 24-h ACs)}}$ × ($\sqrt{5}$/10) $_{\text{(small n factor)}}$ = 2,000 mg/m^3

Flush Response

In Volume 3, ACs were developed that were protective against the flush response that is frequently observed in alcohol-sensitive subpopulations. This flush response is evidenced by visible facial flushing, an increase in pulse rate, blood pressure changes, elevated skin temperature, and other physical symptoms (Shibuya et al. 1989). These effects are believed to be related to a buildup of acetaldehyde, a main metabolic product of ethanol (Chan et al. 1986), as peak BECs do not differ among flushing and nonflushing subjects (Mizoi et al. 1979). Due to genetic differences related to differences in aldehyde dehydrogenase activity, 50-80% of Asians are susceptible to this adverse response to ethanol, compared with only 5-10% of Caucasians (Wolff 1972, Zeiner et al. 1979). Given these statistics, a fair percentage of crew members may exhibit this sensitivity, and final ACs should ensure that the potential for these adverse effects is minimized.

Volume 3 set the flush response ACs by using breath acetaldehyde concentrations observed in a study by Zeiner et al. (1979) in which Caucasian and Asian volunteers were exposed to ethanol at 0.7 mL/kg of body weight (about 40 g of ethanol for a 70-kg individual). As Shibuya et al. (1989) reported that ethanol exposures from 0.3 to 0.5 mL/kg were sufficient to induce the flush re-

sponse in flush-sensitive individuals, flushing was expected in the Zeiner et al. (1979) subjects. Among Asian volunteers, 6 of 7 experienced flushing symptoms, whereas only 1 of 10 Caucasians reported these effects. In Volume 3, a NOAEL was estimated from this study by observing that peak breath acetaldehyde concentrations below 10 ng/mL (Figure 11-2) did not correspond with flushing in either exposure group. As the peak individual breath acetaldehyde concentration for any test subject was 60 ng/mL, the ethanol dose was downwardly adjusted by a factor of 6 to estimate an oral NOAEL. With standard exposure assumptions, this dose was extrapolated to an inhalation exposure that resulted in an AC of 2,000 mg/m^3 for the 7-, 30-, and 180-d time frames. A doubling of the exposure concentration was deemed acceptable for short-term exposures (1- and 24-h ACs of 4,000 mg/m^3).

As part of this reevaluation it was determined that there was an error in the calculation for flush response ACs in Volume 3 (p. 196). In determining the ACs, the assumed inhalation absorption percentage (62%) was applied in the numerator rather than in the denominator of the AC equation, which resulted in an underestimation of the allowable air concentration by a factor of 2.5. Recalculating the ACs based on the correct application of the absorption fraction yields long-term ACs of 5,000 mg/m^3 and corrected 1- and 24-h ACs of 10,000 mg/m^3.

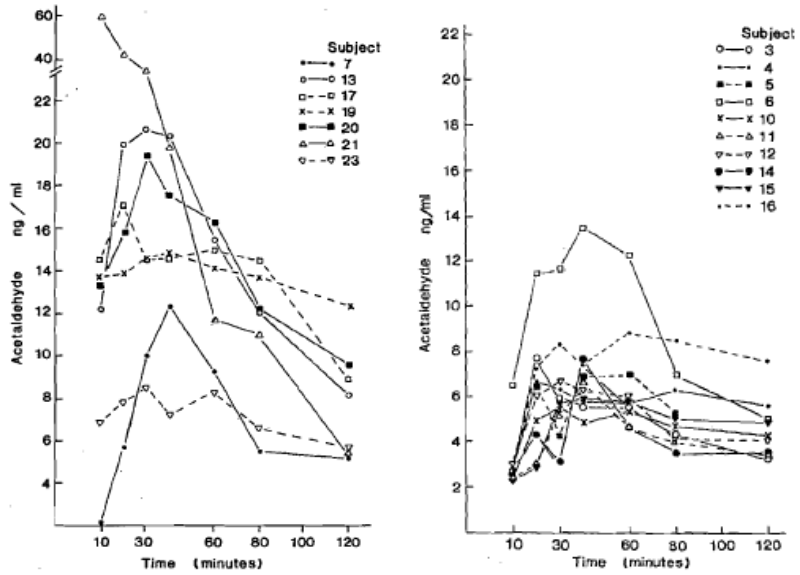

FIGURE 11-2 Breath acetaldehyde concentrations (ng/mL) in Asian (*left*) and Caucasian (*right*) volunteers. Source: Zeiner et al. 1979. Reprinted with permission; copyright 1979, *Alcoholism: Clinical and Experimental Research*.

New Data

As stressed in Volume 3, it would be preferable to have a study that demonstrates that flushing is relevant to inhalation exposures to ethanol. Unfortunately, an inhalation study that evaluated the induction of the flush response in sensitive and nonsensitive populations was not located in the scientific literature as part of this reevaluation. Tardif et al. (2004) evaluated breath acetaldehyde concentrations (although the flush response was not specifically evaluated) in five nonsmoking Caucasian volunteers exposed to ethanol at 50, 200, and 2,000 mg/m^3. In evaluating inhalation exposures (2-6 h) to ethanol at 2,000 mg/m^3, the authors observed a maximum breath acetaldehyde concentration of 2.6 ng/mL among the five volunteers (Robert Tardif, University of Montreal, personal communication, October 11, 2006). Although this level is well below the NOAEL of 10 ng/mL of Zeiner et al. (1979), the Tardif et al. (2004) group did not appear to include any flush-sensitive individuals, and it is not unusual for flush-sensitive populations to exhibit breath acetaldehyde concentrations 3-5 times higher than nonflushers (Zeiner et al. 1979, Jones 1995). Thus, it is reasonable to conclude that flush-sensitive individuals may approach the 10-ng/mL NOAEL in association with inhalation exposure to ethanol at 2,000 mg/m^3.

AC Development

With joint consideration of the Tardif et al. (2004) breath acetaldehyde measurements and the NOAEL estimated from Zeiner et al. (1979), an AC of 2,000 mg/m^3 is established. Given that data for multiple doses are not available for the critical study, benchmark dose analysis was not pursued for this end point.

7-, 30-, 180-, and 1,000-d ACs = 2,000 mg/m^3

With regard to the short-term ACs (1 and 24 h), mild flushing should be tolerable during temporary off-nominal situations. Where flushing is induced in the scientific literature (ethanol dose of 0.3-0.5 mL/kg per Shibuya et al. (1989)), the level of discomfort most test subjects experienced appears to be consistent with the degree of impairment allowable for short-term SMACs. Thus, the short-term ACs were set at the lower end of the ethanol dose used to elicit the flushing effect in sensitive individuals (0.3 mL/kg or 17 g for a 70-kg person). A short-term AC is calculated by making the same route-to-route extrapolation assumptions as in Volume 3.

1- and 24-h AC = 17 g $_{(\text{ethanol})}$/0.62$_{(\text{absorption by inhalation})}$/(0.015 m^3/min $\times$ 120 min) $_{(\text{inhaled air volume})}$ = 15 g/m^3

1- and 24-h AC = 15 g/m^3= 15 mg/L= 15,000 mg/m^3

UPDATED RECOMMENDATIONS

Literature not reviewed in Volume 3, in addition to new literature published since 1996, was considered in the preceding sections. This was done in an effort to update, if necessary, the recommendations for acceptable concentrations for ethanol inhalation exposure. These updated recommendations are summarized in Table 11-3. Also included in Table 11-3 are NASA's proposed SMACs. These values were determined based on the lowest AC for the endpoints in consideration: neurotoxicity, sensory irritation, flush response, and hepatotoxicity.

TABLE 11-3 Updated Acceptable Concentrations for Ethanol

		Uncertainty Factor				Acceptable Concentration (mg/m^3)					
End Point, Data	Species	Species	Time	Small n	Space-flight	1 h	24 h	7 d	30 d	180 d	1,000 d
Neurotoxicity											
Estimated NOAEL of 15,000 mg/m^3 (Lester and Greenberg 1951); based on target BEC of 26 mg/dL (Kennedy et al.1993)	Human	1	1	√27/10 (applied to target BEC)	1	15,000	15,000	15,000	15,000	15,000	15,000
Sensory Irritation											
NOAEL of 10,000 mg/m^3 (Lester and Greenberg 1951)	Human	1	1	√3/10	1	10,000	10,000	2,000	2,000	2,000	2,000
Flush Response											
LOAEL of 0.3 mL/kg (Shibuya et al. 1989)	Human	1	1	1	1	15,000	15,000	-	-	-	-
NOAEL of 2,000 mg/m^3 (Tardif et al. 2004) based on 10 ng/dL target breath acetaldehyde level (Zeiner et al. 1979)	Human	1	1	1	1	-	-	2,000	2,000	2,000	2,000
Hepatotoxicity											
NOAEL, 20,000 mg/m^3, (Di Luzio and Stege 1979)	Rat	10	1	1	1	-	-	2,000	2,000	2,000	2,000
SMACs						*10,000*	*10,000*	*2,000*	*2,000*	*2,000*	*2,000*

-, Flush Response: Mild flushing should be tolerable during temporary off-nominal situations, so short-term ACs were set at the lower end of the ethanol dose used to elicit the flushing effect in sensitive individuals.

-, Hepatotoxicty: Non-narcotic exposures could not cause the relevant effects during these exposure timeframes.

REFERENCES

Ahmed, F.E. 2001. Toxicology and human health effects following exposure to oxygenated or reformulated gasoline. Toxicol. Lett. 123(2-3):89-113.

Chan, A.W.K. 1986. Racial difference in alcohol sensitivity. Alcohol Alcoholism 21(1):93-104.

Chu, I., R. Poon, V. Valli, A. Yagminas, W.J. Bowers, R. Seegal, and R. Vincent. 2005. Effects of an ethanol-gasoline mixture: Results of a 4-week inhalation study in rats. J. Appl. Toxicol. 25(3):193-199.

Cometto-Muniz, J.E., and W.S. Cain. 1990. Thresholds for odor and nasal pungency. Physiol. Behav. 48(5):719-725.

Cometto-Muniz, J.E., and W.S. Cain. 1996. Relative sensitivity of the ocular trigeminal, nasal trigeminal, and olfactory systems to airborne chemicals. Chem. Senses. 20(2):191-198.

Di Luzio, N.R., and T.E. Stege. 1979. Influence of chronic ethanol vapor inhalation on hepatic parenchymal and Kupffer cell function. Alcohol Clin. Exp. Res. 3(3):240-247.

Hempel-Jorgensen, A., S.K. Kjaergaard, L. Molhave, and H.K. Hudnell. 1999. Time course of sensory irritation in humans exposed to N-butanol and 1-octene. Arch. Environ. Health 54(2):86-94.

James, J.T. 1996. Ethanol. Pp. 171-207 in Spacecraft Maximum Allowable Concentrations for Selected Airborne Contaminants, Vol. 3. Washington, DC: National Academy Press.

Jones, A.W. 1995. Measuring and reporting the concentrations of acetaldehyde in human breath. Alcohol Alcoholism 30(3):271-285.

Kennedy, R.S., W.P. Dunlap, J.J. Turnage, and J.E. Fowlkes. 1993. Relating alcohol-induced performance deficits to mental capacity: A suggested methodology. Aviat. Space Environ. Med. 64(12):1077-1085.

Lester, D., and L. Greenberg. 1951. The inhalation of ethyl alcohol by man. I. Industrial hygiene and medicolegal aspects. II. Individuals treated with tetraethylthiuram disulfide. Q.J. Stud. Alcohol 12(2):167-178.

Mizoi, Y., I. Ijiri, Y. Tatsuno, T. Kijima, S. Fujiwara, J. Adachi, and S. Hishida. 1979. Relationship between facial flushing and blood acetaldehyde levels after alcohol intake. Pharmacol. Biochem. Behav. 10(2):303-311.

Nadeau, V., D. Lamoureux, A. Beuter, M. Charbonneau, and R. Tardif. 2003. Neuromotor effects of acute ethanol inhalation exposure in humans: A preliminary study. J. Occup. Health 45(4):215-222.

Pastino, G.M., B. Asgharian, K. Roberts, M.A. Medinsky, and J.A. Bond. 1997. A comparison of physiologically based pharmacokinetic model predictions and experimental data for inhaled ethanol in male and female B6C3GF1 mice, F344 rats, and humans. Toxicol. Appl. Pharmacol. 145(1):147-157.

Seeber, A., C. van Thriel, K. Haumann, E. Kiesswetter, M. Blaszkewicz, and K. Golka. 2002. Psychological reactions related to chemosensory irritation. Int. Arch. Occup. Environ. Health 75(5):314-325.

Shibuya, A., M. Yasunami, and A. Yoshida. 1989. Genotypes of alcohol dehydrogenase and aldehyde dehydrogenase loci in Japanese alcohol flushers and nonflushers. Hum. Genet. 82(1):14-16.

Tardif, R., L. Liu, and M. Raizenne. 2004. Exhaled ethanol and acetaldehyde in human subjects exposed to low levels of ethanol. Inhal. Toxicol. 16(4):203-207.

Winebrake, J.J., M.Q. Wang, and D. He. 2001. Toxic emissions from mobile sources: A total fuel-cycle analysis of conventional and alternative fuel vehicles. J. Air Waste Manage. Assoc. 51(7):1073-1086.

Wise, P.M., T.M. Canty, and C.J. Wysocki. 2006. Temporal integration in nasal lateralization of ethanol. Chem. Senses 31(3):227-235.

Wolff, P. 1972. Ethnic differences in alcohol sensitivity. Science 175(20):449-450.

Zeiner, A.R, A. Paredes, and H.D. Christensen. 1979. The role of acetaldehyde in mediating reactivity to an acute dose of ethanol among different racial groups. Alcohol. Clin. Exp. Res. 3(1):11-18.

12

Formaldehyde

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RATIONALE FOR REASSESSMENT

Spacecraft maximum allowable concentrations (SMACs) for formaldehyde were established by the National Research Council (NRC) Committee on Toxicology and documented in Volume 1 of the SMAC documents published by the NRC (Wong 1994). Since that time, formaldehyde has become an air pollutant of increasing interest for the Space Shuttle as well as the International Space Station (ISS). With the deployment of air-monitoring devices (e.g., passive formaldehyde monitoring badges) in both the orbiter and ISS beginning in the mid-1990s, NASA now has reliable data on concentrations of formaldehyde in the space environment. Also, several ground-based, closed environment studies conducted by NASA have demonstrated airborne accumulation of formaldehyde. This information was not available to NASA or the NRC in developing the formaldehyde SMACs in 1994.

Experience with formaldehyde has shown that its concentration in the spacecraft atmosphere can often approach or exceed the 180-d formaldehyde SMAC of 0.04 parts per million (ppm). In evaluating these measurements in a crew health context, it is important for NASA to be confident that the SMAC is set at an appropriate level that will minimize the potential for significant crew health effects, but not falsely indicate cause for concern.

A preliminary review of the scientific literature suggested that there may be useful information on formaldehyde that is more recent than the studies used in deriving the formaldehyde SMACs in 1994. In addition, several comprehensive reviews of formaldehyde have been conducted since 1994, including assessments by the World Health Organization (WHO 2002), the Agency for Toxic Substances and Disease Registry (ATSDR 1999), Health Canada (Health Canada 2001), and the Chemical Industry Institute of Toxicology (CIIT 1999). (See Table 12-1 for occupational exposure limits and related guidelines set by

other organizations). Review of the formaldehyde SMACs also identified an opportunity for refinement with respect to the approach taken in developing acceptable concentrations (ACs) for the critical end points.

TABLE 12-1 Occupational Exposure Limits and Other Established Limits for Formaldehyde

Organization, Standard	Limit, ppm	Basis	References
ACGIH			
ceiling	0.3	Protective of sensory irritation	ACGIH 1991
OSHA PEL			
TWA	0.75	Protective of sensory irritation, other adverse respiratory effects	29 CFR § 1910.1048 [2008]
STEL	2	Protective of sensory irritation, other adverse respiratory effects	
NIOSH			
REL (TWA)	0.016	Based on lowest reliable analytical detection limit, based on NIOSH consideration as a carcinogen	NIOSH 2005
STEL	0.1	Protective against sensory irritation	
NIOSH			
IDLH	20	Respiratory tract damage, severe irritation	NIOSH 1996
ATSDR			
Acute MRL	0.4	Protective of sensory irritation. Based on observations from Pazdrak et al. (1993)	ATSDR 1999
Intermediate (15-365 d) MRL	0.03	Degenerative effects on nasal epithelium. Based on work of Rusch et al. (1983).	
NRC			
EEGL, 1 h CEGL, 24 h CEGL, 90 d	2 ppm 1 ppm 0.3 ppm	Weight-of-evidence evaluation of sensory irritation	NRC 2007
NAC			
AEGL-1	0.9 ppm (all time frames)	Weight-of-evidence evaluation of sensory irritation	NAC 2004

Abbreviations: ACGIH, American Conference of Governmental Industrial Hygienists; AEGL-1, acute exposure guideline level (nondisabling); ATSDR, Agency for Toxic Substances and Disease Registry; CEGL, continuous exposure guidance level; EEGL, emergency exposure guidance level; IDLH, immediately dangerous to life and health; MRL, minimal risk level; NAC, National Advisory Council; NIOSH, National Institute for Occupational Safety and Health; NRC, National Research Council; OSHA, Occupational Safety and Health Administration; PEL, permissible exposure limit; REL, recommended exposure limit; STEL, short-term exposure limit; TWA, time-weighted average.

After weighing these factors, NASA decided there was merit in updating and reconsidering critical toxicologic information on formaldehyde in the spacecraft atmosphere. This is the first time a SMAC has been reassessed, and therefore it is worth clarifying that this process is better viewed as a refinement than as a wholesale SMAC evaluation. This reassessment is intended to complement the existing SMAC for formaldehyde, and efforts will be made to avoid duplicating information already presented and approved by the NRC. In terms of organization, this document will do the following:

- Provide formaldehyde air-monitoring data relevant to the spacecraft environment.
- Summarize the approach taken in developing the existing formaldehyde SMACs.
- Evaluate whether toxicologic data exist that support development of ACs for end points not evaluated in the original SMAC document.
- Reevaluate studies relevant to critical end points considered in SMAC development, including considering more recent data that may be available.
- Provide justification for refined ACs and SMACs as appropriate; and
- Set limits for the 1,000-d exposure time frame (not addressed in the original 1994 document), consistent with NASA needs in anticipation of longer-term lunar or martian missions.

Review of Physical and Chemical Properties

Formaldehyde is a colorless gas with a strong, pungent odor (Sax 1984).

Synonyms:	Formic aldehyde, methyl aldehyde, methanal
Formula:	HCHO
CAS number:	50-00-0
Molecular weight:	30.0
Boiling point:	–19.5°C
Melting point:	–92°C
Lower explosive limit:	7%
Upper explosive limit:	73%
Vapor pressure:	10 mm Hg at –88°C
Vapor density:	1.08
Conversion factors at 25°C, 1 atm:	1 ppm = 1.23 mg/m^3, 1 mg/m^3 = 0.82 ppm

Formaldehyde Air Measurements in the Spacecraft Environment

Formaldehyde is a very common indoor air pollutant, as it can be off-gassed from textiles, foam insulation, resins, epoxys, and a myriad of other substances commonly encountered in the indoor environment (both ground based and on orbit). Health Canada (2000) pooled indoor air measurements across five different ground-based indoor air studies and found that the average formaldehyde concentration in indoor air was roughly 0.03 ppm. However, concentrations can vary significantly depending on the types of materials used in construction; Hare et al. (1996) reported that average indoor air concentrations of formaldehyde monitored in newly constructed homes range between 0.04 and 0.4 ppm. Formaldehyde can also be formed through secondary reactions of other indoor air pollutants (e.g., methane, pinene), especially in the presence of higher temperatures or chemical oxidizers. Studies by NASA have frequently observed formaldehyde releases from delrin, melamine foam, and other commonly used industrial materials.

Formaldehyde air monitoring data are available for both Space Shuttle and ISS, as well as for ground-based habitations designed to mimic enclosed-environment conditions that might be experienced on the moon or on Mars. These experiences have yielded information that is highly relevant to the SMAC process, as briefly described in the following sections.

Shuttle Orbiter Monitoring

In an effort to gain scientific perspective on the challenges posed by extended duration spaceflight, NASA conducted the Extended Duration Orbiter Medical Project from 1989 to 1995. As part of this project, formaldehyde measurements were collected on three STS Missions. Both area and personal monitors (passive dosimetry monitors, 8-h durations) were used to collect representative air samples during shuttle flight. Table 12-2 presents results from this monitoring. Formaldehyde concentrations were below the current 24-h SMAC (0.1 ppm) but were consistently measured at concentrations that approached or exceeded the current 180-d SMAC (0.04 ppm). For STS-59, every 8-h measurement for both area and personal monitors was at or above the 0.04-ppm SMAC, with a maximum 8-h measurement of 0.064 ppm (NASA 1999). No crew symptoms were reported in association with these measured formaldehyde concentrations (J. James, National Aeronautics and Space Administration, Houston, TX, personal communication, 2004).

ISS Monitoring

Consistent with shuttle monitoring, passive dosimetry badges are used to monitor formaldehyde concentrations on ISS. Area monitoring occurs in both the U.S. lab and the service module; samples are collected over 24 h. Given the

TABLE 12-2 Shuttle Orbiter Data on Formaldehyde Concentrations (ppm) in Spacecraft Air

STS Mission	Type of Sample	Range of Concentrations, ppm
56	Area	0.030-0.052
	Personal	0.038-0.045
59	Area	0.040-0.058
	Personal	0.045-0.064
67	Area	0.026-0.031
	Personal	0.034-0.059

relative consistency of the formaldehyde readings over time, it is appropriate to compare the results with the 180-d SMAC of 0.04 ppm. Figure 12-1 presents results from this monitoring for bimonthly time periods between 2001 and 2004 (note that discontinuity in the graph line results from occasional data gaps). These data indicate that, for the U.S. laboratory, formaldehyde has frequently been measured above the 0.04-ppm SMAC. No adverse effects on crew health have been reported in association with these measured formaldehyde concentrations. As described in Figure 12-1, relatively higher amounts of formaldehyde have generally been found in the U.S. lab, although that disparity is significantly less in the more recent measurements. NASA is investigating reasons for this disparity, and preliminary results point to a slightly higher formaldehyde generation rate in the U.S. lab and a greater amount of condensate removal in the service module, among other factors (J. Perry, National Aeronautics and Space Administration, Houston, TX, personal communication, 2004).

Lunar-Mars Life Support Test Project

NASA conducted the Lunar-Mars Life Support Test Project from 1995 to 1997 (James et al. 2002). The primary goal was to create and test an integrated closed-loop habitation that included systems for water recycling, waste processing, and air revitalization. This unique system enabled NASA to better understand human factors inherent to isolation and confinement and to develop, test, and improve capabilities to maintain the closed-loop environment.

Crew volunteers lived in the habitation for the duration of several extended tests (30, 60, and 90 d) conducted as part of this project. Air was monitored for volatile organic compounds and other pollutants and trace gases. In a 60-d test (Phase IIa), formaldehyde was monitored in the air through passive dosimetry badges and was verified by U.S. Environmental Protection Agency (EPA) impinger sampling. Formaldehyde was identified as a compound of particular concern, as air concentrations increased to 0.2 ppm by Day 15 of the test. At this concentration, one of four crew members experienced eye and upper respiratory tract irritation. Source assessment determined that off-gassing of

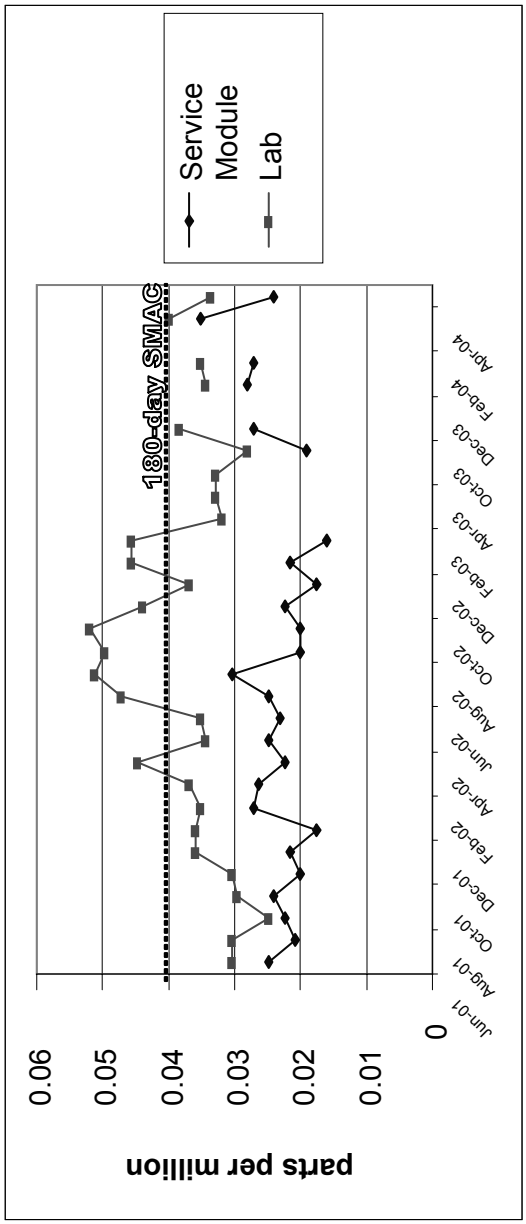

FIGURE 12-1 Formaldehyde concentration measured in the ISS atmosphere. Source: Data from Johnson Space Center Toxicology Laboratory.

formaldehyde from materials that had not been adequately tested for their off-gassing properties was at least partially responsible for the measured formaldehyde concentrations. After poster murals and other possible sources were removed from the habitation, formaldehyde concentrations decreased to 0.12 ppm on Day 18. Reported symptoms did not persist for the crew member at this concentration (although this reaction may be due to adaptation rather than to the reduced concentration) and the individual was able to continue for the 60-d test duration. No other irritants were identified at notable concentrations. Post-test evaluation (including a high-temperature "bake-out" study) indicated that melamine acoustic tiles may have been additional sources of formaldehyde during the habitation testing.

A 90-d test followed (Phase III), with adjustments made to the trace contaminant control devices and replacement of the melamine foam tiles. Air was monitored for formaldehyde in a manner consistent with the previous 60-d testing. The removal of potential formaldehyde sources appeared to be successful, as formaldehyde remained below the 0.04-ppm SMAC until approximately Day 60 of the test. Formaldehyde concentrations increased from that point to a maximum of 0.07 ppm, although this increase was thought to be due to an anomaly in a catalyst bed rather than to an additional off-gassing source. Although the 0.04-ppm long-term SMAC was exceeded for approximately 30 days, crew reported no symptoms in association with these measured formaldehyde concentrations.

SUMMARY OF EXISTING FORMALDEHYDE SMACS

The existing SMACs for formaldehyde were established in 1994 after review and concurrence by the NRC (Wong 1994). Following an assessment of toxicologic information available in the literature, two toxicologic end points were identified as being critical (mucosal irritation and nasal carcinogenesis) and ACs were developed for these end points. The ACs for mucosal (sensory) irritation were significantly lower than those for nasal cancers and formed the basis for the formaldehyde SMACs for all exposure times. Refer to Table 12-3 for a summary of the ACs for these end points and the final SMACs established in 1994.

UPDATE AND RECONSIDERATION OF CRITICAL TOXICOLOGIC DATA

The following sections present the results of a review of the scientific literature with regard to the inhalation toxicology of formaldehyde. This review is not meant to duplicate or replace the efforts involved in establishing the existing formaldehyde SMACs; the 1994 write-up presented in Volume 1 should also be referenced to obtain a more complete characterization of toxicokinetics, metabolism, and available toxicologic information on formaldehyde.

TABLE 12-3 Current Acceptable Concentrations and SMACs for Formaldehyde

	Acceptable Concentration, ppm				
Toxic End Point	1 h	24 h	7 d	30 d	180 d
Mucosal Irritation	0.4	0.1	0.04	0.04	0.04
Nasal cancers	3,400	164	23	6	0.9
SMAC	0.4	0.1	0.04	0.04	0.04

In updating the SMAC evaluation, this review does not focus solely on the two critical end points (mucosal irritation and risk of nasal cancer) used to develop ACs for the existing SMAC document. Other potential toxicologic effects were described and considered in developing the existing SMACs, and it is important to determine whether more recent data suggest that it is appropriate to establish ACs for other end points.

The results of this review are presented in the following sections, with additional information on critical toxicologic studies reported in Table 12-4. Although Table 12-4 includes critical studies pertinent to this reassessment, it is not meant to characterize the wealth of toxicologic information on formaldehyde available in the literature. For more comprehensive references, see Bender (2002), ATSDR (1999), and WHO (2002).

Pertinent occupational exposure limits for formaldehyde and calculated ACs based on this reassessment of formaldehyde are provided later in this document.

Assessment of Possible Additional Toxicologic End Points of Concern

The scientific literature was reviewed to assess whether there were any recent data that needed to be considered with regard to toxicologic end points not fully evaluated in setting the 1994 formaldehyde SMACs. The type of situation envisioned was one in which an end point may now need to be considered, although little supporting information was available in 1994 to suggest the need for an AC.

Three toxicologic end points were identified as having some recent information in the literature that at least warranted closer evaluation; neurotoxicity, reproductive and developmental effects, and immunologic effects. With regard to neurologic effects, the overall body of evidence suggests that low-level exposures (<1 ppm) to formaldehyde are unlikely to result in any neurologic impairment. Similarly, the weight of evidence from an evaluation of reproductive and developmental toxicity suggests that these effects will not occur in association with exposures relevant to the spacecraft environment. With respect to immunologic effects, it does not appear necessary to develop specific ACs to protect people with asthma from formaldehyde exposures at the low levels relevant to

TABLE 12-4 Summary of Critical Toxicologic Studies on Formaldehyde Inhalation

Dose/Route	Exposure Duration[a]	Species	Effects	Reference
Neurotoxicity				
10 ppm (NOAEL)	6 h/d, 5 d/wk for 13 wk	Wistar rats,10 of each sex	Three groups exposed to 1, 10, and 20 ppm of formaldehyde. Uncoordinated movement and wall-climbing were reported during the first 30 min of exposure for the 20-ppm exposure group only. No histopathologic evidence of lesions or damage to the brain.	Woutersen et al. 1987
10 ppm (NOAEL)	6 h/d, 5 d/wk for 13 wk	Wistar rats, male, 40	Exposure groups included 0, 2, 4, 10, 20, and 40 ppm of formaldehyde. No gross neurologic effects were observed in the 0-, 2-, 4-, or 10-ppm groups. Rats in the 20-ppm group were observed to be listless and to have a hunched posture. The same effects were observed at 40 ppm, along with ataxia.	Maronpot et al. 1986
2.6 ppm (LOAEL)	10 min/d for 90 d	Wistar rats, 2 groups with 13 rats each	Exposed animals (either 2.6 or 4.6 ppm) took longer to complete a maze and made more mistakes than controls. No dose-response was exhibited for the two groups.	Pitten et al. 2000
1.6 ppm (NOAEL)	5 h	Human, 16	Exposure to 0.2, 0.4, 0.8, and 1.6 ppm resulted in no cognitive performance impairments during testing.	Andersen and Molhave 1983
1.0 ppm (LOAEL)	5 h	Human, 16	Exposure to 0, 0.12, 0.33, and 1 ppm. In 5 of 6 tests, no dose-related effects were observed. Performance on the digit symbol test was impaired at 1 ppm relative to the lower exposures. There was some uncertainty about the degree to which other variables were properly controlled in establishing the exposure groups.	Bach et al. 1990
Reproductive/developmental effects				
40 ppm (NOAEL for reproductive effects)	6 h/d gestation day 6-20	SD rats, female, 25	No reproductive or developmental effects observed at 0, 5, 10, 20, or 40 ppm. Maternal toxicity in the 40-ppm group was observed.	Saillenfait et al. 1989

10 ppm (NOAEL)	6 h/d gestation day 6-15	SD rats, female, 25	No reproductive or developmental effects observed with formaldehyde exposures of 0,2, 5, or 10 ppm.	Martin 1990
Immunologic effects				
3 ppm	20 minutes	Human, 13	No asthmatic response or changes in pulmonary function parameters in groups of individuals with reported formaldehyde-related asthma who were exposed to up to 3 ppm.	Frigas et al. 1984
1.6 ppm	6 h/d for 10 d, and 6 h/d once a wk for 7 wk	BALB/c mice, 2 groups with10 mice each	Preexposure to 1.6 ppm for 10 d resulted in higher serum titers of IgE in response to ovalbumin administration. This effect was not observed in the 7-wk experiment.	Tarkowski and Gorski 1995
1.0 ppm	3 h	Human, 23	No differences in evaluated immunologic parameters among two groups of asthmatics (one control and one with reported sensitivity to urea-formaldehyde foam) exposed to 1 ppm of formaldehyde.	Pross et al. 1987
0.25 ppm (LOAEL) 0.13 ppm (NOAEL)	8 h/d, 5 consecutive days	DH guinea pigs, 12	After preexposure to formaldehyde, 10 of 12 animals in the 0.25-ppm group were found to exhibit a heightened immune response to an allergen (ovalbumin), vs. 3 of 12 with the control group. The response in the 0.13-ppm group was not different from the control.	Riedel et al. 1996
80 ppb (LOAEL)	16 h/d, 5 d/wk, 12 wk	C3H/He mice, 5/group	After preexposure to formaldehyde at 0, 80, 400, and 2,000 ppb, NGF production was reduced in the lowest two exposure groups compared with controls, but not in the 2,000-ppb group. No dose-response was noted.	Fujimaki et al. 2004
Nasal epithelial damage and nasal cancer				
3 ppm (LOAEL) 1 ppm (NOAEL)	22 h/d, 7 d/wk for 26 wk	Cynomolgus monkeys male, 12; F344 rats, 20 male and 20 female; GS hamsters, 10 male and 10 female	Exposure to 3 ppm resulted in statistically significant amounts of squamous metaplasia/hyperplasia in the nasal epithelium in monkeys and rats. At this concentration monkeys also exhibited hoarseness and other signs of irritation. 1 ppm was established as a NOAEL.	Rusch et al. 1983

(Continued)

TABLE 12-4 Continued

Dose/Route	Exposure Duration[a]	Species	Effects	Reference
0.3 ppm (NOAEL) 2 ppm (LOAEL)	6 h/d, 5 d/wk for 28 mo	F344 rats, male, 32	Statistically significant epithelial cell hyperplasia was observed in the 2- and 15-ppm exposure groups.	Kamata et al. 1997
2 ppm (NOAEL) 6 ppm (LOAEL)	6 h/d, 5 d/wk, 6 wk	F344 rats, male, 36	Exposure to 6, 10, and 15 ppm resulted in statistically significant increases in epithelial hyperplasia and squamous metaplasia. Increased cell proliferation was also observed with these groups.	Monticello et al. 1991
2 ppm (NOAEL) 6 ppm (LOAEL)	6 h/d, 5 d/wk, 24-mo	F344 rats, male, n = 327 (n = 90 in 6-ppm group, n = 90 in 10-ppm group, n = 147 in 15-ppm group)	Exposure to 6, 10, and 15 ppm resulted in statistically significant increases in epithelial hyperplasia and squamous metaplasia. Increased cell proliferation was also observed with the highest two exposure groups. Nasal tumors were reported in the 6-, 10-, and 15-ppm groups. Squamous cell carcinoma were found in 1 of 90 rats at 6 ppm, 20 of 90 rats at 10 ppm, and 69 of 147 rats at the 15-ppm exposure level.	Monticello et al. 1996
2-14.3 ppm	6 h/d, 5 d/wk, 24-mo	F344 rats, male and female, 232-236	Nasal polyploidy adenomas were observed in 1 of 232, 8 of 236, 6 of 235, and 5 of 232 rats, and squamous cell carcinomas were found at 0 of 232, 0 of 236, 2 of 235, and 106 of 232 for the 0-, 2-, 5.6-, and 14.3-ppm groups, respectively.	Kerns et al. 1983
Sensory irritation				
0.3 ppm (LOAEL)	5 min	Human, 5	A concentration response relationship was observed for mild eye irritation between 0.3 and 1 ppm. However, the degree of irritation at 0.5 ppm was essentially identical to that at 0.05 ppm.	Schuck et al. 1966
0.4 ppm	2 h	Human, 9 with skin sensitive to formaldehyde, 11 controls	Increased incidence of transient eye irritation, rhinitis. Change in nasal lavage fluid (increased eosinophil counts and protein levels. A major limitation of this study is the lack of detailed monitoring of the formaldehyde levels generated in the exposure chamber. As calibration was not conducted on the day of testing, there is a	Pazdrak et al. 1993

			potential for formaldehyde levels to have exceeded the 0.4-ppm target by an unknown amount (Bender 2002).	
0.4 ppm	2 h	Human, 10 with asthma, 10 controls	Increased sneezing, rhinorrhea, itching, and changes in nasal lavage fluid (increased eosinophil counts and protein levels). No differences in nasal response between asthmatics and controls. A major limitation of this study is the lack of detailed monitoring of the formaldehyde levels generated in the exposure chamber. As calibration was not conducted on the day of testing, there is a potential for formaldehyde levels to have exceeded the 0.4-ppm target by an unknown amount (Bender 2002).	Krakowiak et al. 1998
0.5 ppm (NOAEL) 1 ppm (LOAEL)	3 h	Human, 2 groups, 19 and 9	None of the nine subjects at 0.5 ppm experienced eye irritation. Half of the subjects noted odors at this concentration. More notable irritant effects were noted at concentrations of 1, 2, and 3 ppm.	Kulle et al. 1987
0.6 ppm (NOAEL)	90 min	Human, 15 with asthma	No differences in sensory irritant or pulmonary effects among groups exposed to 0.01, 0.1, and 0.6 ppm.	Harving et al. 1990
0.8 ppm (LOAEL)	5 h	Human, 16	Exposure to 0.2, 0.4, 0.8, and 1.6 ppm. For the lower two exposure groups none of the subjects reported irritation or discomfort for the first 2 h of the exposure. However, irritation (characterized as "slight discomfort") did not appear to be dose related or significantly different than exposure to clean air. A direct relationship between concentration and irritant response (mainly eye irritation) was observed at 0.8 ppm and above.	Andersen and Molhave 1983
1 ppm (NOAEL)	5 h	Human, 16	At formaldehyde exposure concentrations up to 1 ppm (0, 0.12, 0.33, and 1 ppm) there was not an observable concentration-response relationship in regard to irritation. However, there was some uncertainty about the degree to which other variables were properly controlled in establishing the exposure groups.	Bach et al. 1990

(Continued)

TABLE 12-4 Continued

Dose/Route	Exposure Duration[a]	Species	Effects	Reference
1 ppm (LOAEL)	6 min	Human, 5-27 volunteers	Exposure concentrations of 0, 0.35, 0.56, 0.7, 0.9, and 1.0 ppm were tested. Only at the highest exposure was the irritant response statistically significant compared with clean air. The authors noted that the smaller sample numbers at 0.7 and 0.9 ppm (5 and 7 volunteers, respectively, as opposed to 27 volunteers with the 1-ppm group) may have limited the power of the test at these concentrations. Also, only at 1 ppm did the reported irritant severity move above "slight."	Bender et al. 1983
2 ppm	40 min	Human, 15 with asthma	The volunteers in this study were people with asthma who were evaluated while at rest and while engaged in moderate exercise during exposure. Odors, sore throat, and eye irritation were reported at this concentration. One volunteer noted severe irritation at 2 ppm, and four others noted moderate irritation; none of them responded during the control period.	Witek et al. 1987
2.1 ppm (LOAEL) 1.2 ppm (NOAEL)	90 s	Human, 33 healthy males	At 2.1 ppm, 33% of the subjects experienced a doubling in their eye blink rate compared with controls. The blink rate was not elevated compared with controls for the 1.2-ppm exposure group.	Weber-Tschopp et al. 1977
3 ppm	1 h	Human, 22 healthy, 16 with asthma	There was no difference between the two groups in the degree of irritation experienced. Both groups reported odors, and eye, nose, and throat irritation. Response ranged from none to severe; 27% of the healthy subjects and 19% of those with asthma scored eye irritation as moderate or above. In both groups, 31% to 32% reported nose or throat irritation as moderate or above.	Green et al. 1987
3 ppm	3 h	Human, 9	There was a statistically significant increase in eye, nose or throat irritation, and odor at 3 ppm compared with clean air. Five of the subjects scored the nose or throat irritation as moderate, whereas only one scored eye irritation as moderate.	Sauder et al. 1986

1-5 ppm	5 min	Human, 13-20/test, multiple tests	In the static testing, 8% of the responses at 1 ppm were positive (per the authors, moderate or severe irritation was considered as a positive response). This was similar to the 7% response rate to clean air. Between 2 and 4 ppm, this rate increased to 33%, whereas at 5 ppm, 67% of the responses were positive.	Stephens et al. 1961

[a]Inhalation, unless otherwise noted.
Abbreviations: LOAEL, lowest-observed-adverse-effect level; NOAEL, no-observed-adverse-effect level.

the spacecraft environment. There is some limited evidence that formaldehyde may increase individual response to other allergens (Tarkowski and Gorski 1995, Riedel et al. 1996, Fujimaki et al. 2004), but more evaluation is needed (e.g., appreciation for mechanism of action, closer evaluation of dose-response relationships) before using these data to establish ACs.

Most of these immunologic studies were available before several comprehensive scientific reviews (ATSDR 1999, WHO 2002, NAC 2004, NRC 2007), although they were not used in developing air guidelines for formaldehyde by these organizations, presumably based on recognition of these uncertainties.

The neurotoxicity, reproductive and developmental, and immunologic end points are addressed in more detail in the following sections.

Neurotoxicity

The 1994 SMAC evaluation does not specifically discuss the potential neurotoxicity of formaldehyde, and evidence generally indicates that eye and upper respiratory tract irritation would be expected to be a more sensitive end point. Woutersen et al. (1987) exposed Wistar rats to 1, 10, and 20 ppm of formaldehyde by inhalation (6 h/d, 5 d/wk for 13 wk). Rats in the 20-ppm group exhibited abnormal behavior, including uncoordinated movement and wall-climbing within the first 30 min of each exposure. No such effects were observed with the lower-exposure groups. Histopathologic examination of the brain did not indicate lesions or other damage at necropsy.

In a similar study, Maronpot et al. (1986) exposed B6C3F1 mice to formaldehyde at 0, 2, 4, 10, 20, and 40 ppm. Mice in the two highest exposure groups exhibited dyspnea, hunched posture, and general listlessness, whereas no effects were observed in the other groups. Histopathologic evaluation did not identify lesions or other damage to brain tissue.

Kerns et al. (1983) did not observe any behavioral abnormalities, and histopathologic findings were negative in a 24-mo study with Fisher rats and B6C3F1 mice exposed by inhalation to formaldehyde at 0, 2, 5.6, or 14.3 ppm.

Pitten et al. (2000) concluded that formaldehyde was "probably neurotoxic" by inhalation based on the results of their study of the performance of Wistar rats in a maze trial, in which they exposed the rats for 10 min/d to formaldehyde at concentrations of 0, 2.6, and 4.6 ppm for 90 consecutive days. Exposed rats in both groups needed more time to complete the maze and made more mistakes than controls. No dose-response was observed among the two exposure groups, and the authors cautioned that further testing with established behavioral trial methods still needs to be performed.

Following a 5-h inhalation exposure to formaldehyde at concentrations of approximately 1 ppm, human volunteers reported fatigue and headache, and exhibited poorer performance on mathematical tests; no effects were observed after exposures to formaldehyde at 0.1 ppm (Bach et al. 1990). However, it is difficult to draw firm conclusions from the study results, as there were conflict-

ing results among the battery of tests, and the experimental design did not fully account for important variables (e.g., age, smoking status) in matching exposed and control subjects.

Andersen and Molhave (1983) evaluated human performance in several tests (speed and accuracy in completing specific tasks, mathematical skills) during 5 h of exposure to formaldehyde at concentrations of approximately 1.6 ppm and did not observe any performance decrements.

There is only limited epidemiologic evidence to suggest that neurologic impairment (e.g., loss of memory, sleep disturbance, lack of concentration, impaired balance) may be a risk for workers chronically exposed to formaldehyde (Kilburn et al. 1987, Kilburn 1994). Although these effects were positively associated with increased daily exposure to formaldehyde in one cohort of histology technicians (with exposures ranging from 0.2 to 1.9 ppm), coexposure to other air pollutants and other limiting factors confound determination as to whether formaldehyde actually caused any of the observed effects (ATSDR 1999, WHO 2002). A longer-term study (Kilburn and Warshaw 1992) of a larger cohort failed to find these effects in association with formaldehyde exposure.

Reproductive and Developmental Effects

Reproductive and developmental effects were not considered to be critical effects in setting the 1994 SMACs for formaldehyde. However, a few studies have evaluated reproductive or developmental end points, and several critical reviews have been published since 1994. In general, only very limited evidence exists (either from studies of laboratory animals or from human epidemiologic studies) to suggest that reproductive or developmental effects could occur in association with inhalation exposures to formaldehyde. Given the reactivity and rapid metabolism of formaldehyde, toxicity at sites distant from the portal of entry is extremely unlikely, especially in association with low-level exposures (Collins et al. 2001). Various organizations have assessed available data on formaldehyde and have come to the same conclusion (WHO 2002, ATSDR 1999, IARC 1995).

Collins et al. (2001) conducted a thorough review and meta-analysis of studies that investigated the reproductive and developmental toxicity of formaldehyde. With respect to evidence from studies of laboratory animals, they found that most of the animal studies did not report positive reproductive or developmental effects associated with formaldehyde exposure.

One inhalation study that did observe reproductive effects with formaldehyde was a Russian study (Guseva 1972) in which groups of male rats were exposed to formaldehyde by inhalation for 4 h/d 5 d/wk for 6 mo. The formaldehyde concentrations were 0, 0.1, and 0.2 ppm. After the rats mated with unexposed females, reproductive effects were assessed. The males in the highest exposure group exhibited a significant decrease in testicular DNA, although

there were no observed effects on the fetus (e.g., abnormalities in fetal weight, litter size, incidence of birth defects).

Several Russian studies have also suggested a relationship between formaldehyde exposure and various developmental effects in rats (Gofmekler 1968, Gofmekler et al. 1968, Pushkina et al. 1968). In these studies, male and female rats were exposed to formaldehyde 24 h/d by inhalation for 10-15 d, at concentrations of 0, 0.01, and 0.83 ppm. These effects included increases in fetal body weight, reduced number of fetuses per litter, reduced amounts of nucleic acids in fetuses, and fetal histopathologic changes. Specific study details have been found to be lacking or inconsistent, and these results have not been duplicated in other studies (Staples 1983, Collins et al. 2001). They are also inconsistent with the findings from Saillenfait et al. (1989), who evaluated SD rats exposed to formaldehyde at up to 40 ppm on gestations days 6-20 and observed no changes in a number of reproductive variables (e.g., number of resorptions, implantation), and with the work of Martin (1990), who evaluated a wide variety of reproductive and developmental end points and observed no effects in SD rats exposed to formaldehyde at concentrations as high as 10 ppm (6 h/d on gestation days 6-15).

Collins et al. (2001) also described the results of a number of human epidemiologic studies. In general, reproductive and developmental effects evaluated included spontaneous abortion rates, congenital malformations, reduced birth weights, and infertility. Among these effects, spontaneous abortion was most often investigated in the epidemiologic studies. Of nine studies identified, four suggested higher rates of spontaneous abortion in formaldehyde-exposed workers (Axelsson et al. 1984; John et al. 1994; Taskinen et al. 1994, 1999). In several cases, there was no attempt to control for confounding factors such as age and smoking status.

Even in those studies that attempted to control for these major confounding factors, coexposures to other reproductive toxicants may be important factors. For example, John et al. (1994) based their assessment on self-reported spontaneous abortion rates (relative risk factor of 2.1, with a 95% confidence interval [CI] of 1.0-4.3) from female cosmetologists who were also exposed to solvents and other potential chemical confounders. In their meta-analysis, Collins et al. (2001) reported a meta-relative risk of only 1.4 when considering reported data across all studies. They also pointed out that reporting bias was a factor in the reported relative risks, as studies that used self-reported exposure data had a relative risk of 2.0 (95% CI of 1.4-2.8), whereas studies in which the work task was specifically evaluated reported a mean relative risk of only 0.7 (95% CI of 0.5-1.0). There was little epidemiologic evidence for an increased risk of congenital malformations or low birth weight in exposed women (ATSDR 1999, Collins et al. 2001).

Similarly, a population-based case-control study of low-birth-weight newborns in Lithuania evaluated a possible association with formaldehyde exposures and found no statistically significant association between incidences of

low-birth-weight newborns and estimates of airborne concentrations of formaldehyde (Grazuleviciene et al. 1998).

Taskinen et al. (1999) evaluated a potential relationship between formaldehyde exposure and infertility. They investigated 235 women exposed to formaldehyde in the woodworking industry (estimated formaldehyde exposure concentrations of 0.01-1.0 ppm) and found that 21% of women in the high-formaldehyde exposure group (>0.33 ppm) experienced longer times to pregnancy, compared with 9% in a control group. However, this study had a number of sources of uncertainty, including the fact that exposure ranges were not measured but were estimated based on expected workplace exposures, paternal exposures were not evaluated, and the study relied exclusively on self-reported data (Collins et al. 2001).

Immunologic Effects

Although asthma would likely be identified in astronaut health screening, the 1994 SMAC document discusses it briefly in the context of sensitive nonasthmatic individuals. Direct exposure to formaldehyde is clearly irritating to the skin, and allergic contact dermatitis related to formaldehyde exposures is not uncommon (NRC 1981, WHO 2002). Exposures to formaldehyde in air can result in immunologically induced sensitization of the respiratory tract (Grammar et al. 1990, Lemiere et al. 1995, Hilton et al. 1996, Kim et al. 2001), although it is uncertain whether formaldehyde should be considered to be an asthmatic agent (Riedel et al. 1996, Frigas et al. 1984). Most immunologic studies of formaldehyde do not support the contention that there is an IgE- or IgG-mediated response to formaldehyde exposure (ACGIH1991, Krakowiak et al. 1998, ATSDR 1999), and various publications have shown that exposures to formaldehyde in the 1- to 3-ppm range are unlikely to trigger an asthmatic reaction in someone who has preexisting asthma (Sheppard et al. 1984, Pross et al. 1987, Uba et al. 1989).

However, in some cases, exposures to formaldehyde can cause the development of asthma (Kim et al. 2001). It has been proposed that formaldehyde may be more likely to act as a direct respiratory irritant when asthmatic reactions are triggered by other causes (NRC 1981, Grammar et al. 1990). Also, some research suggests that the development of asthma may be more likely to be associated with particulate forms (e.g., resin dust) than with gaseous formaldehyde (Lemiere et al. 1995) and that these particulate forms may contain complex mixture of other chemicals that could also be causing the response. In their 1997 review, a panel of the Industrial Health Foundation concluded that, at lower exposures up to 3 ppm, people with asthma were no more sensitive to formaldehyde than other individuals (Paustenbach et al. 1997), a conclusion consistent with the observation that most inhaled formaldehyde at these exposures is retained in the upper respiratory tract because of the reactivity and high water solubility of the molecule (Egle 1972).

Several studies have emerged since the 1994 SMAC that suggest formaldehyde may interact with the immune system to enhance the response to other inhaled allergens. Tarkowski and Gorski (1995) preexposed mice to 0 and 1.6 ppm of formaldehyde by inhalation (6 h/d) for 10 d. They exposed a separate group for 6 h/d once a week over 7 wk. When challenged with ovalbumin, neither the control group nor the group exposed intermittently over 7 wk demonstrated an effect on serum IgE ovalbumin. However, significantly higher serum titers were seen in the group of mice preexposed to formaldehyde for 10 consecutive days.

This type of immune effect was also evaluated in guinea pigs (Riedel et al. 1996). In this study, two groups of animals were exposed to 0.13 and 0.25 ppm of formaldehyde by inhalation (8 h/d over 5 consecutive days). Three control groups were also evaluated. The animals were then exposed to 0.5% ovalbumin in air, and their immune response (evidence of airway obstruction, circulating IgG) was evaluated again in a challenge 3 wk after the preexposure. In the group preexposed to 0.25 ppm of formaldehyde, 10 of 12 animals displayed sensitization to the ovalbumin compared with only 3 of 12 in the control group, and anti-ovalbumin IgG titers were significantly higher than in controls. The 0.13-ppm exposure group did not differ from the control group in either of these tests. The authors cautioned that the exact mechanism by which formaldehyde interacts with the immune system to exhibit these effects is unclear and noted that guinea pigs may be more sensitive than humans to the effects of formaldehyde (consistent with greater formaldehyde sensitivity demonstrated for pulmonary resistance).

In a longer-term study, Fujimaki et al. (2004) exposed female mice for 12 wk (16 h/d, 5 d/wk) to formaldehyde at 0, 80, 400, and 2,000 parts per billion (ppb) and investigated the effects of this exposure on allergic inflammatory responses. They observed that in ovalbumin-immunized mice, production of nerve growth factor (NGF) (measured in both plasma and broncho-alveolar lavage (BAL) fluid) decreased for mice preexposed to formaldehyde at 80 and 400 ppb, but not for those exposed at 2,000 ppb. The authors hypothesized that formaldehyde may inhibit the immunologically mediated augmentation of NGF and IgG3 production or that reduction in NGF may affect its anti-inflammatory properties. However, there was no dose-response in NGF reductions for the three formaldehyde exposure groups, and the authors noted that further studies are needed to better understand these findings.

EVALUATION OF ADDITIONAL DATA AND REFINEMENT OF ACs

Sensory Irritation

Summary of Previous Approach

There are a wealth of data on the sensory irritant effects of exposure to formaldehyde, including animal studies, controlled human exposure studies,

occupational findings, and community health surveys (e.g., mobile home studies). In developing ACs for mucosal irritancy, the 1994 SMAC document used a mobile home study (Hanrahan et al. 1984) as a primary reference. This study investigated formaldehyde exposures in 65 mobile homes in Wisconsin with airborne formaldehyde concentrations between 0.1 and 0.8 ppm. Approximately half of the individuals exposed to indoor air at formaldehyde concentrations above 0.4 ppm reported mild eye irritation, and this concentration formed the basis for the 1-h AC (Table 12-5).

For the 24-h AC, a smaller likelihood of irritation was desired, and the AC was set at 0.1 ppm, the concentration that produced eye irritation in only 4% of the subjects in the Wisconsin mobile home study. For the longer-term ACs (7, 30, and 180 d), there was a desire to set the guideline at a level that would be nonirritating for almost all the population. However, because of difficulty in establishing an irritation threshold and lacking a better approach, the mean outdoor air concentration reported by Hanrahan et al. (1984) for the Wisconsin mobile home study was used as the basis for the limit (0.04 ppm). This is likely to be a relatively high estimate for typical ambient air and is more in line with central tendency estimates of indoor air concentrations of formaldehyde in conventional homes that are not newly constructed. For example, Health Canada (2000) recently pooled indoor air measurements across five different indoor air studies and found that the average formaldehyde concentration in indoor air was roughly 0.03 ppm, with a 95th percentile of 0.07 ppm.

The 1994 SMAC document did not develop ACs based on loss of pulmonary function, presumably based on the conclusion that mucosal irritation would be a more sensitive end point. This conclusion is supported by evidence from the scientific literature, which generally indicates that pulmonary function is not impaired even at formaldehyde concentrations as high as 3 ppm with sensitive humans (asthmatic) (Sheppard et al. 1984) or in humans with chronic occupational exposures (Horvath et al. 1988, Paustenbach et al. 1997). These observations are consistent with the relatively high water solubility of formaldehyde, which makes it amenable to removal in the upper respiratory tract.

Refinement of Approach and Review of Pertinent Data

The mobile home studies have certain strengths. For example, the exposures are continuous and often focus on exposure concentrations that are especially relevant to SMAC development. However, it has been recognized that the potential for confounding coexposures to other irritant gases and the general lack of sufficient control groups can significantly limit the usefulness of these studies (Paustenbach et al. 1997, Bender 2002). In addition, adaptation can potentially mask the acute health effects these residents experienced. Unlike many other chemicals, there are a number of controlled human exposure studies for formaldehyde, and they are widely viewed as providing the most reliable infor-

TABLE 12-5 ACs for Sensory Irritation in the 1994 SMAC Document

1-h AC	24-h AC	7-d AC	30-d AC	180-d AC
0.4 ppm[a]	0.1 ppm[b]	0.04 ppm[c]	0.04 ppm[c]	0.04 ppm[c]

[a]AC is based on the work of Hanrahan et al. (1984) based on incidences of reported eye irritation in a mobile home study.
[b]AC is based on the work of Hanrahan et al. (1984) based on incidences of reported eye irritation in a mobile home study. A lower response rate was deemed acceptable for 24 h compared with the 1-h AC.
[c]AC is based on the mean ambient formaldehyde concentration for the Hanrahan et al. (1984) mobile home study.

mation on the irritancy of formaldehyde (Paustenbach et al. 1997, Bender 2002, NAC 2004, NRC 2007). Accordingly, for the purposes of SMAC development, a focus was placed on using controlled human exposure study results where possible, with other sources of data (e.g., animal studies, mobile home surveys) used as supporting evidence where applicable.

A number of controlled human exposure studies for formaldehyde irritation are available, and they vary in several important ways. For example, whereas many studies focus on healthy individuals, others have also sought to evaluate people with asthma and other potentially sensitive individuals in an attempt to better characterize the wide range of individual susceptibility to formaldehyde irritation (Bender 2002). Other variables include the metric for reporting irritation, the exposure durations used in the testing, and the formaldehyde exposure concentrations.

With regard to exposure concentration, those studies that evaluate formaldehyde concentrations in the 1- to 3-ppm range can generally be separated from those primarily designed to evaluate low levels of formaldehyde (≤1 ppm). With respect to the former category, these studies were generally designed to assess pulmonary effects after acute exposures to formaldehyde, particularly in people with asthma and exercising individuals, although sensory irritation was also evaluated and reported. For example, Green et al. (1987) exposed 22 healthy subjects and 16 subjects with asthma to 3 ppm of formaldehyde for 1 h, along with clean air controls. Both groups were exposed during moderate to intense sessions of exercise. With respect to sensory irritation, both groups reported similar incidences of odors, eye irritation, and nose or throat irritation (irritation was not observed with clean air). The amount of irritation varied from none to severe, demonstrating the wide range of sensitivity to formaldehyde. Even at 3 ppm, however, most individuals did not experience any notable irritant effects.

Witek et al. (1987) conducted a similar study, in which 15 volunteers with asthma were exposed to clean air and formaldehyde at 2 ppm for 40 min. The groups were evaluated while at rest and while engaged in moderate exercise during the exposures. Although exercise did not appear to affect the reported irritation, the volunteers reported higher incidences of moderate irritation with

formaldehyde at 2 ppm (4 of 15 volunteers reported moderate eye irritation, with another individual reporting severe, but not incapacitating, irritation) than when exposed to clean air. The irritant effects did not persist after the exposures were discontinued. One individual noted moderate irritation in the absence of formaldehyde but reported no effects when exposed to 2 ppm.

Sauder et al. (1986) evaluated slightly longer exposures (3 h) to 3 ppm of formaldehyde with nine healthy volunteers. Exposures to clean air were evaluated on the first day of the testing as a control. Eye and nose or throat irritation were statistically significant for the 3-ppm exposures. At 3 ppm, five of the nine volunteers reported moderate nose and throat irritation, but only one reported moderate eye irritation. The authors speculated that this may have been due to adaptation.

In another 3-h study, Kulle et al. (1987) exposed 10 healthy volunteers to 0, 0.5, 1, and 2 ppm of formaldehyde at rest, with an additional 9 volunteers exposed to 0, 1, 2, and 3 ppm of formaldehyde while exercising. No exercise effect was noted with regard to the irritancy of formaldehyde. At 3 ppm, all the individuals reported eye irritation, with 44% of them characterizing it as moderate. With the 2-ppm exposures, 32% of the volunteers reported mild eye irritation and 21% reporting moderate eye irritation when the group data were pooled. At 1 ppm, 16% of the volunteers reported mild eye irritation, with one individual still reporting moderate irritation at this concentration. No eye irritation was reported for the 0.5-ppm exposure group. With respect to nose or throat irritation, 37% reported mild irritation at 2 ppm, but only 22% reported similar irritation at 3 ppm. Frequencies of nose or throat irritation for the 1- and 0.5-ppm groups were not greater than in the control group.

Weber-Tschopp et al. (1977) assessed the eye irritation attributable to formaldehyde by evaluating blink rates in healthy human subjects exposed to formaldehyde at 0, 1.2, and 2.1 ppm for 90-s durations. There was no difference in eye blink rates between the lower exposure group and the controls, although the short-term duration of the testing may limit its usefulness in defining an eye irritation threshold. At 2.1 ppm, the blink rate doubled in a third of the subjects evaluated in the testing, as compared with controls.

Stephens et al. (1961) exposed groups of 13-20 students to formaldehyde at concentrations ranging from 1 to 5 ppm over 5 min to evaluate eye irritation. Several replicate exposures were evaluated at each concentration so that total responses of self-reported irritation observations varied between 27 and 75 depending on the concentration. Observations of moderate to severe irritation were recorded as positive responses. At 1 ppm, only 8% of the exposures resulted in a positive response (at least moderate irritation), which was similar to the rate observed with clean air (7%). Between 2 and 4 ppm, 33% of the responses were positive; at 5 ppm, 67% were positive. This study is somewhat limited by the subjective evaluation of the degree of irritation, the lack of characterization of slight irritation, and the short exposure.

Taken together these results suggest that the likelihood of a subject experiencing notable sensory irritation increases as concentrations rise above 1 ppm and that moderate irritation may occur in some individuals (although not a majority) exposed to formaldehyde in the range of 2 to 3 ppm. Although mild, transient irritation may be acceptable for certain SMAC time frames, moderate irritation is inappropriate for use as a basis for SMAC development. For this reason, studies involving exposures ≤ 1 ppm were generally viewed as being the most applicable for SMAC purposes, and these studies are described in more detail in the following section.

AC Development

The following factors should be emphasized in setting exposure guidelines based on the sensory irritation produced by formaldehyde:

- There are a tremendous number of published studies related to the irritancy of formaldehyde, and it is not surprising that some of the data do not agree. This lack of agreement may be due to different descriptions and interpretations of the intensity of sensory irritation, among other factors.
- There is a wide range of individual variability with regard to human sensitivity to formaldehyde irritation (NRC 1981, Paustenbach et al. 1997, WHO 2002), and a subset of individuals (e.g., 10% to 20% of the population) may exhibit effects at exposures that would be unnecessarily stringent for most of the population (Loomis 1979). It may not be possible to protect against mild irritant effects in all sensitive individuals, but any irritant effect that would impair crew performance should be precluded.
- Relatively few studies provide reliable irritant information on exposures to low concentrations of formaldehyde (0.1 to 0.3 ppm). In assessing exposures at these low concentrations, it is important to recognize that control groups exposed to clean air can often experience relatively high irritant response rates (5% to 20%) (Kulle et al. 1987, Paustenbach et al. 1997).
- In evaluating the relevancy of any controlled human exposure study to SMAC development for formaldehyde, the degree of irritation that is appropriate for each exposure time frame should be kept in mind. Both 1- and 24-h SMACs are useful in making informed decisions in contingency situations and may allow for transient and mild irritation and discomfort. This irritation should not be notable enough to impair crew performance. Although any sensory irritation with formaldehyde is likely to be observed well before 24 h (Andersen and Molhave 1983), 24-h ACs are generally lower than 1-h ACs to minimize the discomfort a crew member would experience for this longer exposure. The 7-, 30-, and 180-d ACs are often identical and should represent a concentration that practically all crew members could be consistently exposed to without an expectation of irritant effects.

1-h AC

Harving et al. (1990) investigated the sensory irritant effects of formaldehyde on 15 volunteers with asthma. They were exposed to formaldehyde at 0.01, 0.1, and 0.6 ppm. The authors did not report any differences in sensory irritation response among these exposure groups, indicating that the threshold for an irritant response attributable to formaldehyde for their study group was above 0.6 ppm.

Schuck et al. (1966) studied eye irritation in humans exposed for 5 min to formaldehyde at 0.01 to 1 ppm. Between 0.3 and 1 ppm, they observed a relationship between concentration and eye irritation. However, for lower exposure concentrations, the association between formaldehyde exposure and irritant effects was more uncertain. For example, the eye irritation intensity at 0.05 ppm reported by the test subjects was identical to the intensity reported at 0.5 ppm. Given that formaldehyde was generated by the photooxidation of hydrocarbons, the likelihood that other irritants were present (e.g., ethylene, peroxyacetyl nitrate) should be noted (Paustenbach et al. 1997). These compounds were not characterized during the study, but their presence may explain the inconsistencies between this study and others in the literature that did not observe irritant effects associated with similar formaldehyde exposures.

Several 2-h controlled human exposure studies from the same laboratory observed mild, transient irritation with exposure to formaldehyde at 0.4 ppm (Pazdrak et al. 1993, Krakowiak et al. 1998) in both sensitive (individuals with asthma and individuals sensitive to formaldehyde) and healthy individuals. Increased sneezing, rhinorrhea, and itching were demonstrated, as well as changes in nasal lavage fluid (increased eosinophil counts and protein), although these effects were generally mild and subsided during the 2-h exposure. A major limitation of these two studies was the lack of detailed monitoring of the formaldehyde concentrations generated in the exposure chamber. According to the authors, the chamber is calibrated only seven times annually (to an average of 0.4 ppm, with an upper range of 0.6 ppm). As calibration was not conducted on the day of the testing, there is the potential for formaldehyde levels to have exceeded the 0.4-ppm target by an unknown amount (Bender 2002), which may explain why all individuals (healthy and potentially sensitive) reported mild irritation at this exposure level, contrary to observations in other studies in the scientific literature.

Andersen and Molhave (1983) conducted a controlled human exposure study involving 16 healthy human volunteers. Exposure to 0.2, 0.4, 0.8, and 1.6 ppm of formaldehyde in air occurred over 5 h. Potential eye and upper respiratory irritant effects were evaluated, along with assessments of pulmonary function and cognitive performance. No adverse pulmonary or cognitive performance effects were observed at any concentration. With the 0.2- and 0.4-ppm exposure groups, none of the 16 volunteers reported irritation or discomfort for the first 2 h. Although subjects in these lower exposure groups reported slight eye irritation by the end of the 5-h exposure period, there is uncertainty about

whether the subjects in these groups were able to confidently distinguish irritation attributable to formaldehyde. This uncertainty is supported by the observation that the individuals reported more discomfort with the 0.2-ppm than with the 0.4-ppm exposures and that a greater percentage of the subjects reported irritation at 0.2 ppm (19%) than at 0.4 ppm (13%). As there was not a similar 5-h control exposure period, it is not possible to confidently attribute any irritation to formaldehyde at these concentrations. In contrast, slight discomfort was experienced within the first 2 h at the 0.8-ppm exposure level and above, and a relationship between dose and irritant effect can be observed for the 0.8- and 1.6-ppm exposure groups. For the 0.8-ppm exposure group, 44% (7 of 16) of the subjects reported slight discomfort, but none of the individual characterizations of the irritation at this concentration noted strong discomfort.

Kulle et al. (1987) evaluated the potential for formaldehyde exposure to result in eye and upper respiratory tract irritation as well as odors. Healthy human volunteers were exposed to formaldehyde at different concentrations for 3 h, with 19 individuals exposed at 0, 1, 2, and 3 ppm, and 10 individuals exposed at 0.5 ppm. Exposures were separated by a week to avoid carryover effects. Above 0.5 ppm, the frequency and intensity of reported irritant symptoms generally increased consistently with the higher formaldehyde exposures. None of the individuals exposed to 0.5 ppm experienced eye irritation, although roughly half of the volunteers discerned odors at this concentration. At 1 ppm, 3 of the 19 volunteers experienced mild eye irritation. One individual reported moderate eye irritation at this concentration.

Bender et al. (1983) studied human volunteers previously determined to be responsive to formaldehyde-induced eye irritation. Exposures included 0.35, 0.56, 0.7, 0.9, and 1.0 ppm, along with a control group. Only at the 1-ppm exposure were the authors able to statistically distinguish the response time for eye irritation (the metric used in this test) from background exposures. With a semiquantitative "severity index," they determined that exposures at 1 ppm on average resulted in slight to moderate irritation and any irritation response for exposures below 1 ppm was characterized as slight. The authors noted that the 0.7- and 0.9-ppm exposure groups had fewer subjects (5 to 7, compared with 27 for the 1-ppm exposure group) and that it is possible that results for these groups would also have been significant if not for this limitation. A strength of this study was its focus on potentially sensitive human subpopulations. One potential shortcoming was that the subjects were exposed to formaldehyde for only 6 min, which may not have been adequate to allow full characterization of the degree of sensory irritant response, particularly for the lower exposure groups.

Bach et al. (1990) exposed different groups of 16 human subjects to formaldehyde over 5.5 h. They observed no concentration-response relationship for sensory irritation in evaluating exposures to formaldehyde at 0, 0.12, 0.33, and 1 ppm. However, the exposure groups consisted of different populations of workers exposed to formaldehyde and control subjects, and it is not clear that other potentially important variables (e.g., smoking status, age) were properly controlled in the experimental design.

The 1-h AC is specifically based on the work of Andersen and Molhave (1983), although studies by Kulle et al. (1987) (also discussed by Kulle 1993) and Bender et al. (1983) provide additional support. The lowest-observed-adverse-effect level (LOAEL) of 0.8 ppm was not adjusted, as the degree of irritation was consistent with the intent of a 1-h SMAC.

$$\text{1-h AC}_{\text{(irriation)}} = 0.8 \text{ ppm}_{\text{(LOAEL)}}$$

24-h AC

No controlled human exposure studies were identified in which 24-h exposures were specifically evaluated for formaldehyde. However, many of the sensory irritation studies described previously are also relevant to the 24-h AC, because specific adjustments (e.g., Haber's rule) to account for differences in time are not considered to be necessary for most irritants, as effects are generally understood to be primarily concentration dependent (Paustenbach et al. 1997, Shusterman et al. 2006).

The work of Andersen and Molhave (1983) demonstrated that the maximum irritant effect for formaldehyde is likely to be observed within the first few hours of testing and that further exposures often result in a diminishing irritant response because of subject adaptation (Paustenbach et al. 1997, Bender 2002). As discussed previously, a lower 24-h limit is generally warranted to minimize discomfort that a crew member would be expected to tolerate over this longer exposure.

The Kulle et al. (1987) no-observed-adverse-effect level (NOAEL) of 0.5 ppm was used as the 24-h AC for formaldehyde. Although none of the individuals reported eye irritation at this concentration during the 3-h exposures, it is reasonable to use this concentration as a 24-h exposure limit, as it likely represents an estimate of the lower limit for eye irritation for most individuals (Kulle et al. 1987, Kulle 1993, Paustenbach et al. 1997, Bender 2002). After thoroughly reviewing the available studies, Bender (2002) concluded that it would be rare for individuals to experience sensory irritation below 0.5 ppm, and that 5-20% of individuals might report mild sensory irritation when concentrations are between 0.5 and 1 ppm. The weight of evidence suggests that the vast majority of crew members could tolerate exposure up to 0.5 ppm of formaldehyde for 24 h without experiencing any notable sensory irritation and without performance decrements or other adverse effects.

$$\text{24-h AC}_{\text{(irritation)}} = 0.5 \text{ ppm}_{\text{(NOAEL)}}$$

7-, 30-, 180-, and 1,000-d ACs

Setting longer-term ACs for formaldehyde irritation is difficult, and a number of studies have concluded that there does not appear to be a clear

threshold for effects (NRC 1981, Bender 2002) and that a small percentage of the population may exhibit mild irritation, even at extremely low exposures (e.g., 0.1 ppm). It is clear that at these low concentrations, the vast majority of human subjects have difficulty consistently being able to differentiate eye irritation induced by the presence of formaldehyde from clean air (Schuck et al. 1966, Anderson and Molhave 1983). Although it is impractical to try to identify longer-term ACs that will definitely eliminate the possibility of an irritant response in all sensitive individuals, it is necessary that the 7-, 30-, 180-, and 1,000-d ACs protect the vast majority of crew members from even slight irritation.

For the 7-, 30-, 180-, and 1,000-d ACs, a formaldehyde concentration of 0.1 ppm is being identified as a reasonable guideline for long-term exposure that is unlikely to result in any irritant effects, even with sensitive individuals. Instead of relying on one particular study, multiple lines of evidence support this AC as appropriate. This evidence, as described in more detail below, includes the findings from a number of controlled human studies, evaluations from several comprehensive scientific reviews, community health surveys, and practical NASA experience with formaldehyde in an enclosed environment designed to mimic conditions relevant to spacecraft exposures.

- NASA experiences with the Lunar-Mars Life Support Test Project. Although exposures to 0.2 ppm produced short-term irritation with one crew member by Day 15 of the 60-d test, irritation did not persist as concentrations dropped to 0.1 ppm, a concentration that was generally maintained for the remainder of the test. There are possible confounding factors with these observations, such as the fact that exposures were not limited to formaldehyde (other unidentified irritants may have been present at low levels) and the subsidence of irritation may have resulted from adaptation rather than from concentration reduction. However, monitoring for this project included other potential irritants, and none was identified at a concentration that might account for the irritant effects (although potential synergistic effects of several irritants present individually at low concentrations cannot be ruled out). Overall, this study provides practical supporting evidence for a formaldehyde AC of about 0.1 ppm. In the 90-d test (following a catalyst anomaly) formaldehyde concentrations persisted for more than 30 d at 0.05 to 0.07 ppm with no reported irritation in the test subjects.
- Benchmark dose analysis (Arts et al. 2006) of eye irritation data from Kulle (1987, 1993) and from Andersen and Molhave (1983). For both of these data sets, Arts et al. (2006) predicted that formaldehyde at 0.1 ppm would result in approximately a 1% excess risk (95% lower confidence limit on the benchmark concentration that would result in 1% excess risk [$BMCL_{01}$] of mild eye irritation or slight discomfort.
- Consistent conclusions from the U.S. Consumer Product Safety Commission (CPSC1997), WHO (2002), NRC (1981), Paustenbach et al. (1997), and

others that 0.1 ppm is a conservative lower limit for formaldehyde with regard to sensory irritation, even when addressing continuous exposure conditions.

- Results from sensory irritation studies in mice (Kane and Alarie 1977) that observed a 50% decrease in the respiratory rate (RD-50) after 10-min exposures to 3 ppm of formaldehyde in air. Alarie (1981a,b) demonstrated that multiplying the rodent RD-50 by a factor of 0.03 (the log midpoint of a range from 0.1 to 0.01) provides a reasonable estimate of a long-term exposure concentration that is protective of sensory irritation in humans. This calculation results in a formaldehyde limit of 0.1 ppm. Although this is a general predictive approach, it has merit in the sense that it avoids some of the subjectivity inherent in evaluating individual irritant response at low formaldehyde concentrations.
- Supporting evidence from community health studies (e.g., mobile home exposure evaluations) regarding the potential irritancy of formaldehyde. Although the limitations of these studies in establishing guidelines have been recognized (e.g., exposure to other irritants, general lack of control groups), they provide some useful information in support of SMAC development. For example, a strength that is particularly beneficial in setting long-term ACs for formaldehyde is that the exposures in these studies are generally continuous. Hanrahan et al. (1984) observed that only 4% of individuals continuously exposed to 0.1 ppm of formaldehyde reported eye irritation. This is an extremely low response rate and is well within the irritation frequency rates reported for clean air in controlled exposure studies (Witek et al. 1987, Bender 2002). Thus, this study provides further evidence that continuous exposure to 0.1 ppm of formaldehyde is very unlikely to result in sensory irritation, even in the presence of other potential irritants.

7-, 30-, 180-, and 1,000-d $AC_{(irritation)}$ = 0.1 ppm

Nasal Epithelial Damage and Tumors

Summary of Previous Approach

The existing 1994 SMAC document reviews the human and animal evidence with respect to the carcinogenic potential of formaldehyde and includes ACs based on risk of developing nasal tumors. These ACs (Table 12-6) were derived from the work of Kerns et al. (1983). The EPA also used this study in a 1987 risk assessment (EPA 1987) for formaldehyde that established the current inhalation unit risk factor for formaldehyde in the Integrated Risk Information System. In this study, the authors exposed 120 F344 rats, male and female, to 0, 2, 5.6, or 14.3 ppm of formaldehyde, 6 h/d, 5 d/wk for 24 mo. Male and female rats in the highest dose group (14.3 ppm) showed increased mortality compared with controls beginning in Month 12. Squamous cell carcinomas were observed in the nasal cavities of 51 of 117 male rats and in 52 of 115 female rats at

TABLE 12-6 Results of Benchmark Dose Risk Analysis Conducted by Schlosser et al. (2003) and Comparison with EPA (1987) Risk Estimate Used as Basis for Existing SMAC

Approach	Rat 95% $BMCL_{01}$, ppm[a]	Human 95% $BMCL_{01}$, ppm	Calculated Inhalation Unit Risk Factor, ppm^{-1}	Acceptable concentrations, (ppm)			
				7 d	30 d	180 d	1,000 d
Flux-DPX extrapolation							
Tumors	5.58 (Weibull)	0.71	1.4×10^{-2}	26	6	1	0.2
Cell proliferation	3.57 (power law)	0.44	2.3×10^{-2}	16	4	0.6	0.1
Direct airflow extrapolation							
Tumors	5.58 (Weibull)	0.71	1.4×10^{-2}	26	6	1	0.2
Cell proliferation	3.57 (power law)	0.46	2.2×10^{-2}	17	4	0.7	0.1
EPA (1987) estimate	Based on linearized multistage modeling	Based on linearized multistage modeling	1.6×10^{-2}	23	6	0.9	0.2

[a]Model being fit is given in parentheses below the BMCL.

Abbreviation: $BMCL_{01}$, Lower statistical confidence limit on the benchmark concentration associated with a 1% response.

14.3 ppm by the end of the experiment. At 5.6 ppm, 1 of 119 male rats and 1 of 116 female rats had squamous cell carcinomas in the nasal cavity, whereas no tumors were found in those exposed at 0 or 2 ppm. Applying linearized multistage modeling to the Kerns et al. (1983) results gives an excess lifetime inhalation cancer risk of 1.6×10^{-2}/ppm for formaldehyde (EPA 1987). Cytotoxic damage to the nasal epithelium is also discussed, although specific ACs are not set for this end point apart from the nasal cancer ACs.

The existing 1994 SMAC document also noted that there is some epidemiologic evidence that formaldehyde could cause lymphohematopoietic cancers and lung cancers, although ACs were not set based on these end points.

Review of Pertinent New Data for Nasal Epithelia Damage and Tumors

Formaldehyde is a very reactive, water-soluble compound that can damage tissues, especially mucous membranes at the portal of entry (e.g., upper respiratory tract with inhalation exposures). Although formaldehyde can be effectively metabolized following low levels of exposure, inhalation of formaldehyde at sufficient concentrations has been shown to cause cytotoxic damage and neoplastic lesions in the nasal epithelium in studies with laboratory animals and in evaluations of human exposures. This epithelial damage has been shown to exhibit regional specificity within the nasal cavity, and species dependencies have been recognized in terms of the pattern of formaldehyde distribution and damage, with rats and monkeys generally more sensitive to damage than mice (Morgan 1997, Kimbell et al. 2001). A summary of recent evaluations of formaldehyde-related cancers is provided in the sections below.

Human Epidemiologic Data

Because of the commercial importance of formaldehyde, there is a wealth of human epidemiologic studies (more than 40 case-control cohort studies, and several meta-analyses) that have assessed chronic exposures to formaldehyde and the potential for these exposures to result in the development of cancer (for a comprehensive review of available studies, see ATSDR 1999, WHO 2002). Certain limitations should be considered in evaluating occupational epidemiologic evidence. First, many of these studies involve coexposure to other air contaminants. Also, the frequency and intensity of peak formaldehyde exposures are often poorly characterized.

Given the reactivity and water solubility of formaldehyde and available evidence from laboratory animal inhalation exposures, many epidemiologic studies have focused on the incidence of upper respiratory tract cancer. Although many of them were summarized in the 1994 SMAC document, several more recent studies warrant discussion. There is occupational evidence that long-term human exposures can result in erosions and lesions in the upper respiratory tract. This cytotoxicity and the cell regeneration that accompanies it have

been implicated as important factors in the formaldehyde carcinogenic process (Conolly et al. 2003, Schlosser et al. 2003). Several epidemiologic studies have found slight histologic changes in the nasal epithelium of workers chronically exposed to formaldehyde at concentrations in the range of 0.1 to 0.6 ppm (Edling et al. 1988, Holmstrom et al. 1989, Ballarin et al. 1992, ATSDR 1999,). In an epidemiologic study of chemical plant workers, furniture workers, and a control population of office workers, Holmstrom et al. (1989) observed statistically significant histopathologic changes (cuboidal and squamous cell metaplasia, loss of cilia, goblet cell hyperplasia) in the nasal epithelium of the chemical workers (exposed to a median airborne formaldehyde concentration of 0.24 ppm, with an average duration of exposure of more than 10 years). The described effects were not observed in the office workers, where the authors reported a median formaldehyde concentration of 0.07 ppm. Formaldehyde concentrations were seasonal with this office worker group, with formaldehyde levels as high as 0.13 ppm measured for parts of the year. Short-term exposures to formaldehyde at concentrations approaching 1 ppm were reported to occur frequently with the chemical workers. They were also exposed to formaldehyde resins, which may not be directly comparable to exposures to gaseous formaldehyde.

With respect to sinonasal and nasopharyngeal cancers, whereas some studies and at least two meta-analyses reported an exposure-response relationship (Blair et al. 1990, Partanen 1993), several recent studies and reviews failed to find such a relationship (Collins et al. 1997, Coggon et al. 2003), and the evidence is somewhat equivocal (ATSDR 1999, WHO 2002). However, IARC (2004) recently upgraded its classification of formaldehyde from Group 2A to Group 1 (sufficient evidence from experimental animals and humans to conclude it is carcinogenic to humans), based to a significant degree on their opinion that there is sufficient epidemiologic evidence that formaldehyde causes nasopharyngeal cancers in humans.

Collins et al. (1997) conducted a meta-analysis of sinonasal and nasopharyngeal cancers reported in 47 occupational epidemiologic studies involving formaldehyde inhalation. They found that, although a few studies reported increased incidences of these cancers, the findings from most studies were negative. The authors concluded that these observations do not support the contention that there is a significant association between formaldehyde inhalation and sinonasal or nasopharyngeal cancers. Hauptmann et al. (2004) conducted a follow-up study of more than 25,000 U.S. industrial workers and found an association between nasopharyngeal cancers and high peak and cumulative formaldehyde exposures (but not average exposure or exposure duration). Marsh et al. (2002) evaluated 7,000 chemical plant workers and found no association between formaldehyde exposure and pharyngeal and nasopharyngeal cancers. Pinkerton et al. (2004) also did not observe an association between formaldehyde and these cancers. Similarly, Coggon et al. (2003) conducted a follow-up evaluation of a cohort of more than 14,000 chemical workers who were exposed to formaldehyde by inhalation. The authors did not find an association between

formaldehyde exposures and nasopharyngeal cancers and concluded "Overall, the epidemiologic evidence now available indicates that if formaldehyde does cause nasopharyngeal cancers, then the increased risk is small."

With regard to lung cancer, Coggon et al. (2003) reported increased incidences for their cohort (standardized mortality ratio of 1.28 for the high-exposure group), but there was no association with duration of exposure and the authors cautioned that these results needed to be further investigated (e.g., closer evaluation of confounding factors). Collins et al. (1997) found no increased incidence of lung cancer for industrial workers in the available cohorts or in the case-control studies and concluded that the available epidemiologic evidence did not support an association between formaldehyde exposure and lung cancer. Hauptmann et al. (2004) reported similar negative findings with respect to formaldehyde and lung cancer.

With respect to lymphohematopoietic cancers, Hauptmann et al. (2003) evaluated a cohort of more than 25,000 workers in formaldehyde-related industries across 10 different U.S. industrial plants. They reported relative risks for leukemia of 1.1 (95% CI = 0.4-3.2) and 2.4 (95% CI = 1-6) when average formaldehyde exposures were 0.5 to 0.9 and >1 ppm, respectively (they reported larger relative risks when grouping by peak formaldehyde exposure concentrations). However, leukemia relative risks were not positively associated with cumulative formaldehyde exposure or duration of exposure, all myeloid tumor types were lumped despite their different etiologies, and leukemia risks for the exposed workers were lower than for the U.S. population. The authors stated that these results should be viewed with caution, as the overall body of evidence for an association between formaldehyde and leukemia is mixed.

As an example, Coggon et al. (2003) did not find increased mortality from leukemia in their cohort, even in evaluating the subset of workers with the highest formaldehyde exposure. Pinkerton et al. (2004) observed an association between myeloid leukemia risks and exposure duration for U.S. garment workers exposed to formaldehyde, although the observed cancer risks were not significantly different than in the U.S. population. WHO (2002) echoed the need for caution in interpreting non-respiratory tract cancers with formaldehyde in stating "Available evidence for these tumors at sites other than the respiratory tract does not, therefore, fulfill traditional criteria of causality (e.g., consistency, biological plausibility) for associations observed in epidemiological studies."

Animal Bioassays

Rusch et al. (1983) studied the degenerative effects of formaldehyde exposure on the nasal epithelium in various species (cynomolgus monkeys, rats, mice, and hamsters). They exposed test and control animals to formaldehyde concentrations of 0.2, 1, and 3 ppm by inhalation (near-continuous 22-h exposures daily for 26 wk) in an exposure chamber. At 3 ppm, they observed mild

lesions in the nasal epithelium (squamous metaplasia) in the monkeys and rats and reported a NOAEL of 1 ppm for all species tested.

Kamata et al. (1997) also observed increased incidence of squamous cell metaplasia in the nasal epithelium of F344 rats when exposed to formaldehyde at 2 ppm for 28 mo. However, several lifetime inhalation exposure studies in rodents, with formaldehyde exposures in the range of 2 ppm, did not report adverse degenerative effects on the nasal epithelium (Woutersen et al. 1989; Monticello et al. 1991, 1996). Monticello et al. (1991) exposed F344 rats to formaldehyde at 0.7, 2, 6, 10, and 15 ppm for 6 h/d for up to 6 wk. They reported no tissue damage at 0.7 and 2 ppm but observed squamous metaplasia and epithelial hyperplasia in the three highest exposure groups. Increased rates of cell proliferation also corresponded to the cytotoxicity observed at these formaldehyde concentrations (Table 12-7).

In a chronic study, Monticello et al. (1996) exposed F344 rats to formaldehyde concentrations of 0, 0.7, 2, 6, 10, and 15 ppm for 6 h/d 5 d/wk for 24 mo. They measured rates of cell proliferation in the nasal epithelium at several interim periods during the study and found that exposures to formaldehyde at concentrations of 6 ppm or less did not result in statistically significant increases in cell proliferation, whereas increases were observed in the two highest exposure groups. They observed polyploid adenomas in the nasal cavity of rats from these two exposure groups but not in any of the other exposure groups or controls. Squamous cell carcinomas were reported in 1 of 90 rats at the 6-ppm exposure, 20 of 90 at 10 ppm, and 69 of 147 at 15 ppm, with no observed incidences in the other exposure groups or controls. These results are generally consistent with the IARC (1995) conclusion that "Acute or subacute exposure of rats to a concentration of 2 ppm appears to cause no detectable damage to the nasal epithelium and does not significantly increase rates of cell turnover. Cell turnover rates in rat nose during subchronic or chronic exposures to formaldehyde do not increase at 2 ppm, increase marginally at concentrations of 3-6 ppm and increase substantially at concentrations of 10-15 ppm. Concentration is more important than length of exposure in determining the cytotoxicity of formaldehyde."

Refinement of ACs

Nasal Cancer

In consideration of the findings of Monticello et al. (1996) and Kerns et al. (1983), the CIIT Centers for Health Research undertook an extensive risk assessment in 1999 in an effort to better characterize human cancer risks associated with inhalation of formaldehyde; the work was published in a series of papers by various authors, including Kimbell et al. (2001), Conolly et al. (2003), and Schlosser et al. (2003). Two main risk assessment approaches were taken.

TABLE 12-7 Time-Weighted, Site-Averaged Unit-Length Labeling Index Data from Schlosser et al. (2003), Derived from Original Work of Monticello et al. (1996)

Formaldehyde Exposure, ppm	ULLI, Number/Length ± Standard Deviation	Number of Animals
0	10.9 ± 3.2	48
0.7	8.2 ± 2.3	46
2	7.7 ± 2.7	47
6	15.0 ± 15.6	48
10	43.8 ± 17.6	48
15	70.7 ± 19.4	47

Abbreviation: ULLI, unit-length labeling index.
Source: Schlosser et al. 2003. Reprinted with permission; copyright 2003, *Risk Analysis*.

The first approach involved using the animal bioassay data in a benchmark dose analysis, where different methodologies were used to extrapolate formaldehyde exposures from rats to humans (see Schlosser et al. 2003). A second approach was based on computational modeling, where species-specific dosimetry of formaldehyde within the rodent and human respiratory tracts was predicted with three-dimensional anatomically accurate computational fluid dynamics (CFD) modeling (Kimbell et al. 2001, Conolly et al. 2003).

With regard to the benchmark dose analysis, a point of departure (95% $BMCL_{01}$) was established to allow assessment of human health risks based on linear extrapolation from this concentration to zero. Two different data sets, generally representing two different cancer end points, were generated to support this analysis. The first data set (tumor end point) was based on combining incidence data on squamous cell carcinoma from both Kerns et al. (1983) and Monticello et al. (1996), along with 94 additional animals that were not previously examined in the latter study. A second data set (cell proliferation end point) was generated based on the cell proliferation data (as quantified by the unit-length labeling index) reported by Monticello et al. (1996). With labeling index data, it is possible to characterize the growth kinetics of a group of targeted cells. This data set was included in recognition of the significant role that cell proliferation is thought to play in formaldehyde carcinogenesis (Schlosser et al. 2003). In utilizing these data, the assumption is made that the increase in unit-length labeling index above background equates to a corresponding increase in cancer risk.

Two different extrapolation methods were then used to estimate human health risks from the rat data. The first approach (direct airflow) used rat and human CFD models to directly estimate the flux to the entire surface of the nasal airway lining. This approach involved no fitted parameters and did not require allometric scaling between rats and humans. A second extrapolation approach was used, based on the assumption that there is a consistent relationship between

DNA-protein crosslink (DPX) formation and tumor development in rats and humans; it is known as the flux-DPX approach. This approach used CFD modeling, but also incorporated a pharmacokinetic modeling of DPX formation in rats and humans. The extrapolation approach assumed that equal amounts of DPX (used as a measure of tissue dose) in rats and humans correspond to equivalent nasal tissue concentrations of formaldehyde.

Schlosser et al. (2003) examined different statistical models for benchmark dose analysis for the tumor end point (Weibull, multistage, log-probit) and cell proliferation end point (power-law, polynomial) and selected the Weibull and power-law models as appropriate for the two end points, respectively. Benchmark concentrations (lower 95% $BMCL_{01}$ values) are presented in Table 12-6. The cell proliferation 95% $BMCL_{01}$ values were slightly lower than for the tumor end point. Given that the 95% $BMCL_{01}$ values across both end points and extrapolation methods vary by less than a factor of 2, there is considerable agreement between the approaches (Schlosser et al. 2003).

ACs were calculated based on the benchmark dose analysis results (95% $BMCL_{01}$) from Schlosser et al. (2003). The following equation, based on Crump and Howe's 1984 multistage model (with only the first stage dose related) was used to calculate the exposure concentrations (D) that would yield a tumor risk of 1×10^{-4} for exposure durations of 7, 30, 180, and 1,000 d (1- and 24-h ACs are no longer calculated for carcinogenic effects based on current NRC policy):

$$D = \frac{d \cdot (25{,}600)^k \cdot (10^{-4}/\text{risk})}{(25{,}600 - 365 \cdot \text{age})^k - [(25{,}600 - 365 \cdot \text{age}) - t]^k}$$

where
d = $BMCL_{01}$,
25,600 = number of days in a 70-y human lifetime,
k = number of stages in the model (1 in this case),
10^{-4} = acceptable risk level,
age = minimum age of an astronaut, in years (30 y in this case),
t = exposure duration, in days (7, 30, 180, and 1,000 d), and
risk = risk of tumor for lifetime exposure to d (10^{-2} in this case).

As indicated in Table 12-6, calculated ACs based on the benchmark dose analysis fall on either side but are very similar to the AC based on the original 1987 EPA risk estimate for formaldehyde. For the purposes of setting an AC for nasal carcinogenesis, the Schlosser et al. (2003) modeled results for the cell-proliferation end point were used in conjunction with the direct airflow extrapolation method. The use of the cell proliferation end point recognizes its importance in the formaldehyde carcinogenic process and results in slightly more conservative ACs compared with the tumor data. The use of the direct airflow extrapolation approach required fewer assumptions and did not rely on allometric scaling compared with the flux-DPX method and was subject to less mecha-

nistic uncertainty in terms of the exact role of DPX formation in formaldehyde-induced tumor development (Schlosser et al. 2003). This approach resulted in ACs of 17, 4, and 0.7 ppm, for the 7-, 30-, 180-, and 1,000-d time frames, respectively. These ACs are very similar to the previous ACs based on the EPA 1987 risk estimate. As described in Table 12-6, the selection of end point (tumor or cell proliferation) or extrapolation method has little real effect on the final ACs for nasal cancers, as there is considerable agreement among approaches.

These ACs based on carcinogenic effects are considerably higher than the calculated ACs based on the irritancy of formaldehyde (Table 12-8). This is consistent with the observations of Connolly et al. (2004) and Arts et al. (2006), who noted that protection against the noncarcinogenic effects of formaldehyde should be sufficient to guard against potential carcinogenic effects. The approach taken with formaldehyde in setting cancer-based ACs is also believed to be conservative, as Schlosser et al. (2003) noted that there is some uncertainty about the appropriateness of linear extrapolation from the point of departure with formaldehyde, as the dose-response for both cell proliferation (resulting from the cytotoxicity of formaldehyde) and tumor induction has been shown to be highly nonlinear.

Conolly et al. (2003) used three-dimensional CDF and clonal growth modeling to evaluate two modes of action for formaldehyde carcinogenesis: mutagenicity mediated through DPX formation and cytotoxicity-induced cell proliferation. Their work suggests that the cell proliferation mode of action is dominant with formaldehyde and that below a certain threshold (e.g., less than about 2 ppm) there is no increase in cancer risk relative to controls. Using the full computational modeling, CIIT estimated an inhalation unit risk factor of approximately 6×10^{-6}/ppm (Schlosser et al. 2003), which is at least 3 orders of magnitude less than the risk estimates based on linear extrapolation (see Table 12-6). This risk estimate is more in line with the low incidences of sinonasal and nasopharyngeal cancers observed in most epidemiologic studies of occupational exposures to formaldehyde (Schlosser et al. 2003).

Nasal Epithelial Damage

It is appropriate to set ACs for the degenerative effects of formaldehyde exposure on the nasal epithelium separate from those for nasal cancer risks. Monticello et al. (1996) exposed F344 rats to formaldehyde concentrations of 0, 0.7, 2, 6, 10, and 15 ppm, 6 h/d 5 d/wk for 24 mo. They measured rates of cell proliferation in the nasal epithelium at several interim periods during the study and found that exposures to formaldehyde at concentrations of 6 ppm or less did not result in statistically significant increases in cell proliferation, whereas increases were observed in the two highest exposure groups.

As discussed previously, the cytotoxicity of inhaled formaldehyde and resulting cell proliferation has been recognized as an important part of the car-

TABLE 12-8 Acceptable Concentrations

End Point, Exposure Data (Reference)		Uncertainty Factor					Acceptable Concentration, ppm					
		NOAEL	Time	Species	Space-flight	Inter-individual	1 h	24 h	7 d	30 d	180 d	1,000 d
Sensory irritation												
0.8 ppm (LOAEL), mild eye irritation, 5-h exposure (Andersen and Molhave 1983)	Human	1	1	1	1	1	0.8	—	—	—	—	—
0.5 ppm (NOAEL) for sensory irritation, 3-h exposure (Kulle et al. 1987)	Human	1	1	1	1	1	—	0.5	—	—	—	—
0.1 ppm (NOAEL) (James et al. 2002, Arts et al. 2006)	Human	1	1	1	1	1	—	—	0.1	0.1	0.1	0.1
Nasal tumors												
Based on linear extrapolation/application of rat 95% $BMCL_{01}$ (3.57 ppm), using cell proliferation data (Monticello et al. 1996), and direct airflow extrapolation (Schlosser et al. 2003)	F344 Rats	—	1[b]	1[a]	1	1	—	—	17	4	0.7	0.1
Nasal epithelial damage												
Benchmark dose analysis of cell proliferation data, rat 95% $BMCL_{01}$ (3.57 ppm) (Monticello et al., 1996) and direct airflow extrapolation to human 95% $BMCL_{01}$ (0.46 ppm) (Schlosser et al. 2003)	F344 rats	—	1[b]	1[a]	1	1	—	—	0.5	0.5	0.5	0.5
SMAC							*0.8*	*0.5*	*0.1*	*0.1*	*0.1*	*0.1*

Abbreviation: —, Not applicable.

[a]This study incorporated specific modeling to extrapolate between rats and humans, so additional adjustment for species differences was not necessary.

[b]Schlosser et al. (2003) adjusted the 24-mo, 6-h/d, 5-d/wk animal study to reflect continuous exposure conditions.

cinogenic process for formaldehyde (Conolly et al. 2003). Although Schlosser et al. (2003) used the cell proliferation data (as represented by the unit-length labeling index) from Monticello et al. (1996) in a cancer risk assessment framework by assuming that a given increase in cell proliferation corresponds to an equivalent increase in cancer risk, noncancer effects can also be assessed with this data set.

There are no reliable data upon which to base an AC for the degenerative effects of formaldehyde for the 1- or 24-h exposures. For these time frames, there is no evidence that damage to the nasal epithelium will occur following exposures to environmentally relevant concentrations of formaldehyde. In addition, it is worth nothing that short-term concentrations will be maintained at concentrations that will minimize the potential sensory irritant effects of formaldehyde. Thus, for these exposure times, formaldehyde ACs protective of sensory irritation should also adequately protect against any damage to the nasal epithelium.

Using the unit-length labeling index results provided in Table 12-7, Schlosser et al. (2003) calculated a rat 95% $BMCL_{01}$ of 3.57 ppm. When related to human exposures through direct airflow extrapolations (as described in the section above), a human 95% $BMCL_{01}$ of 0.46 ppm is derived. Given that Schlosser et al. (2003) incorporated detailed species extrapolation modeling, it is not necessary to incorporate an additional uncertainty factor for species extrapolation. In addition, further exposure time corrections are not necessary for the study, as the authors adjusted the 95% $BMCL_{01}$ concentration for the 24-mo study to reflect continuous exposure conditions**.** This approach is likely to be conservative, as some have suggested that increased cell proliferation is better related to concentration than to cumulative formaldehyde exposure (McGregor et al. 2006) (cytotoxicity and resulting cell proliferation are likely to exhibit a clear threshold). The nasal cancer and noncancer proliferation approaches result in slightly different long-term ACs (0.5 ppm for noncancer and 0.1 ppm for cancer ACs) because of inherent differences in risk modeling, even though the two end points are thought to be biologically related.

7-, 30-, and 1,000-d ACs = human 95% $BMCL_{01}$
= 0.46 ppm, rounded to 0.5 ppm

REFERENCES

ACGIH (American Conference of Governmental Industrial Hygienists). 1991. Formaldehyde. Pp. 664-688 in Documentation of the Threshold Limit and Biological Exposure Limits, 6th Ed. American Conference of Governmental Industrial Hygienists, Cincinnati, OH.

Alarie, Y. 1981a. Dose-response analysis in animal studies: Prediction of human responses. Environ. Health Perspect. 42:9-13.

Alarie, Y. 1981b. Bioassay for evaluating the potency of airborne sensory irritants and predicting acceptable levels of exposure in man. Food Cosmet. Toxicol. 19(5):623-626.

Andersen, I., and L. Molhave. 1983. Controlled human studies with formaldehyde. Pp. 155-165 in Formaldehyde Toxicity, J.E. Gibson, ed. Washington, DC: Hemisphere Publishing Corporation.

Arts, J.E., M.A. Rennen, and C. de Heer. 2006. Inhaled formaldehyde: Evaluation of sensory irritation in relation to carcinogenicity. Regul. Toxicol. Pharmacol. 44(2):144-160.

ATSDR (Agency for Toxic Substances and Disease Registry). 1999. Toxicological Profile for Formaldehyde. U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry, Atlanta, GA. July 1999 [online]. Available: http://www.atsdr.cdc.gov/toxprofiles/tp111.pdf [accessed Dec. 20, 2007].

Axelsson, G., C. Lutz, and R. Rylander. 1984. Exposure to solvents and outcomes of pregnancy in university laboratory employees. Br. J. Ind. Med. 41(3):305-312.

Bach, B., O.F. Pederson, and L. Molhave. 1990. Human performance during experimental formaldehyde exposure. Environ. Int. 16(2):105-113.

Ballarin, C., F. Sarto, L. Giacomelli, G.B. Bartolucci, and E. Clonfero. 1992. Micronucleated cells in nasal mucosa of formaldehyde-exposed workers. Mutat. Res. 280(1):1-7.

Bender, J. 2002. The use of noncancer endpoints as a basis for establishing a reference concentration for formaldehyde. Regul. Toxicol. Pharmacol. 35(1):23-31.

Bender, J.R., L.S. Mullin, G.J. Graepel, and W.E. Wilson. 1983. Eye irritation response of humans to formaldehyde. Am. Ind. Hyg. Assoc. J. 44(6):463-465.

Blair, A., R. Saracci, P.A. Stewart, R.B. Hayes, and C. Shy. 1990. Epidemiologic evidence on the relationship between formaldehyde and cancer. Scand. J. Work Environ. Health 16(6):381-393.

CIIT (Chemical Industry Institute of Toxicology). 1999. Formaldehyde: Hazard Characterization and Dose-Response Assessment for Carcinogenicity by the Route of Inhalation, Rev. Ed. Research Triangle Park, NC: Chemical Industry Institute of Toxicology.

Coggon, D., E.C. Harris, J. Poole, and K.T. Palmer. 2003. Extended follow-up of a cohort of British chemical workers exposed to formaldehyde. J. Natl. Cancer Inst. 95(21):1608-1615.

Collins, J.J., J.F. Acquavella, and N.A. Esmen. 1997. An updated meta-analysis of formaldehyde exposure and upper respiratory tract cancers. J. Occup. Environ. Med. 39(7):639-651.

Collins, J.J., R. Ness, R.W. Tyl, N. Krivanet, N.A. Esmen, and T.A. Hall. 2001. A review of adverse pregnancy outcomes and formaldehyde exposure in human and animal studies. Regul. Toxicol. Pharmacol. 34(1):17-34.

Conolly, R.B., J.S. Kimbell, D. Janszen, P.M. Schlosser, D. Kalisak, J. Preston, and F.J. Miller. 2003. Biologically-motivated computational modeling of formaldehyde carcinogenicity in the F344 rat. Toxicol. Sci. 75(2):432-447.

Conolly, R.B., J.S. Kimbell, D. Janszen, P.M. Schlosser, D. Kalisak, J. Preston, and F.J. Miller. 2004. Human respiratory tract cancer risks of inhaled formaldehyde: Dose-response predictions derived from biologically-motivated computational modeling of a combined rodent and human dataset. Toxicol. Sci. 82(1):279-296.

CPSC (U.S. Consumer Product Safety Commission). 1997. An Update on Formaldehyde. Washington DC: U.S. Consumer Product Safety Commission [online]. Available: http://www.cpsc.gov/CPSCPUB/PUBS/725.pdf [accessed Dec. 26, 2007].

Edling, C., H. Hellquist, and L. Ödkvist. 1988. Occupational exposure to formaldehyde and histopathological changes in the nasal mucosa. Br. J. Ind. Med. 45(11):761-765.

Egle, J.L. 1972. Retention of inhaled formaldehyde, propionaldehyde, and acrolein in the dog. Arch. Environ. Health 25(2):119-124.

EPA (U.S. Environmental Protection Agency). 1987. Assessment of Health Risks to Garment Workers and Certain Home Residents from Exposure to Formaldehyde. Office of Prevention, Pesticides and Toxic Substances, Office of Toxic Substances, U.S. Environmental Protection Agency, Washington DC.

Frigas, E., W.V. Filley, and C.E. Reed. 1984. Bronchial challenge with formaldehyde gas: Lack of bronchoconstriction in 13 patients suspected of having formaldehyde-induced asthma. Mayo Clin. Proc. 59(5):295-299.

Fujimaki, H. Y. Kurokawa, and M. Kunugita. 2004. Differential immunogenic and neurologic inflamatory response in an allergic mouse model exposed to low levels of formaldehyde. Toxicology. April 1 197(1), pp 1-13.

Gofmekler, V.A. 1968. The embryotrophic action of benzene and formaldehyde in experimental administration by inhalation. Gig. Sanit. 33(3):12-16.

Gofmekler, V.A., N.N. Pushkina, and G.N. Klevtsova. 1968. Various biochemical shifts during a study of the embryotrophic effect of benzene and formaldehyde, based on data on morphological studies. Gig. Sanit. 33(7):96-98.

Grammar, L.C., K.E. Harris, M.A. Shaughnessy, P. Sparks, G.H. Ayars, L.C. Altman, and R. Patterson. 1990. Clinical and immunologic evaluation of 37 workers exposed to gaseous formaldehyde. J. Allergy Clin. Immunol. 86(2):177-181.

Grazuleviciene, R., V. Dulskiene, and J. Vencloviene. 1998. Formaldehyde exposure and low birth weight incidence. J. Occup. Health 40:61-67.

Green, D.J., L.R. Sauder, T.J. Kulle, and R. Bascom. 1987. Acute response to 3.0 ppm formaldehyde in exercising healthy nonsmokers and asthmatics. Am. Rev. Respir. Dis. 135(6):1261-1266.

Guseva, V. 1972. Study of the gonadotropic effect in male rats exposed to the action of formaldehyde simultaneously present in the air and water [in Russian]. Gig. Sanit. 37(10):102-103.

Hanrahan, L., K.A. Dally, H.A. Anderson, M.S. Kanarek, and J. Rankin. 1984. Formaldehyde vapor in mobile homes: Cross-sectional survey of concentrations and irritant effects. Am. J. Public Health 74(9):1026-1027.

Hare, D.A., W.H. Groah, L.G. Schweer, R.L. Margosian, S.W. Abel III, and M.D. Koontz. 1996. Evaluating the Contribution of UF-Bonded Building Materials to Indoor Formaldehyde Levels in a Newly Constructed House. Presentation at 30th Annual Particle Board/Composite Materials Symposium, April 17, 1996, Pullman WA [online]. Available: http://www.ecobind.com/research/Evaluating_the_Contribution.pdf [accessed Dec. 20, 2007].

Harving, H., J. Korsgaard, O.F. Pederson, L. Molhave, and R.Dahl. 1990. Pulmonary function and bronchial reactivity in asthmatics during low-level formaldehyde exposure. Lung 168(1):15-21.

Hauptmann, M., J.H. Lubin, P.A. Stewart, R.B. Hayes, and A. Blair. 2003. Mortality from lymphohematopoietic malignancies among workers in formaldehyde industries. J. Natl. Cancer Inst. 95(21):1615-1623.

Hauptmann, M., J.H. Lubin, P.A. Stewart, R.B. Hayes, and A. Blair. 2004. Mortality from solid cancers among workers in formaldehyde industries. Am. J. Epidemiol. 159(12):1117-1130.

Health Canada. 2000. Draft Supporting Documentation for PSL2 Assessments. Human Exposure Assessment for Formaldehyde. Health Canada, Health Protection Branch, Priority Substances Section, Ottawa, Ontario. January 2000.

Health Canada. 2001. Canadian Environmental Protection Act, 1999. Priority Substances List Assessment Report-Formaldehyde. Minister of Public Works and Government Services, Ottawa, Ontario [online]. Available: http://www.ec.gc.ca/substances/ese/eng/psap/final/reports/formaldehyde.pdf [accessed Dec. 21, 2007].

Hilton, J., R.J. Dearman, D.A. Basketter, E.W. Scholes, and I. Kimber. 1996. Experimental assessment of the sensitizing properties of formaldehyde. Food Chem. Toxicol. 34(6):571-578.

Holmstrom, M., B. Wilhelmsson, and H. Hellquist. 1989. Histological changes in the nasal mucosa in rats after long-term exposure to formaldehyde and wood dust. Acta Otoloryngol. 108(3-4):274-283.

Horvath Jr., E.P., H. Anderson Jr., W.E. Pierce, L. Hanrahan, and J.D. Wendlick. 1988. Effects of formaldehyde on the mucous membranes and lungs. A study of an industrial population. JAMA 259(5):701-707.

IARC (International Agency for Research on Cancer). 1995. Formaldehyde. Pp. 217-362 in Wood Dust and Formaldehyde. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 62. International Agency for Research on Cancer, World Health Organization, Lyon, France.

IARC (International Agency for Research on Cancer). 2004. Formaldehyde (Group 1). IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 88. International Agency for Research on Cancer, World Health Organization, Lyon, France.

James, J.T., T.F. Limero, S.W. Beck, M. Martin, P.A. Covington, L. Yang, D. Lind, and J.F. Boyd. 2002. Environmental monitoring air quality. Pp. 177-191 in Isolation: NASA Experiments in Closed-Environment Living, H. Lane, R. Sauer, and D. Feeback, eds. Science and Technology Series Vol. 104. San Diego, CA: American Astronautical Society [online]. Available: http://lsda.jsc.nasa.gov/books/ground/chambers.pdf [accessed Dec. 21, 2007].

John, E.M., D.A. Savitz, and C.M. Shy. 1994. Spontaneous abortions among cosmetologists. Epidemiology 5(2):147-155.

Kamata, E., M. Nakadate, O. Uchida, Y. Ogawa, S. Suzuki, T. Kaneko, M. Saito, and Y. Kurokawa. 1997. Results of a 28-month chronic inhalation toxicity study of formaldehyde in male Fischer-344 rats. J. Toxicol. Sci. 22(3):239-254.

Kane, L.E., and Y. Alarie. 1977. Sensory irritation to formaldehyde and acrolein during singles and repeated exposures in mice. Am. Ind. Hyg. Assoc. J. 38(10):509-522.

Kerns, W.D., K.L. Pavkov, D.J. Donofrio, E.J. Gralla, and J.A. Swenberg. 1983. Carcinogenicity of formaldehyde in rats and mice after long-term inhalation exposure. Cancer Res. 43(9):4382-4392.

Kilburn, K.H. 1994. Neurobehavioral impairment and seizures from formaldehyde. Arch. Environ. Health 49(1):37-44.

Kilburn, K.H., and R.H. Warshaw. 1992. Neurobehavioral effects of formaldehyde and solvents on histology technicians; Repeated testing across time. Environ. Res. 58(2):134-136.

Kilburn, K.H., R. Warshaw, and J.C. Thornton. 1987. Formaldehyde impairs memory, equilibrium, and dexterity in histology technicians: Effects which persist for days after exposure. Arch. Environ. Health 42(2):117-120.

Kim, C.W., J.S. Song, Y.S. Ahn, S.H. Park, J.W. Park, J.H. Noh, and C.S. Hong. 2001. Occupational asthma due to formaldehyde. Yonsei Med. J. 42(4):440-445.

Kimbell, J.S., R.P. Subramaniam, E.A.Gross, P.M. Schlosser, and K.T. Morgan. 2001. Dosimetry modeling of inhaled formaldehyde: Comparisons of local flux predictions in the rat, monkey, and human nasal passages. Toxicol. Sci. 64(1):100-110.

Krakowiak, A., P. Gorski, K. Pazdrak, and U. Ruta. 1998. Airway response to formaldehyde inhalation in asthmatic subjects with suspected respiratory formaldehyde sensitization. Am. J. Ind. Med. 33(3):274-281.

Kulle, T.J. 1993. Acute odor and irritation response in healthy nonsmokers with formaldehyde exposure. Inhal. Toxicol. 5(5):323-332.

Kulle, T.J., L.R. Sauder, J.R. Heber, D.J. Green, and M.D. Chatham. 1987. Formaldehyde dose-response in healthy nonsmokers. JAPCA 37(8):919-924.

Lemiere, C., A. Desjardins, Y. Cloutier, D. Drolet, G. Perrault, A. Cartier, and J.L. Malo. 1995. Occupational asthma due to formaldehyde resin dust with and without reaction to formaldehyde gas. Eur. Respir. J. 8(5):861-865.

Loomis, T.A. 1979. Formaldehyde toxicity. Arch. Pathol. Lab. Med. 103(7):321-324.

Marsh, G.M, A.O. Youk, J.M. Buchanich, L.D. Cassidy, L.J. Lucas, N.A. Esmen, and I.M. Gathuru. 2002. Pharyngeal cancer mortality among chemical plant workers exposed to formaldehyde. Toxicol. Ind. Health 18(6):257-268.

Maronpot, R.R., R.A. Miller, W.J. Clarke, R.B. Westerberg, J.R. Decker, and O.R. Moss. 1986. Toxicity of formaldehyde vapor in B6C3F1 mice exposed for 13 weeks. Toxicology 41(3):253-266.

Martin, W.J. 1990. A teratology study of inhaled formaldehyde in the rat. Reprod. Toxicol. 4(3):237-239.

McGregor, D., H. Bolt, V. Cogliano, and H.B. Richter-Reichhelm. 2006. Formaldehyde and glutaraldehyde and nasal cytotoxicity: Case study within the context of the 2006 IPCS human framework for the analysis of a cancer mode of action for humans. Crit. Rev. Toxicol. 36(10):821-835.

Monticello, T.M., F.J. Miller, and K. Morgan. 1991. Regional increases in rat nasal epithelial cell proliferation following acute and subchronic inhalation of formaldehyde. Toxicol. Appl. Pharmacol. 111(3):409-421.

Monticello, T.M., J.A. Swenberg, E.A. Gross, J.R. Leininger, J.S. Kimbell, S. Seilkop, T.B. Starr, J.E. Gibson, and K.T. Morgan. 1996. Correlation of regional and nonlinear formaldehyde-induced nasal cancer with proliferating populations of cells. Cancer Res. 56(5):1012-1022.

Morgan, K. 1997. A brief review of formaldehyde carcinogenesis in relation to rat nasal pathology and human health risk assessment. Toxicol. Pathol. 25(3):291-307.

NAC (National Advisory Committee). 2004. Proposed Acute Exposure Guideline Levels (AEGLs) for Formaldehyde. Draft11/2004. National Advisory Committee/AEGL, U.S. Environmental Protection Agency, Washington, DC.

NASA (National Aeronautics and Space Administration). 1999. Extended Duration Orbiter Medical Project: Final Report 1989-1995. NASA/SP-1999-534. National Aeronautics and Space Administration, Johnson Space Center, Houston, TX [online]. Available: http://ntrs.nasa.gov/archive/nasa/casi.ntrs.nasa.gov/20040201524_2004207498.pdf [accessed Dec. 21, 2007].

NIOSH (National Institute for Occupational Safety and Health). 2005. NIOSH Pocket Guide to Chemical Hazards. DHHS (NIOSH) Publication No. 2005-149. National

Institute for Occupational Safety and Health, Centers for Disease Control and Prevention, U.S. Department of Health and Human Services, Cincinnati, OH [online]. Available: http://www.cdc.gov/niosh/npg/ [accessed July 15, 2008].

NIOSH (National Institute for Occupational Safety and Health). 1996. Formaldehyde. In Documentation for Immediately Dangerous to Life or Health Concentrations (IDLH): NIOSH Chemical Listing and Documentation of Revised IDLH Values (as of 3/1/95). National Institute for Occupational Safety and Health [online]. Available: http://www.cdc.gov/niosh/idlh/50000.html [accessed July 15, 2008].

NRC (National Research Council). 1981. Health effects of formaldehyde. Pp. 175-220 in Formaldehyde and Other Aldehydes. Washington, DC: National Academy Press.

NRC (National Research Council). 2007. Emergency and Continuous Exposure Guidance Levels for Selected Submarine Contaminants, Vol. 1. Washington, DC: The National Academies Press.

Partanen, T. 1993. Formaldehyde exposure and respiratory cancer- a meta-analysis of the epidemiologic evidence. Scand. J. Work Environ. Health 19(1):8-15.

Paustenbach, D., Y. Alarie, T. Kulle, N. Schachter, R. Smith, J. Swenberg, H. Witschi, and S.B. Horowitz. 1997. A recommended occupational exposure limit for formaldehyde based on irritation. J. Toxicol. Environ. Health. 50(3):217-263.

Pazdrak, K., P. Gorski, A. Krakowiak, and U. Ruta. 1993. Changes in nasal lavage fluid due to formaldehyde inhalation. Int. Arch. Occup. Environ. Health 64(7):515-519.

Pinkerton, L.E, M.J. Hein, and L.T. Stayner. 2004. Mortality among a cohort of garment workers exposed to formaldehyde: An update. Occup. Environ. Med. 61(3):193-200.

Pitten, F.A., A. Kramer, K. Herrmann, J. Bremer, and S. Koch. 2000. Formaldehyde neurotoxicity in animal experiments. Pathol. Res. Pract. 196(3):193-198.

Pross, H.F., J.H. Day, R.H. Clark, and R.E. Lees. 1987. Immunologic studies of subjects with asthma exposed to formaldehyde and urea-formaldehyde foam insulation (UFFI) off products. J. Allergy Clin. Immunol. 79(5):797-810.

Pushkina, N.N., V.A. Gofmekler, and G.N. Klevtsova. 1968. Changes in content of ascorbic acid and nucleic acids produced by benzene and formaldehyde [in Russian]. Bull. Exp. Biol. Med. 66(8):51-53.

Riedel, F., E. Hasenauer, P.J. Barth, A. Koziorowski, and C.H. Riedel. 1996. Formaldehyde exposure enhances inhalative allergic sensitization in the guinea pig. Allergy 51(2):94-96.

Rusch, G.M., J.J. Clary, W.E. Rinehart, and H.F. Bolte. 1983. A 26-week inhalation toxicity study with formaldehyde in the monkey, rat, and hamster. Toxicol. Appl. Pharmacol. 68(3):329-343.

Saillenfait, A.M., P. Bonnet, and J. de Ceaurriz. 1989. The effects of maternally inhaled formaldehyde on embryonal and foetal development in rats. Food Chem. Toxicol. 27(8):545-548.

Sauder, L.R., M.D. Chathan, D.J. Green, and T.J. Kulle. 1986. Acute pulmonary response to formaldehyde exposure in healthy nonsmokers. J. Occup. Med. 28(6):420-424.

Sax, N.I. 1984. Dangerous Properties of Industrial Materials, 6th Ed. New York: Van Nostrand Reinhold.

Schlosser, P.M., P.D. Lilly, R.B. Conolly, D.B. Janszen, and J.S. Kimbell. 2003. Benchmark dose risk assessment for formaldehyde using airflow modeling and a single compartment DNA-protein cross-line dosimetry model to estimate human equivalent doses. Risk Anal. 23(3):473-487.

Schuck, E.A., E.R. Stephens, and J.T. Middleton. 1966. Eye irritation response at low concentrations of irritants. Arch. Environ. Health 13(5):570-575.

Sheppard, D., W.L. Eschenbacher, and J. Epstein. 1984. Lack of bronchomotor response to up to 3 ppm formaldehyde in subjects with asthma. Environ Res. 35(1):133-139.

Shusterman, D., E. Matovinovic, and A. Salmon. 2006. Does Haber's law apply to human sensory irritation? Inhal. Toxicol. 18(7):457-471.

Staples, R.E. 1983. Teratology of formaldehyde. Pp. 51-59 in Formaldehyde Toxicity, J.E. Gibson, ed. New York: Hemisphere Press.

Stephens, E.R., E.F. Darley, O.C. Taylor, and W.E. Scott. 1961. Photochemical reaction products in air pollution. Int. J. Air Water Pollut. 4(1):79-100.

Tarkowski, M., and P. Gorski. 1995. Increased IgE antiovalbumin level in mice exposed to formaldehyde. Int. Arch. Allergy Immunol. 106(4):422-424.

Taskinen, H., P. Kyyrrönen, K. Hemminki, M. Hoikkala, K. Lajunen, and M.L. Lindbohm.1994. Laboratory work and pregnancy outcome. J. Occup. Med. 36(3): 311-319.

Taskinen, H.K., P. Kyyrönen, M. Sallmén, S.V. Virtanen, T.A. Liukkonen, O. Huida, M.L. Lindbohm, and A. Anttila. 1999. Reduced fertility among female wood workers exposed to formaldehyde. Am. J. Ind. Med. 36(1):206-212.

Uba, G., D. Pachorek, J. Bernstein, D.H. Garabrant, J.R. Balmes, W.E. Wright, and R.B. Amar. 1989. Prospective study of respiratory effects of formaldehyde among healthy and asthmatic medical students. Am. J. Ind. Med. 15(1):91-101.

Weber-Tschopp, A., T. Fischer, and E. Grandjean. 1977. Irritating effects of formaldehyde on men. Int. Arch. Occup. Environ. Health 39(4):207-218.

WHO (World Health Organization). 2002. Formaldehyde. Concise International Chemical Assessment Document 40. Geneva: World Health Organization [online]. Available: http://www.inchem.org/documents/cicads/cicads/cicad40.htm [accessed Dec. 26, 2007].

Witek Jr., T.J., E.N. Schachter, T. Tosun, G.J. Beck, and B.P. Leaderer. 1987. An evaluation of respiratory effects following exposure to 2.0 ppm formaldehyde in asthmatics: Lung function, symptoms, and airway reactivity. Arch. Environ. Health 42(4):230-237.

Wong, K.L. 1994. Formaldehyde. Pp. 91-120 in Spacecraft Maximum Allowable Concentrations for Selected Airborne Contaminants, Vol. 1. Washington, DC: National Academy Press.

Woutersen, R.A., L.M. Appelman, J.W. Wilmer, H.E. Falke, and V.J. Feron. 1987. Subchronic (13-week) inhalation toxicity study of formaldehyde in rats. J. Appl. Toxicol. 7(1):43-49.

Woutersen, R.A., A. van Garderen-Hoetmer, J.P. Bruijntjes, A. Zwart, and V.J. Feron. 1989. Nasal tumours in rats after severe injury to the nasal mucosa and prolonged exposure to 10 ppm formaldehyde. J. Appl. Toxicol. 9(1):39-46.

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Limonene

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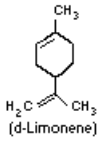
PHYSICAL AND CHEMICAL PROPERTIES

Synonym:	1-Methyl-4-(1-methylethenyl) cyclohexene; *p*-mentha-1,8-diene; enthadiene; carvene; cinene; citrus terpenes; and orange oil terpenes (NICNAS 2002)
CAS number:	5989-27-5
Molecular weight:	136.24
Boiling point:	176°C
Density:	0.84
Vapor pressure:	<3 mm Hg at 14°C
Solubility in water:	Sparingly soluble (13.8 mg/L at 25°C)
Concentration conversion:	1 ppm = 5.6 mg/m³, 1 mg/m³ = 0.18 ppm



(d-Limonene)

OCCURRENCE AND USE

Natural Occurrence and Commercial Uses

Limonene, chemically belonging to the terpene family and bearing an asymmetric carbon atom, exists in two enantiomers, *l*-limonene [also referred to as (–)-limonene or *S*-(–)-limonene] and *d*-limonene [also referred to as (+)-limonene or *R*-(+)-limonene]. *l*-Limonene has a piney and turpentine smell, whereas *d*-limonene has a pleasant lemon-like fragrance and a fresh citrus taste. The former monoterpene is present in pine oils and some trees; the latter is pre-

sent in lemon, orange, other citrus fruits, and to a lesser extent in vegetables and plants.

d-Limonene is the major constituent of lemon and orange oils; it is also present in other essential oils. Besides being naturally present in fruits, vegetables, and their products (such as orange juice, which contains 100 ppm of *d*-limonene), it is used as a flavoring agent and is found in common food items, such as ice cream (68 ppm), baked goods (120 ppm), gelatins and puddings (48 to 400 ppm), and nonalcoholic beverages (31 ppm) (NTP 1990, NICNAS 2002). *d*-Limonene is a food additive on the Food and Drug Administration's Generally Recognized as Safe List (Opdyke 1978). Consumption of *d*-limonene has been estimated to be 0.2 to 2 mg/kg body weight per day (or 14 to 140 mg/70 kg/d) (IARC 1999). Because *d*-limonene can be isolated from a large number of natural sources and has a desirable odor and taste, it is the isomer that is commercially produced and is mainly used in soap, personal hygiene products, medicinal cosmetics, and perfume, in addition to its wide use in foods and beverages. In 1976, 68,000 kg of *d*-limonene was produced and used in the United States mainly as a fragrance and flavoring agent; by 1984, the consumption increased to 254,000 kg. The use of limonene has continued to increase because consumers prefer natural and organic food additives to synthetic products. *d*-Limonene seems to have some medicinal properties. It has been shown experimentally to have protective effects against certain types of cancer and was evaluated in phase I clinical trials with advanced cancer patients (Gould 1997).

Because it has good solvent properties, relatively low toxicity, and a pleasant odor, *d*-limonene is used increasingly as an industrial solvent to replace chlorinated hydrocarbons as a remover/stripper for wax, paints, ink, and adhesives and in degreasing operations and other applications (NICNAS 2002). It has been substituted for xylene in slide preparation in many histopathology laboratories. Because of its widespread presence in botanical and commercial products and its increasing industrial uses, *d*-limonene is released into the environment from biogenic and anthropogenic sources (NICNAS 2002).

Occurrence and Use in Spacecraft

d-Limonene has been considered for use on the International Space Station (ISS) as a cleansing solvent. On the ISS, low-toxicity water-soluble solvents (especially alcohols such as ethanol and isopropanol) are used in medical applications and for hardware cleansing. These volatile, highly soluble, and low-molecular-weight compounds, which are released into the ISS air after their use, are readily removed together with water vapor by the humidity removal system as water condensate. In the ISS water purification system, the contaminants in the condensate are removed by charcoal filtration and catalytic oxidation. This water recycling system has a limited capacity for removing these water-borne organics. Thus, the ISS program has placed a restriction on the use of water-soluble volatile organic compounds (VOCs).

d-Limonene, a compound with low solubility in water and relatively low toxicity, has been proposed as a cleansing agent to reduce the use of alcohols and other water-soluble VOCs on the ISS. Replacing these compounds with the water-insoluble terpene would increase *d*-limonene concentration in the ISS atmosphere. Increased industrial applications of *d*-limonene would increase its presence in nonmetallic materials as well as increase the potential for off-gassing of limonene into the ISS atmosphere if some of these materials are used for construction of ISS components or flight hardware. Limonene has been commonly and constantly found in the ISS atmosphere. ISS air samples collected in 2003 showed limonene concentrations ranging from 0.036 to 0.13 mg/m^3 (JSC 2003); this range is similar to that (0.035 to 0.18 mg/m^3) found in the samples collected more recently (JSC 2008). The variation in limonene concentrations in the samples collected at different times during the same ISS mission increment probably reflects the extent the air was circulated through the activated charcoal bed in the ISS trace contaminant removal system.

This document has been drafted to set the airborne exposure limits of *d*-limonene on the ISS and should also provide the data needed for deciding whether to increase *d*-limonene uses as a degreaser or cleaner associated with space mission operations.

TOXICOKINETICS AND METABOLISM

Absorption, Distribution, and Excretion

d-Limonene, a small lipophilic compound, has high blood/air, oil/water, and oil/blood partition coefficients ($\lambda_{blood/air} = 42$, $\lambda_{oil/water} = 3{,}167$, $\lambda_{oil/blood} = 140$) (Falk et al. 1990); it is expected to be taken up readily from the lungs and from the gastrointestinal tract. The toxicokinetics of *d*-limonene were studied in a group of eight human volunteers engaged in light exercise and exposed for 2 h to the vapor at 10, 225, and 450 mg/m^3 (corresponding to ~2, 40, and 80 ppm) (Falk-Filipsson et al. 1993). The average pulmonary uptake of the vapor was 65%, 68%, and 68%, respectively. Measurements obtained in subjects exposed to *d*-limonene at 450 mg/m^3 showed that ~1% of the total uptake was eliminated unchanged in expired air after exposure ended, whereas ~0.003% was eliminated unchanged in the urine. The finding that very little of the compound was excreted unchanged in breath and urine indicates that the compound is extensively metabolized or avidly taken up by fat. The half-lives of the triphasic elimination of limonene from blood were 3, 30, and 750 min (Falk-Filipsson et al. 1993). When humans and laboratory animals (rat, guinea pig, hamster, rabbit, and dog) were given oral doses of *d*-[^{14}C]limonene (which was completely absorbed), 75% to 95% of the radioactivity was recovered in urine within 2 or 3 d after they ingested the compound. Most of the radioactivity was recovered in the first 24 h; less than 10% was found in feces (Kodama et al. 1976). After administration of radiolabeled *d*-limonene orally to male Wistar rats, Igimi et al. (1974)

found that radioactivity in the blood reached a maximum 2 h after the treatment. The blood concentrations remained relatively high for 10 h and then declined. A negligible amount of radioactivity was found in the blood 48 h post-treatment. Study of the distribution of radioactivity in the tissues of these rats showed that the liver had the highest concentration, followed by the kidneys and blood (fat was not examined). Autoradiograms also showed that negligible radioactivity remained in the body 48 h after ingestion of *d*-[^{14}C]limonene. These results show that only small amounts of limonene or its metabolites accumulate in the body. The recovery of radioactivity in urine, feces, and breath (as CO_2) was 60%, 5%, and 2%, respectively (Igimi et al. 1974).

Metabolism

The finding that only small amounts of absorbed airborne limonene were eliminated in the urine (0.003%) and breath (~1%) of exposed humans indicates that the compound was extensively metabolized (Falk-Filipsson et al. 1993). As mentioned above, in humans and laboratory animals given *d*-[^{14}C]limonene, 75% to 95% of the radioactivity was recovered in urine within 2 or 3 d, with most recovered in the first 24 h after dosing (Kodama et al. 1976). Depending on the animal species, the metabolism of limonene could occur by hydroxylation (or epoxidation followed by hydroxylation) of the ethenyl group, hydroxylation of the cyclohexene ring, and/or oxidation of the 1-methyl group. Most of these metabolites are further conjugated by phase II enzymes before they are eliminated in the urine (Igimi et al. 1974, Kodama et al. 1976). Kodama et al. (1976) proposed the metabolic pathway shown in Figure 13-1 based on the information of the metabolites they identified (Table 13-1). In the two human test subjects, about 30% of the administered dose was found in the urine as *d*-limonene-8,9-diol and its glucuronide; about 10% was identified as perillic acid (M-III; Figure 1). In cancer patients who received oral *d*-limonene therapy for up to 1 year, the major urinary metabolites were glucuronide conjugates of perillic acid, dihydroperillic acid, limonene-8,9-diol, and monohydroxylimonene (Vigushin et al. 1998). In male rats, some of the *d*-limonene-1,2-epoxide produced was bound reversibly to $_{2u}$-globulin, a protein produced exclusively by the livers of these rodents (Lehman-McKeeman et al. 1989).

TOXICITY SUMMARY

With its pleasant odor and taste, *d*-limonene dwarfs its levorotary isomer in industrial production and commercial uses. Toxicity studies on limonene have been conducted almost exclusively on the former compound. *d*-Limonene is ubiquitous in fruits and vegetables; it is a food additive on the Food and Drug Administration's approved list (Opdyke 1978). This natural flavoring agent has low irritancy and toxicity (Falk-Filipsson et al. 1993). For these reasons, very

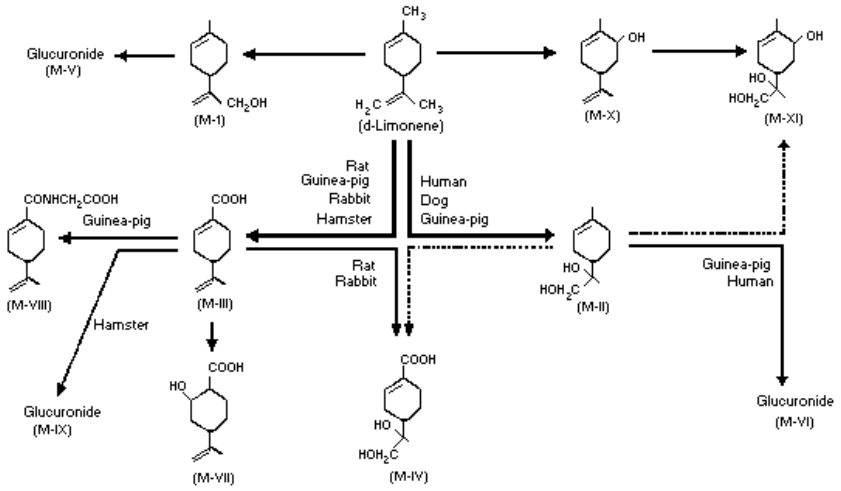


FIGURE 13-1 Major Pathways for *d*-Limonene Metabolism. Source: Kodama et al. 1976. Reprinted with permission; copyright 1976, *Xenobiotica*.

few inhalation studies have been conducted to assess the toxicity of this compound. A 2-year carcinogenesis bioassay conducted by the National Toxicology Program (NTP 1990) on rodents gavaged with doses of *d*-limonene up to 1,000 mg/kg/d showed no histopathology in female rats or both sexes of mice, but kidney lesions and neoplasms were found in male rats (Table 13-2). The lesions were found to be associated with hyaline droplets, which contained α_{2u}-globulin. Because the formation of α_{2u}-globulin in the liver is unique to the male rat and does not occur in humans, and because this protein played a crucial role in the pathogenesis and carcinogenesis of *d*-limonene, the results for kidney cancer and lesions in these animals are not considered relevant to assessment of the risk to humans of *d*-limonene exposure (IARC 1999). Table 13-3 summarizes the inhalation toxicity of *d*-Limonene, while the data on oral toxicity of this compound are summarized in Table 13-4 and 13-5.

Acute and Short-Term Toxicity Studies

Human Exposures

In a study to investigate the pulmonary uptake of inhaled *d*-limonene in humans (mentioned above), eight test subjects were exposed for 2 h on three occasions at concentrations of approximately 10, 225, and 450 mg/m³ (about 2, 40, or 80 ppm) (Falk-Filipsson et al. 1993). The subjects did not have any irritation or symptoms related to the central nervous system. A slight (~ 2%), but

TABLE 13-1 Metabolites in Urine

		Species in Which Metabolites Have Been Detected					
Number[a]	Limonene Metabolites	Rat	Guinea Pig	Hamster	Rabbit	Dog	Human
M-I	*p*-Mentha-1,8-dien-10-ol	—	X	X	X	X	X
M-II	*p*-Mentha-1-ene-8,9-diol	X	X	X	X	X*	X
M-III	Perillic acid	X	X	X	X	X	X
M-IV	Perillic acid-8,9-diol	X*	X	X	X*	X	X
M-V	*p*-Mentha-1,8-dien-10-yl-β-D-glucopyranosiduronic acid	X	X	X	X	X	X
M-VI	8-Hydroxy-*p*-menth-1-en-9-yl-β-D-glucopyranosiduronic acid	X	X*	X	X	X	X*
M-VII	2-Hydroxy-*p*-menth-8-en-7-oic acid	X	X	X	X	X	X
M-VIII	Perillylglycine	X	X	X	X	X	X
M-IX	Perillyl-β-D-glucopyranosiduronic acid	X	X	X*	X	X	X
M-X	*p*-menth-1-ene-6,8,9-triol	X	X	X	X	X	—
M-XI	*p*-Mentha-1,8-dien-6-ol	X	X	X	X	X	X

[a]Reference to structures shown in Figure 13-1.
Abbreviations: *, major metabolite; —, metabolite not found; X, metabolite found.
Source: Kodama et al. 1976. Reprinted with permission; copyright 1976, *Xenobiotica*.

statistically significant ($p < 0.01$), decrease in vital capacity measurement was noted after an exposure to *d*-limonene at the highest concentration, but no clinical symptoms were observed in the test subjects. The exposure duration was very short (total of 6 h) and was unlikely to produce lung lesions that could produce pulmonary restriction reflected clinically as reduction in vital capacity. The assessment that *d*-limonene is very unlikely to elicit lung lesions after such a short time is supported by the results of other lung function tests, including forced expiratory volumes after 1 second, residual volume, total lung capacity, peak expiratory flow, mean expiratory flow, airway resistance, and airway conductance, which showed no significant changes after the *d*-limonene exposure. Although it was statistically significant, the authors concluded that the 2% change in vital capacity had no functional significance. The decrease in forced vital capacity was not consistent with the absence of change in total lung capacity and airway conductance, which are two more reliable measures of restriction.

In a study to assess eye irritancy and odor threshold of VOCs, including *l*-limonene, released by Nordic coniferous trees that produce the characteristic wood odor (when the wood is used for building), 12 volunteers were exposed to four monoterpenes separately (Molhave et al. 2000). With goggle instrumentation, *l*-limonene was administered to the eyes for 2 min and the threshold of irritation was found to be 3,400 mg/m³ (600 ppm). The odor threshold was found to be 12 mg/m³.

d-Limonene had been tested for toxicity to determine the maximum tolerated doses in cancer patients before it was used as a therapeutic agent to suppress cancer growth on these patients. Thirty-two patients were given *d*-limonene ranging from 0.5 to 12 g/d/m^2 of body surface for 21 d or more; some were dosed for up to 1 year. The maximum tolerated dose was 8 g/d/m^2 or about 190 mg/kg/d. The toxicities were limited to gastrointestinal effects (nausea, vomiting, and diarrhea) in a dose-dependent fashion. Vigushin et al. (1998) concluded that *d*-limonene had low toxicity. In another study, therapeutic administration of 20 g of *d*-limonene orally to patients with gallstones resulted in diarrhea and painful contractions, but no changes in blood biochemical parameters (Igimi et al. 1976).

TABLE 13-2 Incidence of Kidney Lesions, Including Cancer, in Male Rats Dosed Orally with *d*-Limonene for 2 Years[a]

Lesions	0 mg/kg/d	75 mg/kg/d	150 mg/kg/d
Papilla mineralization	7/50	43/50	48/50
Papilla epithelial hyperplasia	0/50	35/50	43/50
Tubular cell hyperplasia	0/50	4/50	7/50
Tubular cell adenoma	0/50	4/50	8/50
Tubular cell adenocarcinoma	0/50	4/50	3/50

[a]*d*-Limonene in corn oil was administered by gavage 5 days per week.

TABLE 13-3 Inhalation Toxicity of *d*-Limonene

Dose, PPM	Exposure Duration	Species	Effects	Reference
Human studies				
610[a]	2 min	Human (n = 12)	Threshold of eye irritation	Molhave et al. 2000
2, 40, or 80	2 h	Human (n = 8)	No irritation No CNS effects No changes in pulmonary function	Falk-Filipsson et al. 1993
Animal studies				
≤2,421[a]	30 min	BALB/ca mouse (n = 4)	No pulmonary irritation detected	Larsen et al. 2000
≤1,600	30 min	BALB/ca mouse (n = 4)	No pulmonary irritation detected	Larsen et al. 2000
1,715[a]	30 min	BALB/ca mouse (n = 4)	RD_{50}[b]	Larsen et al. 2000
1,163	30 min	BALB/ca mouse (n = 4)	RD_{50}	Larsen et al. 2000
199[a]	30 min	BALB/ca mouse (n = 4)	RD_0[c]	Larsen et al. 2000
125	30 min	BALB/ca mouse (n = 4)	RD_0	Larsen et al. 2000
111	10-30 min	BALB/ca mouse (n = 4)	Respiratory rate depression not detected	Wolkoff et al. 2000
47	1 h	BALB/ca mouse (n = 4)	No effects on respiratory system	Rohr et al. 2002

[a]Study conducted on *l*-limonene.
[b]RD_{50}, respirable rate depression by 50% due to pulmonary irritation in the exposed animals.
[c]RD_0, no-observed-effect level for respirable irritation in the exposed animals.

TABLE 13-4 Oral Toxicity of *d*-Limonene in Rodents

Dosage, mg/kg/d	Exposure Duration[a]	Species[b]	Effects
6,000	16 d	10 rats, 10 mice	10/10 rats died, 10/20 mice died
3,000	16 d	10 rats, 10 mice	2/10 rats died, 1/20 mice died
1,650	16 d	10 rats, 10 mice	No deaths; decrease in body weight gain
825	16 d	10 rats, 10 mice	No observable effects (no histologic exam)
413	16 d	10 rats, 10 mice	No observable effects (no histologic exam)
75-1,200[c]	21 d	24 rats (12 M, 12 F)	Dose-related granules, contained α_{2u}-globulin in kidneys of male rats

(Continued)

TABLE 13-4 Continued

Dosage, mg/kg/d	Exposure Duration[a]	Species[b]	Effects
2,400	13 wk	20 rats (10 M, 10 F)	14/20 died; lethargy, excessive lacrimation nephropathy (in male rats only)
2,000	13 wk	20 mice (10 M, 10 F)	3/20 died; lethargy, excessive lacrimation nephropathy (in male rats only)
1,200	13 wk	20 rats (10 M, 10 F)	Rough hair coats, decreased activity, lethargy, excessive lacrimation nephropathy (in male rats only)
1,000	13 wk	20 mice (10 M, 10 F)	Rough hair coats and decreased activity
600	13 wk	20 rats (10 M, 10 F)	Nephropathy (in male rats only)
500	13 wk	20 mice (10 M, 10 F)	None
300	13 wk	20 rats (10 M, 10 F)	Effects (nephropathy) only in male rats
250	13 wk	20 mice (10 M, 10 F)	None
150	13 wk	20 rats (10 M, 10 F)	Effects (nephropathy) only in male rats
125	13 wk	20 mice (10 M, 10 F)	None
1,000	2 y	50 mice (F)	10% less body weight gain, no other effects
500	2 y	50 mice (F)	No effects, decrease in incidence of cytomegaly and multinucleated cells in liver
500	2 y	50 mice (M)	Increase in incidence of cytomegaly and multinucleated cells in liver
250	2 y	50 mice (M)	Decrease in incidence of cytomegaly and multinucleated cells in liver
600	2 y	50 rats (F)	5% less body weight gain, no other effects
300	2 y	50 rats (F)	No effects
150	2 y	50 rats (M)	5% less body weight gain, decrease in survival, renal lesions and tumors[c,d]
75	2 y	50 rats (M)	Decrease in survival, renal lesions and tumors[c,d]

[a]Daily doses given by gavage 5 d per week.
[b]All these NTP studies used Fischer 344 rats and B6C3F1 mice.
[c]Doses: 75, 150, 300, 600, and 1200 mg/kg/d.
[d]See details in Table 13-2.
Abbreviations: M, male; F, female.
Source: NTP 1990.

TABLE 13-5 Oral Toxicity of *d*-Limonene (Non-NTP Studies)

Dose, mg/kg/d	Exposure Duration	Species	Effects	Reference
1,000	6 mo	Dog (5 M, 5 F)	No hyaline droplets or kidney lesions, kidney weight ca. 30% higher than controls	Webb et al. 1990
100	6 mo	Dog (5 M, 5 F)	No hyaline droplets or kidney lesions	Webb et al. 1990
2,363	Gestation days 7-12	15 mice	↓ maternal body weight ↑ fetal skeletal abnormality	Kodama et al. 1977a
591	Gestation days 7-12	15 mice	No effects in dams and fetuses	Kodama et al. 1977a
1,000	Gestation days 6-18	10-18 rabbits	Some fetuses died ↓ maternal body weight	Kodama et al. 1977b
500	Gestation days 6-18	10-18 rabbits	Some fetuses died ↓ maternal body weight	Kodama et al. 1977b
250	Gestation days 6-18	10-18 rabbits	No effects in dams and fetuses	Kodama et al. 1977b

Abbreviations: M, male; F, female.

Animal Exposures

Effects of *d*-limonene on sensory irritation, pulmonary irritation, and expiratory airflow limitation were investigated in mice; the results showed that an exposure to 47 ppm of limonene for 1 h produced no measurable changes. Wolkoff et al. (2000) reported that, in BALB/c mice exposed to 111 ppm for 30 min, sensory irritation did not occur. Larsen et al. (2000) conducted a study on *d*-limonene and its enantiomer *l*-limonene by monitoring respiratory rate, tidal volume, and mid-expiratory flow rate of mice exposed to the compounds for 30 min. Pulmonary irritation and bronchoconstriction were not observed at concentrations ≤ 1,600 ppm for *d*-limonene and ≤ 2,421 ppm for *l*- limonene. The concentration that decreased respiratory rate by 50% (RD_{50}) in a 30-min exposure was estimated to be 1,163 ppm for *d*-limonene and 1,715 ppm for *l*-limonene; the no-observed-effect levels for sensory irritancy for these two limonene compounds were estimated to be 125 and 199 ppm, respectively.

In a dose-finding study on *d*-limonene for a subsequent NTP carcinogenesis bioassay, groups of 10 Fisher 344 rats and 10 B6C3F1 mice (5 of each sex) were gavaged with the compound (in corn oil) at 413, 825, 1,650, 3,000, or 6,600 mg/kg/d for 16 d (5d/wk). In the groups that received 3,300 or 6,600 mg/kg/d, 18 rats and 19 mice died. Body weight gain decreased at 1,650 mg/kg/d. No compound-related signs of toxicity were observed in animals administered <1,650 mg/kg/d (NTP 1990).

Long-Term Toxicity Studies

Long-term human exposures to limonene have not been reported. In an NTP 13-wk (5 d/wk) study, groups of 20 rats (10 males, 10 females) were gavaged with *d*-limonene at 0, 150, 300, 600, 1,200, or 2,400 mg/kg/d; groups of 20 mice (10 males, 10 females) were dosed at 0, 125, 250, 500, 1,000, or 2,000 mg/kg/d. In the 2,400-mg/kg groups, 14 rats died; in the 2,000-mg/kg groups, 3 mice died. Body weight gain decreased in a dose-related fashion in the male rats starting at 600 mg/kg/d; in male mice, the decrease was observed in the 1,000- and 2,000-mg/kg groups. Male rats administered 1,200 or 2,400 mg/kg/d showed lethargy and excessive lacrimation; lethargy was also observed in male mice treated with the two highest doses (1,000 and 2,000 mg/kg/d). The only compound-related pathologic effect noted was nephropathy in male rats (NTP 1990).

Kanerva et al. (1987) initiated a study to investigate the time and dose relationship of limonene-induced renal lesions, specifically hyaline droplet formation. Groups of five F344 male rats were given (by gavage) *d*-limonene at 75, 150, or 300 mg/kg/d for up to 4 wk (5 d/wk). The animals were killed at the end of the 1st or 4th week; at these times, an increase in liver weight was noted in the highest dose group. The increase in number of protein droplets in the kidneys of rats was dose dependent. Formation of granular casts and chronic nephrosis were found only in the rats that received 4 wk of limonene treatment. The nephropathy was found to associate with α_{2u}-globulin in the kidney.

In an effort to assess whether nephropathy is unique to male rats, Webb et al. (1990) dosed groups of 10 dogs (5 males, 5 females) with *d*-limonene at 0, 100, or 1,000 mg/kg/d; the dose was divided and given by gavage twice daily for 6 months. The body weights of the animals were not affected. In the 1,000-mg/kg/d group, the average kidney weight of the female dogs and the absolute kidney weight of male and female dogs were all about 30% higher than that of the controls. The 100-mg/kg/d group showed no effects on kidney weight. Microscopic examination of the kidneys revealed no hyaline droplet nephropathy or other lesions associated with the *d*-limonene treatment. The authors did not explain or speculate about the reasons for the kidney weight increase in the high-dose group but did point out that this high dose "is more than 10 times higher than that causes frank nephrotoxicity and significant increase in renal cancer in male rats (70 mg/kg body weight, National Toxicology Program, 1990)."

After completing its 13-wk study, NTP (1990) conducted a 2-year carcinogenesis study in which groups of 50 male rats were gavaged daily (5 d/wk) with *d*-limonene at 0, 75, or 150 mg/kg; female rats were dosed with the compound at 0, 300, or 600 mg/kg. The doses for groups of male mice were 0, 250, and 500 mg and those for female mice were 0, 500, and 1,000 mg/kg. Female mice exposed to the highest dose had 5% to 15% lower mean body weights

than their respective vehicle controls after week 28 of the study; no other compound-related clinical signs of toxicity were noted in either sex. The survival of the female rats administered 600 mg/kg was significantly lower than that of the vehicle controls. Compared with controls (8/49), the incidence of multinucleated cells in the livers of male mice was lower in the 250-mg/kg group (4/36) but higher in the 600-mg/kg group (32/50). The incidence of hepatic cytomegaly in these three groups of male mice followed the same pattern, with corresponding rates of 23/49, 11/36, and 38/50. No differences from controls were observed in treated female mice.

Similar to the findings of the NTP (1990) 13-wk study that histopathologic lesions in male rats treated with *d*-limonene were found exclusively in the kidney, results of the 2-year toxicology and carcinogenesis studies revealed kidney lesions in male rats, which were the only compound-related histopathology noted. In both dosage groups, pathologic manifestations in the male rats were deposition of mineral in the renal medulla and papilla and hyperplasia of the transitional epithelium of the papilla. Uncommon tubular cell adenomas and adenocarcinomas of the kidney were also observed in some of the rats chronically dosed with 75 or 150 mg/kg/d. Tubular cell hyperplasia and neoplasia were observed at increased incidences with positive trends in dosed male rats, which are shown in Table 13-2. Tubular cell hyperplasia, adenomas, and adenocarcinomas were part of a continuous morphologic spectrum. Hyperplasia is a proliferative lesion characterized by enlarged renal tubules with stratification of the tubular epithelium. Tubular cell adenomas are characterized by enlarged tubules and with proliferative epithelial cellular mass up to 1 cm in diameter. Adenomas consisted of relatively well-differential epithelium and exhibited solid, cystic, or papillary patterns of growth. Adenocarcinomas showed growth patterns similar to those in adenomas but generally were larger and exhibited cellular pleomorphism and anaplasia.

Carcinogenicity

As mentioned above, NTP conducted a 2-year bioassay in F344 rats (50 rats per sex per dose) and B6C3F1 mice gavaged with *d*-limonene. Doses as high as 500 mg/kg/d in female rats and 1,000 mg/kg/d in male and female mice did not increase cancer incidences in these rodents. The incidence of neoplasms of the anterior pituitary gland in high-dose female mice was lower than that in the vehicle-control mice (adenomas and carcinomas combined: vehicle control, 12/49; high dose, 2/48). These results are consistent with tumor suppression activity of *d*-limonene observed by others (Crowell et al. 1994). However, male rats dosed with 75 or 150 mg/kg/d had uncommon forms of tubular cell adenomas and adenocarcinomas of the kidney, which are summarized in Table 13-2. No chemical-related increases in other forms of cancer were observed.

Mechanism of Limonene-Induced Renal Pathogenesis and Carcinogenesis in Male Rats, and the Irrelevancy of the Renal Lesions of These Animals for Assessment of Human Risk from Limonene Exposures

Several studies were launched to investigate the mechanism of pathogenesis after NTP found that limonene caused kidney cancer and other renal lesions in male rats. One of these studies was conducted by NTP (1990), in which male and female F344/N rats were administered *d*-limonene at 75 to 1,200 mg/kg for 21 d. Microscopic examination of kidney sections from these rats indicated a compound-related increase in intracytoplasmic granules in the proximal convoluted tubules of dosed male rats but not of female rats. Immunohistochemical staining of the kidney tissue revealed that the granules contained α_{2u}-globulin. An enzyme-linked immunosorbent assay also showed that the amount of α_{2u}-globulin increased in kidney homogenates from *d*-limonene-dosed male rats. These results established the links between the male unique α_{2u}-globulin and nephropathy.

Tsuji et al. (1975) investigated nephrotoxicity of *d*-limonene in another strain of rats. Male and female Sprague-Dawley rats were gavaged with *d*-limonene at 277, 554, or 1,385 mg/kg/d daily for 6 months. Like that in the results with F344 rats in the NTP study, nephropathy was observed in the male but not in the female rats similarly exposed. The kidney lesions also consisted of granular casts characteristic of α_{2u}-globulin.

The association of *d*-limonene with α_{2u}-globulin in the kidney of exposed rats was investigated in a study in which Sprague-Dawley rats were exposed to radiolabeled *d*-limonene (Lehman-McKeeman et al. 1989). About 40% and 5% of the radioactivity found in the kidneys of males and females, respectively, bound to proteins. The kidney protein that contained the radioactivity in male rats was identified as α_{2u}-globulin, and 82% of the radiolabeled moiety was associated with *d*-limonene-1,2-epoxide. No α_{2u}-globulin-radiolabeled complex was detected in kidneys of *d*-limonene-exposed female rats.

α_{2u}-Globulin is synthesized under androgenic control in the liver of mature male rats, secreted into the blood, and excreted in large amounts in urine. This small-molecular-weight protein is found in minute quantities in female rats. In the acute stage of male-rat-specific and chemical-induced nephropathy, accumulations of protein droplets (consisting chiefly of α_{2u}-globulin) were found in the lysosomes of cells in the P_2 segment of the proximal convoluted tubules. As discussed above, in male rats, *d*-limonene-1,2-epoxide binds reversibly to this protein (Lehman-McKeeman et al. 1989). The α_{2u}-globulin bound to *d*-limonene-1,2-epoxide resists degradation by lysosomal enzymes, resulting in an accumulation of α_{2u}-globulin-limonene-derived complex. The accumulated protein complex caused tubular cell degeneration and necrosis with some degree of cell regeneration (Saito et al. 1991).

d-Limonene pathogenesis in the kidneys of male rats was further investigated by Dietrich and Swenberg (1991), who used male F344 rats and α_{2u}-

globulin-deficient NCI-NBR rats. The rats, pretreated with 0 or 500 ppm of a preneoplastic initiator (*N*-ethyl-*N*-hydroxyethylnitrosamine) in drinking water for 2 wk, were gavaged with *d*-limonene at 0 or 150 mg/kg/d for 30 wk (5 d/wk). A 5-fold increase in DNA labeling was observed in the P2 cells of the kidneys of the *d*-limonene-treated F344 rats, but no increase was observed in the *d*-limonene-treated NBR rats. A 10-fold increase in renal adenomas and atypical hyperplasia was found in the initiator-pretreated F344 rats that were dosed with *d*-limonene, but no increase was observed in the NBR rats treated with both the initiator and promotor. It is noteworthy that the incidence of nitrosamine-induced liver cancer in F344 rats decreased with *d*-limonene treatment (54.8% versus 86.7% treated with corn oil), which is consistent with the tumor suppression activity observed by others (Crowell et al. 1994). The findings that *d*-limonene promoted preneoplastic lesions and renal tumors only in the presence of α_{2u}-globulin and that this protein-limonene metabolite complex caused both the cytotoxic and the carcinogenic response in male rats led Dietrich and Swenberg (1991) to conclude that extrapolation of *d*-limonene carcinogenicity data from rat studies to humans is probably not warranted.

Another piece of data reported by Lehman-McKeeman and Caudill (1992) supports the conclusion that carcinogenesis induced by *d*-limonene in male rats is not relevant to humans for assessing risk of exposures to *d*-limonene. They found that *d*-limonen-1,2-oxide, a putative metabolite, binds to α_{2u}-globulin (a lipocalin specifically synthesized in livers of male rats) but not to other lipocalin proteins found in other species, including human-derived α1-acid glycoprotein and human protein 1.

The International Agency for Research on Cancer Working Group concluded that *d*-limonene produces renal tubular tumors in male rats by a non-DNA-reactive mechanism, through an α_{2u}-globulin-associated response. Therefore, the mechanism by which *d*-limonene increases the incidence of renal tubular tumors in male rats is not relevant to humans. *d*-Limonene is therefore not classifiable as carcinogenic to humans (IARC 1999). The Risk Assessment Forum of the U.S. Environmental Protection Agency reached a similar conclusion that α_{2u}-globulin-induced nephropathy in male rats would not be an appropriate end point to determine noncancer effects potentially occurring in humans (EPA 1991). The data on nephropathy and cancer in limonene-exposed male rats will not be used for setting exposure limits.

Genetic Toxicology

d-Limonene, tested at concentrations from 0.3 to 3,333 μg/plate, was found not to be mutagenic in four strains of *Salmonella typhimurium* (TA 98, TA 100, TA 1535, and TA 1537) in the presence or absence of liver metabolic enzymes (S9). Testing concentrations of *d*-limonene from 10 to 80 μL/mL produced negative results in a mouse L5178Y/TK$^{+/-}$ assay. At concentrations up to 100 μL/mL, the compound did not induce sister-chromatid exchanges in the

absence or presence of liver microsomes. Chromosomal aberrations were not observed in cultured Chinese hamster ovary cells treated with *d*-limonene at concentrations up to 162 μL/mL (NTP 1990).

Developmental and Reproductive Toxicity

Two developmental studies were identified, as described below; however, no reproductive toxicity studies were found.

In a developmental toxicity study of *d*-limonene in ICR mice, groups of 15 pregnant dams were gavaged with the compound at 0, 591, or 2,363 mg/kg/d on gestation days 7 to 12 (Kodama et al. 1977a). The high dose caused a significant decrease in maternal body weight; it also caused a significant increase in the number of fetuses with skeletal abnormalities, including lumbar ribs, fused ribs, and delayed ossification of several bones in the paws. No maternal or fetal effects were observed at the low dose.

Kodama et al. (1977b) also conducted a study on groups of 10 to 18 pregnant Japanese white rabbits gavaged with *d*-limonene at 0, 250, 500, or 1,000 mg/kg/d on gestation days 6 to 18 (Kodama et al. 1977b). The dams that received the two highest doses had significant reductions in food consumption and body weight; death occurred in the group that received the highest dose. Developmental toxicity in the litters was not observed at any dose.

Allergenicity

To demonstrate that the allergenicity of limonene is due to the oxidative products of limonene, Karlberg et al. (1992) stirred *d*-limonene (96% pure) in open air for 1 h four times daily for 2 to 4 h and up to 2 to 4 months to induce oxidative products (only 40% *d*-limonene was left after 2.5 months). Air-unexposed and air-exposed *d*-limonene together with *d*-limonene oxide were tested for skin allergenicity on guinea pigs; only *d*-limonene that had not been exposed to air was shown to be void of allergenicity. A skin sensitization test of *d*-limonene conducted on 25 volunteers was negative (Grief 1967). Two of 470 patients showed a positive response to a skin patch test carried out in Australia in 1999 (Fewings, personal communication, 2001, as cited in NICNAS 2002). A case report exists on asthma in subjects exposed to perfume containing *d*-limonene (Jensen and Petersen 1991). These data are insufficient to establish that d-*d*-limonene, per se, is an allergen and to quantitate a dose response; therefore the data will not be used to calculate an acceptable concentration (AC).

Effects on the Immune System

The effects of *d*-limonene on the immune system of BALB/c mice were studied by Evans et al. (1987). They prepared the material in buffered saline as an emulsion and made a series of dilutions to attain a dilution as low as 1:16,384

(no concentrations were given; doses were expressed as dilutions). At a dilution of 1:16,384 and with a daily dose of 0.1 mL per mouse, lipopolysaccharide-induced proliferation of spleen cells in mice was significantly suppressed after 4 wk of treatment, but proliferation increased significantly after 8 wk of treatment. This dilution also produced significant effects on concanavalin A responses, and on phytohemagglutinin-induced proliferation. It could be calculated that mice dosed with *d*-limonene at this dilution would receive 0.006 mg (or 6 μg) per mouse or a *d*-limonene concentration of 0.2 mg/kg/d. This dosage of *d*-limonene would be equivalent to a human drinking half a cup of orange juice daily. It seems unlikely that such a small amount of this low-toxicity compound has such immunologic effects in mice. Before conducting their immunologic study, Evans et al (1987) conducted a dose-finding experiment for the study and reported a 50% lethal dose (LD_{50}) of "0.080 mg *d*-limonene/kg (corrected for 82% purity)." As stated above, the International Agency for Research on Cancer (IARC 1999) estimated human consumption of *d*-limonene to be 0.2 to 2 mg/kg/d (or 14 to 140 mg/70 kg/d); these values are greater than the lethal dose in Evans's study. Vigushin et al. (1998) reported that cancer patients tolerated *d*-limonene at 189 mg/kg/d and showed no symptoms of toxicity. This human therapeutic dosage is 2,368 times higher than the mouse LD_{50} reported by Evan et al. In an NTP study involving large numbers of mice, no deaths or other sign of toxicity, other than decreased body weight gain, were observed in B6C3F1 mice exposed to *d*-limonene at 1,600 mg/kg/d for 16 days (NTP 1990). The dose in this NTP mouse study is 20,000 times higher than the Evans mouse LD_{50}. The data of Evans are not consistent with the findings of others and should not be considered for setting exposure limits.

RATIONALE FOR ACCEPTABLE VALUES

The American Industrial Hygiene Association (AIHA 1993) recommends a Workplace Environmental Exposure Level (WEEL) for *d*-limonene of 30 ppm. In providing the rationale for reaching this WEEL value, AIHA states that "in the 2-year NTP study, liver effects were noted in male mice at 500 mg/kg, and reduced survival was noted in female rats at 600 mg/kg. The no observable effect levels (NOELs) were 250 and 300 mg/kg, respectively. A WEEL of 30 ppm (165.6 mg/m^3) as an 8-h time-weighted average (TWA) is recommended to protect against these effects." No information is provided about how the WEEL, a recommended airborne concentration for humans, was derived from the rodent oral NOELs.

The Threshold Limit Value (TLV) Committee of the American Conference of Industrial Hygienists Association (ACGIH) has not set an occupational exposure limit on *d*-limonene. However, for sensory irritants, ACGIH recommends occupational exposure limits of $0.03 \times RD_{50}$ based on mouse data (Caldwell 2002). This approach was initially proposed by Alarie (1981); he and his colleagues reported an RD_{50} for limonene in mice of 1,163 ppm, as previously

discussed (Larsen et al., 2000), and proposed a TLV of 30 to 45 ppm, based on $0.03 \times RD_{50}$.

Several Nordic countries have occupational exposure limits on limonene of 25 to 75 ppm (see Table 13-6), but no rationales for these values could be found.

RATIONALE FOR SMAC VALUES

As mentioned above, *d*-limonene has a pleasant odor and dwarfs its levorotary isomer in the amount of industrial production and number of uses. If limonene is found in the spacecraft atmosphere, it is most likely to be the dextrorotary isomer. Because toxicity studies on limonene have been conducted almost exclusively on *d*-limonene, the exposure limits, based on these data, are also for *d*-limonene.

As also discussed above, *d*-limonene is one of the few compounds shown to induce a unique syndrome of nephropathy in male rats after subchronic or chronic exposure. After reviewing the literature on these effects of limonene and other hydrocarbons (EPA 1991), EPA's Risk Assessment Forum concluded that nephropathy in male rats associated with α_{2u}-globulin accumulation in hyaline droplets is not an appropriate end point to determine potential effects occurring in humans exposed to limonene. Therefore, the results for male rats would not be considered in setting exposure limits. Spacecraft maximum allowable concentrations (SMACs) are derived by using relevant toxicologic data and following the guidelines developed by the Subcommittee on Guidelines for Developing Spacecraft Maximum Allowable Concentrations for Space Station Contaminants of the Committee on Toxicology (NRC 1992). The irritation study (Falk-Filipsson et al. 1993) conducted in humans exposed to limonene for 2 h was the longest inhalation study on limonene and will be used to set the ACs for all lengths of time under consideration (1 h, 24 h, 7 d, 30 d, 180 d, and 1,000 d). Because there are no long inhalation exposure studies, the data on 13 wk and 2 yr histopathology gavage studies in rodents will be used to set ACs for exposures of 7 d and longer. The SMACs (Table 13-7) are chosen from the lowest AC at each time point summarized in Table 13-8.

ACs Based on Short-Term Studies of Eye and Pulmonary Irritation and Human Test Subjects

When eight human subjects engaged in light activity were exposed to *d*-limonene at concentrations up to 80 ppm (450 mg/m³) for 2 h, no irritation or symptoms related to the central nervous system were noted. No changes in pulmonary function variables were detected (Falk-Filipsson et al. 1993). If limonene were to cause irritation, it would be the nonreactive type (Alarie, personal communication, 2004). Nonreactive irritation generally causes an effect that is

TABLE 13-6 Limonene Occupational Exposure Limits Set, Recommended, or Proposed by Other Organizations

Organization/Agency	Limits, ppm	Remarks	Reference
American Industrial Hygiene Association	30 (168 mg/m^3)	8-h WEEL TWA[a]	AIHA 1993
Swedish National Board of Occupational Safety	25 (150 mg/m^3) 50 (300 mg/m^3)	8 hr TWA STEL[b]	SWEA 2005
Danish Environmental Protection Agency	75	Occupational exposure limit (tentative)	Madsen et al. 2001
Finnish Institute of Occupational Health	25	8-h TWA	NICNAS 2002
Norway	25	8-h TWA	RTECS 2006

[a]TWA, 8 h/d, 40 h/wk.
[b]TWA for 15 min.

TABLE 13-7 Spacecraft Maximum Allowable Concentrations for Limonene[a]

Duration	ppm	mg/m^3	Target Toxicity
1 h	80	450	Eye and pulmonary irritation
24 h	80	450	Eye and pulmonary irritation
7 d	20	115	Eye and pulmonary irritation
30 d	20	115	Eye and pulmonary irritation
180 d	20	115	Eye and pulmonary irritation
1,000 d	20	115	Eye and pulmonary irritation

[a]Chosen from the lowest AC at each time point summarized in Table 13-8.

relatively independent of exposure duration. According to Alarie, the maximum response to a nonreactive irritant will occur within the first 10 to 30 min of exposure (Nielson and Alarie 1982). Once this maximum is reached, the degree of irritation response is either maintained at this level or it gradually fades. This observation has been reported for many volatile compounds, including substituted benzenes, alcohols, and pinenes tested by Alarie's group (Kane et al. 1980, Nielson and Alarie 1982, Kasanen et al. 1998); especially relevant to the assessment of limonene is their study on pinenes. Pinenes, like limonene, are cyclic monoterpenes. Falk-Filipsson showed that a 2-h exposure to 80 ppm of *d*-limonene did not cause irritation to the eyes and lungs, so it is valid to conclude that this concentration would not cause irritation at any given length of exposure. Therefore, 80 ppm is recommended as the AC for 1 and 24 h. However, the study was conducted in only eight human subjects; it is possible that sensi-

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TABLE 13-8 Acceptable Concentrations and Proposed SMACs for Limonene

Toxicity End Points	Exposure Data	Safety Factor for Exposure Extrapolation			Acceptable Concentration, ppm					
		Species	Time	Small size sample (n)	1 h	24 h	7 d	30 d	180 d	1,000 d
No irritation in human study[a]	8 human subjects exposed to 450 mg/m^3 or 80 ppm experienced no irritation	1	1	√(100/n)	80	80	20	20	20	20
NOAEL in NTP's 13-wk toxicity study[b]	Rats (10 females) and mice (10 males, 10 females) dosed at 500 and 600 mg/kg/d for 13 wk showed no clinical signs or histopathology. The dose of 500 mg/kg/d is equivalent to an acceptable daily inhalation concentration of ~40 ppm for humans.	10	1 or 2[c]	--	--	--	40	40	20	--
NOAEL in NTP's 2-year toxicity and carcino-genesis bioassay.[b]	Mice (male and female; 50/group) dosed for 2 years with 250 or 300 mg/kg/d showed no clinical signs or organ toxicity. A dose of 250 mg/kg/d is equivalent to an acceptable daily inhalation concentration of ~20 ppm for humans.	10	1	--	--	--	--	--	20	20
SMACs[d]					80	80	20	20	20	20

[a]Study of Falk-Filipsson et al (1993).
[b]Study of NTP (1990).
[c]See text for details.
[d]Based on lowest acceptable concentrations.
Abbreviation: NOAEL, no-observed-adverse-effect level; --, not calculated.

tive individuals could find 80 ppm mildly irritating. Although mild irritation is acceptable for up to 24 h, for longer exposure durations (7, 30, and 180 d), it is necessary to ensure that limonene at the AC will not be irritating to essentially all crewmembers. According to the NRC guidelines (NRC 2000), a NOAEL for a large population could be derived from the NOAEL from a study involving a small number (N) of test subjects by incorporating a factor of √(100/N) (NRC 2000). Therefore, the acceptable limit of 20 ppm for long-term (7, 30, and 180 d) exposure is obtained as shown below.

1- and 24-h $AC_{(\text{eye and pulmonary irritation})} = 80 \text{ ppm}_{(\text{NOAEL})}$

7-, 30-, and 180-d $AC_{(\text{eye and pulmonary irritation})} = 80 \text{ ppm}_{(\text{NOAEL})} \div \sqrt{(100/8)}_{(\text{small n factor})} = 22.6$ ppm, rounded to 20 ppm

7-, 30-, and 180-d ACs Based on No-Observed-Adverse-Effect Level of NTP 13-wk Studies

In the 13-wk NTP study (NTP 1990), at a dose of 1,200 mg/kg/d, *d*-limonene caused a decrease in activity, lethargy, and excessive lacrimation in rats. In mice, rough hair coat and decreased activity were observed at 1,000 mg/kg/d. Doses of 500 and 600 mg/kg/d produced no symptoms, thus, 500 mg/kg/d (the lower of these two doses) is considered a no-observed-adverse-effect-level (NOAEL). In order to extrapolate toxicity data from rodent to human, a species-extrapolating factor of 10 is applied, making the NOAEL for humans 50 mg/kg/d. For a 70-kg person, this dose would be equivalent to 3,500 mg/d.

Thus,

500 mg/kg/d (NOAEL) × 1/10 (species factor) × 70 kg = 3,500 mg/d.

In order to use this 13-wk NTP gavage study data to extrapolate equivalent risk of inhalation exposure, two assumptions are made. The first is that a person would inhale 20 m^3 of air per day (NRC 1992) and the second is that limonene uptake in the lung is 76%. This second assumption is based on results of the study by Falk-Filipsson et al. (1993), who reported that when two groups (eight per group) of human subjects were exposed to *d*-limonene at concentrations of 450 and 225 mg/m^3, the pulmonary uptake for each group was 68% ± 4% (Mean + SD). The upper value of 76% (68% + 2 × SD or 68% + 8%), an estimate of the 95^{th} percentile of the pulmonary uptake distribution, was used to calculate the AC. Thus, in order for a person to absorb 3,500 mg of limonene per day (human NOAEL), he or she would have to breathe air containing limonene at 230 mg/m^3 for 24 h.

The AC for 7 and 30 d is set at 230 mg/m^3 or 40 ppm; the AC for 180 d is set at 115 mg/m^3 or 20 ppm by applying a time factor of 2. The calculation is shown below:

$$3{,}500 \text{ mg/d}_{(\text{NOAEL})} = 230 \text{ mg/m}^3{}_{(\text{equiv. inhal. conc.})} \times 20 \text{ m}^3\text{/d}_{(\text{air inhal. rate})} \times 76\%_{(\text{pulmonary uptake})}$$

$$\text{7- and 30-d AC}_{(\text{eye and pulmonary irritation})} = 230 \text{ mg/m}^3{}_{(\text{equiv. inhal. conc.})} \times (0.18 \text{ ppm} \div 1 \text{ mg/ m}^3)_{(\text{conversion})} = 41.4\text{ppm, rounded to 40 ppm}$$

Because the NTP data came from a 13-wk (90-d) study, the AC for 180 d is obtained by applying a time-extrapolating factor of 2.

$$\text{180-d AC}_{(\text{eye and pulmonary irritation})} = 230 \text{ mg/m}^3{}_{(\text{equiv. inhal. conc.})} \div 2_{(\text{time extrapolation factor})} \times (0.18 \text{ ppm} \div 1 \text{ mg/ m}^3)_{(\text{conversion})} = 20.7 \text{ ppm, rounded to 20 ppm}$$

In the NTP studies, rodents were gavaged with bolus doses. As mentioned above, when humans and laboratory animals (rat, guinea pig, hamster, rabbit, and dog) were given oral doses of *d*-[^{14}C]limonene, it was completely absorbed (Kodama et al. 1976). In a human inhalation study, the average pulmonary uptake of *d*-limonene vapor was 68%. Generally, systemic effects produced by a chemical given by bolus dose (in gavage) are more marked than those produced by an equivalent dose absorbed gradually from an inhalation exposure. In other words, an oral dose (*x* mg/kg) would likely be more toxic than an equivalent dose (*x* mg/kg) given by inhalation exposure. Therefore, the 3,500 mg/d of limonene absorbed into the body from inhalation exposure will have lower toxicity than that given by gavage, and extrapolation of a gavaged dose to an inhalation dose provides another safety margin.

Similarly, at an equivalent amount of chemical absorbed from an exposure, a short-term exposure to a high concentration generally poses a greater toxicologic risk than a longer exposure to a lower concentration. Haber's rule ($c \times t = k$) is used to extrapolate data from a shorter exposure to a longer exposure but not the other way around.

AC for 180-d Based on NOAEL of the NTP 2-Year Toxicology and Carcinogenesis Studies

The noncarcinogenic results of the NTP 2-year toxicology and carcinogenesis studies showed that three groups of 50 male mice dosed with *d*-limonene at 0, 250, and 500 mg/kg/d and female mice dosed with *d*-limonene at 0, 500, and 1,000 mg/kg all had no compound-related clinical signs of toxicity. After week 28 of the study, the high-dose group of female mice had 5% to 15% lower mean body weights than the control group. In male mice, the incidence of multi-

nucleated cells in the livers for the control, low-dose, and high-dose groups was 8/49, 4/36, and 32/50, respectively; the incidence of liver cytomegaly in these three groups followed the same pattern, with corresponding rates of 23/49, 11/36, and 38/50. Because the low-dose group showed less effect than the control group, a benchmark dose approach was not used. Therefore, the NOAEL approach is used for AC setting. The mouse dose of 250 mg/kg/d was the NOAEL from the study. Applying a rodent-to-human extrapolation factor of 10 would provide an acceptable human oral exposure dose of 25 mg/kg/d (1,750 mg/70kg/d). Using an approach similar to the one used above for the 13-wk NTP study (pulmonary uptake of 76% and daily inhalation of 20 m^3), we can calculate that a person breathing air containing 115 mg/m^3 of limonene will absorb 1,750 mg of limonene per day. The equivalent inhalation exposure concentration of 115 mg/m^3 or 20 ppm is the AC for 180 d; the calculation is shown below:

$$1{,}750 \text{ mg/d}_{(\text{NOAEL})} = 115 \text{ mg/m}^3{}_{(\text{equiv. inhal. conc.})} \times 20 \text{ m}^3\text{/d}_{(\text{air inhal. rate})} \times 76\%_{(\text{pulmonary uptake})}$$

$$180\text{-d AC}_{(\text{carcinogenicity})} = 115 \text{ mg/m}^3{}_{(\text{equiv. inhal. conc.})} \times (0.18 \text{ ppm} \div 1 \text{ mg/ m}^3)_{(\text{conversion})} = 20.7 \text{ ppm, rounded to 20 ppm}$$

AC for 1,000-d Based on NOAEL of the NTP 2-Year Toxicology and Carcinogenesis Studies

As discussed in the section on long-term toxicity studies and the preceding section, when groups of mice were gavaged with *d*-limonene at daily doses of 0, 250, and 500 mg/kg for 2 years, the incidence of multinucleated cells was 8/49, 4/36, and 32/50, respectively. The incidence of cytomegaly in these three groups followed the same non-dose-dependent pattern, with corresponding rates of 23/49, 11/36, and 38/50. No similar lesions were observed in rats exposed to 300 or 600 mg/kg/d. The chronic treatment dose of 250 mg/kg/d is considered a NOAEL. An acceptable human oral exposure dose of 25 mg/kg/d (1,750 mg/70kg/d) is obtained after applying a rodent-to-human extrapolation factor of 10. As calculated above, 25 mg/kg/d is equivalent to a daily inhalation exposure concentration of 115 mg/m^3 or 20 ppm, which is chosen as the AC for 1,000 d. The 2 years (730 days) in the rodent bioassay study is about the lifetime of the mice and rats. For using data from the lifetime bioassay for setting a 1,000-d SMAC, the NRC SMAC committee agrees that no time factor would be needed.

REFERENCES

AIHA (American Industrial Hygiene Association). 1993. Workplace Environmental Exposure Level Guides on d-Limonene. American Industrial Hygiene Association, Fairfax, VA.

Alarie, Y. 1981. Dose-response analysis in animal studies: Prediction of human responses. Environ. Health Perspect. 42:9-13.

Caldwell, D.J. 2002. Current Issues of Interest to the TLV-Chemical Substances Committee. Forum 214: TLV Chemical Substances Committee: The Process for Decision Making, American Industrial Hygiene Conference and Exposition, June 1-6, 2002, San Diego, CA [online]. Available: http://www.acgih.org/tlv/CurrentIssuesTLVCS Comm.pps [accessed Oct. 7, 2004].

Crowell, P.L., C.E. Elson, H.H. Bailey, J.A. Elegbede, J.D. Haag, and M.N. Gould. 1994. Human metabolism of experimental cancer therapeutic agent d-limonene. Cancer Chemother. Pharmacol. 35(1):31-37.

Dietrich, D.R., and J.A. Swenberg. 1991. NCI-Black-Reiter (NBR) male rats fail to develop renal disease following exposure to agents that induce alpha$_{2u}$-globulin nephropathy. Fundam. Appl. Toxicol. 16(4):749-762.

EPA (U.S. Environmental protection Agency). 1991. Alpha2u-Globulin: Association with Chemically-Induced Renal Toxicity and Neoplasia in the Male Rat. EPA/625/3-91/019F. Office of Health and Environmental Assessment, U.S. Environmental Protection Agency, Washington, DC.

Evans, D.L., D.M. Miller, K.L. Jacobsen, and P.B. Bush. 1987. Modulation of immune responses in mice by d-limonene. J. Toxicol. Environ. Health 20(1-2):51-66.

Falk, A., E. Gullstrand, A. Löf, and E. Wigaeus-Hjelm. 1990. Liquid/air partition coefficients of four terpenes. Br. J. Ind. Med. 47(1):62-64.

Falk-Filipsson, A., A. Löf, M. Hagberg, E.W. Hjelm, and Z. Wang. 1993. d-Limonene exposure to humans by inhalation: Uptake, distribution, elimination, and effects on the pulmonary function. J. Toxicol. Environ. Health 38(1):77-88.

Gould, M.N. 1997. Cancer chemoprevention and therapy by monoterpenes. Environ. Health Perspect. 105(Suppl. 4):977-979.

Grief, N. 1967. Cutaneous safety of fragrance material as measured by the Maximization test. Am. Perfumer Cosmetics 82:54-57 (as cited in NICNAS 2002).

IARC (International Agency for Research on Cancer). 1999. d-Limonene. Pp. 307-328 in Some Chemicals that Cause Tumours of the Kidney or Urinary Bladder in Rodents and Some Other Substances: Summary of Data Reported and Evaluation, IARC Monographs on the Evaluation of Carcinogenic Risks to Humans Vol. 73. World Health Organization, International Agency for Research on Cancer [online]. Available: http://monographs.iarc.fr/ENG/Monographs/vol73/volume73.pdf [accessed Oct. 7, 2004].

Igimi, H., M. Nishimura, R. Kodama, and H. Ide. 1974. Studies on the metabolism of d-limonene (p-mentha-1,8-diene). I. The absorption, distribution and excretion of d-limonene in rats. Xenobiotica 4(2):77-84.

Igimi, H., T. Hisatsugu, and M. Nishimura. 1976. The use of d-limonene preparation as a dissolving agent of gallstones. Am. J. Dig. Dis. 21(11):926-939.

Jensen, O.C., and I. Petersen. 1991. Occupational asthma caused by scented gravel in cat litter boxes [in Danish]. Ugeskr. Laeger. 153(13):939-940.

JSC (Johnson Space Center). 2003. Analytical Results of ISS 11A, Lab Samples Returned on Soyuz 5 and STS-113 Container Air Samples. NASA Johnson Space Center Toxicology Group, Houston, TX [online]. Available: http://hefd.jsc.nasa.gov/files/STS113-ISS11Aconc.pdf [accessed July 9, 2008].

JSC (Johnson Space Center). 2008. Analytical Results of IE Grab Sample Container Air Samples. NASA Johnson Space Center Toxicology Group, Houston, TX [online]. Available: http://hefd.jsc.nasa.gov/files/Table_1B.1E_conc.xls [accessed July 9, 2008].

Kane, L.E, R. Dombroske, and Y. Alarie. 1980. Evaluation of sensory irritation from some common industrial solvents. Am. Ind. Hyg. Assoc. J. 41(6):451-455.

Kanerva, R.L., G.M. Ridder, F.R. Lefever, and C.L. Alden. 1987. Comparison of short-term renal effects due to oral administration of decalin or d-limonene in young adult male Fischer 344 rats. Food Chem. Toxicol. 25(5):345-353.

Karlberg, A.T., K. Magnusson, and U. Nilsson. 1992. Air oxidation of d-limonene (the citrus solvent) creates potent allergens. Contact Dermatitis 26(5):332-340.

Kasanen, J.P, A.L. Pasanen, P. Pasanen, J. Liesivuori, V.M. Kosma, and Y. Alarie. 1998. Stereospecificity of the sensory irritation receptor for nonreactive chemicals illustrated by pinene enantiomers. Arch. Toxicol. 72(8):514-523.

Kodama, R., T. Yano, K. Furukawa, K. Noda, and H. Ide. 1976. Studies on the metabolism of d-limonene (p-mentha-1,8-diene). IV. Isolation and characterization of new metabolites and species differences in metabolism. Xenobiotica 6(6):377-389.

Kodama, R., A. Okubo, E. Araki, K. Noda, H. Ide, and T. Ikeda. 1977a. Studies on d-limonene as a gallstone solubilizer: VII. Effects on development of mouse fetuses and offsprings. Oyo Yakuri 13(6):863-873 (as cited in EPA 1993).

Kodama, R., A. Okubo, K. Sato, E. Araki, K. Noda, H. Ide, and T. Ikeda. 1977b. Studies on d-limonene as a gallstone solubilizer: IX. Effects on development of rabbit fetuses and offsprings. Oyo Yakuri 13(6):885-898 (as cited in EPA 1993).

Larsen, S.T., K.S. Hougaard, M. Hammer, Y. Alarie, P. Wolkoff, P.A. Clausen, C.K. Wilkins, and G.D. Nielsen. 2000. Effects of R-(+)- and S-(-)-limonene on the respiratory tract in mice. Hum. Exp. Toxicol. 19(8):457-466.

Lehman-McKeeman, L.D., and D. Caudill. 1992. Alpha 2u-globulin is the only member of the lipocalin protein superfamily that binds to hyaline droplet inducing agents. Toxicol. Appl. Pharmacol. 116(2):170-176.

Lehman-McKeeman, L.D., P.A. Rodriguez, R. Takigiku, D. Caudill, and M.L. Fey. 1989. d-Limonene-induced male rat-specific nephrotoxicity: Evaluation of the association between d-limonene and alpha 2u-globulin. Toxicol. Appl. Pharmacol. 99(2):250-259.

Madsen, T., H.B. Boyd, D. Nylén, A.R. Pedersen, G.I. Petersen, and S. Flemming. 2001. Fragrances. Chapter 12 in Environmental and Health Assessment of Substances in Household Detergents and Cosmetic Detergent Products. Environmental Project 615. Danish Environmental Protection Agency, Denmark [online]. Available: http://www.mst.dk/udgiv/publications/2001/87-7944-596-9/html/kap12_eng.htm [acessed Oct. 7, 2004].

Molhave, L., S.K. Kjergaard, A. Hempel-Jorgensen, J.E. Juto, K. Andersson, G. Stridh, and J. Falk. 2000. The eye irritation and odor potencies of four terpenes which are major constituents of the emissions of VOCs from Nordic soft woods. Indoor Air 10(4):315-318.

NICNAS (National Industrial Chemicals Notification and Assessment Scheme). 2002. Limonene. Priority Existing Chemical Assessment Report No. 22. National Industrial Chemicals Notification and Assessment Scheme, Commonwealth of Australia. May 2002 [online]. Available: http://www.nicnas.gov.au/publications/car/pec/pec22/pec_22_full_report_pdf.pdf [accessed Oct. 7, 2004].

Nielsen, G.D., and Y. Alarie. 1982. Sensory irritation, pulmonary irritation, and respiratory stimulation by airborne benzene and alkylbenzenes: Prediction of safe industrial exposure levels and correlation with their thermodynamic properties. Toxicol. Appl. Pharmacol. 65(3):459-477.

NRC (National Research Council). 1992. Guidelines for Developing Spacecraft Maximum Allowable Concentrations for Space Station Contaminants. Washington, DC: National Academy Press.

NRC (National Research Council). 2000. Methods for Developing Spacecraft Water Exposure Guidelines. Washington, DC: National Academy Press.

NTP (National Toxicology Program). 1990. NTP Technical Report on the Toxicology and Carcinogenesis Studies of d-Limonene (CAS No. 5989-27-5) in F344/N Rats and B6C3F_1 Mice (Gavage Studies). Technical Report NTP TR 347. NIH 90-2802. National Toxicology Program, U.S. Department of Health and Human Services, National Institutes of Health, Research Triangle Park, NC [online]. Available: http://ntp.niehs.nih.gov/ntp/htdocs/LT_rpts/tr347.pdf [accessed Apr. 18, 2008].

Opdyke, D.L. 1978. Monographs on fragrance raw materials: d-limonene. Food Cosmet. Toxicol. 16(Suppl. 1):809.

Rohr, A.C., C.K. Wilkins, P.A. Clausen, M. Hammer, G.D. Nielsen, P. Wolkoff, and J.D. Spengler. 2002. Upper airway and pulmonary effects of oxidation products of (+)-alpha-pinene, d-limonene, and isoprene in BALB/c mice. Inhal. Toxicol. 14(7):663-684.

RTECS (The Registry of Toxic Effects of Chemical Substances). 2006. p-Mentha-1,8-diene. RTECS No. OS8100000. CAS No. 138-86-3. The Registry of Toxic Effects of Chemical Substances, National Institute for Occupational Safety and Health [online]. Available: http://www.cdc.gov/niosh/rtecs/os7b98a0.html [accessed Apr. 18, 2008].

Saito, K., S. Uwagawa, H. Kaneko, and A. Yoshitake. 1991. Behavior of alpha 2u-globulin accumulating in kidneys of male rats treated with d-limonene: Kidney-type alpha 2u-globulin in the urine as a marker of d-limonene nephropathy. Toxicology 70(2):173-183.

SWEA (Swedish Work Environment Authority). 2005. Occupational Exposure Limit Values and Measures against Air Contaminants. Statute Book. AFS 2005:17. Swedish Work Environment Authority, Solna, Sweden [online]. Available: http://www.av.se/dokument/inenglish/legislations/eng0517.pdf [accessed Apr. 18, 2008].

Tsuji, M., Y. Fujisaki, Y. Arikawa, et al. 1975. Studies on d-limonene as a gallstone solubilizer. (III). Chronic toxicity in rats. Oyo Yakuri 9(3):403-412 (as cited in EPA 1993).

Vigushin, D.M., G.K. Poon, A. Boddy, J. English, G.W. Halbert, C. Pagonis, M. Jarman, and R.C. Coombes. 1998. Phase I and pharmacokinetic study of D-limonene in patients with advanced cancer. Cancer Chemother. Pharmacol. 42(2):111-117.

Webb, D.R., R.L. Kanerva, D.K. Hysell, C.L. Alden, and L.D. Lehman-McKeeman. 1990. Assessment of the subchronic oral toxicity of d-limonene in dogs. Food Chem. Toxicol. 28(10):669-675.

Wolkoff, P., P.A. Clausen, C.K. Wilkins, and G.D. Nielsen. 2000. Formation of strong airway irritants in terpene/ozone mixtures. Indoor Air 10(2):82-91.

14

Methanol

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Methanol (H_3COH, CAS no. 67-56-1), the simplest alcohol, is a colorless, volatile, highly flammable liquid with a mild, characteristic, agreeable odor; it is completely miscible in water. One part per million (ppm) of methanol = 1.31 milligrams per cubic meter (mg/m^3). An extremely wide range of odor threshold values for methanol vapor has been reported in the literature, from 5.5 mg/m^3 (NLM 2007) to 26,840 mg/m^3 (Ruth 1986).

In anticipation of longer-duration exploration missions, the purpose of this document is to establish a spacecraft maximum allowable concentration (SMAC) value for methanol for an extended exposure of 1,000 d and to revise the previous SMAC values based on recently available data.

OCCURRENCE AND USE

Methanol occurs naturally in humans and animals, in plants, including fresh fruits and vegetables, and in fermented products, including wine and other spirits (see Tables 14-1 and 14-2). It is produced from the distillation of wood or is synthesized catalytically from crude petroleum. It is used industrially in the manufacture of other chemicals and as a solvent. It is added to a variety of commercial and consumer products, including windshield washing solutions, deicing solutions, glass cleaners, duplicating fluids, solid canned fuels, paint thinners and removers, model airplane fuels, embalming fluids, lacquers, inks, and some formulations of gasohol motor fuel.

Methanol vapor concentrations in the atmosphere of the Shuttle and the International Space Station have rarely exceeded 1 mg/m^3 and are generally less than 0.6 mg/m^3.

TABLE 14-1 Methanol Concentrations in Foods and Beverages

Source	Concentration
Fresh and canned fruit juices (orange and grapefruit juices)	1-43 mg/L 11-80 mg/L 12-640 mg/L (Average of 140 mg/L)
Neutral spirits	<1.5 g/L
Beer	6-27 mg/L
Wines	96-329 mg/L
Distilled spirits	16-220 mg/L
Bourbon	55 mg/L
50% grain alcohol	1 mg/L
Brandies (United States, Canada, and Italy)	6,000-7,000 mg/L
Beans	1.5-7.9 mg/kg
Split peas	3.6 mg/kg
Lentils	4.4 mg/kg
Carbonated beverages	~56 mg/L

Abbreviations: kg, kilogram; L, liter.
Source: Data from NTP CERHR 2003.

TABLE 14-2 Background Blood Methanol and Formate Concentrations in Humans

Subjects	mg of methanol/L, mean ± SD (range)	mg of formate /L, mean ± SD (range)
Twelve males on restricted diet (no methanol-containing or methanol-producing foods) for 12 h	0.570 ± 0.305 (0.25-1.4)	3.8 ± 1.1 (2.2-6.6)
Twenty-two adults on restricted diet (no methanol-containing or methanol-producing foods) for 24 h	1.8 ± 2.6 (no range data)	11.2 ± 9.1 (no range data)
Three males who ate a breakfast with no aspartame-containing cereals and no juice	1.82 ± 1.21 (0.57-3.57)	9.08 ± 1.26 (7.31-10.57)
Five males who ate a breakfast with no aspartame-containing cereals and no juice (second experiment)	1.93 ± 0.93 (0.54-3.15)	8.78 ± 1.82 (5.36-10.83)
Twelve adults who drank no alcohol for 24 h	1.8 ± 0.7 (no range data)	No data
Twelve adults who drank no alcohol for 24 h	1.7 ±0.9 (0.44.7)	No data
Thirty fasted adults	<4 (no range data)	19.1 (no range data)
Twenty-four fasted infants	<3.5 (no range data)	No data

Abbreviation: SD, standard deviation.
Source: Data from NTP CERHR 2003.

SUMMARY OF ORIGINAL APPROACH

SMAC values for methanol were previously published in volume 1 of this series, *Spacecraft Maximum Allowable Concentrations for Selected Airborne Contaminants*, for exposure durations of 1 h, 24 h, 7 d, 30 d, and 180 d (Wong 1994). These methanol SMACs were based on data in a report (Frederick et al. 1984) published by the National Institute for Occupational Safety and Health that described visual disturbances in 66 teacher aides exposed to methanol vapors from "spirit" duplicating machines during a work week, with exposure durations in individual aides varying from 1 to 40 h/wk. The 1-h SMAC was calculated by estimating a no-observed-adverse-effect level (NOAEL) from the 391-ppm average concentration at which visual effects were reported. This was done by multiplying 391 ppm by 0.1 (lowest-observed-adverse-effect level [LOAEL] to NOAEL factor) and again by $(\sqrt{66})/\sqrt{100}$ as a "small n" factor to achieve a value of 32 ppm, rounded to 30 ppm. The calculations did not take into account the exposure duration beyond 7 d.

To calculate a 24-h SMAC, Wong assumed that "unlike headaches, methanol's ocular toxicity might not be entirely dependent on the blood concentration of formic acid and might not have a threshold" (Wong 1994, P. 162), but instead may depend on the total dose (concentration times duration). Therefore, he used Haber's rule to reduce the 1-h SMAC by a factor of 8 h/24 h to a value of 11 ppm, rounded to 10 ppm. For the 7-, 30-, and 180-d SMACs, Wong applied a "small n" factor, a 0.1 LOAEL-to-NOAEL factor, and a time factor of 40/168 to the 392 ppm LOAEL to extrapolate from a work week to a continuous 7-d exposure. He calculated an acceptable concentration of 7 ppm for 7 d, which he applied for exposure durations of 7, 30, and 180 d.

CHANGES IN FUNDAMENTAL APPROACHES RECOMMENDED BY THE NATIONAL RESEARCH COUNCIL

The original SMAC values for methanol, set in 1994 by King Lit Wong, were calculated using safety factors applied to a LOAEL. More recently, the National Research Council has recommended using a benchmark dose analysis for setting the point of departure and ten Berge's generalization ($C^N \times T = K$) of Haber's rule for extrapolating ACs based on short-duration studies to longer durations (when data permit). Although Wong applied a "small n" factor to a reported LOAEL to estimate a NOAEL, NASA and the National Research Council have since recommended (James and Gardner 1996) the use of a "small n" factor only when the data include an apparent NOAEL.

NEW DATA SINCE 1994

Table 14-3 is a compilation of data from a selected list of currently available studies (published up through and since 1994) on methanol toxicity. Since

TABLE 14-3 Toxicity Summary

Concentration/dose and chemical form, route	Exposure duration	Species and strain	Effects	Reference
Acute and short-term exposure (≤10 d)				
Unknown high dose	Single bolus	Humans (n = 2)	Severe metabolic acidosis, optic disc edema, death in 1 of 2.	McMartin et al. 1980
Unknown high dose	Single bolus	Humans (n = 2)	Severe metabolic acidosis, optic disc edema, blindness.	Hayreh et al. 1977, 1980
Unknown high dose	Single bolus	Humans (n = 2)	Severe metabolic acidosis, optic disc edema, blindness.	Jacobsen and McMartin 1986
800 ppm	0.5, 1, 2, 8 h	Humans (n = 4, 4, 4, 15)	MeOH concentration in breath, blood, and urine measured. No toxicity reported.	Batterman et al. 1998
200 ppm	6 h	Humans (n = 6)	No toxicity reported. Mean blood MeOH concentration increased from 1.8 μg/mL background to 7.0 μg/mL at rest or 8.1 μg/mL with light exercise. No increase in blood formate concentration at rest or exercising.	Lee et al. 1992
100 ppm	2 h	Humans (n = 8)	No toxicity reported. Mean blood MeOH concentration increased from 20 to 116 μM.	Ernstgard et al. 2005
200 ppm	2 h	Humans (n = 8)	No toxicity reported. Mean blood MeOH concentration increased from 20 to 244 μM.	Ernstgard et al. 2005

192 ppm	75 min	Humans (n = 12)	No significant effects on tests of sensory, behavioral, and reasoning performance.	Cook et al. 1991
200 ppm	4 h	Humans (n = 26)	No significant effects on neurobehavioral performance. Serum MeOH concentration increased but serum formate concentration did not.	Chuwers et al. 1995
200 ppm	4 h	Humans (n = 26)	No significant effects on neurobehavioral performance. Serum MeOH concentration increased, but serum formate concentration did not.	D'Alessandro et al. 1994
200 ppm	4 h	Humans (n = 22)	Serum MeOH increased from 0.9 to 6.5 μg/mL. No toxicity reported.	Osterloh et al. 1996
Subchronic exposure (11–100 d)				
365- to 3,080-ppm vapors	1-40 h/wk	Teacher aides, female	Dose-dependent incidence of blurred vision, headache, dizziness, and nausea.	Frederick et al. 1984
Chronic exposure (≥100 d)				
32.7 mg of methanol/dose (as metabolite of 900 mg of aspartame/d)	3 doses/d, 7 d/wk, 6 mo	Human (n = 53)	NOAEL for standard lab tests or symptoms experienced.	Leon et al. 1989

Abbreviations: MeOH, methanol; μM, micromolar.

1994, published studies on methanol have examined the metabolic, toxicokinetic, histopathologic, neurobehavioral, and ocular effects of methanol in humans and other animals after short-term inhalation of vapors or by other routes of exposure and the kinetics of elimination of methanol or formate from blood and urine (D'Alessandro et al. 1994, Chuwers et al. 1995, Osterloh et al. 1996, Batterman et al. 1998, Ernstgard et al. 2005). The results of these studies reinforce and expand upon the findings reported before 1994 but do not support Wong's assumption that the ocular toxicity of methanol might not have a threshold.

Studies and case reports before 1994 show that ocular effects are associated only with blood formate concentrations in excess of 5 to 10 millimolar (225-450 mg/liter [L]) and are observed only after a latent, symptom-free period of about a day (6-30 h). Presumably, during this latent period, methanol is metabolized to formaldehyde, formate, and carbon dioxide (CO_2) until the body's stores of tetrahydrofolate are depleted and increasing blood formate concentrations lead to anoxia in the retinal cells, due to their relatively low concentrations of mitochondrial cytochrome oxidase (Nicholls 1975; Martin-Amat et al. 1977, 1978; Kavet and Nauss 1990). In the initial stages, developing ocular toxicity can be reversed, consistent with a mechanism involving the reversible inhibition of cytochrome *c* oxidase by formate and the need for prolonged retinal cell hypoxia to gradually produce symptoms of impaired vision (Nicholls 1975). Anoxia leads to swelling of the retinal ganglion cells and progressive loss of vision if not reversed within about 24 h of the initiation of methanol intoxication. The delays seen in development of ocular toxicity in humans who ingest significant quantities of methanol can be attributed to two factors: the time necessary to deplete the body's stores of tetrahydrofolate so that formate metabolism to CO_2 is greatly decreased and formate begins to accumulate to concentrations that inhibit mitochondrial cytochrome oxidase in the retinal ganglia, and the time needed for the axonal swelling caused by the inhibition of cytochrome oxidase to reach a point at which vision is affected.

Studies of controlled inhalation exposure of humans to methanol vapors, most of which have been published since 1994, describe only brief durations and relatively low concentrations—for example, 12 volunteers for 75 min at 192 ppm (Cook et al. 1991), 8 volunteers for 2 h at 100 and 200 ppm (Ernstgard et al. 2005), 22-26 volunteers for 4 h at 200 ppm (D'Alessandro et al. 1994, Chuwers et al. 1995, Osterloh et al. 1996), 6 volunteers for 6 h at 200 ppm (Lee et al. 1992), and 15 volunteers for up to 8 h at 800 ppm (Batterman et al. 1998). For all of these studies, the doses and exposure durations are insufficient for ocular effects to be manifested. Although increases were observed in the concentration of methanol in blood and urine, the formate concentrations in blood and urine increased only negligibly compared with background levels.

Similar results were reported in monkeys, whose metabolism of methanol is believed to be similar to that of humans, with no increase in blood formate concentrations above background for 6-h exposures to methanol of up to 2,000 ppm (Medinsky and Dorman 1995). Similarly, in both normal and folate-

deficient monkeys exposed for 2 h to up to 900 ppm of [^{14}C]methanol, [^{14}C]methanol-derived formate concentrations in the blood remained below background formate concentrations (Medinsky et al. 1997). Unfortunately, none of the published studies, either in humans or in primates, reports continuous exposures of sufficient duration to provide confidence that steady state had been achieved, particularly in the blood concentration of formate. It is not known whether longer exposures at the tested concentrations could have led to depletion of the stores of tetrahydrofolate, resulting in saturation of formate metabolism and increasing blood and tissue concentrations of formate.

A biologically based dynamic model of the disposition of methanol in humans and animals, based on and validated against published studies on methanol exposures in humans and animals, predicted that near steady-state concentrations of methanol in blood would be reached within 20 h after the start of a continuous inhalation exposure (Bouchard et al. 2001). This model implies that steady-state concentrations of methanol in blood had not been reached in any of the published studies in human volunteers. At the end of a hypothetical 5-d exposure to 200 ppm of methanol, Bouchard's model predicted that the blood methanol concentration in humans would be 0.55 mg/deciliter (dL) (a >5-fold increase over mean background values), but that the methanol-derived increase in formate concentration in the blood would be only 0.016 mg/dL, which is small compared with the background value of 0.49 mg/dL in unexposed subjects but is in accord with experimental data from methanol exposures in primates and humans.

Because there are no published studies of controlled exposures in humans involving the dose range at which formate metabolism to CO_2 becomes saturated and blood formate concentrations begin to increase, the model did not include a saturation term for formate metabolism, and its usefulness is limited to situations in which tetrahydrofolate is not limiting. The amount of tetrahydrofolate in tissues is known to vary considerably among individuals and depends on their nutritional status. A healthy individual has 500-20,000 micrograms (μg) of folate in body stores. Humans need to absorb 50-100 μg of folate per day to replenish the daily degradation and loss through urine and bile. Otherwise, signs and symptoms of deficiency can manifest after 4 months (Gentili et al. 2007). Data from the National Health and Nutrition Examination Survey 1999-2000 (Pfeiffer et al. 2005) indicate that the prevalence of low serum folate concentrations (<6.8 nmol/L = <3 μg/L) in the U.S. population decreased from 16% before to 0.5% after the U.S. began requiring folic acid fortification of cereal-grain products in November 1998. U.S. astronauts on missions to the International Space Station lasting 128-195 d were found to have 20% lower folate concentrations in red blood cells at landing than before launch (Johlin et al. 1989). The folate concentration in red blood cells of many astronauts after landing approached the lower limit of the normal range. It is not known whether this decrease in folate would level off or continue to decrease with even longer missions (Johlin et al. 1989), but the reduction is believed to be due to inadequate food intake while in orbit.

Simulations using Bouchard's model suggest that an 8-h inhalation exposure to 500-2,000 ppm of methanol without physical exercise would be required just for the methanol-derived increase in formate concentration in the blood to equal the levels normally seen as backgrounds in humans (thus, presumably doubling the blood concentration of formate) (Bouchard et al. 2001). Note, however, that, according to the model, a steady-state concentration of methanol in the blood would not have been achieved after 8 h of exposure (Bouchard et al. 2001). Note also that data from Lee et al. suggest that, although the amount of methanol volunteers inhaled during light exercise is 1.8 times the amount inhaled at rest, the concentration of methanol in the blood is only slightly, but not significantly, increased by light exercise (8.1 μg/mL compared with 7.0 μg/mL at rest and 1.8 μg/mL pre-exposure), and no increase in blood formate concentration was observed after a 6-h exposure to 200 ppm of methanol either at rest or during exercise (Lee et al. 1992).

NEW RISK ASSESSMENT APPROACHES

Neither the data available when the original SMACs were set in 1994 nor the data available since then include suitable dose-response data amenable to analysis using benchmark dose or ten Berge methodology. The published data on which the original SMACs were based did not include the raw data for individual subjects.

RATIONALE FOR THE 1,000-D SMAC AND FOR REVISED 1-H, 24-H, 7-D, 30-D, AND 180-D SMACS

Table 14-4 lists standards developed by government and nongovernmental agencies for methanol vapors. Table 14-5 lists the SMAC values for methanol whose derivation is described below.

D'Allesandro and colleagues (D'Alessandro et al. 1994, Chuwers et al. 1995) reported no significant neurobehavioral effects in 26 volunteers exposed for 4 h to 200 ppm of methanol, whose peak serum methanol concentrations were 6.5 mg/L. A similar lack of effects was reported for 200-ppm exposures in 22 subjects exposed for 4 h (Osterloh et al. 1996), 12 subjects exposed for 75 min (Cook et al. 1991), 8 subjects exposed for 2 h (Ernstgard et al. 2005), and 6 subjects exposed for 6 h (Lee et al. 1992).

Because no adverse effects were reported, the data could not be subjected to a benchmark dose analysis or to a ten Berge analysis. Therefore, the 1-h SMAC can be set equal to the NOAEL of 200 ppm reported in the studies cited above.

1-h SMAC = 200 ppm

TABLE 14-4 Air Standards for Methanol Vapors Set by Other Organizations

Organization, Standard	Amount	Reference
NIOSH		NIOSH 2005
IDLH	6,000 ppm (7,860 mg/m^3)	
REL TWA	200 ppm (260 mg/m^3)	
ST, skin	250 ppm (325 mg/m^3)	
OSHA		NIOSH 2005
PEL TWA	200 ppm (260 mg/m^3)	
ACGIH		ACGIH 1997
TLV TWA, skin	200 ppm (260 mg/m^3)	
TLV STEL, skin	250 ppm (325 mg/m^3)	

Abbreviations: ACGIH, American Conference of Governmental Industrial Hygienists; IDLH, immediately dangerous to life and health; NIOSH, National Institute for Occupational Safety and Health; OSHA, Occupational Safety and Health Administration; PEL, permissible exposure limit; REL, recommended exposure limits; ST, short-term exposure limit (15 min); STEL, short-term exposure limit; TLV®, threshold limit value; TWA, time-weighted average.

TABLE 14-5 SMACs for Methanol Vapors

Duration	ppm	mg/m^3	Target toxicity
1 h	200	260	Ocular effects
24 h	70	90	Ocular effects
7 d	70	90	Ocular effects
30 d	70	90	Ocular effects
180 d	70	90	Ocular effects
1,000 d	23	30	Ocular effects

The mathematical model developed by Bouchard predicts that about 20 h of continuous inhalation exposure is needed for blood methanol concentrations to achieve near steady state at an atmospheric concentration of 200 ppm of methanol (Bouchard et al. 2001). The model predicts that 5 d of continuous exposure to methanol at 200 ppm would result in blood formate concentrations in humans of 0.16 mg/L, a value well below experimental mean background concentrations in unexposed subjects (4.9-10.3 mg/L) reported by various authors. The finding that blood methanol concentrations should be near steady state but formate concentrations remain near background after the first 20 h implies that continuous exposure to methanol vapors at 200 ppm could be maintained indefinitely without risk of formate toxicity. The model explicitly assumes, however, that folate has not been depleted, because no saturation of formate metabolism was apparent in the experimental data used to validate the model. It is unknown, however, whether tetrahydrofolate concentrations would continue to decrease at exposure durations >20 h. Because 200 ppm of methanol differs by only about a

factor of 2 from the 390-ppm average concentration reported to cause visual disturbances in teacher's aides who worked with spirit duplicating machines (Frederick et al. 1984), and because Bouchard's model has not been validated for exposure durations beyond 8 h, a safety factor for uncertainty should be applied to the 200-ppm NOAEL for exposure durations exceeding 8 h. Given that formate's visual toxicity is known to be a threshold response (Nicholls 1975; Martin-Amat et al. 1977, 1978; Kavet and Nauss 1990), a factor of 3 was applied. Thus,

$$\text{24-h, 7-d, 30-d, and 180-d SMACs} = 200 \text{ ppm}_{\text{(NOAEL)}} \div 3_{\text{(safety factor)}}$$
$$= 66.7 \text{ ppm, rounded to } 70 \text{ ppm}$$

For setting a SMAC for a 1,000-d exposure, an additional factor of 3 was applied because of the potential for folate deficiency to develop in crewmembers on long-duration missions based on their tendency not to eat all the balanced diet provided to them. Thus,

$$\text{1,000-d SMAC} = 70 \text{ ppm}_{\text{(24-h, 7-, 30- , and 180-d SMACs)}} \div 3_{\text{(safety factor)}}$$
$$= 23.3 \text{ ppm, rounded to } 23 \text{ ppm}$$

The calculated SMAC values are summarized in Table 14-6.

SPACEFLIGHT EFFECTS

None of the reported adverse effects of methanol exposures is known to be affected by spaceflight, but the 1.7% to 12% reduction in total body water associated with prolonged microgravity would proportionately increase the blood concentration of methanol inhaled. However, the clinical ramifications of such a small effect on the toxicity of methanol would be expected to be negligible. Crews on long-duration missions have been found to have reduced folate concentrations in blood cells compared with their preflight concentrations. If folate concentrations were to continue to decrease on longer missions, they could become low enough to limit the metabolism of formate derived from methanol, thereby increasing the toxicity of methanol.

RECOMMENDATIONS FOR ADDITIONAL RESEARCH

There are no published studies that elucidate the rate (mg/h) of continuous methanol vapor inhalation at which formate metabolism to CO_2 begins to become saturated and blood formate concentrations begin to increase. After oral ingestion of liquid methanol, numerous case reports describe symptom-free latent periods of 6-30 h before vision impairment is manifested. To confidently predict a vapor concentration of methanol that would not cause ocular toxicity

TABLE 14-6 Acceptable Concentrations for Methanol (ppm)

			Uncertainty factor					Acceptable concentration, ppm					
Effect	Exposure data	Species and reference	NOAEL	Species	Time	Space-flight	Folate defi-ciency	1 h	24 h	7 d	30 d	180 d	1,000 d
Visual disturbance	200 ppm, 75 min-6 h = NOAEL	Human Cook et al. 1991, D'Alessandro et al. 1994, Chuwers et al. 1995, Ernstgard et al. 2005, Lee et al. 1992, Osterloh et al. 1996	1	1	1	1	1	200	–	–	–	–	–
Visual disturbance	200 ppm, 5 d (modeled) = NOAEL	Human Bouchard et al. 2001	3	1	1	1	1	–	70	70	70	70	–
Visual disturbance	200 ppm, 5 d (modeled) = NOAEL	Human Bouchard et al. 2001	3	1	1	1	3	–	–	–	–	–	23
SMACs								*200*	*70*	*70*	*70*	*70*	*23*

Abbreviations: –, not calculated.

during chronic exposures, data are needed from continuous exposures at a range of concentrations of animals or humans in both folate-deficient and folate-replete states to a range of concentrations of methanol vapors for least 1 d and preferably for several days.

REFERENCES

ACGIH (American Conference of Governmental Industrial Hygienists). 1997. 1997 TLVs and BEIs-Threshold Limit Values for Chemical Substances and Physical Agents; Biological Exposure Indices. Cincinnati, OH: American Conference of Governmental Industrial Hygienists.

Batterman, S.A., A. Franzblau, J.B. D'Arcy, N.E. Sargent, K.B. Gross, and R.M. Schreck. 1998. Breath, urine, and blood measurements as biological exposure indices of short-term inhalation exposure to methanol. Int. Arch. Occup. Environ. Health 71(5):325-335.

Bouchard, M., R.C. Brunet, P.O. Droz, and G. Carrier. 2001. A biologically based dynamic model for predicting the disposition of methanol and its metabolites in animals and humans. Toxicol. Sci. 64(2):169-184.

Chuwers, P., J. Osterloh, T. Kelly, A. D'Alessandro, P. Quinlan, and C. Becker. 1995. Neurobehavioral effects of low-level methanol vapor exposure in healthy human volunteers. Environ. Res. 71(2):141-150.

Cook, M.R., F.J. Bergman, H.D. Cohen, M.M. Gerkovich, C. Graham, R.K. Harris, and L.G. Siemann. 1991. Effects of Methanol Vapor on Human Neurobehavioral Measures. Research Report No. 42. Boston, MA: Health Effects Institute.

D'Alessandro, A., J.D. Osterloh, P. Chuwers, P.J. Quinlan, T.J. Kelly, and C.E. Becker. 1994. Formate in serum and urine after controlled methanol exposure at the threshold limit value. Environ. Health Perspect. 102(2):178-181.

Ernstgard, L., E. Shibata, and G. Johanson. 2005. Uptake and disposition of inhaled methanol vapor in humans. Toxicol. Sci. 88(1):30-38.

Frederick, L.J., P.A. Schulte, and A. Apol. 1984. Investigation and control of occupational hazards associated with the use of spirit duplicators. Am. Ind. Hyg. Assoc. J. 45(1):51-55.

Gentili, A., M. Vohra, V. Subir, D. Chen, and W. Siddiqi. 2007. Folic acid deficiency. eMedicine Topic 802 [online]. Available: http://www.emedicine.com/med/topic802.htm [accessed Jan. 16, 2008].

Hayreh, M.S., S.S. Hayreh, G.L. Baumbach, P. Cancilla, G. Martin-Amat, T.R. Tephly, K.E. McMartin, and A.B. Makar. 1977. Methyl alcohol poisoning. III. Ocular toxicity. Arch. Ophthalmol. 95(10):1851-1858.

Hayreh, M.S., S.S. Hayreh, G. Baumbach, P. Cancilla, G. Martin-Amat, and T.R. Tephly. 1980. Ocular toxicity of methanol: An experimental study. Pp. 35-53 in Neurotoxicity of the Visual System, W. Merrigan, and B. Weiss, eds. New York: Lippincott-Raven.

Jacobsen, D., and K.E. McMartin. 1986. Methanol and ethylene glycol poisonings. Mechanism of toxicity, clinical course, diagnosis and treatment. Med. Toxicol. 1(5):309-334.

James, J.T., and D.E. Gardner. 1996. Exposure limits for airborne contaminants in spacecraft atmospheres. Appl. Occup. Environ. Hyg. 11(12):1424 -1432.

Johlin, F.C., E. Swain, C. Smith, and T.R. Tephly. 1989. Studies on the mechanism of methanol poisoning: Purification and comparison of rat and human liver 10-formyltetrahydrofolate dehydrogenase. Mol. Pharmacol. 35(6):745-750.

Kavet, R., and K.M. Nauss. 1990. The toxicity of inhaled methanol vapors. Crit. Rev. Toxicol. 21(1):21-50.

Lee, E.W., T.S. Terzo, J.B. D'Arcy, K.B. Gross, and R.M. Schreck. 1992. Lack of blood formate accumulation in humans following exposure to methanol vapor at the current permissible exposure limit of 200 ppm. Am. Ind. Hyg. Assoc. J. 53(2):99-104.

Leon, A.S., D.B. Hunninghake, C. Bell, D.K. Rassin, and T.R. Tephly. 1989. Safety of long-term large doses of aspartame. Arch. Intern. Med. 149(10):2318-2324.

Martin-Amat, G., T.R. Tephly, K.E. McMartin, A.B. Makar, M.S. Hayreh, S.S. Hayreh, G. Baumbach, and P. Cancilla. 1977. Methyl alcohol poisoning. II. Development of a model for ocular toxicity in methyl alcohol poisoning using the rhesus monkey. Arch. Ophthalmol. 95(10):1847-1850.

Martin-Amat, G., K.E. McMartin, S.S. Hayreh, M.S. Hayreh, and T.R. Tephly. 1978. Methanol poisoning: Ocular toxicity produced by formate. Toxicol. Appl. Pharmacol. 45(1):201-208.

McMartin, K.E., J.J. Ambre, and T.R. Tephly. 1980. Methanol poisoning in human subjects. Role for formic acid accumulation in the metabolic acidosis. Am. J. Med. 68(3):414-418.

Medinsky, M.A., and D.C. Dorman. 1995. Recent developments in methanol toxicity. Toxicol. Lett. 82-83:707-711.

Medinsky, M.A., D.C. Dorman, J.A. Bond, O.R. Moss, D.B. Janszen, and J.I. Everitt. 1997. Phamacokinetics of Methanol and Formate in Female Cynomolgus Monkeys Exposed to Methanol Vapors. Research Report No. 77. Boston, MA: Health Effects Institute.

Nicholls, P. 1975. Formate as an inhibitor of cytochrome c oxidase. Biochem. Biophys. Res. Commun. 67(2):610-616.

NIOSH (National Institute for Occupational Safety and Health). 2005. NIOSH Pocket Guide to Chemical Hazards. DHHS (NIOSH) No. 2005-151. National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention, U.S. Department of Health and Human Services, Cincinnati, OH.

NLM (U.S. National Library of Medicine). 2007. Methyl Alcohol. Haz-Map Occupational Exposure to Hazardous Agents. U.S. National Library of Medicine, Bethesda, MD [online]. Available: http://hazmap.nlm.nih.gov/cgi-bin/hazmap_generic?tbl=TblAgents&id=13 [accessed Nov. 15, 2007]

NTP-CERHR (National Toxicology Program-Center for the Evaluation of Risks to Human Reproduction). 2003. NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Methanol. NIH Publication No. 03-4478. U.S. Department of Health and Human Services, National Toxicology Program-Center for the Evaluation of Risks to Human Reproduction. September 2003 [online]. Available: http://cerhr.niehs.nih.gov/chemicals/methanol/Methanol_Monograph.pdf [accessed Jan. 17, 2008].

Osterloh, J.D., A. D'Alessandro, P. Chuwers, H. Mogadeddi, and T.J. Kelly. 1996. Serum concentrations of methanol after inhalation at 200 ppm. J. Occup. Environ. Med. 38(6):571-576.

Pfeiffer, C.M., S.P. Caudill, E.W. Gunter, J. Osterloh, and E.J. Sampson. 2005. Biochemical indicators of B vitamin status in the U.S. population after folic acid fortification: Results from the National Health and Nutrition Examination Survey 1999-2000. Am. J. Clin. Nutr. 82(2):442-450.

Ruth, J.H. 1986. Odor thresholds and irritation levels of several chemical substances: A review. Am. Ind. Hyg. Assoc. J. 47(3):A142-A151.

Wong, K.L. 1994. Methanol. 149-167 in Spacecraft Maximum Allowable Concentrations for Selected Airborne Contaminants, Vol. 1. Washington, DC: National Academy Press.

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Methylene Chloride

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Spacecraft maximum allowable concentration (SMAC) values for methylene chloride (also called 1,2-dichloromethane, DCM) were previously published in volume 2 of the series, *Spacecraft Maximum Allowable Concentrations (SMAC) for Selected Airborne Contaminants*, for exposure durations of 1 h, 24 h, 7 d, 30 d, and 180 d (Wong 1996). With NASA's current focus on exploration missions beyond low Earth orbit to the moon and Mars, there is a need to derive acceptable concentrations (ACs) for long-duration missions, such as for 1,000 d.

GENERAL APPROACH GUIDELINES

The effort consists of identifying new toxicology literature on DCM toxicity since the previous SMAC document was prepared (Wong 1996) and suitable studies that may have been missed during the preparation of that earlier document, in order to derive ACs for 1,000 d and other durations. Another objective is to determine if the previous SMAC (approved by the previous SMAC committee) needs to be updated based on new data that have become available or novel approaches have been developed, such as the benchmark dose (BMD) approach, which can be used on the data from the principal studies that were used before.

GENERAL PROPERTIES AND OCCURRENCE

Physical and chemical properties and occurrence have already been discussed (Wong 1996). The formula weight of DCM is 84.9 and conversion factors for DCM are 1 part per million (ppm) = 3.47 milligrams per cubic meter (mg/m^3) and 1 mg/m^3 = 0.29 ppm. DCM has been detected in the Shuttle spacecraft atmosphere in 28 of 33 missions at concentrations ranging from 0.029 to

0.29 ppm (0.1-1 mg/m^3) and also has been detected in the International Space Station atmosphere at about 0.5 mg/m^3. The odor threshold for DCM in air is 250 ppm.

BACKGROUND AND SUMMARY OF ORIGINAL APPROACH

Studies in human volunteers show that DCM is well absorbed (up to 70%) by resting subjects during inhalation exposures, and exercise changes the absorption (DiVincenzo et al. 1972, Astrand et al. 1975, DiVincenzo and Kaplan 1981). Animal studies indicate that inhaled DCM is distributed in the liver, kidneys, lungs, brain, muscle, adipose tissues, and adrenals about 1 h after inhalation exposure, with the highest concentrations found in white adipose tissue and the next highest in liver (McKenna et al. 1982).

Systemically absorbed DCM is metabolized by two pathways. One pathway is via the microsomal mixed function oxidase (MFO) in the cytochrome P-450 system (cytochrome P-450 2E1 or CYP 2E1) (Gargas et al. 1986, Guengerich et al. 1991). The oxidative dehalogenation yields hydrogen chloride, carbon monoxide (CO), and carbon dioxide, with formyl chloride as an intermediate. At low exposures, this pathway predominates, and it is saturable at about 300 to 500 ppm (Gargas et al. 1986). The CO from this pathway binds reversibly to hemoglobin, forming carboxyhemoglobin (COHb). COHb reduces the oxygen-carrying capacity of the blood and also impairs the release of O_2 from oxyhemoglobin, thus leading to tissue oxygen deficiency. In six sedentary human subjects exposed to DCM at 50, 100, 150, or 200 ppm for 7.5 h on 5 consecutive days, concentrations of COHb in blood were 1.9%, 3.4%, 5.3%, and 6.8%, respectively (DiVincenzo and Kaplan 1981). Numerous investigations have shown that CO is toxic to the cardiovascular system (changes heart rate and minute volume) and also to the central nervous system (CNS), where it has adverse effects such as impairing vigilance and performance in addition to causing headache, decreased vision, and other symptoms.

The second pathway is the glutathione (GSH)-dependent cytosolic pathway via glutathione *S*-transferase theta 1 (GSTT1) (Kubic and Anders 1975, Ahmed and Anders 1976, Andersen et al. 1987, Reitz et al. 1989). This pathway is a low-affinity first-order pathway that metabolizes DCM to hydrogen chloride, formaldehyde, and carbon dioxide. In the GSTT1 pathway, the haloalkane is metabolized to produce the reactive *S*-chloromethylglutathione intermediate, which has the capacity to interact with cellular DNA. The chloromethyl glutathione is short-lived; it undergoes rapid hydrolysis to yield formaldehyde. This GSH pathway is not saturable and is linear up to 10,000 ppm (Gargas et al. 1986). Carcinogenicity of DCM in long-term inhalation exposure of rodents has been attributed to metabolism of the compound via the GST-dependent pathway. Andersen et al. (1987) reported that large quantities of GSH-DCM conjugates in vivo may increase the frequency of lung and liver tumors that develop in some species of animals (such as B6C3F1 mice). DCM metabolism via the GSH

pathway in the target tissue has been the subject of several studies as the basis for species sensitivity to DCM-induced tumor incidence. It has also been suggested that formaldehyde produced from DCM metabolism via the same GST pathway may be responsible for the observed tumors due to the formation of DNA-protein crosslinks mediated by formaldehyde (Casanova et al. 1997). Describing and discussing numerous studies that attempted to explore the mechanism of DCM-induced tumors and its specificity in organs and species are far beyond the scope of this document.

Several reports have appeared on physiologically based pharmacokinetic (PBPK) modeling of these various metabolic pathways using in vivo and in vitro metabolic rates obtained from animal and human tissue samples and validating the kinetic data from human subjects exposed to DCM (Andersen et al. 1987, Reitz et al. 1989, Andersen et al. 1991, Clewell 1995, and many others). These earlier PBPK models for DCM have gone through several refinements and developments, including integrating the statistical models of the parameters for uncertainty and population distributions with the toxicokinetic models. In addition, to obtain target concentrations of DCM, PBPK models have been described for DCM uptake and distribution during rest and exercise (Dankovic and Bailer 1994, Jonsson et al. 2001). These models use blood flow and perfusion changes that occur during rest and exercise, accounting for changes in ventilation rate and cardiac output. Using statistical methods and PBPK models, investigators have attempted to estimate interindividual and population variability in the rate of metabolizing DCM.

To obtain a more accurate assessment of human health risk from synthetic halomethanes in the last few years, investigators have attempted to correlate and explain the interindividual variations and species sensitivities to DCM-induced carcinogenicity by the existence of polymorphisms in theta-class isoforms of GST (GSTT1). In humans, GSH-dependent conjugation of halomethane is polymorphic, with 60% of the population classed as conjugators and 40% classed as nonconjugators, implying that conjugators will be more sensitive to DCM than nonconjugators. Pemble et al. (1994), Hallier et al. (1994), Nelson et al. (1995), Katoh et al.(1996), Thier et al. (1998), and El-Masri et al. (1999) have discussed the importance of GSST1 polymorphism in many different ethnic groups in the risk assessment of haloalkanes such as DCM. GSTT1 can catalyze the GSH conjugation of DCM via a metabolic pathway that has been shown to be mutagenic in *Salmonella typhimurium* mutagenicity tester strains and was believed to be responsible for the carcinogenicity of DCM reported in the NTP (1986) DCM inhalation bioassay study in mice. Thus, concerns have been raised that this polymorphism is an important factor that will affect the risk estimates for DCM (El-Masri et al. 1999, Jonsson and Johanson 2001).

SUMMARY OF ORIGINAL APPROACH AND ACs

The SMACs for exposure durations of 1 h to 7 d were based on CNS de-

pression. The 30- and 180-d SMACs were based on hepatotoxicity. Wong et al. (1996) also estimated ACs based on the end point of carcinogenicity.

Wong (1996) derived a 1-h AC based on the adverse CNS effects of DCM vapors reported by many investigators in humans, both in an occupational setting and in controlled studies. He based the critical effect on reports by Peterson (1978), Putz et al. (1979), Winneke (1974, 1981), Winneke and Fodor (1976), and Stewart et al. (1972) in which impaired hand-eye coordination, increased tracking error, and impaired vigilance were reported in human volunteers exposed to different concentrations of DCM for different lengths of time. Many of these studies had measured COHb, and the effects correlated with the concentrations of serum COHb resulting from DCM oxidative metabolism. Wong adopted a strategy of using the previously established NASA SMACs for CO (Wong 1994) as a basis for deriving some ACs for DCM. On that basis, the 1- and 24-h ACs for blood COHb level were set at 3% (Wong 1994). Winneke (1974) stated that the observed CNS effects are directly related to DCM and not COHb formed from its metabolism, because there were no CNS effects at 100 ppm, in spite of the formation of COHb in blood. If one assumes a threshold concentration of COHb that will not produce any such effects, Wong's approach (Wong 1996) is reasonable. He collated the data from the human volunteer studies in which concentrations of COHb were measured after various concentrations and durations of DCM exposures and derived a linear regression of the total dose of DCM versus the percent increase of COHb concentrations. From the slope of the fitted regression line, a concentration of DCM was calculated that will produce an increase of 2.4% COHb over the background nominal concentration of 0.6% COHb, which is produced by endogenous CO production in the human body. This corresponded to a DCM concentration of 100 ppm as the 1- and 24-h no-observed-adverse-effect levels NOAELs.

Wong (1996) used another method to derive a 24-h AC; the COHb concentration was used as a surrogate variable for CO formation from DCM. He used the data for CO and COHb formation computed from the PBPK model developed by Andersen et al. (1991), which modeled the parent compound and its metabolites, CO and COHb, in rats and humans. The human model was validated with human volunteers exposed to DCM at 100 or 350 ppm for 6 h. Wong (1996) calculated that an exposure of 35 ppm for 24 h would produce a final COHb of 3%. It was indirectly implied that neurotoxicity (CNS depression, visual performance, and perception of time) was the adverse end point used, as that is based on the levels of COHb.

For deriving the 7-d AC, Wong considered neurotoxicity and hepatotoxicity to be critical effects. For ACs for 7 d and longer, Wong adopted the 7-, 30-, and 180-d AC he had derived for CO, with a target COHb concentration of 1.6%. For CNS effects, he followed the approach mentioned above using the Andersen et al. (1991) PBPK model and computed an AC of 14 ppm for DCM, which will lead to increased COHb concentrations from the background 0.6% to 1.6%. Wong (1996) rounded the 14 ppm to 15 ppm in the AC summary table (see Table 15-1 for a summary 1996 SMACs).

TABLE 15-1 Summary of Previously Published SMACS for DCM (Wong 1996)

Duration	ppm	mg/m^3	Critical effect	Principal studies
1 h	100	350	CNS depression	Various human data
24 h	35	120	CNS depression	Andersen et al. 1991(PBPK)
7 d	15	50	CNS depression	Andersen et al. 1991
30 d	5	20	Hepatotoxicity	Burek et al. 1984
180 d	3	10	Hepatotoxicity	Burek et al. 1984

The 7-d AC was also derived using hepatotoxicity as the end point. In a 13-wk study (NTP 1986) in which groups of 10 rats per gender (F344/N) and 10 mice per gender (B6C3F1) were exposed to air containing 0, 525, 1,050, 2,100, 4,200, or 8,400 ppm cytoplasmic vacuolization and necrosis of the liver as well as hemosiderosis and focal granulomatous inflammation were noted in mice after repetitive exposures to DCM greater than 2,100 ppm for 6 h/d, 5 d/wk for 13 wk. Using the NOAEL of 2,100 ppm, a 7-d AC of 210 ppm was derived after a species factor of 10 was applied.

For a 30-d AC derivation, Wong used the 2-y NTP (1986) study (in which rats and mice were exposed to 0, 1,000, 2,000, or 4,000 ppm of DCM for 6 h/d, 5 d/wk for 102 wk) for cytoplasmic vacuolization, hemosiderosis, and focal granulomatous inflammation in liver. The Burek et al. (1984) 2-y study looked for similar effects at a lower dose of 500 ppm. Using the LOAEL of 500 ppm, and after applying factors of 10 for LOAEL to NOAEL and species, each author arrived at a 30-d AC of 5 ppm (see Wong 1996 for details).

For a 180-d AC derivation, Wong used the same hepatotoxicity as the end point reported in the Burek et al. (1984) study in which 500 ppm was identified as the LOAEL. The author derived an AC of 3.6 ppm as a 180-d AC after adjusting for the LOAEL for discontinuous to continuous exposure and applying factors of 10 for LOAEL to NOAEL and for species extrapolation. The author rounded the value of 3.6 ppm to 3.0 in the AC summary table (see Table 15-1).

For deriving a 180-d AC for carcinogenicity risk, the 2-y NTP carcinogenesis bioassay data, as summarized by Mennear et al. (1988), were used. The results of the 2-y carcinogenicity bioassay conducted in various species exposed to DCM by inhalation are summarized in Table 15-2.

Several epidemiologic studies have been conducted of workers exposed to DCM in the manufacturing of triacetate fibers (Lanes et al. 1990), photographic film, and paint and varnish. The collected data do not demonstrate a strong, statistically significant excess cancer risk associated with occupational exposures to DCM below 500 ppm (Ott et al. 1983; Hearne et al. 1987, 1990 [Kodak workers study]; Lanes et al. 1993). However, positive results from the animal carcinogenicity tests have driven some regulatory agencies to declare that DCM may be carcinogenic to humans.

TABLE 15-2 Summary of Rodent Carcinogenicity Bioassays for Exposure to DCM by Inhalation

Route and Dosing	Dosage (Number of Animals)	Species/ Strain	Comments	Reference
Inhalation 6 h/d, 5 d/wk, 2 y	0, 2,000, 4,000 ppm; 50 mice/gender/ dose	B6C3F1 mouse	Dose-related increases in both hepatocellular adenomas and hepatocellular carcinomas; increased incidence of alveolar/bronchiolar adenomas in lungs of both genders at both doses; also, increases in the incidence of animals bearing multiple lung tumors.	NTP 1986
Inhalation 6 h/d, 5 d/wk, 2 y	0, 1,000, 2,000, 4,000 ppm; 50 rats/gender/ dose	F344 rat	Mammary and integumentary fibromas and fibrosarcomas in both genders; increased incidence of leukemia in females—thus, clear evidence of carcinogenicity in females; some evidence of carcinogenicity in males.	NTP 1986
Inhalation 6 h/d, 5 d/wk, 2 y	0, 500, 1500, 3,500 ppm; 95 rats/gender/ dose	Sprague-Dawley rat	Number of female rats with a benign tumor did not increase, but total number of these tumors increased; in male rats, the number of sarcomas near the salivary gland increased.	Burek et al. 1984
Inhalation 6 h/d, 5 d/wk, 2 y	0, 500, 1500, 3,500 ppm; 90 hamsters/ gender/dose	Syrian golden hamster	No malignant tumors observed.	Burek et al. 1984
Inhalation 6 h/d, 5 d/wk, 2 y	0, 50, 200, 500 ppm; 70 rats/gender/ dose	Sprague-Dawley rat	No increase in malignant tumors even at 500 ppm.	Nitschke et al. 1988b

In the NTP study (1986), exposure to DCM for 2 y at 0, 2,000, and 4,000 ppm produced 3 of 50, 30 of 48, and 41 of 48 cases of lung tumors and 3 of 50, 16 of 48, and 40 of 48 cases of liver tumors, respectively, in female B6C3F1 mice. Instead of using the airborne DCM concentrations to calculate the 1 in 10,000 tumor risk, Wong used the equivalent concentration of active metabolite produced by the GST pathway (dose metrics) in the lung and liver, as estimated by a PBPK model (Andersen et al. 1987). The exposure concentrations of 2,000 and 4,000 ppm were substituted by the corresponding values of the metabolites produced in the liver and lung in the multistage linearized model as the doses against tumor incidence. According to the Andersen et al. (1987) PBPK model,

DCM exposure concentrations of 6 and 12 ppm for lung and liver, respectively, for humans will result in a lifetime excess tumor risk of 1 in 10,000. The lower concentration of 6 ppm (for lung) was then used in the final risk assessment. After adjusting the 6 ppm for the discontinuous to continuous exposure, the author arrived at a risk value of 1.1 ppm for a lifetime excess lung tumor incidence of 1 in 10,000. For calculating the 180-d cancer risk, Wong used the NRC (1992) recommended formula for calculating a cancer risk from a lifetime exposure to less than lifetime durations that resulted in a factor of 146.7. By multiplying 1.1 ppm with this factor, a value of 160 ppm was established as the exposure that would produce an excess risk of lung tumor incidence of 1 in 10,000 after 180 d of continuous exposure to DCM (see Wong 1996 for details).

CHANGES IN FUNDAMENTAL NRC-RECOMMENDED APPROACHES

The original SMACs were published in 1996 before the current NRC approaches to data analysis (BMD and ten Berge approach) were commonly used. Values were derived using the approach of identifying and modifying LOAEL and NOAEL by default safety factors.

New Data Since 1995 and Data Not Discussed in the Wong (1996) Document

A survey of the literature around and after 1995 for any DCM toxicity studies yielded no new experimental data that could change the previous SMACs or that could be used to develop a 1,000-d AC. However, numerous papers have been published on the PBPK modeling and simulation of DCM metabolism to active intermediates, which have refined the model or addressed the variability of the parameters used, their distribution in the population, and the uncertainty associated with the distribution of model parameters. Some publications recommended factors that should be considered and incorporated in the PBPK model simulation to derive meaningful risk estimates; for example, including the effect of exercise (Dankovic and Bailer 1994, Jonsson et al. 2001) and changes in target tissue kinetics because of aging (Thomas et al. 1996a,b).

Additional Studies Not Discussed by Wong (1996)

Nitschke et al. (1988a) conducted a 2-y inhalation toxicity and oncogenicity study in which they exposed male and female Sprague-Dawley rats to 0, 50, 200, or 500 ppm of DCM for 6 h/d, 5 d/wk for 2 y. These doses were chosen to identify a NOAEL, because the Burek et al. (1984) study determined only a LOAEL of 500 ppm for adverse hepatic effects. Furthermore, because the MFO system, and thus COHb formation, becomes saturated at 500 ppm, the authors

chose these doses to be close to the saturation region to obtain a monotonic dose-response relationship. Liver lesions included increased incidence of hepatocellular vacuolization in male and female rats exposed to DCM at 500 ppm and an increased incidence of multinucleated hepatocytes in female rats. These effects were not seen in the 200-ppm DCM exposure treatment group. In the 2-y study (Nitschke et al.1988a), these authors identified 200 ppm of DCM as a NOAEL and 500 ppm as a LOAEL for hepatotoxicity in Sprague-Dawley rats.

Gross pathology in all tissues and detailed histopathology were examined. Histopathologic lesions in DCM-treated female rats (up to 500 ppm) indicated that the observed incidences of benign mammary tumors (adenomas, fibromas, and fibroadenomas) with no progression to malignancy were comparable to historical control values. The authors also measured DNA synthesis (using [^{3}H]thymidine incorporation) in the livers of female rats exposed to 200 or 500 ppm of DCM for 6 or 12 mo. The results were comparable to those for the control groups.

COHb was also measured during the interim durations of 6 and 12 mo. Though the levels increased as a function of dose, the rate of increase was less than linear with dose.

Another study not discussed in the 1996 document on DCM was a 1988 study by Nitschke et al. (1988b), who conducted a two-generation DCM inhalation reproductive study in F344 rats. Male and female rats (30 each), approximately 7 wk old, were exposed to DCM at 0, 100, 500, or 1,500 ppm for 6 h/d, 5 d/wk for 14 wks and then mated (within the same treatment group) to produce F_1 litters. The F_0 rats continued to be exposed. Fertility, litter size and neonatal growth, and survival were determined as reproductive indices, and these were done for two successive generations. Also, after weaning, 30 F_1 pups per gender per group (randomly selected) were exposed to DCM for 17 wk under the same schedule. They were mated to produce F_2 litters. All animals were examined for visible lesions, and tissues were examined histopathologically. No changes were reported in any of the reproductive parameters measured and no abnormal tissue histopathology was observed in any of the F_0, F_1, or F_2 weanlings. So, one might identify at least 1,500 ppm as the NOAEL for reproductive effects for up to 31 wk. As the NOAEL dose is rather high, NASA decided not to derive an AC for this end point.

RATIONALE FOR THE 1,000-d SMAC

In general, the ACs were determined according to the NRC guidelines for developing SMACs for space station contaminants (NRC 1992).

The exposure limits set or recommended by other organizations are presented in Table 15-3. Occupational Safety and Health Administration (OSHA) reduced the 8-h time-weighted average (TWA) permissible exposure level (PEL) and the short-term exposure limit in 1997, as it believed that strong evidence existed for a risk of human cancer incidence from occupational exposure to DCM.

TABLE 15-3 Exposure Limits Recommended or Set by Other Organizations

Organization, Standard	Concentration, ppm	Concentration, mg/m^3	Reference
ACGIH			ACGIH 1986
TLV TWA, 8 h[a]	50	174	
OSHA			OSHA (62 Fed.
PEL TWA, 8 h[a,]	25	87	Reg.1491 [1997])
Action level	12.5	44	
EPA carcinogenicity risk			EPA 1995
1 in 10,000	0.06	0.2	
1 in 100,000	0.006	0.02	
1 in 1,000,000	0.0006	0.002	
ATSDR			ATSDR 2000
Acute duration inhalation MRL	0.6	2.1	
Intermediate duration inhalation MRL (150-364 d)	0.3	1.0	
Chronic duration inhalation MRL (≥365 d)	0.3	1.0	

[a]OSHA reduced the PEL from 500 ppm to 25 ppm.This is based on an upper 95th percentile of human internal dose distribution; if a mean of this distribution of this analysis were used, then the maximum likelihood estimate of extra cancer risk would be 1.24/1,000 for 25 ppm of DCM of an occupational lifetime exposure (8 h/d, 5 d/wk, for 45 y). The OSHA estimated risk at the previous PEL of 500 ppm is 126 excess cancer deaths per 1,000 workers; the revised standard of 25 ppm will effect a substantial reduction to a risk of 3.62 deaths per 1,000 workers occupationally exposed to DCM for a working lifetime.
Abbreviations: ACGIH, American Conference of Governmental Industrial Hygienists; ATSDR, Agency for Toxic Substances and Disease Registry; EPA, U.S. Environmental Protection Agency; MRL, minimal risk level; TLV, Threshold Limit Value; TWA, time-weighted average.

STATUS OF CARCINOGENICITY CLASSIFICATION FOR DCM

- International Agency for Research on Cancer (IARC) classification: Group 2B. Possibly carcinogenic to humans (inadequate evidence for the carcinogenicity of DCM in humans and sufficient evidence in experimental animals) (IARC 1999).
- U.S. Environmental Protection Agency (EPA) classification: B2 carcinogen (a probable human carcinogen) (EPA 1995).
- Health Canada classification: Group II (probably carcinogenic to humans) (Health Canada 1993).

The Agency for Toxic Substances and Disease Registry (ATSDR) acute inhalation minimal risk level (MRL) was derived from the behavioral and performance effects of DCM in a human volunteer study by Winneke (1974). Auditory and visual performance and psychomotor task performance were decreased.

The inhalation MRL for the intermediate duration was based on cytoplasmic vacuolization and nonspecific tubular degeneration changes in the kidney reported in rats in a 14-wk DCM exposure study by Haun et al. (1972). The MRL for the chronic exposure duration was derived from the 2-y inhalation toxicity study in rats, in which hepatocellular cytoplasmic vacuolization with fatty changes and multinuclear hepatocytes were reported (Nitschke et al. 1988b).

DERIVATION OF 1,000-d ACs

A toxicity summary for neoplastic and non-neoplastic adverse end points from studies used for deriving the 1,000-d AC are shown in Tables 15-2 and 15-4.

The 1,000-d AC for DCM by inhalation is based on liver toxicity reported in three studies that used different ranges of concentrations. The NTP (1986) study reported cytoplasmic vacuolization, hemosiderosis, and focal granulomatous inflammation in liver in rats exposed to DCM at 1,000, 2,000, or 4,000 ppm, 6 h/d, 5 d/wk, for 2 y. Burek et al. (1984), who exposed male and female rats (90 each) to DCM at 0, 500, 1,500, or 3,500 ppm for 6 h/d, 5 d/wk, for 2 y, similarly reported cytoplasmic vacuolization, indicative of fatty liver, at concentrations as low as 500 ppm. This study identified a LOAEL of 500 ppm for hepatotoxicity. A NOAEL concentration was not identified in either of the above studies. In the third study, a 2-y inhalation toxicity and oncogenicity study by Nitschke et al. (1988a), male and female Sprague-Dawley rats were exposed to 0, 50, 200, or 500 ppm of DCM for 6 h/d, 5 d/wk for 2 y. These doses were chosen to identify a NOAEL and to possibly obtain a monotonic dose-response relationship. Liver lesions observed included increased incidence of hepatocellular vacuolization with fatty liver in male and female rats exposed to 500 ppm of DCM as well as increased incidence of multinucleated hepatocytes in female rats. These effects were not observed in the 200-ppm DCM exposure group. A NOAEL for hepatotoxic effects could be identified as 200 ppm in this study. A 1,000-d AC was derived from this study with supporting observations of similar liver toxicity end points from the NTP and Burek studies described above.

Nitschke et al. (1988b) used three exposure concentrations. The incidence of hepatic vacuolization data was used in the EPA BMD software (EPA 2007) to derive the benchmark concentration (BMC) and the 95% lower confidence boundary on the BMC (the BMCL) for benchmark responses (BMR) of 10% ($BMCL_{10}$), 5% ($BMCL_{05}$), and 1% ($BMCL_{01}$). Upon review of the results, it was decided to use a BMR of 10% as the most appropriate point of departure, on the advice of the statistician expert of the NRC committee who reviewed this document. The choice of a BMR of 10% was primarily based on the calculated added risk and biological plausibility of detecting such a change without being too conservative. Six different dose-response models offered in the EPA software were used, and BMC_{10} and $BMCL_{10}$ were summarized for all models. No single

TABLE 15-4 Summary of Noncancer Effects of Chronic Inhalation Exposures to DCM

Route and Dosing	Dosage (Number. of Animals)	Species/Strain	Description of Effects	Reference
Inhalation 6 h/d, 5 d/wk, 2 y	0, 2,000, 4,000 ppm; 50 mice/gender/dose	B6C3F1 mouse	2,000 ppm: increased cytoplasmic vacuolization in liver, consistent with fatty liver; 4,000 ppm: hepatic cytologic degeneration in both genders; for controls, 2,000, and 4,000 ppm dose groups, respectively, testicular atrophy in male mice (0/50, 4/50, 31/50) and ovarian atrophy (6/50, 28/47, 32/43) and atrophy of the uterus (0/50, 1/48, 8/47) in female mice.	NTP 1986, Mennear et al. 1988
Inhalation 6 h/d, 5 d/wk, 2 y	0, 1,000, 2,000, 4,000 ppm; 50 rats/gender/ dose	F344 rat	1,000 and 2,000 ppm: hemosiderosis, hepatomegaly, cytoplasmic vacuolization, focal necrosis, focal granulomatous inflammation in liver (female rats) and bile ducts (male rats); 4,000 ppm: all the above plus reduced survival, squamous metaplasia of nasal cavity, benign papillary mesothelioma of tunica vaginalis in males, mononuclear cell leukemia in females.	NTP 1986; data summarized by Mennear et al. 1988
Inhalation 6 h/d, 5 d/wk, 2 y	0, 1,000, 2,000, 4,000 ppm; 50 rats/gender/dose	F344/N rat	1,000-4,000 ppm: renal tubular cell degeneration in female rats.	Mennear et al. 1988, NTP 1986
Inhalation 6 h/d, 5 d/wk	0, 500, 1,500, 3,500 ppm; 95 rat/gender/ dose	Sprague-Dawley rat	500 ppm: cytoplasmic vacuolization in liver cells, multinucleated hepatocytes in female rats, 13% increase in COHb. 1,500 ppm: in addition to the above, necrosis of hepatocytes and chronic glomerulonephropathy were seen in male rats.	Burek et al. 1984

(Continued)

TABLE 15-4 Continued

Route and Dosing	Dosage (Number. of Animals)	Species/Strain	Description of Effects	Reference
Inhalation 6 h/d, 5 d/wk, 2 y	0, 500, 1,500, 3,500 ppm; 90 hamsters/gender/dose	Syrian golden hamster	500-3,500 ppm: no injuries in hamsters; 27% increase in COHb.	Burek et al. 1984
Inhalation 6 h/d, 5 d/wk, 2 y	0, 50, 200, 500 ppm; 70 rats/gender/dose; end points were also evaluated at 6, 12, 15, and 18 mo.	Sprague-Dawley rat	500 ppm: (at terminal sacrifice) histopathologic lesions in liver (multinucleated hepatocytes in females) and mammary tissues of rats (increased spontaneous benign mammary tumors). NOAEL = 200 ppm; COHb concentrations increased (less than linear rate) with the DCM concentrations but leveled off after 6 mo of the 2-y study.	Nitschke et al. 1988b

model or detailed algorithm to choose a particular one was used to derive the final BMC or BMCL. A summary of the data from Nitschke et al. (1988b) used for obtaining the BMCs are shown in Table 15-5. Some of the output values from using the BMD software on this data are summarized in Table 15-6. Of the two end points, cytoplasmic vacuolization and multinucleated hepatocytes, the former was used because of its extensive use as an adverse end point in the toxicology literature. NASA calculated the BMC for both end points, but only one is shown here.

The model-averaged BMC was calculated as a weighted average of the individual BMCs using the posterior probabilities as weights to account for dose-response model uncertainty. However, the NASA statistician advised NASA that this weighted-average method should not be applied to lower confidence bounds for BMCs—that is, to BMCLs—as BMCL does not represent model uncertainty (A. Feiveson, NASA, personal communication, 2004). Therefore, it was decided to estimate the BMCL from the model averaged BMC and the weighted mean of the BMC/BMCL ratio.

Similar calculations done using the incidences of multinucleated hepatocytes gave an estimated $BMCL_{10}$ comparable to that of the $BMCL_{10}$ for hepatic vacuolization; the data are not included here.

TABLE 15-5 Incidence of Hepatic Vacuolization in Rats from DCM Inhalation

DCM, ppm	Number of Rats	Incidence	% affected
0	70	41	59
50	70	42	60
200	70	41	59
500	70	53	76

Source: Data from Nitschke et al. 1988b. Reprinted with permission; copyright 1988, *Applied Toxicology*.

TABLE 15-6 Summary of BMC and BMCL for Hepatic Vacuolization for Various Models

BMD model	AIC	*P* value	BMC_{10}, ppm	$BMCL_{10}$, ppm	$BMC_{10}/BMCL_{10}$ ratio
Gamma	365.80	0.980	383	69.7	5.49
Weibull	367.82	0.843	442	69.7	6.34
Multistage	366.12	0.945	292	69.0	4.23
Probit	366.92	0.559	119	74.2	1.60
Probit (log)	367.82	0.843	400	121.7	3.29
Logistic	366.90	0.551	117	72.3	1.63
Log-logistic	367.80	0.843	434	58.2	7.46

Abbreviation: AIC, Akaike information criterion.

The models were averaged using weights derived from the Akaike information criterion. Weighted means were calculated for BMC_{10} and the ratio, which were 307 ppm and 4.10, respectively. The weighted mean for BMC_{10} was divided by the weighted ratio to obtain the estimated $BMCL_{10}$ of 75 ppm. This value was used as the point of departure DCM concentration for calculating the AC for liver toxicity.

The 1,000-d AC for hepatotoxicity will use the $BMCL_{10}$ concentration, which will be adjusted from a discontinuous to a continuous duration. The NRC Committee on Spacecraft Exposure Guidelines suggested that, although adjustment factors for daily exposure and number of daily exposures per week should be used to arrive at a dose rate for continuous exposures, an additional correction of 728 d (104 wk) to 1,000 d is not necessary, because 2 y is a greater fraction of a rat's lifetime than 1,000 d is of a human lifetime. Thus, the value derived without a time extrapolation factor will still be protective for a long duration. The $BMCL_{10}$ concentration will be adjusted for discontinuous to continuous exposure by using the adjustment factors of (adjusted) 6h/24h and 5d/7d.

$$BMCL_{10(adjusted)} = 75\ ppm_{(estimated\ BMCL10)} \times [6\ h/24\ h \times 5\ d/7\ d]_{(discont.\ to\ contin.)}$$
$$= 13.39, \text{ rounded to} 13.5\ ppm$$

$$1{,}000\text{-d } AC_{(hepatotoxicity)} = BMCL_{10\ (adjusted)} \times (1/10)_{(species\ factor)}$$
$$= 1.35\ ppm, \text{ rounded to } 1.4\ ppm$$

1,000-d AC for Nephrotoxicity Using Data from NTP (1986)

Another end point was used to derive a 1,000-d AC for a non-neoplastic effect reported in the NTP (1986) study. This study reported that female F344/N rats showed treatment-related squamous metaplasia of the nasal cavity, degeneration of kidney tubules, and fibrosis of the spleen when exposed to 1,000, 2,000, or 4,000 ppm of DCM by inhalation for 6 h/d, 5 d/wk for 102 wk (Table 15-7). The LOAEL appears to be 1,000 ppm and no NOAEL could be identified. As the data showed a reasonable dose-response profile, they were processed by the BMD method and the LOAEL-NOAEL method was, therefore, not used.

TABLE 15-7 Non-neoplastic Changes in Female F344/N Rats Exposed to DCM for 2 y

	Incidence			
Lesions	Control	At 1,000 ppm	At 2,000 ppm	At 4,000 ppm
Renal tubular cell degeneration	14/50	20/50	22/50	25/49
Splenic fibrosis	0/50	2/50	4/50	4/49
Nasal cavity squamous metaplasia	1/50	2/50	3/50	9/50

Sources: Data from NTP 1986 and Mennear et al. 1988.

The authors did not say whether these data were statistically analyzed. NASA assumes, based on the trend, that 1,000 ppm is the LOAEL. NASA reviewed the data and, judging by the excess risk at 1,000 ppm for splenic fibrosis and the effect on the nasal cavity, it was clear that the AC that would be calculated for renal tubular degeneration would drive the 1,000-d AC for the nonneoplastic lesions reported for the NTP study.

A BMC was derived using the BMD method with the EPA BMD software. For this derivation, a BMR was set at 1% excess risk. The BMC_{01} and $BMCL_{01}$ and the ratio summary data are presented in Table 15-8.

The probit (log) model and the quantal-quadratic model BMDs were omitted as the estimated curve did not fit the data well.

The weighted $BMCL_{01}$ was computed from the weighted mean of the BMC_{01} and the weighted mean of the ratios of BMC_{01} and $BMCL_{01}$ as described previously. The model weighted mean for BMC_{01} was 94 ppm and the weighted mean of the BMC/BMCL ratio was 1.485. The estimated $BMCL_{01}$ is 63 ppm.

A 1,000-d AC for renal tubular cell degeneration was calculated after ascertaining a $BMCL_{01}$ (adjusted), which are dose estimates obtained for a continuous exposure from a discontinuous exposure by multiplying $BMCL_{01}$ by 6 h/24 h and 5 d/7 d. As explained earlier, no factor is used to account for the extrapolation for 1,000 d from 2 y (730 d).

$$BMCL_{01(adjusted)} = 63 \text{ ppm}_{(\text{estimated BMCL01})} \times [6 \text{ h}/24 \text{ h} \times 5 \text{ d}/7 \text{ d}]_{(\text{discontin. to contin.})} = 11.25 \text{ ppm}$$

$$1{,}000\text{-d } AC_{(\text{nephrotoxicity})} = BMCL_{01\ (\text{adjusted})} \times 1/10_{(\text{species factor})} = 1.12 \text{ ppm, rounded to } 1 \text{ ppm}$$

Thus, the 1,000-d AC for nephrotoxicity is 1.0 ppm.

1,000-d AC for Carcinogenicity Risk

In the NTP (1986) study, the incidence of lung neoplasms in B6C3F1 mice exposed to DCM for 2 y was as follows: in the males, the incidence of adenomas and carcinomas combined was 5/50 in controls, 27/50 in the 2,000-ppm group, and 40/50 in the 4,000-ppm group; in the females, the incidence of adenomas and carcinomas combined was 3/50 in controls, 30/48 in the 2,000-ppm group, and 41/48 in the 4,000-ppm group.

The incidence of hepatocellular neoplasms in B6C3F1 male mice exposed to DCM for 2 y was as follows: in male mice, adenomas and carcinomas combined were 22/50 in controls, 24/49 in the 2,000-ppm group, and 33/49 in the 4,000-ppm group; in female mice, they were 3/50 in controls, 16/48 in the 2,000-ppm group, and 40/48 in the 4,000-ppm group. According to the NTP report, the historical controls for this end point in this strain of mice are “Male:

TABLE 15-8 DCM and Renal Tubular Degeneration (NTP 1986): Summary of Results from the BMD Method

Model[a]	*P* value	AIC	BMC_{01}	$BMCL_{01}$	$BMC_{01}/BMCL_{01}$ ratio
Gamma	0.799	267.5	102.58	58.55	1.75
Weibull	0.799	267.5	102.58	58.55	1.75
Probit (no log)	0.706	267.8	138.15	91.99	1.50
Probit (log)	0.482	268.6	646.22	393.30	1.64
Logistic (no log)	0.699	267.8	141.33	94.67	1.49
Log-logistic	0.868	267.4	82.50	41.98	1.97
Multistage 2	0.799	267.5	102.58	58.55	1.75
Quantal linear	0.799	267.5	102.58	58.55	1.75
Quantal quadratic	0.413	268.9	699.57	500.45	1.40

[a]Both multistage degrees 2 and 3 gave the same values. Data from quantal linear and quantal quadratic were not included in the model averaging, as these two models are a variation of the multistage model. Data from the probit (log) model were also not included in the model averaging.
Abbreviation: AIC, Akaike information criterion.

33% ± 8% and for females it is 2.7% ± 2.99%," which are not very different from the numbers reported in this study.

There were only two treatment groups in this study. Initially, NASA contemplated doing BMD modeling for all these data. However, it was decided to adopt the carcinogenicity excess risk determination with suitable factors for NASA extended-duration and exploration missions, because it is based on the target tissue dose metrics. The advanced PBPK model that was developed for these data from NTP (1986) should be preferable to values that could be derived from BMD analysis based on exposure concentrations.

Data were collected in carcinogenicity bioassays using DCM exposure to three different species (rat, mouse, and hamster) by two routes of administration—oral and inhalation. NTP concluded that there was some evidence of carcinogenicity in male rats and there was clear evidence of lung and liver tumors in male and female mice as a result of exposure to DCM. OSHA believed that there was a significant risk of carcinogenicity to humans in an occupational setting and that the current PEL (time-weighted average PEL) is too high.

OSHA (62 Fed. Reg.1491 [1997]) based its risk analysis on two PBPK models that represented substantial refinement over the conventional risk estimates based on applied dose. While incorporating animal and human metabolic parameters in their risk analysis, OSHA extensively addressed the concepts of uncertainty, variability, and sensitivity of the model parameters used.

The choice of OSHA's risk estimate value is very sound, because OSHA (62 Fed. Reg.1491[1997]) used state-of-the-art advanced computational methods for PBPK modeling of the NTP lung carcinogenesis data with extrapolation to

humans for an occupational environment. This was the result of a collaborative effort of several experts in toxicology, pharmacokinetics, and mathematics. The derivation was extensively scrutinized and commented on by the scientific community.

OSHA (62Fed. Reg.1491 [1997]) developed two PBPK models using the 95th percentile of the distribution of GST metabolites from the Bayesian analysis as the input to the multistage model, instead of using the 95th percentile of the Monte Carlo simulation distribution of GST metabolites, as the input recommended by Clewell et al. (1993). The PBPK analysis showed a final estimate of risk of 3.62 deaths per 1,000 workers occupationally exposed to 25 ppm DCM for a working lifetime. An occupational lifetime here means exposure to DCM for 8 h/d, 5 d/wk for 45 y (as opposed to 70 y of human lifetime in EPA risk estimation procedures). Because of high confidence in the overall procedures that OSHA followed in developing and applying the PBPK model, NASA decided to use the new PEL value and modify it with factors that are applicable to NASA SMAC derivations.

NASA assumed 25 ppm as the acceptable risk concentration (based on OSHA PEL) for the exposure conditions. This AC is adjusted to reflect spacecraft exposure duration as follows:

$$AC_{(adjusted)} = 25\ ppm_{(OSHA\ PEL} \times [8\ h/24\ h \times 5\ d/7\ d]_{(discontin.\ to\ contin.)}$$
$$= 5.95, \text{ rounded to } 6\ ppm$$

NASA accepts a cancer risk factor of 1 in 10,000. OSHA's excess risk at 25 ppm is 3.62 deaths in 1,000, which may also mean 36.2 deaths in 10,000. Assuming a linear response relationship, the calculated exposure was divided by 36.2 to give a 1 in 10,000 risk, which is equal to 0.165 ppm if exposure continues for 45 y. This approach is very conservative and may overestimate the cancer risk.

In 1992, the NRC SMAC committee recommended that NASA use time-compression factors to derive carcinogenicity risk for durations shorter than a lifetime. In this case, the duration is 1,000 d and the time that OSHA calculated is 45 y. Using the formula and approach that NRC (1992) provided to NASA, this factor can be calculated in the following way.

According to NRC (1992), setting $k = 3$ (the number of stages in the carcinogenic process affected by DCM) and $t = 16{,}425$ d (occupational lifetime of 45 y) to 10,950 d (an initial exposure age of 30 y), the adjustment factor for 1,000 d can be calculated to be 61.92 d (NRC 1992).

Thus, the 1,000-d exposure that would produce a 1 in 10,000 excess cancer risk is as follows:

$$0.165\ ppm \times 61.92 = 10\ ppm$$

If NASA uses 70 y as a lifetime, then the time-compression factor for 1,000 d becomes 27.953. Then, the 1,000-d AC is as follows:

$$1{,}000\text{-d AC}_{(\text{carcinogenicity risk } 1/10{,}000)} = 0.165 \text{ ppm} \times 27.953_{(\text{time extrapolation, 70 y})}$$
$$= 4.6 \text{ ppm, rounded to 5 ppm}$$

Therefore, the 1,000-d AC for carcinogenicity risk at 1 in 10,000 is 5 ppm.

As carcinogenicity to humans has not been conclusively ascertained, the GST activity in the mouse lung appears to be greater than in rats and humans, and since the exposure time used here is only 1,000 d, there is a large enough margin of safety at the 1,000-d AC of 5 ppm.

Along the same lines, the compression factor for 180 d can be calculated as 146.7 (based on NRC 1992 guidelines), and the 180-d AC based on OSHA estimated values is 0.165 ppm × 146.7 = 24 ppm. Therefore, the 180-d AC for carcinogenicity risk for 1 in 10,000 will be 24 ppm.

A summary of the 1,000-d ACs derived is shown in Table 15-9.

REDERIVATION OF 30-d AND 180-d ACs

A summary of SMACs (Wong, 1996) is shown in Table 15-1. One-hour, 24-h, 7-d, and 30-d ACs were determined on the basis of CO generation and formation of COHb, and the values from different investigations were fitted into a regression curve to calculate the ACs for DCM. Also, Wong (1996) used the PBPK model developed by Andersen et al. (1991) to compute the AC for 7, 30, and 180 d.

Rederivation of 30-d AC Based on Hepatotoxicity

The 30-d AC was based on the hepatotoxicity of DCM. Wong (1996) used the same study that he had used for 180 d (the NTP and the Burek et al. study), basing the calculation on the LOAEL. This document uses the Nitschke et al. (1988b) study in which a NOAEL is identified.

Using BMD methodology on the dose-response data for hepatotoxicity, a $BMCL_{10}$ of 75 ppm was derived, as described in the section on 1,000-d AC derivation, which was used to derive a 30-d AC. First, this concentration was adjusted for discontinuous to continuous exposure as follows.

$$BMCL_{10(\text{adjusted})} = 75 \text{ ppm}_{(BMCL10)} \times [6 \text{ h}/ 24 \text{ h} \times 5 \text{ d}/7 \text{ d}]_{(\text{discontin. to contin.})}$$
$$= 13.39 \text{ ppm, rounded to } 13.4 \text{ ppm rounded}$$

NASA initially considered that it may not be appropriate to use a ten Berge interpolation factor from 2 y to 30 d and hence had derived the 30-d AC after using a species factor of 10 on the 2-y BMCL value of 75 ppm for hepatotoxicity without adjusting for discontinuous to continuous exposure. NASA thought that there might be enough margin of safety in this approach. However,

TABLE 15-9 Summary of 1,000-d ACs

Critical effect	AC, ppm	Principal Studies
Hepatotoxicity	1.4	Nitschke et al. 1988a
Nephrotoxicity	1.0	NTP 1986, Mennear et al. 1988
Carcinogenicity	5.0	NTP 1986, OSHA (62 Fed. Reg. 1491 [1997]) PBPK model extrapolation

the NRC Committee on spacecraft exposure guidelines (SEG) disagreed with NASA and concluded that NASA should use the ten Berge approach.

As the committee recommended, ten Berge's time conversion method was used (NRC guideline document, NRC 2000) to derive a concentration for 30 d from the 730-d data. The ten Berge approach (ten Berge et al. 1986) for time interpolation (from longer duration to shorter duration) uses a default exponent of 2, as suggested by the National Advisory Committee for Acute Exposure Guideline Levels for Hazardous Substances in 1997 when no relevant duration versus response data were available to calculate the value for the exponent. The ten Berge approach is as follows.

The ten Berge equation is $C^N \times T = K$

where C = concentration,13.4 ppm, N = exponent, default factor of 2, T = exposure days, 730 d, and $K = (13.4)^2 \times 730$ d

$C_{\text{(for 30 d)}} = (K/30)^{1/2}$ = approximately 66 ppm

Thus, the ten Berge adjusted concentration is 66 ppm.

30-d $AC_{\text{(hepatotoxicity)}}$ = 66 ppm × $1/10_{\text{(species factor)}}$
= 6.6 ppm, rounded to 7 ppm

Thus, 30-d AC = 7 ppm

NASA decided to use only hepatotoxicity as the adverse end point for derivation of the 30-d AC and for the 180-d AC and not the nephrotoxicity data reported in the NTP study (NTP 1986), because, in the 13-wk rat study conducted as a part of the 2-y study, NTP did not observe any nephrotoxicity even at a dose as high as 8,400 ppm.

Rederivation of 180-d AC Based on Hepatotoxicity

Wong derived the 180-d AC based on the NOAEL-LOAEL method using the NTP (1986) study and the Burek et al. (1984) study in which a NOAEL was not identified for hepatotoxicity. Wong applied a LOAEL-to-NOAEL factor and

a species factor and obtained a 180-d AC of 3.6 ppm for hepatotoxicity. In this document, the AC is updated using the Nitschke et al. (1988b) data, which indicated a NOAEL of 200 ppm for hepatic effects. Furthermore, the dose-response data is processed by the benchmark dose method and an AC is derived from the $BMCL_{10}$ using a BMR of 10% excess risk.

For calculating the 180-d AC, the $BMCL_{10}$ of 75 ppm for hepatic vacuolization derived earlier is adjusted for discontinuous to continuous exposure.

$$BMCL_{10(adjusted)} = 75 \text{ ppm}_{(\text{estimated BMCL10})} \times [6 \text{ h}/24 \text{ h} \times 5 \text{ d}/7 \text{ d}]_{(\text{discontin. to contin.})} = 13.39 \text{ ppm, rounded to } 13.4 \text{ ppm rounded.}$$

The ten Berge approach (ten Berge et al. 1986), as mentioned above for time interpolation, was used as follows. $C^N \times T = K$ where N is the exponent, T is exposure days (730 d), and K is a constant. After calculating K, it was used to derive C with $T = 180$ d (target exposure duration).

$$\text{180-d ten Berge time adjusted } BMCL_{10} = 28 \text{ ppm}$$

$$\text{180-d } AC_{(\text{hepatotoxicity})} = 28 \text{ ppm} \times 1/10_{(\text{species factor})} = 2.8 \text{ ppm, rounded to } 3 \text{ ppm}$$

Thus, the 180-d AC for hepatotoxicity is 3 ppm.

A final summary of all SMACs for DCM is shown in Tables 15-10 and 15-11, with updated values incorporated as discussed in this document.

ADDITIONAL INFORMATION

During the development of this document, several articles were published on the risk assessment using PBPK modeling, especially for DCM. Most of these focused on using advanced statistical tools to address the probability distributions of variability and uncertainty of the input parameters used in the PBPK modeling so one can use the data of the outcome to estimate the effective dose (predict the target dose) in humans more accurately. For example, Marino et al. (2006) refined the mouse PBPK model used to characterize the dose response of the tumor incidence in lung and liver of male and female B6C3F1 mice noted in the NTP (1986) carcinogen bioassay study for DCM. The authors used the Bayesian Markov chain Monte Carlo (MCMC) analysis. They showed that the internal dosimetrics (the target organ dose, the reactive GSH metabolite, *S*-(chloromethyl)glutathione) was 3- to 4-fold higher than doses that support the EPA cancer risk assessment, meaning a decrease in the magnitude of the dose response (that is, EPA was 4 times more conservative).

Similarly, using the same methodology, David et al. (2006) refined and calibrated the human PBPK model for human DCM exposure (using human

TABLE 15-10 Summary of Spacecraft Maximum Allowable Concentration

Duration	ppm	mg/m^3	Critical effect	Principal studies
1 h	100	350	CNS depression	Various human data
24 h	35	120	CNS depression	Andersen et al. 1991
7 d[a]	14	49	CNS depression	Andersen et al. 1991
30 d	7	24	Hepatotoxicity	Nitschke et al. 1988b
180 d	3	10	Hepatotoxicity	Nitschke et al. 1988b
1,000 d	1	3.5	Nephrotoxicity	NTP 1986

[a]Wong (1996) rounded the 7-d SMAC of 14 to 15. This committee insisted that NASA not round this value to 15.

TABLE 15-11 Acceptable Concentrations for Cancer Risk of 1 in 10,000

Duration	ppm	mg/m^3	Critical effect	Principal studies
180 d	24	84	Cancer	NTP 1986, OSHA (62 Fed. Reg.1491 [1997])
1,000 d	5	18	Cancer	NTP 1986, OSHA (62 Fed. Reg.1491 [1997])

DCM exposure studies composed of 43 subjects from five published studies) by incorporating the human genetic GSTT1 polymorphism (20% nonconjugators in the U.S. population) into an MCMC PBPK model. The authors concluded that the unit risk for cancer from DCM (for lung and liver together) is only 9.33×10^{-10} (50th percentile), which is about 500 times lower than the current EPA unit risk of 4.7×10^{-7}. That means EPA is 500 times more conservative than what these advanced probabilistic PBPK modeling methodologies can estimate for human carcinogenic risk for inhaled DCM. Note that unit risk is defined as the risk of cancer from exposure to DCM at 1 microgram/m^3 over a lifetime. These studies and others were discussed in a forum on the reassessment of the cancer risk of DCM in humans (Starr et al. 2006).

REFERENCES

ACGIH (American Conference of Governmental Industrial Hygienists). 1986. Methylene chloride. Documentation of the Threshold Limit Values and Biologic Exposure Indices. American Conference of Governmental Industrial Hygienists, Cincinnati, Ohio.

Ahmed, A.E., and M.W. Anders. 1976. Metabolism of dihalomethanes to formaldehyde and inorganic halide. I. In vitro studies. Drug Metab. Dispos. 4(4):357-361.

Andersen, M.E., H.J. Clewell III, M.L. Gargas, F.A. Smith, and R.H. Reitz. 1987. Physiologically based pharmacokinetics and the risk assessment process for methylene chloride. Toxicol. Appl. Pharmacol. 87(2):185-205.

Andersen, M.E., H.J. Clewell III, M.L. Gargas, M.G. MacNaughton, R.H. Reitz, R.J. Nolan, and M.J. McKenna. 1991. Physiologically based pharmacokinetic modeling with dichloromethane, its metabolite, carbon monoxide, and blood carboxyhemoglobin in rats and humans. Toxicol. Appl. Pharmacol. 108(1):14-27.

Astrand, I., P. Ovrum, and A. Carlsson. 1975. Exposure to methylene chloride. I. Its concentration in alveolar air and blood during rest and exercise and its metabolism. Scand. J. Work Environ. Health 1(2):78-94.

ATSDR (Agency for Toxic Substances and Disease Registry). 2000. Toxicological Profile for Methylene Chloride. U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry, Atlanta, GA. September 2000 [online]. Available: http://www.atsdr.cdc.gov/toxprofiles/tp14.pdf [accessed Jan. 17, 2008].

Burek, J.D., K.D. Nitschke, T.J. Bell, D.L. Wackerle, R.C. Childs, J.E. Beyer, D.A. Dittenber, L.W. Rampy, and M.J. McKenna. 1984. Methylene chloride: A two year inhalation toxicity and oncogenicity study in rats and hamsters. Fundam. Appl. Toxicol. 4(1):30-47.

Casanova, M., D.A. Bell, and H.D. Heck. 1997. Dichloromethane metabolism to formaldehyde and reaction of formaldehyde with nucleic acids in hepatocytes of rodents and humans with and without glutathione S-transferase T1 and M1 genes. Fundam. Appl. Toxicol. 37(2):168-180.

Clewell, H.J. 1995. Incorporating biological information in quantitative risk assessment: An example with methylene chloride. Toxicology 102(1-2):83-94.

Clewell, H.J., J.M. Gearhart, and M.E. Andersen. 1993. Analysis of the Metabolism of Methylene Chloride in the B6C3F1 Mouse and its Implications for Human Carcinogenic Risk. Submission to OSHA Docket No. H-071, Exhibit No. 96. January 15, 1993.

Dankovic, D.A, and A.J. Bailer. 1994. The impact of exercise and intersubject variability on dose estimates for dichloromethane derived from a physiologically based pharmacokinetic model. Fundam. Appl. Toxicol. 22(1):20-25.

David, R.M., H.J. Clewell, P.R. Gentry, T.R. Covington, D.A. Morgott, and D.J. Marino. 2006. Revised assessment of cancer risk to dichloromethane. II. Application of probabilistic methods to cancer risk determinations. Regul. Toxicol. Pharmacol. 45(1):55-65.

DiVincenzo, G.D., and C.J. Kaplan. 1981. Uptake, metabolism, and elimination of methylene chloride vapor by humans. Toxicol. Appl. Pharmacol. 59(1):130-140.

DiVincenzo, G.D., F.J. Yanno, and B.D. Astill. 1972. Human and canine exposure to methylene chloride vapor. Am. Ind. Hyg. Assoc. J. 33(3):125-135.

El-Masri, H.A, D.A. Bell, and C.J. Portier. 1999. Effects of glutathione transferase theta polymorphism on the risk estimates of dichloromethane to humans. Toxicol. Appl. Pharmacol. 158(3):221-230.

EPA (U.S. Environmental Protection Agency). 1995. Dichloromethane (CASNR 75-09-02). Integrated Risk Information System, U.S. Environmental Protection Agency [online]. Available: http://www.epa.gov/NCEA/iris/subst/0070.htm [accessed Jan. 17, 2008].

EPA (U.S. Environmental Protection Agency). 2007. Benchmark Dose Software (BMDS) Version 1.4.1c. National Center for Environmental Assessment, U.S. En-

vironmental Protection Agency [online]. Available: http://www.epa.gov/ncea/bmds/ [accessed July 22, 2008].

Gargas, M.L., H.J. Clewell III, and M.E. Anderson. 1986. Metabolism of inhaled dihalomethanes in vivo: Differentiation of kinetic constants for two independent pathways. Toxicol. Appl. Pharmacol. 82(2):211-223.

Guengerich, F.P., D.H. Kim, and M. Iwasaki. 1991. Role of human cytochrome P-450 IIE1 in the oxidation of many low molecular weight cancer suspects. Chem. Res. Toxicol. 14(2):168-179.

Hallier, E., K.R. Schroder, K. Asmuth, A. Dommermuth, B. Aust, and H.W. Goergens. 1994. Metabolism of dichloromethane (methylene chloride) to formaldehyde in human erythrocytes: Influence of polymorphism of glutathione transferase theta (GST T1-1). Arch. Toxicol. 68(7):423-427.

Haun, C.C., E.H. Vernot, K.I. Darmer, and S.S. Diamond. 1972. Continuous animal exposure to low levels of dichloromethane. Paper No. 12. Pp. 199-208 in Proceedings of the Third Annual Conference on Environmental Toxicology. AMRL-TR-72-130. Wright-Patterson Air Force Base, Dayton, OH.

Health Canada. 1993. Canadian Environmental Protection Act (CEPA). Priority Substances List (PSL) Assessment Report: Dichloromethane. Ottawa: Ministry of Public Works and Government Services [online]. Available: http://www.hc-sc.gc.ca/ewh-semt/alt_formats/hecs-sesc/pdf/pubs/contaminants/psl1-lsp1/dichloromethane/dichloromethane-eng.pdf [accessed July 14, 2008].

Hearne, F.T., F. Grose, J.W. Pifer, B.R. Friedlander, and R.L. Raleigh. 1987. Methylene chloride mortality study: Dose-response characterization and animal model comparison. J. Occup. Med. 29(3):217-228.

Hearne, F.T., J.W. Pifer, and F. Grose. 1990. Absence of adverse mortality effects in workers exposed to methylene chloride: An update. J. Occup. Med. 32(3):234-240.

IARC (International Agency for Research on Cancer). 1999. Dichloromethane. Pp. 251-315 in Re-Evaluation of Some Organic Chemicals, Hydrazine, and Hydrogen Peroxide Part 1. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Lyon, France: IARC [online]. Available: http://www.inchem.org/documents/iarc/vol71/004-dichloromethane.html [accessed Jan. 18, 2008].

Jonsson, F., and G. Johanson. 2001. A Bayesian analysis of the influence of GSTT1 polymorphism on the cancer risk estimate for dichloromethane. Toxicol. Appl. Pharmacol. 174(2):99-112.

Jonsson, F., F. Bois, and G. Johanson. 2001. Physiologically based pharmacokinetic modeling of inhalation exposure of humans to dichloromethane during moderate to heavy exercise. Toxicol. Sci. 59(2):209-218.

Katoh, T., N. Nagata, Y. Kuroda, H. Itoh, A. Kawahara, N. Kuroki, R. Ookuma, and D.A. Bell. 1996. Glutathione S-transferase M1 (GSTM1) and T1 (GSTT1) genetic polymorphism and susceptibility to gastric and colorectal adenocarcinoma. Carcinogenesis 17(9):1855-1859.

Kubic, V.L., and M.W. Anders. 1975. Metabolism of dihalomethanes to carbon monoxide. II. In vitro studies. Drug Metab. Dispos. 3(2):104-112.

Lanes, S.F., A. Cohen, K.J. Rothman, N.A. Dreyer, and K.J. Soden. 1990. Mortality of cellulose fiber production workers. Scand. J. Work Environ. Health 16(4):247-251.

Lanes, S.F., K.J. Rothman, N.A. Dreyer, and K.J. Soden. 1993. Mortality update of cellulose fiber production workers. Scand. J. Work Environ. Health 19(6):426-428.

Marino, D.J, H.J. Clewell, P.R. Gentry, T.R. Covington, C.E. Hack, R.M. David, and D.A. Morgott. 2006. Revised assessment of cancer risk to dichloromethane: Part I

Bayesian PBPK and dose-response modeling in mice. Regul. Toxicol. Pharmacol. 45(1):44-54.

McKenna, M.J., J.A. Zempel, and W.H. Braun. 1982. The pharmacokinetics of inhaled methylene chloride in rats. Toxicol. Appl. Pharmacol. 65(1):1-10.

Mennear, J.H., E.E. McConnell, J.E. Huff, R.A. Renne, and E. Giddens. 1988. Inhalation toxicity and carcinogenesis studies of methylene chloride (dichloromethane) in F344/N rats and B6C3F1 mice. Ann. N.Y. Acad. Sci. 534:343-351.

Nelson, H.H., J.K. Wiencke, D.C. Christiani, T.J. Cheng, Z.F. Zuo, B.S. Schwartz, B.K. Lee, M.R. Spitz, M. Wang, X.P. Xu, and K.T. Kelsey. 1995. Ethnic differences in the prevalence of the homozygous deleted genotype of glutathione S-transferase theta. Carcinogenesis 16(5):1243-1245.

Nitschke, K.D., D.L. Eisenbrandt, L.G. Lomax, and K.S. Rao. 1988a. Methylene chloride: Two-generation inhalation reproductive study in rats. Fundam. Appl. Toxicol. 11(1):60-67.

Nitschke, K.D., J.D. Burek, T.J. Bell, R.J. Kociba, L.W. Rampy, and M.J. McKenna. 1988b. Methylene chloride: A 2-year inhalation toxicity and oncogenicity study in rats. Fundam. Appl. Toxicol. 11(1):48-59.

NRC (National Research Council). 1992. Guidelines for Developing Spacecraft Maximum Allowable Concentrations for Space Station Contaminants. Washington, DC: National Academy Press.

NRC (National Research Council). 2000. Methods for Developing Spacecraft Water Exposure Guidelines. Washington, DC: National Academy Press.

NTP (National Toxicology Program). 1986. Toxicology and Carcinogenesis Studies of Dichloromethane (Methylene Chloride) in F344/N Rats and B6C3F1 Mice (Inhalation Studies). NTP Technical Report 306. NIH Publication No. 86-2562. U.S. Department of Health and Human Services, Public Health Services, National Institute of Health, National Toxicology Program, Research Triangle Park, NC.

Ott, M.G., L.K. Skory, B.B. Holder, J.M. Bronson, and P.R. Williams. 1983. Health evaluation of employees occupationally exposed to methylene chloride. Scand. J. Work Environ. Health 9(Suppl. 1):8-16.

Pemble, S., K.R. Schroeder, S.R. Spencer, D.J. Meyer, E. Hallier, H.M. Bolt, B. Ketterer, and J.B. Taylor. 1994. Human glutathione S-transferase theta (GSST1): cDNA cloning and the characterization of a genetic polymorphism. Biochem. J. 300(Pt.1): 271-276.

Peterson, J.E. 1978. Modeling the uptake, metabolism and excretion of dichloromethane by man. Am. Ind. Hyg. Assoc. J. 39(1):41-47.

Putz, V.R., B.L. Johnson, and J.V. Setzer. 1979. A comparative study of the effects of carbon monoxide and methylene chloride on human performance. J. Environ. Pathol. Toxicol. 2(5):97-112.

Reitz, R.H., A.L Mendrala, and F.P. Guengerich. 1989. In vitro metabolism of methylene chloride in human and animal tissues: use in physiologically based pharmacokinetic models. Toxicol. Appl. Pharmacol. 97(2):230-246.

Starr, T.B., G. Matanoski, M.W. Anders, and M.E. Andersen. 2006. Workshop overview: Reassessment of the cancer risk of dichloromethane in humans. Toxicol. Sci. 91(1):20-28.

Stewart, R.D., T.N. Fisher, M.J. Hosko, J.E. Peterson, E.D. Baretta, and H.C. Dodd. 1972. Experimental human exposure to methylene chloride. Arch. Environ. Health 25(5):342-348.

ten Berge, W.F., A. Zwart, and L.M. Appelman. 1986. Concentration-time mortality response relationship of irritant and systemically acting vapours and gases. J. Hazard. Mater. 13(3):301-309.

Their, R., F.A. Wiebel, A. Hinkel, A. Burger, T. Bruning, K. Morgenroth, T. Senge, M. Wilhelm, and T.G. Schulz. 1998. Species differences in the glutathione transferase GSTT1-1 activity towards the model substrates methyl chloride and dichloromethane in liver and kidney. Arch. Toxicol. 72(10):622-629.

Thomas, R.S., R.S. Yang, D.G. Morgan, M.P. Moorman, H.R. Kermani, R.A. Sloane, R.W. O'Connor, B. Adkins Jr., M.L. Gargas, and M.E. Andersen. 1996a. PBPK modeling/Monte Carlo simulation of methylene chloride kinetic changes in mice in relation to age and acute, subchronic, and chronic inhalation exposure. Environ. Health Perspect. 104(8):858-865.

Thomas, R.S., P.L. Bigelow, T.J. Keefe, and R.S. Yang. 1996b. Variability in biological exposure indices using physiologically based pharmacokinetic modeling and Monte Carlo simulation. Am. Ind. Hyg. Assoc. J. 57(1):23-32.

Winneke, G. 1974. Behavioral effects of methylene chloride and carbon monoxide as assessed by sensory and psychomotor performance. Pp. 130-144 in Behavioral Toxicology, C. Xinitaras, B.L. Johnson, and I. deGroot, eds. Washington DC: U.S. Government Printing Office**.**

Winneke, G. 1981. The neurotoxicity of dichloromethane. Neurobehav. Toxicol. Teratol. 3(4):391-395.

Winneke, G., and G.G. Fodor. 1976. Dichloromethane produces narcotic effect. Occup. Health Saf. 45(2):34-49.

Wong, K.L. 1994. Carbon monoxide. Pp. 61-90 in Spacecraft Maximum Allowable Concentrations for Selected Airborne Contaminants, Vol. 1. Washington, DC: National Academy Press.

Wong, K.L. 1996. Methylene chloride. Pp. 277-305 in Spacecraft Maximum Allowable Concentrations for Selected Airborne Contaminants, Vol. 2. Washington, DC: National Academy Press.

16

Propylene Glycol

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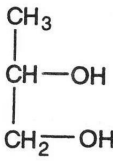
BACKGROUND AND PURPOSE

On the Mir space station, several gallons of ethylene glycol were used as a coolant. In one incident, gallons of coolant leaked out and ethylene glycol vapors were detected in the air. A high concentration of ethylene glycol was also found in the humidity condensate that would be used as a source of water for recycling on the International Space Station. On the basis of extensive literature on the toxicity of ethylene glycol, its use was not recommended. Propylene glycol (PG) is generally believed to be less toxic than ethylene glycol (LaKind et al. 1999). NASA is planning to use PG-based coolant for the Orion crew exploration vehicle, which is part of the Constellation Program to send human explorers back to the moon and onward to Mars and other destinations in the solar system.

The purpose of this document is to review the existing inhalation toxicology literature on PG and develop maximum acceptable air concentrations for 1 h, 24 h, 7 d, 30 d, 180 d, and 1,000 d of potential exposure to vapors of PG.

STRUCTURE OF PROPYLENE GLYCOL



PG is a colorless, practically odorless and tasteless, and somewhat viscous liquid (see Table 6-1 for physical and chemical properties of propylene glycol).

TABLE 16-1 Physical and Chemical Properties of Propylene Glycol

Chemical formula:	$CH_3CHOHCH_2OH$ or $C_3H_8O_2$
Chemical name:	Propylene glycol
Synonyms:	1,2-propanediol, 1,2-dihydroxypropane, methyl glycol
Molecular weight:	78
CAS number:	57-55-6
Boiling point:	187°C
Vapor pressure:	0.07 mm Hg at 20°C; 0.13 mm Hg at 25°C
Concentration in air at saturation:	170 ppm[a] at 25°C
Conversion factor:	1 ppm = 3.2 mg/m^3, 1 mg/m^3 = 0.31 ppm, 1 mg/L = 313 Ppm

[a]Calculated from the vapor pressure at that temperature.
Source: Data from Rowe and Wolf 1982.

OCCURRENCE AND USE

PG is commonly used as an additive in cosmetics and in medicinal agents. It is thought to have low toxicity and is used as a vehicle for intravenous (IV) medications, topical medications, and cosmetics. The Food and Drug Administration considers it safe for use in medication and cosmetics. It is also antibacterial, which makes it useful as a preservative and disinfectant. PG is the principal component of aircraft deicing and anti-icing fluids and of motor vehicle antifreeze. As the general weight of evidence in the toxicology literature supports the conclusion that PG will be less toxic than ethylene glycol, PG-based coolant is strongly considered for use in NASA Constellation Program transport vehicles.

PHARMACOKINETICS AND METABOLISM

No data, human or animal, describing the toxicokinetics of PG exposure through inhalation are available. Because the solubility of PG in water is high, one might expect that any inhaled vapor reaching the lungs would be very well absorbed by the lung and metabolized by the liver in a fashion similar to its metabolism from an ingested dose, although one might expect some quantitative differences. Cavender and Sowinski (1994) described a work in which humans were exposed to 10% PG in a mist tent with labeled deionized water. Less than 5% of the mist entered the body and, of this amount, 90% lodged in the nasopharynx and disappeared in the stomach; very little was found in the lungs. It appears that most of the inhaled PG aerosol becomes trapped in the upper respiratory tract and does not reach the lungs.

For orally administered PG, the metabolites are lactic acid and pyruvic acid, which the body uses as an energy source (either through oxidation by the

tricarboxylic acid cycle or through the generation of glycogen and glucose by the glycolytic pathway) (Ruddick 1972). One-third of absorbed PG is excreted via the kidneys (Browning 1965a,b).

GENERAL TOXICITY INFORMATION ON PG

No reports of human toxicity from environmental or occupational exposure to PG vapors have been published. However, there are several clinical case reports of PG-associated toxicities, such as hyperlactatemia, metabolic acidosis, hyperosmolality, and renal toxicity occurring when patients received certain sedatives such as benzodiazepines (diazepam or lorazepam, etomidate) by continuous IV infusion for several hours (LaKind et al. 1999, Zar et al. 2007). Surprisingly, almost all reports of PG-associated toxicity come from cases treated with lorazepam by IV infusion, although a long list of medications contain PG as a suspension medium. In all these medications, the solvent contained several milligrams to grams of PG (see Yaucher et al. 2003, Wilson et al. 2005, Zar et al. 2007), which amounted to moderate to high doses of PG, the concentrations one is least likely to receive via inhalation. These studies may not be directly relevant to an inhalation route of exposure; however, the observations indicate the toxicity potential of PG.

ACUTE EXPOSURE

Human case studies reporting death caused by exposure to PG (including exposure through industrial use) were not found in the scientific literature (Cavender and Sowinski 1994).

Wieslander et al. (2001) studied the acute ocular and respiratory effects of experimental exposure to PG in an aviation emergency training simulator. Nonasthmatic volunteers (22 men and 5 women) were exposed in an aircraft simulator to PG mist over 1 min to concentrations ranging from 176 to 851 milligrams per cubic meter (mg/m^3) (geometric mean concentration of PG was 309 mg/m^3). Tests conducted within 15 min after the exposures included an estimate of tear-film stability breakup time, nasal patency by acoustic rhinometry, dynamic spirometry, and a symptom questionnaire with 23 yes-or-no questions for ocular and respiratory symptoms (nasal and throat irritation, difficulty in breathing); smell; dermal symptoms; and symptoms of headache, nausea, fatigue, dizziness, and intoxication. After exposure to PG mist for 1 min, tear-film stability decreased, ocular and throat symptoms increased, forced expiratory volume in 1 s per forced vital capacity was slightly reduced, and self-rated severity of dyspnea was slightly increased. Subjects exposed to the higher concentrations had a more pronounced increase in throat symptoms and a more pronounced decrease in tear-film stability. The four subjects who reported developing an irritative cough during exposure to PG also had an increased perception of mild dyspnea (shortness of breath, difficult or labored breathing).

In an animal study, Konradova et al. (1978) exposed rabbits to 10% PG by aerosol inhalation for 20 and 120 min to examine its effect on tracheal epithelium. After 20 min of exposure, no noteworthy alterations in the epithelia were observed. After prolonged exposure, pathologic alteration of the cilial cells was noted. In addition, both exposure durations produced alterations in goblet cells (increased number of degenerated mucus-discharging goblet cells in the rabbits' tracheal lining).

Short-Term and Subchronic Inhalation Exposure Studies

No human data on the effects of short-term or subchronic duration exposure to PG vapors were found in the literature.

In a 90-d nose-only inhalation exposure animal study, the frequency of certain clinical signs was measured every week, so the results are used here as short-term study observations. Suber et al. (1989) conducted a subchronic nose-only inhalation exposure study of PG in male and female Sprague-Dawley rats. They exposed rats to PG aerosol for 6 h/d, 5 d/wk for 90 d at concentrations of 0.16, 1.01, and 2.18 mg per liter (L) (160, 1,000, and 2,200 mg/m^3 or 50, 313, and 688 parts per million [ppm]). These levels were measured and were not target concentrations. The mass median aerodynamic diameters of the diluted aerosols were less than 2.22 and 1.96 micrometers for the medium- and high-concentration groups, with geometric standard deviations of 1.44 and 1.57, respectively. Statistically significant nasal hemorrhaging and ocular discharge were observed beginning the second week of exposure. The reported incidence of nasal hemorrhaging and ocular discharge in the second week of exposure to the lowest test concentration, 50 ppm, was only 3%. The authors attributed the observed nasal hemorrhaging and ocular discharge to dehydration of the nasal passages and eyes. In males the incidence of these symptoms remained essentially constant throughout the exposure (69.9% at 2 wk versus 65.8% at 13 wk), but in females the incidence dropped dramatically from 65.1% at 2 wk to 0.0% at 13 wk.

Respiratory rates and tidal volumes were also measured in four rats per group per sex on day 7; measurements were repeated on days 42 and 84. The measured respiratory parameters were found to be unaltered. The authors also measured hematology and clinical chemistry before the experiment and before necropsy, but not during the weeks of exposure.

Although statistically significant differences were reported between the control groups and the highest-dose group (2.18 mg/L or 688 ppm) for certain hematologic parameters, such as white blood cell count and lymphocyte count, and for the activity of serum enzymes such as serum sorbitol dehydrogenase (all decreases), no dose-dependent relationship was observed. The observed trend of decreases in aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and gamma-glutamyl transferase were hard to interpret.

Chronic Inhalation Exposure Studies

No published toxicity data exist on humans exposed chronically to PG vapors either occupationally or from environmental exposures. Data were available from only one rodent study. Robertson et al. (1947) exposed rats (for 18 months) and monkeys (for 12 months) to PG vapor (at various percentages of atmospheric saturation in the case of monkeys). They used the following concentrations: rats, 0.17 to 0.35 mg/L (53 to 110 ppm); monkeys, 0.23 to 0.35 mg/L (72 to 110 ppm).

In rats, general behavior and appearance; body weight (growth rate) of males; and gross and microscopic examination of lungs, kidneys, liver, and spleen were determined. Kidney function was also evaluated. No significant PG-related adverse effects could be found in the rats. There was no sign of eye irritation in any of the exposed animals.

Monkeys were exposed to 60% saturated or supersaturated PG concentrations for 12 months (Robertson et al. 1947). General behavior and appearance, eye irritation, appetite, blood counts and hemoglobin, gross and microscopic organ lesions, and kidney function were assessed. PG-related increases in numbers of red blood cells and hemoglobin content of blood were the only changes reported. Kidney function was not affected. It was noted that monkeys (both treated and untreated) had infections, to a variable extent, with parasites (roundworms) and lung mites. Many had anemia and were sick or dying during the experiment. Because of these adverse health conditions, only limited confidence can be placed in these data (see Table 16-2).

CARCINOGENICITY

There is no reported incidence of cancer from occupational exposures to PG. Neither the International Agency for Research on Cancer nor the National Toxicology Program has reported that this chemical is a potential carcinogen (NTP 2004). No chronic inhalation exposure study exists in which the carcinogenic potential of PG was evaluated. In several chronic oral ingestion studies (2-y feed studies; see Gaunt et al. 1972), no evidence of tumor induction was found in any of the tissues.

GENOTOXICITY

No in vivo genotoxicity studies have been conducted in humans or animals exposed to PG by inhalation. In vitro tests using various strains of *Salmo-*

TABLE 16-2 Toxicity Studies of Propylene Glycol (Inhalation Exposures)

Exposure Concentration	Form of Administration	Species	General Effects	References
Range, 176 ppm to 851 mg/m^3; geometric mean, 309 mg/m^3; 1- min exposure at several different times	Aerosol spray	Human volunteers	Data on tear-film stability, rhinometry, lung function tests (dynamic spirometry), symptom assessments were collected; reported significant ocular irritation and throat irritation; some breathing difficulty.	Wieslander et al. 2001
0.16 mg/L (50 ppm), 1.0 mg/L (313 ppm), 2.18 mg/L (688 ppm); exposures were for 6 h/d, 5 d/wk for 90 d.	Inhalation (nose only) (aerosol)	Rat	Observations: a high incidence of nasal hemorrhaging and ocular discharge; significantly reduced body weights in medium- and high-dose females; reduced red blood cells in high-dose females. No changes in respiratory rates, tidal volume, or minute volume; unremarkable gross pathology of tissues (at necropsy); thickening of respiratory epithelium with increased number of goblet cells in medium- and high-dose groups.	Suber et al. 1989
230 mg/m^3 (72 ppm) for 12 to 18 months, continuous exposure	Inhalation (vapor)	Monkey	No effects on any of the parameters measured for systemic effects; high mortality in control and treated groups due to various infections.	Robertson et al. 1947
170 mg/m^3 (53 ppm) for 12 to 18 months, continuous exposure	Inhalation (vapor)	Rat	No effects on any of the parameters measured for systemic effects.	Robertson et al. 1947

nella typhimurium with and without metabolic activation were negative (Clark et al. 1979, Pfeiffer and Dunkelberg 1980). In vitro studies using mammalian cells (human fibroblasts, Chinese hamster ovary cells, and Chinese hamster lung cells) that measured chromosome aberrations and DNA damage in cells exposed to PG were negative (Swenberg et al. 1976, Sasaki et al. 1980 as cited in Abe and Sasaki 1982).

IMMUNOTOXICITY

There are no reports of immunotoxicity from inhalation of PG by humans. The published animal inhalation studies have not specifically looked at changes in immune system parameters.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

There are no published reports of reproductive or developmental toxicity in humans from inhalation of PG vapors in either an occupational setting or by environmental exposure. There are also no published animal data on this subject.

RATIONALE

Acceptable concentrations (ACs) were determined following the guidelines of the National Research Council (NRC 1992) Subcommittee on Guidelines for Developing Spacecraft Maximum Allowable Concentrations (SMACs) for Space Station Contaminants 1992. In the following paragraphs, derivation of ACs for durations of 1 h, 24 h, 7 d, 30 d, 180 d, and 1,000 d are shown for various effects as available and the SMAC for each duration will be determined based on the lowest AC for that duration.

As a part of this process, NASA will also review the existing proposed guidelines, advisories, and regulatory values from various organizations, both regulatory and nonregulatory.

There are no standards or health values for PG. The U.S. Environmental Protection Agency reference dose/reference concentration work group did not derive an inhalation reference concentration for PG. The airborne exposure limits set by the American Industrial Hygiene Association (AIHA) workplace environmental exposure level (WEEL) (8-h time-weighted average workplace environmental exposure level) for PG are 50 ppm for vapor and aerosol, 10 mg/m^3 for aerosol only, and 400 ppm for PG mist and PG vapors (AIHA 1985).

The Agency for Toxic Substances and Disease Registry (ATSDR) did not derive an acute-duration inhalation minimal risk level (MRL) for PG because no adequate studies were found. ATSDR derived an intermediate-duration inhalation MRL of 0.009 ppm for nasal hemorrhaging (ATSDR 1997) based on observations in rats in the Suber et al. (1989) study. Because details were lacking, the ATSDR did not derive a chronic-duration inhalation MRL for PG after review-

ing the only data available from one animal study of chronic-duration exposure in monkeys and rats (Robertson et al. 1947).

Table 16-3 summarizes SMACs for various durations. The principal studies selected, the adverse end point chosen, the rationale, and detailed calculations have been presented in the preceding paragraphs.

Derivation of 1- and 24-h ACs for Inhalation of PG

For deriving the 1-h AC, the human subject experiment by Wieslander et al. (2001) was considered. Nonasthmatic volunteers were exposed to PG mist during aviation emergency training and several measurements were collected after 1-min exposures to PG mist at a mean concentration of 309 mg/m^3 (about 96 ppm). Data collected on symptoms (average ratings on 10 questions, data collected on visual analog scale of 1 to 100) indicated that ocular irritation and throat irritation were significantly higher than preexposure responses. Dyspnea was higher but only of marginal significance (P = 0.048). The ocular irritation was not from redness of the eye or swollen eyes. On the basis of this study, it was considered that the concentration of 309 mg/m^3 is a minimal lowest-observed-adverse-effect level (LOAEL) (mild adverse effect). As such sensory effects are based on concentration, and the symptoms were minor, it was decided to use this value for 1 h. Because exposures were just for 1 min, it was decided to use an uncertainty factor of 3 (or LOAEL to NOAEL factor) to derive a 1-h AC as follows:

$$\text{1-h AC}_{\text{(throat and ocular irritation)}} = 96\ \text{ppm}_{\text{(LOAEL)}} \times 1/3_{\text{(LOAEL to NOAEL)}} = 32\ \text{ppm}$$

TABLE 16-3 Spacecraft Maximum Allowable Concentrations for PG

Duration	SMAC, ppm	Target Toxicity	Principal Study
1 h	32.0	Eye, throat, and respiratory system irritation	Wieslander et al. 2001
24 h	17.0	Nasal hemorrhage and ocular discharge	Suber et al. 1989
7 d	9.0	Nasal hemorrhage and ocular discharge	Suber et al. 1989
30 d	3.0	Nasal hemorrhage and ocular discharge	Suber et al. 1989
180 d	1.5	Thickening of respiratory epithelium with increased goblet cells and increased mucin	Suber et al. 1989
1,000 d	1.5	Thickening of respiratory epithelium with increased goblet cells and increased mucin	Suber et al. 1989

For deriving the 24-h AC, the preceding study could not be used, primarily because of the very brief duration of exposure (1 min). Although the discomfort of ocular irritation and throat dryness at 32 ppm may be acceptable for 1 h, these symptoms may not be appropriate for extending the 1-min exposure data to 24 h. Therefore, on the recommendation of the NRC Spacecraft Exposure Guidelines (SEG) committee, the ten Berge method (ten Berge et al. 1986) of extrapolation from the 7-d AC (168 h) to 24 h was used as follows (see below for details of the 7-d AC derivation):

$8.9^3 \times 168$ h = $C^3 \times 24$ h, where 8.9 ppm is the 7-d AC, 3 is a default factor for the chemical-specific exponent, and C is the concentration to be determined for 24 h, which can be calculated as 17 ppm.

Thus, the 24-h AC = 17 ppm

Derivation of 7-d AC for Inhalation of PG

Suber et al. (1989) conducted a 90-d subchronic nose-only inhalation exposure study of PG in male and female Sprague-Dawley rats (19 males and 19 females each). In this study, the rats were exposed 6 h/d, 5 d/wk for 90 d to PG as an aerosol at concentrations of 0.16, 1.01, and 2.18 mg/L (160, 1,000, and 2,200 mg/m^3 equivalent to 50, 313, and 688 ppm, respectively). During this study, the frequency of certain clinical signs was measured every week. Statistically significant nasal hemorrhaging (all exposed groups) and ocular discharge were seen beginning with the second week of exposure. By the end of the first week of exposure to the lowest test concentration, 50 ppm, the incidence of these effects was only 3% (males). The authors attributed the observed nasal hemorrhaging and ocular discharge to dehydration by PG of the nasal passages and eyes. Regardless of the mechanism of action, ACs have been calculated on the basis of such end points. Based of these data, a no-observed-adverse-effect level (NOAEL) of 50 ppm for up to 1 wk can be identified.

The review of the effects clearly shows that by the second week, 69% of the animals had nasal hemorrhaging and ocular discharge, even in the 50-ppm group. Thus, it appears that with longer exposure times, the incidence rate will increase for at least up to 2 wk (as the percent incidence did not change from 2 wk to 13 wk). Therefore, the exposure concentration has to be adjusted with a factor for discontinuous-to-continuous exposure. As far as the use of a species factor is concerned, it was considered that these effects may be due to the dehydrating effect of PG aerosol (physicochemical effect). Known differences between rats and humans in the structure of the nasal region may be important when considering effects on the respiratory tract and lung or when the metabolite of a compound affects the nasal mucosa. As this (dehydration) is a localized effect, the severity of the effect for a particular exposure concentration can be

expected to be similar for rodents and humans. Therefore, a species factor is not needed. Thus, the 7-d AC can be calculated as follows:

$$\text{7-d AC}_{\text{(nasal hemorrhaging)}} = 50\ \text{ppm}_{\text{(NOAEL)}} \times [6\ \text{h}/24\ \text{h} \times 5\ \text{d}/7\ \text{d}]_{\text{(discontin. to contin.)}} = 8.9\ \text{ppm, rounded to 9 ppm}$$

Thus, the 7-d AC for effects on the nasal region is 9 ppm.

Derivation of 30-d AC for Inhalation of PG

The same adverse end points of nasal hemorrhaging and ocular discharge from the Suber et al. study were used to derive a 30-d AC, as no other data were presented in this study that could be used. From weeks 2 to 14, the average incidence of these effects in males, as stated in the body of the text of the authors' document, was <1% in controls and 65%, 74%, and 75% in low-, medium-, and high-concentration exposure groups. As significant effects were noted at 2 wk, 50 ppm is the LOAEL for 2 wk. Because the data reflect the increased frequency of occurrence and not the severity of the effects, a factor of 3 from LOAEL to NOAEL for these sensory effects would be justifiable. As these effects are primarily due to the dehydrating effect of the chemical rather than to progressive tissue injury, a factor of 10 from LOAEL to NOAEL is not needed. In addition, it must be pointed out that, in a study by Robertson et al. (1947), described earlier under "Chronic Inhalation Exposure Studies," no nasal hemorrhaging, ocular discharge, or systemic toxicity was reported in rats or monkeys during a 12- to 18-month continuous whole-body exposure to at least 53 and 72 ppm of PG vapor, respectively (note that this study was not used to derive the AC for subchronic and chronic durations). The concentrations of PG in the Robertson et al. study (described earlier in this document) are comparable to that used in the Suber et al. study, but the exposure methods used in these studies were different. No species factor is used, as explained earlier. The 30-d AC is calculated as shown below.

$$\text{30-d AC}_{\text{(nasal hemorrhaging)}} = 50\ \text{ppm}_{\text{(LOAEL)}} \times 1/3_{\text{(LOAEL to NOAEL)}} \times [6\ \text{h}/24\ \text{h} \times 5\ \text{d}/7\ \text{d}]_{\text{(discontin. to contin.)}} = 2.98\ \text{ppm, rounded to 3 ppm}$$

Thus, the 30-d AC for nasal hemorrhaging is 3 ppm.

Derivation of 180-d AC for Inhalation of PG

For derivation of the 180-d AC, the same 90-d nose-only subchronic study by Suber et al. (1989) was used. At the end of the 90-d exposure, body weight changes, food consumption, organ weight changes (especially of the kidneys), and other variables were also determined. According to NRC guidelines (NRC 1992, 2000), in general, changes in such variables are not to be considered in the

AC derivations. The important measurements that will reflect the systemic effects of PG are the changes in hematologic and clinical biochemistry variables. Hemoglobin concentration, white blood cell count and lymphocyte numbers, serum sorbitol dehydrogenase and gamma-glutamyl transferase activity, and total serum protein were measured in the study. However, these changes did not follow a definite dose-dependent pattern. There were no histopathologic changes. However, light microscopy of the respiratory epithelium showed thickening reflected in an increased number of goblet cells and an increase in their mucin content in both female and male animals with medium- and high-dose treatment groups. Because the authors reported that there were no histologic changes in the trachea, lungs, or larynx and minute volume, tidal volume, and respiratory rate were not significantly altered, the epithelial changes are considered mild adverse effects, and thus one could identify 50 ppm as the NOAEL for these effects.

For deriving the AC for 180 d, a factor for adjusting the exposure duration will be used. Although a species factor was not used for the nasal hemorrhaging end point earlier, a species factor of 3 will be used in this case. The factor is for the uncertainty in the severity of effects due to the differences between humans and rats in the nasal passage respiratory epithelium and the nature of the goblet cells. Because the AC is calculated for 180 d with data from a 90-d study, a time extrapolation factor of 180 d/90 d is used; 50 ppm is a NOAEL for this effect.

$$\text{180-d AC}_{\text{(histologic changes)}} = 50\ \text{ppm}_{\text{(NOAEL)}} \times [6\ \text{h}/24\ \text{h} \times 5\ \text{d}/7\ \text{d}]_{\text{(discontin. to contin.)}} \times 1/3_{\text{(species factor)}} \times 90\ \text{d}/180\ \text{d}_{\text{(time extrapolation)}} = 1.49\ \text{ppm, rounded to } 1.50\ \text{ppm}$$

Thus, the 180-d AC for histologic changes in the nasal passages is 1.5 ppm.

The chronic whole-body exposure study of Robertson et al. (1947), described in detail earlier in this document, was also evaluated. Briefly, in the Robertson et al. study, monkeys and rats were continuously exposed to PG supersaturated vapor in chambers for 12 to 18 months at the following concentrations: rats, 0.17 to 0.35 mg/L (53 to 110 ppm) for 18 months; monkeys, 0.23 to 0.35 mg/L (72 to 110 ppm) for 12 months. During the exposure period, there were no differences between the unexposed and the exposed groups in the general condition of the animals or in their general activity or behavior. There was no sign of eye irritation in any of the exposed animals. There was also no impairment of kidney function. The red blood cell counts and hematocrit values were greater than those of untreated controls. Several of the exposed female rats bore normal-sized litters; all the offspring appeared to be normal. However, in the study, the author reported that the overall health of the monkeys was undesirable, and they had a high mortality rate. In the rats, although PG had caused no generalized or localized inflammation of the bronchi or lungs, microscopic examination of the lungs revealed a localized infectious process. This was also noted in 25% of the control rats. Even though this effect was seen only in rats

kept 8 months or longer (18 months), the NRC SEG committee recommended that this study not be used for AC derivation because of the high incidence of infection in control rats, as evidenced by the microscopic changes in the lungs.

Derivation of 1,000-d AC for Inhalation of PG

Although in the Robertson et al. (1947) study an 18-month chronic exposure to PG vapors did not lead to any overt systemic effects after rats were exposed to 53 to 100 ppm of PG, the study could not be considered for 1,000-d AC derivation because of reported infectious activity (small to large areas of intra-alveolar accumulation of polymorphonuclear leukocytes) in the lungs of 25% of the controls and in exposed rats.

To arrive at a 1,000-d AC, it was decided to use the 180-d AC derived from the Suber et al. (1989) study. As the nature of the effects (epithelial changes in the nasal passages) seemed to be adaptive, the NRC SEG committee recommended using the 180-d AC without any time extrapolation factors from 180 to 1,000 d.

Thus, the 1,000-d AC for nasal morphology change is 1.5 ppm

Table 16-4 summarizes the ACs derived for various end points for various durations from 1 h to 1,000 d and the SMACs for each of these durations.

.

TABLE 16-4 Summary of Acceptable Concentrations and SMACs for Various Durations

Adverse End Point	Exposure Data	Species and Reference	LOAEL/NOAEL	Acceptable Concentrations, ppm					
				1h	24 h	7 d	30 d	180 d	1,000 d
Ocular and throat irritation and slight dyspnea	Inhalation of PG; 1 min; mean concentration = 309 mg/m^3 (~ 96 ppm)	Human, Wieslander et al. 2001	LOAEL = 96 ppm	32	—	—	—	—	—
Nasal hemorrhage and ocular discharge	Inhalation of PG; 6 h/d, 5 d/wk; 0.16, 1.01, 2.18 mg/L (160, 1,000, 2,200 mg/m^3 or 50, 313, 688 ppm)	Sprague-Dawley rat, Suber et al. 1989	NOAEL for 1 wk = 50 ppm; ten Berge method used	—	17	—	—	—	—
Nasal hemorrhage and ocular discharge	Inhalation of PG; 6 h/d, 5 d/wk; 0.16, 1.01, 2.18 mg/L (160, 1,000, 2,200 mg/m^3 or 50, 313, 688 ppm)	Sprague-Dawley rat, Suber et al. 1989	NOAEL for 1 wk = 50 ppm	—	—	9	—	—	—
Nasal hemorrhage and ocular discharge	Inhalation of PG; 6 h/d, 5 d/wk; 0.16, 1.01, 2.18 mg/L (160, 1,000, 2,200 mg/m^3 or 50, 313, 688 ppm)	Sprague-Dawley rat, Suber et al. 1989	LOAEL for 2 wk = 50 ppm	—	—	—	3	—	—
Thickening of respiratory epithelium with increased goblet cells and their mucin	Inhalation of PG; 6 h/d, 5 d/wk; 0.16, 1.01, 2.18 mg/L (160, 1,000, 2,200 mg/m^3 or 50, 313, 688 ppm)	Sprague-Dawley rat, Suber et al. 1989	NOAEL = 50 ppm	—	—	—	—	1.5	1.5
SMACs				*32*	*17*	*9*	*3*	*1.5*	*1.5*

Abbreviations: LOAEL, lowest-observed-adverse-effect level; NOAEL, no-observed-adverse-effect level; —, not calculated.

REFERENCES

AIHA (American Industrial Hygiene Association). 1985. Workplace Environmental Exposure Level (WEEL) Guide for Propylene Glycol. Fairfax (VA): American Industrial Hygiene Association.

ATSDR (Agency for Toxic Substances and Disease Registry). 1997. Toxicological Profile for Ethylene Glycol and Propylene Glycol. U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry, Atlanta, GA. September 1997.

Abe, S., and M. Sasaki. 1982. SCE as an index of mutagenesis and/or carcinogenesis. Pp. 461-514 in Sister Chromatid Exchange. Progress and Topics in Cytogenetics Vol. 2, A.A. Sandberg, ed. New York: A.R. Liss.

Browning, E. 1965a. Ethylene glycol. Pp. 594-600 in Toxicity and Metabolism of Industrial Solvents. New York: Elsevier.

Browning, E. 1965b. Propylene glycol. Pp. 642-644 in Toxicity and Metabolism of Industrial Solvents. New York: Elsevier.

Cavender, F.L., and E.J. Sowinski. 1994. Glycols. Pp. 4645-4719 in Patty's Industrial Hygiene and Toxicology, Vol. 2F. Toxicology, 4th Ed., G.D. Clayton, and F.E. Clayton, eds. New York: Wiley.

Clark, C.R., T.C. Marshall, B.S. Merickel, A. Sanchez, D.G. Brownstein, and C.H. Hobbs. 1979. Toxicological assessment of heat transfer fluids proposed for use in solar energy applications. Toxicol. Appl. Pharmacol. 51(3):529-535.

Gaunt, I.F., F.M. Carpanini, P. Grasso, and A.B. Landsdown. 1972. Long-term toxicity of propylene glycol in rats. Food Cosmet. Toxicol. 10(2):151-162.

Konradova, V., V. Vavrova, and J. Janota. 1978. Effect of the inhalation of a surface tension-reducing substance (propylene glycol) on the ultrastructure of epithelium of the respiratory passages in rabbits. Folia Morphol. (Praha) 26(1):28-34.

LaKind, J.S., E.A. McKenna, R.P. Hubner, and R.G. Tardiff. 1999. A review of the comparative mammalian toxicity of ethylene glycol and propylene glycol. Crit. Rev. Toxicol. 29(4):331-365.

NRC (National Research Council). 1992. Guidelines for Developing Spacecraft Maximum Allowable Concentrations for Space Station Contaminants. Washington, DC: National Academy Press.

NRC (National Research Council). 2000. Methods for Developing Spacecraft Water Exposure Guidelines. Washington, DC: National Academy Press.

NTP (National Toxicology Program). 2004. NTP-CERHR Expert Panel report on the reproductive and developmental toxicity of propylene glycol. Reprod. Toxicol. 18(4):533-579.

Pfeiffer, E.H, and H. Dunkelberg. 1980. Mutagenicity of ethylene oxide and propylene oxide and of the glycols and halohydrins formed from them during the fumigation of foodstuffs. Food Cosmet. Toxicol. 18(2):115-118.

Robertson, O.H., C.G. Loosli, T.T. Puck, H. Wise, H.M. Lemon, and W. Lester. 1947. Test for the chronic toxicity of propylene glycol and triethylene glycol on monkeys and rats by vapor inhalation and oral administration. J. Pharmacol. Exper. Therap. 91(1):52-76.

Rowe, V.K., and M.A. Wolf. 1982. Glycols. Pp. 3817-3908 in Patty's Industrial Hygiene and Toxicology, Vol. IIC. Toxicology, 3rd rev. Ed., G.G. Clayton, and F.E. Clayton, eds. New York: John Wiley & Sons.

Ruddick, J.A. 1972. Toxicology, metabolism, and biochemistry of 1, 2-propanediol. Toxicol. Appl. Pharmacol. 21(1):102-111.

Sasaki, M., K. Sugimura, M.A. Yoshida, and S. Abe. 1980. Cytogenetic effects of 60 chemicals on cultured human and Chinese hamster cells. Kromosomo II. 20:574-584.

Suber, R.L., R. Deskin, I. Nikiforov, X. Fouillet, C.R. Coggins. 1989. Subchronic nose-only inhalation study of propylene glycol in Sprague-Dawley rats. Food Chem. Toxicol. 27(9):573-583.

Swenberg, J.A., G.L. Petzold, and P.R. Harbach. 1976. In vitro DNA damage/alkaline elution assay for predicting carcinogenic potential. Biochem. Biophys. Res. Commun. 72(2):732-738.

Ten Berge, W.F., A. Zwart, and L.M. Appelman. 1986. Concentration-time mortality response relationship of irritant and systematically acting vapours and gases. J. Hazard. Mater. 13(3):301-309.

Wieslander, G., D. Norbeck, and T. Lindgren. 2001. Experimental exposure to propylene glycol mist in aviation emergency training: Acute ocular and respiratory effects. Occup. Environ. Med. 58(10):649-655.

Wilson, K.C., C. Reardon, A.C. Theodore, and H.W. Farber. 2005. Propylene glycol toxicity: A severe iatrogenic illness in ICU patients receiving IV benzodiazepines: A case series and prospective, observational pilot study. Chest 128(3):1674-1681.

Yaucher, N.E., J.T. Fish, H.W. Smith, and J.A. Wells. 2003. Propylene glycol-associated renal toxicity from lorazepam infusion. Pharmacotherapy 23(9):1094-1099.

Zar, T., C. Graeber, and M.A. Perazella. 2007. Recognition, treatment, and prevention of propylene glycol toxicity. Semin. Dial 20(3):217-219.

17

Toluene

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Toluene is a clear, colorless, noncorrosive, flammable liquid with a sweet, pungent, "aromatic" odor. Values reported for the odor threshold range from 0.2 to 16 parts per million (ppm) (Sandmeyer 1981). One ppm of toluene = 3.77 milligrams per cubic meter (mg/m^3).

Spacecraft maximum allowable concentrations (SMACs) for toluene were published in Volume 2 of this series, *Spacecraft Maximum Allowable Concentrations for Selected Airborne Contaminants*, for exposure durations of 1 h, 24 h, 7 d, 30 d, and 180 d (Garcia 1996). In anticipation of longer exploration missions, this document establishes a SMAC for toluene for an extended exposure of 1,000 d and revises the values for some shorter exposures based on data published since 1996.

OCCURRENCE AND USE

Toluene has been measured in urban air at 0.01 to 0.05 ppm, probably from production facilities, automobile and coke oven emissions, gasoline evaporation, and cigarette smoke; it can occur in human respiratory air in smokers and nonsmokers (Sandmeyer 1981). It is used extensively as a component of gasoline; as a solvent in the chemical, rubber, paint, and drug industries; as a thinner for inks, perfumes, and dyes; and as a nonclinical thermometer liquid and suspension solution for navigation instruments (Sandmeyer 1981). Intentional inhalation of toluene vapors from glue has been an abuse problem among youth during the last few decades because of toluene's effects on the central nervous system. Measurements of toluene in spacecraft air have indicated trace (<0.05 mg/m^3 or <0.013 ppm) concentrations for almost all missions, but, on rare occasions, individual measurements have found up to 64 ppm of toluene in one International Space Station module (after a malfunction of the Elektron oxygen generation system), whereas other modules registered only trace amounts.

SUMMARY OF ORIGINAL APPROACH

The toluene SMACs for exposure durations of 1 h to 180 d (Table 17-1) were set in 1996 based on the lack of neurotoxicity (eight tests measuring 20 parameters) and the lack of irritation of the eyes and nose reported in 16 subjects exposed for 6 h to toluene vapors at 40 . During the same experiment, exposures of 100 ppm resulted in reports of headache, dizziness, and a feeling of intoxication significantly more often than when exposed to clean air (Andersen et al. 1983). Acceptable concentrations (ACs) for neurotoxicity (headache, dizziness, and a feeling of inebriation) were calculated as follows:

The ACs for 7, 30, and 180 d were based on the 40-ppm no-observed-adverse-effect level (NOAEL), using the factor (√n)/10 to adjust for the small number (n) of subjects.

$$\text{7-, 30-, and 180-d AC}_{\text{(neurotoxicity)}} = 40_{\text{(NOAEL)}} \times \frac{\sqrt{16}}{10}_{\text{(small n factor)}} = 16 \text{ ppm}$$

In setting ACs for 1- and 24-h exposures, mild effects such as headaches, irritation, and not-quite-significant decrements in psychometric tests would be acceptable for short-term contingency exposures, but dizziness would not be acceptable, even for brief exposures during contingency operations. Thus, the ACs for 1 and 24 h were also based on the 40-ppm NOAEL, adjusting for the number of subjects.

$$\text{1- and 24-h AC}_{\text{(neurotoxicity)}} = 40_{\text{(NOAEL)}} \times \frac{\sqrt{16}}{10}_{\text{(small n factor)}} = 16 \text{ ppm}$$

A NOAEL of 40 ppm of toluene vapor was reported for irritation of the eyes and nose during a 6-h exposure was reported in the same study in 16 young male volunteers. Because irritation depends on concentration but not on exposure duration, the ACs for all exposure durations from 7 to 180 d were based on the 40-ppm NOAEL, adjusting for the small number of subjects by a factor equal to 1/10th the square root of the number of subjects tested.

$$\text{7-, 30-, and 180-d AC}_{\text{(irritation)}} = 40_{\text{(NOAEL)}} \times \frac{\sqrt{16}}{10}_{\text{(small n factor)}} = 16 \text{ ppm}$$

Some irritation is acceptable for short-term SMACs; therefore, the irritancy ACs for 1 and 24 h were set equal to the 100-ppm lowest-observed-adverse-effect level (LOAEL).

$$\text{1- and 24-h AC}_{\text{(irritation)}} = 100 \text{ ppm}_{\text{(LOAEL)}}$$

TABLE 17-1 Spacecraft Maximum Allowable Concentrations for Toluene (Garcia 1996)

SMAC Duration	ppm	mg/m^3	Target Toxicity
1 h	16	60	Neurotoxicity
24 h	16	60	Neurotoxicity
7 d	16	60	Neurotoxicity, irritation
30 d	16	60	Neurotoxicity, irritation
180 d	16	60	Neurotoxicity, irritation

Source: Garcia 1996.

Because the 1- and 24-h ACs for neurotoxicity are lower than the 1- and 24-h ACs for irritation, the ACs for neurotoxicity were used to set the 1- and 24-h SMACs.

CHANGES IN FUNDAMENTAL APPROACHES RECOMMENDED BY THE NATIONAL RESEARCH COUNCIL

The original SMACs for toluene, set in 1996, were calculated by using a NOAEL as the point of departure to which a "small n" factor was applied. More recently, instead of a NOAEL, the National Research Council has recommended using a benchmark dose analysis (preferred) for estimating an AC and ten Berge's generalization ($C^N \times T = K$) of Haber's rule for exposure duration adjustments when the data permit.

RECENT AND ADDITIONAL DATA ON TOLUENE TOXICITY

Overview

Numerous articles have been published since 1996 describing toluene toxicity in humans and animals. Various end points have been studied including transient renal tubular acidosis (at high doses) (Tang et al. 2005), effects on color vision in workers (Zavalic et al. 1998a,b,c; Cavalleri et al. 2000; Gobba 2000; Gobba and Cavalleri 2003), ototoxicity in workers (Vrca et al. 1996, 1997; Morata et al. 1997; Schaper et al. 2003) and in chinchillas (Davis et al. 2002), effects of chronic exposures on monoamine biosynthesis in rat brains (Berenguer et al. 2003; Soulage et al. 2004), behavioral hypersensitivity in rats and humans (Benignus et al. 1998; Lees-Haley 2000; Lopez-Rubalcava et al 2000; Wiaderna and Thomas 2002; Berenguer et al. 2003; Paez-Martinez et al. 2003; Berenguer et al. 2004), reproductive effects in men and women (Luderer et al. 1999; Yilmaz et al. 2001; Nakai et al. 2003; Hooiveld et al. 2006), effects on anxiety and nociception in mice (Lopez-Rubalcava et al 2000; Cruz et al. 2001; Paez-Martinez et al. 2003), genotoxicity in workers (Nakai et al. 2003),

and bone mass toxicity in toluene abusers (Atay et al. 2005). Effects at doses at or below the current threshold limit value of 50 ppm (ACGIH 1999) include impaired color vision and effects on auditory and visual evoked potentials in workers, subtle changes in serum concentrations of luteinizing hormone (LH) in humans, behavioral changes in rats, and changes in the rate of synthesis of neurochemical transmitters in discrete areas of the rat brain.

Ototoxicity

In two studies, ototoxicity, based on pure-tone audiometry and immittance audiometry, was reported in cohorts of Brazilian rotogravure workers exposed for 1 to 25 y to a mixture of organic solvents (toluene, ethyl acetate, and ethanol). The concentration of toluene vapor in this mixture ranged from 75 to 600 ppm in one study (Morata et al. 1993) and from 0.037 to 243 ppm in another study (Morata et al. 1997).

Epidemiologic studies in Croatian printing press workers exposed for an average of 20.3 y to toluene vapors at concentrations estimated from 40 to 60 ppm (based on concentrations of toluene in blood and metabolites in urine) showed changes in auditory evoked potentials including decreased amplitudes, longer P1 latency and interpeak latencies (Vrca et al. 1996), which correlated with the duration of exposure (Vrca et al. 1997). An earlier study had shown altered auditory evoked potentials but no clinical effects in rotogravure workers exposed to toluene at unstated concentrations (Abbate et al. 1993). Pure tone audiometry measurements of ototoxicity, however, failed to show ototoxicity in workers exposed for >5 y to toluene vapors at concentrations of 45 ± 17 ppm (Schaper et al. 2003).

Pryor et al. (1984) conducted seven experiments with young male Fischer rats to examine concentration and exposure parameters necessary and sufficient to cause toluene-induced ototoxicity. They found a complicated pattern for the dependence of ototoxicity on exposure concentration and duration (Pryor et al. 1984). Hearing loss, measured by behavioral and electrophysiologic methods, was repeatedly observed after as few as 2 wk of exposure to 1,000 ppm of toluene for 14 h/d, but lower concentrations (400 and 700 ppm) had no effect even after 16 wk of exposure. Three-day exposures to 1,500 ppm of toluene for 14 h/d or to 2,000 ppm for 8 h/d were ototoxic, whereas single exposures to 4,000 ppm for 4 h or to 2,000 ppm for 8 h were without effect. Intermittent exposure to 3,000 ppm for 30 min every hour for 8 h/d caused hearing loss within 2 wk, but a similar exposure schedule for 4 h/d had no effect even after 9 wk. The results from the Pryor et al. study are summarized and the calculated total doses, in ppm-h, to which the rats were exposed are shown in Table 17-2.

Based on Table 17-2 and the discussion of results from Pryor et al. (1984), the lack of an apparent dose-response relationship may be explained by results obtained in studies by the U.S. Environmental Protection Agency that showed

TABLE 17-2 Summary of Dose-Response Data for Ototoxicity

Exposure, Duration	ppm-H
Ototoxic	
3,000 ppm, 30 min/h × 8 h/d × 2 wk	168,000
2,000 ppm, 8 h/d × 3 d	48,000
1,500 ppm, 14 h/d × 3 d	63,000
1,000 ppm, 14 h/d × 2 wk	196,000
Nonototoxic	
3,000 ppm, 4 h/d × 9 wk	756,000
2,000 ppm × 8 h, once	16,000
4,000 ppm × 4 h, once	16,000
700 ppm, 14 h/d × 16 wk	1,097,600

Source: Pryor et al. 1984. Reprinted with permission; copyright 1984, *Neurobehavioral Toxicology and Teratology*.

that, although a poor dose-response was observed when the exposure concentration of toluene in air was used as the dose metric, good correlation was obtained using the concentration of toluene in arterial blood, as calculated with a physiologically based pharmacokinetic model, for rats and humans, measuring behavioral effects as the response (Benignus et al. 1998). The mechanism of the toluene-induced hearing loss involves permanent loss of outer hair cells in the cochlea, beginning with those involved in hearing high frequencies (Pryor and Rebert 1984; Sullivan et al. 1989). Exposure to noise at levels > 85 decibels can also induce hearing loss, and exposure to both toluene and noise can induce additive effects if toluene exposure occurs after noise exposures or synergistic effects if toluene exposure precedes noise exposures (Johnson 1993; Morata et al. 1993, 1997; Sliwinska-Kowalska et al. 2004).

Renal Tubular Acidosis

Although there have been several clinical case reports of renal tubular acidosis after exposures to abuse concentrations of toluene (Patel and Benjamin 1986; Batlle et al 1988; Tang et al. 2005), a controlled clinical trial involving 86 subjects exposed to 100 ppm of toluene for 6.5 h found no renal toxicity and concluded that no causal relationship exists between moderate exposure to organic solvents and renal injury (Nielsen et al. 1985).

Visual Effects

Toluene exposure has been shown to decrease the ability of subjects to discriminate among shades of colors (Nakatsuka et al. 1992; Vrca et al. 1995,

1997; Zavalic et al. 1998a,b,c; Cavalleri et al. 2000; Gobba 2000; Gobba and Cavalleri 2003). Workers occupationally exposed to toluene at concentrations of 11.3 to 49.3 ppm (median 35 ppm) showed no effect on color discrimination, whereas workers exposed to concentrations of 66.0 to 250 ppm (median 156 ppm) had significantly higher color confusion index scores and alcohol intake-adjusted color confusion index scores—that is, decreased ability to discriminate shades of color (Zavalic et al. 1998a).

Neurochemical and Behavioral Effects

Numerous studies have confirmed the toxicity of toluene to the nervous system as measured by either changes in behavior (rearing frequency, avoidance of noxious stimuli in rodents; choice reaction time, mood in humans) or changes in neurochemistry (Weiss et al. 1979; Kostas and Hotchin 1981; Dyer et al. 1984; Juntunen et al. 1985; Taylor and Evans 1985; Alho et al. 1986; Kishi et al. 1988; Mattsson et al. 1989; Foo et al. 1990; Abbate et al. 1993; Murata et al. 1993; Boey et al. 1997; Benignus et al. 1998; Eller et al. 1999; Lopez-Rubalcava et al. 2000; Cruz et al. 2001; Berenguer et al. 2003, 2004; Paez-Martinez et al. 2003; Soulage et al. 2004; Wiaderna and Tomas 2002). Eller et al. (1999) studied rotogravure workers and reported a LOAEL of >100 ppm for >12-y exposures and a NOAEL of <20 ppm for <13-y exposures for deficits in cognitive functions (learning, memory, attention, concentration, eye-hand coordination) and neurologic effects (tremor and sway). Vrca et al. (1995, 1996, 1997) found changes in both auditory and visual evoked potentials in workers occupationally exposed to ≥40 ppm of toluene for an average of 21.4 y. Similarly, rats exposed to 40 ppm toluene for 16 wk showed significant changes in the biosynthesis rates of catecholamine and 5-hydroxytryptamine in specific regions of the brain (Soulage et al. 2004). The dose-response relationship for toluene exposures is inconsistent and difficult to interpret when dosimetry is measured by exposure concentration and duration, but it correlates well with the concentration of toluene in arterial blood estimated by the physiologically based pharmacokinetic method in both rats and humans (Benignus et al. 1998).

Effects on Reproductive Hormones

Luderer et al. (1999) reported subtle effects on the secretion of LH in both male and female (during the luteal phase of the menstrual cycle) volunteers exposed for 3 h to 50 ppm of toluene. No abnormalities were observed in the pulsatile secretion profiles of LH or follicle-stimulating hormone (FSH), but mean concentrations of LH in men were significantly decreased during exposure without any effect on testosterone concentrations. In women, the LH pulse frequency showed a trend toward decreasing concentrations during exposures.

Svensson et al. (Svensson et al. 1992) reported decreased concentrations of FSH (3.2 versus 4.9 international units per liter [IU/L]) and LH (6.4 versus 7.2 IU/L) in plasma and decreased (7.8 versus 86.8 pmol/L) free testosterone in the serum of 20 rotogravure printers exposed to 8 to 111 ppm (median = 36 ppm) of toluene compared with 44 unexposed referents. Reduced plasma concentrations of prolactin were related to blood toluene concentrations. In 8 printers, concentrations of LH and FSH increased during a 4-wk vacation, indicating that the effect was reversible.

Such effects on the plasma concentrations of reproductive hormones, although some are subtle, can have profound adverse effects that develop gradually over several months. When circulating testosterone concentrations in mammalian males and 17β-estradiol concentrations in mammalian females fall, the hypothalamic-pituitary-gonadal axis feedback loop responds by releasing FSH from the pituitary into the circulation, which acts on the hormone-producing cells of the testis (or ovary) to stimulate testosterone (or 17β-estradiol) production. When the gonads do not respond by producing steroid sex hormone, the pituitary continues to release FSH in response to continuing low concentrations of testosterone (or 17β-estradiol). In women, this profile (high FSH, low 17β-estradiol) is considered indicative of incipient menopause, and, over time, results in loss of estrous cycling, premature menopause, weight gain and fluid retention, and neurobehavioral changes such as mood swings (including increased irritability), headaches, loss of concentration, and depression.

In men, this profile (high FSH, low testosterone), over time, results in reduced upper body strength, reduced size and weight of the testes, reduced male-pattern hair loss, reduced sexual arousal and function, reduced aggressive and assertive behavior, and reduced executive functions (command and control, decision making).

Because of the effects of long-term exposure to toluene on FSH and testosterone (and presumably 17β-estradiol) serum concentrations in mammalian animal models and the potential (but highly likely) long-term consequences of such changes in the crew, the data for these end points are used to set 180- and 1,000-d ACs.

NEW RISK ASSESSMENT APPROACHES

Neither the data available when the original SMACs were set in 1996 nor the data available since then are amenable to analysis by benchmark dose methodology. The published data on which the original SMACs were based did not include the raw data for individual subjects and included only one dose (100 ppm) at which significant effects were reported. For the only statistically significant effects reported (headache, dizziness, and feeling of intoxication at 100 ppm but not at 40 or 10 ppm), the only description in the published report was: "This phenomenon was experienced by about half of the subjects, and the intensity was slight to moderate." Table 17-3 lists selected data published since 1996

TABLE 17-3 Toxicity Summary

Concentration/Dose and Chemical Form, Route	Exposure Duration	Species and Strain	Reported Adverse Effects	Reference
Acute and short–term exposures (<10 d)				
50 ppm, inhalation (only dose tested)	3 h	Humans, Male	Significantly decreased pulsatile secretion of LH into blood during exposure. No change in testosterone concentration in blood.	Luderer et al. 1999
50 ppm, inhalation (only dose tested)	3 h	Humans, female	Trend toward decreased pulsatile secretion of LH into blood during exposure. No other effects were significant.	Luderer et al. 1999
50 ppm, inhalation (only dose tested)	3 h	Men and women	No abnormal episodic LH or FSH secretion profiles; however, subtle effects on LH secretion seen in men and in women in the luteal phase.	Luderer et al. 1999
500-8,000 ppm, inhalation	30 min	Mice	Increased nociception using the hot plate test.	Cruz et al. 2001
1,000-4,000 ppm, inhalation	30 min	Mice	Increased nociception and anxiety (conditioned defensive burying test) and impaired learning.	Paez-Martinez et al. 2003
1,000-4,000 ppm, inhalation	30 min	Mice	Increased nociception and anxiety (conditioned defensive burying test) and impaired learning.	Lopez-Rubalcava et al. 2000
Subchronic exposures (10-90 d)				
50 mg/kg/d, subcutaneous injection	10 d	Rats, male	8-oxo-7,8-dihydro-2'-deoxyguanosine formation in testes, the biological marker for oxidative DNA damage, was increased by toluene.	Nakai et al. 2003
100 and 250 ppm, inhalation	6 h/d, 5 d/wk 4 wk	Rats	Long-term behavioral changes linked to reduced functional tonus of the dopaminergic system.	Wiaderna and Tomas 2002
300 ppm, inhalation	8 wk	Mice	Reduced bone mineral density in femoral neck.	Atay et al. 2005

2,000 ppm, inhalation	8 or 12 h/d 10 d	Chinchillas	No change in auditory brainstem response.	Davis et al. 2002
3,000 ppm	15 d	Rats, male	Significantly suppressed serum LH.	Yilmaz et al. 2001
Chronic exposures (>90 d)				
<20 ppm, TWA, inhalation	<13 y (rotogravure)	Workers n = 30	NOAEL for neurologic and neuropsychological effects.	Eller et al. 1999
35 ppm, average, inhalation	Occupational	Workers n = 41	NOAEL for impaired color vision	Zavalic et al. 1998a,c
40 ppm, inhalation	104 h/wk, 16 wk	Rats	Significant and gender-dependent alteration in both catecholamine and 5-hydroxytryptamine biosynthesis rate in brainstem catecholaminergic cell groups and hypothalamus.	Soulage et al. 2004
40 ppm, inhalation	104 h/wk, 16 wk	Rats	Alterations in dopamine and serotonin turnover in rat brain, decreased rearing activity, sensitization to toluene-induced narcosis.	Berenguer et al. 2003
40 ppm, inhalation	104 h/wk, 16 wk	Rats	Decreased rearing activity, sensitization to toluene-induced narcosis. NOAEL for ototoxicity.	Berenguer et al. 2004
40-60 ppm, measured by blood toluene concentration and urine metabolite concentrations	20.3 y, average	Workers n = 49	Increased amplitude and longer latency in N75, P100, and N145 waves of pattern reversal visual evoked potentials.	Vrca et al. 1995
40-60 ppm, measured by blood toluene concentration and urine metabolite concentrations	20.3 y, average	Workers n = 49	Decreased amplitude, longer P1 latency and interpeak latencies (P3-P5) in auditory evoked potentials.	Vrca et al. 1996

(Continued)

TABLE 17-3 Continued

Concentration/Dose and Chemical Form, Route	Exposure Duration	Species and Strain	Reported Adverse Effects	Reference
40-60 ppm, measured by blood toluene concentration and urine metabolite concentrations	20.3 y, average	Workers n = 49	Effects on auditory and visual evoked potentials were correlated with duration of exposure.	Vrca et al. 1997
45 ± 17 ppm (mean ± SD), Inhalation	Occupational ≥5 y	Workers n = 333	No ototoxicity (by pure tone audiometry).	Schaper et al. 2003
45 ppm, lifetime weighted average exposure	Occupational 21 y	Workers n = 192	No significant effects on attention, memory, or psychomotor functions by repeated measures analysis over four examinations in 5 y.	Seeber et al. 2004
46 ppm, geometric mean, predominantly toluene	Occupational	Human n = 174	No color vision deficits.	Nakatsuka et al. 1992
66-250 ppm	Occupational	Workers n = 32	Significantly impaired color vision.	Zavalic et al. 1998a
75-600 ppm	Occupational (rotogravure) 1-25 y	Workers n = 39	Ototoxicity.	Morata et al. 1993
0.037-243 ppm	Occupational (rotogravure) 1-25 y	Workers n = 124	Ototoxicity.	Morata et al. 1997
88 ppm, TWA, blood toluene = 1.25 mg/L	Occupational	Workers n = 30	Altered manual dexterity (grooved peg board), visual scanning (trail making, visual reproduction, Benton visual retention, and digit symbol), and verbal memory (digit span) but no clinical signs or symptoms.	Foo et al. 1990

>100 ppm, TWA	>12 y (rotogravure)	Workers n = 49	Impaired neuropsychological function (visuospatial function, number learning, and word recognition).	Eller et al. 1999
120 ppm	Occupational	Workers n = 45	Significantly impaired color vision persists >64 h.	Zavalic et al. 1998b
156 ppm, average, inhalation	Occupational	Workers n = 32	Significantly impaired color vision.	Zavalic et al. 1998c
1,000 ppm	5 h/d, 5 d/wk	Mice	Impaired behavior in T-maze test and decreased cell density and thickness of nasal epithelium.	Jacquot et al. 2006
Inhalation dose estimated by measuring urinary toluene	Occupational (rubber)	Humans n = 33	Subclinical reduction in color vision.	Cavalleri et al. 2000
36 ppm, median 8-111 ppm, range	Occupational (rotogravure)	Humans n = 20	Decreased LH, FSH, and testosterone in plasma.	Svensson et al. 1992
Inhalation dose not stated	Occupational (rotogravure)	Humans n = 40	Altered auditory evoked potentials but no clinical effects.	Abbate et al. 1993
Inhalation dose not stated	Occupational (offset printing)	Humans n = 26	Slightly increased cytogenetic damage in lymphocytes.	Aksoy et al. 2006
Inhalation dose not stated. mean blood toluene = 1.25 µg/mL standard deviation = 0.37 µg/mL	Occupational	Humans n = 29	Impaired short-term memory, sustained attention and concentration, visual scanning, perceptual motor speed, and finger dexterity.	Boey et al. 1997
Dose not stated for various paint solvents	Occupational (painting)	Workers n = 398	Increased risk of congenital malformations.	Hooiveld et al. 2006

Abbreviations: mg/kg/d, milligrams per kilogram of body weight per day; TWA, time-weighted average; SD, standard deviation; µg/mL, micrograms per milliliter.

and some earlier supporting data. Table 17-4 lists standards other organizations have published for toluene vapors. Table 17-5 lists updated SMACs as determined in this document. Table 17-6 lists the ACs calculated in the Rationale section for each end point and exposure duration.

RATIONALE

ACs were determined following the guidelines of the National Research Council (NRC 1992).

ACs are calculated for the following effects of toluene, which have been reported for exposure concentrations below the current regulatory levels: auditory and visual toxicity, neurotoxicity, and decreased blood concentrations of reproductive hormones.

TABLE 17-4 Air Standards for Toluene Vapors Set by Other Organizations

Organization, Standard	Amount	Reference
NIOSH,		NIOSH 2005
IDLH	500 ppm	
REL TWA, 10 h	100 ppm (377 mg/m^3)	
STEL, 15 min	150 ppm (566 mg/m^3)	
OSHA,		NIOSH 2005
PEL TWA, 8 h	200 ppm (754 mg/m^3)	
PEL TWA, ceiling	300 ppm (1,130 mg/m^3)	
PEL TWA, 10 min max peak	500 ppm (1,880 mg/m^3)	
ACGIH,		ACGIH 2008
TLV TWA, skin	20 ppm (75 mg/m^3)	
TLV STEL	None set	

Abbreviations: NIOSH, National Institute for Occupational Safety and Health; IDLH, immediately dangerous to life and health; REL, recommended exposure limits; TWA, time-weighted average; STEL, short-term exposure limit; OSHA, Occupational Safety and Health Administration; PEL, permissible exposure limit; ACGIH, American Conference of Governmental Industrial Hygienists; TLV, threshold limit value; STEL, short-term exposure limit.

TABLE 17-5 2008 Spacecraft Maximum Allowable Concentration for Toluene Vapors

Duration	ppm	mg/m^3	Target Toxicity
1 h	16	60	Neurotoxicity (dizziness)
24 h	16	60	Neurotoxicity (dizziness)
7 d	4	15	Auditory and visual toxicity
30 d	4	15	Auditory and visual toxicity
180 d	4	15	Auditory, visual, and hormonal effects
1,000 d	4	15	Auditory, visual, and hormonal effects

Auditory and Visual Effects

Vrca et al. (1995, 1996, 1997) found changes in both auditory and visual evoked potentials in workers occupationally exposed to toluene concentrations of 40 to 60 ppm for an average of 20.3 y. Schaper et al. (2003) reported a 45-ppm NOAEL for ototoxicity in workers exposed for 5 y. Zavalic et al. (1998a,c) found NOAELs for impaired color vision at an average toluene concentration of 35 ppm in workers. Assuming that 40 ppm is a LOAEL for auditory and visual effects, an AC can be calculated by dividing the LOAEL by a default safety factor of 10 to estimate a NOAEL. Because the LOAEL is from multiyear (occupational) exposures, the calculated AC will be applicable to 1,000-d exposures. Because no published studies were found that examined auditory and visual effects in humans after short-term exposures to toluene vapors (which might have provided data to support the calculation of higher ACs for short-term exposures), the AC calculated for 1,000-d exposures will be used for all exposure durations ≥7 d.

$$\text{7-, 30-, 180-, 1,000-d AC}_{\text{(auditory, visual effects)}} = 40\ \text{ppm}_{\text{(LOAEL)}} \div 10_{\text{(LOAEL to NOAEL)}} = 4\ \text{ppm}$$

No factors are needed to adjust for species or spaceflight effects.

Neurotoxicity

Seeber et al. (2004) report a NOAEL in workers for effects on attention, memory, and psychomotor functions at an average exposure concentration of 45 ppm for 21 y.

The weight of evidence from the studies listed in Table 17-3 supports treating a concentration of 20 ppm (Eller et al. 1999) as a human NOAEL for neurotoxicity. Because this study included only 30 workers exposed at <20 ppm, the NOAEL is multiplied by a factor of ($\sqrt{30}$)/10 to adjust for the small sample size in calculating an AC. The resulting AC, based on effects measured in workers exposed for years, will be conservatively applied for exposure durations as short as 7 d. Although no data are available for neurologic and neuropsychological effects during or after short-term exposures (1 to 24 h), the severity of the effects reported for long-term exposures is mild enough to be acceptable in a short-term emergency situation. Thus,

$$\text{1- and 24-h AC}_{\text{(neurotoxicity)}} = 20\ \text{ppm}$$

$$\text{7-, 30-, 180-, and 1,000-d AC}_{\text{(neurotoxicity)}} = 20\ \text{ppm}_{\text{(NOAEL)}} \times \sqrt{30}/10_{\text{(small n factor)}} = 10\ \text{ppm}$$

Reproductive Hormone Effects

Decreased circulating concentrations of LH and FSH were reported both for short (3 h) exposures to 50 ppm of toluene and for chronic (occupational) exposures to a median of 36 ppm of toluene and decreased testosterone and prolactin were reported for the occupational exposures. Because the reproductive effects of altered serum hormone concentrations develop gradually over several months and are reversible upon cessation of exposure to toluene, ACs for reproductive effects will be calculated only for exposure durations of 180 and 1,000 d. Normal monthly cycling of serum hormones in women is known to affect mood and behavior, but NASA does not consider it necessary to protect the crew against such normal behavioral effects. Thus, with 36 ppm used as a LOAEL, a NOAEL for long-term reproductive hormone effects can be estimated by reducing the LOAEL 10-fold:

$$\text{180- and 1,000-d AC}_{\text{(reproductive hormone effects)}} = 36\ \text{ppm}_{\text{(LOAEL)}} \div 10_{\text{(LOAEL to NOAEL)}} = 3.6\ \text{ppm, rounded to 4 ppm}$$

No factors are needed to adjust for species, exposure duration, or spaceflight effects.

Neurochemical Effects

Rats exposed to 40 ppm of toluene for 16 wk showed significant changes (both increases and decreases) in the biosynthesis rates of catecholamine and 5-hydroxytryptamine in specific regions of the brain (Soulage et al. 2004). These changes could not be correlated with any signs, symptoms, or clinical effects. Thus, although these changes may have some functional effects, the clinical magnitude, relevance, and adverse nature of such putative functional effects cannot be determined from the available data. Therefore, no AC will be set using the results of the study of Soulage et al.

Spaceflight Effects

None of the reported adverse effects of toluene exposures is known to be affected by spaceflight.

TABLE 17-6 Acceptable Concentrations for Toluene

			Uncertainty Factors				Acceptable Concentration, ppm					
Effect	Exposure Data	Species and Reference	NOAEL	Species	Time	Space Flight	1 h	24 h	7 d	30 d	180 d	1,000 d
Auditory and visual evoked potentials	40-60 ppm, 20.3 y, occupational	Human (Vrca et al. 1995, 1996, 1997)	10	1	1	1	—	—	4	4	4	4
NOAEL: neurologic and neuropsychological effects	<20 ppm, occupational	Human, n = 30 (Eller et al. 1999)	1	1	1	1	20	20	—	—	—	—
NOAEL: neurologic and neuropsychological effects	<20 ppm, occupational	Human, n = 30 (Eller et al. 1999)	$\frac{\sqrt{30}}{10}$	1	1	1	—	—	10	10	10	10
Decreased LH, FSH, testosterone, prolactin	50 ppm, 3 h 36 ppm, occupational	Human (Luderer et al. 1999) Human n = 20 (Svensson et al. 1992)	10	1	1	1	—	—	—	—	4	4
Neurotoxicity, irritation	40 ppm, 6 h NOAEL	Human, n = 16 (Andersen et al. 1983)	$\frac{\sqrt{16}}{10}$	1	1	1	16	16	16	16	16	—
SMACs							*16*	*16*	*4*	*4*	*4*	*4*

Abbreviation: —, not calculated.

REFERENCES

Abbate, C., C. Giorgianni, F. Munao, and R. Brecciaroli. 1993. Neurotoxicity induced by exposure to toluene: An electrophysiologic study. Int. Arch. Occup. Environ. Health 64(6):389-392.

ACGIH (American Conference of Governmental Industrial Hygienists). 1999. TLVs and BEIS: Threshold Limit Values for Chemical Substances and Physical Agents and Biological Exposure Indices. Cincinnati, OH: American Conference of Governmental Industrial Hygienists.

ACGIH (American Conference of Governmental Industrial Hygienists). 2008. Guide to Occupational Exposure Values. Cincinnati, OH: American Conference of Governmental Industrial Hygienists.

Alho, H., H. Tahti, J. Koistinaho, and A. Hervonen. 1986. The effect of toluene inhalation exposure on catecholamine content of rat sympathetic neurons. Med. Biol. 64(5):285-288.

Andersen, I., G.R. Lundqvist, L. Molhave, O.F. Pedersen, D.F. Proctor, M. Vaeth, and D.P. Wyon. 1983. Human response to controlled levels of toluene in six-hour exposures. Scand. J. Work Environ. Health 9(5):405-418.

Atay, A.A., E. Kismet, T. Turkbay, M. Kocaoglu, E. Demirkaya, S.U. Sarici, A. Congologlu, and E. Gokcay. 2005. Bone mass toxicity associated with inhalation exposure to toluene. Biol. Trace Elem. Res. 105(1-3):197-203.

Batlle, D.C., S. Sabatini, and N.A. Kurtzman. 1988. On the mechanism of toluene-induced renal tubular acidosis. Nephron 49(3):210-218.

Benignus, V.A., W.K. Boyes, and P.J. Bushnell. 1998. A dosimetric analysis of behavioral effects of acute toluene exposure in rats and humans. Toxicol. Sci. 43(2):186-195.

Berenguer, P., C. Soulage, D. Perrin, J.M. Pequignot, and J.H. Arbraini. 2003. Behavioral and neurochemical effects induced by subchronic exposure to 40 ppm toluene in rats. Pharmacol. Biochem. Behav. 74(4):997-1003.

Berenguer, P., C. Soulage, A. Fautrel, J.M. Pequignot, and J.H. Arbraini. 2004. Behavioral and neurochemical effects induced by subchronic combined exposure to toluene at 40 ppm and noise at 80 dB-A in rats. Physiol. Behav. 81(3):527-534.

Boey, K.W., S.C. Foo, and J. Jeyaratnam. 1997. Effects of occupational exposure to toluene: A neuropsychological study on workers in Singapore. Ann. Acad. Med. Singapore 26(2):184-187.

Cavalleri, A., F. Gobba, E. Nicali, and V. Fiocchi. 2000. Dose-related color vision impairment in toluene-exposed workers. Arch. Environ. Health 55(6):399-404.

Cruz, S.L., N. Paez-Martinez, F. Pellicer, L.A. Salazar, and C. Lopez-Rubalcava. 2001. Toluene increases acute thermonociception in mice. Behav. Brain Res. 120(2): 213-220.

Davis, R.R., W.J. Murphy, J.E. Snawder, C.A. Striley, D. Henderson, A. Khan, and E.F. Krieg. 2002. Susceptibility to the ototoxic properties of toluene is species specific. Hear. Res. 166(1-2):24-32.

Dyer, R.S., K.E. Muller, R. Janssen, C.N. Barton, W.K. Boyes, and V.A. Benignus. 1984. Neurophysiological effects of 30 day chronic exposure to toluene in rats. Neurobehav. Toxicol. Teratol. 6(5):363-368.

Eller, N., B. Netterstrom, and P. Laursen. 1999. Risk of chronic effects on the central nervous system at low toluene exposure. Occup. Med. 49(6):389-395.

Foo, S.C., J. Jeyaratnam, and D. Koh. 1990. Chronic neurobehavioural effects of toluene. Br. J. Ind. Med. 47(7):480-484.

Garcia, H.D. 1996. Toluene. Pp. 373-393 in Spacecraft Maximum Allowable Concentrations for Selected Airborne Contaminants, Vol.2. Washington, DC: National Academy Press.

Gobba, F. 2000. Color vision: A sensitive indicator of exposure to neurotoxins. Neurotoxicology 21(5):857-862.

Gobba, F., and A. Cavalleri. 2003. Color vision impairment in workers exposed to neurotoxic chemicals. Neurotoxicology 24(4-5):693-702.

Hooiveld, M., W. Haveman, K. Roskes, R. Bretveld, I. Burstyn, and N. Roeleveld. 2006. Adverse reproductive outcomes among male printers with occupational exposure to organic solvents. Occup. Environ. Med. 63(8):538-544.

Jacquot, L., G. Pourie, G. Buron, J. Monnin, and G. Brand. 2006. Effects of toluene inhalation exposure on olfactory functioning: Behavioral and histological assessment. Toxicol. Lett. 165(1):57-65.

Johnson, A.C. 1993. The ototoxic effect of toluene and the influence of noise, acetyl salicylic acid, or genotype: A. study in rats and mice. Scand. Audiol. Suppl. 39:1-40.

Juntunen, J., E. Matikainen, M. Antti-Poika, H. Souranta, and M. Valle. 1985. Nervous system effects of long-term occupational exposure to toluene. Acta Neurol. Scand. 75(5):512-517.

Kishi, R., I. Harabuchi, T. Ikeda, H. Yokota, and H. Miyake. 1988. Neurobehavioral effects and pharmacokinetics of toluene in rats and their relevance to man. Br. J. Ind. Med. 45(6):396-408.

Kostas, J., and J. Hotchin. 1981. Behavioral effects of low-level perinatal exposure to toluene in mice. Neurobehav. Toxicol. Teratol. 3(4):467-469.

Lees-Haley, P.R. 2000. Methodology in epidemiological studies of human neurobehavioral toxicity: A case study with critical review. Psychol. Rep. 86(1): 85-101.

Lopez-Rubalcava, C., R. Hen, and S.L. Cruz. 2000. Anxiolytic-like actions of toluene in the burying behavior and plus-maze tests: Differences in sensitivity between 5-HT(1B) knockout and wild-type mice. Behav. Brain Res. 115(1):85-94.

Luderer, U., M.S. Morgan, C.A. Brodkin, D.A. Kalman, and E.M. Faustman. 1999. Reproductive endocrine effects of acute exposure to toluene in men and women. Occup. Environ. Med. 56(10):657-666.

Mattsson, J.L., R.R. Albee, and S.J. Gorzinski. 1989. Similarities of toluene and o-cresol neuroexcitation in rats. Neurotoxicol. Teratol. 11(11):71-75.

Morata, T.C., D.E. Dunn, L.W. Kretschmer, G.K. Lemasters, and R.W. Keith. 1993. Effects of occupational exposure to organic solvents and noise on hearing. Scand. J. Work Environ. Health 19(4):245-254.

Morata, T.C., A.C. Fiorini, F.M. Fischer, S. Colacioppo, K.M. Wallingford, E.F. Krieg, D.E. Dunn, L. Gozzoli, M.A. Padrao, and C.L. Cesar. 1997. Toluene-induced hearing loss among rotogravure printing workers. Scand. J. Work Environ. Health 23(4):289-298.

Murata, K., S. Araki, K. Yokoyama, T. Tanigawa, K. Yamashita, F. Okajima, T. Sakai, C. Matsunaga, and K. Suwa. 1993. Cardiac autonomic dysfunction in rotogravure printers exposed to toluene in relation to peripheral nerve conduction. Ind. Health 31(3):79-90.

Nakai, N., M. Murata, M. Nagahama, T. Hirase, M. Tanaka, T. Fujikawa, N. Nakao, K. Nakashima, and S. Kawanishi. 2003. Oxidative DNA damage induced by toluene is involved in its male reproductive toxicity. Free Radical. Res. 37(1):69-76.

Nakatsuka, H., T. Watanabe, Y. Takeuchi, N. Hisanaga, E. Shibata, H. Suzuki, M.Y. Huang, Z. Chen, Q.S. Qu, and M. Ikeda. 1992. Absence of blue-yellow color vision loss among workers exposed to toluene or tetrachloroethylene, mostly at levels below occupational exposure limits. Int. Arch. Occup. Environ. Health 64(2):113-117.

Nielsen, H.K., L. Krusell, J. Beaelum, G. Lundqvist, O. Omland, M. Vaeth, S.E. Husted, C.E. Mogensen, and E. Geday. 1985. Renal effects of acute exposure to toluene: A controlled clinical trial. Acta Med. Scand. 218(3):317-321.

NIOSH (National Institute for Occupational Safety and Health). 2005. NIOSH Pocket Guide to Chemical Hazards. DHHS (NIOSH) No. 2005-151. National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention, U.S. Department of Health and Human Services, Cincinnati, OH

NRC (National Research Councel). 1992. Guidelines for Developing Spacecraft Maximum Allowable Concentrations for Space Station Contaminants. Washington, DC: National Academy Press.

Paez-Martinez, N., S.L. Cruz, and C. Lopez-Rubalcava. 2003. Comparative study of the effects of toluene, benzene, 1,1,1-trichloroethane, diethyl ether, and flurothyl on anxiety and nociception in mice. Toxicol. Appl. Pharmacol. 193(1):9-16.

Patel, R., and J.J. Benjamin. 1986. Renal disease associated with toluene inhalation. J. Toxicol. Clin. Toxicol. 24(3):213-223.

Pryor, G.T., and C.S. Rebert. 1984. Hearing loss in rats caused by inhalation of toluene, xylenes, and styrene. Abstract No. 315 in Arbetarskyddsverket, International Conference on Organic Solvent Toxicity, Stockholm.

Pryor, G.T., C.S. Rebert, J. Dickenson, and E.M. Feeney. 1984. Factors affecting toluene-induced ototoxicity in rats. Neurobehav. Toxicol. Teratol. 6(3):223-238.

Sandmeyer, E.E. 1981. Aromatic hydrocarbons. Pp. 3283-3291 in Patty's Industrial Hygiene and Toxicology, Vol. 2B, G.D. Clayton and F.E. Clayton, eds. New York: John Wiley & Sons.

Schaper, M., P. Demes, M. Zupanic, M. Blaszkewicz, and A. Seeber. 2003. Occupational toluene exposure and auditory function: Results from a follow-up study. Ann. Occup. Hyg. 47(6):493-502.

Seeber, A., M. Schäper, M. Zupanic, M. Blaszkewicz, P. Demes, E. Kiesswetter, and C. van Thriel. 2004. Toluene exposure below 50 ppm and cognitive function: A follow-up study with four repeated measurements in rotogravure printing plants. Int. Arch. Occup. Environ. Health 77(1):1-9.

Sliwinska-Kowalska, M., E. Zamyslowska-Szmytke, W. Szymczak, P. Kotylo, M. Fiszer, W. Wesolowski, M. Pawlaczyl-Luszczynska, M. Bak, and A. Gajda-Szadkowska. 2004. Effects of coexposure to noise and mixture of organic solvents on hearing in dockyard workers. J. Occup. Environ. Med. 46(1):30-38.

Soulage, C., D. Perrin, P. Berenguer, and J.M. Pequignot. 2004. Sub-chronic exposure to toluene at 40 ppm alters the monoamine biosynthesis rate in discrete brain areas. Toxicology 196(1-2):21-30.

Sullivan, M.J., K.E. Rarey, and R.B. Conolly. 1989. Ototoxicity of toluene in rats. Neurotoxicol. Teratol. 10(6):525-530.

Svensson, B.G., G. Nise, E.M. Erfurth, A. Nilsson, and S. Skerfving. 1992. Hormone status in occupational toluene exposure. Am. J. Ind. Med. 22(1):99-107.

Tang, H.L., K.H. Chu, A. Cheuk, W.K. Tsang, H.W. Chan, and K.L. Tong. 2005. Renal tubular acidosis and severe hypophosphataemia due to toluene inhalation. Hong Kong Med. J. 11(1):50-53.

Taylor, J.D., and H.L. Evans. 1985. Effects of toluene inhalation on behavior and expired carbon dioxide in macaque monkeys. Toxicol. Appl. Pharmacol. 80(3):487-495.

Vrca, A., D. Bozicevic, V. Karacic, R. Fuchs, D. Prpic-Majic, and M. Malinar. 1995. Visual evoked potentials in individuals exposed to long-term low concentrations of toluene. Arch. Toxicol. 69(5):337-340.

Vrca, A., V. Karacic, D. Bozicevic, V. Bozikov, and M. Malinar. 1996. Brainstem auditory evoked potentials in individuals exposed to long-term low concentrations of toluene. Am. J. Ind. Med. 30(1):62-66.

Vrca, A., D. Bozicevic, V. Bozikov, R. Fuchs, and M. Malinar. 1997. Brainstem evoked potentials and visual evoked potentials in relation to the length of occupational exposure to low levels of toluene. Acta Med. Croatica 51(4-5):215-219.

Weiss, B., R.W. Wood, and D.A. Macys. 1979. Behavioral toxicology of carbon disulfide and toluene. Environ. Health Perspect. 30:39-45.

Wiaderna, D., and T. Tomas. 2002. Assessment of long-term effects of exposure to toluene based on the analysis of selected behavioral responses with particular reference to the ability to trigger behavioral hypersensitivity in rats. Int. J. Occup. Med. Environ. Health 15(3):239-245.

Yilmaz, B., S. Kutlu, S. Canpolat, S. Sandal, A. Ayar, R. Mogulkoc, and H. Kelestimur. 2001. Effects of paint thinner exposure on serum LH, FSH and testosterone levels and hypothalamic catecholamine contents in the male rat. Biol. Pharm. Bull. 24(2):163-166.

Zavalic, M., Z. Mandic, R. Turk, A. Bogadi-Sare, and D. Plavec. 1998a. Quantitative assessment of color vision impairment in workers exposed to toluene. Am. J. Ind. Med. 33(3):297-304.

Zavalic, M., Z. Mandic, R. Turk, A. Bogadi-Sare, D. Plavec, M. Gomzi, and L.J. Skender. 1998b. Assessment of colour vision impairment in male workers exposed to toluene generally above occupational exposure limits. Occup. Med. 48(3):175-180.

Zavalic, M., Z. Mandic, R. Turk, A. Bogadi-Sare, D. Plavec, and L.J. Skender. 1998c. Qualitative color vision impairment in toluene-exposed workers. Int. Arch. Occup. Environ. Health 71(3):194-200.

18

Trimethylsilanol

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BACKGROUND AND SUMMARY OF ORIGINAL APPROACH

No inhalation toxicity data were available on trimethylsilanol (TMS) at the time the original spacecraft maximum allowable concentrations (SMACs) were set; however, there were limited data from oral, intraperitoneal, and intravenous doses administered primarily to rodent species (Kaplan et al. 1994). The longest study was an oral dominant lethal study in Sprague-Dawley (S-D) rats given TMS doses of 20, 100, or 200 milligrams per kilogram of body weight per day (mg/kg/d), 5 d/wk for 8 wk (Isquith et al. 1988). The SMACs were based on various limited studies in which central nervous system (CNS) depression was an observed clinical sign in the dosed animals. Abstract data on the disposition of TMS (absence of silicon compounds in the urine) suggest that rodents eliminate it within 48 h (Dow Corning Corp. 1972). Further factors considered in setting the SMACs included an interspecies extrapolation factor of 10, the uptake efficiency of TMS in the respiratory system (50%), and the minute volume of a crewmember over the putative exposure period. The details for each SMAC calculation are given below and the SMAC values are presented in Table 18-1.

An intravenous study in rats showed that TMS caused mild anesthesia at 100 mg/kg and moderate anesthesia at 200 mg/kg (A.J. Isquith, Dow Corning Corp., unpublished material, 1991). From these data, it was estimated that 50 mg/kg would be a no-observed-adverse-effect level (NOAEL). It was further assumed that the inhalation uptake was 50% in a human breathing 0.02 m^3/min and weighing 70 kg. The calculation, which used a 10-fold species-extrapolation factor, was as follows:

$$AC_{(CNS)} = 50\ mg/kg_{(NOAEL)} \times 1/10_{(species)} \times [70\ kg/(0.5 \times 0.02\ m^3/min \times 60\ min)]_{(inhalation\ uptake)} = 580\ mg/m^3$$

$580\ mg/m^3 \times (0.27\ ppm / 1\ mg/m^3)_{(conversion)}$
$= 156.6\ ppm$, rounded to 150 ppm

where AC is the acceptable concentration and ppm is parts per million.

The 24-h SMAC was set with the same starting point except that it was assumed that 50% of the TMS was eliminated in 24 h and the average breathing rate was lower (0.015 m^3/min) because of sleep periods. The calculation was as follows:

$24\text{-h AC}_{(CNS)} = 50\ mg/kg_{(NOAEL)} \times 1/10_{(species)} \times [70\ kg/(0.5 \times 0.5 \times 0.015\ m^3/min \times 1{,}440\ min)]_{(inhalation\ uptake)} = 65\ mg/m^3$

$65\ mg/m^3 \times (0.27\ ppm / 1\ mg/m^3)_{(conversion)} = 17.6\ ppm$, rounded to 20 ppm

The long-term SMACs were set from the oral CNS-depression NOAEL of 100 mg/kg observed in rats given doses over 31 d (Dow Corning Corp. 1972). The calculation was as follows:

$7\text{-, 30-, 180-, and 1,000-d AC}_{(CNS)} = 100\ mg/kg_{(NOAEL)} \times 1/10_{(species)} \times [70\ kg/(0.015\ m^3/min \times 1{,}440\ min/d)]_{(inhalation\ uptake)} = 32\ mg/m^3$

$32\ mg/m^3 \times (0.27\ ppm / 1\ mg/m^3)_{(conversion)} = 8.6\ ppm$, rounded to 10 ppm

Because the relative uptake of an oral and an inhalation dose was unknown, no factor was used to relate the oral and inhalation routes. Basically, it was assumed that the two routes of exposure have comparable uptakes.

Experience with samples of air obtained in the International Space Station shows that the concentration of TMS is typically about 0.1 mg/m^3 (0.03 ppm) or less, with occasional excursions up to 0.3 mg/m^3 (1.1 ppm) and one excursion to 0.65 mg/m^3 (2.4 ppm). The most likely source is silicone lubricants, which would have in episodic entry into the atmosphere. TMS is commonly found in off-gas tests of flight hardware, presumably from lubricants in hardware joints or from off-gassing from silicone-based seals.

TABLE 18-1 Previously Set SMACs for TMS

Duration of exposure	SMAC, ppm	Target toxicity to prevent
1 h	150	CNS depression
24 h	20	CNS depression
7 d	10	CNS depression
30 d	10	CNS depression
180 d	10	CNS depression

Source: Data from Kaplan et al. 1994.

CHANGES IN ORIGINAL APPROACH

The studies upon which the SMACs were based were reported in abstract form by Dow Corning Corp. 1972. As noted in the section on new risk assessment approaches, a structure-activity comparison with the CNS effects of *t*-butanol is considered.

RELEVANT NEW DATA SINCE 1991

No new data were found as a result of a search of the open literature. Dow Corning was contacted and reported that they have not developed any new data since 1991 (M. Andriot, Dow Corning Corp., personal communication). However, an industry consortium, the Silicon Environmental Safety and Health Council, expects to initiate a research effort in 2008 to build the database on TMS toxicity.

NEW RISK ASSESSMENT APPROACHES

The limited data on TMS toxicity, available primarily in abstract form, consist of nonquantitative clinical observations of the anesthetic effects of orally or intravenously administered TMS. Such nonquantitative data are not suitable for benchmark dose analysis because a dose-response relationship is not reported. One important observation not considered in the original assessment was that "the CNS effect is qualitatively the same as noted for similar carbon analogs, e.g. t-butanol; however, quantitatively TMS appears to be more potent" (A.J. Isquith, Dow Corning Corp., unpublished material, 1991). To avoid CNS effects, the AC for *t*-butanol has been set at 50 ppm; thus, one must expect that the SMACs for TMS ought to be lower than 50 ppm.

RATIONALE FOR REVISIONS TO THE PREVIOUS APPROACHES

The previous SMACs have four critical weaknesses. They were based on a noninhalation route of administration, there were no prolonged exposures even approaching a subchronic study, the data were reported mostly in the form of company abstracts, and the means of assessment of CNS effects was insensitive. There is potential for considerable bias in the reporting of data under these conditions. On the basis of these considerations, a factor should be applied to compensate for the uncertainty these weaknesses cause. A factor of 10 is proposed for this uncertainty. Thus, each of the original SMACs has been reduced by a factor of 10 because of these database limitations.

STRUCTURE-ACTIVITY RELATIONSHIP AND LIPOPHILICITY

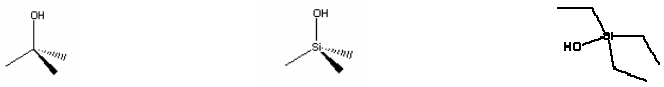
An approach using structure-activity relationships was considered. Unfor-

tunately, there are few toxicity data on structurally similar compounds. No useful data on dimethylsilanol could be found except that it does not appear to be a CNS depressant like TMS (Bennett and Statt 1973). A study designed to characterize the antimicrobial activity of trialkylsilanol compounds compared the antimicrobial activity of TMS, *t*-butanol, and triethylsilanol (TES) in two bacterial strains (Kim et al. 2006). The structures are presented in Figure 18-1.

The average antimicrobial activity in the bacterial strains was inversely related to the octanol-water partition coefficient as shown in Table 18-2.

Kim et al. (2006) speculate that the increased antimicrobial activity of silanols is due to the higher lipophilicity of the silanols compared with the alcohol. This was especially true for the larger, more lipophilic silanol, TES. The most important observation from Table 18-2 is that *t*-butanol is approximately 5-fold less toxic than TMS, which is reasonably consistent with the observation that TMS is roughly 3 times as potent as *t*-butanol in inducing CNS depression, a phenomenon known to depend on lipophilicity. The relationship between lipophilicity and anesthetic potency, called the Meyer-Overton correlation, has been known for more than a century. At least for small compounds such as those considered here, the mechanism of action is basically that the compounds act by partitioning into the lipid bilayer of membranes and crossing the blood-brain barrier. The differences in potency are due to the degree of lipophilicity.

Once the 10-fold factor has been applied for database weaknesses, the TMS SMACs are much lower than the *t*-butanol SMACs, which is consistent with the observation that *t*-butanol is approximately 3-fold less potent than TMS in inducing CNS depression (A.J. Isquith, Dow Corning Corp., unpublished material, 1991) and 5-fold less potent in antimicrobial activity (Kim et al. 2006).



t-butanol *TMS* *TES*

FIGURE 18-1 Structures of compounds tested by Kim et al. (2006). Abbreviation: TES, triethylsilanol; TMS, trimethylsilanol. Source: Data from Kim et al. 2006.

TABLE 18-2 Lipophilicity (Octanol-Water Partition Coefficient) of Three Compounds Compared with Their Antimicrobial Activity in Two Strains of Bacteria

Compound/parameter	Log OWPC	Minimum lethal concentration, %
TES	2.62	0.2
TMS	1.14	2.5
t-butanol	0.73	12.5

Abbreviation: OWPC, octanol-water partition coefficient; TES, triethylsilanol; TMS, trimethylsilanol.
Source: Kim et al. 2006.

RATIONALE FOR THE 1,000-D SMAC

With the addition of a factor of 10 for the limited nature of the database, the 7- to 180-d SMAC was reduced from 10 to 1 ppm. Given the demonstration that this compound does not accumulate and that no pathological effects have been found in rats repeatedly exposed to the compound by gavage (Dow Corning Corp. 1972), there is no need to reduce the limit further for 1,000 d of exposure.

COMPARISON OF SMACS WITH OTHER AIR-QUALITY LIMITS

The state of Michigan has set an initial threshold screening level (ITSL) based on analysis by the Air Quality Division of the Department of Environmental Quality. The value was set at 0.065 mg/m^3. The starting point was the NOAEL of 100 mg/kg from a 31-d gavage study (Dow Corning Corp. 1972, M.L. Hultin, Michigan Department of Natural Resources, personal communication, 1993). A safety factor of 15 was used (10 for the short duration of the study, plus 5 for the lack of controls in the study). The calculation was as follows:

$$\text{ITSL} = 100 \text{ mg/kg}_{\text{(NOAEL)}} \times 0.97_{\text{(inhalation rate conversion)}} \times 1/15_{\text{(safety factors)}} \times 1/100 = 0.065 \text{ mg/m}^3 \sim 0.02 \text{ ppm}$$

where 0.97 is the conversion factor for inhalation rate per kg of body weight (rats to humans), and a default value of 1 was used for unknown relative absorption efficiency. The genesis of the factor of 100 is unclear but is probably for inter- and intraspecies differences.

The U.S. Department of Energy (DOE) has declared temporary emergency exposure limits (TEELs) for TMS. Table 18-3 compares the TEELs for TMS and *t*-butanol (DOE 2005).

As indicated in Table 18-3, the method used to derive the TEELs for TMS was the structure-activity relationship. The specific method for *t*-butanol, however, was not provided. It is apparent from the footnote material that TEELs are a work in progress and that they are default values with minimal expert judgment used to derive the values. No rational explanation was given as to why TMS should be 2,000 (300/0.15) times as toxic as *t*-butanol based on a Si atom in place of a C atom.

In Table 18-4, the new SMACs are presented in comparison to the previous SMACs. The proposed long-term SMACs for TMS are generally above either the Michigan ITSL or the DOE TEEL-0. There is good consistency between the TEEL-1 of 1.5 ppm and the 24-h SMAC of 2 ppm. Both are set to avoid anything more than mild transient effects.

If the factor of 100 were dropped from the ITSL, the value would be 2 ppm, which is close to the long-term SMAC of 1 ppm. The application of this

factor of 100 seems excessive as TMS does not accumulate, the intravenous study is likely to exaggerate any CNS effects when compared with the same dose inhaled over 24 h, and there is no evidence that the compound is metabolized, even in the liver. The long-term SMAC is 7 times higher than the TEEL-0 of 0.15 ppm; however, the TEEL-0 seems excessively low compared with the *t*-butanol TEEL-0 of 300 ppm and the observation that TMS is 3-5 times as potent as *t*-butanol. This is based on the comparative observations of CNS depression in rats (A.J. Isquith, Dow Corning Corp., unpublished material, 1991) and the relative antimicrobial activity in two strains of bacteria (Kim et al. 2006).

RECOMMENDATIONS FOR ADDITIONAL RESEARCH

An inhalation database is needed to develop evidence-based exposure standards. It should consist of short-term exposures in animals and perhaps even in humans to determine irritancy and odor thresholds and to more thoroughly characterize CNS effects. Inhalation exposures in animals for at least 13 wk are needed to more precisely develop longer term inhalation standards.

TABLE 18-3 Comparison of TEELs for TMS and *t*-Butanol

TEEL[a]	Explanation	TMS, ppm	*t*-Butanol, ppm
0	The threshold concentration below which most people will experience no appreciable risk of health effects	0.15	300
1	The maximum concentration in air below which it is believed nearly all individuals could be exposed without experiencing other than mild transient adverse health effects or perceiving a clearly defined objectionable odor	1.5	400
2	The maximum concentration in air below which it is believed nearly all individuals could be exposed without experiencing or developing irreversible or other serious health effects or symptoms that could impair their abilities to take protective action	7.5	5,000
3	The maximum concentration in air below which it is believed nearly all individuals could be exposed without experiencing or developing life-threatening health effects	15	5,000

[a]It is recommended that, for application of TEELs, the concentration at the receptor point of interest be calculated as the peak, 15-min time-weighted average concentration. It should be emphasized that TEELs are default values, following the published methodology (on Subcommitte on Consequence Assessment and Protective Actions' web page) explicitly. The only judgment involved is that exercised in extracting data that are entered in the Excel Workbook used to automatically calculate the recommended TEELs (DOE 2007).

TABLE 18-4 Previous and Revised SMACs for TMS

		Uncertainty Factors			Acceptable Concentrations, ppm					
Effect, exposure route	Species / Sex, Reference	NOAEL, mg/m^3	Species	Critical weakness in original approach [a]	1 h	24 h	7 d	30 d	180 d	1,000 d
Original Approach										
CNS depression, IV	Rats/unknown (A.J. Isquith, unpublished material, 1991)	50	10	N/A	150	—	—	—	—	—
CNS depression , IV	Rats/unknown (A.J. Isquith, unpublished material, 1991)	50	10	N/A	—	20	—	—	—	—
CNS depression, Oral 100 mg/kg/d	Sprague-Dawley rats/M and F (Dow Corning 1972)	100	10	N/A	—	—	10	10	10	10
Original SMACs					*150*	*20*	*10*	*10*	*10*	*10*
Revised Approach[a]										
CNS depression, IV	Rats/unknown (A.J. Isquith, unpublished material, 1991)	50	10	10	15	—	—	—	—	—
CNS depression , IV	Rats/unknown (A.J. Isquith, unpublished material, 1991)	50	10	10	—	2	—	—	—	—
CNS depression, Oral 100 mg/kg/d	Sprague-Dawley rats/M and F (Dow Corning 1972)	100	10	10	—	—	1	1	1	1
Revised SMACs					*15*	*2*	*1*	*1*	*1*	*1*

[a]Because the original SMACs were based on a noninhalation route of administration, a lack of prolonged exposure, data reported in the form of company abstracts, and an insensitive means of assessment of CNS effects, a factor of 10 is applied to compensate for uncertainty.
Abbreviations: F, female; IV, intravenous; M, male; N/A, not applicable.

REFERENCES

Bennett, D.R., and H. Statt. 1973. Primate absorption and elimination balance studies including pulmonary, urinary, biliary and fecal excretion of t-butanol, trimethylsilanol, dimethylsilanol and hexamethyldisiloxane. Toxicol. Appl. Pharmacol. 25:445.

DOE (U.S. Department of Energy). 2007. Protective Action Criteria (PAC) with AEGLs, ERPGs, & TEELs: Rev. 23 for Chemicals of Concern. Chemical Safety Program, Office of Health, Safety and Security, U.S. Department of Energy [online]. Available: http://www.hss.energy.gov/HealthSafety/WSHP/chem_safety/teel.html [accessed Jan. 16, 2008].

Dow Corning Corp. 1972. A Toxicological Evaluation of Trimethylsilanol and Dimethylsilanol in the Rat. Dow Corning Internal Research Report No. 4006. Dow Corning Corp., Midland, MI.

Isquith, A.R., R. Slesinski, and D. Matheson. 1988. Genotoxicity studies on selected organosilicon compounds: In vivo assays. Food Chem. Toxicol. 26(3):263-266.

Kaplan, H.L., M.E. Coleman, and J.T. James. 1994. Trimethylsilanol. Pp. 177-184 in Spacecraft Maximum Allowable Concentrations for Selected Airborne Contaminants, Vol. 1. Washington, DC: National Academy Press.

Kim, Y.M., S. Farrah, and R.H. Baney. 2006. Silanol—A novel class of antimicrobial agents. Electron. J. Biotechnol. 9(2) [online]. Available: http://www.ejbiotechnology.info/content/vol9/issue2/full/4/index.html [accessed Jan. 15, 2008].

19

Xylenes

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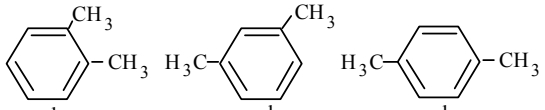
BACKGROUND

Xylene, also known as dimethylbenzene (formula: C_6H_4 $(CH_3)_2$) exists as three isomers—*ortho*-(1,2-dimethylbenzene), *meta*-(1,3-dimethylbenzene), and *para*-(1,4-dimethylbenzene)—whose structures are shown below. Commercial xylenes (also called mixed xylenes) are a mixture of the three forms with *meta*- (abbreviated "*m*-") being the major component and *ortho*- (abbreviated "*o*-") and *para*- (abbreviated "*p*-") present in minor amounts; usually, the proportions are 40% *m*-isomer and 20% each of the *p*- and *o*-isomers. Technical-grade xylenes also contain ethylbenzene. In general, they have similar physical, chemical, and toxicologic properties. Although the odor threshold is listed as 1 part per million (ppm) in air, it seems to vary among the isomers, being 3.7, 0.17, and 0.47 ppm for *m*-, *o*-, and *p*-xylene, respectively (ATSDR 2007). The vapor pressures for the three are comparable (8.29, 6.61, and 8.84 mm Hg for *m*-, *o*-, and *p*-xylenes, respectively (ATSDR 2007).



o-xylene *m*-xylene *p*-xylene

Conversion factors at 25°C and 1 standard unit of atmospheric pressure (atm) are as follows: 1 ppm = 4.34 milligrams per cubic meter (mg/m^3) and 1 mg/m^3 = 0.23 ppm. Partition coefficients for *m*-xylene are as follows: blood:air = 46 for male Wistar rats and 26.4 for humans (Gargas et al. 1989, Tardif et al. 1997).

OBJECTIVE

With NASA's current focus on exploration missions beyond low Earth orbit to the Moon and Mars, there is a need to derive an acceptable concentration (AC) of xylene in spacecraft atmospheres for 1,000 d, which has not previously been derived. Typical spacecraft concentrations have been 0.48 ppm, and it is not known what the concentrations will be for the newer spacecraft being designed for Mars and Moon missions (NASA, personal communication, May 19, 2008). Spacecraft maximum allowable concentrations (SMACs) for xylene were originally developed and published in Volume 3 of this series, *Spacecraft Maximum Allowable Concentrations for Selected Airborne Contaminants*, for exposure durations of 1 h, 24 h, 7 d, 30 d, and 180 d (Garcia 1996). This document reviews all available subchronic and chronic duration xylene exposure studies and derives exposure limits acceptable for exposures up to 1,000-d (1,000-d AC). At the same time, NASA will evaluate the need to update the previously published SMACs for up to 180 d based on toxicologic studies published since the last evaluation. NASA will also consider whether the previously used data are suitable for processing by current risk assessment methodologies (such as benchmark dose computations) recommended by the National Research Council (NRC) Committee on Spacecraft Exposure Guidelines.

PHARMACOKINETICS AND METABOLISM

A detailed summary of the pharmacokinetics and metabolism of xylene was presented in the 1996 SMAC document (Garcia 1996). A brief summary is presented here. It has been reported that humans absorb about 60% of the xylene from inhalation exposures over concentrations ranging from 46 to 200 ppm, and the percent retained is independent of the duration of exposure time from 15 min to 8 h in a single day, or after 5 d of 6-h/d exposure. The percent retention varies only slightly among individuals and among the three isomers of xylene (Sedivec and Flek 1976, Riihimäki et al. 1979). Postexposure, 4% to 6% of the amount retained is expired as unchanged xylene. In humans exposed to 100 ppm, the half-lives for elimination of xylene were 0.8 h for the initial phase, 7.7 h for the intermediate phase, and 17.7 h for the slowest phase (Sedivec and Flek 1976).

Monitoring of urine from humans exposed to xylene both experimentally and in the workplace indicates that almost all the absorbed xylene undergoes oxidation of the methyl group by the microsomal mixed function oxidases to yield methylhippuric acid (MHA), which is excreted in the urine (Ogata et al. 1970, Sedivec and Flek 1976, Senczuk and Orlowski 1978, Ogata and Fujii 1979, Riihimäki et al. 1979). Metabolism of xylene does not appear to saturate even at 200 ppm of exposure (Riihimäki et al. 1979). Urinary excretion of MHA correlates well with xylene uptake, so that one can use it to estimate exposure. Physiologically based pharmacokinetic (PBPK) models indicate that the elimination of MHA is linear up to an exposure concentration of 500 ppm (Kaneko et

al. 1991). In humans, there appear to be some small gender differences for excretion of MHA, with men excreting more than women do (Ernstgard et al. 2002, 2003).

SUMMARY OF ORIGINAL APPROACH

The xylene SMACs for exposures of 1 h to 180 d were primarily based on neurotoxicity end points such as headache, dizziness, and central nervous system (CNS) depression; subjective measures of irritation of the nose and eyes; and throat discomfort. Several controlled human exposure studies and several animal studies were reviewed (Garcia 1996).

The 1- and 24-h ACs were derived for various end points based on reports on acute effects, which included both human and animal data. Lethality in rats and humans (Morley et al. 1970, Carpenter et al. 1975), mild throat irritation and eye irritation in humans (Carpenter et al. 1975), narcosis in rats (Molnar et al. 1986), and cardiac depression in dogs (Kobayashi et al. 1989) were used as end points. Both the 1- and 24-h SMACs of 100 ppm were driven by mild throat irritation in humans and narcosis in rats.

Garcia (1996) calculated 1- and 24-h ACs based on mild throat irritation and discomfort reported by Carpenter et al. (1975) for one of seven human volunteers during the first minute of inhalation to 230 ppm and again during the seventh minute of a 15-min inhalation to 230 ppm *m*-xylene. The degree of irritation reported was minor. Such mild irritation would be acceptable for a brief contingency exposure; thus, in calculating the ACs for 1- and 24-h exposures, the 106-ppm value was considered a no-observed-adverse-effect level (NOAEL) and was not adjusted for exposure duration. The value was rounded to 100 ppm. As the NOAEL for throat irritation is much lower than that for eye irritation, eye irritation did not drive the SMAC for 1- and 24-h exposure durations. Similarly, as the NOAEL for throat irritation is much lower, the end point of dizziness did not drive the SMAC for these durations.

Garcia (1996) also derived 1- and 24-h ACs from the results of a rat study by Molnar et al. (1986) in which narcosis of rats was noted when they were exposed for 4 h at 2,100 ppm but not at 1,000 ppm. Using 1,000 ppm as a NOAEL, Garcia (1996) derived 1- and 24-h ACs for narcosis of 100 ppm after applying a species factor of 10.

ACs and SMACs for 7, 30, and 180 d (Garcia 1996) were calculated on the basis of eye irritation and mild throat irritation in humans (Carpenter et al. 1975); narcosis in rats (Molnár et al. 1986); reduced cardiac output in dogs (Kobayashi et al. 1989); absence of abnormal serum clinical chemistry, hematology, and urine chemistry in rats and dogs; and electrocardiogram in rats and dogs (Carpenter et al. 1975). For calculating the SMACs for these short and long durations (7-, 30-, and 180-d), 106 ppm from the human study was considered a lowest-observed-adverse-effect level (LOAEL). Because prolonged irritation of eyes and throat would not be acceptable, the LOAEL was divided by 2 to obtain

the NOAEL. Garcia (1996) derived the same SMAC of 50 ppm (217 mg/m^3) for 7-, 30-, and 180-d durations, driven by the lowest of ACs for mild throat irritation reported by humans in the Carpenter et al. (1975) study.

Garcia (1996) also evaluated an animal experiment by Carpenter et al. (1975) in which a subchronic intermittent inhalation exposure study of dogs and rats (180, 460, or 810 ppm of commercial mixed xylenes for 6 h/d and 5 d/wk for 13 wk) resulted in no changes from controls in blood and urine chemistry, hematology, and histopathology of several tissues. As the NOAEL of 810 ppm for rats and dogs for systemic effects is much higher (even after considering the species factor) than that for throat irritation, the end points for systemic effects in rodents and dogs did not drive the AC for 30 and 180 d.

CARCINOGENIC RISK ASSESSMENT

Xylenes are not classifiable as to their carcinogenicity to humans (Group 3) (IARC 1999, p. 1189). The U.S. Environmental Protection Agency (EPA) categorized xylene as D, meaning there is no evidence of carcinogenicity in human or animal studies. In vivo genotoxicity of xylenes in humans occupationally exposed or experimentally exposed for short durations, or in rats or mice intraperitoneally injected with xylene, were all found to be negative. Thus, Garcia (1996) calculated no carcinogenic risk factor for xylene. A summary of SMACs for xylene presented by Garcia (1996) is shown in Table 19-1.

STUDIES NOT COVERED IN THE 1996 SMAC DOCUMENT FOR XYLENE

Acute Exposure Studies

Sixteen men were studied in an exposure chamber to assess the effect of 4 h of exposure to 70 ppm of *p*-xylene and a control condition. Subjects performed computer-administered tests of simple reaction time, short-term memory, and choice reaction time immediately after entering the chamber and after 2 and 4 h of exposure. Xylene exposure did not affect their performance on these tests (Olson et al. 1985).

In a similar study, Dudek et al. (1990) assessed CNS functions in 10 male volunteers aged 22 to 35 y by means of a battery of nine psychological tests, during an experimental exposure to 100 ppm of pure xylene (purity not specified) for 4 h compared with clean air. Each individual served as his or her own control and the treatments were randomized. This produced a statistically significant effect at $P \leq 0.01$ on two of the nine tests—namely, simple reaction time (SRT) (prolongation of simple reaction time test) and choice reaction time (ChRT) (22% and 13% longer, respectively, than controls). No adverse responses to the other tests were observed. The only concentration used (100 ppm)

TABLE 19-1 A Summary of SMACs for Xylene

Duration	ppm	mg/m^3	Adverse End Point	Principal Study
1 h	100	435	Throat irritation, narcosis	Carpenter et al. 1975, Molnar et al. 1986
24 h	100	435	Throat irritation, narcosis	Carpenter et al. 1975
7 d	50	217	Throat irritation	Carpenter et al. 1975
30 d	50	217	Throat irritation	Carpenter et al. 1975
180 d	50	217	Throat irritation	Carpenter et al. 1975

Source: Garcia 1996.

seems to be the LOAEL (compared with no effect seen at 70 ppm in the Olson et al. study [1985]) for neurologic effects and should be considered for 1- and 24-h AC derivations. Thus, a NOAEL of 70 ppm based on the Olson study and a LOAEL of 100 ppm based on the Dudek study could be identified.

Ernstgard et al. (2002) conducted an acute exposure study in which 56 healthy volunteers (28 of each sex) were exposed to 50 ppm of *m*-xylene for 2 h at rest. This study involved measuring the adverse effects by both subjective assessment and objective measurements. The subjects rated symptoms (perceived level of discomfort) for 10 questions on a visual analog scale (VAS) before exposure, during exposure (at 3, 60, and 118 min), and after exposure (20 min and about 4 h after exposure). The VAS results were rated on a scale of 0 to 100 mm where the level of perceived discomfort was rated as follows: 0 mm (none at all) to 6 mm (hardly at all) to 26 mm (somewhat) to 48 mm (rather) to 71 mm (quite) to 90 mm (very) to 100 mm (unbearable). Increased symptom ratings were rated by both sexes for nearly all 10 questions during exposure to *m*-xylene; most increases were statistically significant for at least one time point (at either 60 or 118 min during the exposure). The rating of "discomfort in the throat or airways" was higher in women. Solvent smell was rated as "rather" by both sexes. Discomfort to the eye, nose, and throat were only just above "hardly at all." Although "fatigue" was rated close to "somewhat," it did not differ from the rating of unexposed controls. Nausea, giddiness, and a feeling of intoxication were below 6 mm in the VAS scale. Although these VAS scores for "discomfort in the throat or airways" were statistically significantly different from those of control subjects exposed to clean air, the absolute numbers indicated only minimal discomfort from xylene exposure. On the basis of these ratings and overall significance and importance, 50 ppm is considered as a minimal LOAEL.

Pulmonary function, nasal swelling, inflammatory markers in nasal lavage, and color vision (color confusion index) were measured before and 0 and 3 h after the exposure. No significant effect on pulmonary function was seen in men after either exposure or 3 h after exposure or in women immediately after exposure. Women had small but significant decreases in forced vital capacity 3 h after exposure to *m*-xylene. An evaluation of the data expressed as percent change indicates that 3 h after exposure the change was less than 4% (Ernstgard

et al. 2002). Thus, these changes are considered unremarkable, as they are not expected to pose any concern.

No significant effects were noted in nasal swelling, in inflammatory markers in the nasal lavage, or in blinking frequency (Ernstgard et al. 2002).

Short-Term and Subchronic Duration Studies

Riihimäki and Savolainen (1980) reported changes in body balance (function of the vestibular system), and psychomotor function (choice reaction time and simple reaction time) in male volunteers exposed to *m*-xylene at 100 to 400 ppm over 5 consecutive days, 6 h/d with a break for 1 h at noon, then for 1 to 3 d after a weekend. Because the exposure regimens (constant exposure levels and fluctuating exposure levels) were very complex, the data could not be interpreted properly to find out the NOAEL or LOAEL and the dose-effect response pattern.

Hake et al. (1981), in a controlled human exposure study, evaluated the effect on men (one to four subjects) of exposure to *p*-xylene for 7.5, 3, or 1 h/d, 5 d/wk, for 4 wk. The exposure concentration was 100 ppm during the first week, then 20, 150, and 100 ppm in the following weeks. Groups of women (two or three per group) were exposed to *p*-xylene for 7.5, 3, or 1 h/d for 5 d. Data on subjective responses and objective responses such as neurologic tests, cognitive tests, and cardiopulmonary function tests were gathered. Except for irritating effects reported at 100 ppm, no serious effects were noted. The sample size was too small to draw any meaningful conclusions.

In a 4-wk inhalation exposure study, rats (10 or 11 per group) were exposed repeatedly to 100 ppm of *m*-xylene for 6 h/d, 5 d/wk (Gralewicz and Wiaderna 2001). Starting 2 wk postexposure, the behavior of the rats was assessed by radial maze performance, spontaneous activity in an open field, and learning and retention of passive and active (two-way) avoidance responses. There was no significant change in radial maze performance. Treated groups showed significantly higher spontaneous locomotor activity in the open field and impaired passive avoidance learning. As the only concentration used (100 ppm) resulted in adverse effects, this concentration would be considered a LOAEL for 4 wk of discontinuous exposure. Because the measurements were made 2 wk after exposure ceased, when all xylene should have been eliminated from the system, it appears that xylene exposure resulted in some potentially persistent neurologic effects on sensorimotor functions.

In subchronic inhalation experiments, male Wistar rats (12 per group) were exposed to *m*-xylene for 6 h/d, 5 d/wk at 1,000 ppm for 3 mo or at 100 ppm for 6 mo. Disturbances in the Rotarod performance test and a decrease in spontaneous motor activity that were observed were significantly different from those in controls (Korsak et al. 1992) for both exposure groups. The authors conducted another animal study using one lower dose (Korsak et al. 1994) in which male Wistar rats (12 per dose group) were exposed to 50 and 100 ppm of

m-xylene, 6 h/d, 5 d/wk for 3 mo. The Rotarod performance test (motor coordination) was done before the exposure, and in each month during the 3 mo of inhalation exposure. Clinical chemistry values were unremarkable. In this study, the NOAEL for decrease in Rotarod performance (motor coordination and balance) was identified as 50 ppm for 3 mo.

Gralewicz et al. (1995) investigated the effects of a 3-mo (6 h/d, 5 d/wk) inhalation exposure of 8-mo-old male Wistar rats to *m*-xylene, at concentrations of 100 and 1,000 ppm, on changes in electroencephalogram (EEG) recordings and on spatial learning in an eight-arm radial maze. EEG recordings were performed before the exposure; on days 28, 56, and 84 of exposure; and again on days 14, 28, 42, and 84 after the exposure. According to the authors, exposure to *m*-xylene did not appear to influence the level of arousal as shown in the EEG, although retarded development of spontaneous neocortical spike and wave discharge activity was seen. The authors stated that large interindividual variations could not explain these results. The testing in the maze (one trial daily for 5 d) performed 2 mo after the exposure indicated that rats exposed to *m*-xylene at 1,000 ppm developed a learning deficit, as reflected by the number of omission errors and response speed even after training in successive trials, compared with the performance of control rats. The maze behavior of the 100-ppm group was similar to that of the 1,000- ppm group, but it was less pronounced. Thus, 100 ppm was identified as a 3-mo LOAEL for neurotoxicity (neurobehavioral) in this study.

Another important adverse effect of xylene exposure is ototoxicity—loss of hearing—and this has been the subject of many studies on exposures to organic solvents. Gagnaire et al. (2001) exposed 13-wk-old male Sprague-Dawley rats to *o*-, *m*-, and *p*-xylene at 450, 900, and 1,800 ppm, 6 h/d, 6 d/wk for 13 wk. Brainstem auditory evoked response, electrophysiologic auditory thresholds, and histologic analysis of the organ of Corti were used to assess the ototoxicity of individual isomers of xylene. Increased auditory thresholds were found at the end of the exposure period (13 wk) in rats exposed to 1,800 ppm. This did not reverse even by 8 wk after exposure. In addition, morphologic investigations conducted 8 wk postexposure revealed moderate to severe losses of outer hair cells of the organ of Corti in animals exposed to 900 and 1,800 ppm of *p*-xylene. However, the *m*- and the *o*-isomers did not exhibit any ototoxicity (no changes in audiometric thresholds or loss of either inner or outer hair cells). A recent study (Maguin et al. 2006) confirmed that only *p*-xylene was ototoxic and all of the xylene isomers were cochleotoxic when rats were exposed to 1,800 ppm for 6 h/d, 5 d/wk. According to the authors, 450 ppm is the NOAEL for ototoxicity for *p*-xylene. Moser et al. (1985) reported that, even though xylene produced pronounced neurobehavioral effects (based on operant performance and inverted screen test) after acute exposures over the concentration ranges of 500 to 7,000 ppm in mice, only slight potency differences among the isomers existed for neurobehavioral effects. Taking into consideration the ototoxic effects of xylene in all of these studies (Pryor et al. 1987, Crofton et al. 1994, Nylen and Hagman 1994, Gagnaire et al. 2001), ototoxicity should be considered an important ad-

verse effect of exposure to xylene and may be useful for AC derivation. However, it is clear that there is a remarkable difference in ototoxic potential among the isomers.

In a recent study, two types of mixed xylene with known proportions of each of the xylene isomers with ethylbenzene were tested for ototoxicity (Gagnaire et al. 2007). The first mixture contained 20% *o*-, 20% *p*-, and 40% *m*-xylenes and 20% ethylbenzene; and the second synthetic mixture contained 30% *o*-, 10% *p*-, and 50% *m*-xylene with 10% ethylbenzene. Male Sprague-Dawley rats (n = 16) were exposed to 250, 500, 1,000, and 2,000 ppm of each of these mixtures for 6 h/d, 6 d/wk for 13 wk. The brain auditory responses (changes in the thresholds) to different frequencies and a morphologic study of the organ of Corti confirmed that exposure to synthetic xylene mixtures resulted in ototoxicity. The confounding effects of ethylbenzene as a more potent ototoxicant than the xylenes make it difficult to interpret the data with respect to the xylenes. The Gagnaire et al. (2001) study would be more useful for deriving ACs.

Pryor et al. (1987) investigated male weanling F344 rats (3 wk old) exposed to mixed xylene (10% of *ortho*, 80% of *meta*, and 10% of *para*-xylene) *at* 800, 1,000, and 1,200 ppm daily for 14 h/d, 7 d/wk for 6 wk. They reported substantial loss of auditory sensitivity (20 to 25 decibels at 12.5 kHz) assessed by behavioral (conditioned avoidance) and electrophysiologic (brainstem auditory evoked response) methods. As rats used in this study were too young, this study will not be useful for deriving ACs.

In another study (Nylen and Hagman 1994), 8-wk-old male albino Sprague-Dawley rats (n = 23) were exposed to mixed xylene (1.5% *o*-xylene, 65% *m*-xylene, 32% *p*-xylene, and 2.5% ethylbenzene) at 1,000 ppm, 18 h/d, 7 d/wk for 61 d (Nylen and Hagman 1994). Neurophysiologic (electrophysiologic) recordings to assess latencies of the flash-evoked potentials and nerve and muscle action potentials were made 2 d, 4 mo, and 10 mo after the end of exposure. The authors characterized the loss of auditory sensitivity in low to middle frequencies in response to a click stimulus, observed 2 d after the exposure ended, as only minor; 4 and 10 mo after exposure, the results were comparable to those for controls (Nylen and Hagman 1994). Though only one concentration (1,000 ppm) was used, this study used the most continuous hours of exposure of any of the subchronic studies reviewed. However, Crofton et al. (1994) reported that, in adult male Long-Evans rats exposed to mixed xylenes at 1,800 ppm, 6 h/d for 5 d, the reflex modification audiometry data collected 5 to 8 wk postexposure indicated a hearing loss in the middle-frequency ranges (8 and 16 kHz). Reflex modification audiometry data at lower- and higher-frequency ranges were comparable to those for controls.

Chronic Duration Study

Uchida et al. (1993) carried out a cross-sectional evaluation of Chinese factory workers who were exposed to vapors that were predominantly xylene

(70% of the total exposures) for about 7 y; 175 xylene-exposed workers (107 men and 68 women) were selected. Monitoring by personal diffusive sampling showed that the concentration of xylene vapor was 14 ppm (as a geometric mean) and 21 ppm (as an arithmetic mean). *m*-Xylene was about 50% of these mixed isomers. Urinary MHA was used to verify the exposure estimate. The authors noted that the subjects were also coexposed to toluene and ethylbenzene at 1 and 3 ppm, respectively. As controls, 241 nonexposed workers (116 men and 125 women) were included. There was an increased prevalence of subjective symptoms (as reported in a questionnaire and self-reported) in the exposed workers; these symptoms were apparently related to CNS effects (dizziness, forgetfulness, anxiety) and to local effects on the eyes, nose, and throat (irritation). Because the intensity of exposure was rather low, a dose-response relationship between level of exposure and severity of reported symptoms was perhaps not evident.

Hematology and serum biochemistry with respect to liver and kidney function were generally unaffected (Uchida et al. 1993). On the basis of these observations (mild subjective symptoms in a small number of cases), 14 ppm could be considered as a LOAEL.

Reproductive Toxicity

Biodynamics (Bio/dynamics 1983) conducted a study in which male and female Sprague-Dawley rats were exposed by inhalation to a mixture of xylenes at concentrations of 0, 60, 250, or 500 ppm, 6 h/d for 131 d before mating and during a 20-d mating period. The mated females were also exposed during gestation from gestation days 1 to 20 and during days 5 to 20 of lactation. Additionally, exposed males from the highest-dose group were mated with unexposed females and vice versa. No mortalities occurred and there were no treatment-related effects on mating, fertility, pregnancy indices, mean duration of gestation, mean litter size, or mean pup weight. No effect on reproductive organs or sperm count of the male rats was observed.

In another long-term exposure study by Nylen et al. (1989), in which male Sprague-Dawley rats were exposed by inhalation to mixed xylenes at 1,000 ppm for 61 d, 18 h/d, 7 d/wk, no alterations in testes, accessory glands, or circulating male hormone levels were noted. All rats exposed to xylene were fertile.

Saillenfait et al. (2003) evaluated the developmental toxicities of *o*-, *m*-, and *p*-xylene and technical xylene in Sprague-Dawley rats after inhalation exposures. Animals were exposed at 100, 500, 1,000, or 2,000 ppm for 6 h/d, during days 6 to 20 of gestation. All the agents tested caused maternal toxicity, expressed as a reduction in maternal body weight gain, at 1,000 and 2,000 ppm. Even at the highest dose tested, no evidence of teratogenic effects was found. Fetal toxicity as evidenced by decreases in fetal body weight occurred only in groups exposed to 1,000 ppm or greater in the case of *m*-xylene and *p*-xylene.

This effect was seen at 500 ppm and was higher in the case of groups exposed to *o*-xylene and mixed (technical) xylene; however, the fetal body weight reductions were only 5% and 4%, respectively, in the case of these two xylenes (Saillenfait et al. 2003).

Several studies reported reproductive and developmental effects of short-term xylene exposures (e.g., see Ungvary and Tatrai 1985, Rosen et al. 1986, Hass et al. 1997). Results of these studies were either negative or indicated that reproductive and developmental toxicity effects can be noted only at high concentrations. Thus, these studies were not used to derive ACs.

Immunologic Effects

Studies of immune system effects in human subjects occupationally exposed to xylene had serious uncertainty with respect to specific association of xylene exposure and changes, because subjects had extensive coexposure to other solvents.

Carpenter et al. (1975) reported no immune system effects in dogs and rats exposed to mixed xylenes for 13 wk at 810 ppm. No specific immunologic adverse end points were measured except for spleen weight, which was unaffected. A study not included in the 1996 SMAC document for xylene was that of Selgrade et al. (1993). They exposed mice to *p*-xylene at 600 or 1,200 ppm, 6 h/d for 4 d, and infected them with a sublethal dose of murine cytomegalovirus (MCMV) after the first exposure to xylene. A death rate of 34% occurred in MCMV-challenged mice exposed to xylene at 1,200 ppm. However, no deaths occurred in other groups, including mice exposed to *p*-xylene at 600 ppm and infected with MCMV. In the group that showed high mortality, spleen natural killer cell activity was unaltered, and so were the virus titers in the liver. Because serum hepatotoxic marker enzymes, which indicate liver damage, increased only in mice exposed to xylene at 1,200 ppm and infected with MCMV, the authors stated that enhanced mortality was caused not by immune suppression but by enhanced liver damage. The mechanism was not understood.

The toxicity literature on xylene exposure discussed in this document is summarized in Table 19-2.

RATIONALE FOR THE 1,000-d AC

ACs were determined according to the Subcommittee on Guidelines for Developing Spacecraft Maximum Allowable Concentrations for Space Station Contaminants (NRC 1992). The resulting ACs for the various end points were compared and the lowest AC at each exposure duration was selected as the SMAC. NASA also reviewed the recommended or permissible exposure levels set by other regulatory and nonregulatory organizations shown in Tables 19-3 and 19-4.

TABLE 19-2 Toxicity Summary of Studies Included in This Document

Concentration, ppm	Exposure	Duration	Species	Adverse End Points	Reference
0, 100, 300 (w/o exercise); 300 (w/exercise)	mixed xylenes	70 min	humans	No effect on performance or subjective symptoms; decreased performance and decreased short-term memory with exercise.	Gamberale et al. 1978
50	*m*-xylene at rest	2 h	humans, male and female volunteers, 28 per sex	Affirmative answers to many subjective symptoms, sensory irritations; increased color confusion index at 0 and 3 h postexposure; significant decreases in pulmonary function 3 h after exposure.	Ernstgard et al. 2002
100	pure xylene (purity not specified)	4 h	humans, male volunteers, n = 10	Significant adverse effect on choice reaction time and simple reaction time (psychomotor efficiency).	Dudek et al. 1990
70	*p*-xylene	4 h	humans, male, n = 16	Computerized tests for simple reaction time, short-term memory, and choice reaction time immediately measured at 0, 2, and 4 h of exposure indicated that performance on the tests was unaffected by exposure.	Olson et al. 1985
600; 1,200	*p*-xylene, infected with a sublethal dose of MCMV after the first exposure to xylene	6 h/d, 4 d	Mice	34 percent death occurred in MCMV-challenged mice exposed to xylene at 1,200 ppm; no deaths in mice exposed to *p*-xylene at 600 ppm and infected with MCMV; liver damage in 1,200-ppm group infected with MCMV. In the 1,200-ppm group challenged with the virus, among those that died, spleen natural killer cell activity was unaltered and liver virus titers were also unaffected. Mortality was not caused by immune suppression.	Selgrade et al. 199
100, 500, 1,000, 2,000	*o*-, *m*-, and *p*-xylene and mixed xylene	6 h/d, during days 6 to 20 of gestation	Sprague-Dawley rats	Maternal toxicity (reduction in maternal body weight gain), at 1,000 and 2,000 ppm; no teratogenic effects found; fetal toxicity (decreases in fetal body weight) seen in 1,000- and 2,000 ppm *m*-xylene and *p*-xylene	Saillenfait et al. 2003

				groups. In the case of *o*-xylene and technical grade xylene this is seen at 500 ppm or higher while these reductions were only 5% and 4%, respectively.	
100	*m*-xylene	6 h/d, 5 d/wk, 4 wk	Wistar rat, male, 11 per group	Altered passive avoidance test, and delayed acquisition of two-way active avoidance were noted.	Gralewicz and Wiaderna 2001
800, 1,000, 1,200	mixed xylenes	7 h/d, 7 d/wk, 6 wk	weanling 3-wk-old Fischer-344 rats	Mixed xylenes at 800 ppm caused marked hearing loss as assessed by behavioral (conditioned avoidance) and electrophysiologic (brainstem auditory-evoked response) methods. A NOAEL could not be identified.	Pryor et al. 1987
1,000	mixed xylenes; 1.5% *o*-, 65% *m*-, and 32% *p*-isomers	18 h/d, 7 d/wk, 61 d	8-wk-old Sprague-Dawley rats, n = 23	A slight decreased auditory sensitivity (hearing loss) only in one frequency (12.5kHz); a NOAEL could not be identified. LOAEL=1000 ppm (minor change).	Nylén and Hagmai 1994
1,000	mixed xylenes	18 h/d, 7 d/wk, 61 d	male Sprague-Dawley rats	No alterations in testes, accessory glands, or circulating male hormone levels were noted. All exposed rats were fertile.	Nylen et al. 1989
50, 100	*m*-xylene	6 h/d, 5 d/wk, 3 mo	Wistar rats, male, 12 per dose	Impaired rotarod performance was noted; 50 ppm is the NOAEL and 100 ppm is the LOAEL for decreased rotarod performance.	Korsak et al. 1994
100, 1,000	*m*-xylene	6 h/d, 5 d/w, 3 mo	Wistar rats, male, 20 per dose	Altered radial maze performance and deficits in learning; 100 ppm is the LOAEL; some changes in EEG (spontaneous neocortical spike and wave discharges).	Gralewicz et al. 1995

(Continued)

TABLE 19-2 Continued

Concentration, ppm	Exposure	Duration	Species	Adverse End Points	Reference
100, 1,000	*m*-xylene	1,000 ppm: 6 h/d, 5 d/w, 3 mo 100 ppm: 6 h/d, 5 d/w, 6 mo	Wistar rat, male, 12 per dose	Impaired rotarod performance and decreased motor activity observed at both doses; 100 ppm was considered a LOAEL.	Korsak et al. 1992
450, 900, 1,800	*o*-, *m*-, and *p*-xylene	6 h/d, 6 d/wk, 13 wk	Sprague-Dawley rats, male, n = 13	Increased auditory threshold at different frequencies in 1,800-ppm *p*-xylene group; moderate loss of outer hair cells at 900 ppm of *p*-xylene; 450 ppm of *p*-xylene is the NOAEL for these effects; the *m*- and the *o*-isomers did not result in ototoxicity.	Gagnaire et al. 2001
250, 500, 1,000, 2,000	two technical grade xylene mixtures	6 h/d, 6 d/wk,13 wk	Sprague-Dawley rats, male, n = 14 per group	Increased auditory thresholds and losses of outer hair cells; 250 ppm is the LOAEL for the mixture that had a higher proportion of ethylbenzene; 1,000 ppm is the LOAEL for the mixture with less ethylbenzene.	Gagnaire et al. 2007
0, 60, 250, 500	xylene mixture	6 h/d for 131 d before mating and during a 20-d mating period, for females also during gestation days 1 to 20 and during lactation	male and female Sprague-Dawley rats	No mortalities and no treatment-related effects on mating, fertility, pregnancy indices, mean duration of gestation, mean litter size, or mean pup weight noted. Also, no effects were observed on reproductive organs or sperm count of male rats.	Biodynamics 1983

14 (geometric mean, sum of all three xylene isomers), 21 (arithmetic mean)	xylene mixture	occupational exposure, average exposure was 7 yr	humans, male, n = 107 and female, n = 68	Increased prevalence of subjective symptoms (CNS-related and local irritative effects) in Chinese factory workers exposed during work; when results were analyzed for 0 to 21 ppm and for >21 ppm, dose-effect correlation was noted only for a few end points.	Uchida et al.1993

Abbreviations: LOAEL, lowest-observed-adverse-effect level; NOAEL, no-observed-adverse-effect level, MCMV, murine cytomegalovirus; w/, with; w/o, without.

TABLE 19-3 Exposure Limits Set or Recommended by Other Organizations

Organization and Standard	ppm	mg/m^3	Source
ACGIH			ACGIH 1997
TLV-TWA	100	435	
STEL	150	655	
OSHA			29 CFR 1910.1010
PEL TWA, 8 h	100	435	Table Z1
NIOSH			NIOSH 2005
REL	100	435	
STEL	150	655	
NRC			NRC 1984
EEGL, 1 h	200	870	
EEGL, 24 h	100	435	
CEGL, 90 d	50	217	

Abbreviations: ACGIH, American Conference of Governmental Industrial Hygienists; CEGL, continuous exposure guidance level; EEGL, emergency exposure guidance level; NRC, National Research Council; NIOSH, National Institute for Occupational Safety and Health; OSHA, Occupational Safety and Health Administration; PEL, permissible exposure limit; REL, recommended exposure limit; STEL, short-term exposure limit; TLV, threshold limit value; TWA, time-weighted average.
Source: Adapted from Garcia 1996.

TABLE 19-4 ATSDR Inhalation Minimal Risk Levels[a]

Duration	Concentration, ppm	Toxicity End Point	Principal Study
Acute duration MRL	2	Subjective respiratory and neurologic effects (human)	Ernstgard et al. 2002
Intermediate duration MRL	0.6	Decreased latency of paw-lick response (rat)	Korsak et al. 1992
Chronic duration MRL	0.05	Mild subjective respiratory and neurologic symptoms in workers	Uchida et al. 1993

[a]MRLs are based on noncancerous health effects.
Abbreviation: MRL, minimum risk level; ATSDR, Agency for Toxic Substances and Disease Registry.

EPA (2003) derived an inhalation reference concentration (RfC) of 0.02 ppm (0.1 mg/m^3) for mixed xylenes. This was based on the decreased Rotarod performance (impaired motor function and coordination) in male rats exposed to

vapors of *m*-xylene. The principal studies used for this critical health effect were those of Korsak et al. (1992, 1994). To derive the RfC, EPA adjusted the concentrations for discontinuous-to-continuous exposure and used factors of 3 for species difference, 10 for human intraindividual variability, and 3 for time extrapolation from subchronic-to-chronic duration (lifetime)

JUSTIFICATION OF USE OF FACTORS OTHER THAN DEFAULT FACTORS

Before deriving the revised ACs for 1 h, 24 h, 7 d, 30 d, 180 d, and 1,000 d, NASA determined some guidelines on what the uncertainty factors applied in the derivations should be. There are no controlled human-exposure studies of subchronic or chronic duration; only one occupational exposure study exists in which the nature of the chemicals the workers were exposed to is known and thus is a study that can be used. Similarly, no chronic animal studies were available to derive a 1,000-d AC for humans. Data from subchronic studies have to be used for extrapolation to chronic durations, which leaves a lot of uncertainty, especially for CNS effects, the critical adverse end point for xylene toxicity.

A default factor of 10 for species extrapolation has been used for many compounds. This interspecies extrapolation factor of 10 has two components: a toxicokinetic component for variability and uncertainty for the difference in toxicokinetics between rats and humans, and a toxicodynamic component for variability and uncertainty in the differences in adverse effects between humans and animals. The risk-assessment community has used a factor of 3.2 for each of these, to make a final factor of 10, if there is a reason to believe that variability and uncertainties exist in both of these components (Renwick 1999, Pelekis and Krishnan 2004). In the case of xylene, the literature indicates that in animals and humans, absorption from the lung is very high, metabolism is high, and excretion rates and urinary excretion products are comparable (see Garcia 1996, for details). For xylene, the blood:air partition coefficients (which determine the alveolar concentration of xylene) are different for rats and humans. However, the fact that this coefficient is much higher for rats (46) than for humans (26.4) indicates that the target dose will be greater in rats than in humans. As the toxicokinetic component is comparable, one need not use a factor for this when extrapolating from animals to humans. In the case of xylene, the overall literature indicates that neurotoxicity is caused by the parent compound, and the neurotoxic effects on humans and animals are quite similar. Thus, the toxicodynamic components are comparable. There is some uncertainty about the comparability of neurologic responses of rodents and humans, and the tests used to assess these responses are not the same. Therefore, a factor of 3.2 or 3 should be kept for this uncertainty. Thus, the use of 3 and not 10 as the interspecies factor is justified. In fact, NRC (2000; see page 87 under interspecies uncertainty) recommended that for interspecies extrapolation for CNS effects a factor of 1 is sufficient.

Another important issue is the time extrapolation factor, from short-term or subchronic exposures to chronic exposure. NASA decided not to use subchronic study data for the 1,000-d AC, although EPA (2003) has used the subchronic data to derive an RfC for chronic exposure duration after using a factor of 3 on the subchronic duration data. Another challenging issue is the need to adjust the concentration for discontinuous-to-continuous exposure, especially when the neurotoxicity effects seem to be a function of concentration rather than exposure duration. The PBPK model EPA used to derive the RfC for xylene (EPA 2003) supports the idea that the discontinuous-to-continuous exposure, at least for neurotoxicity end points, may not need to be adjusted. EPA used the time-weighted blood concentrations of xylene from the discontinuous exposure protocol used in the Korsak et al. study and applied to the human xylene PBPK to derive an exposure concentration of xylene that will lead to comparable blood concentrations in a continuous exposure scenario and result in the same level of neurotoxicity as found in the rat. That derived human equivalent concentration was comparable to the exposure concentration used in the rat discontinuous exposure study.

NRC (2000) also recommended that a factor of 3 to 5 would be more appropriate for LOAEL to NOAEL (Abdel-Rahman and Kadry 1995). Dourson (1996) and Dourson et al. (1996) also proposed that a science-based approach to the use of uncertainty factors would indicate the use of less than default factors of 10 for uncertainties usually considered in risk extrapolation.

Before deriving a 1,000-d AC, the ACs previously derived for durations less than 1,000-d were reviewed to assess whether revisions are needed because of results in the literature not originally considered in the Garcia (1996) document.

A summary of SMACs from the revised derivations for 1-h to 180-d durations and the new SMAC for 1,000 d are shown in Table 19-5. The paragraphs following Table 19-5 describe in detail the selection of studies and the adverse end points and the calculations leading to the derivation of ACs for various durations and various adverse end points. Final SMACs were based on the ACs of individual durations.

SUMMARY OF SMACS FOR VARIOUS DURATIONS

RATIONALE FOR THE REVISION OF SMACS PUBLISHED IN 1996

SMACs developed by Garcia (1996) for xylenes were based on sensory end points such as irritation. Carpenter et al. (1975) based the data on well-designed, controlled human exposure studies. However, this study is several years old and consisted of exposure to a mixture of xylene isomers with a large amount of ethylbenzene (19%). In addition, the study was carried out on only 6 to 12 subjects. The exposure duration was just 15 min at each concentration. The

TABLE 19-5 Summary of Revised SMACs for Xylene for Various Durations of Exposure

Duration	SMAC, ppm	Adverse End Point	Principal Study
1 h	50	Reported symptoms of irritation of eye, nose, and throat and headache.	Ernstgard et al. 2002
24 h	17	Reported symptoms of irritation of eye, nose, and throat and headache.	Ernstgard et al. 2002
7 d	17	Neurotoxicity (motor function).	Korsak et al. 1994
30 d	17	Neurotoxicity (motor function).	Korsak et al. 1994
180 d	8.5	Ototoxicity.	Nylen and Hagman 1994
1,000 d	1.5	Ototoxicity.	Nylen and Hagman 1994

measurements were based on self-reported subjective symptoms. Thus, the toxicology literature published since then has provided additional data useful to revise the previous values or to support them. In the earlier parts of this document, these studies were described.

All data used for the derivation of ACs in this document were first evaluated for dose-response information and then for suitability for application of the benchmark dose (BMD) methodology. In the studies cited in this document, where there was only one treatment group, the treatment group had to be considered either a NOAEL or a LOAEL. In other studies, there was more than one treatment group; however, the dose-response profile was not robust enough to obtain a reliable BMD or the data were expressed as charts. For these reasons, the BMD methodology could not be applied to any of the studies cited in this document. The NRC committee suggested that some of the charts be digitized to extract data suitable for using the BMD method. NASA believed that it may not be beneficial to do so with the graphic data included in the studies considered in this document.

Revised Derivations of 1-h AC

Three human subject studies were considered for deriving ACs for acute exposure (Olson et al. 1985, Dudek et al. 1990, Ernstgard et al. 2002). These studies were chosen because the effects were objectively measured in terms of neurologic functional parameters and were not simply self-reported subjective symptoms. Olson et al. found no change in the SRT, ChRT, or short-term memory tests in subjects exposed to *p*-xylene at 70 ppm for 4 h. The authors seem to have used a single xylene isomer and 70 ppm can be identified as the NOAEL.

Dudek et al., using only one concentration (100 ppm) of pure xylene (purity not specified), found prolonged reaction time (in both SRT and ChRT) in 10 male volunteers exposed for 4 h. When 15 volunteers were exposed to 100 and 299 ppm of mixed xylenes, 100 ppm seemed to be a threshold for the increased ChRT and impaired short-term memory (Gamberale et al. 1978), whereas when the subjects were exposed to the same concentrations during exercise, the effects were seen at 100 ppm. When these studies are considered together, a NOAEL of 70 ppm and a LOAEL of 100 ppm could be identified. The NOAEL concentration for a 4-h exposure will be protective for a 1-h exposure.

Thus, the 1-h AC for neurologic function is set at 70 ppm.

Ernstgard et al. (2002) reported that, according to the self-reported degree of discomfort on a VAS scale, several subjective symptoms increased when 56 volunteers (28 per sex) were exposed to 50 ppm of *m*-xylene for 2 h. This study was described in detail in the section "Acute Exposure Studies." With the exception of the solvent smell, scores for irritation and other symptoms in subjects exposed to xylene were about 15 compared with a score of 6 for controls, indicating that 50 ppm of xylene resulted in only minor adverse symptoms. Pulmonary function, nasal swelling, and inflammatory markers were also measured. Pulmonary function parameters, measured immediately after the exposure, were not significantly different from those of controls. When they were assessed 3 h postexposure, a few changes were statistically significant, but the change from the controls was less than 4%. Nasal swelling and inflammatory markers were unaffected by exposure to xylene. Because of the details provided and the fact that both subjective and objective measurements were made on sufficient numbers of subjects of both genders, this study was chosen to rederive the 1- and 24-h ACs using 50 ppm as the LOAEL based on self-reported symptoms.

However, as NASA allows some minor effects that will not interfere with duties during an emergency, a LOAEL of 50 ppm for 2 h is considered acceptable as an AC for 1 h.

Thus, the 1-h AC for minor effects (sensory irritation) is 50 ppm.

The safety of the use of 50 ppm as a 1-h AC is also supported by the observation that exposure to 200 ppm (868 mg/m^3) of xylene for up to 5 h did not result in CNS disturbances measured by increased body sway (Laine et al. 1993). Only minor electroencephalographic effects were noted on 4-h exposures to *m*-xylene at 200 ppm, and no other adverse effects were noted (Seppalainen et al. 1991).

Revised Derivation of 24-h AC

The reported minor increases in subjective symptoms (Ernstgard et al.

2002) noted at 50 ppm may not be acceptable for 24 h. As the end point is sensory irritation, which is concentration dependent and does not depend on the duration of exposure, no factors are needed for continuous exposure for 24 h. However, a factor of 3 is applied to reduce the 1-h ACs, as prolonged exposure at this concentration for 24 h would not be acceptable.

$$\text{24-h AC}_{\text{(minor irritation)}} = 50 \text{ ppm}_{\text{(LOAEL)}} \times 1/3_{\text{(LOAEL to NOAEL)}} = 16.7 \text{ ppm, rounded to 17 ppm}$$

Thus, the 24-h AC for minor irritation is 17 ppm.

Revised Derivation of 7-d AC

Two human-exposure controlled studies (Riihimäki and Savolainen 1980, Hake et al. 1981) described earlier in the text (see "Short-Term and Subchronic Duration Studies") could not be used because the very small sample size, lack of details, and complex exposure design made it difficult to identify a NOAEL or a LOAEL.

As no other suitable human exposure study for derivation of a 7-d AC was available, a short-term animal study was considered. Gralewicz and Wiaderna (2001) reported that exposure of male rats to 100 ppm of *m*-xylene for 4 wk (6 h/d, 5 d/wk) resulted in significantly greater spontaneous locomotor activity in the open field and impaired passive avoidance learning. These measurements were made at different times starting 2 wk after exposure. The only concentration used resulted in adverse effects. Another rodent study, in which similar effects were also observed and in which a NOAEL could be identified, was preferred.

Korsak et al. (1992) reported that male Wistar rats (12 per dose) exposed to *m*-xylene at 100 ppm for 6 mo at 6 h/d, 5 d/wk showed a 35% decrease in Rotarod performance and 50% decreased spontaneous motor activity. In another study by the same authors in 1994, rats were exposed to *m*-xylene at 50 and 100 ppm for 3 mo. This study indicated that 50-ppm exposure for up to 3 mo did not affect the Rotarod performance. This parameter is potentially relevant to changes in motor coordination in humans. A NOAEL of 50 ppm for 3 mo for neurotoxic effects was identified.

This NOAEL for 1 or 3 mo will be protective for a 7-d AC, and no factors are needed for time adjustment. The results also indicated that in the 100-ppm group the same degree of effects were seen at 1 and 3 mo. Because of the existing margin of safety, it was decided not to adjust for discontinuous-to-continuous exposure. Furthermore, the species factor used will be only 3, as the pharmacokinetics of xylene is about the same in rats and humans. This factor is used because of uncertainty about how comparable the relationship between neurologic responses and exposure concentration is in rodents and humans. The 7-d AC can be calculated as follows:

7-d AC $_{(\text{neurotoxicity})}$ = 50 ppm $_{(\text{NOAEL})}$ × 1/3 $_{(\text{species factor})}$ = 16.70 ppm, rounded to 17 ppm

Thus, the 7-d AC for neurotoxicity is 17 ppm.

Revised Derivation of 30-d AC

No controlled human-exposure study could be found that was conducted for a longer time and was suitable for calculating the 30- and 180-d ACs. Thus, animal studies carried out for 3 mo were chosen.

First, data from the Korsak et al. (1992, 1994) studies, described above, were considered. Male Wistar rats (12 per dose group) were exposed to *m*-xylene at 50 and 100 ppm, 6 h/d, 5 d/wk for 3 mo (Korsak et al. 1994) and 100 ppm for 6 mo (Korsak et al. 1992). The disturbances in the Rotarod performance test and the decrease in spontaneous motor activity observed in the group exposed to 100 ppm (Korsak et al. 1992) were not seen in rats exposed to 50 ppm for 3 mo. Even though the measurements were taken at 1, 2, and 3 mo, the percent of failure in the Rotarod test did not increase with the length of exposure, which appears to indicate that it is the concentration that matters. Thus, for the 30-d AC derivation, the concentration is not adjusted for discontinuous-to-continuous exposures or for the duration of exposure for this end point. Thus, a 3-mo NOAEL of 50 ppm for motor coordination disturbance was chosen for AC calculations. A species factor of 3 was used. The 30-d AC can be calculated as follows:

30-d AC $_{(\text{motor coordination disturbance})}$ = 50 pm $_{(\text{NOAEL})}$ × 1/3 $_{(\text{species factor})}$ = 16.67, rounded to 17 ppm

Thus, the 30-d AC for motor coordination disturbance is 17 ppm.

Another study considered for deriving the AC for 30 d is a 13-wk study in which ototoxicity was observed (Gagnaire et al. 2001). Male Sprague-Dawley rats (13 wk old) were exposed to *o*-, *m*-, and *p*-xylene separately at 450, 900, and 1,800 ppm, 6 h/d, 6 d/wk for 13 wk. Electrophysiologic measurements for brainstem auditory evoked response recordings at threshold frequencies of 2, 4, 8, and 16 kHz revealed increased auditory thresholds (indicating loss of hearing) in rats exposed to 1,800 ppm. In addition, morphologic investigations conducted 8 wk postexposure revealed moderate to severe losses of outer hair cells of the organ of Corti in animals exposed to 900 and 1,800 ppm of *p*-xylene. It is important to note that the *m*- and the *o*-isomers of xylene did not exhibit ototoxicity. For *p*-xylene, 450 ppm could be identified as a NOAEL for ototoxicity.

Though three concentrations were used, the data were presented as graphs and electron micrographs; hence, BMD methodology could not be used. The NRC committee suggested that NASA look at the possibility of digitizing the

graphic data to obtain numerical data that could be used with the BMD method. In the present case, the only study with various concentrations is the ototoxicity study by Gagnaire et al. (2001) in which 450, 900, and 1,800 ppm were used as exposure concentrations. According to the literature, *p*-xylene is the only ototoxic agent and not the *m*- and *o*-isomers. Even in the case of *p*-xylene, only one concentration produced a change. If there are four groups including untreated controls and only the fourth concentration gives a change, obtaining a reliable BMD response curve that can be used to obtain a point-of-departure dose is not very accurate. Hence, NASA decided not to extract data by digitizing the charts created by the investigators.

A review of data on ototoxicity by xylene clearly indicated that it is not only concentration dependent but also duration dependent. Therefore, it was decided to adjust the concentration for discontinuous-to-continuous exposure. The use of this adjustment factor is considered for this end point in contrast to the neurotoxicity end point because the published data indicate that the mechanism of ototoxicity is different from the neurotoxicity mechanism (e.g., morphologic organ changes reported). As a 13-wk exposure study is used, it will be protective of a 30-d AC and no time factor is needed. A species factor of 3 is used as in other cases. The 30-d AC for ototoxicity can be calculated as follows after adjusting for intermittent exposure to continuous exposure:

$$\text{NOAEL}_{(\text{adjusted})} = 450 \text{ ppm}_{(\text{NOAEL})} \times [6 \text{ h}/24 \text{ h} \times 6 \text{ d}/7 \text{ d}]_{(\text{discontin. to contin.})} = 96.4 \text{ ppm}$$

$$\text{30-d AC}_{(\text{ototoxicity})} = 96.4_{(\text{NOAEL adjusted})} \times 1/3_{(\text{species factor})} = 32.13 \text{ ppm, rounded to } 32 \text{ ppm}$$

Thus, the 30-d AC for ototoxicity is 32 ppm.

Another study considered for 30- and 180-d ACs was a rat ototoxicity study by Nylen and Hagman (1994). They exposed male Sprague-Dawley rats to 1,000 ppm of mixed xylenes (consisting of 1.5% *o*-xylene, 65% *m*-xylene, 32% *p*-xylene, and 2.5% ethylbenzene) for 18 h/d, 7 d/wk for 61 d. Two days postexposure, the loss of auditory sensitivity in response to a click stimulus and latencies and amplitudes in auditory brain stem response of treated animals were no different from those of controls. However, when loss of auditory sensitivity was filtered by frequency, at 12 kHz, treated animals had a significant loss compared with controls. Even this minor change might have been caused by the small amounts of ethylbenzene in the sample. The flash-evoked potential of nerve or muscle was unaltered. Thus, 1,000 ppm was considered a LOAEL. At 4 and 10 mo postexposure, the flash-evoked potentials were no different from controls. The study design involved much longer daily and weekly exposure duration protocols than many other studies in the literature, and it was decided to use the study even though only one dose was used. In this study, the authors did not evaluate morphologic changes in the organ of Corti.

With a LOAEL of 1,000 ppm for decreased auditory sensitivity, a 30-d AC can be calculated. The concentration is adjusted for discontinuous exposure. No time extrapolation is required to derive a 30-d AC, as the data used are from a 61-d exposure study. A factor of 3 is used for species extrapolation.

$$\text{LOAEL}_{(\text{adjusted})} = 1{,}000 \text{ ppm}_{(\text{LOAEL})} \times [18 \text{ h}/24 \text{ h}]_{(\text{discontin. to contin.})} = 750 \text{ ppm}$$

$$30\text{-d AC}_{(\text{ototoxicity})} = 750 \text{ ppm}_{(\text{LOAEL adjusted})} \times 1/10_{(\text{LOAEL to NOAEL})} \times 1/3_{(\text{species factor})} = 25 \text{ ppm}$$

Thus, the 30-d AC for ototoxicity is = 25 ppm.

Another study considered for deriving the 30- and 180-d ACs was that of Gralewicz et al.(1995), in which exposure of rats to *m*-xylene at concentrations of 100 and 1,000 ppm for 3 mo, 6 h/d, 5 d/wk resulted in a deficit in spatial learning in an eight-arm radial maze in rats exposed to 1,000 ppm. The radial maze test was run 2 mo after the exposure ended. In rats exposed to *m*-xylene at 100 ppm, the effects on maze behavior were similar to those in the 1,000-ppm treated group, but they were less pronounced. Of the five trials during the testing, only the last two trials showed differences from the controls, and the results are somewhat difficult to interpret. As a NOAEL was not seen in this study, nor was a clear concentration-dependent response, it is difficult to use a proper factor for LOAEL to NOAEL. Thus, this study was not considered for AC derivation for 30 or 180 d.

Revised Derivation of 180-d AC

No suitable human exposure study was found. Three rodent studies with two different end points—neurotoxicity and ototoxicity—were used to derive the 180-d AC (Korsak et al. 1992, Nylen and Hagman 1994, Gagnaire et al. 2001).

First, the rodent study by Korsak et al. (1992) described earlier was considered. The authors had used *m*-xylene exposure concentrations of 1,000 ppm for 3 mo and 100 ppm for 6 mo. Rotarod performance was measured after 1, 2, and 3 mo of exposure (1,000- and 100-ppm groups) and at 6 mo (100-ppm group). At the end of 6 mo, the rats exposed to 100 ppm showed decreased performance in the Rotarod test. A LOAEL of 100 ppm for 6 mo was identified. The effect appeared to be more concentration dependent than duration dependent, and thus no factor was used for discontinuous-to-continuous exposure. The use of a factor of only 3 for LOAEL to NOAEL was used based on the observation of a 3-mo NOAEL of 50 ppm (Korsak et al. 1994). If one were to use the same data for 180 d, a factor of 2 (Haber's rule) would have been applied on 50 ppm to give a NOAEL of 25 ppm for 180 d. A species factor of 3 has also been used.

Thus, with a LOAEL of 100 ppm for changes in Rotarod performance, the 180-d AC is derived as follows using factors of 3 for LOAEL to NOAEL and 3 for species extrapolation.

$$\text{180-d AC}_{\text{(neurotoxicity)}} = 100 \text{ pm}_{\text{(LOAEL)}} \times 1/3_{\text{(LOAEL to NOAEL)}} \times 1/3_{\text{(species factor)}} = 11.11 \text{ ppm, rounded to 11 ppm}$$

Thus, the 180-d AC for neurotoxicity is 11ppm.

A second study was also used to derive a 180-d AC using ototoxicity as the end point. This AC was based on the results from Gagnaire et al. (2001), as described earlier in this chapter. A NOAEL of 450 ppm of *p*-xylene for ototoxicity was identified in this study. For derivation of the 180-d AC, in addition to the adjustment for discontinuous-to-continuous exposure, a species factor of 3 and a time extrapolation factor of 91 d/180 d following Haber's rule were used. The 180-d AC for ototoxicity can be calculated as follows:

$$\text{NOAEL}_{\text{(adjusted)}} = 450 \text{ ppm}_{\text{(NOAEL)}} \times [6 \text{ h}/24 \text{ h} \times 6 \text{ d}/7 \text{ d}]_{\text{(discontin. to contin.)}} = 96.4 \text{ ppm}$$

$$\text{180-d AC}_{\text{(ototoxicity)}} = 96.4 \text{ ppm}_{\text{(NOAEL adjusted)}} \times 1/3_{\text{(species factor)}} \times (91 \text{ d}/180 \text{ d})_{\text{(time extrapolation)}} = 16.2, \text{ rounded to 16 ppm}$$

Thus, the 180-d AC for ototoxicity is 16 ppm.

The third study considered for the 180-d AC was that of Nylen and Hagman (1994), described above, in which 1,000 ppm could be identified as a LOAEL for loss of auditory sensitivity at a frequency of 12 kHz. Rats were exposed to mixed xylenes at 1,000 ppm for 18 h/d, 7 d/wk for 61 d. After adjusting the LOAEL for discontinuous to continuous exposure (18 h/24 h), and after applying factors of 10 for LOAEL-to-NOAEL, 3 for interspecies extrapolation, and a time extrapolation factor of 61 d/180 d following Haber's rule, the 180-d AC for ototoxicity is derived as follows:

$$\text{LOAEL}_{\text{(adjusted)}} = 1{,}000 \text{ ppm}_{\text{(LOAEL)}} \times 18 \text{ h}/24 \text{ h}_{\text{(discontin. to contin.)}} = 750 \text{ ppm}$$

$$\text{180-d AC}_{\text{(ototoxicity)}} = 750 \text{ ppm}_{\text{(LOAEL adjusted)}} \times 1/10_{\text{(LOAEL to NOAEL)}} \times 61 \text{ d}/180 \text{ d}_{\text{(time extrapolation)}} \times 1/3_{\text{(species factor)}} = 8.47 \text{ ppm, rounded to 8.5 ppm}$$

Thus, the 180-d AC for ototoxicity is 8.5 ppm.

Derivation of 1,000-d ACs

A human subject study was first considered for deriving a 1,000-d AC. In this human cross-sectional study conducted in a Chinese production factory by Uchida et al. (1993) workers were exposed to a mixture of solvent vapors, mostly xylene, with some level of exposure to toluene and ethylbenzene. The study included 107 men and 68 women exposed to xylene and 200 unexposed control subjects (116 men and 125 women). The time-weighted average for 7 y for xylene exposure was 21 ppm (arithmetic mean) with a calculated geometric mean of 14 ppm. The data were collected from a self-reported questionnaire for various symptoms of irritations of the eye and nose, dizziness, and other symptoms that represent neurotoxicity.

However, there are limitations in using this study for deriving a 1,000-d AC. First, data were collected from subjects only once. Even though exposure was measured before data of the symptom assessment were collected, the data may not truly represent a correlation between exposure and response; exposure to a higher concentration on several occasions before the test was administered is possible. In addition, the study involves exposures to 30% of mixed vapors (exposure to toluene and ethylbenzene) and not just xylene isomers. The end points were also subjective. No objective neurologic tests were conducted.

Many rodent studies have documented neurologic and ototoxic effects of xylenes, although the duration of each of them was less than subchronic (Korsak et al. 1992, 1994; Gralewicz et al. 1995; Gagnaire et al. 2001). Because these effects have been well documented in several studies, and in the absence of robust chronic exposure data in humans or in rodents, it was decided to use a time extrapolation factor from subchronic studies to chronic studies.

Thus, a 1,000-d AC was derived with the ototoxicity results of Nylen and Hagman (1994) with supporting observations from Gagnaire et al. (2001) and applying a time extrapolation factor. A 180-d AC for ototoxicity was derived earlier as 8.5 ppm (based on Nylen and Hagman 1994). A time factor of 1,000 d/180 d is applied to the 180-d AC to reduce the dose for 1,000 d. Thus, the 1,000-d AC is calculated as follows:

$$\text{1,000-d AC}_{\text{(ototoxicity)}} = 8.5 \text{ ppm}_{\text{(180-d AC, ototoxicity)}} \times 180 \text{ d}/1{,}000 \text{ d}_{\text{(time extrapolation)}} = 1.53 \text{ ppm, rounded to } 1.5 \text{ ppm}$$

Spaceflight Effects

The national standard for exposure to noise in the occupational environment is an 8-h equivalent continuous A-weighted sound pressure level of 85 decibels (dB)A. For peak noise, the national standard is a C-weighted peak sound pressure level of 140 dBC. From the National Institute for Occupational Safety and Health criteria document for noise (NIOSH 1998), one can find that

an exposure level of 80 dBA may be acceptable for as long as 25 h and 24 min. The flight rules established by NASA for the International Space Station (ISS) and the Shuttle established that a noise level of 65 dBA on ISS could be tolerated for 24 h. The ISS acoustics office provides support for acoustic measurement devices, on-orbit testing, and real-time remedial actions to protect crewmember's hearing.

There is an extensive literature aimed at establishing whether exposure to neurotoxic solvents causes ototoxicity in humans and whether there is any significant level of interaction between exposure to neurotoxic solvents and noise levels, for example additive or synergistic effects on ototoxicity. In an industrial setting, coexposure to various solvents limits any interpretations or conclusions.

An evaluation of the ACs derived for xylene for various durations, especially the 30-d AC (32 and 25 ppm form two different studies) and the 180-d AC (16 and 8.5 ppm) do not drive the SMAC for the respective durations, especially for 30 d, even if one were to apply a customary default factor of 3. In addition, based on consistent data that only *p*-xylene appears to be ototoxic, and not *m*- and *o*-xylene, and the fact that the ACs were derived based on the assumption that all the xylene vapors are *p*-xylene indicates that the ACs for ototoxicity end points are conservative. Therefore, consideration of any interaction of noise with xylene induced ototoxicity is not necessary.

A summary of revised SMACs for various durations and effects are shown in Table 19-5. Updated ACs, new ACs, and final SMACs for 1 h to 1,000 d are listed in Table 19-6.

RESEARCH NEEDS

Data that establish a relationship between a target tissue concentration (dose), instead of an exposure concentration, and two critical adverse end points of xylene toxicity—namely, the neurobehavioral effects and ototoxic effects in humans are needed. Although PBPK modeling studies based on the pharmacokinetics of xylene in rats and humans have been published, pharmacodynamic-based models are needed. Savolainen et al. (1985) reported a correlation between venous blood xylene concentrations and changes in both average and maximal body sway along the sagittal axis and along the lateral axis (an indication of vestibular system balance) in nine human volunteers exposed to constant or fluctuating concentrations of *m*-xylene. The literature indicates that this response seems to be biphasic, making it difficult for dosemetric analysis. Hence, data are needed for neurobehavioral end points (such as ChRT and SRT that can be assessed by a computerized test battery), so that a physiologically based pharmacodynamic model can be developed to predict reasonable exposure concentrations for various durations of expected exposures using blood levels as surrogates for target (brain) dose. Research is also needed to understand certain persistent neurobehavioral effects seen in many studies several weeks after ex-

TABLE 19-6 A Summary of Updated and New ACs and SMACs for Various Durations

	Acceptable Concentrations, ppm					
Adverse End Point and Principal Study	1 h	24 h	7 d	30 d	180 d	1,000 d
Reported symptoms of irritation of eye, nose, and throat and headache (Ernstgard et al. 2002).	50	17	—	—	—	—
Neurologic function: decreased SRT, ChRT, and short-term memory (Olson et al. 1985).	70	—	—	—	—	—
Neurotoxicity: Decreased neuromotor function (Korsak et al. 1994).	—	—	17	—	—	—
Neurotoxicity: Decreased neuromotor function (Korsak et al. 1994).	—	—	—	17	—	—
Ototoxicity: Decreased auditory threshold and loss of hair cells of organ of Corti (Gagnaire et al. 2001).	—	—	—	32	—	—
Ototoxicity: Loss of auditory sensitivity/response (ototoxicity) (Nylen and Hagman 1994).	—	—	—	25	—	—
Neuromotor function (Korsak et al. 1994).	—	—	—	—	11	—
Ototoxicity (Gagnaire et al. 2001).	—	—	—	—	16	—
Loss of auditory sensitivity/response (ototoxicity) (Nylen and Hagman 1994).	—	—	—	—	8.5	—
Loss of auditory sensitivity/response (ototoxicity) (Nylen and Hagman 1994).	—	—	—	—	—	1.5
SMAC,[a] ppm	*50*	*17*	*17*	*17*	*8.5*	*1.5*

[a]SMAC is the lowest of the ACs for that particular duration.
Abbreviation: —, not derived for the endpoint for this duration.

posure ceased when xylene is undetectable in the blood, even though these effects are generally believed to depend on circulating blood concentrations of the parent compound. This is important in reducing uncertainties in AC derivations.

REFERENCES

Abdel-Rahman, M.S., and A.M. Kadry. 1995. Studies on the use of uncertainty factors in deriving RfDs. Hum. Ecol. Risk Assess. 1(5):614-624.

ACGIH (American Conference of Governmental Industrial Hygienists). 1997. Threshold Limit Values for Chemical Substances and Physical Agents. Biological Exposure Indices. ACGIH, Cincinnati, OH

ATSDR (Agency for Toxic Substances and Disease Registry). 2007. Toxicological Profile for Xylene. U.S. Department of Health and Human Services, Public Health

Service, Agency for Toxic Substances and Disease Registry, Atlanta, GA [online]. Available: http://www.atsdr.cdc.gov/toxprofiles/tp71.html [accessed April 29, 2008].

Bio/dynamics Inc. 1983. Parental and Fetal Reproduction Inhalation Toxicity Study in Rats with Mixed Xylene. Project # 80-2520. Project # 80-2520. Prepared for U.S. Environmental Protection Agency under TSCA, by Bio/dynamics Inc., East Millstone, NJ.

Carpenter, C.P., E.R. Kinkead, D.L. Geary Jr., L.J. Sullivan, and J.M. King. 1975. Petroleum hydrocarbon toxicity studies. V. Animal and human response to vapors of mixed xylene. Toxicol. Appl. Pharmacol. 33(3):543-558.

Crofton, K.M., T.L. Lassiter, and C.S. Rebert. 1994. Solvent-induced ototoxicity in rats: An atypical selective mid-frequency hearing deficit. Hear Res. 80(1):25-30.

Dourson, M. 1996. Uncertainty factors in noncancer risk assessment. Regul. Toxicol. Pharmacol. 24(2 Pt 1):107.

Dourson, M.L., S.P. Felter, and D. Robinson. 1996. Evolution of science-based uncertainty factors in noncancer risk assessment. Regul. Toxicol. Pharmacol. 24(2 Pt 1):108-120.

Dudek, B., K. Gralewicz, M. Jakubowski, P. Kostrzewski, and J. Sokal. 1990. Neurobehavioral effects of experimental exposure to toluene, xylene and their mixture. Pol. J. Occup. Med. 3(1):109-116.

EPA (U.S. Environmental Protection Agency). 2003. Xylenes (CASRN 1330-20-7). Integrated Risk Information System, U.S. Environmental Protection Agency [online]. Available: http://www.epa.gov/NCEA/iris/subst/0270.htm [accessed May 5, 2008].

Ernstgard, L., E. Gullstrand, A. Lof, and G. Johanson. 2002. Are women more sensitive than men to 2-propanol and m-xylene vapours? Occup. Environ. Med. 59(11):759-767.

Ernstgard, L., B. Sjogren, M. Warholm, and G. Johanson. 2003. Sex differences in the toxicokinetics of inhaled solvent vapors in humans 1. m-Xylene. Toxicol. Appl. Pharmacol. 193(2):147-157.

Gagnaire, F., B. Marignac, C. Langlais, and P. Bonnet. 2001. Ototoxicity in rats exposed to ortho-, meta- and para-xylene vapours for 13 weeks. Pharmacol. Toxicol. 89(1):6-14.

Gagnaire, F., C. Langlais, S. Grossmann, and P. Wild. 2007. Ototoxicity in rats exposed to ethylbenzene and to two technical xylene vapours for 13 weeks. Arch. Toxicol. 81(2):127-143.

Gamberale, F., G. Annwall, and M. Hultengren. 1978. Exposure to xylene and ethylbenzene. III. Effects on central nervous functions. Scand. J. Work Environ. Health 4(3):204-211.

Garcia, G.D. 1996. Xylene. Pp. 321-344 in Spacecraft Maximum Allowable Concentrations for Selected Airborne Contaminants, Vol. 3. Washington, DC: National Academy Press.

Gargas, M.L., R.J. Burgess, D.E. Voisard, G.H. Cason, and M.E. Andersen. 1989. Partition coefficients of low-molecular-weight volatile chemicals in various liquids and tissues. Toxicol. Appl. Pharmacol. 98(1):87-99.

Gralewicz, S., and D. Wiaderna. 2001. Behavioral effects following subacute inhalation exposure to m-xylene or trimethylbenzene in the rat: A comparative study. Neurotoxicology 22(1):79-89.

Gralewicz, S., D. Wiaderna, and T. Tomas. 1995. Development of spontaneous, age-related nonconvulsive seizure electrocortical activity and radial-maze learning after exposure to m-xylene in rats. Int. J. Occup. Med. Environ. Health 8(4):347-360.

Hake, C.L., R.D. Stewart, A. Wu, S.A. Graff, H.S. Forster, W.H. Keeler, A.J. Lebrun, and P.E. Newton. 1981. p-xylene: Development of a Biological Standard for the Industrial Worker by Breath Analysis. PB82-152844. Prepared for National Institute for Occupational Safety and Health, by Medical College of Wisconsin, Department of Environmental Medicine, Milwaukee, WI.

Hass, U., S.P. Lund, and L. Simonsen. 1997. Long-lasting neurobehavioral effects of prenatal exposure to xylene in rats. Neurotoxicology 18(2):547-551.

IARC (International Agency for Research on Cancer). 1999. Xylenes. Pp. 1189-1208 in Re-Evaluation of Some Organic Chemicals, Hydrazine and Hydrogen Peroxide (Part Three). IARC Monographs on the Evaluation of the Carcinogenic Risk to Humans, Vol. 71. Lyon, France: International Agency for Research on Cancer.

Kaneko, T., K. Endoh, and A. Sato. 1991. Biological monitoring of exposure to organic solvent vapors. II. Simulation studies using a physiological pharmacokinetic model for m-xylene. Yamanshi Med. J. 6:137-149.

Kobayashi, H., R. Hobara, and T. Sakai. 1989. Effects of inhalation of several organic solvents on left ventricular dp/dt. Sangyo Igaku 31(3):136-141.

Korsak, Z., J.A. Sokal, and R. Gorny. 1992. Toxic effects of combined exposure to toluene and m-xylene in animals. III. Subchronic inhalation study. Pol. J. Occup. Med. Environ. Health 5(1):27-33.

Korsak, Z., J. Wisniewska-Knypl, and R. Swiercz. 1994. Toxic effects of subchronic combined exposure to n-butyl alcohol and m-xylene in rats. Int. J. Occup. Med. Environ. Health 7(2): 155-166.

Laine, A., K. Savolainen, V. Riihimäki, E. Matikainen, T. Salmi, and J. Juntunen. 1993. Acute effects of m-xylene inhalation on body sway, reaction times, and sleep in man. Int. Arch. Occup. Environ. Health 65(3):179-188.

Maguin, K., R. Lataye, P. Campo, B. Cossec, M. Burgart, and D. Waniusiow. 2006. Ototoxicity of the three xylene isomers in the rat. Neurotoxicol. Teratol. 28(6):648-656.

Molnar, J., K.A. Paksy, and M. Naray. 1986. Changes in the rat's motor behaviour during 4-hr inhalation exposure to prenarcotic concentrations of benzene and its derivatives. Acta Physiol. Hung. 67(3):349-354.

Morley, R., D.W. Eccleston, C.P. Douglas, W.E. Greville, D.J. Scott, and J. Anderson. 1970. Xylene poisoning: A report on one fatal case and two cases of recovery after prolonged unconsciousness. Brit. Med. J. 3(5720):442-443.

Moser, V.C., E.M. Coggeshall, and R.L. Balster. 1985. Effects of xylene isomers on operant responding and motor performance in mice. Toxicol. Appl. Pharmacol. 80(2):293-298.

NIOSH (National Institute for Occupational Safety and Health). 1998. Criteria for a Recommended Standard: Occupational and Noise Exposure. NIOSH Publication No. 98-126. National Institute for Occupational Safety and Health [online]. Available: http://www.cdc.gov/niosh/docs/98-126/chap1.html#11 [accessed May 20, 2008].

NIOSH (National Institute for Occupational Safety and Health). 2005. NIOSH Pocket Guide to Chemical Hazards. m-xylene. National Institute for Occupational Safety and Health. [online]. Available: http://www.cdc.gov/niosh/npg/npgd0669.html [accessed May 20, 2008].

NRC (National Research Council). 1984. Emergency and Continuous Exposure Guidance Levels for Selected Airborne Contaminants, Vol. 2. Washington, DC: National Academy Press.

NRC (National Research Council). 1992. Guidelines for Developing Spacecraft Maximum Allowable Concentrations for Space Station Contaminants. Washington, DC: National Academy Press.

NRC (National Research Council). 2000. Methods for Developing Spacecraft Water Exposure Guidance. Washington, DC: National Academy Press.

Nylen, P., and M. Hagman. 1994. Function of the auditory and visual systems, and of peripheral nerve, in rats after long-term combined exposure to n-hexane and methylated benzene derivatives. II. Xylene. Pharmacol. Toxicol. 74(2):124-129.

Nylen, P., T. Ebendal, M. Eriksdotter-Nilsson, T. Hansson, A. Henschen, A.C. Johnson, T. Kronevi, U. Kvist, N.O. Sjostrand, G. Hoglund, and L. Olson. 1989. Testicular atrophy and loss of nerve growth factor-immunoreactive germ cell line in rats exposed to n-hexane and a protective effect of simultaneous exposure to toluene or xylene. Arch. Toxicol. 63(4):296-307.

Ogata, M., and T. Fujii. 1979. Urinary excretion of hippuric acid and m-methylhippuric acid after administration of toluene and m-xylene mixture to rats. Int. Arch. Occup. Environ. Health 43(1):45-51.

Ogata, M., K. Tomokuni, and Y. Takatsuka. 1970. Urinary excretion of hippuric acid and m- or p-methylhippuric acid in the urine of persons exposed to vapours of toluene and m- or p-xylene as a test of exposure. Br. J. Ind. Med. 27(1):43-50.

Olson, B.A., F. Gamberale, and A. Iregren. 1985. Coexposure to toluene and p-xylene in man: Central nervous functions. Br. J. Ind. Med. 42(2):117-122.

Pelekis, M., and K. Krishnan. 2004. Magnitude and mechanistic determinants of the interspecies toxicokinetic uncertainty factor for organic chemicals. Regul. Toxicol. Pharmacol. 40(3):264-271.

Pryor, G.T., C.S. Rebert, and R.A. Howd. 1987. Hearing loss in rats caused by inhalation of mixed xylenes and styrene. J. Appl. Toxicol. 7(1):55-61.

Renwick, A.G. 1999. Subdivision of uncertainty factors to allow for toxicokinetics and toxicodynamics. Hum. Ecol. Risk Assess. 5(5):1035-1050.

Riihimäki, V. 1979. Conjugation and urinary excretion of toluene and m-xylene metabolites in a man. Scand. J. Work Environ. Health 5(2):135-142.

Riihimäki, V., and K. Savolainen. 1980. Human exposure to m-xylene. Kinetics and acute effects on the central nervous system. Ann. Occup. Hyg. 23(4):411-422.

Riihimäki, V., P. Pfaffli, K. Savolainen, and K. Pekari. 1979. Kinetics of m-zylene in man: General features of absorption, distribution, biotransformation and excretion in repetitive inhalation exposure. Scand. J. Work Environ. Health 5:217-231.

Rosen, M.B., K.M. Crofton, and N. Chernoff. 1986. Postnatal evaluation of prenatal exposure to p-xylene in the rat. Toxicol. Lett. 34(2-3):223-229.

Saillenfait, A.M., F. Gallissot, G. Morel, and P. Bonnet. 2003. Developmental toxicities of ethylbenzene, ortho-, meta-, para-xylene and technical xylene in rats following inhalation exposure. Food Chem. Toxicol. 41(3):415-429.

Savolainen, K., V. Riihimäki, R. Luukkonen, and O. Muona. 1985. Changes in the sense of balance correlate with concentrations of m-xylene in venous blood. Br. J. Ind. Med. 42(11):765-769.

Sedivec, V., and J. Flek. 1976. The absorption, metabolism, and excretion of xylenes in man. Int. Arch. Occup. Environ. Health 37(3):205-217.

Selgrade, M.K., M.J. Daniels, R.H. Jaskot, B.L. Robinson, and J.W. Allis. 1993. Enhanced mortality and liver damage in virus-infected mice exposed to p-xylene. J. Toxicol. Environ. Health 40(1):129-144.

Senczuk, W., and J. Orlowski. 1978. Absorption of *m*-xylene vapours through the respiratory tract and excretion of *m*-methylhippuric acid in urine. Br. J. Ind. Med. 35(1):50-55.

Seppalainen, A.M., A. Laine, T. Salmi, E. Verkkala, V. Riihimäki, and R. Luukkonen. 1991. Electroencephalographic findings during experimental human exposure to m-xylene. Arch. Environ. Health 46(1):16-24.

Tardif, R., G. Charest-Tardif, J. Brodeur, and K. Krishnan. 1997. Physiologically based pharmacokinetic modeling of a ternary mixture of alkyl benzenes in rats and humans. Toxicol. Appl. Pharmacol. 144(1):120-134.

Uchida, Y., H. Nakatsuka, H. Ukai, T. Watanabe, Y.T. Liu, M.Y. Huang, Y.L. Wang, F.Z. Zhu, H. Yin, and M. Ikeda. 1993. Symptoms and signs in workers exposed predominantly to xylenes. Int. Arch. Occup. Environ. Health 64(8):597-605.

Ungvary, G., and E. Tatrai. 1985. On the embryotoxic effects of benzene and its alkyl derivatives in mice, rats and rabbits. Arch. Toxicol. Suppl 8:425-430.