

Overview information for

Ammonia

TOXICOLOGICAL PROFILE FOR AMMONIA

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

September 2004

DISCLAIMER

The use of company or product name(s) is for identification only and does not imply endorsement by the Agency for Toxic Substances and Disease Registry.

UPDATE STATEMENT

A Toxicological Profile for Ammonia, Draft for Public Comment, was released in September 2002. This edition supersedes any previously released draft or final profile.

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

Agency for Toxic Substances and Disease Registry
Division of Toxicology/Toxicology Information Branch
1600 Clifton Road NE,
Mailstop F-32
Atlanta, Georgia 30333

FOREWORD

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

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

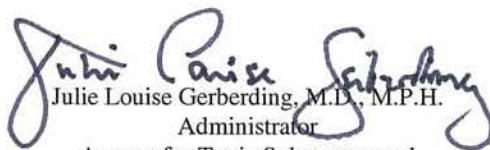
The focus of the profiles is on health and toxicologic information; therefore, each toxicological profile begins with a public health statement that describes, in nontechnical language, a substance's relevant toxicological properties. Following the public health statement is information concerning levels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

Each profile includes the following:

- (A) The examination, summary, and interpretation of available toxicologic information and epidemiologic evaluations on a hazardous substance to ascertain the levels of significant human exposure for the substance and the associated acute, subacute, and chronic health effects;
- (B) A determination of whether adequate information on the health effects of each substance is available or in the process of development to determine levels of exposure that present a significant risk to human health of acute, subacute, and chronic health effects; and
- (C) Where appropriate, identification of toxicologic testing needed to identify the types or levels of exposure that may present significant risk of adverse health effects in humans.

The principal audiences for the toxicological profiles are health professionals at the Federal, State, and local levels; interested private sector organizations and groups; and members of the public.

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


Julie Louise Gerberding, M.D., M.P.H.
Administrator
Agency for Toxic Substances and
Disease Registry

*Legislative Background

The toxicological profiles are developed in response to the Superfund Amendments and Reauthorization Act (SARA) of 1986 (Public law 99-499) which amended the Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (CERCLA or Superfund). This public law directed ATSDR to prepare toxicological profiles for hazardous substances most commonly found at facilities on the CERCLA National Priorities List and that pose the most significant potential threat to human health, as determined by ATSDR and the EPA. The availability of the revised priority list of 275 hazardous substances was announced in the *Federal Register* on November 17, 1997 (62 FR 61332). For prior versions of the list of substances, see *Federal Register* notices dated April 29, 1996 (61 FR 18744); April 17, 1987 (52 FR 12866); October 20, 1988 (53 FR 41280); October 26, 1989 (54 FR 43619); October 17, 1990 (55 FR 42067); October 17, 1991 (56 FR 52166); October 28, 1992 (57 FR 48801); and February 28, 1994 (59 FR 9486). Section 104(i)(3) of CERCLA, as amended, directs the Administrator of ATSDR to prepare a toxicological profile for each substance on the list.

QUICK REFERENCE FOR HEALTH CARE PROVIDERS

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

Primary Chapters/Sections of Interest

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

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

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

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

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

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

Other Sections of Interest:

Section 3.8	Biomarkers of Exposure and Effect
Section 3.11	Methods for Reducing Toxic Effects

ATSDR Information Center

Phone: 1-888-42-ATSDR or (404) 498-0110	Fax: (770) 488-4178
E-mail: atsdric@cdc.gov	Internet: http://www.atsdr.cdc.gov

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

Case Studies in Environmental Medicine: Taking an Exposure History—The importance of taking an exposure history and how to conduct one are described, and an example of a thorough exposure history is provided. Other case studies of interest include *Reproductive and Developmental*

Hazards; Skin Lesions and Environmental Exposures; Cholinesterase-Inhibiting Pesticide Toxicity; and numerous chemical-specific case studies.

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

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

Other Agencies and Organizations

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

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

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

Referrals

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

The American College of Occupational and Environmental Medicine (ACOEM) is an association of physicians and other health care providers specializing in the field of occupational and environmental medicine. Contact: ACOEM, 55 West Seegers Road, Arlington Heights, IL 60005 • Phone: 847-818-1800 • FAX: 847-818-9266.

CONTRIBUTORS

CHEMICAL MANAGER(S)/AUTHOR(S):

Nickolette Roney, MPH
ATSDR, Division of Toxicology, Atlanta, GA

Fernando Lladós, Ph.D.
Syracuse Research Corporation, North Syracuse, NY

Susan S. Little, Ph.D.
Syracuse Research Corporation, North Syracuse, NY

David B. Knaebel, Ph.D.
Syracuse Research Corporation, North Syracuse, NY

THE PROFILE HAS UNDERGONE THE FOLLOWING ATSDR INTERNAL REVIEWS:

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

PEER REVIEW

A peer review panel was assembled for ammonia. The panel consisted of the following members:

1. Dr. Finis Cavender, Private Consultant, Greer, South Carolina;
2. Dr. Jerold Last, Professor, Internal Medicine, University of California, Davis;
3. Dr. Frederick Oehme, Professor of Toxicology, Medicine/Physiology, Kansas State University;
and
4. Dr. Martin Alexander, Professor, Department of Soil, Crop, and Atmospheric Sciences, Cornell University, New York.

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

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

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

CONTENTS

DISCLAIMER	ii
UPDATE STATEMENT	iii
FOREWORD	v
QUICK REFERENCE FOR HEALTH CARE PROVIDERS.....	vii
CONTRIBUTORS	ix
PEER REVIEW	xi
CONTENTS.....	xiii
LIST OF FIGURES	xvii
LIST OF TABLES	xix
 1. PUBLIC HEALTH STATEMENT.....	 1
1.1 WHAT IS AMMONIA?.....	1
1.2 WHAT HAPPENS TO AMMONIA WHEN IT ENTERS THE ENVIRONMENT?.....	3
1.3 HOW MIGHT I BE EXPOSED TO AMMONIA?	3
1.4 HOW CAN AMMONIA ENTER AND LEAVE MY BODY?	5
1.5 HOW CAN AMMONIA AFFECT MY HEALTH?	6
1.6 HOW CAN AMMONIA AFFECT CHILDREN?	7
1.7 HOW CAN FAMILIES REDUCE THE RISK OF EXPOSURE TO AMMONIA?	7
1.8 IS THERE A MEDICAL TEST TO DETERMINE WHETHER I HAVE BEEN EXPOSED TO AMMONIA?	8
1.9 WHAT RECOMMENDATIONS HAS THE FEDERAL GOVERNMENT MADE TO PROTECT HUMAN HEALTH?.....	9
1.10 WHERE CAN I GET MORE INFORMATION?	10
 2. RELEVANCE TO PUBLIC HEALTH	 13
2.1 BACKGROUND AND ENVIRONMENTAL EXPOSURES TO AMMONIA IN THE UNITED STATES.....	13
2.2 SUMMARY OF HEALTH EFFECTS	15
2.3 MINIMAL RISK LEVELS	18
 3. HEALTH EFFECTS.....	 23
3.1 INTRODUCTION	23
3.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE	23
3.2.1 Inhalation Exposure	25
3.2.1.1 Death	25
3.2.1.2 Systemic Effects	26
3.2.1.3 Immunological and Lymphoreticular Effects.....	55
3.2.1.4 Neurological Effects.....	55
3.2.1.5 Reproductive Effects	56
3.2.1.6 Developmental Effects	56
3.2.1.7 Cancer.....	56
3.2.2 Oral Exposure.....	57
3.2.2.1 Death	57
3.2.2.2 Systemic Effects	58
3.2.2.3 Immunological and Lymphoreticular Effects.....	67
3.2.2.4 Neurological Effects.....	68
3.2.2.5 Reproductive Effects	69

3.2.2.6	Developmental Effects	69
3.2.2.7	Cancer.....	69
3.2.3	Dermal Exposure.....	70
3.2.3.1	Death	70
3.2.3.2	Systemic Effects	71
3.2.3.3	Immunological and Lymphoreticular Effects.....	75
3.2.3.4	Neurological Effects.....	75
3.2.3.5	Reproductive Effects	75
3.2.3.6	Developmental Effects	75
3.2.3.7	Cancer.....	75
3.2.4	Other Routes of Exposure	76
3.3	GENOTOXICITY	76
3.4	TOXICOKINETICS.....	78
3.4.1	Absorption.....	79
3.4.1.1	Inhalation Exposure.....	79
3.4.1.2	Oral Exposure.....	79
3.4.1.3	Dermal Exposure	80
3.4.2	Distribution	81
3.4.2.1	Inhalation Exposure.....	81
3.4.2.2	Oral Exposure.....	81
3.4.2.3	Dermal Exposure	82
3.4.2.4	Other Routes of Exposure	82
3.4.3	Metabolism.....	83
3.4.4	Elimination and Excretion.....	83
3.4.4.1	Inhalation Exposure.....	83
3.4.4.2	Oral Exposure.....	86
3.4.4.3	Dermal Exposure	86
3.4.4.4	Other Routes of Exposure	87
3.4.5	Physiologically Based Pharmacokinetic (PBPK)/Pharmacodynamic (PD) Models	87
3.5	MECHANISMS OF ACTION	90
3.5.1	Pharmacokinetic Mechanisms.....	90
3.5.2	Mechanisms of Toxicity.....	90
3.5.3	Animal-to-Human Extrapolations	91
3.6	TOXICITIES MEDIATED THROUGH THE NEUROENDOCRINE AXIS	92
3.7	CHILDREN'S SUSCEPTIBILITY	93
3.8	BIOMARKERS OF EXPOSURE AND EFFECT	96
3.8.1	Biomarkers Used to Identify or Quantify Exposure to Ammonia.....	97
3.8.2	Biomarkers Used to Characterize Effects Caused by Ammonia.....	97
3.9	INTERACTIONS WITH OTHER CHEMICALS	98
3.10	POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE	99
3.11	METHODS FOR REDUCING TOXIC EFFECTS.....	100
3.11.1	Reducing Peak Absorption Following Exposure	100
3.11.2	Reducing Body Burden.....	101
3.11.3	Interfering with the Mechanism of Action for Toxic Effects	101
3.12	ADEQUACY OF THE DATABASE.....	101
3.12.1	Existing Information on Health Effects of Ammonia.....	102
3.12.2	Identification of Data Needs	102
3.12.3	Ongoing Studies.....	110

4. CHEMICAL AND PHYSICAL INFORMATION.....	113
4.1 CHEMICAL IDENTITY.....	113
4.2 PHYSICAL AND CHEMICAL PROPERTIES.....	113
5. PRODUCTION, IMPORT/EXPORT, USE, AND DISPOSAL	117
5.1 PRODUCTION	117
5.2 IMPORT/EXPORT	121
5.3 USE.....	122
5.4 DISPOSAL	122
6. POTENTIAL FOR HUMAN EXPOSURE	123
6.1 OVERVIEW.....	123
6.2 RELEASES TO THE ENVIRONMENT	127
6.2.1 Air	131
6.2.2 Water.....	133
6.2.3 Soil	133
6.3 ENVIRONMENTAL FATE.....	134
6.3.1 Transport and Partitioning.....	134
6.3.2 Transformation and Degradation	136
6.3.2.1 Air.....	136
6.3.2.2 Water	137
6.3.2.3 Sediment and Soil.....	138
6.3.2.4 Other Media.....	138
6.4 LEVELS MONITORED OR ESTIMATED IN THE ENVIRONMENT	138
6.4.1 Air	139
6.4.2 Water.....	141
6.4.3 Sediment and Soil	143
6.4.4 Other Environmental Media.....	143
6.5 GENERAL POPULATION AND OCCUPATIONAL EXPOSURE	143
6.6 EXPOSURES OF CHILDREN	145
6.7 POPULATIONS WITH POTENTIALLY HIGH EXPOSURES	146
6.8 ADEQUACY OF THE DATABASE.....	147
6.8.1 Identification of Data Needs	147
6.8.2 Ongoing Studies	149
7. ANALYTICAL METHODS	153
7.1 BIOLOGICAL MATERIALS	153
7.2 ENVIRONMENTAL SAMPLES.....	156
7.3 ADEQUACY OF THE DATABASE.....	160
7.3.1 Identification of Data Needs	160
7.3.2 Ongoing Studies	161
8. REGULATIONS AND ADVISORIES	163
9. REFERENCES	171
10. GLOSSARY	217
APPENDICES	
A. ATSDR MINIMAL RISK LEVELS AND WORKSHEETS	A-1

B. USER'S GUIDE	B-1
C. ACRONYMS, ABBREVIATIONS, AND SYMBOLS.....	C-1
D. INDEX	D-1

LIST OF FIGURES

3-1.	Levels of Significant Exposure to Ammonia and Ammonium Compounds—Inhalation	41
3-2.	Levels of Significant Exposure to Ammonia and Ammonium Compounds—Oral.....	63
3-3.	Glutamine Cycle	84
3-4.	The Urea Cycle Showing the Compartmentalization of its Steps Within Liver Cells.....	85
3-5.	Conceptual Representation of a Physiologically Based Pharmacokinetic (PBPK) Model for a Hypothetical Chemical Substance	89
3-6.	Existing Information on Health Effects of Ammonia	103
6-1.	Frequency of NPL Sites with Ammonia Contamination	124
6-2.	Simplified Schematic for the Microbial Processes of the Nitrogen Cycle.....	125

LIST OF TABLES

3-1.	Levels of Significant Exposure to Ammonia and Ammonium Compounds—Inhalation	27
3-2.	Levels of Significant Exposure to Ammonia and Ammonium Compounds—Oral.....	59
3-3.	Levels of Significant Exposure to Ammonia and Ammonium Compounds—Dermal.....	72
3-4.	Genotoxicity of Ammonia <i>In Vitro</i> and <i>In Vivo</i>	77
3-5.	Ongoing Studies on the Health Effects of Ammonia.....	111
4-1.	Chemical Identity of Ammonia	114
4-2.	Physical and Chemical Properties of Ammonia	115
5-1.	Facilities that Produce, Process, or Use Ammonia	119
6-1.	Releases to the Environment from Facilities that Produce, Process, or Use Ammonia.....	129
6-2.	Ongoing Studies on Environmental Fate and the Potential for Human Exposure to Ammonia	150
7-1.	Analytical Methods for Determining Ammonia in Biological Samples	154
7-2.	Analytical Methods for Determining Ammonia in Environmental Samples.....	157
7-3.	Ongoing Studies on the Development of Analytical Approaches to the Study of Ammonia	162
8-1.	Regulations and Guidelines Applicable to Ammonia.....	165

1. PUBLIC HEALTH STATEMENT

This public health statement tells you about ammonia and the effects of exposure.

The Environmental Protection Agency (EPA) identifies the most serious hazardous waste sites in the nation. These sites are then placed on the National Priorities List (NPL) and are targeted for long-term federal clean-up activities. Ammonia has been found in at least 137 of the 1,647 current or former NPL sites. Although the total number of NPL sites evaluated for this substance is not known, the possibility exists that the number of sites at which ammonia is found may increase in the future as more sites are evaluated. This information is important because these sites may be sources of exposure and exposure to this substance may harm you.

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

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

1.1 WHAT IS AMMONIA?

Ammonia is a chemical that is made both by humans and by nature. It is made up of one part nitrogen (N) and three parts hydrogen (H₃). The amount of ammonia manufactured every year by humans is almost equal to the amount produced by nature every year. However, when ammonia is found at a level that may cause concern, it was likely produced either directly or indirectly by humans.

1. PUBLIC HEALTH STATEMENT

Ammonia is a colorless gas with a very sharp odor. Ammonia in this form is also known as ammonia gas or anhydrous (“without water”) ammonia. Ammonia gas can also be compressed and becomes a liquid under pressure. The odor of ammonia is familiar to most people because ammonia is used in smelling salts, household cleaners, and window cleaning products.

Ammonia easily dissolves in water. In this form, it is also known as liquid ammonia, aqueous ammonia, or ammonia solution. In water, most of the ammonia changes to the ionic form of ammonia, known as ammonium ions, which are represented by the formula NH_4^+ (an ion is an atom or a group of atoms that has acquired a net electric charge by gaining or losing one or more electrons). Ammonium ions are not gaseous and have no odor. Ammonia and ammonium ions can change back and forth in water. In wells, rivers, lakes, and wet soils, the ammonium form is the most common. Ammonia can also be combined with other substances to form ammonium compounds, including salts such as ammonium chloride, ammonium sulfate, ammonium nitrate, and others.

Ammonia is very important to plant, animal, and human life. It is found in water, soil, and air, and is a source of much needed nitrogen for plants and animals. Most of the ammonia in the environment comes from the natural breakdown of manure and dead plants and animals.

Eighty percent of all manufactured ammonia is used as fertilizer. A third of this is applied directly to soil as pure ammonia. The rest is used to make other fertilizers that contain ammonium compounds, usually ammonium salts. These fertilizers are used to provide nitrogen to plants. Ammonia is also used to manufacture synthetic fibers, plastics, and explosives. Many cleaning products also contain ammonia in the form of ammonium ions.

For detailed information on the chemical properties of ammonia, see Chapter 4. Details on the production and use of ammonia are in Chapter 5, and more information on the environmental fate of ammonia and sources of human exposure is in Chapter 6.

1. PUBLIC HEALTH STATEMENT

1.2 WHAT HAPPENS TO AMMONIA WHEN IT ENTERS THE ENVIRONMENT?

Since ammonia occurs naturally in the environment, we are regularly exposed to low levels of ammonia in air, soil, and water. Ammonia exists naturally in the air at levels between 1 and 5 parts in a billion parts of air (ppb). It is commonly found in rainwater. The ammonia levels in rivers and bays are usually less than 6 parts per million (ppm; 6 ppm=6,000 ppb). Soil typically contains about 1–5 ppm of ammonia. The levels of ammonia vary throughout the day, as well as from season to season. Generally, ammonia levels are highest in the summer and spring. Ammonia is essential for mammals and is necessary for making DNA, RNA, and proteins. It also plays a part in maintaining acid-base balance in tissues of mammals.

Ammonia does not last very long in the environment. Because it is recycled naturally, nature has many ways of incorporating and transforming ammonia. In soil or water, plants and microorganisms rapidly take up ammonia. After fertilizer containing ammonia is applied to soil, the amount of ammonia in that soil decreases to low levels in a few days. In the air, ammonia will last about 1 week.

Ammonia has been found in air, soil, and water samples at hazardous waste sites. In the air near hazardous waste sites, ammonia can be found as a gas. Ammonia can also be found dissolved in ponds or other bodies of water at a waste site. Ammonia can be found attached to soil particles at hazardous waste sites. The average concentration of ammonia reported at hazardous waste sites ranges from 1 to 1,000 ppm in soil samples and up to 16 ppm in water samples.

See Chapter 6 for more detailed information on the environmental fate of ammonia, ammonia levels in the environment, and exposure to ammonia.

1.3 HOW MIGHT I BE EXPOSED TO AMMONIA?

Ammonia is naturally produced and used by all mammals in their normal metabolism. Ammonia is produced within a person's body each day. Most of this ammonia is produced by organs and tissues, but some is produced by bacteria living inside our intestines.

1. PUBLIC HEALTH STATEMENT

Ammonia is found naturally in the environment. You may be exposed to ammonia by breathing air, eating food, or drinking water that contains it, or through skin contact with ammonia or ammonium compounds. Exposure to ammonia in the environment is most likely to occur by breathing in ammonia that has been released into the air.

Ammonia has a very strong odor that is irritating and that you can smell when it is in the air at a level higher than 50 ppm. Therefore, you will probably smell ammonia before you are exposed to a concentration that may harm you. Levels of ammonia in air that cause serious effects in people are much higher than levels you would normally be exposed to at home or work. However, low levels of ammonia may harm some people with asthma and other sensitive individuals.

You can taste ammonia in water at levels of about 35 ppm. Lower levels than this occur naturally in food and water. Swallowing even small amounts of liquid ammonia in your household cleaner might cause burns in your mouth and throat. A few drops of liquid ammonia on the skin or in the eyes will cause burns and open sores if not washed away quickly. Exposure to larger amounts of liquid ammonia or ammonium ion in the eyes causes severe eye burns and can lead to blindness.

Outdoors, you may be exposed to high levels of ammonia gas in air from leaks and spills at production plants and storage facilities, and from pipelines, tank trucks, railcars, ships, and barges that transport ammonia. Higher levels of ammonia in air may occur when fertilizer with ammonia or ammonium compounds is applied to farm fields. After fertilizer is applied, the concentration of ammonia in soil can be more than 3,000 ppm; however, these levels decrease rapidly over a few days.

Indoors, you may be exposed to ammonia while using household products that contain ammonia. Some of these products are ammonia-cleaning solutions, window cleaners, floor waxes, and smelling salts.

1. PUBLIC HEALTH STATEMENT

Household and industrial cleaning solutions may contain ammonia, and use of these products at home or work may lead to exposure to ammonia. Both types of ammonia cleaning solutions are made by adding ammonia gas to water to form liquid ammonia. Household ammonia cleaners typically contain lower levels of ammonia (between 5 and 10%) compared to industrial cleaning solutions, which can contain higher levels of ammonia (up to 25%).

Farmers can be exposed to ammonia when they work with or apply fertilizers containing ammonia to fields. Farmers, cattle ranchers, and people who raise other types of livestock and/or poultry can be exposed to ammonia from decaying manure. Some manufacturing processes also use ammonia. Some older refrigeration units used ammonia as the refrigerant.

For more information on levels of exposure associated with effects, see Chapter 3.

1.4 HOW CAN AMMONIA ENTER AND LEAVE MY BODY?

Ammonia can enter your body if you breathe in ammonia gas or if you swallow water or food containing ammonium salts. If you spill a liquid containing ammonia on your skin, a small amount of ammonia might enter your body through your skin; however, more ammonia will probably enter as you breathe ammonia gas from the spilled ammonia. After you breathe in ammonia, you breathe most of it out again. The ammonia that is retained in the body is changed into ammonium compounds and carried throughout the body in seconds. If you swallow ammonia in food or water, it will get into your bloodstream and be carried throughout your body in seconds. Most of the ammonia that enters your body from food or water rapidly changes into other substances that will not harm you. The rest of this ammonia leaves your body in urine within a couple of days. For more information on how ammonia can enter and leave your body, see Chapter 3.

1. PUBLIC HEALTH STATEMENT

1.5 HOW CAN AMMONIA AFFECT MY HEALTH?

Scientists use many tests to protect the public from harmful effects of toxic chemicals and to find ways for treating persons who have been harmed.

One way to learn whether a chemical will harm people is to determine how the body absorbs, uses, and releases the chemical. For some chemicals, animal testing may be necessary. Animal testing may also help identify health effects such as cancer or birth defects. Without laboratory animals, scientists would lose a basic method for getting information needed to make wise decisions that protect public health. Scientists have the responsibility to treat research animals with care and compassion. Scientists must comply with strict animal care guidelines because laws today protect the welfare of research animals.

Ammonia is a corrosive substance and the main toxic effects are restricted to the sites of direct contact with ammonia (i.e., skin, eyes, respiratory tract, mouth, and digestive tract). For example, if you spilled a bottle of concentrated ammonia on the floor, you would smell a strong ammonia odor; you might cough, and your eyes might water because of irritation. If you were exposed to very high levels of ammonia, you would experience more harmful effects. For example, if you walked into a dense cloud of ammonia or if your skin comes in contact with concentrated ammonia, your skin, eyes, throat, or lungs may be severely burned. These burns might be serious enough to cause permanent blindness, lung disease, or death. Likewise, if you accidentally ate or drank concentrated ammonia, you might experience burns in your mouth, throat, and stomach. There is no evidence that ammonia causes cancer. Ammonia has not been classified for carcinogenic effects by EPA, Department of Health and Human Services (DHHS) (NTP), or the International Agency for Research on Cancer (IARC). Ammonia can also have beneficial effects, such as when it is used as a smelling salt. Certain ammonium salts have long been used in veterinary and human medicine. For more information on how ammonia can affect your health, see Chapter 3.

1. PUBLIC HEALTH STATEMENT

1.6 HOW CAN AMMONIA AFFECT CHILDREN?

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

Children are less likely than adults to be exposed to concentrated ammonia because most exposures to concentrated ammonia occur in occupational settings. Children can still be exposed in the same way as adults to ammonia gas from spills or leaks from ammonia tanks or pipelines, especially on farms where it is used as a fertilizer. Children can also be exposed to dilute ammonia solutions from household cleaners containing ammonia.

The effects of ammonia on children are likely to be the same as for adults. Ammonia is an irritant and the solution and gas can cause burns of the skin, eyes, mouth, and lungs. If a spill occurs, children may be exposed to ammonia for a longer time than adults because they may not leave the area as quickly.

There is no evidence that exposure to the levels of ammonia found in the environment causes birth defects or other developmental effects. It is not known whether ammonia can be transferred from a pregnant mother to a developing fetus through the placenta or from a nursing mother to her offspring through breast milk. One study in animals showed that exposure of mothers to very high levels of ammonia during pregnancy caused their newborn offspring to be smaller than normal, but this occurred at levels of ammonia that also affected the mothers.

1.7 HOW CAN FAMILIES REDUCE THE RISK OF EXPOSURE TO AMMONIA?

If your doctor finds that you have been exposed to significant amounts of ammonia, ask whether your children might also be exposed. Your doctor might need to ask your state health department to investigate.

You can reduce your risk of exposure to ammonia by carefully using household products and by avoiding areas where ammonia is used or produced. At home, you can reduce your risk of

1. PUBLIC HEALTH STATEMENT

exposure to ammonia by careful handling of any household products that contain ammonia. For example, some cleaning products contain ammonia; so when you use them, you should be sure that rooms are adequately ventilated during the time you are using them. Avoid ammonia-containing products in glass bottles since breakage could lead to a serious exposure. You should wear proper clothing and eye protection, because ammonia can cause skin burns and damage eyes if it is splashed on them. To lower the risk of your children being exposed to ammonia, you should tell them to stay out of the room when you are using it. While use of ammonia by a child is not recommended, any use by a child should be closely supervised by an adult.

You can also reduce your risk of exposure to ammonia by avoiding areas where it is being used. Ammonia is used to fertilize crops, so you can lower your exposure to ammonia by avoiding these areas when it is being applied. You can also lower your exposure to ammonia by avoiding places where it is produced. Ammonia is found in many animal wastes, and it may be present in high concentrations in the air in livestock buildings. You can lower your exposure to ammonia by avoiding these buildings, especially if large numbers of animals are inside.

If you are a worker who uses or applies ammonia for farming, you can reduce your exposure by using it according to the instructions and wearing proper clothing and protective gear. Be sure to follow all instructions and heed any warning statements.

1.8 IS THERE A MEDICAL TEST TO DETERMINE WHETHER I HAVE BEEN EXPOSED TO AMMONIA?

There are tests that measure ammonia/ammonium ion in blood and urine; however, these tests would probably not tell you whether you have been exposed because ammonia is normally found in the body. If you were exposed to harmful amounts of ammonia, you would notice it immediately because of the strong, unpleasant, and irritating smell, the strong taste, and because of skin, eye, nose, or throat irritation. Exposure detection levels and methods for determining ammonia levels in biological materials are discussed in Chapters 3 and 7.

1. PUBLIC HEALTH STATEMENT

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

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

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

Recommendations and regulations are also updated periodically as more information becomes available. For the most current information, check with the federal agency or organization that provides it. Some regulations and recommendations for ammonia include the following:

EPA regulates the ammonia content in waste water released by several industries. Any discharges or spills of ammonia of 100 pounds or more, or of ammonium salts of 1,000 or 5,000 pounds (depending upon the compound), must be reported to EPA.

Some restrictions have been placed on levels of ammonium salts allowable in processed foods. FDA states that the levels of ammonia and ammonium compounds normally found in food do not pose a health risk. Maximum allowable levels in processed foods are as follows: 0.04–3.2% ammonium bicarbonate in baked goods, grain, snack foods, and reconstituted vegetables; 2.0% ammonium carbonate in baked goods, gelatins, and puddings; 0.001% ammonium chloride in

1. PUBLIC HEALTH STATEMENT

baked goods and 0.8% in condiments and relishes; 0.6–0.8% ammonium hydroxide in baked goods, cheeses, gelatins, and puddings; 0.01% monobasic ammonium phosphate in baked goods; and 1.1% dibasic ammonium phosphate in baked goods, 0.003% in nonalcoholic beverages, and 0.012% in condiments and relishes.

OSHA has set an 8-hour exposure limit of 25 ppm and a short-term (15-minute) exposure limit of 35 ppm for ammonia in the workplace. NIOSH recommends that the level in workroom air be limited to 50 ppm for 5 minutes of exposure.

Further information on governmental recommendations can be found in Chapter 8.

1.10 WHERE CAN I GET MORE INFORMATION?

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

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

Toxicological profiles are also available on-line at www.atsdr.cdc.gov and on CD-ROM. You may request a copy of the ATSDR ToxProfiles™ CD-ROM by calling the toll-free information and technical assistance number at 1-888-42ATSDR (1-888-422-8737), by e-mail at atsdric@cdc.gov, or by writing to:

Agency for Toxic Substances and Disease Registry
Division of Toxicology
1600 Clifton Road NE
Mailstop F-32
Atlanta, GA 30333
Fax: 1-770-488-4178

1. PUBLIC HEALTH STATEMENT

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

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

2. RELEVANCE TO PUBLIC HEALTH

2.1 BACKGROUND AND ENVIRONMENTAL EXPOSURES TO AMMONIA IN THE UNITED STATES

Ammonia is a natural compound, as well as a manufactured compound. In nature, most ammonia probably comes from decomposing animal excreta, with the decay of organic materials from plants, dead animals, and the like also contributing significant amounts. It is also exhaled by animals. Production of fixed nitrogen (NH_3) by plants and microorganisms is estimated at 90 to 130 metric tons annually. Manufacture of ammonia within the United States was 9.5 million metric tons in 2001, which is down from 16.6 million metric tons in 1999. Commercially produced ammonia is used primarily as fertilizer, with plastics, synthetic fibers and resins, explosives, and other uses accounting for most of the remainder.

Ammonia is released to the atmosphere by natural processes such as the decay of organic matter and animal excreta, or by volcanic eruptions. It can also be released to the atmosphere by anthropogenic activities such as fertilizer use; spillage or leakage from storage or production facilities; or loss from waste water effluents. The average global ammonia concentration in the atmosphere ranges from 0.3 to 6 ppb, with concentrations sometimes higher in the vicinity of agricultural or industrial areas. For example, near industrial sources or manure heaps in Germany, ammonia concentrations ranged from 10.3 to 89 ppb. Concentrations may be orders of magnitude higher near some types of livestock areas, such as pigpens, where local atmospheric concentrations have been reported to be as high as 47 ppm.

Elevated concentrations of ammonia in water are usually due to effluent discharges from sewage treatment plants or industrial processes, or runoff from fertilized fields or livestock areas. Ammonia concentrations can therefore vary widely in aquatic environments, with concentrations being lower in bodies of water that are unimpacted by residential, industrial, or farming effluents, compared to those that are impacted (where concentrations can be orders of magnitude higher). In unimpacted waterways, ammonia concentrations have been reported to range from 8.5 to 43 ppb, whereas in impacted waterways, concentrations as high as 16 ppm have been reported.

Soils usually obtain additional ammonia from natural or synthetic fertilizer application, animal excreta, decaying organic matter, or natural fixation from the atmosphere. Soils have been reported to have background concentrations of ammonia ranging from 1 to 5 ppm. Immediately following application of

2. RELEVANCE TO PUBLIC HEALTH

fertilizer or manure, however, ammonia concentrations can rise to 2–3,000 ppm, with levels dropping after 5 days to 2–850 ppm. These high ammonia concentrations are usually limited to the upper few centimeters of topsoil.

In the atmosphere, ammonia can react with acidic substances in the air to produce ammonium aerosols, which can be subject to dry or wet deposition. The best estimate of the half-life of atmospheric ammonia is a few days. In water, ammonia can volatilize to the atmosphere, be removed by microbial processes, or adsorb to sediment and suspended organic material. In soil, ammonia can volatilize to the atmosphere, adsorb to soil particles, undergo microbial transformation to nitrate or nitrite anions, or be taken up by plants.

For the general population, the most likely source of exposure to elevated levels of ammonia is from the use of household cleaners containing ammonia or ammonium salts. People who live near farms, who visit farms during the application of fertilizer, or who live near cattle feedlots, poultry confinement buildings, or other areas where animal populations are concentrated can also be exposed to ammonia. Local atmospheric concentrations in these agricultural settings have been reported to range from 280 to 88,000 ppb.

There is also the possibility for exposure to ammonia via water and food ingestion. If untreated surface water is ingested, the average uptake would be 0.36 mg/day (assuming an ammonia concentration in untreated water of 0.18 mg/L and a consumption rate of 2 L/day). For most sources of drinking water, however, adsorption, nitrification, and the conversion of ammonia to chloramines upon chlorination will result in negligible levels of ammonia in most drinking water supplies. Food ingestion can also lead to an exposure to ammonia, primarily due to the use of various ammonium salts as food stabilizers; the estimated exposure from these food additives is 18 mg/day.

Populations that live or work near a hazardous waste site that contains ammonia or ammonium salts could be exposed to above-average levels of ammonia in soil, water, or air in similar concentrations as those in agricultural settings. While these exposures may occur, the half-life of ammonia in nature is probably very short. Ammonia has been identified in at least 137 of 1,647 National Priority List (NPL) hazardous waste sites.

2. RELEVANCE TO PUBLIC HEALTH

2.2 SUMMARY OF HEALTH EFFECTS

Ammonia is an essential mammalian metabolite for DNA, RNA, and protein synthesis and is necessary for maintaining acid-base balance. Ammonia is produced and used endogenously in all mammalian species. It has been estimated that up to 17 grams of ammonia are produced in humans daily. Of these 17 grams, approximately 4 grams are produced in the gut by intestinal bacteria, where it enters the portal circulation and is metabolized rapidly in the liver to urea. Ammonia is excreted primarily as urea and urinary ammonium compounds through the kidneys. Levels of ammonia in the blood from healthy humans range from 0.7 to 2 mg/L.

The most important injurious effects of exposure to excessive amounts of ammonia on humans are due to its irritative and corrosive properties. Exposures to ammonia gas cause chemical burns of the respiratory tract, skin, and eyes. Ammonia dissolves in the water present in skin, mucous membranes, and eyes and becomes ammonium hydroxide, which is a highly ionized weak base that causes necrosis of the tissues. Specifically, ammonium hydroxide causes saponification of cell membrane lipids resulting in cell disruption and death. Additionally, it extracts water from the cells, and initiates an inflammatory response, which further damages the surrounding tissues. Contact with liquid ammonia (not ammonium salts) results in cryogenic injury in addition to the alkali burns. Airway blockage and respiratory insufficiency may be lethal outcomes of exposure to anhydrous ammonia vapors or concentrated aerosols. Ingestion of concentrated ammonium solutions may produce severe burns and hemorrhage of the upper gastrointestinal tract. Survival of the initial insult may be compromised by infections, scarring, and other complications that may develop days or weeks following inhalation or ingestion. Effects that have been observed in humans exposed to ammonia gas and ammonium salt aerosols have also been observed in animals. Hepatic and renal effects have also been reported in animals and humans; however, ammonia does not appear to be a primary liver or kidney toxicant.

Increased systemic ammonia/ammonium salts/ion, or hyperammonemia, is generally not seen following inhalation or dermal exposure, but can result from ingestion and from certain disease states such as cirrhosis of the liver, acute liver failure, and congenital deficiencies of any of the urea cycle enzymes. Liver disease can result in decreased metabolism of ammonia with resultant increased levels of ammonia in the bloodstream and in the brain, which can produce neurological effects such as seizures and coma, and eventually death. In chronic liver failure, arterial ammonia concentrations may reach approximately 3.6 mg/L, whereas arterial ammonia in acute liver failure may rise as high as 8 mg/L. The most likely and significant effects of exposure to elevated levels of ammonia are discussed below.

2. RELEVANCE TO PUBLIC HEALTH

Respiratory Effects. Ammonia is an upper respiratory irritant in humans. Exposures to levels exceeding 50 ppm result in immediate irritation to the nose and throat; however, tolerance appears to develop with repeated exposure. Exposure to an air concentration of 250 ppm is bearable for most persons for 30–60 minutes. Acute exposure to higher levels (500 ppm) have been shown to increase respiratory minute volume. Accidental exposures to concentrated aerosols of ammonium salts or high concentrations of ammonia gas have resulted in nasopharyngeal and tracheal burns, airway obstruction and respiratory distress, and bronchiolar and alveolar edema. Ammonia vapor readily dissolves in the moisture present on the skin, eyes, oropharynx and lungs forming ammonium hydroxide which dissociates to yield hydroxyl ions. Chronic occupational exposure to low levels of airborne ammonia (<25 ppm) had little effect on pulmonary function or odor sensitivity in workers at some factories, but studies of farmers exposed to ammonia and other pollutants in livestock buildings indicated an association between exposure to pollutants, including ammonia, and an increase in respiratory symptoms (such as bronchial reactivity/hyperresponsiveness, inflammation, cough, wheezing, or shortness of breath) and/or a decrease in lung function parameters. The contribution of ammonia to these respiratory symptoms is unclear.

Dermal Effects. Skin is extremely sensitive to airborne ammonia or ammonia dissolved in water. The topical damage caused by ammonia is probably due mainly to its reactivity and irritation properties. Its high water solubility allows it to dissolve in moisture on these surfaces, react with fatty substances, be absorbed into deeper layers, and inflict extensive damage. Reports of skin damage in humans are numerous, but good quantitative data are lacking. The severity of the damage is proportional to the concentration and duration of exposure; flushing with water immediately after contact alleviates or prevents effects.

Dermal exposures to liquid ammonia or concentrated solutions and/or ammonia gas are frequently occupationally related and produce cutaneous burns, blisters, and lesions of varying degrees of severity. Unlike acid burns, which cause a coagulation necrosis, ammonia causes alkali burns, resulting in liquification of the tissue and deeper penetrations. Burns can be severe enough to require skin grafting, and loss of the epidermal layer increases body fluid loss and incidence of infection. While most ammonia exposures are occupational, household products containing ammonia can also cause dermal injury. Several cases of young children (2–3 years old) who bit into ammonia pellets/capsules and sustained oral and esophageal lesions have been reported in the literature.

2. RELEVANCE TO PUBLIC HEALTH

Very limited animal data regarding dermal effects of exposure to ammonia support the findings in humans.

Ocular Effects. Reported ocular effects in humans following ammonia gas exposure increased in severity with dose and duration. Good quantitative data are lacking, but symptoms progress as follows: inflamed eyes, lacrimation, swelling of the eyelids, hyperemic conjunctiva, blurred vision, possible transient blindness, corneal abrasions, and sustained corneal damage. Ammonia is slightly irritating to human eyes in a brief exposure at concentrations of 100 ppm, and immediately irritating to the eyes and throat at 698 ppm. Exposure to an air concentration of 250 ppm is bearable for most persons for 30–60 minutes.

Limited animal data regarding ocular effects of exposure to ammonia support the findings in humans.

Neurological Effects. Neurological effects in humans following inhalation or dermal exposure to ammonia are usually limited to blurred vision, most likely due to direct contact, but more severe exposures, which result in significant elevation of blood ammonia levels (hyperammonemia), can result in diffuse nonspecific encephalopathy, muscle weakness, decreased deep tendon reflexes, and loss of consciousness. Hyperammonemia in humans can result from certain disease states such as cirrhosis of the liver, acute liver failure, and congenital deficiencies of any of the urea cycle enzymes; hyperammonemia may lead to encephalopathy. Some have suggested that ammonia may be involved in the generation of the symptomatology and progression of Alzheimer's disease as a result of pathological ammonia metabolism in the brain. Cerebral edema and herniation and intracranial hypertension have been noted in animal models of hyperammonemia. The mechanism of ammonia-induced encephalopathies has not been definitively elucidated. It is thought to involve the alteration of glutamate metabolism in the brain with resultant increased activation of N-methyl-D-aspartate (NMDA) receptors, which causes decreased protein kinase C-mediated phosphorylation of Na^+/K^+ ATPase, increased activity of Na^+/K^+ ATPase, and depletion of ATP. Additional evidence of altered energy levels includes changes in some TCA cycle-associated components including acetoacetate, and NAD^+/NADH ratio, 2-oxoglutarate, and 3-hydroxybutyrate. This reduced ATP level may be involved in ammonia-induced coma and death. A disruption in neurotransmission has also been suggested by alteration of brain tubulin, which is an essential component of the axonal transport system.

2. RELEVANCE TO PUBLIC HEALTH

2.3 MINIMAL RISK LEVELS***Inhalation MRLs***

- An MRL of 1.7 ppm has been derived for acute-duration inhalation exposure (14 days or less) to ammonia.

This MRL is based on a lowest-observed-adverse-effect level (LOAEL) of 50 ppm for mild irritation to the eyes, nose, and throat in humans exposed to ammonia as a gas for 2 hours (Verberk et al. 1977). In that study, a group of 16 subjects were tested, 8 of them (experts) knew the effects of ammonia from the literature, but had no personal contact, whereas the remaining 8 subjects (non-experts) were students from a non-science faculty and were not familiar with ammonia or experiments in laboratory situations. All members of a group were exposed on the same day to one of the concentrations tested (50, 80, 110, or 140 ppm). The testing was repeated with a 1-week interval. Immediately before and after exposure, vital capacity, forced expiratory volume, and forced inspiratory volume were measured. During exposure, each subject recorded subjective feelings every 15 minutes as no sensation (0), just perceptible (1), distinctly perceptible (2), nuisance (3), offensive (4), or unbearable (5). No statistical analysis was performed and there was no group exposed to air only. Results of the pulmonary function tests after exposure were not statistically significantly different from pre-exposure values. For the non-experts, there was a clear increase in the number of reported symptoms for smell, eye irritation, throat irritation, cough, and general discomfort as the exposure concentration increased. The latter was not as clear for the experts. It should also be mentioned that the subjective responses appeared more pronounced in the non-expert group than in the expert group. The LOAEL was divided by an uncertainty factor of 30 (10 to protect sensitive individuals and 3 for the use of a minimal LOAEL). A study of piggerie workers exposed to a mean level of 7.9 ppm ammonia measured pulmonary function change over a workshift; a small but borderline significant decrease in pulmonary function was noted (Heederik et al. 1990). This study was not used as a basis for MRL derivation because the workers were also exposed to other potential respiratory toxicants (dust and endotoxins). Although the Verberk et al. (1977) study has limitations (no statistical analysis, subjective end points, no control group), it demonstrates that concentrations of 50 ppm ammonia produce minimal discomfort in healthy members of the general population and therefore, should be avoided. A more detailed discussion of additional information supporting the findings of Verberk et al. (1977) is presented in Appendix A.

No intermediate-duration inhalation MRL was derived for ammonia. The only available intermediate-duration inhalation study in humans is that of Ferguson et al. (1977). In that study, a group of 6 healthy

2. RELEVANCE TO PUBLIC HEALTH

volunteers, not previously accustomed to working in an ammonia environment, were exposed 5 days/week to 25 ppm (2 hours/day), 50 ppm (4 hours/day), or 100 ppm (6 hours/day) of ammonia, or to 50 ppm of ammonia 6 hours/day for 6 weeks. End points monitored included subjective and objective measures of eye and throat irritation as well as pulse rate, respiration rate, pulmonary function (FVC, FEV), assessment of neurological function (reflex, balance, and coordination), and body weight. The exposure protocol consisted of a pre-exposure evaluation by a physician, 3 hours of exposure (this conflicts with exposure data on table 2 of the study and mentioned above), a mid-point physician's observation, lunch break, 3 additional hours of exposure, and a third physician's observation 30 minutes after exposure ceased. The conjunctiva and mucosa of the nose and throat were examined by a physician before and after each daily exposure and the degree of irritation noted was described as mild, moderate, or marked. Exposure to ammonia had no significant effect on the measures of respiratory function or in the neurological tests conducted. The results of the evaluations of irritation conducted by the physician showed no significant differences between the exposure groups, including the 0 ppm exposure group (pre-exposure). All subjects experienced some watering of the eyes and a sensation of dryness in the nose and throat, and there was one observation of definite redness in the mucosa of the nose after a 6-hour exposure to 100 ppm during which time, there was an excursion to 200 ppm ammonia. No redness was observed in this subject the following morning. Throughout the study, the physician observed 6 cases of eye irritation, 20 of nose irritation, and 9 of throat irritation, and most cases appeared to have occurred the first week of the study during exposure to 50 ppm. It is difficult to determine in this study a no-observed-adverse-effect level (NOAEL) or LOAEL for irritation due to the different exposure durations experienced by the subjects. In general, studies in animals have used higher exposure concentrations and the overall quality of the studies is less than desirable. For ammonia, a corrosive irritant gas that affects the portal of entry and produces irritation of eyes and respiratory tract, use of human data should be preferred over animal studies.

- An MRL of 0.1 ppm has been derived for chronic-duration inhalation exposure (365 days or more) to ammonia.

This MRL supersedes the previous chronic inhalation MRL of 0.3 ppm derived in the 2002 draft for public comment version of this profile. The MRL is based on a NOAEL of 9.2 ppm for sense of smell, prevalence of respiratory symptoms (cough, bronchitis, wheeze, dyspnea, and others), eye and throat irritation, and lung function parameters (FVC, FEV₁, FEV₁/FVC, FEF₅₀, and FEF₇₅) in humans exposed for an average of 12.2 years in a soda ash plant (Holness et al. 1989); no LOAEL was determined. The cohort consisted of 52 workers and 35 controls. The subjects were assessed on two workdays: on the first workday of their workweek and on the last workday of their workweek. Spirometry was performed at the

2. RELEVANCE TO PUBLIC HEALTH

beginning and end of each work shift, so that each worker had four tests done. To determine the exposure levels, exposed and control workers were sampled over one work shift; the average sample collection period was 8.4 hours. All of the participants in the study were males. Analysis of the results showed no significant differences in the prevalence of reported symptoms, but the exposed workers reported that exposure in the plant aggravated some of their reported symptoms (cough, wheeze, nasal complaints, eye irritation, and throat discomfort). Odor threshold was not affected by exposure to ammonia and there were no significant differences in baseline lung functions between exposed and control subjects. Analysis of each worker separately showed no significant relationship between the level of ammonia exposure and changes in lung function. Also, when the workers were divided into groups of individuals that were exposed to low (<6.25 ppm), medium (6.25–12.5 ppm), and high (>12.5 ppm) ammonia levels, no significant association was found between reporting of symptoms, decline in baseline function, or increasing decline in function over the work shift and exposure to ammonia. Furthermore, no association was evident between increasing years of exposure and decreasing lung function. However, the power of the indices of both level and length of exposure is low because only eight workers were in areas with relatively high ammonia exposure. The MRL was calculated by adjusting the mean TWA exposure concentration of 9.2 ppm for continuous exposure (8/24 hours x 5/7 days) and dividing by an uncertainty factor of 10 to protect sensitive individuals. A modifying factor of 3 was added for the lack of reproductive and developmental studies.

Oral MRLs

No oral MRLs were derived for ammonia because of two main reasons. In the first place, the overall quality and/or usefulness of the oral database is limited. The only human acute oral studies available were case reports with no exposure levels (Klein et al. 1985; Klendshoj and Rejent 1966; Lopez et al. 1988). Animal studies were limited to a food intake study (Noda and Chikamori 1976), single-exposure studies with no effect, serious effects, or unsupported effects (Benyajati and Goldstein 1975; Koenig and Koenig 1949), a gavage study that lacked study details (Boyd and Seymour 1946), and a 6-day drinking water study with effects at high levels (Barzel 1975). Rats exposed to 3,102 mg NH_4^+ /kg/day in the diet and drinking water for 7 days had statistically significantly reduced body weight gain (64% less) compared to a control group that consumed only 22 mg NH_4^+ /kg/day (Boyano-Adáñez et al. 1996). Such a high dose of ammonium (as acetate) is equivalent to a 70 kg human ingesting approximately 1.4 lb of ammonium acetate daily. No human studies or reports of intermediate-duration oral exposure to ammonia were located. Intermediate-duration animal studies have reported decreases in body weight gain in rats exposed via drinking water (Gupta et al. 1979) or diet (Boyano-Adáñez et al. 1996). It should be

2. RELEVANCE TO PUBLIC HEALTH

mentioned that Gupta et al. (1979) administered ammonium sulfamate to the rats. Ammonium sulfamate is an herbicide whose herbicide properties reside in the sulfamate portion of the salt and for which there is little toxicity information in the open literature. The EPA (IRIS 2004) has derived an oral RfD for the sulfamate moiety based on the results of Gupta et al. (1979). Following gavage administration of ammonium salts, bone, blood pressure, adrenal gland, and renal effects have been observed in early studies, generally inadequate by current standards (Bodansky et al. 1932; Fazekas 1939; Seegal 1927). No chronic-duration oral data were located.

An additional reason not to derive oral MRLs for ammonia is because of the role played by the anion of the salt administered. Briefly, in many animal studies, the animals were administered ammonium chloride. Ammonium chloride is commonly used to induce metabolic acidosis in experimental animals. The acidosis is due to the formation of hydrogen ions from the metabolism of ammonium ions to urea. WHO (1986) notes that the ingestion of ammonium chloride in doses around 500–1,000 mg/kg/day for 1–8 days (longer treatment would worsen the condition) has induced metabolic acidosis in mice, guinea pigs, rats, rabbits, and dogs. Metabolic acidosis can result in a variety of nonspecific changes in neurological, cardiovascular, pulmonary, gastrointestinal, and musculoskeletal function, as well as in changes in hematological and clinical chemistry parameters. Acidosis following the administration of ammonium chloride is due to the formation of hydrogen chloride and although it will occur with any ammonium salt, the degree of acidosis (and associated consequences) will be determined by the ability of the kidneys to excrete the specific anion. DeSousa et al. (1974) showed that administration of hydrochloric acid to dogs induced a significantly greater decrease in plasma bicarbonate than administration of equivalent quantities of H^+ as nitric or sulfuric acid. This means that it would be inappropriate to extrapolate findings obtained with ammonium chloride (or any ammonium salt) to equivalent amounts of ammonium, but derived from a different salt.

Finally, as discussed by WHO (1986), the amount of excess ammonia (over and above the amount normally produced in the body) that can be safely ingested and assimilated is difficult to define. However, data from human and animals suggest that that amount may be substantial based on the existence of various efficient ways by which the body can dispose of ammonia.

3. HEALTH EFFECTS

3.1 INTRODUCTION

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

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

3.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

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

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

3. HEALTH EFFECTS

considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear. LOAELs or NOAELs should also help in determining whether or not the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

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

Estimates of exposure levels posing minimal risk to humans (Minimal Risk Levels or MRLs) have been made for ammonia. An MRL is defined as an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects (noncarcinogenic) over a specified duration of exposure. MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration within a given route of exposure. MRLs are based on noncancerous health effects only and do not consider carcinogenic effects. MRLs can be derived for acute, intermediate, and chronic duration exposures for inhalation and oral routes. Appropriate methodology does not exist to develop MRLs for dermal exposure.

Although methods have been established to derive these levels (Barnes and Dourson 1988; EPA 1990), uncertainties are associated with these techniques. Furthermore, ATSDR acknowledges additional uncertainties inherent in the application of the procedures to derive less than lifetime MRLs. As an example, acute inhalation MRLs may not be protective for health effects that are delayed in development or are acquired following repeated acute insults, such as hypersensitivity reactions, asthma, or chronic bronchitis. As these kinds of health effects data become available and methods to assess levels of significant human exposure improve, these MRLs will be revised.

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

3. HEALTH EFFECTS

3.2.1 Inhalation Exposure**3.2.1.1 Death**

There are many reports in the literature of human deaths resulting from inhalation of ammonia (Arwood et al. 1985; Burns et al. 1985; Close et al. 1980; Couturier et al. 1971; George et al. 2000; Heifer 1971; Price et al. 1983; Sobonya 1977; Walton 1973; Weiser and Mackenroth 1989; Yang et al. 1987). Most of these reports are of acute accidental exposure to ammonia gas. A review of the early literature on ammonia toxicity cites acute exposure to 5,000–10,000 ppm as being rapidly fatal in humans (Henderson and Haggard 1927; Mulder and Van der Zalm 1967) and exposure to 2,500–4,500 ppm as being fatal in about 30 minutes (Helmers et al. 1971; Millea et al. 1989). Immediate deaths resulting from acute exposure to ammonia appear to be caused by airway obstruction while infections and other secondary complications are lethal factors among those who survive for several days or weeks. Chemical burns and edema of exposed tissues, including the respiratory tract, eyes, and exposed skin, are often observed after exposure to lethal levels. Post-mortem findings in the fatal case described by Walton (1973) included extensive edema and burns affecting the mouth, faces, trunk, arms, and upper part of the trunk. The airway at the larynx was almost blocked and the lungs were greatly distended and congested. Histological examination of the lungs showed acute congestion and edema. The bronchial walls were stripped of their epithelial lining, and some smaller bronchi contained plugs of debris, which included epithelial cells, red blood cells, and dust cells. No reports of human death due to intermediate or chronic exposure to ammonia were located.

Studies in animals indicate that the acutely lethal exposure concentration depends on the exposure duration. The lethal concentration in rats and mice increases 5–10 times as the exposure duration decreases from 16 hours to several minutes (Hilado et al. 1977, 1978; Kapeghian et al. 1982; Morgan 1997; Prokop'eva et al. 1973; Weedon et al. 1940). Exposure frequency also appears to be an important factor in determining lethality. Continuous exposure to 653 ppm for 25 days resulted in nearly 64% lethality in rats, whereas intermittent exposure (5 days/week, 8 hours/day) to nearly twice this concentration was tolerated for 42 days (Coon et al. 1970). It appears that male rats are more sensitive than female rats to the lethal effects of ammonia (Appelman et al. 1982; Stupfel et al. 1971). Animals exposed to acutely lethal concentrations show severe lesions in the respiratory tract that are similar to those observed in humans. Less severe lesions of the liver, heart, and kidney have been observed following continuous long-term exposure to lethal concentrations in rats, guinea pigs, rabbits, and dogs (Coon et al. 1970). However, these may represent secondary complications from chronic respiratory tract injury.

3. HEALTH EFFECTS

3.2.1.2 Systemic Effects

Respiratory Effects. Ammonia is an upper respiratory irritant in humans. Exposures to levels exceeding 30 ppm result in immediate irritation to the nose and throat (Industrial Bio-Test Laboratories 1973; MacEwen et al. 1970; Sekizawa and Tsubone 1994; Verberk 1977). Four out of six human subjects described moderate irritation of the nose and eyes when exposed to 50, but not 30, ppm ammonia gas for 10 minutes (MacEwen et al. 1970). Twenty to 30% of subjects exposed to 72, but not 50, ppm ammonia gas for 5 minutes experienced eye, nasal, and throat irritation (Industrial Bio-Test Laboratories 1973). However, tolerance appears to develop with repeated exposure (Sekizawa and Tsubone 1994; Verberk 1977). Thus, subjects exposed to 50 ppm ammonia 6 hours/day, 5 days/week for 6 weeks experienced nose and throat irritation only during the first week (Ferguson et al. 1977). Acute exposure to higher levels (500 ppm) has been shown to alter respiratory minute volume (Cole et al. 1977; Silverman et al. 1949). Buff and Koller (1974) suggest that this is due to an effect on "irritant receptors" in the lungs resulting in increased activity of reflex respiratory muscles. This mechanism is also suggested by Cole et al. (1977), who exposed men to 100–331 ppm ammonia gas for 8–11 minutes while they were exercising on a stationary bicycle. Respiratory minute volume was decreased at concentrations of 150–331 ppm (but not at 100 ppm), and tidal volume was increased at 100 ppm ammonia, but decreased at higher concentrations (Cole et al. 1977). Accidental exposures to concentrated aerosols of ammonium solutions, high concentrations of ammonia gas, or anhydrous ammonia fumes have resulted in nasopharyngeal and tracheal burns, airway obstruction and respiratory distress, and bronchiolar and alveolar edema (Burns et al. 1985; Close et al. 1980; Couturier et al. 1971; de la Hoz et al. 1996; George et al. 2000; Hatton et al. 1979; Heifer 1971; Kass et al. 1972; Kerstein et al. 2001; Latenser and Lucktong 2000; Leduc et al. 1992; Millea et al. 1989; Morgan 1997; O’Kane 1983; Price et al. 1983; Sobonya 1977; Taplin et al. 1976; Walton 1973; Weiser and Mackenroth 1989). Chronic occupational exposure (about 14 years) to low levels of airborne ammonia (12.5 ppm) had no significant effect on pulmonary function or odor sensitivity in a group of workers at a soda ash factory compared to a control group from the same factory that was not exposed to ammonia (Holness et al. 1989). An acute-duration inhalation MRL of 1.7 ppm was derived from the Verberk (1977) study, and a chronic inhalation MRL of 0.1 ppm was derived from the Holness et al. (1989) study; MRLs are presented in Table 3-1 and Figure 3-1 and are discussed in Section 2.3.

One human study with somewhat controlled exposure to ammonia showed that pulmonary function was not affected by low levels (25–100 ppm) of ammonia (Ferguson et al. 1977). Transient nose and throat

Table 3-1 Levels of Significant Exposure to Ammonia And Ammonium Compounds - Inhalation

Key to figure ^a	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form
					Less Serious (ppm)	Serious (ppm)	
ACUTE EXPOSURE							
Death							
1	Human	1 d 0.5 hr/d				5000 (rapidly fatal)	Henderson & Haggard 1927
2	Rat	1 d 15 min/d				17401 (LC50)	Prokop'eva et al. 1973
3	Rat	1 d 16 hr/d				1000 (LC50)	Weedon et al. 1940
4	Mouse	1 d 30 min/d				21430 (LC50)	Hilado et al. 1977
5	Mouse	1 d 1 hr/d				4230 (LC50)	Kapeghian et al. 1982
6	Mouse	1 d 60 min/d				11299 (LC50)	Prokop'eva et al. 1973
7	Mouse	1 d 16 hr/d				1000 (LC50)	Weedon et al. 1940

Table 3-1 Levels of Significant Exposure to Ammonia And Ammonium Compounds - Inhalation (continued)

Key to figure ^a	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form
					Less Serious (ppm)	Serious (ppm)	
8	Rabbit	1 d 1 hr/d				5025 (LC50)	Boyd et al. 1944
9	Cat	1 d 1 hr/d				5025 (LC50)	Boyd et al. 1944
10	Human	8-11 min	Resp	100 M	150 M (decreased minute volume; increased tidal volume)		Cole et al. 1977
11	Human	5 min	Resp	50	72 (nasal and throat irritation)		Industrial Bio-Test Laboratories, Inc. 1973
12	Human	10 min	Resp	30	50 (moderate nasal irritation)		MacEwen et al. 1970

Table 3-1 Levels of Significant Exposure to Ammonia And Ammonium Compounds - Inhalation (continued)

Key to figure ^a	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form
					Less Serious (ppm)	Serious (ppm)	
13	Human	1 d 30 min/d	Resp		500 M (nasal and throat irritation; increased minute volume and respiratory rate)		Silverman et al. 1949
			Cardio	500 M			
			Hemato	500 M			
			Ocular		500 M (lacrimation)		
14	Human	1 d 2 hr/d	Resp		50 ^b	(urge to cough; irritation to nose and throat)	Verberk 1977
			Ocular		50	(irritation to eyes)	
15	Rat (Sherman)	4 wk 24 hr/d	Ocular		100	(eye irritation)	Broderson et al. 1976

Table 3-1 Levels of Significant Exposure to Ammonia And Ammonium Compounds - Inhalation

(continued)

Key to figure ^a	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form
					Less Serious (ppm)	Serious (ppm)	
16	Rat (OFA)	1 wk 24 hr/d	Resp		500	(irritation)	Richard et al. 1978a
			Renal		500	(increased kidney weight)	
			Bd Wt		500	(body weight and food intake decreased 21%)	
17	Rat	7 d	Resp	714			Schaerdel et al. 1983
			Gastro	714			
			Hemato		15	(slight increase blood pO ₂)	
			Renal	714			
			Dermal	714			
			Other	714			

Table 3-1 Levels of Significant Exposure to Ammonia And Ammonium Compounds - Inhalation (continued)

Key to figure ^a	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form
					Less Serious (ppm)	Serious (ppm)	
18	Mouse (ICR)	1 d 1 hr/d	Resp		3440 M (dyspnea; nasal irritation)	4220 M (congestive intraalveolar hemorrhage, 24% increased relative lung weight)	Kapeghian et al. 1982
			Hepatic			3440 M (degenerative changes; increased relative liver weight)	
			Bd Wt	3440 M	4220 M (12% reduction in body weight)		
19	Dog (Beagle)	1 wk 5 d/wk 8 hr/d	Resp	218.6 M	1085.7 M (temporary dyspnea during first week of exposure)		Coon et al. 1970
20	Rabbit (New Zealand)	1 wk 5 d/wk 8 hr/d	Resp	218.6 M	1085.7 M (temporary dyspnea during first week of exposure)		Coon et al. 1970
			Ocular	218.6 M	1085.7 M (temporary lacrimation)		
21	Rabbit	1 d 1 hr/d	Resp			5000 (acute pulmonary edema)	Richard et al. 1978b
			Cardio		2500 (bradycardia)	5000 (hypertension, acidosis, EKG change)	

Table 3-1 Levels of Significant Exposure to Ammonia And Ammonium Compounds - Inhalation (continued)

Key to figure ^a	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form
					Less Serious (ppm)	Serious (ppm)	
22	Cat (Mongrel)	1 x 10 min (IT)	Resp		1000	(dyspnea; rhonchi; rales)	Dodd and Gross 1980
23	Pig (Belgian Landrace)	6 d	Resp	50 M	100 M	(decreased pulmonary vascular response to endotoxin challenge)	Gustin et al. 1994
			Cardio	50 M	100 M	(decreased pulmonary vascular response to endotoxin challenge)	
			Hemato	100 M			
			Endocr	100 M			
			Bd Wt	25 M	50 M	(3% weight loss)	
24	Pig (Duroc)	1-2 wk	Resp	10	50	(frequent coughing)	Stombaugh et al. 1969
			Dermal	10	50	(oral and nasal irritation)	
			Bd Wt	10	50	(reduced weight gain and food intake)	

Table 3-1 Levels of Significant Exposure to Ammonia And Ammonium Compounds - Inhalation

(continued)

Key to figure ^a	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form
					Less Serious (ppm)	Serious (ppm)	
Immuno/ Lymphoret							
25	Mouse	7 d 24 hr/d			500 M (decreased resistance to infection)		Richard et al. 1978a
26	Pig (Belgian Landrace)	6 d		50 M	100 M (decreased pulmonary response to endotoxin challenge)		Gustin et al. 1994
Neurological							
27	Rat	1 d 6 hr/d			100 (sensory irritation)		Tepper et al. 1985
28	Mouse	1 d 6 hr/d			100 (sensory irritation)		Tepper et al. 1985
INTERMEDIATE EXPOSURE							
Death							
29	Rat	90 d				641.6 (98% lethality)	Coon et al. 1970

Table 3-1 Levels of Significant Exposure to Ammonia And Ammonium Compounds - Inhalation

(continued)

Key to figure ^a	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form
					Less Serious (ppm)	Serious (ppm)	
Systemic							
30	Human	6 wk 5 d/wk 6 hr/d	Resp	25	50	(transient irritation of nose and throat)	Ferguson et al. 1977
			Cardio	100			
			Ocular	25	50	(transient eye irritation)	
31	Monkey (Squirrel monkey)	6 wk 5 hr/wk 8 hr/d	Resp		218.6 M	(focal pneumonitis)	Coon et al. 1970
			Cardio	1085.7 M			
			Hemato	1085.7 M			
			Hepatic	1085.7 M			
			Renal	1085.7 M			
			Dermal	1085.7 M			
32	Rat (Sherman)	5-10 wk 24 hr/d	Resp		250	(nasal lesions, epithelial hyperplasia)	Broderson et al. 1976

Table 3-1 Levels of Significant Exposure to Ammonia And Ammonium Compounds - Inhalation (continued)

Key to figure ^a	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form
					Less Serious (ppm)	Serious (ppm)	
33	Rat	6 wk 5 d/wk 8 hr/d	Resp	218.6	1085.7	(nonspecific inflammation)	Coon et al. 1970
			Cardio	1085.7			
			Hemato	1085.7			
			Hepatic	1085.7			
			Renal	1085.7			
			Ocular	1085.7			
34	Rat (Sprague- Dawley)	90 or 114 d	Resp	179.1	369.4	(mild nasal discharge in 25% of animals)	Coon et al. 1970
			Cardio	369.4		641.6 (myocardial fibrosis)	
			Hepatic	369.4	641.6	(fatty changes of liver plate cells)	
			Renal	369.4		641.6 (renal tubular calcification)	

Table 3-1 Levels of Significant Exposure to Ammonia And Ammonium Compounds - Inhalation (continued)

Key to figure ^a	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form
					Less Serious (ppm)	Serious (ppm)	
35	Gn Pig	6 wk 5 d/wk 8 hr/d	Resp	218.6	1085.7	(non specific inflammation)	Coon et al. 1970
			Cardio	1085.7			
			Hemato	1085.7			
			Hepatic	1085.7			
			Renal	1085.7			
			Dermal	1085.7			
36	Gn Pig (Hartley)	3 wk 24 hr/d	Resp	90			Targowski et al. 1984
			Hemato	90			
			Ocular	90			
			Bd Wt	90			

Table 3-1 Levels of Significant Exposure to Ammonia And Ammonium Compounds - Inhalation

(continued)

Key to figure ^a	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form
					Less Serious (ppm)	Serious (ppm)	
37	Gn Pig	18 wk 5 d/wk 6 hr/d	Resp	170			Weatherby 1952
			Cardio	170			
			Gastro	170			
			Hemato		170	(increased hemosiderin)	
			Hepatic		170	(congestion)	
			Renal		170	(congestion)	

Table 3-1 Levels of Significant Exposure to Ammonia And Ammonium Compounds - Inhalation

(continued)

Key to figure ^a	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form
					Less Serious (ppm)	Serious (ppm)	
38	Rabbit	114 d	Resp	57			Coon et al. 1970
			Cardio	57			
			Hemato	57			
			Hepatic	57			
			Renal	57			
			Dermal	57			
39	Pig (NS)	4 wks	Resp		100	(excessive nasal secretion, coughing, tracheal inflammation)	Drummond et al. 1980
			Ocular		50	(excessive lacrimation)	
			Bd Wt	50	100	(18.6% reduction in final body weight)	
40	Rat (Sherman and Fischer)	4 wk 24 hr/d	Immuno/ Lymphoret		25	(increased severity of infection by mycoplasma)	Broderson et al. 1976

Table 3-1 Levels of Significant Exposure to Ammonia And Ammonium Compounds - Inhalation (continued)

Key to figure ^a	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form
					Less Serious (ppm)	Serious (ppm)	
41	Rat	3 wk 24 hr/d			500 M (reduced resistance to infection)		Richard et al. 1978a
42	Gn Pig (Hartley)	3 wk 24 hr/d			90 (significantly reduced delayed-type response to tuberculin)		Targowski et al. 1984
				50			
43	Pig (NS)	31-45 d 24 hr/d			100 (decreased serum concentration of gamma globulin)		Neumann et al. 1987
44	Neurological Monkey (Squirrel monkey)	6 wk 5 hr/wk 8 hr/d		1085.7 M			Coon et al. 1970
45	Gn Pig	6 wk 5 d/wk 8 hr/d		1085.7			Coon et al. 1970

Table 3-1 Levels of Significant Exposure to Ammonia And Ammonium Compounds - Inhalation (continued)

Key to figure ^a	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form
					Less Serious (ppm)	Serious (ppm)	
46	Pig	4 wks		50	100 (lethargy)		Drummond et al. 1980
CHRONIC EXPOSURE							
Systemic							
47	Human	12.2 yr 5 d/wk 8 hr/d	Resp	9.2 ^c			Holness et al. 1989
			Ocular	9.2			

a The number corresponds to entries in Figure 3-1.

b Used to derived an acute-duration inhalation MRL of 1.7 ppm; the MRL was derived by dividing the LOAEL of 50 ppm by an uncertainty factor of 30 (10 for variation in sensitivity among humans and 3 for use of a minimal LOAEL).

c Used to derive a chronic-duration inhalation MRL of 0.1 ppm; the MRL was derived by adjusting the NOAEL of 9.2 ppm for continuous exposure (9.2 x 8/24 hours x 5/7 days) and dividing by an uncertainty factor of 30 (10 for the protection of sensitive individuals, and 3 for the lack of reproductive and developmental studies).

Bd Wt = body weight; Cardio = cardiovascular; d = day(s); Endocr = endocrine; F = female; gastro = gastrointestinal; hemato = hematological; hr = hour(s); LOAEL = lowest-observed-adverse-effect level; M = male; metab = metabolic; min = minute; mo = month(s); NOAEL = no-observed-adverse-effect level; NS = not specified; Resp = respiratory; wk = week(s)

AMMONIA

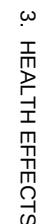
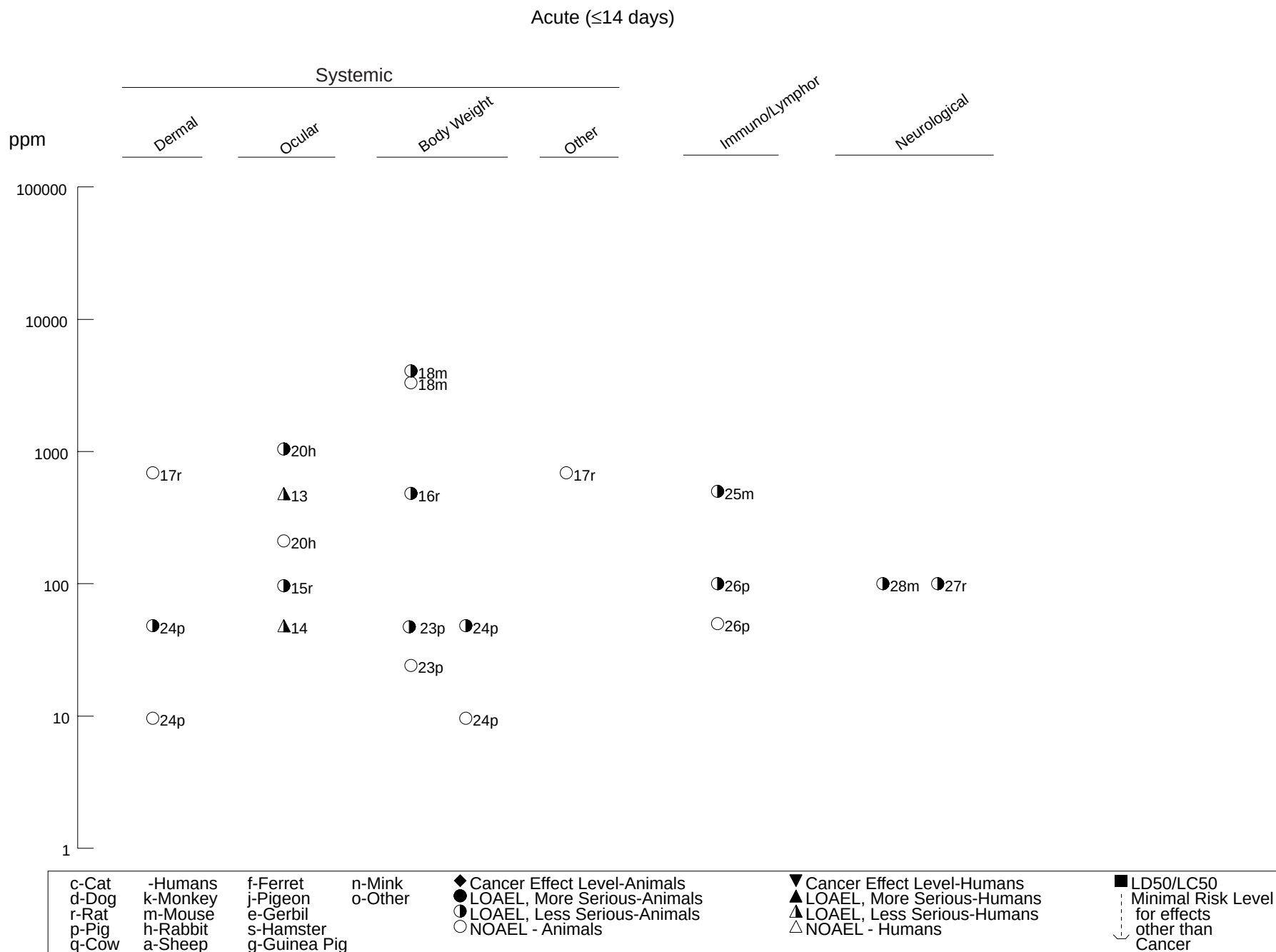


Figure 3-1. Levels of Significant Exposure to Ammonia And Ammonium Compounds - Inhalation (*Continued*)

AMMONIA

3. HEALTH EFFECTS



Figure 3-1. Levels of Significant Exposure to Ammonia And Ammonium Compounds - Inhalation (*Continued*)

Intermediate (15-364 days)

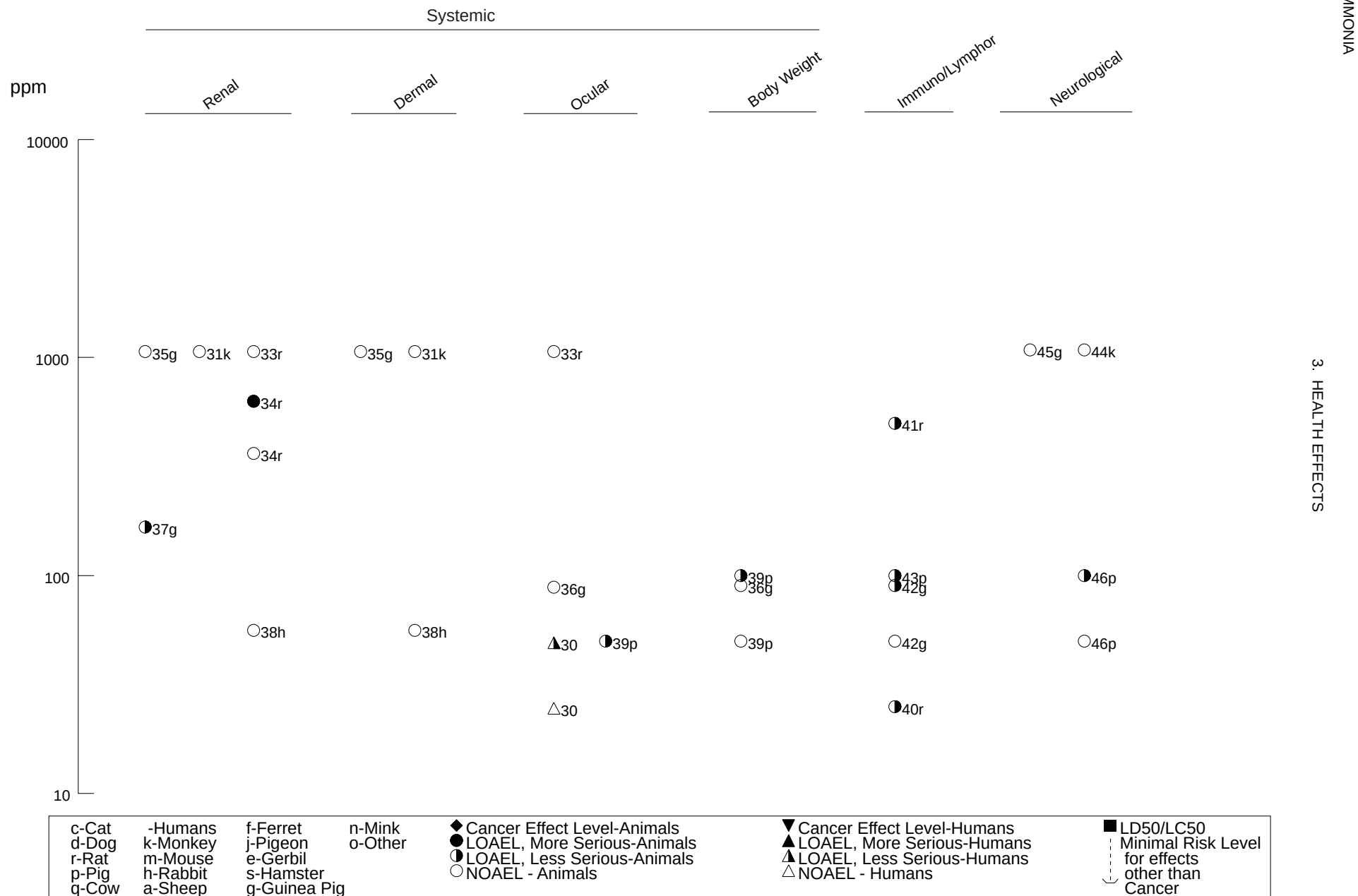
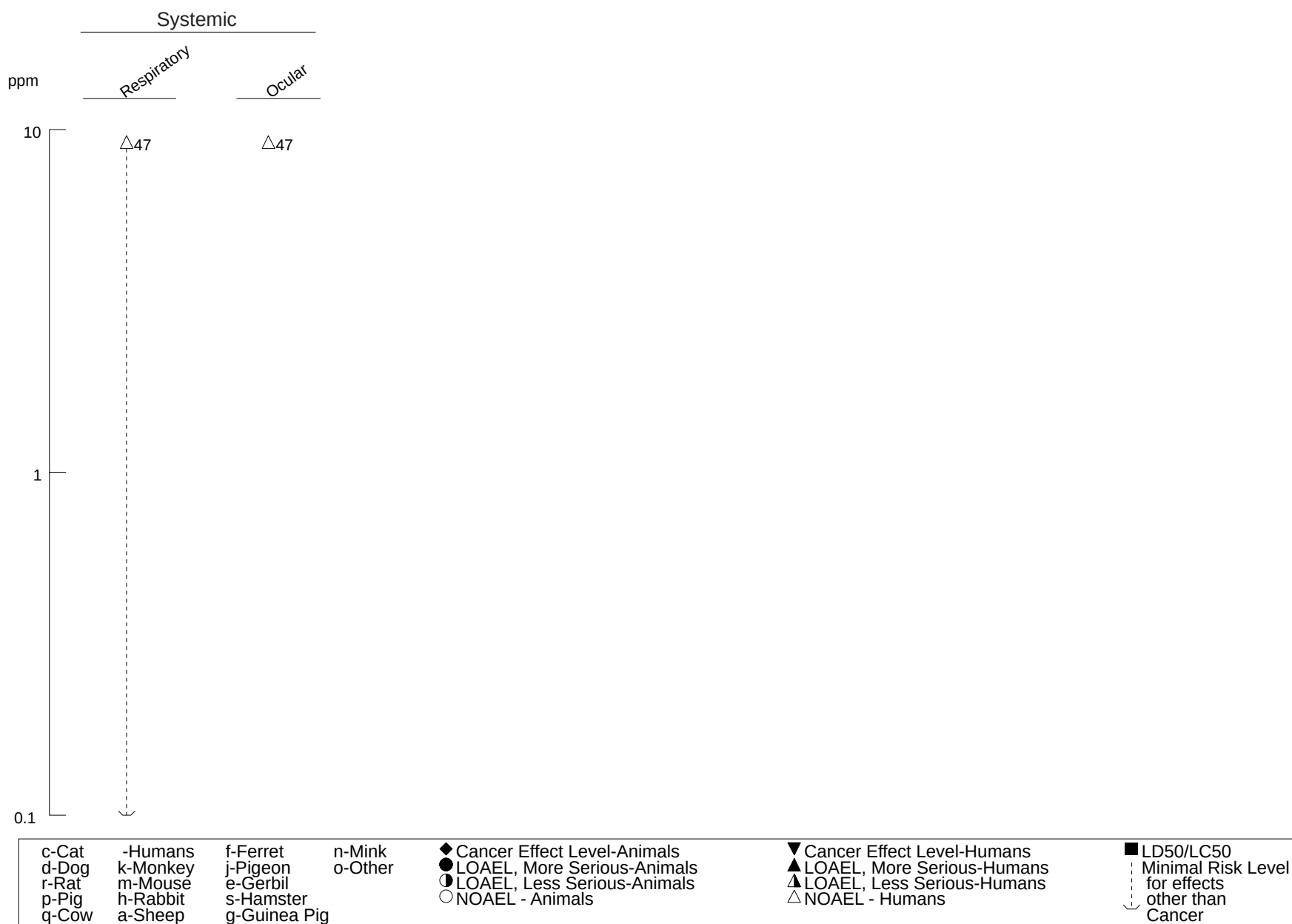


Figure 3-1. Levels of Significant Exposure to Ammonia And Ammonium Compounds- Inhalation (*Continued*)Chronic (≥ 365 days)

3. HEALTH EFFECTS

irritation was noted the first week of exposure in male and female volunteers exposed to 50 ppm, but not 25 ppm ammonia 5 days/week, for 6 weeks. No significant differences were noted between exposed and control groups for pulmonary function tests, physical examinations, or performance of normal job duties (Ferguson et al. 1977).

A number of occupational cohort studies that examined farmers who worked in enclosed livestock buildings have been conducted. These studies all included measurements of ammonia in the livestock confinement buildings, as well as measurements of one or more of the following: total dust, respirable dust, carbon dioxide, total endotoxins, respirable endotoxins, fungi, bacteria, and molds (Choudat et al. 1994; Cormier et al. 2000; Donham et al. 1995, 2000; Heederik et al. 1990, 1991; Melbostad and Eduard 2001; Reynolds et al. 1996; Vogelzang et al. 1997, 2000). Of the pollutants measured, ammonia and dust were most frequently associated with respiratory effects, many of which were temporary and disappeared with cessation of exposure. Ammonia levels ranged from 2.3 to 20.7 ppm and total dust levels from 0.04 to 5.64 mg/m³. Most of these studies reported an association between exposure to pollutants, including ammonia, in livestock confinement buildings and an increase in respiratory symptoms (such as bronchial reactivity/hyperresponsiveness, inflammation, cough, wheezing, or shortness of breath) and/or a decrease in pulmonary function (such as forced expiratory volume in the first second [FEV_{1.0}], maximum expiratory flow rates [MEF₅₀ and MEF₇₅], and maximal mid-expiratory flow rate [MMEF]) (Choudat et al. 1994; Cormier et al. 2000; Donham et al. 1995, 2000; Heederik et al. 1990; Reynolds et al. 1996; Vogelzang et al. 1997, 2000). One study, however, reported correlations only between total dust, fungal spore, and endotoxin mean exposure levels and task-specific prevalences (Melbostad and Eduard 2001). Another study reported no significant correlations between lung function or chronic respiratory symptoms and dust or ammonia levels, but suggested that endotoxins and bacteria levels may play a role (Heederik et al. 1991). Most studies adjusted for confounding factors, such as smoking and number of years worked on a farm, in their statistical analyses. All of the studies concluded that prevalence of respiratory symptoms of some type was higher in the farmer cohort than in the respective control group. It is not clear from these studies what the contribution of ammonia is to the respiratory changes, but the cumulative data indicate that ammonia may contribute to transient respiratory distress in farmers working in enclosed livestock facilities.

A cross-sectional study of male workers at two fertilizer factories in Saudi Arabia showed a significant association between exposure to ammonia gas and respiratory symptoms including bronchial asthma (Ballal et al. 1998). Workers in factory one were exposed to air ammonia levels of 2.82–183.86 ppm (2.0–130.4 mg/m³), and workers in factory two were exposed to 0.03–9.87 ppm (0.02–7.0 mg/m³).

3. HEALTH EFFECTS

However, continuous exposure levels for workers could not be calculated because the number of days worked per week was not provided by the study authors. Logistic regression analysis showed that ammonia concentration was significantly related to cough, phlegm, wheezing (with and without shortness of breath), and asthma, whereas smoking was only a factor for wheezing and phlegm. Additionally, those workers exposed to ammonia levels above the American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit value (TLV) of 25.4 ppm (18 mg/m³) had significantly higher relative risks for cough, phlegm, wheezing, dyspnea, and asthma than workers exposed to levels below the TLV. Incidence of wheezing was also elevated in workers exposed to ammonia levels below the TLV. Cumulative ammonia concentration (CAC) of >50 mg/m³-years also showed a significantly increased relative risk for all of the above symptoms compared to workers with a CAC of ≤50 mg/m³-years. None of the relative risks for workers in the second factory (ammonia levels <25.4 ppm) were significant.

Other occupational studies also evaluated the effects of ammonia exposure and pulmonary function. Firefighters who reported exposure to ammonia while working had a rate of decline of FEV₁ of 1.7 times that of nonexposed firefighters over a period of 6–10 years (Tepper et al. 1991).

Children (8–9 years old) who attended two schools in the vicinity of a fertilizer plant had higher incidences of acute respiratory diseases than children of the same age who attended a school 20 kilometers away (Gomzi and Šarić 1997). Incidence was related to levels of measured pollutants (ammonia, hydrogen fluoride, nitrogen dioxide, total suspended particulate matter, and smoke) in the inside and outside air. Forced expiratory volumes were not statistically different between the three schools. These results indicate that exposure to low levels of ammonia (0.04–0.23 ppm) and other air-borne pollutants may not cause functional respiratory deficits, but may lower the resistance to respiratory pathogens in children. These effects may be due in part or in whole to toxicants other than ammonia, such as nitrogen dioxide.

Case reports of individuals acutely exposed to anhydrous or aqueous ammonia reported respiratory effects including nasal irritation; epiglottic, laryngeal, pharyngeal, tracheal, and pulmonary edema; dyspnea; wheezing; coughing; rhonchi; pneumonia; and cardio-respiratory arrest (de la Hoz et al. 1996; George et al. 2000; Kerstein et al. 2001; Latenser and Lucktong 2000; Leduc et al. 1992; Lee et al. 1993; Millea et al. 1989; Morgan 1997; Prudhomme et al. 1998; Weiser and Mackenroth 1989). de la Hoz et al. (1996) described the initial and residual effects of three adult males who had been acutely exposed to ammonia

3. HEALTH EFFECTS

gas in separate incidents. All three men complained of burning eyes, throat, and skin, cough, and wheezing, and all had been treated at hospitals shortly after exposure. Follow-up examinations 2–2½ years later showed persistent dyspnea, cough, and wheezing at rest and/or on exertion, which is consistent with restrictive lung disease secondary to acute ammonia inhalation injury. Another man exposed to anhydrous ammonia gas experienced pharyngeal and laryngeal edema, dyspnea, chest tightness, copious bronchial secretions, and wheezing (Leduc et al. 1992); 12 years postexposure, he continued to have cough, exertional dyspnea, and recurrent bronchial infections. Similar cases were reported by Kerstein et al. (2001) and Latenser and Lucktong (2000); no follow-up reports were available.

A more severe exposure was reported by George et al. (2000). An adult male was found unconscious next to a burst pipe carrying liquefied ammonia. He had ocular and cutaneous burns and severe difficulty breathing. Over the next 27 days, he suffered many medical setbacks, including attacks of bradycardia, a complete circulatory collapse from which he was resuscitated, and finally, a fatal cardiac arrest from severe bleeding. Another similar severe exposure resulted in the death of the patient 13 days postexposure due to treatment-resistant bronchopneumonia; histological examination showed massive, hemorrhagic pulmonary edema, regions of emphysema, and edema of the epiglottis and glottis (Weiser and Mackenroth 1989).

Reports of apparently rare effects have been found. A man exposed occupationally for 5 months to low levels of ammonia gas (8–15 ppm) from ammonia-containing silver polish developed asthma-like symptoms (Lee et al. 1993). Separate specific bronchial provocation tests to the silver polish and to 12 ppm ammonia produced asthmatic reactions, implicating the ammonia in the silver polish as the cause. Another study reported hyposmia (loss of the sense of smell) in a man following acute inhalation exposure (for several hours) to an unknown concentration of ammonia gas; the hyposmia had not resolved 30 months after exposure (Prudhomme et al. 1998).

Studies in animals have demonstrated similar dose-effect and duration-effect patterns for the respiratory tract. Acute exposures (1 hour to 1 week) to low concentrations of ammonia ($\leq 1,000$ ppm) irritate the upper respiratory tract whereas exposures (3 hours to 2 weeks) to high concentrations ($\geq 4,000$ ppm) result in severe damage to the upper and lower respiratory tract and alveolar capillaries (Coon et al. 1970; Kapeghian et al. 1982; Mayan and Merilan 1972; Richard et al. 1978a, 1978b; Schaerdel et al. 1983; Stombaugh et al. 1969). Prolonged or repeated exposures to lower levels (≥ 150 ppm) produce inflammation and lesions of the upper respiratory tract (Broderson et al. 1976; Coon et al. 1970).

3. HEALTH EFFECTS

Clinical and histological effects have been seen in the lungs of animals following exposure to ammonia gas (Dodd and Gross 1980; Gaafar et al. 1992; Sjöblom et al. 1999). Cats exposed to 1,000 ppm ammonia gas for 10 minutes and observed for up to 35 days showed a biphasic course of respiratory pathology (Dodd and Gross 1980). Effects seen at 24 hours post-exposure included severe dyspnea, anorexia, and dehydration, with rhonchi and coarse rales evident upon auscultation. Microscopy of lung samples on day 1 showed necrotizing bronchitis in the large conducting airways, and necrosis and sloughing of the epithelium and acute inflammatory reaction in the bronchi. On day 7, the mucosal lesions had resolved, but on day 35, varying degrees of bronchitis and early bronchopneumonia with areas of bulbous emphysema were seen. Gross pathology revealed varying degrees of congestion, hemorrhage, edema, interstitial emphysema, and collapse of the lungs at all time points. Pulmonary resistance was increased throughout the study (Dodd and Gross 1980). Swiss mice exposed to 909 ppm, but not 303 ppm, ammonia gas 6 hours/day, 5 days/week for 4–14 days had histological lesions in the respiratory epithelium in the nasal cavity (Zissu 1995); no lesions were observed in the trachea or lungs. Nasal mucosa was adversely affected in adult male mice exposed to vapor of 12% ammonia solution for 15 minutes/day, 6 days/week for 4, 5, 6, 7, or 8 weeks (Gaafar et al. 1992). Histological changes progressed from weeks 4–8 from crowding of cells forming crypts and irregular arrangements to epithelial hyperplasia, patches of squamous metaplasia, loss of cilia, and dysplasia of the nasal epithelium. One animal that had loss of polarity of the epithelium, hyperchromatism, and mitotic figures with an intact basement membrane also had a carcinoma *in situ* in one nostril. At week 8, one mouse had an invasive adenocarcinoma of the nasal mucosa. Histochemical results were also abnormal. The levels and cell locations of succinic dehydrogenase, acid phosphatase, alkaline phosphatase, and nonspecific esterase activities were altered, indicating altered cell metabolism and energy production, cell injury, proliferation, and possibly chronic inflammation and neoplastic transformation (Gaafar et al. 1992).

Anesthetized, mechanically ventilated rabbits exposed to high levels of nebulized ammonia (2 mL of 23–27% ammonia solution; estimated by the study authors as peak ammonia concentrations of 35,000–39,000 ppm) for 4 minutes had a decrease in blood oxygen saturation and an increase in airway pressure (a measure of changes in airway resistance) (Sjöblom et al. 1999). Arterial oxygen tension decreased from 23.3 (± 3.6) to 11.0 (± 3.6) kPa and peak airway pressure increased from 13 (± 2) to 17 (± 2) cm H₂O. At baseline and 5 and 15 minutes after ammonia administration, measurements were taken via a catheter in the left auricular artery, which monitored pressure and sampled for arterial blood gases, and via transducers in the ventilator. Thirty and 150 minutes after ammonia exposure, rabbits received inhalation therapy of either 0.5 mg budesonide (a steroid) or a placebo, and airway pressure, hemodynamics, and gas exchange were measured every 30 minutes for 6 hours. Slight, gradual

3. HEALTH EFFECTS

improvement of blood gas parameters was noted over the 6-hour observation period in all rabbits, with or without steroid treatment; however, no parameters approached normal during that time period.

Other studies examined pigs in normal swine production facilities (Donham 1991) or in environmentally regulated enclosures (Diekman et al. 1993; Gustin et al. 1994; Urbain et al. 1994). Donham (1991) investigated the correlation of housing air environment (in the finishing barn) to swine diseases and productivity over 12 months on 28 swine farms. Total dust, respirable dust, endotoxin activity of the dust, and hydrogen sulfide levels were determined, and area dust and microbial counts were monitored at 1.2 meters above the floor (the human breathing zone). Ammonia and carbon dioxide levels were determined 1.2 meters and 20 cm (swine breathing zone) above the floor. The average ammonia concentration in the human breathing zone for all farms was 9.1 ppm; the mean concentration in the swine breathing zone was 14.5 ppm. The mean concentrations of environmental contaminants were calculated for the most productive farrowing operations and the least productive ones and compared with lower production variables (Donham 1991). Ammonia concentration was related to number of pigs weaned per litter, and total and respirable dust concentrations were related to prolonged age to reach a weight of 25 kg. Another comparison involved the stratification of the finishing farms into quartiles according to percentage of pigs with specified disease conditions and comparison of mean concentrations of various environmental contaminants for each farm in each strata (Donham 1991). Levels of ammonia (in the animal breathing zone) greater than 25, 29, and 23 ppm were associated with buildings in which pneumonia, pleuritis, and arthritis, respectively, were greater than the mean value for the group. Overall, respirable dust ($>0.8 \text{ mg/m}^3$), ammonia ($>23 \text{ ppm}$ in the animal breathing zone), and carbon dioxide ($>2,000 \text{ ppm}$) levels were most often associated with increased disease. Possible study shortcomings noted by the study author were that pre-existing conditions could have been present in the pigs (before they entered the finishing barns) and that nasal turbinates were not routinely examined for abnormalities. These data suggest that ammonia may contribute to respiratory and other pathological conditions in pigs raised in crowded, enclosed conditions, but the exact contribution of ammonia is difficult to assess.

The lungs of young pigs exposed continuously to 0, 25, 50, or 100 ppm ammonia gas for 6 days in air-pollutant exposure chambers were removed, ventilated, and perfused, and the pulmonary vascular hemodynamics and permeability and the endotoxin-induced vascular response were assessed (Gustin et al. 1994). In lungs from pigs exposed to 100 ppm, but not 25 or 50 ppm ammonia, the endotoxin-induced vascular response seen in lungs from control pigs was abolished. The study authors suggested that this is due to a modification of the balance between vasodilators (such as cyclooxygenase products and platelet activating factor) and vasoconstrictors (such as prostacyclin). Since vasoconstriction, as induced by

3. HEALTH EFFECTS

endotoxin, may serve as a protective mechanism in the lungs and attenuate edema formation, the effective abolishment of this effect by ammonia may be detrimental.

Young pigs exposed continuously to ammonia vapors (0, 25, 50, or 100 ppm) for 6 days in air-pollutant exposure chambers had increased numbers of neutrophils in nasal lavage fluid in all exposure groups (Urbain et al. 1994) and increased porcine serum albumin at 100 ppm.

Not all studies have shown adverse respiratory effects from intermediate exposure to ammonia vapors. Groups of gilts (virgin female pigs) were raised from the age of 2–4.5 months in a conventional grower unit where they were naturally exposed to mycoplasmal and bacterial pathogens that cause enzootic pneumonia and atrophic rhinitis (Diekman et al. 1993). The pigs were then transferred to environmentally regulated rooms, where they were exposed continuously to low (mean 7 ppm) or moderate (mean 35 ppm) levels of ammonia for 6 weeks. No statistically significant differences were seen in the percent of lung tissue containing lesions or in snout grade (Diekman et al. 1993). Ninety-five percent of all gilts had lung lesions, with a wide range of degree of severity. Snouts were graded at the level of the second deciduous premolar as having normal turbinates (grade of 0), slight to moderate degeneration (grade of 1–3), or severe degeneration to complete loss of turbinates (grade of 4 or 5). Some of the gilts were continuously exposed through puberty and breeding (around 205 days of age) and the lungs and turbinates were examined at 30 days of gestation. No statistically significant differences were observed in percent of lung tissue containing lesions or in snout grade (Diekman et al. 1993).

A number of cattle were acutely exposed to anhydrous ammonia when a pipeline running through their pasture ruptured and leaked 1,800 barrels of ammonia in a short period of time (Morgan 1997). The ammonia combined with moisture and formed a white cloud (ammonia aerosol), which drifted south across two additional fields containing cattle. In the field where the rupture occurred, four head of cattle were found dead and two others were euthanized because of blindness and respiratory distress. Cattle in the adjacent pasture had runny eyes and noses and were coughing and wheezing. Eight days after the pipeline rupture, the cloud of ammonia aerosol, which had apparently settled in a low-lying protected area, blew back up the valley and exposed the remaining cattle again and also exposed a horse in the same pasture. All animals had respiratory distress, elevated body temperatures, and one cow and the horse had swollen tongues and enlarged lymph glands. All cattle were given antibiotics and some were treated specifically for respiratory problems. No measurements or estimations of ammonia concentrations were provided and no follow-up examinations were available to assess long-term effects from the exposures.

3. HEALTH EFFECTS

All reliable LOAELs and highest NOAELs are presented in Table 3-1 and Figure 3-1.

Cardiovascular Effects. Acute exposure to highly concentrated aerosols of ammonium compounds may cause elevated pulse and blood pressure, bradycardia, and cardiac arrest in humans (George et al. 2000; Hatton et al. 1979; Montague and Macneil 1980; White 1971). These effects did not occur after acute exposure to 500 ppm ammonia or repeated exposure to 100 ppm ammonia (Ferguson et al. 1977; Silverman et al. 1949).

Cardiovascular changes that may be analogous to those observed in humans have been observed in rabbits exposed to high concentrations of ammonia (Richard et al. 1978b). Bradycardia was seen at 2,500 ppm, and hypertension and cardiac arrhythmias leading to cardiovascular collapse followed acute exposures to concentrations exceeding 5,000 ppm. Pathological correlates for these effects have not been demonstrated. Atrophy of pericardial fat has been observed in mice exposed to 4,000 ppm ammonia (Kapeghian et al. 1982). Myocardial fibrosis has been observed in rats, guinea pigs, rabbits, dogs, and monkeys after prolonged (90 days) continuous exposure to 653 ppm (Coon et al. 1970). The contribution of these lesions to the morbidity and mortality of affected animals has not been determined.

Exposure of pigs *in vivo* to up to 100 ppm ammonia for 6 days did not alter the baseline values of any hemodynamic or permeability parameters (arterial, pre- or postcapillary, or venous blood flow resistance, or total pulmonary blood flow resistance), but did eliminate the hemodynamic response to *Escherichia coli* endotoxins in the lungs (Gustin et al. 1994). This may affect the ability of the lungs to resist bacterial infection. The pulmonary blood flow resistance measurements were taken *in vitro* in ventilated and perfused lungs from pigs exposed to ammonia *in vivo* (Gustin et al. 1994). Reliable LOAELs and highest NOAELs for cardiovascular effects are presented in Table 3-1 and Figure 3-1.

Gastrointestinal Effects. Exposure to highly concentrated aerosols of ammonium compounds can produce burns of the lips, oral cavity, and pharynx, along with edema of these areas (Hatton et al. 1979; Kass et al. 1972; Leduc et al. 1992; Levy et al. 1964; Price et al. 1983; Stroud 1981; Ward et al. 1983; Yang et al. 1987). Gastrointestinal effects of ammonia in animals have not been reported. As shown in Table 3-1, pathological changes in the gastrointestinal tract were not observed in guinea pigs exposed repeatedly to 170 ppm ammonia (Weatherby 1952).

3. HEALTH EFFECTS

Hematological Effects. Cyanosis, elevated white blood cell count, and pulmonary artery thrombosis have been observed in humans exposed to highly concentrated aerosols of ammonium compounds (Sobonya 1977; Taplin et al. 1976; Voisin et al. 1970; Ward et al. 1983; White 1971).

Standard hematological measurements, including blood hemoglobin and differential cell counts, have been reported for a few animal species. As shown in Table 3-1 and Figure 3-1, acute hematological effects of ammonia have not been demonstrated (Doig and Willoughby 1971; Gustin et al. 1994). Pigs exposed to up to 100 ppm ammonia for 6 days had no statistically significant differences from controls in total leukocytes or percent lymphocytes, neutrophils, or eosinophils (Gustin et al. 1994). Repeated exposure to 1,100 ppm had no effect on hematological parameters in guinea pigs, rats, and rabbits (Coon et al. 1970). Weatherby (1952) reported increased concentrations of hemosiderin in the spleen of guinea pigs exposed to 170 ppm ammonia for 18 weeks. This suggests the possibility of increased turnover of red blood cells; however, this has not been corroborated.

Musculoskeletal Effects. Spasms of muscles of the extremities have resulted from an acute exposure of a man to anhydrous ammonia gas (White 1971), but this was probably caused by an effect of ammonia on the nervous system.

Hepatic Effects. Hemorrhagic necrosis of the liver was observed in an individual exposed to a lethal concentration of ammonia gas and liquid for a short period of time (<45 minutes) (Heifer 1971). No other cases of hepatic effects have been reported in humans. Hepatic effects are usually not seen in animals exposed to ammonia gas. As shown in Table 3-1, liver necrosis has been observed following acute lethal exposure of mice to 3,440 ppm ammonia for 1 hour (Kapeghian et al. 1982). Fatty changes of liver plate cells were seen in rats following continuous long-term exposure to 642 ppm ammonia for 90 days, but no such changes were seen in rats, squirrel monkeys, and guinea pigs exposed to 1,086 ppm ammonia 8 hours/day, 5 days/week for 6 weeks (Coon et al. 1970).

Renal Effects. No studies were located regarding renal effects in humans after inhalation exposure to ammonia. In animals, renal effects do not appear to be an important feature of the toxicity of inhaled ammonia. Effects reported have not been corroborated or cannot be interpreted. Mild abnormalities in the renal tubules have been described in guinea pigs exposed to 170 ppm for 12 weeks, 5 days/week, 6 hours/day; however, renal effects at this relatively low level have not been corroborated (Weatherby 1952). Exposure to more than 6 times this concentration for 6 weeks, 5 days/week, 8 hours/day did not

3. HEALTH EFFECTS

result in pathological changes to the kidney (Coon et al. 1970). Renal tubular calcification (severity not reported) has been reported in rats continuously exposed to near lethal levels (Coon et al. 1970).

Endocrine Effects. Adrenaline levels in urine, 17-oxycorticosteroids in the urine, and 11-oxycorticosteroid levels in blood were increased in humans exposed to 3.0 ppm ammonia for 37 days (Kalandarov et al. 1984). Exposure to 7.2 ppm for 17 days also increased adrenaline levels in urine and 17-oxycorticosteroids in the urine, and increased free, but not total, 11-oxycorticosteroid levels in blood (Kalandarov et al. 1984). Experimental details were lacking in this study; additionally, no clinical or histological data were provided for this or other end points in this study and no supporting data are available in the literature. Therefore, the significance of these effects is unclear. Exposure of pigs to up to 100 ppm ammonia for 6 days did not significantly alter the plasma cortisol concentration (Gustin et al. 1994). No statistically significant difference was seen in adrenal gland weight of female pigs exposed to about 35 ppm ammonia for 6 weeks or for 6 weeks plus through day 30 of gestation compared to pigs exposed for similar time frames to about 7 ppm ammonia (Diekman et al. 1993). No unexposed controls were included in that study. The endocrine system does not appear to be a primary target of inhaled ammonia.

Dermal Effects. Ammonia gas and aerosols of ammonium compounds derived from anhydrous ammonia are dermal irritants in humans and animals. These effects are described in the discussion of dermal effects associated with dermal exposure (Section 3.2.3.2).

Ocular Effects. Ammonia gas and aerosols of ammonium compounds derived from anhydrous ammonia are ocular irritants in humans and animals. These effects are described in the discussion of ocular effects associated with dermal exposure (Section 3.2.3.2).

Body Weight Effects. Reduced body weight has been observed in rats exposed via inhalation to 500 ppm (Richard et al. 1978a) and in pigs exposed to 50 ppm or more ammonia for 6 days (Gustin et al. 1994; Urbain et al. 1994). Pigs gained less weight and showed decreased food consumption when exposed to 100 ppm ammonia for 4 or 5 weeks (Drummond et al. 1980; Stombaugh et al. 1969). Female pigs exposed to about 35 ppm for 6 weeks gained less weight than those exposed to only about 7 ppm (Diekman et al. 1993). However, females that were continuously exposed to about 7 or 35 ppm ammonia from 6 weeks before breeding until day 30 of gestation had no statistically significant difference in body weight (Diekman et al. 1993); however, no controls were included in this study.

3. HEALTH EFFECTS

3.2.1.3 Immunological and Lymphoreticular Effects

Several case reports describe occupational asthma that developed due to exposure to aerosols that contained ammonium compounds (Ballal et al. 1998; Lee et al. 1993; Weir et al. 1989).

Secondary infections often complicate the clinical outcome of burns and respiratory lesions related to exposure to highly concentrated aerosols derived from anhydrous ammonia (Sobonya 1977; Taplin et al. 1976). However, there is no evidence that the decreased immunological resistance represents a primary impairment of the immune system in humans following exposure to ammonia. Nevertheless, as shown in Table 3-1 and Figure 3-1, studies in animals have shown that acute and long-term exposure to ammonia can decrease the resistance to bacterial infection and decrease immune response to infection. A significant increase in mortality was observed in mice exposed to ammonia for 168 hours followed by exposure to the LD₅₀ of *Pasteurella multocida* (Richard et al. 1978a). Exposure of rats to ammonia at ≥ 25 ppm for 4–6 weeks following inoculation with *Mycoplasma pulmonis* intranasally significantly increased the severity of respiratory signs characteristic of murine respiratory mycoplasmosis (Broderson et al. 1976). Guinea pigs exposed to 90 ppm ammonia for 3 weeks developed a significant decrease in the cell-mediated immune response to challenge with a derivative of tuberculin (Targowski et al. 1984). Furthermore, the response of blood and bronchial lymphocytes to mitogens (phytohemagglutinin, concanavalin A, purified protein derivative of tuberculin) was markedly reduced. The hemodynamic response (increased total pulmonary blood flow resistance) to *E. coli* endotoxins in the lungs of pigs was eliminated by exposure to up to 100 ppm ammonia for 6 days, which may affect the ability of the lungs to resist bacterial infection (Gustin et al. 1994). Also, a reduction in gamma globulin concentration was reported in pigs exposed to 100 ppm ammonia for 31–45 days (Neumann et al. 1987).

3.2.1.4 Neurological Effects

Case reports of accident victims exposed to highly concentrated aerosols derived from anhydrous ammonia describe blurred vision, diffuse nonspecific encephalopathy, loss of consciousness, muscle weakness, and decreased deep tendon reflexes (George et al. 2000; Hatton et al. 1979; Latenser and Lucktong 2000; White 1971). Acute exposure to low levels of ammonia (100 ppm) has been shown to depress free-access wheel running behavior in rodents (Tepper et al. 1985). No overt symptoms of neurological disorders were reported in guinea pigs or monkeys that were exposed to up to 1,105 ppm ammonia for 6 weeks (Coon et al. 1970). Exposure of the nasal mucosa to ammonia-saturated air elicited vasodilatation and corresponding increased blood flow and reflex hypertension in the lower lip of cats

3. HEALTH EFFECTS

(Izumi and Karita 1993). Stimulation of the nasal mucosa by chemical irritants has been shown to elicit changes in the respiratory system, such as apnea, laryngeal spasm, and bronchoconstriction, and in the cardiovascular system, such as bradycardia and variable blood pressure changes (Izumi and Karita 1993). Brain glutamine levels have also been shown to increase in rats that inhaled 25 or 300 ppm ammonia vapor for 6 hours/day for 5 days, which is likely a result of ammonia metabolism by the astrocytic glutamate-glutamine cycle (Manninen and Savolainen 1989; Manninen et al. 1988).

3.2.1.5 Reproductive Effects

No studies were located regarding reproductive effects in humans after inhalation exposure to ammonia. No statistically significant differences were noted in ovarian or uterine weights of pigs exposed to about 7 or 35 ppm ammonia for 6 weeks (Diekman et al. 1993). Female pigs that were continuously exposed to about 35 ppm ammonia from 6 weeks before breeding until day 30 of gestation had no statistically significant differences in age at puberty, number of live fetuses, or fetus-to-corpus luteum ratio compared to pigs exposed to only about 7 ppm (Diekman et al. 1993). No unexposed controls were included in that study.

3.2.1.6 Developmental Effects

No information was located regarding developmental effects of ammonia in humans following inhalation exposure. No statistically significant difference in fetal length was evident at 30 days of gestation in offspring of pig dams that were continuously exposed to about 7 or 35 ppm ammonia from 6 weeks before breeding until day 30 of gestation (Diekman et al. 1993).

3.2.1.7 Cancer

Carcinogenic potential of ammonia by the inhalation route has not been assessed in humans or animals. One case report was found of an individual who developed epidermal carcinoma of the nasal septum 6 months after being badly burned by accidental contact with a refrigeration ammonia-oil mixture (Shimkin et al. 1954). However, the role of ammonia is impossible to ascertain and no conclusion can be drawn from this study. Shimkin et al. (1954) indicated that “no single case can prove a general principle, and it is only by the publication of additional reports of similar cases that enough data can become available for critical analysis.” No other such reports were located, although other cases of inhalation

3. HEALTH EFFECTS

exposure to ammonia from spills have been followed for more than 6 months after exposure. One of 10 adult male mice exposed to ammonia gas for 15 minutes/day 6 days/week for 8 weeks had mitotic figures with an intact basement membrane and a carcinoma *in situ* in one nostril and one mouse had an invasive adenocarcinoma of the nasal mucosa (Gaafar et al. 1992). Again, there is no conclusive evidence that ammonia played a role in the induction of the carcinoma. Gaafar et al. (1992) provided an alternate explanation by stating that “prolonged exposure to ammonia may interfere with the normal protective reflexes of the respiratory nasal mucosa resulting in the accumulation of particulate matter initiating or promoting a neoplastic process.” However, the plausibility of tumor formation in only 8 weeks by a weak carcinogen such as particulate matter is debatable.

3.2.2 Oral Exposure

As discussed in Chapter 4, ammonia in aqueous solution exists in equilibrium with ammonium hydroxide, a weak base, which is partially ionized in water. Degree of ionization is dependent on pH; at physiological pH, ammonium hydroxide is 99% ionized, but at pH 9.25, is only 50% ionized. Information available for humans exposed to ammonia by the oral route usually involved case reports of people who swallowed household ammonia (ammonium hydroxide). Studies by the oral route in animals generally have used ammonium salts or ammonium hydroxide. For these reasons, oral doses are expressed as mg NH_4^+ /kg/day, given as the particular ammonium compound.

In many animal studies, the animals were administered ammonium chloride. Ammonium chloride is commonly used to induce metabolic acidosis in experimental animals. The acidosis is due to the formation of hydrogen ions from the metabolism of ammonium ions to urea. WHO (1986) notes that the ingestion of ammonium chloride in doses around 500–1,000 mg/kg/day for 1–8 days (longer treatment would worsen the condition) has induced metabolic acidosis in mice, guinea pigs, rats, rabbits, and dogs. Metabolic acidosis can result in a variety of nonspecific changes in neurological, cardiovascular, pulmonary, gastrointestinal, and musculoskeletal function, as well as in changes in hematological and clinical chemistry parameters.

3.2.2.1 Death

Human deaths due to ingestion of household ammonium salts have been reported (Klein et al. 1985; Klendshoj and Rejent 1966), but no quantitative data for oral exposure in humans were located. A

3. HEALTH EFFECTS

69-year-old woman who ingested an unknown quantity of lemon ammonia (3% ammonium ion) was found semi-conscious and making gurgling respiratory sounds (Klein et al. 1985). Radiographic results were consistent with aspiration pneumonia, and endoscopy showed laryngeal and epiglottic edema and a friable, erythematous esophagus with severe corrosive injury. The woman died several days later after developing acute respiratory distress syndrome and renal failure (Klein et al. 1985). A 57-year-old man was found dead with a glass containing dilute ammonium hydroxide (2.4% ammonium ion) nearby (Klendshoj and Rejent 1966); autopsy showed hemorrhagic esophagus, stomach, and duodenum. As shown in Table 3-2 and Figure 3-2, 303 mg ammonium/kg as ammonium chloride is a lethal dose in guinea pigs when given as single gavage dose (30/40 died) (Koenig and Koenig 1949). Death, in this study, resulted from pulmonary edema. No deaths were seen in cats, rabbits, guinea pigs, or rats after a similar dose of ammonium (337 mg ammonium/kg given as ammonium chloride) (Boyd and Seymour 1946).

3.2.2.2 Systemic Effects

No information was located regarding dermal or ocular effects of ammonia or ammonium compounds in humans or animals following oral exposure.

Respiratory Effects. No information was located regarding respiratory effects of ammonia or ammonium compounds in humans following oral exposure. Guinea pigs that received a single gavage dose of ammonium chloride developed serious respiratory effects including increased rate and depth of respiration, pulmonary edema, and death by respiratory failure (Koenig and Koenig 1949). Because the blood pH of the guinea pigs decreased after administration of ammonium chloride, adjustments in respiratory rate and depth may have been a compensatory mechanism for acidosis. Similarly, administration of ammonium chloride in doses of approximately 100 mg of NH_4^+ /kg/day (as ammonium chloride) or higher for up to a year to rabbits resulted in metabolic acidosis and compensatory changes in respiratory rate and tidal volume (Seegal 1927). The low blood pH results in increased lung ventilation, which increases the elimination of carbon dioxide from the blood, and therefore, can be considered a compensatory response to acidosis rather than a direct effect of ammonium ion on the lungs or respiratory system.

Cardiovascular Effects. No information was located regarding cardiovascular effects of ammonia or ammonium compounds in humans following oral exposure. No pathological abnormalities were noted in the hearts of adult and weanling rats fed doses of up to 79 mg ammonium/kg/day as ammonium

Table 3-2 Levels of Significant Exposure to Ammonia And Ammonium Compounds - Oral

Key to figure ^a	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
ACUTE EXPOSURE							
Death							
1	Gn Pig	1 d				286 (death due to pulmonary edema)	Koenig & Koenig 1949 NH4CL
Systemic							
2	Rat (Sprague-Dawley)	6 d (W)	Hemato		2325 (elevated serum calcium)		Barzel 1975 NH4Cl
3	Rat (Wistar)	3 or 7 d (F)	Bd Wt	22 F	3150.4 F (10% reduction in final body weight)		Bodega et al. 1993 ammonium acetate
4	Rat (Wistar)	3 or 7 d (F)	Bd Wt	22	3102.2 (final body weight decreased by 15%)		Boyano-Adanez et al. 1996 ammonium acetate
5	Rat (Sprague-Dawley)	7 d Gavage - NS	Renal		433 (renal enlargement due to cell hypertrophy)		Janicki 1970 NH4Cl
6	Gn Pig	1 d Gavage - NS	Resp			303 (pulmonary edema)	Koenig & Koenig 1949 NH4CL

Table 3-2 Levels of Significant Exposure to Ammonia And Ammonium Compounds - Oral

(continued)

Key to figure ^a	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
Neurological							
7	Rat (Wistar)	3 or 7 d (F)		22	3102.2	(decreased binding of somatostatin to receptors in frontoparietal cortex and hippocampus)	Boyano-Adanez et al. 1996 ammonium acetate
INTERMEDIATE EXPOSURE							
Systemic							
8	Rat	330 d (W)	Musc/skel		991	(reduced calcium less fat-free solid)	Barzel & Jowsey 1969 NH4Cl
			Bd Wt		991	(reduced body weight)	
9	Rat (Wistar)	90 d (F)	Bd Wt	22 F	3150 F	(15% reduction in final body weight after 90 days)	Bodega et al. 1993 ammonium acetate
10	Rat (Wistar)	15 d (F)	Bd Wt	22	3102.2	(final body weight gain decreased by 10%)	Boyano-Adanez et al. 1996 ammonium acetate
11	Rat	3 wk (W)	Renal	412			Freedman & Beeson 1961 NH4CL

Table 3-2 Levels of Significant Exposure to Ammonia And Ammonium Compounds - Oral

(continued)

Key to figure ^a	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	LOAEL			Reference Chemical Form
				NOAEL (mg/kg/day)	Less Serious (mg/kg/day)	Serious (mg/kg/day)	
12	Rat (albino)	90 d 6 d/wk (W)	Cardio	79 F			Gupta et al. 1979 NH4NH2SO3
			Gastro	79 F			
			Hemato	79 F			
			Hepatic	79 F			
			Renal	79 F			
			Bd Wt	39.5 F	79 F (body weight decreased by 16%)		
13	Dog	11 wk Gavage - NS	Musc/skel			337 (bone deformity and softening)	Bodansky et al. 1932 NH4Cl
14	Rat (Wistar)	15 d (F)		22	3102.2 (decreased binding of somatostatin to receptors in frontoparietal cortex and hippocampus)		Boyano-Adanez et al. 1996 ammonium acetate

Neurological

Table 3-2 Levels of Significant Exposure to Ammonia And Ammonium Compounds - Oral

(continued)

Key to figure ^a	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	LOAEL			Reference Chemical Form
				NOAEL (mg/kg/day)	Less Serious (mg/kg/day)	Serious (mg/kg/day)	
Developmental							
15	Rat (Wistar)	Gd 1-ppd 21 (F)				4293 (BW decreased by 16-27%; decreased NMDA receptor function in neurons)	Minana et al. 1995 NH3CH3CO2

a The number corresponds to entries in Figure 3-2.

Bd Wt = body weight; Cardio = cardiovascular; d = day(s); DW = drinking water; (F)= feed; F = female; Gd = gestation day; G = gavage; gastro = gastrointestinal; hemato = hematological; hr = hour(s); LOAEL = lowest-observed-adverse-effect level; min = minute(s); Musc/skel = musculoskeletal; NOAEL = no-observed-adverse-effect level; NS = not specified; Resp = respiratory; NS = not specified; wk = week(s)

Figure 3-2. Levels of Significant Exposure to Ammonia And Ammonium Compounds - Oral
Acute (≤ 14 days)

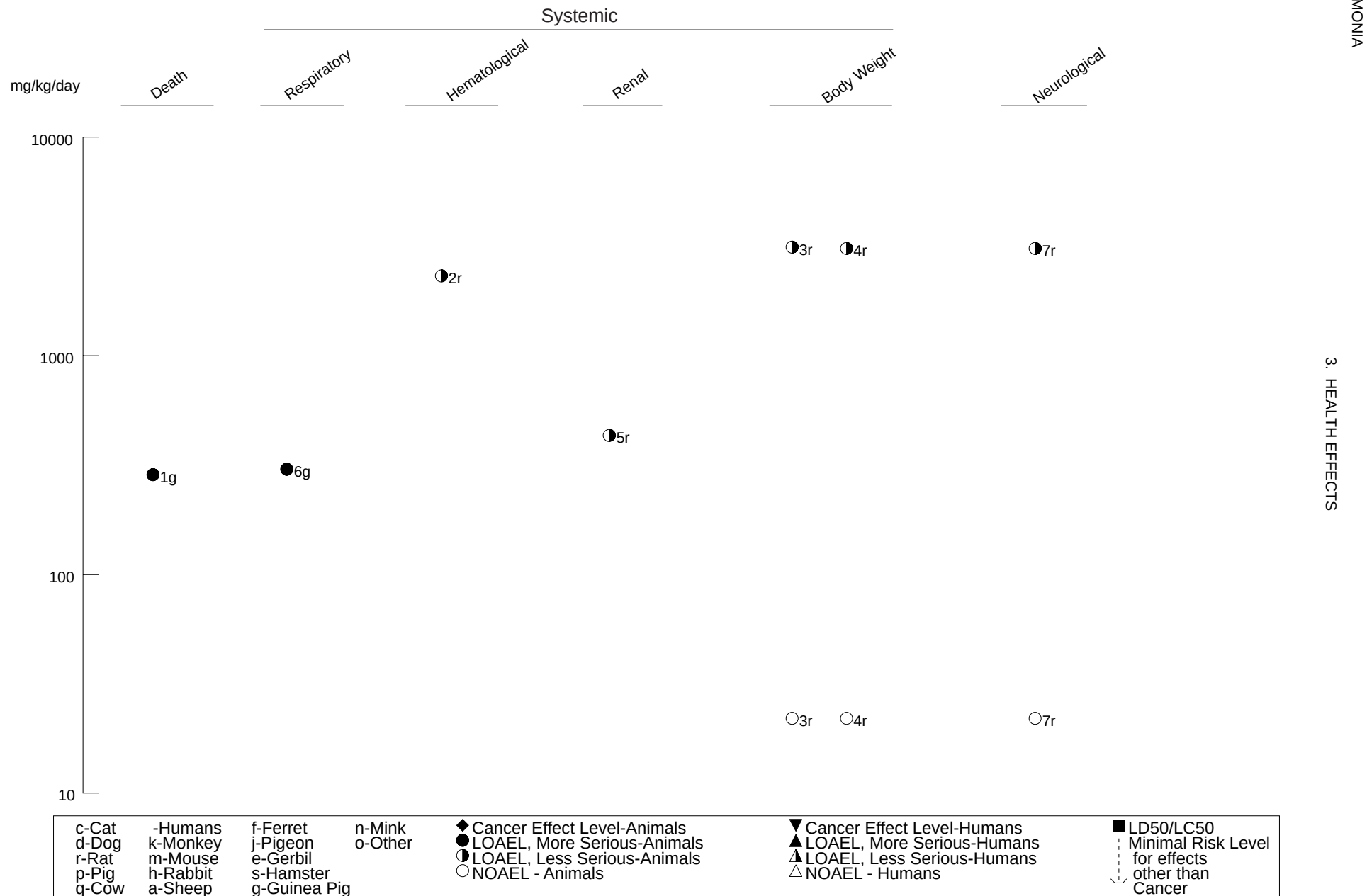
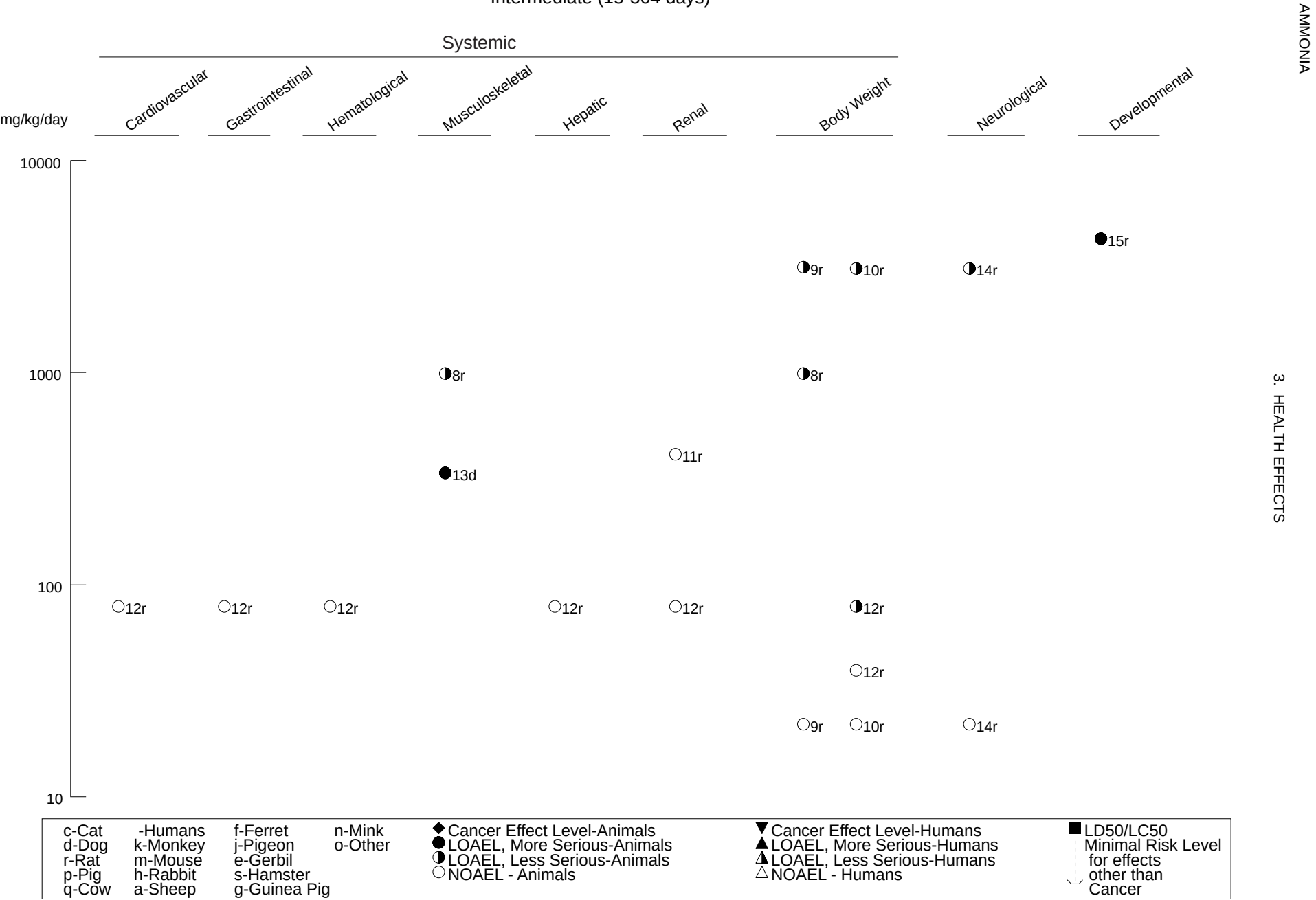


Figure 3-2. Levels of Significant Exposure to Ammonia And Ammonium Compounds - Oral (Continued)
Intermediate (15-364 days)



3. HEALTH EFFECTS

sulfamate for 90 days in drinking water (Gupta et al. 1979). These data are presented in Table 3-2 and Figure 3-2.

Gastrointestinal Effects. Several cases have been described of young children (2–3 years old) who bit into ammonia pellets/capsules (Lopez et al. 1988; Rosenbaum et al. 1998). Two of the children drooled and had ulcerative lesions on the tongue and/or on the buccal mucosa; one child had superficial ulcerations on the posterior esophageal wall and the other child had edematous, erythematous upper and lower lips with areas of desquamation, eschar of the hard palate, and edema and erythema of the supraglottic structures and upper trachea (Rosenbaum et al. 1998). All of the children experienced one or more of the following symptoms: vomiting, drooling, dysphagia, cough, or oral or pharyngeal burns (Lopez et al. 1988; Rosenbaum et al. 1998). None of the children had esophageal or respiratory burns and all healed within a few days. Esophageal lesions and edema were reported in five persons who ingested household ammonia (ammonium hydroxide), one of whom experienced acute respiratory obstruction (Christesen 1995; Klein et al. 1985). These observations were not quantified. The effects are probably due to the alkaline nature of ammonium hydroxide. A single case report described a self-administered ammonia solution enema that resulted clinically in anal pain, diffuse abdominal colic, and tenesmus (da Fonseca et al. 1998). Sigmoidoscopy showed diffuse erythematous friable mucosa with large ulcerations covered by yellowish exudate that receded in a few days, but chronic inflammation and fibrosis of the rectum and sigmoid colon was noted 3 months postexposure (da Fonseca et al. 1998).

No histopathological abnormalities of the gastrointestinal tract were observed in adult or weanling rats administered doses of up to 79 mg ammonium/kg/day as ammonium sulfamate for 90 days via drinking water (Gupta et al. 1979). Likewise, a 3% solution of ammonium chloride administered to rats via gastric tube produced no gastric mucosal damage in 1 hour and a 10% solution produced only a minimum of hemorrhagic lesions (about 9 mm²) (Takeuchi et al. 1995). However, similar administration of 1 or 3% ammonium hydroxide in rats produced severe hemorrhagic lesions (about 26.6 or 97.7 mm², respectively) (Takeuchi et al. 1995). Gavage administration in rats of 0.3% ammonia (33.3 mg/kg) produced gastric mucosal lesions within 5 minutes with corresponding decreases in gastric wall immunoreactive endothelin-1 (ET-1) and immunoreactive thyrotropin-releasing hormone (TRH) concentrations and increases in gastric juice ET-1 and TRH concentrations (Mori et al. 1998). *In situ* gastric exposure has also shown ammonia-induced gastric mucosal damage (Murakami et al. 1995; Nagy et al. 1996). These lesions are exacerbated by neutrophil products, especially hypochlorous acid (Murakami et al. 1995) and cysteine proteases, such as some of the cathepsins (Nagy et al. 1996). Administration of 0.01% ammonia in drinking water to rats (approximately 42 mg/kg/day) for 8 weeks resulted in acceleration of cell

3. HEALTH EFFECTS

migration leading to mucosal atrophy in the stomach antrum, increased labeling indices, and enlargement of the proliferative zone in the antral and body mucosa (Tsujii et al. 1993).

Hematological Effects. No information was located regarding the hematological effects of ammonia or ammonium compounds in humans following oral exposure. Repeated exposure to ammonium chloride in animals resulted in metabolic acidosis with related changes in bone metabolism and serum calcium. For example, rats fed diets containing high levels of ammonium chloride had increased serum calcium (Barzel 1975). The increased serum calcium resulted from enhanced demineralization of bone in response to chronic acidosis. This effect was not found to be a specific effect of ammonium and was reported to occur in states of chronic metabolic acidosis produced from repeated doses of acidifying agents (e.g., hydrochloric acid, sulfuric acid). Decreased blood pH was seen in cats fed an acidifying diet containing ammonium chloride for several weeks (Kienzle and Wilms-Eilers 1994). As shown in Table 3-2 and Figure 3-2, no effects on blood hemoglobin or blood cell counts were observed in adult or weanling rats that received doses of up to 79 mg ammonium/kg/day administered as ammonium sulfamate in drinking water (Gupta et al. 1979).

Musculoskeletal Effects. No information was located regarding musculoskeletal effects of ammonia or ammonium compounds in humans following oral exposure. Guinea pigs and rats that received lethal gavage doses of ammonium chloride developed muscle weakness, fasciculation, and incoordination (Koenig and Koenig 1949). In other animal studies, repeated ingestion of ammonium salts resulted in metabolic acidosis, which stimulated bone demineralization. As is shown in Table 3-2 and Figure 3-2, repeated ingestion of ammonium chloride in drinking water resulted in net bone resorption in rats and bone deformities in dogs (Barzel and Jowsey 1969; Bodansky et al. 1932). This effect can be anticipated with repeated exposure to any acidifying agent.

Hepatic Effects. No information was located regarding hepatic effects of ammonia or ammonium compounds in humans following oral exposure. As shown in Table 3-2 and Figure 3-2, no toxic effects were noted in livers of adult or weanling rats fed doses of up to 79 mg ammonium/kg/day as ammonium sulfamate for 90 days in drinking water (Gupta et al. 1979).

Renal Effects. Renal failure was identified as the cause of death in humans after ingestion of an unknown amount of household ammonia (ammonium hydroxide) (Klein et al. 1985). It is not certain if this represents a primary effect of ammonium or is secondary to massive burns to the gastrointestinal tract.

3. HEALTH EFFECTS

Renal effects have been observed in animals following repeated oral doses of ammonium chloride. These effects may be secondary to chronic acidosis produced from the interaction of ammonium chloride with water (which results in an increased H^+ concentration) rather than from a direct effect of ammonium ion on the kidney. Renal enlargement, increased blood ammonia content, and increased urinary ammonia have been reported in rats exposed to 180–433 mg/kg/day for 3–7 days (Benyajati and Goldstein 1975; Janicki 1970; Lotspeich 1965;), but are unlikely to be indicative of renal pathology. The highest NOAELs and LOAELs are presented in Table 3-2 and Figure 3-2.

Endocrine Effects. No information was located regarding endocrine effects of ammonia or ammonium compounds in humans following oral exposure. Enlarged adrenal glands were observed in rabbits that received 124 mg ammonium/kg/day as ammonium hydroxide by gavage in water for 17 months (Fazikas 1939). These limited data suggest that the endocrine system is not a primary target for ammonia or ammonium compounds.

Body Weight Effects. Decreased body weight or weight gain has been observed in animals following oral exposure to ammonium ion (Barzel and Jowsey 1969; Bodega et al. 1993; Boyano-Adánez et al. 1996; Gupta et al. 1979; Noda and Chikamori 1976). Rats exposed to ammonium ion *in utero* and during lactation (dams received 4,293 mg ammonium/kg/day in the diet from gestational day 1 through lactation day 21) and then received a normal diet had showed reduced body weight gain (Miñana et al. 1995); body weight gain was reduced by 25 and 16% in male and female offspring, respectively, at 120 days of age. Rats that were continued on the same ammonia diet as their dams had an even greater reduction in body weight gain (27 and 26% for males and females, respectively) (Miñana et al. 1995). Birth weights were not reported in the Miñana et al. (1995) study. Gupta et al. (1979) noted increased water intake and reduced food intake in weanling rats, and decreased body weight in adults but not weanlings fed 79 mg ammonium/kg/day in drinking water for 90 days as ammonium sulfamate. This represents the LOAEL for this effect. A NOAEL of 39.5 mg ammonium/kg/day was also identified in this study (see Table 3-2 and Figure 3-2).

3.2.2.3 Immunological and Lymphoreticular Effects

No information was located regarding immunological effects of ammonia or ammonium compounds in humans or animals after oral exposure.

3. HEALTH EFFECTS

3.2.2.4 Neurological Effects

No information was located regarding neurological effects of ammonia or ammonium compounds in humans after oral exposure.

Guinea pigs that received lethal gavage doses of ammonium chloride (303.5–404.7 mg NH_4^+ /kg) developed neuromuscular effects including fasciculation; incoordination; hyperexcitability to tactile, auditory, and painful stimuli; and tonic convulsions (Koenig and Koenig 1949).

A number of studies have indicated that increased ammonium ion levels in the brain may disrupt energy production and modify the availability of some receptors that are involved in neurotransmission. Administration of 20% ammonium acetate in the diet of rats for 20 days resulted in statistically significant increases in brain ammonium ion (12.8-fold), glutamine (37%), and alanine (93%) and in some TCA cycle-associated components in the brain including glucose, lactate, and pyruvate, and decreases in brain cytosolic NAD^+/NADH ratio, β -hydroxybutyrate, and ATP content (Kosenko et al. 1993). Rats with high NH_4^+ intake from administration of 20% ammonium acetate in the diet and 5 mM ammonium acetate in the water for up to 15 days had a decreased number of available somatostatin receptors in the frontoparietal cortex and hippocampus (Boyano-Adánez et al. 1996). Since somatostatin hyperpolarizes neurons in the cerebral cortex, the study authors speculated that this reduction in available receptors may contribute to the alteration of electrophysiological properties of neural tissue caused by excess NH_4^+ (Boyano-Adánez et al. 1996). Binding of [H^3]MK-801 (an NMDA receptor antagonist) to NMDA receptors was reduced by approximately 60% in cerebellar cell cultures from 8-day-old rats exposed to NH_4^+ *in utero* and during lactation (dams received 4,293 mg ammonium/kg/day in the diet from gestational day 1 through lactation day 8) (Miñana et al. 1995). Additionally, aspartate aminotransferase (AST) induction was absent in treated neurons (occurred in neurons from control rats), which also indicates impairment of NMDA receptors. Treated neurons were much more resistant to the toxic effects of glutamate than control neurons; since glutamate toxicity is mediated by NMDA receptors, attenuation of glutamate toxicity is indicative of impaired NMDA receptor function (Miñana et al. 1995). Loss of glial fibrillary acidic protein (GFAP) has been shown to occur in human spontaneous hyperammonemia (Kimura and Budka 1986; Kretschmar et al. 1985; Sobel et al. 1981) and in other hyperammonemia models, such as portacaval shunt rats (Bodega et al. 1991; Suárez et al. 1992), but was not evident after exposure of rats to ammonium acetate (20% ammonium acetate in the diet and 5 mM ammonium acetate in the water for up to 90 days) (Bodega et al. 1993). Rats fed 19.5% ammonium acetate in the diet had increased NH_4^+ levels in the brain and altered assembly and disassembly of tubulin, an essential

3. HEALTH EFFECTS

component of the axonal transport system in the brain (Miñana et al. 1989a). The amount of polymerized tubulin increased but the amount of free tubulin was not affected. *In vitro* experiments using brains of rats fed a diet high in NH_4^+ indicated that the cause of the alteration might be a modification of the tubulin (and not the microtubule-associated proteins [MAPs], which modulate polymerization of the tubulin), which may result in a disruption in neurotransmission (Miñana et al. 1989a). Additional studies in rats brain showed that tubulin was significantly increased specifically in the septum, ventral hippocampus, dorsal hippocampus, hypothalamus, reticular formation, and frontal cortex, but not in the temporal amigdala, mammillary nucleus, locus coeruleus, caudate nucleus, or cingulate cortex after 2 months on the high ammonia diet (Miñana et al. 1989b).

3.2.2.5 Reproductive Effects

No information was located regarding reproductive effects of ammonia or ammonium compounds in humans or animals following oral exposure.

3.2.2.6 Developmental Effects

No information was located regarding the developmental effects of ammonia or ammonium compounds in humans. Rats exposed to NH_4^+ *in utero* and during lactation (dams received 4,293 mg ammonium/kg/day in the diet from gestational day 1 through lactation day 21), which then received a normal diet, had a statistically significant reduction in body weight gain (Miñana et al. 1995); body weight was reduced by 25 and 16% in male and female offspring, respectively, at 120 days of age. Rats that were continued on the same ammonia diet as their dams had an even greater decrease in body weight gain (27 and 26% for males and females, respectively) at 120 days of age (Miñana et al. 1995). No information was provided in the study regarding the health of the dams, but it is likely that the high ammonium dietary concentration made them hyperammonemic. Body weights and food consumption by the dams throughout the study were not reported.

3.2.2.7 Cancer

No information was located regarding carcinogenic effects of ammonia or ammonium compounds in humans following oral exposure. Exposure of mice to 193 mg ammonium/kg/day as ammonium hydroxide in drinking water for 2 years did not produce carcinogenic effects, nor did it affect spontaneous

3. HEALTH EFFECTS

development of breast cancer, which is common to C3H female mice (Toth 1972). No evidence of a carcinogenic effect was found in mice treated by gavage with ammonia dissolved in water alone at a dose of 42 mg NH_4^+ /kg/day for 4 weeks or with diethyl pyrocarbonate alone, but 9 of 16 mice treated with a combination of ammonium and pyrocarbonate developed lung tumors (Uzvolgyi and Bojan 1980). The ammonia and pyrocarbonate may have reacted *in vivo* to form the carcinogen, urethane. In a group of mice treated with urethane, the incidence of lung tumors was 9 of 9. Data from studies *in vitro* cited by Uzvolgyi and Bojan (1980) demonstrated the formation of urethane from diethyl pyrocarbonate added to beverages containing ammonia. No lung tumors were observed in the offspring of mice exposed similarly to NH_4^+ and diethyl pyrocarbonate during pregnancy or during lactation (Uzvolgyi and Bojan 1985). In another study, Tsujii et al. (1992a, 1995) tested the hypothesis that ammonia produced in the stomach in humans infected with *Helicobacter pylori* may play a role in the development of gastric cancer. Rats pretreated with the initiator N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) in the drinking water for 24 weeks before receiving 0.01% ammonia solution in the drinking water for 24 weeks had a statistically significantly greater incidence of gastric cancer (70% of rats) and number of tumors per tumor-bearing rat (2.1) than rats receiving only MNNG and tap water (31% and 1.3 tumors/rat) (Tsujii et al. 1992a). Additionally, the size, depth, and metastasis of the MNNG-initiated tumors were enhanced in the rats treated with ammonia (Tsujii et al. 1995). It can be estimated that the dose of ammonia received by the rats was approximately 42 mg/kg/day (the daily intake from food and water for the general population is approximately 0.3 mg/kg/day [WHO 1986]). The relevance of these studies to assess the cancer risk of oral exposure to ammonia is uncertain.

3.2.3 Dermal Exposure

Dermal exposure to ammonia may also result in some inhalation exposure. Therefore, based on the available data, it is not always clear to what extent each route of exposure contributes to the toxicity observed in dermal exposure studies.

3.2.3.1 Death

Human and animal deaths involving dermal exposure to ammonia and ammonium have been reported (Prokop'eva et al. 1973; Slot 1938; Sobonya 1977), but the extent of exposure is not known, and effects were probably due to inhalation exposure as well. A 25-year-old woman exposed to ammonia gas from a broken pipe had burns on her face, arms, and torso, and had difficulty breathing and swallowing (Slot

3. HEALTH EFFECTS

1938). She was treated symptomatically and with supportive treatment, but died about a month after exposure (Slot 1938). Autopsy showed edematous, inflamed, hemorrhagic epiglottis, trachea, and lungs. Petechial hemorrhages were found on the heart and the kidneys were congested with hemorrhagic nephritis. A 25-year-old man died after a tank of anhydrous ammonia exploded near him while he was farming (Sobonya 1977). Immediately after exposure he had mild bilateral conjunctival edema, burns over about 30% of his body surface, bilateral pulmonary edema, and severe respiratory distress. He developed pneumonia and died on the sixtieth day post-exposure. In rats, LC_{50} values of 112, 71.9, and 48.4 mg ammonia/L were determined for exposures of 15, 30, and 60 minutes, respectively (Prokop'eva et al. 1973). These data are presented in Table 3-3.

3.2.3.2 Systemic Effects

No information was located on hematological, musculoskeletal, hepatic, endocrine, or body weight effects in humans or animals after dermal exposure to ammonia or ammonium.

Dermal or multiple route exposure to ammonia or ammonium has produced respiratory, cardiovascular, gastrointestinal, renal, dermal, and ocular effects.

Respiratory Effects. Respiratory effects have been reported in humans from exposure to massive amounts of ammonia gas, but no quantitative data were located. It is also unclear as to what extent the effects were a result of inhalation and dermal exposure. Tracheitis, bronchitis, edema, and bronchopneumonia were reported by Slot (1938). Lung infection and respiratory distress were reported in one case (Sobonya 1977). Dyspnea, rales, rhonchi, and blocked airways were found by Levy et al. (1964). The effects probably resulted from concurrent inhalation and dermal exposure. No information was located regarding respiratory effects of ammonia or ammonium in animals following dermal or ocular exposure.

Cardiovascular Effects. Elevated pulse, shock, and cardiac failure were reported in humans from accidental exposures to massive amounts of ammonia gas, but the extent of exposure was not quantified (Slot 1938). No information was located regarding cardiovascular effects of ammonia or ammonium in animals following dermal or ocular exposure.

Gastrointestinal Effects. Persistent vomiting was noted by Slot (1938) in human accidental massive exposure cases, but the extent of exposure was not quantified. Oral and pharyngeal burns and edema

Table 3-3 Levels of Significant Exposure to Ammonia And Ammonium Compounds - Dermal

Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL	LOAEL		Reference Chemical Form
				Less Serious	Serious	
ACUTE EXPOSURE						
Death						
Rat	1 d 30 min/d				71.9 mg/L (LC50)	Prokop'eva et al. 1973
Rat	1 d 60 min/d				48.4 mg/L (LC50)	Prokop'eva et al. 1973
Systemic						
Human	5 min	Ocular	50 ppm	72 ppm	(eye irritation)	Industrial Bio-Test Labora
Human	10 min	Ocular	30 ppm	50 ppm	(moderate ocular irritation)	MacEwen et al. 1970
Pig (Duroc)	5 wk min/d	Ocular	10 ppm	50 ppm	(ocular irritation)	Stombaugh et al. 1969
INTERMEDIATE EXPOSURE						
Systemic						
Human	6 wk 5 d/wk 6 hr/d	Ocular	25 ppm	50 ppm	(transitory eye irritation)	Ferguson et al. 1977

d = day(s); hr = hour; LOAEL = lowest-observed-adverse-effect level; min = minute(s); NOAEL = no-observed-adverse-effect level; wk = week(s)

3. HEALTH EFFECTS

were reported by Levy et al. (1964) in four human males accidentally exposed to an unknown quantity of anhydrous ammonia. Inhalation exposure may have contributed to these effects also.

Renal Effects. Renal congestion and hemorrhagic nephritis were reported by Slot (1938) in six cases of accidental human exposures to highly concentrated aerosols of ammonium derived from anhydrous ammonia. The exposure level cannot be determined from the available data.

Dermal Effects. Skin and eyes are extremely sensitive to airborne ammonia or ammonium in water. The topical damage caused by ammonia is probably due mainly to its alkaline properties. Its high water solubility allows it to dissolve in moisture on these surfaces, react with fatty substances in the corneal layer, be absorbed into deeper layers, and inflict extensive damage (Jarudi and Golden 1973). Reports of skin damage in humans are numerous, but good quantitative data are lacking. The severity of the damage is proportional to concentration and duration of exposure; flushing with water immediately after contact alleviates or prevents effects. Burns, blisters, and lesions of the skin have been reported (Close et al. 1980; Flury et al. 1983; Shimkin et al. 1954; Slot 1938; Taplin et al. 1976; Walton 1973). Exposure levels associated with dermal effects are presented in Table 3-3.

Several case reports described exposure of individuals to ammonia liquid and/or gas that resulted in cutaneous burns (Amshel et al. 2000; da Fonseca et al. 1998; George et al. 2000; Kerstein et al. 2001; Latenser and Lucktong 2000; Leduc et al. 1992; Rosenbaum et al. 1998; Weiser and Mackenroth 1989). All exposures were occupationally related. Total body surface area burned ranged from 14 to 45% and most had at least small areas of full-thickness burns that required skin grafting. A summary of 12 case reports of liquid anhydrous ammonia injuries reported a range of percent body surface area burned of 3–22%, with 25% of the patients having full-thickness burn injuries (Millea et al. 1989). One case report included a skin litmus paper test that showed the pH of the skin to be 10 at the time of hospital admission (Amshel et al. 2000). Infection of the burn wounds was not uncommon, with most of the patients responding to antibiotic treatment. One person had facial and neck hyperemia, erythematous petechiae on one ear, and edematous and peeling lips (Latenser and Lucktong 2000). The individual with 45% total body surface area burned had additional severe injuries, including respiratory and ocular, and developed circulatory and hematological problems, which led to his death (George et al. 2000).

Rosenbaum et al. (1998) described two cases of young children (2–3 years old) who bit into ammonia pellets/capsules. Both children drooled and had ulcerative lesions on the tongue and/or on the buccal mucosa. One child had superficial ulcerations on the posterior esophageal wall and the other child had

3. HEALTH EFFECTS

edematous, erythematous upper and lower lips with areas of desquamation, eschar of the hard palate, and edema and erythema of the supraglottic structures and upper trachea. Both children recovered without incident.

A single case report described a self-administered ammonium solution enema that resulted in anal pain, diffuse abdominal colic, and tenesmus (da Fonseca et al. 1998). Sigmoidoscopy showed diffuse erythematous friable mucosa with large ulcerations covered by yellowish exudate. Six days later, the ulcers had receded, but the colon was still erythematous. Three months postexposure, biopsies showed chronic inflammation and fibrosis of the rectum and sigmoid colon, but no stenosis.

Animal data regarding dermal and ocular effects of exposure to ammonia support the findings in humans. A number of cattle were acutely exposed to anhydrous ammonia fumes when a pipeline running through their pasture ruptured and leaked 1,800 barrels of ammonia in a short period of time (Morgan 1997). The ammonia combined with moisture in the air and formed a white cloud, which drifted south across two additional fields containing cattle. The noses of the cattle in the field with the pipeline turned black and peeled and the horns of cattle in an adjacent field turned black and peeled. Hair coats on all livestock within a 2-mile radius of the rupture were singed.

Ocular Effects. Reported ocular effects in humans following ammonia or ammonium exposure increase in severity with dose and duration. Good quantitative data are lacking, but symptoms progress as follows: inflamed eyes, lacrimation, swelling of the eyelids (Beare et al. 1988; Caplin 1941; Close et al. 1980; Ferguson et al. 1977; Jarudi and Golden 1973; Legters et al. 1981; Montague and Macneil 1980; O’Kane 1983; Price et al. 1983; Silverman et al. 1949; Stombaugh 1969; Verberk 1977; Ward et al. 1983), hyperemic conjunctiva (Caplin 1941; Hatton et al. 1979; Levy et al. 1964; Slot 1938; Sobonya 1977), transient blindness, blurred vision, and corneal abrasions (Latenser and Lucktong 2000), and sustained corneal damage (Caplin 1941; Grant 1974; Kass et al. 1972; McGuinness 1969; Stroud 1981; Yang et al. 1987). Ammonia is slightly irritating to human eyes at concentrations of 100 ppm (Ferguson et al. 1977), and immediately irritating to the eyes and throat at 698 ppm (Henderson and Haggard 1927). Exposure to an air concentration of 250 ppm is bearable for most persons for 30–60 minutes (Withers et al. 1986). Exposure levels associated with ocular effects are presented in Table 3-3.

Animal data regarding ocular effects of exposure to ammonium support the findings in humans. Corneal opacity has been observed in rabbits following brief exposures (2 seconds) to a solution of 28.5% ammonium hydroxide (Grant 1974). Volume administered was not reported. Cattle in an adjacent field

3. HEALTH EFFECTS

to a pipeline that ruptured and released 1,800 barrels of ammonia had runny eyes; two cows in the same field with the ruptured pipeline were euthanized because of blindness and respiratory distress (Morgan 1997).

3.2.3.3 Immunological and Lymphoreticular Effects

Secondary infections often complicate the clinical outcome of burns and respiratory lesions related to exposures to highly concentrated aerosols derived from anhydrous ammonia in which dermal and ocular exposure accompanies inhalation exposure (Sobonya 1977; Taplin et al. 1976). However, there is no evidence that the decreased immunological resistance represents a primary impairment of the immune system in humans. No information was located regarding the immunological effects of ammonia or ammonium in animals following dermal or ocular exposure.

No information was located regarding the following effects of ammonia or ammonium compounds in humans or animals following dermal or ocular exposure:

3.2.3.4 Neurological Effects

3.2.3.5 Reproductive Effects

3.2.3.6 Developmental Effects

3.2.3.7 Cancer

Carcinogenic potential of ammonia has not been established in humans or animals by the dermal route of exposure. One case report was found of a person who developed epidermal carcinoma of the nasal septum 6 months after being badly burned by accidental contact with a refrigeration ammonia-oil mixture (Shimkin et al. 1954). It is unclear whether ammonia played a role in this tumor development. No other reports were located, although many cases of contact with ammonia from spills have been followed for more than 6 months after exposure.

3. HEALTH EFFECTS

3.2.4 Other Routes of Exposure

There are limited *in vitro* data suggesting that ammonium ion may affect fetal development (Lane and Gardner 1994). Mouse embryos (conceived *in vivo*) were cultured in modified mouse tubal fluid medium (mMTF) or mMTF supplemented with 300 $\mu\text{mol/L}$ ammonium ion for 48, 69, or 93 hours before being transferred to pseudopregnant mouse dams (Lane and Gardner 1994). Examination on gestational day 15 showed an apparent relationship between the duration of exposure and the incidence of exencephaly. Embryos that were cultured with various concentrations of ammonium ion before being transferred to recipient dams showed increased incidence of exencephaly with increased ammonium concentration (38–300 $\mu\text{mol/L}$) and decreased percentage of implantation sites with increased ammonium concentration. It is unclear how embryos might be exposed to ammonia or ammonium *in vivo* or if *in vivo* exposure would affect fetal development and implantation in a way similar to that described in the Lane and Gardner (1994) study.

3.3 GENOTOXICITY

A single study examined the genotoxic effect of ammonia in humans (Yadav and Kaushik 1997). Analysis of blood samples from 22 workers exposed to ammonia in a fertilizer factory and 42 control workers not exposed to ammonia showed increased frequency of chromosomal aberrations (CAs) and sister chromatid exchanges (SCEs), increased mitotic index (MI), and increased frequency of CAs and SCEs with increasing length of exposure.

Swiss albino mice administered a single dose of 12, 25, or 50 mg/kg ammonium intraperitoneally had an increased frequency of micronuclei compared to controls (Yadav and Kaushik 1997).

All remaining tests of ammonia's mutagenicity consist of studies in *E. coli*, chick fibroblast cells, and *Drosophila melanogaster* (Table 3-4). Demerec et al. (1951) noted positive effects in a reverse mutation test in *E. coli*, but only in treatments using toxic levels of NH_4^+ (98% lethality). Lobasov and Smirnov (1934) found slight mutagenic activity in *Drosophila* following exposure to ammonia gas, but once again, survival after treatment was <2%. Auerbach and Robson (1947) tested Lobasov and Smirnov's results and noted 0.5% sex-linked lethals. The authors concluded that although their data did not support the earlier study's findings, it is possible that ammonia has a very slight mutagenic action. In their data presentation, however, they report their findings as negative, qualifying it as doubtful and probably negative.

3. HEALTH EFFECTS

Table 3-4. Genotoxicity of Ammonia *In Vitro* and *In Vivo*

Species (test system)	End point	Form	Activation system	Results		Reference
				With activation	Without activation	
<i>In vitro:</i>						
<i>Escherichia coli</i>	Reverse mutation	NH ₃		NT	+ (at toxic levels)	Demerec et al. 1951
Chick fibroblasts	Chromosomal aberrations	NH ₄ Cl ⁺ NH ₄ OH buffer		NT	+	Rosenfeld 1932
Mouse fibroblasts	Reduced cell division	NH ₃ ⁺ NH ₄ Cl		NT	+	Vissek et al. 1972
Mouse fibroblasts (3T3)	Reduced cell division			NT	+	Capuco 1977
Mouse fibroblasts	DNA repair inhibition	NH ₄ Cl		NT	+	Capuco 1977
<i>In vivo:</i>						
<i>Drosophila melanogaster</i>	Mutagenic lethality	NH ₃		+	NT	Lobasov and Smirnov 1934
<i>D. melanogaster</i>	Sex-linked recessive lethal mutations	NH ₃		– (doubtful, probably negative)	NT	Auerbach and Robson 1947
<i>D. melanogaster</i>	Dominant lethality	NH ₃		–	NT	Auerbach and Robson 1947
Mouse ileal and colonic mucosa cells	Decreased rate of DNA synthesis	NH ₄ Cl		NT	+	Zimber and Vissek 1972a

– = negative result; + = positive result; (+) = weakly positive result; DNA = deoxyribonucleic acid; NH₃ = ammonia; NH₄Cl = ammonia chloride; NH₄OH = ammonium hydroxide; NT = not tested

3. HEALTH EFFECTS

In vitro tests of chick fibroblast cells showed that buffered ammonia-ammonium chloride solutions can induce clumping of chromosomes, inhibit spindle formation, and result in polyploidy (Rosenfeld 1932). Visek et al. (1972) noted reduced cell division in mouse fibroblasts cultured in media to which ammonia and ammonium chloride were added. The effect was noted in cultures irrespective of pH. Decreased rate of DNA synthesis was noted in mouse mucosal cells in the ileum and colon when serum NH_4^+ levels were significantly elevated over normal levels; these elevated levels were induced by intraperitoneal injection of urease or infusion of ammonium chloride (Zimber and Visek 1972a).

Iwaoka et al. (1981), responding to controversy regarding mutagenicity in fried hamburgers, found that extraction of organic ingredients from fried hamburger and refrigerated biscuit products with ammonium hydroxide or ammonium sulfate increased mutagenic activities in *Salmonella typhimurium* T98 and TA1538 Ames' microsomal systems, while negative results were obtained from extraction with sodium sulfate. The mode of action is unclear; ammonium salts may in some way affect the mutagenic activities of some agents, or they may simply be more efficient extractors of mutagenic components from these foods.

Taken together, the data indicate that ammonia and ammonium ion may have clastogenic and mutagenic properties.

3.4 TOXICOKINETICS

Studies suggest that ammonia can be absorbed by the inhalation and oral routes of exposure, but there is less certainty regarding absorption through the skin. Absorption through the eye has been documented. Most of the inhaled ammonia is retained in the upper respiratory tract and is subsequently eliminated in expired air. Almost all of the ammonia produced endogenously in the intestinal tract is absorbed. Exogenous ammonia is also readily absorbed in the intestinal tract. Ammonia that reaches the circulation is widely distributed to all body compartments although substantial first pass metabolism occurs in the liver where it is transformed into urea and glutamine. Ammonia or ammonium ion reaching the tissues is taken up by glutamic acid, which participates in transamination and other reactions. The principal means of excretion of ammonia that reaches the circulation in mammals is as urinary urea; minimal amounts are excreted in the feces and in expired air.

3. HEALTH EFFECTS

3.4.1 Absorption**3.4.1.1 Inhalation Exposure**

Experiments with volunteers show that ammonia, regardless of its tested concentration in air (range, 57–500 ppm), is almost completely retained in the nasal mucosa (83–92%) during short-term exposure, i.e., up to 120 seconds (Landahl and Herrmann 1950). However, longer-term exposure (10–27 minutes) to a concentration of 500 ppm resulted in lower retention (4–30%), with 350–400 ppm eliminated in expired air by the end of the exposure period (Silverman et al. 1949), suggesting an adaptive capability or saturation of the absorptive process. Nasal and pharyngeal irritation, but not tracheal irritation, suggests that ammonia is retained in the upper respiratory tract. Unchanged levels of blood-urea-nitrogen (BUN), non-protein nitrogen, urinary-urea, and urinary-ammonia are evidence of low absorption into the blood. Exposure to common occupational limits of ammonia in air (25 ppm) with 30% retention (and assuming this quantity is absorbed into the blood stream) would yield an increase in blood ammonium concentration of 0.09 mg/L (calculated by WHO 1986). This calculated rise is only 10% above fasting levels, as reported by Conn (1972).

Animal data provide supporting evidence for high-percentage nasal retention, thus protecting the lower respiratory tract from exposure (Dalhamn [1963] and Boyd et al. [1944], rabbit; Egle [1973], dog). Continuous exposure of rats for 24 hours to concentrations up to 32 ppm resulted in significant increase in blood ammonia levels (Schaerdel et al. 1983). Exposures to 310–1,157 ppm led to significantly increased blood concentrations of ammonia within 8 hours of exposure initiation, but blood ammonia returned to pre-exposure values within 12 hours of continuous exposure and remained so over the remaining of the 24-hour exposure period. This suggests an adaptive response mechanism may be activated with longer-term exposure (Schaerdel et al. 1983).

3.4.1.2 Oral Exposure

Case reports of human ingestion of household ammonia (ammonium hydroxide) provide evidence of its absorption by this route, but few provide quantitative data. For example, in a fatal case of a man who drank an unknown amount of a 2.4% solution of ammonium hydroxide, analysis of the contents of the stomach and blood showed ammonium ion concentrations of 153 and 33 ppm, respectively (Klendshoj and Rejent 1966). In a study of volunteers, ingestion of a single ammonium chloride tablet (approximately 15 mg NH_4^+ /kg/day) led to a small transient increase (33% above fasting levels) in arterial

3. HEALTH EFFECTS

blood concentrations of ammonium ion in 11 out of 20 subjects (Conn 1972); no change was noted in the remaining nine subjects in this group. Among 50 cirrhotic patients, increases of about 150% were noted in arterial blood concentrations of ammonium ion and return to normal levels was slow (Conn 1972). These data indicate that ingested ammonia is readily absorbed from the digestive tract and that the liver plays a large role in removing it from the blood (Conn 1972). Analysis of urine samples from subjects on high and low protein diets and given ^{15}N -ammonium chloride, showed that 30–65% of labeled nitrogen from ^{15}N -ammonium chloride is absorbed and metabolized (Richards et al. 1975). Oral administration of $^{15}\text{NH}_4\text{Cl}$ to a group of six subjects for six days resulted in absorption of at least 38.7% of the administered radioactivity as determined by the amount of ^{15}N that appeared in urinary urea within 24 hours of the last $^{15}\text{NH}_4\text{Cl}$ ingestion (Metges et al. 1999).

Ammonium ion is endogenously produced in the human digestive tract, much of it arising from the bacterial degradation of nitrogenous compounds from ingested food. About 4,200 mg/day are produced, greater than 70% of which is synthesized or liberated within the colon and its fecal contents. The total amount absorbed is about 4,150 mg/day, or 99% of the amount produced (Summerskill and Wolpert 1970); absorption after oral loading of NH_4^+ is similarly complete (Fürst et al. 1969). Evidence from Castell and Moore (1971) and Mossberg and Ross (1967) suggests that absorption of NH_4^+ increases as the pH of the contents of the lumen increases, and that the ammonium ion is actively transported at the lower pH levels (pH 5 was lowest detected absorption). Ammonium ion absorbed from the gastrointestinal tract travels via the hepatic portal vein directly to the liver, where in healthy individuals, most of it is converted to urea and glutamine. Human and animal data show that little of it reaches the systemic circulation as ammonia or ammonium compounds, but that it is a normal constituent of plasma at low levels (Brown et al. 1957; Pitts 1971; Salvatore et al. 1963; Summerskill and Wolpert 1970). Analysis of plasma drawn from 10 healthy young male subjects yielded endogenously derived NH_4^+ concentrations ranging from 30 to 55 $\mu\text{g NH}_3/100\text{ mL}$, with a mean of 39 $\mu\text{g}/100\text{ mL}$ (Brown et al. 1957).

3.4.1.3 Dermal Exposure

Quantitative data on absorption from exposure by the dermal route were not located in the available literature. Human case reports of dermal exposure describe local damage (burns, irritations). One report of case histories of five persons exposed to an exploding, bursting anhydrous ammonia gas pipe indicated there was systemic toxicity (vomiting, renal congestion, delirium), but exposure was by inhalation as well as dermal route, and it is impossible to delineate a systemic dermal exposure contribution (Slot 1938).

3. HEALTH EFFECTS

WHO (1986) concluded that systemic effects from skin and eye exposure are not quantitatively important. Ammonia is readily absorbed into the eye; it was found to diffuse within seconds into cornea, lens, drainage system, and retina (Beare et al. 1988; Jarudi and Golden 1973). However, amounts absorbed were not quantified, and absorption into systemic circulation was not investigated.

3.4.2 Distribution

3.4.2.1 Inhalation Exposure

No quantitative reports of distribution of ammonia from inhalation exposure were found in the available literature. Absorption data from human inhalation exposure suggest that only small amounts of ammonia are absorbed into the systemic circulation (Silverman et al. 1949; WHO 1986). Initial retention of inhaled ammonia in the mucus of the upper respiratory tract may be 80% or more, but after equilibrium is established (within 30 minutes) 70–80% of inspired ammonia is expired in exhaled air (Silverman et al. 1949). The lack of change in blood nitrogen compounds and urinary-ammonia compounds lends further support to a limited absorption into the systemic circulation (Silverman et al. 1949). Toxic effects reported from inhalation exposure suggest local damage, or changes resulting from necrotic tissue degradation, rather than the presence of elevated levels of NH_4^+ , *per se*, in tissues other than the respiratory/pharyngeal tissues. Information on the distribution of endogenously-produced ammonia suggests that any NH_4^+ absorbed through inhalation would be distributed to all body compartments via the blood, where it would be used in protein synthesis or as a buffer, and that excess levels would be reduced to normal by urinary excretion, or converted by the liver to glutamine and urea. If present in quantities that overtax these organs, NH_4^+ is distributed to other tissues and is known to be detoxified in the brain (Takagaki et al. 1961; Warren and Schenker 1964).

3.4.2.2 Oral Exposure

Human oral exposure data for NH_4^+ clearly indicate that it readily enters the portal circulation and is delivered to the liver (Conn 1972; Fürst et al. 1969), as has been shown to be the case for endogenously produced NH_4^+ (Pitts 1971; Summerskill and Wolpert 1970). In nitrogen-deficient persons, NH_4^+ (as ammonium acetate) administered orally was absorbed and carried directly to the liver where most of it was converted to urea and excreted in the urine; little change in the negative nitrogen balance was observed (Fürst et al. 1969). Output of urea from the liver corresponded to the amount of NH_4^+ ingested (Fürst et al. 1969).

3. HEALTH EFFECTS

Un-ionized ammonia is freely diffusible, whereas the ammonium ion is less so and is relatively confined to the extracellular compartment (Stabenau et al. 1958). However, ammonium ion is in dynamic equilibrium with dissolved ammonia. Therefore, ammonium compounds that enter the circulatory system or other body fluids can thus freely penetrate tissue cells as ammonia. In hypophysectomized rats that were administered ^{15}N -ammonium citrate orally by gavage, labeled protein was found in liver, kidney, spleen, heart, and skeletal muscle 6–72 hours after ^{15}N -ammonium citrate administration (Vitti et al. 1964). The percentages of ingested label absorbed and then excreted as urea in the urine were not provided (Vitti et al. 1964).

3.4.2.3 Dermal Exposure

No quantitative data on distribution of ammonia from dermal exposure were located in the available literature. Toxic effects from dermal exposure suggest that little or no ammonia gains entry into the systemic circulation by this route.

3.4.2.4 Other Routes of Exposure

Intravenous administration of NH_4^+ (as ammonium salts) to people with a nitrogen deficiency (in negative nitrogen balance) resulted in an increase in the peripheral blood NH_4^+ level and a shift in the nitrogen balance from negative to positive; no increase in urinary urea was seen (Fürst et al. 1969). The nitrogen from NH_4^+ , which gains entry into the general circulation, is distributed to cells throughout the body and incorporated into tissues (Fürst et al. 1969; Vitti et al. 1964). After intraperitoneal injection of ammonium chloride in mice, ammonia distributes to brain tissues within 20 seconds (Warren and Schenker 1964), and in rats, brain concentrations increase dramatically within 5 minutes (Salvatore et al. 1963). Tissues other than blood and brain were not analyzed by these researchers. Comparative patterns of distribution of ^{15}N -labeled ammonium citrate indicate that the amount of NH_4^+ taken up by tissues other than the liver is greatest by subcutaneous injection, less by intraperitoneal injection, and least following intragastric administration. Intravenous administration of ^{15}N -labeled ammonium salts leads to rapid distribution of ^{15}N -labeled metabolites throughout the body, with the highest levels of labeled urea appearing in the kidney and liver, and lesser amounts in heart, spleen, brain, testes, and carcass. Highest levels of labeled glutamine were found in heart and liver, with lesser amounts in brain, spleen, carcass, kidney, and testes (Duda and Handler 1958).

3. HEALTH EFFECTS

3.4.3 Metabolism

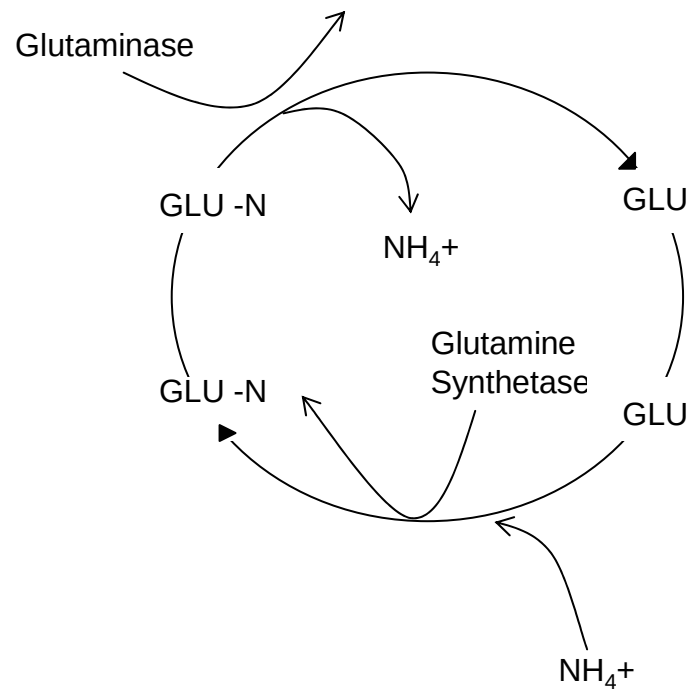
Quantitative data on human metabolism of exogenously introduced ammonia were not located in the available literature. Ammonia and ammonium ion are metabolized to urea and glutamine mainly in the liver by the process diagrammed in Figures 3-3 and 3-4 and described by Fürst et al. (1969) and Pitts (1971). However, it can be rapidly converted to glutamine in the brain and other tissues as well (Takagaki et al. 1961; Warren and Schenker 1964). The nitrogen is released from glutamine within tissue cells and used for protein synthesis as needed (Duda and Handler 1958; Fürst et al. 1969; Richards et al. 1975; Vitti et al. 1964). Ingestion of ammonium salts leads to almost complete conversion of ammonium ion into urea in the liver, whereas exposure by other routes may lead to its metabolism in body tissues to glutamine or tissue protein (Fürst et al. 1969; Vitti et al. 1964).

Duda and Handler (1958) administered 0.03 mg/kg body weight of ^{15}N -ammonium acetate intravenously to rats and noted that 90% was converted to glutamine and urea within 30 minutes, with glutamine being the major early product. Labeled nitrogen was also found in amino acids, purines, pyrimidines, and other nitrogenous compounds. Morimoto et al. (1988) found that the amount of ^{15}N from an intravenous injection of ammonium chloride to rats that was taken up into glutamine-amide-N and urea-N reached a peak at 5 minutes and decreased gradually from 15 to 60 minutes after the injection. This finding suggests that urea synthesis and glutamine synthesis occurred simultaneously within minutes after the injection, and glutamine-amide-N is gradually transferred to the urea cycle from 15 to 60 minutes following dosing. Low amounts (0.008% of a 17 mg oral dose) of ^{15}N -ammonium chloride administered repeatedly to rats were converted to ^{15}N -nitrate in the urine (Saul and Archer 1984).

3.4.4 Elimination and Excretion**3.4.4.1 Inhalation Exposure**

Studies using low levels of ammonia show that inhaled ammonia is temporarily dissolved in the mucus of the upper respiratory tract, and then a high percentage of it is released back into the expired air. Following exposure to 500 ppm ammonia for 10–27 minutes, healthy male subjects eliminated 70–80% of the inspired ammonia by this route (Silverman et al. 1949). Analysis of endogenous ammonia levels in the expired air of rats showed concentrations ranging from 10–353 ppb (mean=78 ppb) in nose-breathing animals (Barrow and Steinhagen 1980).

3. HEALTH EFFECTS

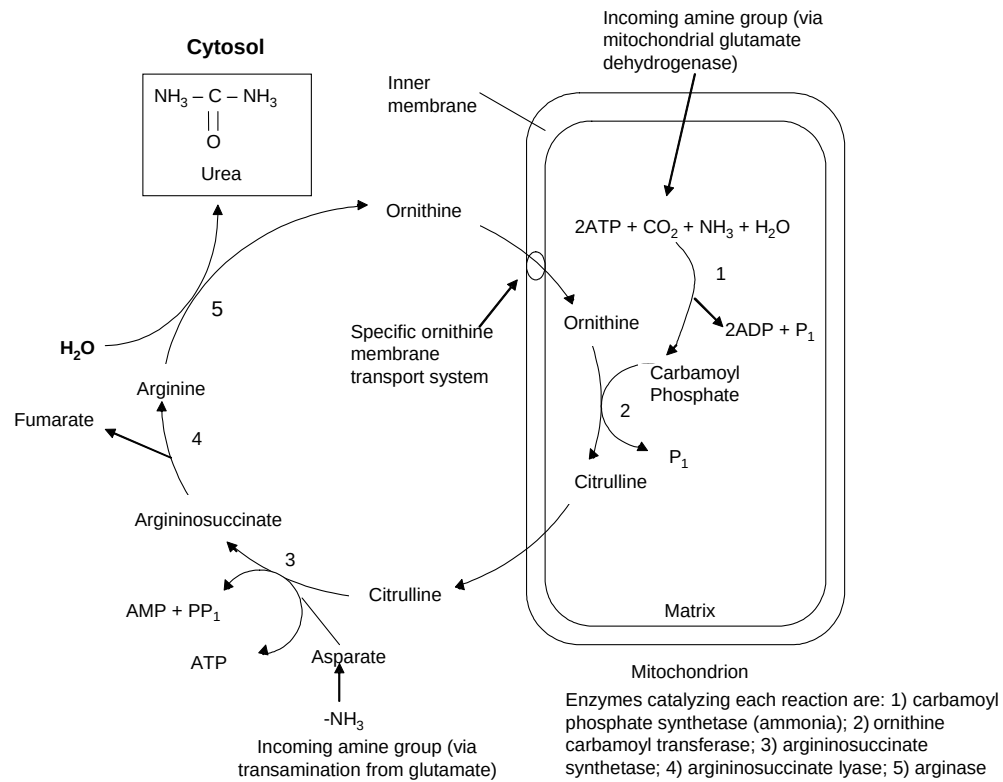
Figure 3-3. Glutamine Cycle

GLU = Glutamate; GLU-N = Glutamine

Source: Brunner and Thaler 1981

3. HEALTH EFFECTS

Figure 3-4. The Urea Cycle Showing the Compartmentalization of its Steps Within Liver Cells



Source: Lehninger 1975

3. HEALTH EFFECTS

The quantitative difference between inspired and expired ammonia suggests that small amounts are absorbed across the nasopharyngeal membranes into the systemic circulation. Absorbed ammonia is excreted by the kidneys as urea and urinary ammonium compounds (Gay et al. 1969; Pitts 1971; Richards et al. 1975; Summerskill and Wolpert 1970), as urea in feces (Richards et al. 1975), and as components of sweat (Guyton 1981; Wands 1981), but quantitative data are lacking. Toxic levels do not develop as a result of chronic inhalation exposure because the body has multiple effective mechanisms for detoxifying and excreting it.

3.4.4.2 Oral Exposure

Excretion data for humans orally exposed to ammonia have been quantified with respect to excretion of isotope from ^{15}N -labeled ammonium salts, thus providing an indication of the turnover rate of the compound within the body and excretion route of its metabolites. Approximately 72% of a dose of ^{15}N was excreted in the urine of three subjects within 3 days of ingestion of ammonium salts in drinking water; 25% (24% urinary urea and 1% urinary NH_4^+) was eliminated within the first 6 hours after exposure. Ammonium salt administered by gavage to humans led to a corresponding increase in blood urea concentration transported out of the liver, leading the authors (Fürst et al. 1969) to conclude that orally ingested ammonium salt is quickly and almost completely converted in the liver and eliminated from the body as urinary urea. Analysis of urine samples from subjects on high and low protein diets showed higher cumulative excretion of ^{15}N (percent of dose) in the urine of the high protein group (approximately 70%) than that of the low protein group (35%). Small amounts of labeled nitrogen were also excreted as urea in feces (Richards et al. 1975).

These data correspond to that for excretion of endogenously produced ammonia (Davies and Yudkin 1952; Muntwyler et al. 1956; Summerskill and Wolpert 1970; Van Slyke et al. 1943). Ammonia is also known to be excreted via sweat (Guyton 1981; Wands 1981) and expired air (Barrow and Steinhagen 1980; Larson et al. 1980; Robin et al. 1959; Utell et al. 1989); quantitative data are unavailable for excretion via sweat.

3.4.4.3 Dermal Exposure

Data regarding excretion of ammonia absorbed following dermal exposure were not located in the available literature.

3. HEALTH EFFECTS

3.4.4.4 Other Routes of Exposure

Data are available on exposure of humans and dogs to ammonium salts by intravenous injection. Excretion of isotope after ^{15}N -ammonium lactate injection in three human subjects yielded 5–7% of isotope excreted as urinary NH_4^+ in the first 6 hours postexposure, and another 2% within 3 days. Approximately 6% of the isotope was excreted as urea in urine in the first 6 hours. An average of approximately 60% of the dose of label was excreted in urine within 3 days. These data are considerably different from that resulting from oral loading (as described in Section 3.4.4.2). Intravenous loading led to decreased labeling of urinary urea and grossly increased labeling of urinary ammonia; the differences are attributed by the authors to a "first pass" effect from oral loading (Gay et al. 1969). The hepatic transformation of ammonium ion to urea is so efficient that relatively little unconverted ammonium salt is released to the general circulation.

Intravenous exposure of seven dogs to 107 mg/kg ammonium acetate led to amounts ranging from 0.044 to 0.073 mg ammonia excreted in expired air. No measurable amount of ammonia was present in expired air during the pre-exposure control period (Robin et al. 1959).

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

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

PBPK/PD models refine our understanding of complex quantitative dose behaviors by helping to delineate and characterize the relationships between: (1) the external/exposure concentration and target tissue dose of the toxic moiety, and (2) the target tissue dose and observed responses (Andersen et al. 1987; Andersen and Krishnan 1994). These models are biologically and mechanistically based and can

3. HEALTH EFFECTS

be used to extrapolate the pharmacokinetic behavior of chemical substances from high to low dose, from route to route, between species, and between subpopulations within a species. The biological basis of PBPK models results in more meaningful extrapolations than those generated with the more conventional use of uncertainty factors.

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

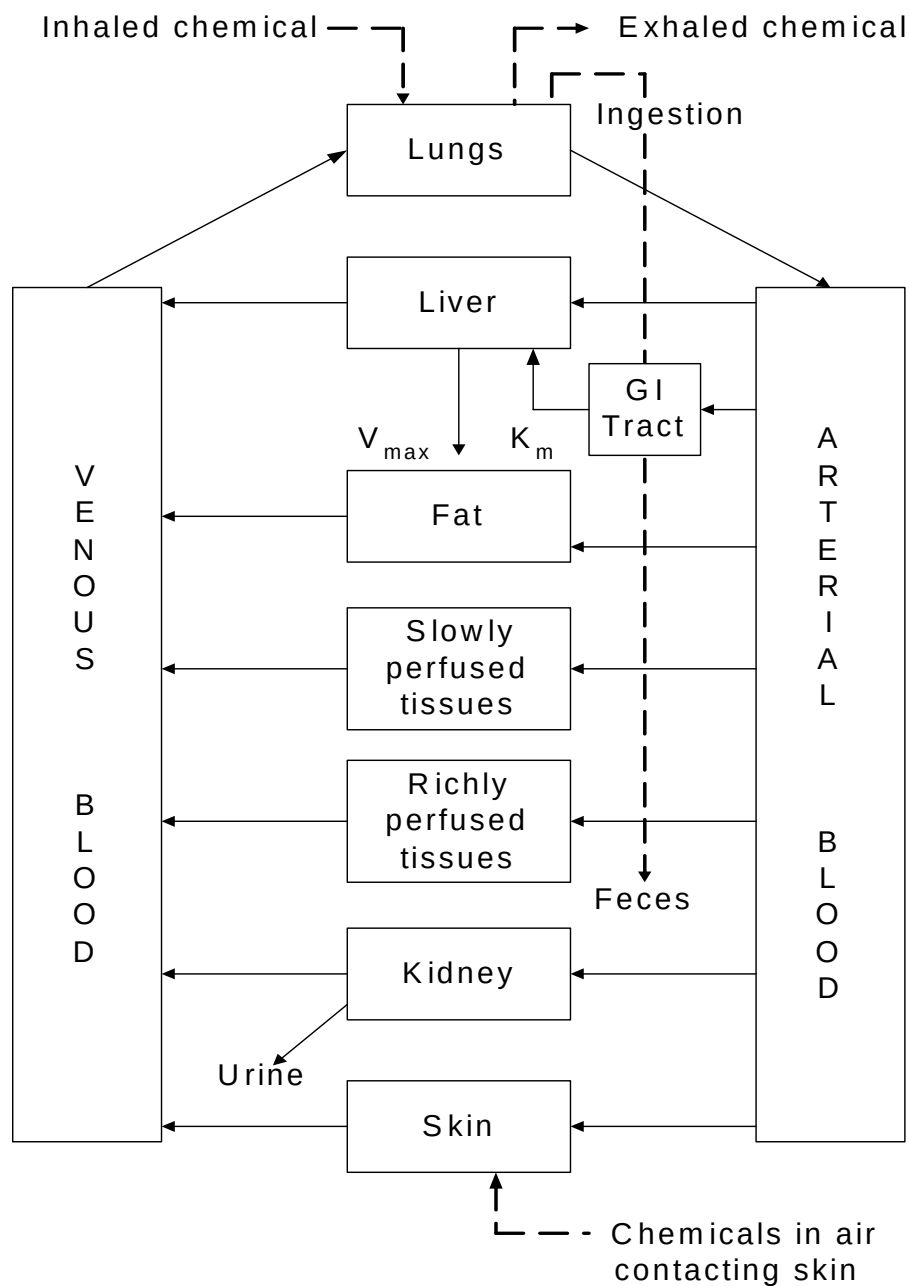
The structure and mathematical expressions used in PBPK models significantly simplify the true complexities of biological systems. If the uptake and disposition of the chemical substance(s) is adequately described, however, this simplification is desirable because data are often unavailable for many biological processes. A simplified scheme reduces the magnitude of cumulative uncertainty. The adequacy of the model is, therefore, of great importance, and model validation is essential to the use of PBPK models in risk assessment.

PBPK models improve the pharmacokinetic extrapolations used in risk assessments that identify the maximal (i.e., the safe) levels for human exposure to chemical substances (Andersen and Krishnan 1994). PBPK models provide a scientifically sound means to predict the target tissue dose of chemicals in humans who are exposed to environmental levels (for example, levels that might occur at hazardous waste sites) based on the results of studies where doses were higher or were administered in different species. Figure 3-5 shows a conceptualized representation of a PBPK model.

No data regarding PBPK models for ammonia were located.

3. HEALTH EFFECTS

Figure 3-5. Conceptual Representation of a Physiologically Based Pharmacokinetic (PBPK) Model for a Hypothetical Chemical Substance



Source: adapted from Krishnan et al. 1994

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

3. HEALTH EFFECTS

3.5 MECHANISMS OF ACTION**3.5.1 Pharmacokinetic Mechanisms**

Data regarding the pharmacokinetic mechanisms of ammonia were not located in the available literature.

3.5.2 Mechanisms of Toxicity

Ammonia is an irritant and the primary and most immediate effect of ammonia exposure is burns to the skin, eyes, and respiratory tract. The topical damage caused by ammonia is probably due mainly to its alkaline properties. Its high water solubility allows it to dissolve in moisture on the mucous membranes, skin, and eyes, forming ammonium hydroxide, which causes liquefaction necrosis of the tissues (Jarudi and Golden 1973). Specifically, ammonium hydroxide causes saponification of cell membrane lipids, resulting in cell disruption and death. Additionally, it extracts water from the cells and initiates an inflammatory response, which further damages the surrounding tissues (Amshel et al. 2000). Contact with liquid ammonia results in cryogenic injury in addition to the alkali burns (Amshel et al. 2000; Wibbenmeyer et al. 1999).

Excess circulating levels of ammonia (hyperammonemia) can cause serious neurological effects. Hyperammonemias can be inherited (i.e., inborn errors of urea cycle enzymes) or acquired (i.e., liver toxicity caused by ingested toxins, viral infections, autoimmune disease) (Felipo and Butterworth 2002). An extensively studied neuropsychiatric disorder known as hepatic encephalopathy develops when liver function is impaired and the organ cannot metabolize ammonia. This results in an increased concentration of ammonia in the blood and brain. The mechanism of ammonia-induced encephalopathies has not been definitively elucidated, but is thought to involve the alteration of glutamate metabolism in the brain and resultant increased activation of NMDA receptors (Felipo et al. 1993; Marcaida et al. 1992), which causes decreased protein kinase C-mediated phosphorylation of Na^+/K^+ ATPase, increased activity of Na^+/K^+ ATPase, and depletion of ATP (Kosenko et al. 1994). Antagonists of NMDA receptors, agonists of metabotropic glutamate receptors, agonists of muscarinic receptors, and inhibitors of protein kinase C, calcineurin, or nitric oxide synthase prevent glutamate toxicity, indicating that all of these play a role in acute ammonia neurotoxicity (Felipo et al. 1998). Additional evidence of altered energy levels include changes in some TCA cycle-associated components including acetoacetate, and NAD^+/NADH ratio, 2-oxoglutarate, and 3-hydroxybutyrate (Kosenko et al. 1993). A disruption in neurotransmission

3. HEALTH EFFECTS

has also been suggested by alteration of brain tubulin, which is an essential component of the axonal transport system (Miñana et al. 1989a, 1989b).

During certain disease states that result in renal tubular injury, NH_4^+ production by renal proximal tubules may increase in order to maintain net acid excretion. However, this may also contribute to further renal damage by modifying the third component of complement and initiating the alternative complement pathway (Clark et al. 1990). Ammonia can chemically interact with an internal thiolester bond of complement 3 (C3), resulting in an amide linkage and a subsequent conformational change of the C3. The altered C3 then activates the alternative complement pathway, which causes the release of chemoattractants and the assembly of the membrane attack complex of complement (Clark et al. 1990). Amidated C3 can also bind directly to phagocyte complement receptors, which causes the release of toxic oxygen species (Clark et al. 1990). It has also been suggested that NH_4^+ depresses protein degradation in renal cells and inhibits renal cell replication, which supports the findings of renal hypertrophy in renal injury and indicates that NH_4^+ may inhibit recovery from injury (Rabkin et al. 1993).

Ammonium ion may also contribute to adverse effects of *Helicobacter pylori* on the stomach. *H. pylori* produces urease, which breaks down urea that is normally present in the stomach into ammonia (Mégraud et al. 1992; Tsujii et al. 1992a). An *in vitro* study that examined the effects of ammonia produced by *H. pylori* on HEP2 cells showed increased cell vacuolation and viability of the cells compared to a urease-negative variant of the same cells (Mégraud et al. 1992). An *in vivo* study suggested that NH_4^+ also causes macroscopic gastric lesions and increases the release of endothelin-1 (ET-1) and thyrotropin releasing hormone (TRH) from the gastric mucosa, probably via an endothelin A (ET_A) receptor, which exerts ulcerogenic action on the gastric mucosa (Mori et al. 1998). Ammonia may also trigger the release of cysteine proteases in the stomach that contribute to the development of gastric hemorrhagic mucosal lesions (Nagy et al. 1996). Neutrophils that migrate to the gastric mucosa in response to the presence of *H. pylori* may release hypochlorous acid, which can interact with NH_4^+ to produce the powerful cytotoxic oxidizing agent monochloramine (Murakami et al. 1995).

3.5.3 Animal-to-Human Extrapolations

The primary effects of ammonia in humans are due to its corrosive and irritative properties. Exposure to ammonia gas causes damage to the respiratory tract, eyes, and skin when the ammonia combines with water to become ammonium hydroxide, which results in liquefaction necrosis of the tissues, cell structural breakdown, and inflammatory damage (Amshel et al. 2000; Wibbenmeyer et al. 1999). Animal studies

3. HEALTH EFFECTS

have indicated similar types of injuries of the respiratory tract (Coon et al. 1970; Kapeghian et al. 1982; Mayan and Merilan 1972; Richard et al. 1978a, 1978b; Schaerdel et al. 1983; Stombaugh et al. 1969), eyes, and skin (Morgan 1997).

Oral exposure of humans to high concentrations of ammonia and ammonium hydroxide has been shown to result in buccal, esophageal, and upper tracheal burns and edema (Christesen 1995; Klein et al. 1985; Rosenbaum et al. 1998), but no reports of the effects of ammonia on the stomach or upper gastrointestinal tract in humans have been found. One report of an ammonia enema in a human showed diffuse erythematous, friable mucosa, and large exudative ulcerations in the sigmoid colon and rectum (da Fonseca et al. 1998). Gavage studies in rats have shown similar lesions of the gastric mucosa with notable histopathological effects (Mori et al. 1998; Takeuchi et al. 1995; Tsujii et al. 1993). Rats, therefore, appear to be an adequate model for the primary effects of ammonia in humans. However, humans are unlikely to be orally exposed to amounts of ammonia that would result in the gastric lesions seen in rats. Elevated levels of endogenously produced ammonia resulting from disease states apparently may cause or contribute to gastric pathology (Mégraud et al. 1992; Mori et al. 1998; Tsujii et al. 1992a).

3.6 TOXICITIES MEDIATED THROUGH THE NEUROENDOCRINE AXIS

Recently, attention has focused on the potential hazardous effects of certain chemicals on the endocrine system because of the ability of these chemicals to mimic or block endogenous hormones. Chemicals with this type of activity are most commonly referred to as *endocrine disruptors*. However, appropriate terminology to describe such effects remains controversial. The terminology *endocrine disruptors*, initially used by Colborn and Clement (1992), was also used in 1996 when Congress mandated the Environmental Protection Agency (EPA) to develop a screening program for “...certain substances [which] may have an effect produced by a naturally occurring estrogen, or other such endocrine effect[s]...”. To meet this mandate, EPA convened a panel called the Endocrine Disruptors Screening and Testing Advisory Committee (EDSTAC), which in 1998 completed its deliberations and made recommendations to EPA concerning *endocrine disruptors*. In 1999, the National Academy of Sciences released a report that referred to these same types of chemicals as *hormonally active agents*. The terminology *endocrine modulators* has also been used to convey the fact that effects caused by such chemicals may not necessarily be adverse. Many scientists agree that chemicals with the ability to disrupt or modulate the endocrine system are a potential threat to the health of humans, aquatic animals, and wildlife. However, others think that endocrine-active chemicals do not pose a significant health risk, particularly in view of the fact that hormone mimics exist in the natural environment. Examples of

3. HEALTH EFFECTS

natural hormone mimics are the isoflavinoid phytoestrogens (Adlercreutz 1995; Livingston 1978; Mayr et al. 1992). These chemicals are derived from plants and are similar in structure and action to endogenous estrogen. Although the public health significance and descriptive terminology of substances capable of affecting the endocrine system remains controversial, scientists agree that these chemicals may affect the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body responsible for maintaining homeostasis, reproduction, development, and/or behavior (EPA 1997). Stated differently, such compounds may cause toxicities that are mediated through the neuroendocrine axis. As a result, these chemicals may play a role in altering, for example, metabolic, sexual, immune, and neurobehavioral function. Such chemicals are also thought to be involved in inducing breast, testicular, and prostate cancers, as well as endometriosis (Berger 1994; Giwercman et al. 1993; Hoel et al. 1992).

No studies were located regarding toxicity mediated through the endocrine axis in humans after exposure to ammonia. Two studies examined the effects of induced hyperammonemia (with infusion of ammonium chloride) in steers on circulating and portal-drained visceral flux of metabolites and on pancreatic hormones (Fernandez et al. 1988, 1990). Plasma glucose increased 12% during infusion of ammonium chloride (Fernandez et al. 1988, 1990). Plasma insulin decreased up to 46% during ammonium chloride infusion, and then increased up to 122% after infusion was halted (Fernandez et al. 1988); portal-drained visceral release of insulin did not increase during ammonium chloride infusion even with the rise in plasma glucose levels, but increased 109% after cessation of infusion (Fernandez et al. 1990). These data indicate that hyperammonemia in steers may cause reduced hepatic glucose output and glucose-mediated pancreatic insulin release.

No *in vitro* studies were located regarding toxicity mediated through the endocrine axis by ammonia.

3.7 CHILDREN'S SUSCEPTIBILITY

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

3. HEALTH EFFECTS

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

Children sometimes differ from adults in their susceptibility to hazardous chemicals, but whether there is a difference depends on the chemical (Guzelian et al. 1992; NRC 1993). Children may be more or less susceptible than adults to health effects, and the relationship may change with developmental age (Guzelian et al. 1992; NRC 1993). Vulnerability often depends on developmental stage. There are critical periods of structural and functional development during both prenatal and postnatal life and a particular structure or function will be most sensitive to disruption during its critical period(s). Damage may not be evident until a later stage of development. There are often differences in pharmacokinetics and metabolism between children and adults. For example, absorption may be different in neonates because of the immaturity of their gastrointestinal tract and their larger skin surface area in proportion to body weight (Morselli et al. 1980; NRC 1993); the gastrointestinal absorption of lead is greatest in infants and young children (Ziegler et al. 1978). Distribution of xenobiotics may be different; for example, infants have a larger proportion of their bodies as extracellular water and their brains and livers are proportionately larger (Altman and Dittmer 1974; Fomon 1966; Fomon et al. 1982; Owen and Brozek 1966; Widdowson and Dickerson 1964). The infant also has an immature blood-brain barrier (Adinolfi 1985; Johanson 1980) and probably an immature blood-testis barrier (Setchell and Waites 1975). Many xenobiotic metabolizing enzymes have distinctive developmental patterns. At various stages of growth and development, levels of particular enzymes may be higher or lower than those of adults, and sometimes unique enzymes may exist at particular developmental stages (Komori et al. 1990; Leeder and Kearns 1997; NRC 1993; Vieira et al. 1996). Whether differences in xenobiotic metabolism make the child more or less susceptible also depends on whether the relevant enzymes are involved in activation of the parent compound to its toxic form or in detoxification. There may also be differences in excretion, particularly in newborns who all have a low glomerular filtration rate and have not developed efficient tubular secretion and resorption capacities (Altman and Dittmer 1974; NRC 1993; West et al. 1948). Children and adults may differ in their capacity to repair damage from chemical insults. Children also have a longer remaining lifetime in which to express damage from chemicals; this potential is particularly relevant to cancer.

Certain characteristics of the developing human may increase exposure or susceptibility, whereas others may decrease susceptibility to the same chemical. For example, although infants breathe more air per kilogram of body weight than adults breathe, this difference might be somewhat counterbalanced by their

3. HEALTH EFFECTS

alveoli being less developed, which results in a disproportionately smaller surface area for alveolar absorption (NRC 1993).

Human and animal data indicate that the primary effects of ammonia are irritation and burns and that the primary targets of ammonia are the respiratory tract, eyes, and skin (Burns et al. 1985; Close et al. 1980; Couturier et al. 1971; de la Hoz et al. 1996; Flury et al. 1983; George et al. 2000; Hatton et al. 1979; Heifer 1971; Holness et al. 1989; Kerstein et al. 2001; Latenser and Lucktong 2000; Leduc et al. 1992; Millea et al. 1989; Morgan 1997; Price et al. 1983; Shimkin et al. 1954; Slot 1938; Sobonya 1977; Taplin et al. 1976; Verberk 1977; Weiser and Mackenroth 1989). There are limited data on the toxicity of ammonia in children and no information on effects in adults who were exposed as children. Children (8–9 years old) who attended two schools in the vicinity of a fertilizer plant had higher incidences of acute respiratory diseases than children who attended a school 20 kilometers away (Gomzi 1999; Gomzi and Šarić 1997). Incidence was related to levels of measured pollutants (ammonia, hydrogen fluoride, nitrogen dioxide, total suspended particulate matter, and smoke) in the inside and outside air (Gomzi and Šarić 1997). Forced expiratory volumes were not statistically different between the three schools (Gomzi and Šarić 1997). These results indicate that exposure to low levels of ammonia (0.04–0.23 ppm) or other airborne pollutants may not cause functional respiratory deficits, but may lower the resistance to respiratory pathogens in children. There is no indication that children are more susceptible to the effects of ammonia than adults. However, children have greater surface area to body weight and lung surface area to body weight ratios, and increased minute volume to weight ratio, so they may receive a higher dose than adults in the same situation. Children may also tend to be exposed longer than adults because they may not be as quick as adults to evacuate a contaminated area.

There are no studies that indicate that metabolism of ammonia differs between children and adults. Ammonia is eliminated from the body mainly by processing through the urea cycle in the liver, and urea is then eliminated in the urine and feces. The urea cycle is fully functional in infants at birth; therefore, it is not expected that infants or children are at greater risk of hyperammonemia. Neurotoxicity resulting from hyperammonemia involves alteration of levels of some components of the citric acid cycle, which leads to depletion of ATP, and starvation of brain cells, and depletion of glutamate, a precursor to the neurotransmitter γ -aminobutyrate (GABA). It is not expected that children are more susceptible than adults to ATP depletion via this mechanism.

Infants under 6 months of age may be more sensitive than adults to the effects of high levels of nitrates (from nitrification of ammonia in fertilizers) that may be present in groundwater and well water (Payne

3. HEALTH EFFECTS

1981). Infants who consume formula and food made with contaminated water from these sources may develop methemoglobinemia, which results in decreased delivery of oxygen to the tissues.

3.8 BIOMARKERS OF EXPOSURE AND EFFECT

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

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

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

3. HEALTH EFFECTS

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

3.8.1 Biomarkers Used to Identify or Quantify Exposure to Ammonia

There are no known specific biomarkers of exposure for ammonia. Identification of biomarkers of exposure to ammonia is confounded because large amounts of ammonia are produced endogenously. Pharmacokinetic studies reveal that after inhalation exposure to low levels of ammonia, BUN, nonprotein nitrogen, urinary-urea, and urinary-ammonia levels do not change (Silverman et al. 1949). Exposure to common occupational limits of ammonia in air (25 ppm) yield increased blood-ammonia levels only 10% above fasting levels (WHO 1986). In one human study, oral ingestion of ammonium chloride tablets (approximately 15 mg NH_4^+ /kg) yielded only a transient increase in blood-ammonia above fasting levels in 11 out of 20 subjects tested; no increase was observed in the remaining 9 subjects (Conn 1972).

3.8.2 Biomarkers Used to Characterize Effects Caused by Ammonia

Effect biomarkers of ammonia exposure are limited to site-of-contact tissue injuries. Upon inhalation exposure, distribution of ammonia is usually limited to the respiratory tract and involves irritation and, at higher concentrations, pulmonary edema and necrosis (Kapeghian et al. 1982; Richard et al. 1978b; Silverman et al. 1949). Oral exposure to high doses of ammonium chloride has produced pulmonary edema in animals (Koenig and Koenig 1949). Dermal exposure to ammonia causes skin and eye irritation and, at higher concentrations, necrosis (Amshel et al. 2000; da Fonseca et al. 1998; George et al. 2000; Kerstein et al. 2001; Latenser and Lucktong 2000; Leduc et al. 1992; Rosenbaum et al. 1998; Weiser and Mackenroth 1989). The severity of injuries by all routes of exposure are dose-related. Unfortunately, these effect biomarkers are not specific for ammonia and can be caused by a variety of caustic substances.

The tissues and organs most sensitive to ammonia exposure are mainly dependent on route of exposure. After inhalation exposure, which can involve a significant dermal exposure, the skin and eyes and the respiratory tract, including the lungs, are most sensitive. Direct dermal exposure produces dose-related effects from irritation to necrosis. Ingestion of ammonium hydroxide has resulted in oral, pharyngeal, and

3. HEALTH EFFECTS

esophageal lesions (Christesen 1995; Klein et al. 1985). The tissue and organ injuries produced by ammonia, however, are of limited value as biomarkers to characterize the effects caused by ammonia because many other caustic chemicals can produce similar injuries.

3.9 INTERACTIONS WITH OTHER CHEMICALS

Exposure to substances that would increase the pH of exposed tissues could be expected to enhance the alkalotic effects of ammonia, and vice versa. Agents acting to elevate the intestinal-tract pH would increase its local irritant effect, and would promote its absorption as well (Castell and Moore 1971).

Co-administration of ammonia and diethyl pyrocarbonate induced lung tumors in Kid:CFLP mice, while neither agent administered intragastrically and separately was carcinogenic; this effect is believed to be a result of a compound, urethane (a known carcinogen), produced by their interaction (Uzvolgyi and Bojan 1980, 1985). Sprague-Dawley rats given intrarectal doses of N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) and ammonium acetate had a higher incidence of tumors than did controls that were administered distilled water in place of ammonium acetate (Clinton et al. 1988). The role of acetate was not ruled out. Ammonia acted synergistically with potassium ions on pyruvate kinase, a known Ehrlich ascites tumor enzyme (Olavarria et al. 1986).

Some compounds play a synergistic role with ammonia in producing hepatic coma. Simultaneous injection of an ammonium salt and a fatty acid in Holtman or Sprague-Dawley rats produced coma at lower plasma levels than did injection of either compound separately. Inhalation of methanethiol or injection with sodium octanoate blocked metabolism of an injected dose of ammonium acetate and led to elevated blood ammonia levels (Zieve et al. 1974).

Data regarding exposure to mixtures of atmospheric contaminants indicate that, contrary to what might be expected, increased carbon dioxide concentration (up to 5% in air) does not alter the hyperventilatory rate induced by hyperammonemia in dogs (Herrera and Kazemi 1980). Ammonia in expired air may neutralize inhaled acid aerosols (EPA 1979; Larson et al. 1980; Utell et al. 1989).

Other substances to which people have been exposed have been shown to alter the toxic effects of ammonia. Methionine sulfoximine, administered by intraperitoneal injection, suppressed the tonic convulsions produced by intravenous injection of ammonium chloride in mice (Hindfelt and Plum 1975; Warren and Schenker 1964). Intraperitoneal injection of alpha-methylglutamic acid also exerts a

3. HEALTH EFFECTS

protective effect against hyperammonemia in rats (Lamar 1970). Nicotino-hydroxamic acid and neomycin administered orally reduce blood ammonia levels and increase excretion of urea in treated rats (Harada et al. 1985). Ethanol exerted a protective effect on acute ammonia intoxication in mice (O'Connor et al. 1982), although ethanol was reported to increase ammonia concentrations in body tissues of treated rats (Mohanachari et al. 1984).

Sodium benzoate decreased urea production in ammonia challenged rats (Maswoswe et al. 1986) and hyperammonemic mice (O'Connor et al. 1987). Valproate, a widely used antiepileptic drug, has a hyperammonemic effect in Wistar rats (Ferrier et al. 1988) and may therefore predispose to ammonia intoxication. Ammonia interferes with the metabolism of pent-4-enoic acid in cultured rat hepatocytes and may dramatically potentiate its toxicity (Coude and Grimber 1984).

3.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

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

Persons who suffer from severe liver or kidney disease may be susceptible to ammonia intoxication, as it is chiefly by the actions of these organs that NH_4^+ is biotransformed and excreted (Córdoba et al. 1998; Gilbert 1988; Jeffers et al. 1988); individuals with hereditary urea cycle disorders are also at risk (Schubiger et al. 1991). In these individuals, the levels produced endogenously are sufficient to produce toxicity. Levels that are likely to be encountered in the environment, with the exception of those resulting from high-level accidental exposures, are insignificant, due to the low absorption rate, in comparison with levels produced within the body (WHO 1986).

Since ammonia is a respiratory tract irritant, persons who are hyperreactive to other respiratory irritants, or who are asthmatic, would be expected to be more susceptible to ammonia inhalation effects. The results of an epidemiological study of a group of workers chronically exposed to airborne ammonia indicate that ammonia inhalation can exacerbate existing symptoms including cough, wheeze, nasal complaints, eye irritation, throat discomfort, and skin irritation (Ballal et al. 1998).

3. HEALTH EFFECTS

3.11 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to ammonia. However, because some of the treatments discussed may be experimental and unproven, this section should not be used as a guide for treatment of exposures to ammonia. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice. The following texts provide general information about treatment following exposures alkalies:

Ellenhorn MJ, Barceloux DG. 1997. Medical toxicology: Diagnosis and treatment of human poisoning. New York, NY: Elsevier.

Haddad LM, Shannon MW, Winchester JF, eds. 1998. Clinical management of poisoning and drug overdose. 3rd ed. Philadelphia, PA: W.B. Sanders Company.

3.11.1 Reducing Peak Absorption Following Exposure

Absorption of ammonia via dermal exposure is not sufficient to be of concern, but immediate flushing of exposed skin with water or saline will limit dermal damage and reduce dermal absorption of ammonia. It is highly unlikely that enough ammonia could be ingested to be of danger via absorption from the intestines; however, in individuals with liver disease, endogenous production of ammonia may cause toxicity. Emesis should not be induced in case of ingestion of ammonia, but administration of activated charcoal, gastric lavage, or neutralization with weak acids is recommended HSDB (2003). Elimination of urease-producing enteric bacteria with oral antibiotics decreases the amount of ammonia absorbed from the gut (Gilbert 1988). Because ammonia is readily soluble in water at low concentrations, very little may reach and be absorbed in the lungs. Instead, ammonia at low concentrations may be absorbed in the mucosa of the upper respiratory tract and swallowed. Movement to an area of fresh air as quickly as possible would limit respiratory damage and absorption via the lungs.

3. HEALTH EFFECTS

3.11.2 Reducing Body Burden

No experimental data regarding methods for reducing the ammonia body burden were located. In healthy people, ammonia is efficiently metabolized via the urea cycle, primarily in the liver, and eliminated in the urine and feces (Fürst et al. 1969; Richards et al. 1975).

3.11.3 Interfering with the Mechanism of Action for Toxic Effects

The primary effects of ammonia are related to its alkalinity and its solubility in water, which results in rapid and severe tissue damage. It is extremely important to get to an area free of ammonia gas and to remove all clothing contaminated with ammonia as quickly as possible. Skin and eyes should be irrigated with water for at least 15–20 minutes at the time of exposure and periodically for 24 hours after exposure (Millea et al. 1989). Irrigation of the eye should continue until the pH of the conjunctival sac is less than 8.5 (Grant 1974). This should be followed with proper medical treatment for respiratory symptoms and dermal and ocular burns.

3.12 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of ammonia is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of ammonia.

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

3. HEALTH EFFECTS

3.12.1 Existing Information on Health Effects of Ammonia

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to ammonia are summarized in Figure 3-6. The purpose of this figure is to illustrate the existing information concerning the health effects of ammonia. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not necessarily imply anything about the quality of the study or studies, nor should missing information in this figure be interpreted as a “data need”. A data need, as defined in ATSDR’s Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles (Agency for Toxic Substances and Disease Registry 1989), is substance-specific information necessary to conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

Information regarding health effects of ammonia in humans consists largely of case reports of fatalities or illnesses following massive inhalation and/or dermal exposures resulting from accidental explosions or leakages. A few controlled studies have been conducted on inhalation and oral exposure effects. Health effects of ammonia in animals have been investigated in numerous inhalation studies, and a few oral and dermal exposure studies. Clearly, ammonia is an acutely toxic chemical in high concentrations. As indicated in Figure 3-6, available data address these concerns, both in humans and animals. The data indicate that airway blockage, edema, burns, and lesions of tissues directly exposed to ammonia or NH_4^+ are the most prominent ammonia-related effects. Secondary effects include liver and kidney damage, along with decreased resistance to infection.

3.12.2 Identification of Data Needs

Acute-Duration Exposure. The available human and animal data provide strong evidence that acute-duration exposure to ammonia can result in site-of-contact lesions primarily of the skin, eyes, and respiratory tract. In some cases, exposure to very high ammonia concentrations has resulted in death (Arwood et al. 1985; Walton 1973). Respiratory tract irritation (Burns et al. 1985; Close et al. 1980; Couturier et al. 1971; de la Hoz et al. 1996; Ferguson et al. 1977; George et al. 2000; Hatton et al. 1979; Heifer 1971; Kerstein et al. 2001; Latenser and Lucktong 2000; Leduc et al. 1992; Millea et al. 1989; Morgan 1997; Price et al. 1983; Sekizawa and Tsubone 1994; Sobonya 1977; Taplin et al. 1976; Verberk 1977; Weiser and Mackenroth 1989) and impaired pulmonary function (Kass et al. 1972; Silverman et al. 1949) have been observed in humans acutely exposed to ammonia gas. Animal studies support the

3. HEALTH EFFECTS

Figure 3-6. Existing Information on Health Effects of Ammonia

	Systemic									
	Death	Acute	Intermediate	Chronic	Immunologic	Lymphoretic	Neurologic	Reproductive	Developmental	Genotoxic
Inhalation	●	●	●	●	●	●			●	●
Oral	●	●								
Dermal	●	●								●

Human

	Systemic									
	Death	Acute	Intermediate	Chronic	Immunologic	Lymphoretic	Neurologic	Reproductive	Developmental	Genotoxic
Inhalation	●	●	●	●	●	●	●			
Oral	●	●	●			●		●	●	●
Dermal	●	●								

Animal

● Existing Studies

3. HEALTH EFFECTS

identification of the respiratory tract as a sensitive target of toxicity (Coon et al. 1970; Kapeghian et al. 1982; Mayan and Merilan 1972; Richard et al. 1978a, 1978b; Schaerdel et al. 1983; Stombaugh et al. 1969). Nonrespiratory tract effects (e.g., cardiovascular effects, renal effects) have also been observed following inhalation exposure. However, these effects were not consistently observed or may be secondary to the respiratory tract damage. Additional studies would be useful to assess the potential toxicity of NH_4^+ to remote tissues. The available acute data were considered adequate to derive an acute-duration inhalation MRL (Verbeck et al. 1977). No acute-duration oral MRL was derived for NH_4^+ . The acute-duration oral database consists of case reports with no dose information (Klein et al. 1985; Klendshoj and Rejent 1966; Lopez et al. 1988) and several animal studies that examined a limited number of end points (Noda and Chikamori 1976), involved a single exposure resulting in no effect, serious effects, or unsupported effects (Benyajati and Goldstein 1975; Koenig and Koenig 1949), and a repeated exposure study that found effects at high dosages (Barzel 1975). In addition, many animal studies have been conducted with ammonium chloride, a substance widely used experimentally to induce metabolic acidosis in animals. Metabolic acidosis is not a consequence of the ammonium ion, but is due to the formation of hydrogen chloride. Metabolic acidosis can affect the lungs, kidney, nervous system, liver, and bone. Ingestion of concentrated ammonia will cause irritation and damage to the mouth, throat, and gastrointestinal tract. Given the levels of ammonia in the environment, such exposure scenario is unlikely. Additional oral exposure studies do not seem warranted at this time. The available data on the dermal toxicity of ammonia suggest that the skin is a sensitive target of toxicity. Cutaneous burns have been reported in humans exposed to ammonia liquid and/or airborne ammonia (Amshel et al. 2000; da Fonseca et al. 1998; George et al. 2000; Kerstein et al. 2001; Latenser and Lucktong 2000; Leduc et al. 1992; Rosenbaum et al. 1998; Weiser and Mackenroth 1989). Ocular effects (inflamed eyes, lacrimation, swelling of the eyelids, transient blindness, blurred vision, and corneal abrasions) have been reported in humans exposed to ammonia (Beare et al. 1988; Caplin 1941; Close et al. 1980; Ferguson et al. 1977; Grant 1974; Hatton et al. 1979; Jarudi and Golden 1973; Kass et al. 1972; Latenser and Lucktong 2000; Legters et al. 1981; Levy et al. 1964; McGuinness 1969; Montague and Macneil 1980; Price et al. 1983; Silverman et al. 1949; Slot 1938; Sobonya 1977; Stombaugh 1969; Stroud 1981; Verberk 1977; Ward et al. 1983; Yang et al. 1987). Additional studies to examine the dermal toxicity of ammonia are unlikely to provide new key information.

Intermediate-Duration Exposure. Information on the toxicity of inhaled ammonia in humans exposed for an intermediate duration is limited to a case report of an individual who developed asthma-like symptoms following exposure to ammonia gas for 5 months (Lee et al. 1993) and a study with volunteers exposed intermittently for 5–6 weeks (Ferguson et al. 1977). The latter study found no

3. HEALTH EFFECTS

significant changes in pulmonary function in subjects exposed to up to 100 ppm ammonia, but eye and throat irritation occurred at ≥ 50 ppm. Because some uncertainties regarding the study design and reporting, the Fergusson et al. (1977) study could not be used as basis for an intermediate-duration inhalation MRL. Several animal studies examined the toxicity of ammonia following intermittent or continuous exposure to ammonia. As with acute-duration exposure, these studies suggest that the respiratory tract is the most sensitive target of toxicity. Symptoms of irritation, nasal lesions, dyspnea, and pulmonary inflammation have been observed in several animal species (Broderick et al. 1976; Coon et al. 1970; Drummond et al. 1980; Gaafar et al. 1992; Sjöblom et al. 1999; Stombaugh et al. 1969). In general, the concentrations used in these studies were higher than the lowest adverse effect levels identified for acute-duration exposure. No intermediate-duration oral MRL was derived for some of the same reasons mentioned under acute-duration exposure. No human studies or reports of intermediate-duration oral exposure to NH_4^+ were located. Animal studies have reported decreases in body weight gain in rats exposed via drinking water (Gupta et al. 1979) or diet (Boyano-Adanez et al. 1996). It should be mentioned that Gupta et al. (1979) administered ammonium sulfamate to the rats. Ammonium sulfamate is an herbicide for which there is little toxicity information in the open literature. The EPA (IRIS 2004) has derived an oral RfD for the sulfamate moiety based on the results of Gupta et al. (1979). Following gavage administration of ammonium salts, bone, blood pressure, adrenal gland, and renal effects have been observed in early studies of generally poor quality (Bodansky et al. 1932; Fazekas 1939; Seegal 1927). Additional intermediate-duration oral low to moderate dose studies are unlikely to provide new valuable information. No intermediate-duration dermal exposure studies were identified. Based on the irritant properties of ammonia, it is reasonable to assume that direct contact of the skin with ammonia for a prolonged time will produce irritation.

Chronic-Duration Exposure and Cancer. Several studies have examined the relationship between chronic exposure to ammonia in the air and respiratory effects. Studies of farmers working in enclosed livestock facilities provide evidence that ammonia may contribute to transient respiratory distress (Choudat et al. 1994; Cormier et al. 2000; Donham et al. 1995, 2000; Heederik et al. 1990, 1991; Melbostad and Eduard 2001; Reynolds et al. 1996; Vogelzang et al. 1997, 2000); however, co-exposure to total dust, respirable dust, carbon dioxide, total endotoxins, respirable endotoxins, fungi, bacteria, and/or molds complicates the interpretation of these studies. A study of workers at a fertilizer production facility found an association between respiratory effects and ammonia exposure at levels of ammonia higher than 25 ppm (Ballal et al. 1998). Another long-term study of workers exposed to an average of 9.2 ppm ammonia did not find respiratory effects (Holness et al. 1989). A chronic-duration inhalation MRL was derived based on the findings of Holness et al. (1989). Animal studies examining the chronic

3. HEALTH EFFECTS

toxicity of inhaled ammonia were not identified. No chronic-duration oral or dermal data were located. A need for such studies is not apparent at this time.

There are limited data to assess the carcinogenic potential of ammonia. Nasal cancer was reported in an individual accidentally exposed to a refrigeration-oil mixture, but the role of ammonia, if any, is unknown (Shimkin et al. 1954). Animal carcinogenicity data consist of several oral exposure studies. Ammonia was not found to increase the occurrence of tumors following oral exposure to relatively low doses (Toth 1972; Uzvolgyi and Bojan 1980). Another study found evidence that ammonia administered as drinking fluid may act as a cancer promoter (Tsujii et al. 1992a, 1995). The dose of ammonia administered can be estimated at 200 mg/day, compared to the estimated 0.36 mg/day from water ingestion for the general population (WHO 1986). The relevance of these studies to exposures in humans is unknown. The available information does not suggest that ammonia is carcinogenic, but well-designed studies in animals have not been conducted and may be warranted.

Genotoxicity. Data on the genotoxicity of ammonia in humans are limited to a study of workers at a fertilizer factory that found an increase in clastogenic effects (Yadav and Kaushik 1997). *In vivo* animal data consist of a study in mice that found alterations in the occurrence of micronuclei (Yadav and Kaushik 1997) and several studies in *D. melanogaster* that resulted in a positive response for mutagenic lethality (Lobasov and Smirnov 1934), but negative responses for sex-linked recessive lethal mutations and dominant lethality (Auerbach and Robson 1947). *In vitro* studies revealed positive responses for genotoxicity in *E. coli* (Demerec et al. 1951), and chick (Rosenfeld 1932) and mouse (Capuco 1977; Vissek et al. 1972) fibroblasts. It would be valuable to further assess the genotoxicity of ammonia with mutagenicity assays in *S. typhimurium* and *in vitro* and/or *in vivo* tests for chromosomal aberrations in mammalian systems.

Reproductive Toxicity. No information was located regarding reproductive effects of ammonia in humans. Reproductive toxicity data in animals are limited to a study in pigs exposed prior to mating and until gestation day 30 (Diekman et al. 1993). This study did not find alterations in fetus-to-corpus luteum ratio, number of live fetuses, or ovarian or uterine weights (6 weeks of exposure only). This study is not adequate for assessing reproductive toxicity because very low concentrations were used, there were no unexposed controls, and only females were exposed to ammonia. Additional studies are needed to assess ammonia's potential to induce reproductive effects.

3. HEALTH EFFECTS

Developmental Toxicity. No information was located regarding developmental effects of ammonia in humans and very limited data were located for animals. No alterations in number of live fetuses or fetal length were observed in a study of pigs exposed to a relatively low concentration of ammonia for 6 weeks prior to mating and until gestation day 30 (Diekman et al. 1993). A reduction in body weight gain was observed in the offspring of rats orally exposed to high doses of ammonia, but no information was provided regarding the health of the dams (Miñana et al. 1995). Developmental toxicity studies are needed to assess the potential of ammonia to damage the developing organism.

Immunotoxicity. Secondary infection has been observed in humans who have received severe burns from exposure to highly concentrated aerosols derived from ammonia (Sobonya 1977; Taplin et al. 1976). It is not known if this represents a primary effect on the immune system in humans since necrosis of exposed tissues facilitates infection by pathogenic organisms. Animal studies have shown that exposure to airborne ammonia may impair immune function (Broderick et al. 1976; Gustin et al. 1994; Richard et al. 1978a; Targowski et al. 1984). No oral immunotoxicity data were located. The available data provide suggestive evidence that ammonia may be an immunotoxicant. It would be valuable to assess the potential for immunotoxicity of ammonia with a battery of immune function tests.

Neurotoxicity. Neurological effects have been observed in humans who received extensive and serious burns from exposure to anhydrous ammonia (George et al. 2000; Hatton et al. 1979; Latenser and Lucktong 2000; White 1971). These effects may be secondary to trauma, rather than direct effects of ammonia on the central nervous system. There are limited data on the potential of NH_4^+ to induce overt neurological effects in animals. A decrease in motor activity has been observed in rodents following an acute exposure to low levels of airborne ammonia (Tepper et al. 1985); no overt signs of neurological impairment were observed following sublethal inhalation exposure (Coon et al. 1970). However, numerous animal studies have found evidence that orally administered ammonia may disrupt normal energy production in the brain and impair neurotransmitter receptors (Bodega et al. 1991; Boyano-Adáñez et al. 1996; Kimura and Budka 1986; Kosenko et al. 1993; Kretzschmar et al. 1985; Miñana et al. 1989b; Sobel et al. 1981; Suárez et al. 1992). Additional studies following inhalation and oral exposure would be useful to determine if the neurochemical alterations would result in clinical impairment. No dermal studies examining neurological end points were identified.

Epidemiological and Human Dosimetry Studies. Several studies have examined the toxicity of airborne ammonia in workers (Ballal et al. 1998; Choudat et al. 1994; Cormier et al. 2000; Donham et al. 1995, 2000; Heederik et al. 1990, 1991; Holness et al. 1989; Melbostad and Eduard 2001; Reynolds et al.

3. HEALTH EFFECTS

1996; Vogelzang et al. 1997, 2000). These studies have primarily focused on the respiratory tract, which is the most sensitive target of toxicity. Interpretation of many of these studies is complicated by co-exposure to other chemicals and microorganisms. In addition to these studies, there are reports of acute-duration exposure to ammonia via inhalation (Burns et al. 1985; Close et al. 1980; Couturier et al. 1971; de la Hoz et al. 1996; Ferguson et al. 1977; George et al. 2000; Hatton et al. 1979; Heifer 1971; Kass et al. 1972; Kerstein et al. 2001; Latenser and Lucktong 2000; Leduc et al. 1992; Millea et al. 1989; Morgan 1997; O’Kane 1983; Price et al. 1983; Sekizawa and Tsubone 1994; Sobonya 1977; Taplin et al. 1976; Verberk 1977; Weiser and Mackenroth 1989), ingestion (Klein et al. 1985; Klendshoj and Rejent 1966; Lopez et al. 1988), dermal contact (Amshel et al. 2000; da Fonseca et al. 1998; George et al. 2000; Kerstein et al. 2001; Latenser and Lucktong 2000; Leduc et al. 1992; Rosenbaum et al. 1998; Weiser and Mackenroth 1989), or ocular contact (Beare et al. 1988; Caplin 1941; Close et al. 1980; Ferguson et al. 1977; Grant 1974; Hatton et al. 1979; Jarudi and Golden 1973; Latenser and Lucktong 2000; Legters et al. 1981; Levy et al. 1964; McGuinness 1969; Montague and Macneil 1980; Price et al. 1983; Silverman et al. 1949; Slot 1938; Sobonya 1977; Stombaugh 1969; Stroud 1981; Verberk 1977; Ward et al. 1983; Yang et al. 1987). These studies suggest that the most sensitive target is the site of contact. The carcinogenic potential of ammonia has not been assessed in humans. There are several subpopulations of individuals exposed to higher than normal levels of ammonia; these groups include farmers and communities living near fertilizer plants. Studies of these groups that involved examination for a variety of potential effects could provide useful information on the toxicity of ammonia in humans. In addition, if the study group included both children and adults, these data would address the issue of age-related differences in toxicity.

Biomarkers of Exposure and Effect.

Exposure. There are no known specific biomarkers of exposure for ammonia in humans or animals. Furthermore, no evidence for alterations in clinical indices of body ammonia or nitrogen levels after exposure to exogenous ammonia has been reported. It does not seem useful at this time to develop biomarkers of exposure for ammonia because after exposure to low levels, ammonia is either rapidly cleared from the body or metabolized to compounds found endogenously at appreciable levels. Exposure to high concentrations is immediately and overtly toxic, which eliminates the need for a more subtle biomarker.

3. HEALTH EFFECTS

Effect. There are no known specific biomarkers of effect for ammonia in humans or animals. Lesions produced by exposure to high concentrations of ammonia are similar to those produced by other caustic substances.

Absorption, Distribution, Metabolism, and Excretion. Measurement of ammonia absorption is complicated by the appreciable levels of endogenously produced ammonia. Although, most of the inhaled ammonia is retained in the tissues of the upper respiratory tract, inhalation exposure to low levels of ammonia can result in a small amount of absorption (Silverman et al. 1949). As the ammonia concentration increases, the ability of the upper respiratory tract to retain ammonia is saturated, and a larger percentage is absorbed into the blood stream (Silverman et al. 1949). Absorption into the systemic circulation after oral exposure is limited (Metges et al. 1999). Ammonium ions absorbed from the gastrointestinal tract travels via the hepatic portal vein directly to the liver where, in healthy individuals, most of it is converted to urea and glutamine. Although it has not been extensively studied, dermal absorption of ammonia does not occur to a great extent; WHO (1986) concluded that systemic effects from skin and eye exposure to ammonia are not quantitatively important. Data are not available to assess the distribution of ammonia in humans or animals. Studies examining the distribution of ammonia would be useful for identifying potential targets of toxicity. Studies on endogenously produced ammonia, however, indicate that it is distributed to most of the organs and tissues of the body. Extensive work has been completed on the metabolism of ammonia and its participation in the glutamine cycle and the urea cycle (Duda and Handler 1958; Fürst et al. 1969; Richards et al. 1975; Vitti et al. 1964). Data regarding excretion are limited but it is known that ammonia inhaled at low levels is excreted primarily unchanged in the expired breath (Silverman et al. 1949); NH_4^+ absorbed from the gastrointestinal tract is excreted primarily in the urine as urea and other urinary nitrogen compounds (Gay et al. 1969; Pitts 1971; Richards et al. 1975; Summerskill and Wolpert 1970). No information regarding excretion after dermal exposure was located.

Comparative Toxicokinetics. Available data indicate that ammonia has similar targets of toxicity in humans and animals. Ammonia is most hazardous as a site-of-contact toxicant; therefore, the respiratory system is most vulnerable after inhalation exposure, the gastrointestinal tract is most vulnerable after oral exposure, and the skin and eyes are most vulnerable after dermal/ocular exposure. Limited human and animal data are available for toxicokinetics; however, these data indicate that humans and animals are probably very similar regarding the toxicokinetic disposition of ammonia. Furthermore, it is reasonable to expect, especially given the biochemical importance of ammonia, that humans and animals would handle this compound similarly.

3. HEALTH EFFECTS

Methods for Reducing Toxic Effects. There are limited specific data on reducing the toxic effects of ammonia. Many of the methods are generic approaches, such as getting to an area with fresh air and removal of contaminated clothing. No methods were identified for reducing the body burden or interfering with the mechanisms of toxicity. Studies designed to identify methods for interfering with the damage associated with direct contact with ammonia would be useful.

Children's Susceptibility. There are limited data on the toxicity of ammonia in children and no information on effects in adults who were exposed as children. A higher incidence of respiratory diseases was found in school children exposed to airborne ammonia and other chemicals (Gomzi 1999; Gomzi and Šarić 1997). There is no indication that children are more susceptible to the effects of ammonia than adults; studies of children and adults exposed to ammonia would be useful for assessing potential age-related differences in ammonia toxicity.

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

3.12.3 Ongoing Studies

Ongoing studies pertaining to ammonia have been identified and are shown in Table 3-5.

3. HEALTH EFFECTS

Table 3-5. Ongoing Studies on the Health Effects of Ammonia

Investigator	Affiliation	Research description	Sponsor
Seashore MR	Yale University	Sodium phenylbutyrate treatment of inborn errors of ammonia metabolism	National Center for Research Resources
Wall SM	University of Texas Health Science Center	NH ₄ ⁺ transport in renal inner medullary collecting duct	National Institute of Diabetes and Digestive and Kidney Diseases
Matthews JB	University of Cincinnati	Intestinal secretion and inflammation; impact of ammonia	National Institute of Diabetes and Digestive and Kidney Diseases
Wall SM	University of Texas Health Science Center	Renal net acid secretion and Na ⁺ /K ⁺ /2Cl ⁻ cotransporter	National Institute of Diabetes and Digestive and Kidney Diseases
Weiner ID	University of Florida	Localization of ammonia transporters in human liver	National Institute of Diabetes and Digestive and Kidney Diseases
Rauschel FM	Texas A&M University System	Mechanism and control of urea biosynthesis	National Institute of Diabetes and Digestive and Kidney Diseases
Hammon DS	Utah State University, Animal Dairy and Vet Science	Gamete and embryo toxic effects of ammonium in cattle	Animal Health Award
Nagami GT	Department of Veterans Affairs, Medical Center West Los Angeles, California	Effect of angiotensin II on ammonia production by tubule cells. Effect of acidosis on renin-angiotensin system	Department of Veterans Affairs, Research and Development, Washington, DC
Nagami GT	Department of Veterans Affairs, Medical Center West Los Angeles, California	Ammonia production and transport by the proximal tubule	Department of Veterans Affairs, Research and Development, Washington, DC
Feldman GM	Department of Veterans Affairs, Medical Center Richmond, Virginia	Colonic transport of bicarbonate, ammonium and small organic anions	Department of Veterans Affairs, Research and Development, Washington, DC
Walsch PJ	University of Miami	Mechanism of tolerance of extreme ammonia	National Institute of Environmental Health Sciences
Weiner ID	Department of Veterans Affairs, Medical Center Gainesville, Florida	Effect of ammonia on IMCD H-K-ATPase. Expression of an ammonia-sensitive protein in brain that mediates ammonia's neurological effects	Department of Veterans Affairs, Research and Development, Washington, DC
Sastrasinh S	Department of Veterans Affairs, Medical Center East Orange, New Jersey	Na ⁺ /H ⁺ antiport in renal mitochondria	Department of Veterans Affairs, Research and Development, Washington, DC

Source: FEDRIP (2003)

4. CHEMICAL AND PHYSICAL INFORMATION

4.1 CHEMICAL IDENTITY

Data pertaining to the chemical identity of ammonia are presented in Table 4-1. These data are for ammonia in its pure gaseous state (i.e., anhydrous ammonia). Ammonia is also commercially and commonly available as an aqueous solution; the most common commercial formulation is 28–30% NH_3 (Weast et al. 1988). At this concentration, ammonia forms a nearly saturated solution in water. Data on ammonia in aqueous solution, ammonium hydroxide, and ammonium ion are also included in Table 4-1 where appropriate.

4.2 PHYSICAL AND CHEMICAL PROPERTIES

Ammonium hydroxide is a weak base that is partially ionized in water according to the equilibrium:



The dissociation constant, K_b , is 1.774×10^{-5} at 25 °C ($\text{p}K_b$ is 4.751) and increases slightly with increasing temperature (Weast et al. 1988). At pH 9.25 half of the ammonia will be un-ionized (NH_3) and half will be ionized (NH_4^+). At pH 8.25 and 7.25, 90, and 99% of the ammonia will be ionized, respectively. Therefore, at most environmentally significant pHs, ammonia will be largely ionized; the fraction of un-ionized ammonia will become increasingly more important at pHs above 7. As a result, many physical and chemical properties will be a function of pH. For example, the solubility of ammonia in water will increase with decreasing pH. The volatility of ammonia increases with increasing pH; therefore, it volatilizes freely from solution at high pH values. Ammonium salts such as chloride, nitrate, and sulfate are strongly dissociated and very soluble in water (Weast et al. 1988); therefore, changes in pH will not normally result in the formation of ammonium precipitates.

The physical and chemical properties of ammonia are presented in Table 4-2. Also included are some chemical and physical properties of ammonia in solution. Ammonia in solution is widely available, and it is often referred to as ammonium hydroxide and has been also historically referred to as “spirit of hartshorn” (Windholz 1983).

4. CHEMICAL AND PHYSICAL INFORMATION

Table 4-1. Chemical Identity of Ammonia

Characteristic	Information	Reference
Chemical name	Ammonia	
Synonym(s)	Anhydrous ammonia, AM-FOL, Ammonia gas, Liquid ammonia, Nitro-sil, R 717, Spirit of hartshorn	EPA 1987a; Windholz 1983
Registered trade name(s)	No data	
Chemical formula	NH ₃	
Chemical structure	$\begin{array}{c} \text{H}-\text{N}-\text{H} \\ \\ \text{H} \end{array}$	
Identification numbers:		
CAS Registry	7664-41-7	HSDB 2003
NIOSH RTECS	B00875000	NIOSH 2002a
anhydrous ammonia	B00875000	
aqueous solution	B00875000	
aqua ammonia	B00875000	
EPA Hazardous Waste	No data	
OHM/TADS	7216584	OHM-TADS 1988
DOT/UN/NA/IMCO shipping		
anhydrous	UN 1005	NIOSH 2002a
solution (10–35%)	UN 2672	
solution (35–50%)	UN 2073	
solution (>50%)	UN 1005	
HSDB	162	HSDB 2003
NCI	No data	

CAS = Chemical Abstracts Services; DOT/UN/NA/IMCO = Department of Transportation/United Nations/North America/International Maritime Dangerous Goods Code; EPA = Environmental Protection Agency; HSDB = Hazardous Substances Data Bank; NCI = National Cancer Institute; NIOSH = National Institute for Occupational Safety and Health; OHM/TADS = Oil and Hazardous Materials/Technical Assistance Data System; RTECS = Registry of Toxic Effects of Chemical Substances

4. CHEMICAL AND PHYSICAL INFORMATION

Table 4-2. Physical and Chemical Properties of Ammonia

Property	Value	Reference
Molecular weight	17.03	LeBlanc et al. 1978
Color	Colorless	LeBlanc et al. 1978
Physical state	Gas at room temperature	LeBlanc et al. 1978
Melting point	-77.7 °C	LeBlanc et al. 1978
Boiling point	-33.35 °C	LeBlanc et al. 1978
Density:		
Gas	0.7710 g/L	Weast et al. 1988
Aqueous solution (28%)	0.89801 (20 °C) g/L	Windholz 1983
Liquid	0.6818 g/L (-33.35 °C, 1 atm)	Windholz 1983
Vapor density	0.5967 (air=1)	Windholz 1983
Specific gravity (25 °C)	0.747 g/L	Lide 1998
Odor	Sharp, intensely irritating	Sax and Lewis 1987
Odor threshold:		
Air	25 ppm (18 mg/m ³)	Amoore and Hautala 1983
	48 ppm (34 mg/m ³)	Leonardos et al. 1969
	53 ppm (38 mg/m ³)	Budavari et al. 1996
Water	1.5 ppm	Amoore and Hautala 1983
pKa	9.25 (25 °C)	Lide 1998
Solubility:		
Water		
at 0 °C	42.8% (w/w)	LeBlanc et al. 1978
	47% (w/w)	Budavari et al. 1996
at 15 °C	38% (w/w)	Budavari et al. 1996
at 20 °C	33.1% (w/w)	LeBlanc et al. 1978
	34% (w/w)	Budavari et al. 1996
at 25 °C	34% (w/w)	LeBlanc et al. 1978
	31% (w/w)	Budavari et al. 1996
at 30 °C	28% (w/w)	Budavari et al. 1996
at 50 °C	18% (w/w)	Budavari et al. 1996
Organic solvent(s)		
at 0 °C	20% (w/w) in absolute ethanol	Budavari et al. 1996
at 25 °C	10% (w/w) in absolute ethanol	Budavari et al. 1996
	16% (w/w) in methanol	Budavari et al. 1996
	Soluble in chloroform and ether	Budavari et al. 1996
Partition coefficients:		
Log K _{ow}	0.23 (estimated)	EPIWIN 2000

4. CHEMICAL AND PHYSICAL INFORMATION

Table 4-2. Physical and Chemical Properties of Ammonia

Property	Value	Reference
Log K_{oc}	1.155 (estimated)	EPIWIN 2000
Vapor pressure:		
Anhydrous NH_3	8.5 atm (20 °C)	Sax and Lewis 1987
	10.2 atm (25 °C)	Daubert and Danner 1989
Aqueous NH_3 (28%)	2.9 atm (25 °C)	Daubert and Danner 1989
Henry's law constant	1.6x10 ⁻⁵ atm-m ³ /mol (25 °C)	Betterton 1992
	7.3x10 ⁻⁶ atm-m ³ /mol (pH 7, 23.4 °C) ^a	Ayers et al. 1985
	1.60x10 ⁻⁵ atm-m ³ /mol (25 °C) ^b	Yoo et al. 1986
	5.01x10 ⁻⁶ atm-m ³ /mol (5 °C)	Brimblecombe and Dawson 1984
Autoignition temperature	650 °C	LeBlanc et al. 1978
Flashpoint	Not available	
Flammability limits in air	16–25%	LeBlanc et al. 1978
Conversion factors		
ppm (v/v) to mg/m ³ in air (20 °C)	1 ppm (v/v) = 0.707 mg/m ³	Verschueren 1983
mg/m ³ to ppm (v/v) in air (20 °C)	1 mg/m ³ = 1.414 ppm (v/v)	
pH in water	11.6 (1 N)	Windholz 1983
	11.1 (0.1 N)	
	10.6 (0.01 N)	
Explosive limits	Not available	

^aUnitless constant extrapolated from cited data.^bUnconverted value of 0.0168 kg-atm/mol was calculated from equation in citation.

pKa = The dissociation constant of the conjugate acid

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

5.1 PRODUCTION

Ammonia is both a natural and a manufactured chemical. It is a key intermediate in the nitrogen cycle in nature, and microbial production is a major source of ammonia in the world. Recent reports, however, have emphasized the significant influence that humans are having on the global nitrogen budget. At the beginning of the 20th century, most nitrogen was fixed into usable forms (e.g., NH_3) by lightning strikes and microbial nitrogen fixation, with an estimated 90–130 teragrams (Tg; 1 teragram is equivalent to one million metric tons) fixed per year. Human production of fixed nitrogen (NH_3) is now estimated to be 140 Tg N per year, an amount that is similar to non-anthropogenic sources (NSF 1999; Socolow 1999). This increase in human-related ammonia emissions is considerably higher than earlier estimates of the total annual commercial production of ammonia, wherein the anthropogenic emission of ammonia represented approximately 1–5% of nature's global ammonia emission budget (ApSimon et al. 1987; Buijsman et al. 1987; Crutzen 1983; Galbally 1985; Rosswall 1981; Socolow 1999).

The largest amount of naturally produced ammonia is thought to arise from soil. Ammonia from decomposing animal excreta probably accounts for the largest proportion of the ammonia produced, with the decay of organic materials from plants, dead animals, and the like contributing significant amounts (Crutzen 1983; Dawson 1977; Dawson and Farmer 1984; Galbally 1985; Irwin and Williams 1988).

Manufacture of ammonia within the United States has declined steadily over the past several years, with one of the outcomes being the closure of several production plants. The U.S. annual commercial production capacity for ammonia was 16.6 million metric tons in 1999 (CMR 1999), 15.7 million metric tons in 2000 (SRI 2000), but only 9.5 million metric tons in 2001 (Kramer 2002). Production levels increased slightly to 10.8 million metric tons in 2002 (Kramer 2003). High natural gas costs, along with weather-related decreases in demands, contributed to the lower production output in the years leading up to 2002. However, natural gas prices became lower in 2002, and ammonia production increased that year. In 1999, four plant closings eliminated a combined production capacity of 1.2 million tons, some of which was replaced by new facilities (CMR 1999). In 2000, an additional seven plants were completely shut down, and five plants were partially closed due to market conditions (Kramer 2000). In 2002, the largest producer of ammonia in the United States filed for Chapter 11 bankruptcy, leading to the

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

permanent closing of three large production plants (with a combined production capacity of just over 980,000 metric tons) (Kramer 2003).

While ammonia production decreased over the past few years and some ammonia plants were closed, the states and companies that have historically produced most of the ammonia remained relatively constant. In 2000, 2001, and 2002, Louisiana, Oklahoma, and Texas were the three major producing states, contributing over 50% of the total U.S. ammonia production. Six companies (Farmland Industries Inc., Terra Industries Inc., PCS Nitrogen Inc., Agrium Inc., CF Industries Inc., and Koch Nitrogen) produced 73% of the nation's ammonia in 2002 (Kramer 2003).

There are 2,338 facilities that manufacture or process ammonia in the United States (Table 5-1). The amounts manufactured or processed range from relatively small production activities (from 0 to 99,999 pounds) in Hawaii to very large formulation and processing activities (up to 499,999,999 pounds) in Alaska, Florida, Iowa, Kansas, Louisiana, Nebraska, and Texas (TRI01 2003). As mentioned previously, three states, Louisiana, Oklahoma, and Texas, produce more than 50% of the nation's total NH_3 output.

The major method for commercial production of anhydrous ammonia is a modified Haber-Bosch process. This process was first demonstrated in 1909 (Kramer 2000), and was commercially developed in 1913 in Germany. The first U.S. plant to use this process was built in Syracuse, New York, in 1921 (DOI 1985). The basic Haber-Bosch methodology was still responsible for 98% of the industrially produced ammonia in the United States in 1979 (EPA 1980; HSDB 2003). In this process, nitrogen (obtained from the atmosphere) and hydrogen (obtained from natural gas) are mixed together in a 1 to 3 ratio and passed over a catalyst at high pressure and high temperature. The ammonia thus produced is collected by various means, and any unreacted feed gases are recirculated through the reactor.

Small amounts of ammonia are produced industrially as a by-product of the coking of coal. The largest proportion of industrial ammonia production occurs in areas where natural gas is cheap and plentiful because ammonia is synthesized using natural gas. Large pipelines stretching from Louisiana to Nebraska and from Texas to Minnesota carry anhydrous ammonia from its site of production to agricultural areas where it is used as fertilizer (LeBlanc et al. 1978). These pipelines are capable of transporting or storing 3 million metric tons of ammonia per year, and have a storage capacity of 1.5 million metric tons (Kramer 2000, 2003). Ammonia can also be shipped in large refrigerated, low

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

Table 5-1. Facilities that Produce, Process, or Use Ammonia

State ^a	Number of facilities	Minimum amount on site in pounds ^b	Maximum amount on site in pounds ^b	Activities and uses ^c
AK	6	1,000	499,999,999	1, 3, 4, 5, 6, 10, 11, 12
AL	69	0	49,999,999	1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13
AR	45	100	99,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
AS	1	10,000	99,999	11
AZ	16	0	9,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12
CA	161	0	99,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
CO	15	0	999,999	1, 5, 6, 7, 9, 11, 12
CT	24	0	999,999	1, 2, 3, 5, 6, 7, 8, 10, 11, 12
DC	2	100,000	999,999	12
DE	14	0	999,999	1, 3, 5, 6, 7, 11, 12
FL	54	100	499,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13
GA	87	0	99,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13
HI	6	0	99,999	1, 3, 5, 6, 10, 12, 13, 14
IA	54	100	499,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12
ID	16	100	49,999,999	1, 2, 3, 4, 5, 6, 8, 9, 10, 11, 12, 13
IL	108	0	99,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13
IN	72	0	49,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13
KS	36	100	499,999,999	1, 2, 3, 4, 5, 6, 7, 8, 10, 11, 12, 13, 14
KY	34	100	9,999,999	1, 2, 3, 5, 6, 7, 9, 10, 11, 12, 13, 14
LA	73	0	499,999,999	1, 2, 3, 4, 5, 6, 7, 10, 11, 12, 13, 14
MA	34	0	999,999	1, 2, 3, 5, 6, 7, 10, 11, 12
MD	16	1,000	999,999	1, 3, 5, 6, 7, 8, 10, 11, 12, 13, 14
ME	13	0	999,999	1, 2, 3, 5, 6, 10, 11, 12, 13
MI	72	0	999,999	1, 2, 3, 5, 6, 7, 8, 10, 11, 12, 13, 14
MN	39	0	9,999,999	1, 2, 3, 5, 6, 7, 8, 9, 10, 11, 12, 13
MO	50	1,000	9,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12
MS	44	100	99,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13
MT	12	0	9,999,999	1, 3, 5, 6, 10, 11, 12
NC	86	0	49,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13
ND	7	0	999,999	1, 3, 5, 6, 10, 11, 12
NE	35	100	499,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12
NH	14	0	9,999,999	1, 3, 4, 5, 6, 7, 10, 11, 12
NJ	58	0	9,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13
NM	6	100	99,999	1, 5, 7, 11, 12
NV	11	100	9,999,999	1, 2, 3, 4, 5, 6, 10, 11, 12, 13
NY	63	0	49,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13
OH	123	0	99,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
OK	26	100	99,999,999	1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14

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

Table 5-1. Facilities that Produce, Process, or Use Ammonia

State ^a	Number of facilities	Minimum amount on site in pounds ^b	Maximum amount on site in pounds ^b	Activities and uses ^c
OR	46	0	99,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13
PA	108	0	99,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13
PR	15	100	999,999	1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12
RI	13	0	9,999,999	1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12
SC	64	0	999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13
SD	4	1,000	999,999	1, 5, 10, 11
TN	56	0	49,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
TX	191	0	499,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
UT	28	100	9,999,999	1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12
VA	57	0	49,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13
VI	1	100,000	999,999	1, 2, 3, 5, 6, 10, 12
VT	2	1,000	99,999	1, 5, 11, 12
WA	38	0	99,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13
WI	68	100	999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
WV	33	0	49,999,999	1, 3, 5, 6, 7, 9, 10, 11, 12, 13
WY	12	0	99,999,999	1, 3, 4, 5, 6, 7, 10, 13

Source: TRI01 2003 (Data are from 2001)

^aPost office state abbreviations used^bAmounts on site reported by facilities in each state^cActivities/Uses:

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

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

pressure tanks (holding between 4 and 30 thousand tons) or smaller (holding approximately 210 tons), pressurized tanks (Farm Chemicals Handbook 1987). Barges are often used for refrigerated shipments because of their lower cost. Ammonia can be stored in refrigerated tanks holding up to 36,000 tons for use in the ammonia market. Smaller amounts of ammonia are stored in pressurized tanks.

The supply of ammonia for domestic uses has been historically met by domestic production. The ability to meet the ammonia demand depends on the amount of crop acres planted, the price of imported fertilizers, the cost of natural gas, and the availability of ammonia via import from abroad. In 2000, 3.9 million metric tons of anhydrous ammonia were imported; in 2001, this amount increased to more than 5.5 million metric tons. In 2000, domestic sources supplied 15.7 million metric tons of ammonia, whereas international sources provided 3.9 million metric tons; imports represented 20% of the total supply. In 2001 by comparison, domestic production dropped to 9.5 million metric tons, while imports increased 5.5 million metric tons; imports in 2001 represented almost 37% of the supply of ammonia (Kramer 2002). The amount of ammonia imported in 2002 held steady—the amount imported was just under 5.7 million metric tons (Kramer 2003)—while domestic production rose slightly to 10.8 million metric tons. Imports in 2002 represented 35% of the domestic need. The increases in imported ammonia, along with decreased domestic production, resulted in a substantial increase in reliance on foreign sources during these 3 years.

5.2 IMPORT/EXPORT

The import and export of ammonia have fluctuated slightly over the last few years. In 2000, the amount of ammonia imported into the United States was slightly less than 3.9 million metric tons. In both 2001 and 2002, the amount of ammonia imported into the United States was slightly more than 5.5 million metric tons (Kramer 2003). These years reflect a 41% increase in the amount of ammonia imported compared to 2000. U.S. exports of ammonia fell during the years following the change in domestic production and import from abroad. Exports in 2000 were approximately 0.66 million metric tons, and in 2001, 0.65 million metric tons were exported. Exports then dropped to 2/3 of those reported in the previous years. In 2002, when domestic production dropped and imports increased, only 0.44 million metric tons of ammonia were exported.

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

5.3 USE

The largest and most significant use of ammonia and ammonium compounds is the agricultural application of fertilizers. Ammonia and ammonium compounds used as fertilizer represent 89–90% of the commercially produced ammonia, with plastics, synthetic fibers and resins, explosives, and other uses accounting for most of the remainder (Kramer 2002, 2004). Direct uses of ammonia as fertilizer can be broken down into the following categories (percentages based on mass of nitrogen in each compound): anhydrous ammonia, 26%; urea/ammonium nitrate solutions, 23%; urea, 20%; ammonium nitrate, 4.5%; ammonium sulfate, 2%; other forms, 3%; and multiple nutrient forms, 21% (Kramer 2003). Most ammonium compounds and nitric acid, which are produced from anhydrous ammonia, are used directly in the production of fertilizers.

The small proportion of commercially produced ammonia not incorporated into fertilizers is used as a corrosion inhibitor, in the purification of water supplies, as a component of household cleaners, and as a refrigerant. It is also used in the pulp and paper, metallurgy, rubber, food and beverage, textile, and leather industries. Ammonia is used in the manufacture of pharmaceuticals and explosives, and in the production of various chemical intermediates (LeBlanc et al. 1978; Sax and Lewis 1987).

5.4 DISPOSAL

Solutions of ammonia can be highly diluted with water, or alternatively, diluted with water and neutralized with HCl and then routed to the sewer system. The amount released to the receiving stream should not exceed the established limits for ammonia. Limited amounts of gaseous ammonia may be discharged to the atmosphere. Federal, state, and local guidelines should be consulted before disposal.

Disposal of liquefied ammonia or of large quantities of gaseous or aqueous ammonia directly into water is not desirable, because of the large amount of heat generated. This generation of heat could increase exposure to personnel involved in the process. Recovery of ammonia from aqueous waste solutions is a viable option for many industries (HSDB 2003).

6. POTENTIAL FOR HUMAN EXPOSURE

6.1 OVERVIEW

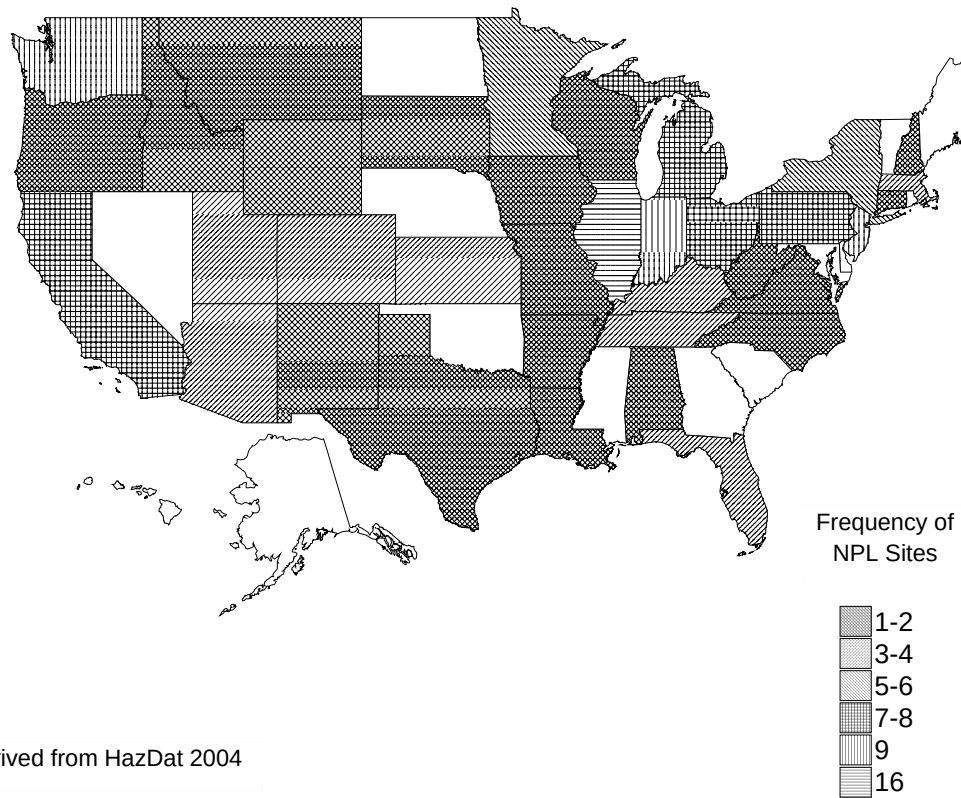
Ammonia has been identified in at least 137 of the 1,647 hazardous waste sites that have been proposed for inclusion on the EPA National Priorities List (NPL) (HazDat 2004). However, the number of sites evaluated for ammonia is not known. The frequency of these sites can be seen in Figure 6-1. Of these sites, 135 are located within the United States and 2 are located in the Commonwealth of Puerto Rico (not shown).

Ammonia is a naturally-occurring compound that is an intermediate in the global nitrogen cycle. It is essential for many biological processes and is a central compound in all living organisms. Nitrogen is converted from atmospheric N_2 to other forms by different processes. Nitrogen fixation (the process of converting atmospheric N_2 to NH_3) occurs naturally due to biological processes. Lightning strikes also “fix” atmospheric nitrogen, but they produce nitrogen oxides, not ammonia. The current amount of nitrogen fixation that occurs by industrial processes equals that of natural, terrestrial nitrogen fixation. Both natural and anthropogenic sources produce a total of approximately 230–270 million metric tons of NH_3 per year.

Because of its role in natural processes and cycles, ammonia is found at low concentrations in most environmental media. When ammonia is found at a local concentration that is higher than these background levels, it is often a result of human influence. Ammonia is hazardous only when exposure is to high levels. In determining the environmental fate of ammonia, several factors should be considered, the primary one being that ammonia is the most abundant alkaline gas in the environment. An acid-base reaction between water and ammonia occurs such that the dominant form of ammonia in water, at environmentally relevant pHs, is the ammonium ion. In media where water is usually present, such as soil, plants, biological tissue, and water itself, ammonia and ammonium are in dynamic equilibrium.

Ammonia is a key intermediate in the nitrogen cycle, a natural cycle that is coupled with other important biological cycles (i.e., the sulfur cycle and carbon cycle). An understanding of the role of ammonia in the nitrogen cycle, at least on a generalized level, is important in determining the environmental fate of ammonia. A simplified schematic of the microbial processes of the nitrogen cycle that involves ammonia can be found in Figure 6-2. Microorganisms perform four processes in the nitrogen cycle that result in

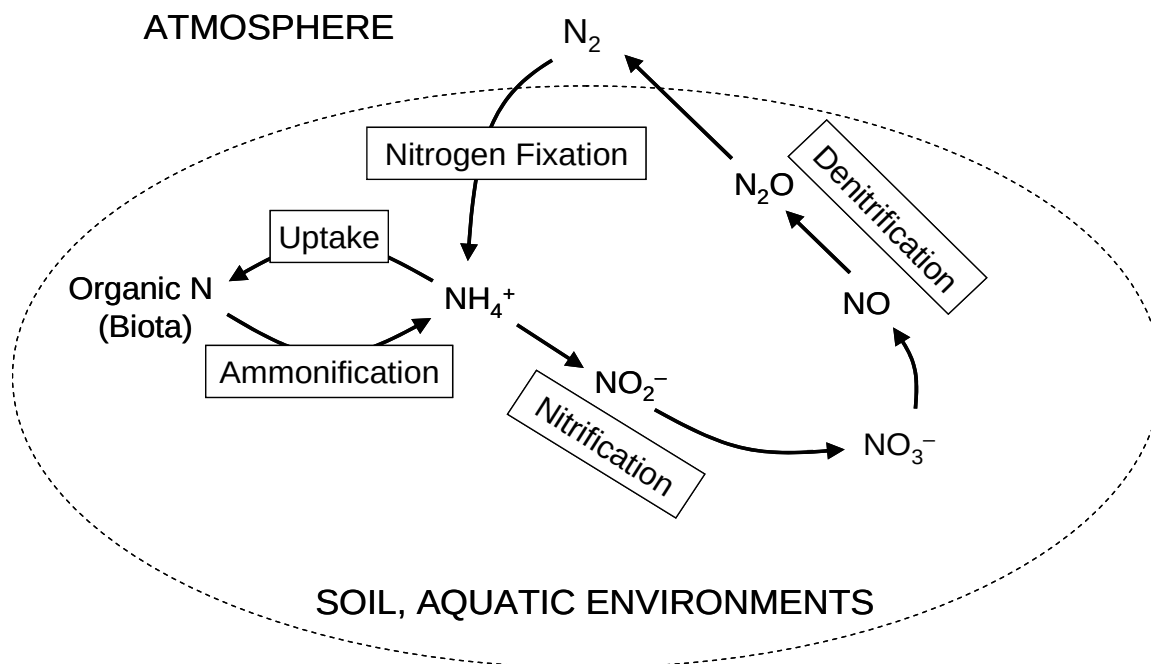
6. POTENTIAL FOR HUMAN EXPOSURE

Figure 6-1. Frequency of NPL Sites with Ammonia Contamination

Derived from HazDat 2004

6. POTENTIAL FOR HUMAN EXPOSURE

Figure 6-2. Simplified Schematic for the Microbial Processes of the Nitrogen Cycle



6. POTENTIAL FOR HUMAN EXPOSURE

production or transformation of ammonia: nitrogen fixation, nitrification, denitrification, and ammonification. As part of this cycle, nitrogen gas and oxidized forms of nitrogen are transformed and returned to the biological world. Nitrogen fixation is the process whereby atmospheric nitrogen gas is converted to ammonia, which is then assimilated into amino acids; it has been found that there is only a small proportion of all genera of microorganisms that can fix nitrogen. Denitrification is the process whereby the nitrogen oxides (i.e., nitrate and nitrite) are reduced under anaerobic conditions to N_2 and N_2O , which can escape to the atmosphere. Nitrification is the biological oxidation of ammoniacal nitrogen to nitrate, with nitrite as the intermediate. Ammonification (or nitrogen mineralization) is the conversion of organic nitrogen into ammonia.

Ammonia may be released to the atmosphere by volatilization from the following sources: decaying organic matter; livestock excreta; fertilizers applied to soils; venting of gas, leaks, or spills during commercial synthesis, production, transportation, or refrigeration equipment failure; sewage or waste water effluent; burning of coal, wood, and other natural products; and volcanic eruptions.

Ammonia may be released to water through effluent from sewage treatment plants, effluent from industrial processes, runoff from fertilized fields, and runoff from areas of concentrated livestock. This usually occurs when the organic N compounds present in these sources enter the water and are converted microbiologically to ammonia.

Ammonia may be released to soils by natural or synthetic fertilizer application, animal (including livestock) excrement degradation, decay of organic material from dead plants and animals, and indirectly from natural fixation of atmospheric nitrogen. In this latter case, ammonia releases can occur following nitrogen fixation by free-living microbes and plants (those that are symbiotic nitrogen-fixing bacteria), which subsequently die and release ammonia (or compounds that are converted to ammonia) to the soils.

In the atmosphere, ammonia can be removed by rain or snow washout. Reactions with acidic substances, such as H_2SO_4 , HCl , HNO_3 , or N oxides (all produced in high concentrations from anthropogenic activities) produce ammonium aerosols, which can undergo dry or wet deposition. The gas phase reaction of ammonia with photochemically produced hydroxyl radicals is thought to contribute about 10% to the overall atmospheric removal process. The best estimate of the half-life of atmospheric ammonia is a few days.

6. POTENTIAL FOR HUMAN EXPOSURE

In water, ammonia volatilizes to the atmosphere, is transformed to other nitrogenous compounds, or may be bound to materials in the water. Volatilization is highly pH-dependent, and can also depend on other factors such as temperature, wind speed, and atmospheric ammonia concentration. Transformation of ammonia in water occurs primarily by the microbial processes of nitrification and to lesser extents, denitrification. Nitrification yields nitrate and nitrite anions; the former species can be responsible for methemoglobinemia in human infants if the contaminated water is ingested. Removal of ammonium from water can also occur by adsorption to sediments or suspended organic material.

In soil, ammonia may either volatilize to the atmosphere, adsorb to particulate matter, or undergo microbial transformation to nitrate or nitrite anions. Uptake by plants can also be a significant fate process. Ammonia at natural concentrations in soil is not believed to have a very long half-life. If ammonia is distributed to soil in large concentrations (e.g., following an application of an ammonia-containing fertilizer), the natural biological transformation processes can be overwhelmed, and the environmental fate of ammonia will become dependent upon the physical and chemical properties of ammonia, until the ammonia concentration returns to background levels.

Occupational exposure to ammonia may occur in industries involved in its synthesis, formulation, processing, transportation, and use. Occupational exposure to ammonia can also occur during the use of an extensive number of cleaning products that contain ammonia. Farmers may be exposed during the application of fertilizers containing anhydrous ammonia or liquid ammonia, or manures high in ammonia. Workers at cattle feedlots, poultry confinement buildings, or other industries that have a high concentration of animals may also be exposed.

Exposure of the general population to elevated levels of ammonia is most commonly from the use of household cleaners that contain ammonia. People who live near farms or who visit farms during the application of fertilizer that contain or release ammonia may also be exposed. People living near cattle feedlots, poultry confinement buildings, or other areas where animal populations are concentrated can also be exposed to ammonia, in addition to other gases generated by putrefaction. Ammonia has been identified at 137 out of 1,647 NPL hazardous waste sites (HazDat 2004).

6.2 RELEASES TO THE ENVIRONMENT

Ammonia is commercially produced for many processes, but most production is for agricultural uses, primarily crop fertilizer. As a result of most it being formulated for agricultural practices, ammonia is

6. POTENTIAL FOR HUMAN EXPOSURE

commonly distributed to the environment during its intended use as a crop fertilizer. Release data generated for the Toxics Release Inventory (TRI) (see Table 6-1) provide detailed information regarding environmental releases related to industrial activities, but should be used with caution because only certain types of facilities are required to report, and data from these reports do not represent an exhaustive list of all commercial releases. It should be noted that for ammonia, since it is one of the most widely-used agricultural fertilizer chemicals in the United States, the TRI data represent only a small fraction of the environmental release, and do not include releases that occur during farming or other agriculture practices.

Table 6-1 shows the 2001 TRI releases of ammonia from manufacturing or processing facilities to different environmental compartments. Most of the ammonia released to the environment from these facilities was the result of air releases, followed by releases via underground injection. The greatest air releases occurred in the state of Louisiana (12,304,532 pounds), which was almost 2 million more pounds released than the second highest releasing state, Ohio (10,505,480 pounds). Texas released the most ammonia via underground injection (15,014,490 pounds), which was more than 3 times the second highest releasing state, Louisiana (4,446,211 pounds). For all on-site releases, the two states releasing the most ammonia were the adjacent states of Louisiana and Texas (Louisiana released 17,742,736 pounds and Texas released 21,354,611 pounds).

Release of ammonia from production and processing facilities has changed from year to year, with amounts generally decreasing since the early 1990s. Reported air releases have ranged from a high of 254,542,289 pounds in 1989 to a low of 122,057,546 pounds in 2001. Surface water releases have ranged from a high of 48,138,279 pounds in 1990 to a low of 6,621,166 pounds in 2001. Land releases (surface releases) have shown a similar trend, with the highest amount (17,782,641 pounds) released in 1990, and the lowest amount (2,868,728 pounds) released in 1999. The general trend is that less and less ammonia has been released to the environment each year, such that the total amount released in 2001 (158,521,046 pounds) was less than a third of the amount released in 1990 (548,828,735 pounds).

The TRI data should be used with caution because only certain types of facilities are required to report. This is not an exhaustive list.

In addition to releases related to agricultural or other anthropogenic usage, ammonia has been identified in several environmental compartments including surface water, groundwater, soil, and sediment collected at 135 of the 1,647 current or former NPL hazardous waste sites in the United States, and in

6. POTENTIAL FOR HUMAN EXPOSURE

Table 6-1. Releases to the Environment from Facilities that Produce, Process, or Use Ammonia^a

State ^c	Number of facilities	Reported amounts released in pounds per year ^b						
		Air ^d	Water	Under-ground injection	Land	Total on-site release ^e	Total off-site release ^f	Total on and off-site release
AK	7	1,427,011	32,428	5	95,950	1,555,394	0	1,555,394
AL	75	3,192,615	180,703	0	57,288	3,430,606	145,305	3,575,911
AR	48	3,672,621	209,122	0	372	3,882,115	18,078	3,900,193
AS	1	6,920	No data	0	0	6,920	0	6,920
AZ	18	107,967	5	0	260	108,232	350	108,582
CA	178	6,362,240	37,470	41,236	264,004	6,704,950	18,728	6,723,678
CO	19	291,922	12,899	0	4,019	308,840	148	308,988
CT	28	169,071	10,035	0	0	179,106	8	179,114
DC	2	0	487	0	0	487	0	487
DE	14	109,356	3,804	0	7,937	121,097	0	121,097
FL	72	5,646,496	148,226	293,106	124,914	6,212,742	122,755	6,335,497
GA	95	8,291,371	171,670	0	13,140	8,476,181	154,582	8,630,763
HI	7	47,492	660	2,059	30,368	80,579	0	80,579
IA	60	4,686,900	133,403	0	3,829	4,824,132	166,223	4,990,355
ID	16	2,705,639	5,874	0	182,369	2,893,882	21,731	2,915,613
IL	136	2,366,356	56,440	0	68,423	2,491,219	1,108,621	3,599,840
IN	82	1,294,431	55,681	824,984	61,545	2,236,641	23,002	2,259,643
KS	38	3,285,064	36,461	22,555	270,459	3,614,539	42,129	3,656,668
KY	40	1,218,869	83,020	0	4,000	1,305,889	1,483	1,307,372
LA	78	12,304,532	917,883	4,446,211	4,816	17,673,442	69,294	17,742,736
MA	48	591,589	6	0	0	591,595	33,235	624,830
MD	17	482,934	25,246	0	18,569	526,749	4,587	531,336
ME	15	813,198	39,047	0	0	852,245	0	852,245
MI	81	1,579,680	141,346	40,307	68,851	1,830,184	12,981	1,843,165
MN	56	1,790,288	43,283	0	36,315	1,869,886	3,315	1,873,201
MO	55	548,485	319,685	0	11,624	879,794	34,796	914,590
MS	49	4,452,540	394,837	0	46,552	4,893,929	255	4,894,184
MT	14	543,332	10,920	0	9,685	563,937	0	563,937
NC	101	3,054,435	287,555	0	30,750	3,372,740	18,711	3,391,451
ND	9	305,283	17,106	0	88,654	411,043	340	411,383
NE	38	804,872	245,587	0	377,539	1,427,998	243,327	1,671,325
NH	15	128,420	551	0	0	128,971	966	129,937
NJ	67	1,144,702	165,642	0	0	1,310,344	18,406	1,328,750
NM	5	9,780	5	29,497	670	39,952	0	39,952
NV	13	428,880	2,710	0	311,810	743,400	0	743,400
NY	71	1,387,982	61,780	0	8,266	1,458,028	1,540	1,459,568

6. POTENTIAL FOR HUMAN EXPOSURE

Table 6-1. Releases to the Environment from Facilities that Produce, Process, or Use Ammonia^a

State ^c	Number of facilities	Reported amounts released in pounds per year ^b						Total on and off-site release
		Air ^d	Water	Under-ground injection	Land	Total on-site release ^e	Total off-site release ^f	
OH	136	10,505,480	98,996	2,006,500	52,964	12,663,940	93,861	12,757,801
OK	28	5,995,915	81,112	0	25,716	6,102,743	1,850	6,104,593
OR	51	1,769,989	43,747	0	106,233	1,919,969	1,882	1,921,851
PA	131	2,346,403	229,268	0	13,469	2,589,140	23,359	2,612,499
PR	16	1,938,727	306	0	0	1,939,033	600	1,939,633
RI	22	84,982	3,573	0	0	88,555	0	88,555
SC	68	2,559,694	162,782	0	39,741	2,762,217	5,627	2,767,844
SD	9	86,361	684	0	225	87,270	29,223	116,493
TN	58	4,865,087	487,650	0	796	5,353,533	78,192	5,431,725
TX	209	5,206,675	518,396	15,014,390	199,710	20,939,171	415,440	21,354,611
UT	29	458,197	8,800	0	911,305	1,378,302	503	1,378,805
VA	64	7,866,819	93,001	0	35,707	7,995,527	129,614	8,125,141
VI	1	77,675	42,154	0	0	119,829	0	119,829
VT	2	47,582	4,450	0	0	52,032	0	52,032
WA	45	857,165	137,956	0	22,488	1,017,609	80,200	1,097,809
WI	83	589,027	89,624	0	3,107	681,758	78,850	760,608
WV	36	961,276	758,661	14,387	0	1,734,324	42,951	1,777,275
WY	12	587,219	8,429	239,000	6,610	841,258	0	841,258
Total	2,668	122,057,546	6,621,166	22,974,237	3,621,049	155,273,998	3,247,048	158,521,046

Source: TRI01 2003 (Data are from 2001)

^aThe TRI data should be used with caution since only certain types of facilities are required to report. This is not an exhaustive list. Data are rounded to nearest whole number.^bData in TRI are maximum amounts released by each facility.^cPost office state abbreviations are used.^dThe sum of fugitive and stack releases are included in releases to air by a given facility.^eThe sum of all releases of the chemical to air, land, water, and underground injection wells.^fTotal amount of chemical transferred off-site, including to publicly owned treatment works (POTW).

6. POTENTIAL FOR HUMAN EXPOSURE

groundwater and soil samples at 2 sites in Puerto Rico (HazDat 2004). Furthermore, ammonia is a key intermediate in nature's nitrogen cycle, and considerable amounts are released to the environment as a result of natural processes. As a result of inputs from natural sources and from anthropogenic sources, ammonia concentrations in nature are in dynamic equilibrium. When ammonia is found at elevated concentrations, however, it is usually a result of anthropogenic activity.

6.2.1 Air

Large amounts of ammonia are released to the atmosphere worldwide by domesticated farm animals (ApSimon et al. 1987; Asman and Janssen 1987; Buijsman et al. 1987; Kramer 2000, 2002; Ryden et al. 1987). Ammonia emissions due to the decay of livestock manure are a source for ammonia release in areas that have artificially high concentrations of animals, such as cattle feedlots and poultry-confinement buildings (Brinson et al. 1994; Hutchinson et al. 1982; Langland 1992; Liao and Bundy 1995; Olivier et al. 1998; Sunesson et al. 2001). In Germany, over 90% of the measured NH_3 emissions originated from agricultural sources (Strogies and Kallweit 1996). In Russia, estimated NH_3 emissions from fertilizer applications and livestock sources accounted for 94% of the total NH_3 emissions from all anthropogenic sources (Tsibulski et al. 1996). The use of high nitrogen content feed for farm animals and the trend toward larger feedlots have been responsible for increased emissions in developed countries.

The application of fertilizer to soil, as ammonia, ammonium compounds, or ammonia precursors (such as urea), is a well documented source of ammonia release to the atmosphere (ApSimon et al. 1987; Beyrouthy et al. 1988; Buijsman et al. 1987; Kucey 1988; Olivier et al. 1998; Reynolds and Wolf 1988). The rate of ammonia emission from ground sources, such as freshly fertilized fields and cattle feedlots, is dependent on variables such as the pH, temperature, soil characteristics, rainfall, method of application, wind speed, etc. (Bouwmeester and Vlek 1981; Brunke et al. 1988; Denmead et al. 1982; Hoff et al. 1981; Kucey 1988; Nason et al. 1988; Reynolds and Wolf 1988). Ammonia can volatilize from sewage sludge that has been spread on the surface of the soil (Beauchamp et al. 1978; Ryan and Keeney 1975) as well as from poultry litter (Brinson et al. 1994). In the latter case, composted poultry litter released far less volatile NH_3 to the atmosphere (0–0.24% of applied) than did fresh poultry litter (17–23%) (Brinson et al. 1994). In contrast, the crops themselves are often minor sources of atmospheric NH_3 . Harper and Sharpe (1995) demonstrated almost no net atmospheric NH_3 flux in corn crops, due to their relatively similar emission and uptake rates of NH_3 over the growing season.

6. POTENTIAL FOR HUMAN EXPOSURE

For much of the history of the Earth, biological activity in soil and natural waters was the primary global source of atmospheric ammonia (Dawson 1977; Dawson and Farmer 1984; National Science Foundation 1999), but this has changed over the last century. Crutzen (1983) suggested that the decay of organic material arising from dead plant, animal, and microbial biomass generates most of the atmospheric ammonia, while Galbally (1985) and Irwin and Williams (1988) suggested that domestic-animal excretions represent the dominant source of atmospheric ammonia. Lee et al. (1997) estimated that grasslands contributed 40% of the total global NH_3 emissions, with domestic animal wastes contributing 42.3% of that. Recent studies, however, provided fairly uniform estimates of ~40% of global NH_3 emissions being due to excreta from domestic animals (Asman et al. 1998; Bouwman et al. 1997; Olivier et al. 1998). Current measurements and estimates, however, indicate that the amount of ammonia produced as a result of anthropogenic activities is equivalent to the amount produced by natural processes (National Science Foundation 1999).

In addition to livestock-related releases, ammonia can be released to the atmosphere through the venting of gases during the production, storage, and transportation of ammonia, and during its formulation or incorporation into secondary products (Buijsman et al. 1987). Long pipelines are used to transport ammonia from its site of manufacture to agricultural areas where it is used as fertilizer (Farm Chemicals Handbook 1987; Kramer 2000; LeBlanc et al. 1978). Releases to the atmosphere could occur at pumping stations and points of transfer along these pipelines, or from leaks. Large refrigerated tanks are used to store ammonia, and release to the environment can occur while venting the pressure in these tanks, or from leaks.

Ammonia can also enter the atmosphere by volatilization from the waste water of industrial processes that involve its production or use, and from the volatilization from the effluent of waste water treatment plants (Buijsman et al. 1987; Langland 1992; Roy and Poricha 1982; Wilkin and Flemal 1980). Ammonia has been found in the exhaust of automobile and diesel engines (Asman et al. 1998; Plerson and Brachaczek 1983). Release to the atmosphere can occur during the burning of coal (Bauer and Andren 1985; Olivier et al. 1998). The latter process, however, is not thought to account for a significant proportion of the total anthropogenic ammonia released to the atmosphere (Olivier et al. 1998; Strogies and Kallweit 1996).

Natural sources of ammonia emissions to the atmosphere are volcanic eruptions, forest fires, and the decomposition of nitrogenous compounds arising from microbially-fixed nitrogen (Galbally 1985; Hegg et al. 1987; National Science Foundation 1999). Excreta from household pets, wild animals, and humans are also contributing sources (Asman and Drukker 1988; Buijsman et al. 1987; Crutzen 1983).

6. POTENTIAL FOR HUMAN EXPOSURE

6.2.2 Water

The major point source of release to surface waters is from the effluents of waste water-treatment plants (Barica 1990; Crumpton and Isenhardt 1988; Wilkin and Flemal 1980). Ammonia can enter surface waters through the effluent of commercial processes in which ammonia is used or produced (Effler et al. 2001; Huddleston et al. 2000; Matthews et al. 2000; Roy and Poricha 1982). Runoff from fertilized farmland and from areas of concentrated livestock production can also result in the transfer of ammonia to surface water (Corsi et al. 2000; Jingsheng et al. 2000; Wilkin and Flemal 1980). Surface water can absorb ammonia directly from the atmosphere near cattle feedlots, areas where the local atmospheric concentration may be high (Fangmeier et al. 1994; Hutchinson and Viets 1969). Ammonium can also be released to water when N_2 -fixing cyanobacteria (also called blue-green algae) die and are decomposed.

6.2.3 Soil

Ammonia enters soil through different processes, primarily human practices (e.g., fertilizer applications, animal husbandry), and natural biological processes. Direct application of fertilizers represents a major influx of ammonia into soils. Of the total U.S. production of anhydrous ammonia, 30% is applied directly to the soil under pressure (Kramer 2000). Approximately 80% of the U.S. production of ammonia is applied to soil in fertilizer formulations designed to release ammoniacal nitrogen. Application of natural fertilizers obtained from livestock excreta will also result in the release of ammonia to the soil (Asman et al. 1998; Beauchamp et al. 1982; Hoff et al. 1981; Olivier et al. 1998). High levels of ammonia in soils can result from the decomposition of animal wastes on cattle feedlots or other confinement areas, as well as from the land disposal of livestock and poultry waste. Ammonia in soil can also arise from the decay of organic material arising from plant, animal, and microbial byproducts and biomass (Dawson 1977; Dawson and Farmer 1984). Microbial fixation of nitrogen from the atmosphere is a natural and continual source of ammonia in soil, which can be released to soil after the microorganisms die (Galbally 1985; National Science Foundation 1999).

In nature, there are many pathways for incorporation of ammonia into soil. Natural sources include microbial decomposition of dead plants and animals, and hydrolysis or breakdown of urea and nitrogenous waste products in animal excretions. Several species of microorganisms can produce ammonia by the fixation of N_2 , and these organisms are widely dispersed throughout the soil (Atlas and

6. POTENTIAL FOR HUMAN EXPOSURE

Bartha 1998; Crutzen 1983); ammonia is released to the soil only after these microorganisms die. While several species of microbes can perform nitrogen fixation, this capability would not be one that is considered common for most microorganisms.

6.3 ENVIRONMENTAL FATE

In considering the environmental fate of ammonia, it is necessary to emphasize that ammonia is very important in nature and in nature's biological cycles. In our limited understanding of fluxes between these cycles, ammonia is considered a key intermediate. Nature has incorporated many mechanisms and “rules” for altering the distribution of ammonia through the biological system, as circumstances dictate. An in-depth discussion of these phenomena is outside the scope of this document; however, it is important to understand that for ammonia, all organisms contribute, either directly or indirectly, to the direction and distribution of the various environmental-fate processes.

An important consideration that affects the transport and partitioning of ammonia in the environment is that ammonia is a base. As a base, the physical and chemical properties of ammonia are pH-dependent, and thus, environmental-fate processes that influence the transport and partitioning of NH_3 will also be pH-dependent. For some environmental fate processes, a change in pH may only affect the relative rate of a process, while for others, it could change the direction or overall result of that process. The influence of pH on the environmental fate of ammonia will be discussed where appropriate. Temperature is also an important consideration in the environmental fate of ammonia. Temperature, although to a lesser extent than pH, affects the ammonia-ammonium equilibrium.

6.3.1 Transport and Partitioning

Atmospheric ammonia can be readily removed from the air by rain or snow washout (Adamowicz 1979; Asman et al. 1998; Kumar 1985). It can dissolve in the water found in clouds (Asman et al. 1998; Brimblecombe and Dawson 1984; Sprenger and Bachmann 1987) or fog (Johnson et al. 1987). Ammonia can be removed from the atmosphere through the direct absorption by surface waters in areas where the local atmospheric concentration is high (Hutchinson and Viets 1969) and by wet deposition onto soils and surface waters (Asman et al. 1998; Cuesta-Santos et al. 1998; Goulding et al. 1998). Uptake of atmospheric ammonia by different species of plants also occurs (Harper and Sharpe 1995; Nason et al. 1988; Rogers and Aneja 1980). Depending on the local atmospheric concentration, however, plants can

6. POTENTIAL FOR HUMAN EXPOSURE

also release ammonia to the atmosphere (Harper and Sharpe 1995; Lee et al. 1997; O'Deen and Porter 1986; Parton et al. 1988). By use of $^{15}\text{NH}_3$, it has been demonstrated that minerals and dry soil can rapidly and effectively adsorb NH_3 from air containing trace quantities of this gas (Bremner 1965). Ammonia is the predominant basic gas in the atmosphere (Allen et al. 1989). As such, it is capable of rapidly reacting with atmospheric H_2SO_4 , HNO_3 , or HCl , forming ammonium aerosols, which can then undergo dry deposition (Allen et al. 1989; Irwin and Williams 1988).

If released to surface water, ammonia can volatilize to the atmosphere or be taken up by aquatic plants. The rate of volatilization of ammonia from water will increase with increasing pH (generally only important above pH values of ~ 7.0) and temperature, and can be influenced by other environmental factors. Gaseous or liquid ammonia added to water will increase the pH of the medium; the rate of volatilization may increase dramatically if large amounts are released to relatively small static bodies of water, such as rice paddies (DeDatta 1995). Agitation will also increase the rate of volatilization. Georgii and Gravenhorst (1977) calculated the equilibrium concentration of ammonia above the Pacific Ocean. Using a constant concentration of 3 $\mu\text{mol/L}$, the ammonia concentration above the ocean as a result of increased volatilization was calculated to change from approximately 2.8 to 7 ppb as the pH was increased from 8.1 to 8.4 (at 25 °C). Volatilization of ammonia from flooded rice paddies was found to increase with increasing ammoniacal nitrogen concentration, pH, temperature, and wind velocity (Bouwmeester and Vlek 1981; DeDatta 1995; Tian et al. 2001). Ammonia can also be taken up by aquatic plants as a source of nutrients (Kemp and Dodds 2002).

In water, adsorption of ammonia to sediment and suspended organic material can be important under proper conditions (Ankley et al. 1990). Adsorption to sediment should increase with increasing organic content, increased metal ion content, and decreasing pH. Ammonia, however, can be produced in, and subsequently released from, sediment (Jones et al. 1982; Malcolm et al. 1986).

The uptake of ammonia by fish can also occur under the proper conditions (Hargreaves 1998; Mitz and Giesy 1985). Ammonia is the final breakdown product of nitrogenous-compound metabolism for catfish, and it is normally released through the gills into the surrounding water, driven by a concentration gradient. If the water concentration is abnormally high, the direction of passive ammonia transport is reversed.

A complete discussion of the factors influencing the transport and partitioning of ammonia in soil is outside the scope of this document. Adsorption of ammonia occurs in most moist or dry soils, and

6. POTENTIAL FOR HUMAN EXPOSURE

ammonia is predominantly, but not exclusively, held as the ammonium ion. Generally, adsorption will increase with increasing organic-matter content of the soil, and will decrease with increasing pH. Other factors that influence the adsorption of ammonia to soil are the presence of metallic ions, the predominant microbial populations and communities present, and its rate of uptake by plants. The ammonia concentration, temperature, and wind speed can also subtly affect the adsorption process by influencing the rate of volatilization (Bouwman et al. 1997; Bouwmeester and Vlek 1981; Brunke et al. 1988; Denmead et al. 1982; Galbally 1985; Goulding et al. 1998; Hoff et al. 1981; Kucey 1988; Nason et al. 1988; Reynolds and Wolf 1988; Socolow 1999). To demonstrate the influence of pH on the volatilization of ammonia (which, as indicated above, influences the potential for ammonia adsorption), ammonia loss was measured in greenhouse experiments using soils that had been adjusted to different pH values. Following the application of manure to the soil surface, ammonia volatilization was found to be 14% of the applied ammonium at a soil pH of 6.4 (manure pH=6.4). At a soil pH of 7.0 (manure pH=7.8), 65% was lost by volatilization (Hoff et al. 1981). In a study of the effects of SO₂ deposition on soils, it was found that the threshold pH at which ammonia volatilization from soil was drastically reduced did not occur until the pH was reduced to between pH 3.5 and 4.0 (Mahendrappa 1982). This is a relatively unrealistic and unrepresentative pH value for most soils; the results, however, indicate that volatilization will be an important process that affects the transport and partitioning of ammonia in most soils.

Because ammonia, as ammonium ion, is the nutrient of choice for many plants (Kramer 2000; Rosswall 1981), uptake of soil ammonia by living plants is an important fate process. The rate of uptake by plants varies with the growing season. At normal environmental concentrations, ammonia does not have a very long residence time in soil. It is either rapidly taken up by plants, bioconverted by the microbial population, or volatilized to the atmosphere. Because of these processes, and because ammonia generally exists in soils as NH₄⁺ (which binds to soils particles), ammonia does not leach readily through soil; thus, it is rarely found as a contaminant of groundwater (Barry et al. 1993). In soil, ammonia that results from the application of fertilizers is usually found in the top 10 inches of the soil (Beauchamp et al. 1982). However, nitrate derived from ammonia may leach to groundwater.

6.3.2 Transformation and Degradation

6.3.2.1 Air

In air, a dominant fate process for ammonia is the reaction with acid air pollutants. Formation of particulate NH₄⁺ compounds by reactions with HNO₃ and H₂SO₄ is rapid (Bouwman et al. 1997; Irwin

6. POTENTIAL FOR HUMAN EXPOSURE

and Williams 1988). The extent to which this process serves as a removal mechanism depends on the concentrations of these acidic compounds (Goulding et al. 1998). Thus, it is likely more important in areas of high industrial activity or a high density of automobile traffic, but of lesser importance over rural areas. These ammonium compounds can then be removed by dry or wet deposition.

The vapor-phase reaction of ammonia with photochemically produced hydroxyl radicals is known to occur. The rate constants for this reaction have been determined to be $1.6 \times 10^{-13} \text{ cm}^3/\text{molecule-sec}$, which translates to a calculated half-life of 100 days at a hydroxyl radical concentration of $5 \times 10^5 \text{ molecules/cm}^3$ (Graedel 1978). This process reportedly removes 10% of atmospheric ammonia (Crutzen 1983). Since ammonia is very soluble in water, rain washout is expected to be a dominant fate process. The half-life for ammonia in the atmosphere was estimated to be a few days (Brimblecombe and Dawson 1984; Crutzen 1983; Dawson 1977; Galbally and Roy 1983; Moller and Schieferdecker 1985). The reaction of atmospheric ammonia with acidic substances in the air results in the formation of ammonium aerosols that can subsequently be removed from the atmosphere by dry or wet deposition. In general, dry deposition processes predominate where there are high amounts of NH_3 emissions; where NH_3 emissions are lower, wet deposition of particulate NH_4^+ predominates (Asman et al. 1998).

6.3.2.2 Water

In surface water, groundwater, or sediment, ammonia can undergo sequential transformation by two processes in the nitrogen cycle, nitrification and denitrification, which would produce ionic nitrogen compounds, and from these, elemental nitrogen. The ionic nitrogen compounds formed from the aerobic process of nitrification, NO_2^- and NO_3^- , can leach through the sediment or be taken up by aquatic plants or other organisms. High concentrations of nitrate in groundwater can cause methemoglobinemia in infants when contaminated water is ingested (Messinga et al. 2003, Payne 1981). Elemental nitrogen formed from the anaerobic process of denitrification is lost by volatilization to the atmosphere.

In water, ammonia is in equilibrium with the ammonium ion, NH_4^+ . The ammonia-ammonium ion equilibrium is highly dependent on the pH and, to a lesser extent, the temperature of the medium. In acidic waters and neutral waters, the equilibrium favors the ammonium ion.

6. POTENTIAL FOR HUMAN EXPOSURE

6.3.2.3 Sediment and Soil

In soil, ammonia can serve as a nutrient source for plants, which can be taken up by plants and microorganisms and converted to organic-nitrogen compounds. Ammonia in soil can be rapidly transformed to nitrate by the microbial population through nitrification (Atlas and Bartha 1998; Payne 1981). The nitrate formed will either leach through the soil or be taken up by plants or other microorganisms. Very high localized concentrations of ammonia, such as those that might occur after a spill, or an excessive application, of ammonia-containing fertilizers can be toxic to plants, other organisms, or microbiota, which if inhibited or killed, will result in a decrease of the rates of any related nitrogen transformation processes. Under these conditions, other fate processes dictated by the physical and chemical properties of ammonia will dominate until the ammonia concentration returns to a background level. These physical and chemical processes include binding to soil particles (including organic carbon) or undergoing volatilization to the atmosphere.

6.3.2.4 Other Media

No data exist for the transformation or degradation of ammonia in other media, apart from biological tissues. These transformations are discussed in more detail in Chapter 3.

6.4 LEVELS MONITORED OR ESTIMATED IN THE ENVIRONMENT

In discussing the concentration of ammonia monitored in the environment, it is important to consider both ammonia and its conjugate acid, the ammonium ion. Independent determination of these compounds cannot always be achieved. In an analysis of the literature, it is difficult to separate aqueous ammonia concentration from aqueous ammonia-ammonium concentrations unless the investigators made a special effort to determine the amount of un-ionized ammonia. In this section of the document, ammonia will refer to the ammonia and ammonium concentration, and un-ionized ammonia will refer specifically to the ammonia concentration.

In the atmosphere, ammonia can exist in its gaseous state, be dissolved in rain, the water of fog, or clouds, or be found as ammonium in particulates and aerosols. These species can be analyzed separately. For this reason, atmospheric ammonia concentrations reported in this document will refer to the concentration of gaseous ammonia, and not to the concentrations of ammonium compounds.

6. POTENTIAL FOR HUMAN EXPOSURE

6.4.1 Air

Ammonia has a worldwide atmospheric background concentration. Estimates of the average global ammonia concentration are approximately 0.6–3 ppb (Aneja et al. 1998; Crutzen 1983; Georgii and Gravenhorst 1977). Dawson and Farmer (1984) reported that the average concentration of ammonia in the southwestern United States is 0.9 ppb, which may be considered a representative background value because at the site of these measurements, the prevalent winds came from the Pacific Ocean and there were no known urban or agricultural ammonia sources nearby. Fangmeier et al. (1994) reported similar values in a review of effects of atmospheric ammonia on vegetation. Values measured at sea or at high altitude provided a background range of 0.06–1.0 ppb (n=9 reports). When atmospheric ammonia levels have been determined to be above background levels, the measurements can often be correlated with industrial, agricultural, or other activities that might occur in nearby areas (Fangmeier et al. 1994).

The concentrations of ammonia in the atmosphere vary across the United States, with concentrations being higher in the Midwest as compared to either the west or east coasts. Based on early data on the concentration of ammonia in rain, Lau and Charlson (1977) determined a trend for the atmospheric ammonia concentration across the United States. The estimation of atmospheric ammonia content increased progressively starting from the east coast to the mid-west and on to the western states. Upon reaching the Pacific coast, the atmospheric ammonia concentration decreases. It should be noted that the values obtained in this study (ppt levels over the eastern seaboard and pacific coasts, and low ppb levels in the midwest and western United States) tend to be lower than those determined by more recent experiments. For example, Aneja et al. (1998) measured concentrations of both NH_3 and NH_4^+ at Mount Mitchell (North Carolina) over 2 years; concentrations averaged 1–2 ppb. Fangmeier et al. (1994) provided atmospheric ammonia values derived from five studies conducted in the United States, with an average NH_3 concentration of 3.3 ppb. The general conclusion of Lau and Charlson (1977) however, appears valid, and is indicative of the trends found for the ammonia concentrations in the atmosphere. Atmospheric ammonia concentrations are expected to be highest near intense agricultural or livestock production areas because of ammonia emissions from fertilizer and animal excreta, respectively. Lower concentrations are generally expected in more industrialized areas because of diminished sources of agricultural emissions and the atmospheric reaction of ammonia with acidic compounds known to be produced in industrial emissions and automobile exhaust. Data summarized in Fangmeier et al. (1994) indicate that industrial regions may have significant ammonia concentrations, but these are orders of magnitude lower than concentrations in regions with some agricultural applications. Concentrations

6. POTENTIAL FOR HUMAN EXPOSURE

determined near industrial sources in Germany (10.3–39.1 ppb) were in the same order of magnitude as concentrations near manure heaps (89 ppb), but were three orders of magnitude lower than emissions near pigpens (4.7×10^4 ppb).

Ground-level ammonia concentrations taken at urban Hampton and rural Langley, Virginia, ranged from 0.2 to 4.0 and from 1.5 to 4.0 ppb, respectively, in the fall of 1979 (Harward et al. 1982). Ammonia concentrations obtained in December of 1979 on Long Island, New York, ranged from approximately 80 to 200 nmol/m³ (1.9–4.8 ppb) (Tanner 1982). The ground level ammonia concentrations in Claremont, Los Angeles, and Anaheim, California, were <25 ppb (Russell et al. 1988). In Riverside and Rubidoux, California, areas near dairy feedlots, the ground level ammonia concentrations were 37–132 ppb and approximately 10–100 ppb, respectively. Rural-area concentrations, however, in Massachusetts and New York were considerably lower (0.2–1.1 ppb) (Fangmeier et al. 1994).

The ambient concentrations of ammonia determined at Whiteface Mountain, New York, in 1982 ranged from approximately 0.3 to 5 ppb, with the hourly median and mean values both determined as 2.2 ppb (Kelly et al. 1984). Ammonia concentrations in rural Thurber, Nevada, ranged from approximately 0.5 to 2 ppb (Farmer and Dawson 1982). In the atmosphere over the world's oceans, ammonia concentrations ranged from approximately 0.28 to 5.6 ppb (Georgii and Gravenhorst 1977).

Several investigators have studied the seasonal variation of ammonia concentrations in the atmosphere. In Hampton, Virginia, the ground level ammonia concentrations during the spring and summer were 10 and 1 ppb, respectively (Levine et al. 1980). The difference in concentration may have been due to volatilization of ammonia resulting from springtime application of fertilizer in nearby agricultural areas. In Warren, Michigan, the average ammonia concentrations measured during the summer, fall, winter, and spring were 0.85, 0.37, 0.10, and 0.16 ppb, respectively. The difference in concentrations was attributed to fluctuations in emissions from livestock excreta, since natural ammonia emissions are much higher in the summer than in the winter (Cadle 1985; NRC 1979). Additionally, in colder weather, microbial activity would be expected to decrease, and thus, ammonia emissions from the decay of organic matter would also be expected to decrease. Ammonia emissions from animal excretions also fluctuate with the time of day (Beauchamp et al. 1982; Brunke et al. 1988).

The concentration of ammonia in the atmosphere decreases with altitude. Levine et al. (1980) found that an ammonia concentration of 10 ppb measured at ground level decreased to a concentration of 1.5–3 ppb at a height of 10 km. In a historical modeling study on the European production of ammonia, levels based

6. POTENTIAL FOR HUMAN EXPOSURE

on ammonia release from livestock, fertilizer production and application, human and domestic animals, and sewage sludge resulted in average atmospheric ammonia concentrations ranging from 0.6 to 1.4 ppb for 1970 and from 0.7 to 5.6 ppb for 1980. The greatest increase occurred between 1950 and 1980, when synthetic fertilizer application and high nitrogen content feed grains were widely used (Asman and Drukker 1988). The ammonia concentration over a field during the application of gaseous ammonia fertilizer was as high as 300 ppb (Denmead et al. 1982). Over cattle feedlots, atmospheric ammonia concentrations have been measured at 520–2,160 ppb (Hutchinson et al. 1982).

6.4.2 Water

The concentration of ammonia in the Ochlocknee River at the head of Ochlocknee Bay, Florida, ranged from approximately 31 to 43 ppb, and concentrations of approximately 8.5 to 26 ppb were determined at the mouth of the bay (Seitzinger 1987). The concentration determined in the Ochlocknee River is consistent with levels reported for unpolluted tropical rivers (Meybeck 1982). Typical ammonia levels in the South Skunk River, Iowa, upstream from a municipal sewage-treatment facility were <1 ppm (Crumpton and Isenhardt 1988). Downstream of the facility, ammonia levels peaked at approximately 16 mg/L (16 ppm), with levels of un-ionized ammonia ranging from <1.0 to 2.2 ppm. The levels of undissociated ammonia were directly related to pH fluctuations in the river. The authors did not discuss why the upstream concentration was so high. In the same study, it was noted that ammonium and un-ionized ammonia concentrations fluctuated in a diurnal pattern in the river, with peaks in ammonia (approximately 1 mg/L) occurring around noon, and low concentrations (0.5 mg/L) occurring usually after midnight (Crumpton and Isenhardt 1988). The mean ammonia concentration in three Illinois rivers ranged from 0.28 mg/L (0.28 ppm) to 6.08 mg/L (6.08 ppm). The lower values were associated with agricultural sampling points and the higher values were associated with urban sampling points (Wilkin and Flemal 1980).

The ammonia concentration measured in Hamilton Harbour, Ontario, Canada was typically 0.1–3 mg/L (0.1–3 ppm) in the early 1980s. This body of water is used for water transportation (e.g., boat and barge traffic), as a source for industrial cooling water, and as a receptor for waste water disposal (Snodgrass and Ng 1985). Measurements made a few years later (1987–1988), in contrast, showed much lower concentrations. Measured concentrations, however, were still greater than the International Joint Commission objective of 20 :g/L (20 ppb) for more than half the year, and concentrations often exceeded the chronic toxicity threshold of 300 :g/L (300 ppb) (Barica 1990). This work reported that ammonia

6. POTENTIAL FOR HUMAN EXPOSURE

loadings into Hamilton Harbor had decreased over the late 1970s and 1980s, and the measured concentrations may reflect that change.

Some representative data regarding the concentration of ammonia in groundwater indicate that natural concentrations are generally low, but that agricultural practices can, at times, lead to higher levels. Low levels of ammonia have been found in groundwater wells under cattle and poultry feed lots, and in shallow wells. Wells 3–6 m deep showed little variation in ammonia concentration over a 3-year period where varying amounts of chicken manure were spread over agricultural plots, except when excessive amounts (54–179 metric ton/ha) were applied (Liebhardt et al. 1979). Groundwater levels of ammonia were also determined in an area in Idaho that had previously been noted as having high nitrogenous compound concentrations in the water. In this study, ammonium concentrations varied from 2.5 ppb in a municipal drinking water well to 3.25 ppm in a deep, private well (Wicherski 2000). Shallow wells in North Carolina had typical ammonia concentrations of 0.1–1 ppm, which were independent of land use, plant type, and amount of fertilization (Gilliam et al. 1974). Water samples from wells on four schoolyards in Michigan that used septic tank sewage systems had ammonia concentrations ranging from 0 to 733 ppb (Rajagopal 1978). In the Netherlands, the ammonium concentration detected in sample cups buried 1.2 m in the ground near forests ranged from 0 to 2.3 ppm (Krajenbrink et al. 1988), but no ammonia was detected in deeper wells (12.6 meters) analyzed in this study. The high adsorptivity of ammonium to soil and the rapid conversion of ammonia to nitrate by microbial action are both consistent with the usual finding of very low ammonia concentrations in groundwater.

Ammonia was measured in rain and snow samples from three sites in northern Michigan in 1978–1979. Concentrations ranged from 23.8 to 3,500 ppb, with mean values for each site of 816, 572, and 632 ppb, respectively. Concentrations were generally greatest in the spring and fall and were lowest during the winter (Munger 1982). Ammonia concentrations in bulk precipitation obtained in the Netherlands had median values ranging from 1.33 ppm in ocean areas to 5.09 ppm in bodies of water near heavily agricultural areas (Schuurkes et al. 1988).

Ammonia concentrations in the influent to sewage-treatment plants, and thus the effluent from sewer systems, typically range from 10 to 20 ppm (Englande et al. 1978; Hauser 1984; U.S. Army Corps of Engineers 1980). Waste water-treatment plant effluent is one of the few types of point sources of ammonia emissions to surface water. In a study of several waste water-treatment plants, eight of nine plants exceeded the guideline ammonia concentration (0.5 mg/L), with measured median values at these sites ranging from 0.08 to 15 mg/L (0.08–15 ppm) (Englande et al. 1978).

6. POTENTIAL FOR HUMAN EXPOSURE

No data were located in the available literature regarding ammonia concentrations in drinking water. This may be attributed to the rapid reaction between ammonia (and ammonium) and the chlorinating agents used in water treatment plants (Morris 1978).

6.4.3 Sediment and Soil

A 4-year study on ammonia levels in the soil (0–10 cm deep) of an open field (samples obtained in early May of each year) showed that ammonia concentrations ranged from 1 to 5 ppm (Beauchamp et al. 1982). The day after application of a slurry of liquid cow manure, the soil concentration ranged from 2 to 3,349 ppm. Five days after application, the concentration of ammonium ranged from 2 to 848 ppm. The greatest ammonia concentration was in the uppermost 4 cm of soil.

Ammonia was found at 137 of 1,647 hazardous waste sites on the NPL of highest priority sites for possible remedial action (HazDat 2004).

6.4.4 Other Environmental Media

The ammonia concentrations measured in the plumes of seven forest fires in the western United States ranged from 7 to 130 ppb; the median value of the 13 measurements was 37 ppb (Hegg et al. 1987, 1988). Fangmeier et al. (1994) reported a slightly higher value for smoke from a forest fire in Canada, 250 ppb. Ammonia has been found in the exhaust of automobile and diesel engines (Plerson and Brachaczek 1983). Ammonia has also been determined to be a component of tobacco and cigarette smoke (Sloan and Morie 1974).

6.5 GENERAL POPULATION AND OCCUPATIONAL EXPOSURE

The most probable routes by which the general population is exposed to ammonia are by the inhalation of ammonia that has volatilized from common household cleaning products and through dermal contact during the use of these products. Inhalation exposure to ammonia by some members of the rural population may occur for those who are near agricultural areas during the fertilizer-application period, those near animal feedlots or confinement areas, and those who apply anhydrous ammonia or ammonia-producing fertilizers to fields. There is also the possibility for exposure to ammonia via water and food

6. POTENTIAL FOR HUMAN EXPOSURE

ingestion. If untreated surface water is ingested, the average uptake would be 0.36 mg/day (assuming an ammonia concentration in untreated water of 0.18 mg/L and a consumption of 2 L/day) (WHO 1986). Food ingestion could lead to an exposure to ammonia, primarily from the use of various ammonium salts as stabilizers; the estimated exposure from these food additives is 18 mg/day (WHO 1986).

In the National Occupational Hazard Survey (NOHS) of 1972–1974, it was statistically estimated that 2,524,678 workers are exposed to ammonia in the United States (RTECS 1988). According to the National Occupational Exposure Survey (NOES), in 1989, 681,780 workers (231,208 of whom were female) were estimated to be exposed to ammonia (NOES 1989). A correlation of data from the EPA Air Toxics Emission Inventory with industrial source codes (SIC codes) shows that volatile emissions of ammonia are associated with 212 different industrial classifications (EPA 1987b).

Workers in swine- and poultry-confinement buildings may be exposed to elevated levels of ammonia (Attwood et al. 1987; Crook et al. 1991; Donham and Pependorf 1985; Jones et al. 1984; Leonard et al. 1984; Liao and Bundy 1995). Average ammonia concentrations in the air of these buildings depend on numerous factors; representative values ranged from 0.28 to 42.2 ppm (280–42,200 ppb) (Attwood et al. 1987; Fangmeier et al. 1994), but in buildings with slow ventilation rates, concentrations exceeded 80 ppm (Liao and Bundy 1995). It should be noted that workers in these buildings may also be exposed to other materials in addition to ammonia, including particulate material (small dessicated manure particles), endotoxin, and others which may lead to combined exposures.

Ammonia levels in air at an ammonium phosphate fertilizer-production plant ranged from 3 to 75 ppm (3,000–75,000 ppb) (NIOSH 1987). In a Finnish plywood factory, short-term ammonia concentrations during the mixing of urea-formaldehyde glue were 50–70 ppm (50,000–70,000 ppb) (Kauppinen 1986). Ammonia concentrations at 42 facilities using a blue-line printing system were 1–40 ppm (1,000–40,000 ppb) (Tuskes et al. 1988). Workers at coal-gasification units may be exposed occupationally to ammonia (Jin et al. 1999; Van Hoesen et al. 1984). Workers at ammonia transportation and storage facilities can be exposed to ammonia during the transfer between facilities, from the venting of built-up pressure in tanks, and during leaks or spills.

Farmers can be exposed to ammonia when applying fertilizer. The ammonia concentration over a field during the application of gaseous anhydrous ammonia fertilizer was as high as 213 $\mu\text{g}/\text{m}^3$ (300 ppb) (Denmead et al. 1982). Workers at cattle production facilities (e.g., feedlots, farms) and those who work under conditions where volatilization from animal excreta would be enhanced may be occupationally

6. POTENTIAL FOR HUMAN EXPOSURE

exposed to ammonia. Over cattle feedlots, atmospheric ammonia concentrations have been measured at 373–1,540 $\mu\text{g}/\text{m}^3$ (520–2,160 ppb) (Hutchinson et al. 1982). Exposure to ammonia can occur by inhalation in the liquid manure-storage facilities of swine-confinement buildings. Ambient air levels have been measured at up to 50 ppm (50,000 ppb) in these facilities (Donham et al. 1982).

6.6 EXPOSURES OF CHILDREN

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

Children are not small adults. A child's exposure may differ from an adult's exposure in many ways. Children drink more fluids, eat more food, breathe more air per kilogram of body weight, and have a larger skin surface in proportion to their body volume. A child's diet often differs from that of adults. The developing human's source of nutrition changes with age: from placental nourishment to breast milk or formula to the diet of older children who eat more of certain types of foods than adults. A child's behavior and lifestyle also influence exposure. Children crawl on the floor, put things in their mouths, sometimes eat inappropriate things (such as dirt or paint chips), and spend more time outdoors. Children also are closer to the ground, and they do not use the judgment of adults to avoid hazards (NRC 1993).

Three recent studies focused on the exposure of children to ammonia or the effects that exposure may have on children, all of which noted little effect on the children's health (Gomzi 1999; Gomzi and Saric 1997; Suh et al. 1992). One of the studies focused on the respiratory effects among people living near a fertilizer plant, another study investigated the effects of living in an urban vs. rural area, and a third study investigated the general effects of acid aerosols on children living in a semi-rural area. In general, these studies noted that exposure to low levels of ammonia had very little impact on the health of the children. The studies did find that other factors, such as parental smoking, had more profound effects on the children's respiratory health.

One study compared the effects of living near a fertilizer factory on the respiratory health of 8–9-year-old children (Gomzi and Saric 1997). The study found that the air quality near a fertilizer plant was within acceptable limits for most of the measurement period, with only a few fluctuations beyond acceptable limits. While these fluctuations correlated somewhat with health parameters measured on children living nearby, the rate of respiratory disease was more influenced by indoor air pollution sources than by

6. POTENTIAL FOR HUMAN EXPOSURE

outdoor sources. No significant effect was observed due to exposure to ammonia at the concentrations seen in the study.

A second study of 223 children (8–10 years old) living in Croatia found that indoor air quality had a slightly greater effect on respiratory health in urban areas compared to those living in rural areas (Gomzi 1999). The differences, however, were not significant. The study found no influence of ammonia on the children's respiratory health, but did find that parental smoking had a significant negative impact on their respiratory health.

A third study evaluated SO_4^{2-} and H^+ exposure to 24 children (ages were not provided) living in Uniontown, Pennsylvania (Suh et al. 1992). This study did not focus on ammonia exposure *per se*, but on other airborne contaminant concentrations in aerosols found outdoors, indoors, and by personal monitors. It sought to determine how personal exposures to these aerosols correlated with indoor and outdoor concentrations. Ammonia concentrations were measured in order to assess their potential for neutralizing H^+ found in aerosols. Ammonia concentrations were found to be highest near the children (detected by the personal monitors), followed by indoor concentrations, and were minimal outdoors. It was proposed that a large proportion of the H^+ found in indoor aerosols are neutralized by NH_3 , and would thus lower the children's exposure to acid aerosols. The authors noted that more research is needed to fully model the influence of factors, including NH_3 , on indoor acid aerosol exposure.

6.7 POPULATIONS WITH POTENTIALLY HIGH EXPOSURES

Workers in industries that commonly use ammonia, especially if there are no adequate safety and/or venting systems, may be at risk for potentially high exposure to ammonia. Examples of these might include farm workers who are employed in inadequately-ventilated, enclosed spaces with high concentrations of animals. Other examples include workers who process ammonia or transfer it from shipping containers to pipelines. The general population is at risk to high levels of exposure if cleaning products containing concentrated solutions of ammonia are used in small, enclosed, or unventilated rooms.

6. POTENTIAL FOR HUMAN EXPOSURE

6.8 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of ammonia is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of ammonia.

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

6.8.1 Identification of Data Needs

Physical and Chemical Properties. The physical and chemical properties of ammonia have all been well documented, and there do not appear to be any data needs in this area.

Production, Import/Export, Use, Release, and Disposal. The large amounts of ammonia produced in nature and in household products indicate that the risk for human exposure to ammonia exists. Data regarding the commercial production, disposal, and use of ammonia are well understood. Data regarding the production of ammonia by natural organisms, and its global and regional concentrations are not as well understood, nor are the influences of different process strategies on livestock ammonia emissions. This information would be useful in determining the contribution of anthropogenic ammonia to the global budget of this compound, which would help in determining the human influence on the global cycle.

According to the Emergency Planning and Community Right-to-Know Act of 1986 (EPCRA), (§313), (Pub. L. 99-499, Title III, 9313), industries are required to submit release information to the EPA. The TRI contains release information for 2001. This database is be updated yearly and provides a more reliable estimate of industrial production and emission.

6. POTENTIAL FOR HUMAN EXPOSURE

Environmental Fate. Since ammonia is a key intermediate in the nitrogen cycle, the environmental fate of ammonia should be interpreted in terms of its involvement in this cycle. Information available on the environmental fate of ammonia is sufficient to define the basic trends, and data are available regarding the direction of changes in these trends resulting from changes in the key variables. There are many subtle facets of the fate of ammonia in the environment that depend on nature and its cycles. Thus, accurately predicting the environmental fate of ammonia is not possible with our present knowledge.

An understanding of the environmental fate of ammonia is important when considering that human contribution to the global ammonia budget has grown over the years. A complete understanding of the environmental fate of ammonia will then allow an understanding of any changes that might occur from the role of ammonia in the nitrogen cycle. Since all living organisms depend on the nitrogen cycle, either directly or indirectly, this information would allow any decisions concerning ammonia to be made in an informed and prudent manner.

Bioavailability from Environmental Media. The bioavailability of ammonia from air and water has been examined rather extensively in animals. Bioavailability from soil has not been studied, although it is not a likely source of exposure.

Food Chain Bioaccumulation. Ammonia is a naturally-occurring compound and a key intermediate in the nitrogen cycle. Since it is continually recycled in the environment, bioaccumulation, as it is usually considered, does not occur. Thus, data on this process are not warranted.

Exposure Levels in Environmental Media. As an intermediate in the nitrogen cycle, ammonia is naturally present in environmental media. Measurements of ammonia in environmental media are sufficient to distinguish between background concentrations and elevated concentrations. Data regarding ammonia levels in soil samples, however, appear not to be as complete as the database for air and water.

Determining low level concentrations of atmospheric ammonia in the presence of ammonium salts is difficult. Recently, investigators have been establishing new methods for the analysis of ammonia in the presence of ammonium compounds (see Chapter 7, Analytical Methods). If highly accurate values for low levels of ammonia are necessary, then a re-evaluation of older literature values might be necessary.

Exposure Levels in Humans. Data regarding the exposure levels of ammonia are sufficient for understanding the sources and approximate magnitudes of human exposure. Quantitative monitoring data

6. POTENTIAL FOR HUMAN EXPOSURE

for specific circumstances, occupations, or events, as reported in the current literature, might be considered to be lacking. Monitoring data for ammonia concentrations in the average household are generally adequate. Reports indicate that while background indoor concentrations of chemicals such as ammonia are sometimes higher inside than outside the home, the levels of exposure do not generally have effects on residents. This exposure, however, would be expected to be higher when ammonia-containing cleaning products are used, or when other ammonia-containing compounds are used in the household, and effects under these conditions would depend on the exposure concentration and duration.

Exposures of Children. Data regarding the exposure levels of ammonia to children were not extensive enough for evaluating the sources and approximate exposures to children. As was found with data in the section for Exposure Levels in Humans above, quantitative monitoring data might be considered lacking. A few recent studies indicate that exposures to, and effects of, ammonia on children are generally minimal, and do not influence the respiratory health of the children studied. However, more studies could be conducted to verify these findings.

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

Exposure Registries. No exposure registries for ammonia were located. This compound is not currently one of the compounds for which a subregistry has been established in the National Exposure Registry (Agency for Toxic Substances and Disease Registry 1999). The compound will be considered in the future when chemical selection is made for subregistries to be established. The information that is amassed in the National Exposure Registry facilitates the epidemiological research needed to assess adverse health outcomes that may be related to exposure to this compound.

6.8.2 Ongoing Studies

The Federal Research in Progress (FEDRIP 2003) database and Current Research Information System (CRIS) database (USDA 2003) provide additional information obtainable from a few ongoing studies that may fill some of the data needs identified in Section 6.8.1. These studies are summarized below and in Table 6-2. Most of the studies are investigating approaches that reduce exposures to ammonia, emissions of ammonia during agricultural practices, and novel systems to reduce those emissions.

6. POTENTIAL FOR HUMAN EXPOSURE

Table 6-2. Ongoing Studies on Environmental Fate and the Potential for Human Exposure to Ammonia

Investigator	Affiliation	Research description	Sponsor
Bazemore RA; Chen TC	Mississippi State University	Evaluation of effectiveness of five substances (copper chlorophyllin complex, chitosan, activated carbon, kenaf, and paper mill sludge) in reducing ammonia emissions from animal waste compost.	Hatch
Harper LA; Sharpe RR	ARS, Athens, Georgia	Investigation of the generation and deposition of ammonia aerosols from swine waste, which is then compared to meteorological fluxes, with the objective being to reduce short-term and long-term ammonia losses that affect the local environment.	USDA in-house
Hristov AN	University of Idaho	This proposal seeks, through dietary means, better capture of ruminal ammonia-nitrogen into microbes and consequently into milk. This will increase the efficiency of utilization of feed N and reducing N excretions in the dairy cow.	NRI comp. grant
Walsh JL Jr.	Georgia Institute of Technology	The objective is to develop an integrated-optics (IO) sensor capable of measuring gaseous ammonia concentrations in the range of 100 ppb. This will be used to measure losses from agricultural croplands after application of nitrogen fertilizers.	U.S. DOE
Wilhelm LR et al.	University of Tennessee at Knoxville	Emission data and production information will be gathered from facilities country-wide for poultry and swine buildings. Evaluation of factors related to ammonia emissions will be conducted, and cost-effective approaches for reducing emissions considered and evaluated.	Hatch

Source: CRIS 2003; FEDRIP 2003

ARS = Agricultural Research Service; NRI = National Research Institute; USDA = U.S. Department of Agriculture; U.S. DOE = U.S. Department of Energy

6. POTENTIAL FOR HUMAN EXPOSURE

Several studies are being conducted to lessen exposures to ammonia. Researchers at the University of Kentucky are investigating methods to reduce emissions of ammonia from poultry houses by improving manure handling. In a different approach, researchers at the University of Idaho are investigating changes in animal diet as a means to improve the abilities of livestock to more completely incorporate ruminal ammonia nitrogen into milk and protein. If successful, both will result in lower exposures to excreted ammonia. Furthermore, both approaches will lead to more knowledge regarding the efficient transformation of ammonia into useful products, either compostable manure or food products.

A considerable number of studies are being conducted to provide better determinations of atmospheric transport and deposition of ammonia, either on a local scale or a global scale, and for reducing ammonia effluents from animal waste. The U.S. Department of Agriculture (USDA) at Watkinsville and Athens, Georgia, in conjunction with the University of Tennessee at Knoxville, is investigating approaches to reduce ammonia emissions from poultry, dairy, and swine facilities, with the objective being to reduce short- and long-term ammonia losses that affect indoor and outdoor air quality. The USDA in Fayetteville, Arkansas is testing different approaches to reduce ammonia emissions from poultry, swine, and dairy facilities via evaluation of the efficacy of alum treatments. Other research efforts being conducted by the USDA include the evaluation of the best use and application practices of animal manures that contain ammonia to reduce emissions and favor nutrient transfer to crop soils. Mississippi State University researchers are evaluating the quality of poultry effluents and swine litter for use as manure-based fertilizers for crops, wherein the efficiency of manure nutrient (including ammonia) transfer to the crop soils is being evaluated. Research is also being conducted at Mississippi State University to evaluate the use of cellulosic materials (i.e., kenaf) to decrease ammonia and odor emissions from poultry waste. Kenaf has several attractive characteristics as a biosorbent for ammonia, and is therefore being evaluated for the removal of odors and ammonia from waste streams. In another study being conducted at Mississippi State University, five treatments (copper chlorophyllin, chitosan, activated carbon, kenaf, and paper mill sludge) are being compared for their effects on reducing emissions from poultry litter. The findings indicate that the materials have different efficiencies for reducing overall odor emissions, but no report has been provided about their specific effects on ammonia emissions.

7. ANALYTICAL METHODS

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

7.1 BIOLOGICAL MATERIALS

When ammonia is found in biological materials at physiological pH (7.2), most of it (99%) will be found as ammonium ion, due to its pK_a of 9.2. This is an important consideration for any subsequent analysis. The determination of ammonia (as dissolved NH_3 and ammonium ion) in blood, plasma, or serum is of value in detecting existing or impending hepatic coma and Reyes Syndrome (Meyerhoff and Robins 1980; Tietz 1970). The determination of ammonia in urine had historically been used as an indicator of the kidney's ability to produce ammonia; however, this procedure has been replaced by more modern and accurate tests for kidney function. Procedures for the determination of ammonia in biological samples are found in Table 7-1. Ammonia is also tested for in calculi (abnormal concretions in the body formed of mineral deposits, often found in the gall bladder, kidney, or bladder) (Tietz 1970); however, the test described therein is not quantitative and is not included in Table 7-1.

The amount of ammonia in collected blood, urine, saliva, or other biological fluid samples can be affected by several mechanisms that may lead to erroneous ammonia concentration determinations. These effects can be minimized by proper sample storage and handling. The ammonia content of freshly drawn blood rises rapidly on standing because of the deamination of labile amides such as glutamine (Henry 1964); at room temperature, the ammonia content can increase by a factor of two or three in several hours. Therefore, it is important to both keep the specimen cold (on ice) and perform the analysis as soon as possible. If the sample cannot be analyzed quickly, it may be frozen (-20 °C). The ammonia content of

7. ANALYTICAL METHODS

Table 7-1. Analytical Methods for Determining Ammonia in Biological Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Urine	24-Hour specimen, add HCl, refrigerate	Colorimetric (Berthelot reaction)	Not reported	Not reported	Tietz 1970
Urine	24-Hour specimen analyzed immediately, or stored up to 8 weeks at -20 °C	Indophenol reaction	Not reported	Not reported	Huizenga et al. 1994
Saliva	Freeze at -20 °C for up to 2 weeks, or store for 1 hour at 4 °C, or analyze immediately	Membrane based ammonia-selective electrode	Not reported	Not reported	Huizenga et al. 1994
Serum, plasma, whole blood	Freeze, then store at -15 °C for several days, or store on ice (4 °C) for 30–60 minutes, or analyze immediately	Colorimetric assay based on indophenol production	Not reported	Not reported	Huizenga et al. 1994
Serum, plasma, whole blood	Freeze, then store at -15 °C for several days, or store on ice (4 °C) for 30–60 minutes, or analyze immediately	Titration	Not reported	Not reported	Huizenga et al. 1994
Serum, plasma, whole blood	Freeze at -30 °C or store on ice, or analyze immediately	Membrane based ammonia-selective electrode	Not reported	-7.0–14% error, 102% average recovery	Meyerhoff and Robins 1980

7. ANALYTICAL METHODS

iced (4 °C) blood samples remains constant for up to 60 minutes, whereas the ammonia content of frozen (-20 °C) blood samples remains constant for several days (Huizenga et al. 1994; Tietz 1970). For blood samples collected from a healthy person (and stored on ice), the ammonia content should be measured within 30–60 minutes of collection. For persons suspected of suffering from liver disease, however, the blood samples should be analyzed within 15 minutes (Huizenga et al. 1994). This more rapid assessment is necessary because some liver diseases result in high levels of γ -glutamyl transferase, an enzyme that hydrolyzes glutamine; the enzyme's activity will increase the concentration of ammonia in the sample to levels higher than was present at the time of collection (Huizenga et al. 1994). Other erroneously high measured ammonia levels can result from ammonia contamination of reagents or pick-up of ammonia from the atmosphere, including from technicians that have recently smoked a cigarette (Huizenga et al. 1994). The presence and activities of bacteria in samples can also cause changes in the concentrations of ammonia. The natural presence of bacteria present in some samples (e.g., saliva) or their presence in infected tissues (e.g., bladder infections) can lead to erroneously high ammonia concentrations, due to the presence of bacterial ureases that hydrolyze urea present in the biological samples. This reaction is the chief cause for the formation of ammonia in unacidified urine on standing (Henry 1964). Furthermore, contamination of samples by environmental bacteria following the collection of the sample may also lead to increases in ammonia concentrations. Therefore, use of sterile collection bottles for sample collection and storage is recommended if there is potential for storage of the sample prior to analysis (Huizenga et al. 1994).

In the determination of ammonia or ammonium ion in biological samples, ammonia is first liberated by distillation, aeration, ion-exchange chromatography, microdiffusion, or deproteinization (Huizenga et al. 1994). Deproteinization methods involve treatment of blood or plasma fluids with trichloroacetic acid (or perchloric acid or tungstate-sulfuric acid), followed by centrifugation. The protein-free supernatant can be assayed colorimetrically for ammonia. Traditionally, Kjeldahl distillation methods have been used to determine ammonia levels in biological tissue, but other methods (e.g., colorimetric or ion-specific electrodes) are also available. In the Kjeldahl distillation, ammonia is distilled and subsequently trapped in acid and analyzed titrimetrically or colorimetrically. High values sometimes result because of the cleavage of protein amino groups and also the formation of ammonia by deamination reactions (Parris and Foglia 1983). Other techniques use the ammonia-selective electrode and enzymatic assays. Discrepancies have been reported between results using electrodes and those using more specific enzymatic procedures because the ammonia electrode responds to both ammonia and volatile amines (Parris and Foglia 1983). Chromatographic separation of ammonia and volatile amines after derivatization has also been used to obtain specificity (Huizenga et al. 1994; Parris 1984). Ammonia in

7. ANALYTICAL METHODS

urine has been measured by Nesslerization, as well as by enzymatic assays and chromatographic approaches (Huizenga et al. 1994).

7.2 ENVIRONMENTAL SAMPLES

Water and waste water samples can be analyzed for ammonia by EPA Test Methods 1689 (EPA 2001a), 1690 (EPA 2001b), and 349.0 (EPA 1997). Analogous procedures (i.e., Method APHA 4500) have been approved and published jointly by the American Public Health Association, American Water Works Association, and Water Pollution Control Association. These methods are suitable for drinking, surface, and saline waters, and domestic and industrial effluent, and can be applied to biosolids. These and other methods for determining ammonia in environmental samples are listed in Table 7-2. Ammonia is reported as ammonia nitrogen. Two methods that are suitable for water employ colorimetric techniques, Nesslerization, and phenate methods. Nessler's reagent, an alkaline mixture of mercuric and potassium iodide, produces a yellow to brown color with ammonia, whereas the phenate reagent, alkaline phenol, and hypochlorite produce a blue color (EPA 2001a, 2001b; Greenberg et al. 1985). In the titrimetric method, the distillate is titrated with standard sulfuric acid with an appropriate indicator. The ammonia electrode employs a hydrophobic gas-permeable membrane to separate the sample solution from an internal ammonium chloride solution: ammonia diffusing through the membrane changes the pH of the internal solution and is sensed by a pH electrode. For determining $\text{NH}_3\text{-N}$ concentrations above 5 mg/L, the titrimetric and ammonia-selective electrode methods are preferred. In contrast, gas chromatography/mass spectrometry methods have been developed that permit NH_3 detection at concentrations near 20 $\mu\text{g/L}$ for environmental waters (Mishra et al. 2001). Methods for determining ammonia in water and soil measure ammoniacal nitrogen, the sum of NH_3 and NH_4^+ . In the determination of ammoniacal nitrogen in soil, exchangeable ammonium should be distinguished from nonexchangeable ammonium. The former is usually defined as that which can be extracted with KCl (or K_2SO_4) at room temperature (Bremner 1965). Nonexchangeable ammonium ion is defined as nitrogen held by clays and not displaced by 2M KCl (Bremner 1965). In the determination of nonexchangeable ammonium, organic forms of ammonia are first removed, the minerals containing the nonexchangeable ammonium are then decomposed with HF, and the ammonium ions released. In colorimetric procedures, turbidity and sample color may lead to interference. To eliminate interference, the pH of the sample may be raised and the ammonia distilled. Care should be taken to prevent losses in water samples due to volatilization and microbial transformation. To prevent such losses, samples should be acidified soon after collection and refrigerated. Care should also be taken during storage and treatment of soil samples to prevent ammonia

7. ANALYTICAL METHODS

Table 7-2. Analytical Methods for Determining Ammonia in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	Passive collection using 0.01 N H ₂ SO ₄ in liquid sorbent badge	Method 6701, ion chromatography, conductivity detection	1 µg NH ₃ /sample	No bias between 6.9 and 48 ppm; +19% at 148 ppm	NIOSH 1987
Air	Air samples from stack emissions collected through an in-stack filter to remove particulates and ammonium salts, and then bubbled through 0.1 N H ₂ SO ₄	EPA Method 30; ion chromatography	1 µg NH ₃ /sample	98.5±1.3%; Bias of 0.996 ppm for a spiked sample of 6.43 ppm. Correction factor of 0.87 needs to be applied	Eaton et al. 1996
Air	0.8 µm prefilter may be used; ammonia trapped on sulfuric acid silica gel	NIOSH method 6015, Colorimetric determination of indophenol by visible light spectrophotometry	0.5 µg NH ₃ /sample	Not determined	NIOSH 1994
Air	0.8 µm prefilter may be used; ammonia trapped on sulfuric acid silica gel	NIOSH method 6016. Ion chromatography	2 µg NH ₃ /sample	102±3.8%	NIOSH 1996
Air	Chromatomembrane cells preextract and preconcentrate sample	Ion chromatography with conductivity detection	6 µg NH ₃ /sample	Not reported	Erxleben et al. 2000
Air	Collection in H ₂ SO ₄ -coated activated carbon beads in sampling tube	Ion chromatography	2 µg NH ₃ /sample	95–110% recovery	Bishop et al. 1986
Air	Known volume of air drawn through prefilter and H ₂ SO ₄ -treated silica gel	NIOSH S347, ammonia-specific electrode	Not reported	97.6% mean recovery	SRI 1988
Water	Sample mixed with borate buffer	Method 1689, ion selective probe	0.1 mg/L	Not reported	EPA 2001a

7. ANALYTICAL METHODS

Table 7-2. Analytical Methods for Determining Ammonia in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Water	Sample collected, preserved with H ₂ SO ₄ , and chilled to 4 °C. Samples should not be stored for >28 days	Method 1690, colorimetric determination of indophenol blue, following reaction of any ammonia with alkaline phenol and hypochlorite	0.2 mg/L	Not reported	EPA 2001b
Water	None	Method 350.1 colorimetric, automated phenate	0.1 mg/L	107 and 99% recoveries at 0.16 and 1.44 mg NH ₃ -N/L, respectively	EPA 1983
Estuarine and coastal water	Samples filtered through 0.45 µm membrane filter, refrigerated and analyzed within 3 hours	Method 349.0, automated colorimetric determination by reactions that form indophenol blue	0.3 µg/L	92.2–109.1% recovery, n=14	EPA 1997a
Water	Removal of residual chlorine with sodium thiosulfate, distillation	Method 350.2 Nessler reagent, colorimetric, titrimetric; or ammonia specific electrode	0.05 mg N/L for colorimetric and potentiometric; 1.0 mg N/L for titrametric	28.12 to -0.46R bias between 0.21 and 1.92 mg N/L	EPA 1983
Water	None	Method 350.3 ion selective electrode	0.03 mg N/L	96 and 91% recoveries at 0.19 and 0.13 mg N/L, respectively	EPA 1983
Soil, exchangeable ammonium	Extract soil with 2N KCl	Method 84-3, steam distillation with MgO, titration	Not reported	Not reported	Bremner 1965
Soil, non-exchangeable (fixed) ammonium	Pretreat soil with KOBBr-KOH, shake with 5 N HF-1N HCl for 24 hours	Method 84-7, steam distillation with KOH, titration	Not reported	Not reported	Bremner 1965

7. ANALYTICAL METHODS

loss or gain. It has been demonstrated that dry soil can rapidly adsorb trace amounts of ammonia from the atmosphere and that extensive amounts of ammonia can be lost during air drying (Bremner 1965). Additionally, in samples containing both ammonium and nitrite, losses during air drying may occur due to the reaction between these ions and the resulting formation and release of nitrogen gas (Bremner 1965).

The detection limit of analytical methods for determining ammonia in air depends on the amount of air collected in a liquid or solid adsorbent. Sampling is performed with passive samplers or by drawing a volume of air through the adsorbent using a pump. Particulate contaminants such as ammonium salts may be removed by a prefilter. For determination of ammonia in the ambient atmosphere, larger volumes of air must be sampled than those appropriate for determinations of ammonia in occupational settings (e.g., industrial, agricultural) where ammonia levels are higher. Improvements in methodologies have led to development of techniques that permit continuous monitoring of atmospheric ammonia down to $0.1 \mu\text{g}/\text{m}^3$ (Pranitis and Meyerhoff 1987). Several passive monitoring systems report detection limits of $0.05\text{--}1.0 \mu\text{g}/\text{m}^3$ and have collection rates ranging from 2.7 to 2,000 mL/minute (Kirchner et al. 1999). One method used for ambient atmospheric sampling employs a specially designed flow-through, ammonia-selective electrode with a sniffer tube, whereas the methods used for occupational settings often use passive collectors with media (usually acids impregnated onto filters) housed within protective cases. Ammonia concentrations on these passive collectors are then determined by a wide range of methods, including colorimetric assays (e.g., indophenol determination), the Berthelot reaction, or ion chromatography (Kirchner et al. 1999).

Ammonia may be present in air in both the vapor phase as ammonia gas and in the particulate phase as ammonium salts. While some analytical methods may distinguish between these phases, most standard methods do not. Methods have been developed that determine gaseous ammonia alone or gaseous and particulate forms of ammoniacal nitrogen separately. These methods use filter packs or sampling tubes coated with a selective adsorbent (denuder tube) to separate the phases (Dimmock and Marshall 1986; Knapp et al. 1986; Rapsomanikis et al. 1988). In these methods, gaseous ammonia is trapped by acids that act as adsorbents (e.g., citric acid, oxalic acid, phosphoric acid) on a coated filter or denuder tube (Kirchner et al. 1999). In filter methods, errors may arise due to ammonia interactions occurring on the filter and volatilization of retained ammonium salt (Dimmock and Marshall 1986; Rapsomanikis et al. 1988). There is evidence that ammonium nitrate in particulate matter is in equilibrium with ammonia. The presence of ammonium nitrate may lead to overestimation of the actual concentration of ammonia, but underestimation of the concentration of ammonium (Doyle et al. 1979). For additional review of the

7. ANALYTICAL METHODS

methodologies for determining ammonia in water and air, see MacCarthy et al. (1987) and Fox (1987), respectively.

7.3 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of ammonia is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of ammonia.

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

7.3.1 Identification of Data Needs

Methods for Determining Biomarkers of Exposure and Effect. No known unique biomarkers for exposure or effects exist for ammonia. Until one has been identified, methodology for the determination of biomarkers must be preceded by an experimental determination of a unique biomarker of human exposure or effect.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Methods for determining ammoniacal nitrogen in environmental media are well developed and adequate. Standardized methods are available from EPA, NIOSH, and other sources. Analytical methods are also well developed for oxidation products of ammonia. Since there are multiple sources of these compounds in the environment, their analysis is not generally used to study the disappearance of ammonia.

7.3.2 Ongoing Studies

The Federal Research in Progress (FEDRIP 2003) database provides additional information obtainable from a few ongoing studies that may fill some of the data needs identified in Section 7.3.1. These studies are summarized below and in Table 7-3. By and large, most of the studies that were reported were related to the detection and measurement of atmospheric ammonia concentrations. Many of these focused on the development of novel sensor devices, which would provide better data for estimating ammonia emission and deposition rates. A company in Atlanta, Georgia is developing an optical ammonia sensor to measure agricultural emissions. This sensor is capable of detecting ammonia concentrations at the 100 ppb range and above. Similarly, the Georgia Institute of Technology is developing an optical sensor that will permit measurements of ammonia in the atmosphere at the 100 ppb range. Another company in Burlington, Massachusetts is developing a diode laser absorption remote sensor for measuring ammonia at trace concentrations, but no detection ranges have been specified. Another company in Massachusetts is developing a solid-state electrochemical sensor that is based on ionomer (i.e., an ion-containing polymer) membrane technology. This technology, however, is not intended for atmospheric ammonia sampling, but for instrument monitoring where ammonia gas may have negative impacts. This particular application seeks to produce these monitors for use in fuel cell systems, where free ammonia can negatively impact performance.

7. ANALYTICAL METHODS

Table 7-3. Ongoing Studies on the Development of Analytical Approaches to the Study of Ammonia

Investigator	Affiliation	Research description	Sponsor
Attar AJ	Perfect View, Inc., Raleigh, North Carolina	Development of a low-cost ammonia detector (a small detector panel, about the size of a credit card, that changes color in response to ammonia in the atmosphere) for use in animal production facilities.	SBIR
Edwards J	Photonic Sensors, Atlanta, Georgia	Development of an optical ammonia sensor to measure agricultural emissions. This sensor is capable of detecting ammonia concentrations at the 100 ppb range.	Cooperative Agreement
Goldstein N	Spectral Sciences, Burlington, Massachusetts	Development of a diode laser absorption sensor for measurement of trace concentrations of ammonia, for potential applications in atmospheric chemistry and pollution monitoring.	U.S. DOE
Laconti AB	Giner, Inc., Waltham, Massachusetts	Development of a solid-state electrochemical sensor that is based on ionomer membrane technology for instrument monitoring where ammonia gas may have negative impacts.	SBIR
Ozanich RM	Northwest Instrument Systems, Richland, Washington	Development of an online system for analyzing ammonia (and other nitrogen containing chemicals) in water systems.	SBIR
Walsh JL Jr.	Georgia Institute of Technology, Atlanta, Georgia	Development of an optical ammonia sensor to measure agricultural emissions. This sensor is capable of detecting ammonia concentrations at the 100 ppb range, and would be useful for real-time monitoring of the injection of anhydrous ammonia fertilizer onto crops.	U.S. DOE

Source: FEDRIP 2002, 2003

SBIR = Small Business Innovative Research; U.S. DOE = U.S. Department of Energy

8. REGULATIONS AND ADVISORIES

International guidelines for ammonia were not located. National and state regulations and guidelines pertinent to human exposure to ammonia are summarized in Table 8-1.

ATSDR has derived an acute-duration inhalation MRL of 1.7 ppm for ammonia based on a minimal LOAEL of 50 ppm for eye, nose, and throat irritation in a study with volunteers (Verberk et al. 1977). No NOAEL was identified in that study. An uncertainty factor of 30 (3 for the use of a minimal LOAEL and 10 to protect sensitive individuals) was applied to the LOAEL.

ATSDR has derived a chronic-duration inhalation MRL of 0.1 ppm for ammonia based on a NOAEL of 9.2 ppm for sense of smell, prevalence of respiratory symptoms (cough, bronchitis, wheeze, dyspnea, etc.), eye and throat irritation, and pulmonary function parameters in workers exposed for approximately 12 years in a soda ash plant (Holness et al. 1989). No LOAEL was defined in that study. The NOAEL was duration-adjusted, and divided by an uncertainty factor of 10 to protect sensitive individuals. A modifying factor of 3 was added for the lack of reproductive and developmental studies. This MRL supersedes the previous chronic inhalation MRL of 0.3 ppm derived in the 2002 draft for public comment version of this profile.

EPA derived an inhalation reference concentration (RfC) of $1\text{E-}1 \text{ mg/m}^3$ (0.14 ppm) for ammonia based on a NOAEL of 6.4 mg/m^3 (9.2 ppm) defined in the Holness et al. (1989) study (IRIS 2004). EPA used an uncertainty factor of 30 (10 to protect sensitive individuals and 3 for data base deficiencies).

Ammonia has not undergone a complete evaluation under EPA's IRIS program for evidence of human carcinogenic potential.

Ammonium ion is regulated by the Clean Water Effluent Guidelines for the following industrial point sources: ferroalloy manufacturing; fertilizer manufacturing; glass manufacturing; inorganic chemicals; iron and steelmaking; landfills; nonferrous metals manufacturing; nonferrous metals forming and metal powder; paper and paperboard; petroleum refining; pharmaceutical manufacturing; pulp, meat products; and transportation equipment cleaning (EPA 2002j).

8. REGULATIONS AND ADVISORIES

The FDA (1973) determined that concentrations of ammonia and ammonium compounds normally present in food do not suggest a health risk; ammonia and ammonium ions are recognized to be integral components of normal metabolic processes. However, some restrictions have been placed on levels of ammonium salts allowable in processed foods. Maximum allowable levels in processed foods are as follows: 0.04–3.2% ammonium bicarbonate in baked goods, grain, snack, foods and reconstituted vegetables; 2.0% ammonium carbonate in baked goods, gelatins and puddings; 0.001% ammonium chloride in baked goods and 0.8% in condiments and relishes; 0.6–0.8% ammonium hydroxide in baked goods, cheeses, gelatins and puddings; 0.01% monobasic ammonium phosphate in baked goods; and 1.1% dibasic ammonium phosphate in baked goods, 0.003% in nonalcoholic beverages, and 0.012% for condiments and relishes.

8. REGULATIONS AND ADVISORIES

Table 8-1. Regulations and Guidelines Applicable to Ammonia

Agency	Description	Information	References
<u>INTERNATIONAL</u>			
Guidelines:			
IARC	Carcinogenicity classification	No data	
WHO	Drinking water quality guideline Ammonia		WHO 2002
	Threshold odor concentration	1.5 mg/L	
	Threshold taste concentration	35 mg/L	
	Health-based guideline	None proposed	
<u>NATIONAL</u>			
Regulations and Guidelines:			
a. Air			
ACGIH	TLV (8-hour TWA) Ammonia	25 ppm	ACGIH 2001
	Ammonium chloride fume	10 mg/m ³	
	STEL (15-minute TWA) Ammonia	35 ppm	ACGIH 2001
	Ammonia chloride fume	20 mg/m ³	
EPA	Accidental release prevention; toxic endpoint		EPA 2002b
	Ammonia (anhydrous)	0.14 mg/L	40CFR68, Appendix A
	Ammonia (>20% concentration)	0.14 mg/L	
	Regulated toxic substance for accidental release prevention under Section 112(r) of the Clean Air Act; threshold quantity		EPA 2002a
	Ammonia (anhydrous)	10,000 pounds	40CFR68.130, Table 1
	Ammonia (>20% concentration)	20,000 pounds	
	AEGL 1 (interim)	30 ppm	EPA 2004
NIOSH	REL (10-hour TWA) Ammonia	25 ppm	NIOSH 2002b
	Ammonium chloride fume	10 mg/m ³	
	STEL (15-minute TWA) Ammonia	35 ppm	NIOSH 2002b
	Ammonium chloride fume	20 mg/m ³	
	IDLH Ammonia	300 ppm	NIOSH 2002b
	Ammonium chloride fume	No data	
OSHA	PEL (8-hour TWA) for general industry		OSHA 2002d 29CFR1910.1000, Table Z-1
	Ammonia	50 ppm	

8. REGULATIONS AND ADVISORIES

Table 8-1. Regulations and Guidelines Applicable to Ammonia

Agency	Description	Information	References
NATIONAL (cont.)			
OSHA	PEL (8-hour TWA) for construction industry		OSHA 2002c 29CFR1926.55, Appendix A
	Ammonia	50 ppm	
	PEL (8-hour TWA) for shipyard industry		OSHA 2002a 29CFR1915.1000
	Ammonia	50 ppm	
	Highly hazardous chemical, toxic, and reactive for general industry; threshold quantity ^a		OSHA 2002e 29CFR1910.119, Appendix A
	Ammonia	10,000 pounds	
	Ammonia solutions (>44% of ammonia by weight)	15,000 pounds	
	Highly hazardous chemical, toxic, and reactive for construction industry; threshold quantity ^a		OSHA 2002f 29CFR1926.64, Appendix A
	Ammonia	10,000 pounds	
	Ammonia solutions (>44% of ammonia by weight)	15,000 pounds	
	Occupational safety and health standards; storage and handling of anhydrous ammonia		OSHA 2002g 29CFR1910.111
	Occupations involved in agriculture that are particularly hazardous for the employment of children below the age of 16	Transporting, transferring, or applying anhydrous ammonia	OSHA 1998 29CFR570.71(a)(11)
b. Water	Safety and health regulations for construction; blasting and use of explosives; common blasting agent is a mixture of ammonium nitrate and carbonaceous combustibles		OSHA 2002b 29CFR1926.914(e)
EPA	Hazardous substance designated pursuant to Section 311(b)(2)(A) of the Clean Water Act		EPA 2002h 40CFR116.4, Table A
	Ammonia		
	Ammonium chloride		
	Ammonium fluoride		
	Ammonium hydroxide		
EPA	Reportable quantity of hazardous substances designated pursuant to Section 311 of the Clean Water Act		EPA 2002i 40CFR117.3
	Ammonia	100 pounds	
	Ammonium chloride	5,000 pounds	
	Ammonium fluoride	100 pounds	
	Ammonium hydroxide	1,000 pounds	

8. REGULATIONS AND ADVISORIES

Table 8-1. Regulations and Guidelines Applicable to Ammonia

Agency	Description	Information	References
<u>NATIONAL</u> (cont.)			
USC	Assurances of availability of adequate supplies of chemicals necessary for treatment of water	Ammonia	USC 2002a 42USC300j
c. Food			
EPA	Residues from ammonium chloride, ammonium hydroxide, and ammonium sulfate are exempted from the requirement of a tolerance when used in accordance with good agricultural practice as inert (or occasionally inactive) ingredients in pesticide formulations applied to growing crops or to raw agricultural commodities after harvest		EPA 2002e 40CFR180.1001(c)
	Ammonium nitrate is exempt from the requirement of a tolerance when used in accordance with good agricultural practice as inert (or occasionally active) ingredients in pesticide formulations applied to growing crops only		EPA 2002e 40CFR180.1001(d)
	The fungicide ammonia is exempted from the requirement of a tolerance when used after harvest on the raw agricultural commodities grapefruit, lemons, oranges, and corn grain for feed use only		EPA 2002f 40CFR180.1003
FDA	Direct food substances affirmed as generally recognized as safe	Ammonium chloride	FDA 2001a 21CFR184.1138
	Direct food substances affirmed as generally recognized as safe	Ammonium hydroxide	FDA 2001b 21CFR184.1139
FDA	Direct food substances affirmed as generally recognized as safe	Ammonium sulfate	FDA 2001c 21CFR184.1143
	Drug products containing certain active ingredients offered over-the-counter	Ammonium chloride	FDA 2001d 21CFR310.545(a)
	Expectorant drug product	Ammonia solution	
	Fever blister and cold sore treatment drug product	Ammonia solution and	
	Insect bite and sting drug products	Ammonium hydroxide	
	Food additives permitted in feed and drinking water of animals	Anhydrous ammonia	FDA 2001e 21CFR573.180
	Substance generally recognized as safe when used in accordance with good manufacturing or feeding practices	Ammonium hydroxide	FDA 2001f 21CFR582.1139

8. REGULATIONS AND ADVISORIES

Table 8-1. Regulations and Guidelines Applicable to Ammonia

Agency	Description	Information	References
<u>NATIONAL</u> (cont.)			
	Substance generally recognized as safe when used in accordance with good manufacturing or feeding practices	Ammonium sulfate	FDA 2001g 21CFR582.1143
d. Other			
CPSC	Federal Caustic Poison Act Ammonia water and any preparation containing free or chemically uncombined ammonia, including ammonium hydroxide and "hartshorn", in a concentration of 5% or more		CPSC 2001 16CFR1500.129(1)
EPA	Ammonia Carcinogenicity classification RfC RfD CERCLA hazardous substance designated pursuant to Section 311(b)(4) of the Clean Water Act Reportable quantity Ammonia Ammonium chloride Ammonium fluoride Ammonium hydroxide	No data 1×10^{-1} mg/m ³ No data	IRIS 2004 EPA 2002d 40CFR302.4(a)
EPA	Extremely hazardous substance Ammonia Reportable quantity Threshold planning quantity Toxic chemical release reporting; Community right-to-know; effective date for reporting Ammonia ^b Ammonium nitrate (solution)	100 pounds 500 pounds	EPA 2002c 40CFR355, Appendix A EPA 2002g
USC	Imposition of Superfund tax on any taxable chemical sold by the manufacturer, producer, or importer Ammonia Refund or credit of Superfund tax paid when ammonia is used as a fertilizer Superfund taxable substance	01/01/87 01/01/87 ^c \$2.64 per ton Ammonium nitrate	40CFR372.65(a) USC 2002d 26USC4661 USC 2002b 26USC4662 USC 2002c 26USC4672
<u>STATE</u>			
Regulations and Guidelines:			
a. Air		No data	

8. REGULATIONS AND ADVISORIES

Table 8-1. Regulations and Guidelines Applicable to Ammonia

Agency	Description	Information	References
<u>STATE (cont.)</u>			
b. Water		No data	
c. Food		No data	
d. Other		No data	
Florida	Toxic substance Ammonia Ammonium chloride Ammonium fluoride Ammonium nitrate Ammonium sulfate		BLR 2002
Massachusetts	Hazardous substance Ammonia Ammonium chloride Ammonium fluoride Ammonium hydroxide Ammonium nitrate Ammonium sulfate		BLR 2002
Minnesota	Hazardous substance Ammonia Ammonium chloride, fume		BLR 2002
New Jersey	Hazardous substance Ammonia		BLR 2002
New York	Hazardous substance Ammonia Ammonium chloride Ammonium fluoride Ammonium hydroxide		BLR 2002
Pennsylvania	Hazardous substance Ammonia Ammonium chloride Ammonium fluoride Ammonium hydroxide		BLR 2002

^aPotential for a catastrophic event at or above the threshold quantity.

^bAmmonia: includes anhydrous ammonia, aqueous ammonia from water, dissociable ammonium salts, and other sources; 10% of total aqueous ammonia is reportable under this listing.

^cAmmonium nitrate (solution) is removed from this listing; the removal is effective 07/02/95, for the 1995 reporting year.

ACGIH = American Conference of Governmental Industrial Hygienists; AEGL = acute exposure guideline value; BLR = Business & Legal Reports, Inc. CERCLA = Comprehensive Environmental Response, Compensation, and Liability Act; CFR = Code of Federal Regulations; CPSC = Consumer Protection Safety Commission; EPA = Environmental Protection Agency; FDA = Food and Drug Administration; IARC = International Agency for Research on Cancer; IDLH = immediately dangerous to life and health; IRIS = Integrated Risk Information System; NIOSH = National Institute of Occupational Safety and Health; OSHA = Occupational Safety and Health Administration; PEL = permissible exposure limit; ppm = parts per million; REL = recommended exposure limit; RfC = inhalation reference concentration; RfD = oral reference dose; STEL = short-term exposure limit; TLV = threshold limit value; TWA = time-weighted average; USC = United States Code; WHO = World Health Organization

9. REFERENCES

ACGIH. 1988. Threshold limit values and biological exposure indices for 1988-1989. Cincinnati, OH. American Conference of Governmental Industrial Hygienists.

ACGIH. 1991. Ammonium chloride fume. Documentation of the threshold limit values: For chemical substances in the work environment. Cincinnati, OH: American Conference of Governmental Industrial Hygienists.

*ACGIH. 2001. Threshold limit values for chemical substances and physical agents and biological exposure indices. Cincinnati, OH: American Conference of Governmental Industrial Hygienists.

*Adamowicz RF. 1979. A model for the reversible washout of sulfur dioxide, ammonia and carbon dioxide from a polluted atmosphere and the production of sulfates in raindrops. *Atmos Environ* 13:105-121.

*Adlercreutz H. 1995. Phytoestrogens: Epidemiology and a possible role in cancer protection. *Environ Health Perspect Suppl* 103(7):103-112.

Aelion CM, Shaw JN, Wahl M. 1997. Impact of suburbanization on ground water quality and denitrification in coastal aquifer sediments. *J Exp Mar Biol Ecol* 213(1):31-51.

*Agency for Toxic Substances and Disease Registry. 1989. Decision guide for identifying substance-specific data needs related to toxicological profiles; Notice. *Fed Reg* 54(174):37618-37634.

*Agency for Toxic Substances and Disease Registry. 1999. ATSDR National Exposure Registry (NER). <http://www.atsdr.cdc.gov/faq/faqner.html>. May 3, 2002.

Agte V, Chiplonkar S, Joshi N, et al. 1994. Apparent absorption of copper and zinc from composite vegetarian diets in young Indian men. *Ann Nutr Metab* 38(1):13-19.

Ahmad I, Ali S, Jan MR. 2000. Physico-chemical analysis of fertilizer industry effluent and its effects on crop plants. *Pak J Sci Ind Res* 43(2):83-89.

Albrecht J. 1998. Roles of neuroactive amino acids in ammonia neurotoxicity. *J Neurosci Res* 51(2):133-138.

Ali BA, Ahmed HO, Ballal SG, et al. 2001. Pulmonary function of workers exposed to ammonia: A study in the Eastern Province of Saudi Arabia. *Int J Occup Environ Health* 7(1):19-22.

Allen AG, Harrison RM, Erisman J-W. 1989. Field measurements of the dissociation of ammonium nitrate and ammonium chloride aerosols. *Atmos Environ* 23(7):1591-1599.

* Cited in text

9. REFERENCES

- Allen WM, Sansom BF, Trower CJ. 1993. Bovine deaths associated with ammoniated straw. *Vet Rec* 132(16):419.
- *Altman PL, Dittmer DS. 1974. *Biological handbooks: Biology data book*. Vol. III. 2nd ed. Bethesda, MD: Federation of American Societies for Experimental Biology, 1987-2008, 2041.
- Ambrose AM. 1943. Studies on the physiological effects of sulfamic acid and ammonium sulfamate. *J Ind Hyg Toxicol* 25(1):26-28.
- *Amoore JE, Hautala E. 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 dilution. *J Appl Toxicol* 3(6):272-290.
- *Amshel CE, Fealk MH, Phillips BJ, et al. 2000. Anhydrous ammonia burns case report and review of the literature. *Burns* 26(5):493-497.
- *Andersen ME, Krishnan K. 1994. Relating in vitro to in vivo exposures with physiologically based tissue dosimetry and tissue response models. In: Salem H, ed. *Animal test alternatives: Refinement, reduction, replacement*. New York: Marcel Dekker, Inc., 9-25.
- *Andersen ME, Clewell HJ III, Gargas ML, et al. 1987. Physiologically based pharmacokinetics and the risk assessment process for methylene chloride. *Toxicol Appl Pharmacol* 87:185-205.
- Andersson BO. 1991. Lithuanian ammonia accident, March 20th 1989. *Inst Chem Eng Symp Ser* 124:15-17.
- *Aneja VP, Murthy AB, Battye W, et al. 1998. Analysis of ammonia and aerosol concentrations and deposition near the free troposphere at Mt. Mitchell, NC, USA. *Atmos Environ* 32(3):353-358.
- Anghileri LJ. 1975. Tumor growth inhibition by ammonium chloride-induced acidosis. *Int J Clin Pharmacol* 12:320-326.
- *Ankley GT, Katko A, Arthur JW. 1990. Identification of ammonia as an important sediment-associated toxicant in the lower Fox River and Green Bay, Wisconsin. *Environ Toxicol Chem* 9:313-322.
- Anonymous. 2003. Final report on the safety assessment of ammonium, potassium, and sodium persulfate. *Int J Toxicol* 20(3):7-21.
- *Appelman LM, ten Berg WF, Reuzel PGJ. 1982. Acute inhalation toxicity of ammonia in rats with variable exposure periods. *Am Ind Hyg Assoc J* 43(9):662-665.
- *ApSimon HM, Kruse M, Bell JNB. 1987. Ammonia emissions and their role in acid deposition. *Atmos Environ* 21:1939-1946.
- *Arwood R, Hammond J, Ward GG. 1985. Ammonia inhalation. *J Trauma* 25 (5):444-447.
- *Asman WAH, Drukker B. 1988. Modelled historical concentrations and depositions of ammonia and ammonium in Europe. *Atmos Environ* 22:725-736.
- *Asman WAH, Janssen AJ. 1987. A long-range transport model for ammonia and ammonium for Europe. *Atmos Environ* 21:2099-2119.

9. REFERENCES

- *Asman WAH, Sutton MA, Schjorring JK. 1998. Ammonia: Emission, atmospheric transport and deposition. *New Phytol* 139(1):27-48.
- *Atlas RM, Bartha R. 1998. Biochemical cycling: nitrogen, sulfur, phosphorus, iron, and other elements. In: Fogel L, Wong G, eds. *Microbial ecology. Fundamentals and applications*. Menlo Park, CA: Benjamin/Cummings Science Publishing, 414-425.
- *Attwood P, Brouwer R, Ruigewaard P, et al. 1987. A study of the relationship between airborne contaminants and environmental factors in Dutch confinement buildings. *Am Ind Hyg Assoc J* 48(8):745-751.
- *Auerbach C, Robson JM. 1947. XXXIII-Test of chemical substances for mutagenic action. *Proc R Soc Edinb (Nat Environ)* 62:284-291.
- *Ayers GP, Gillett RW, Caesar ER. 1985. Solubility of ammonia in water in the presence of atmospheric CO₂. *Tellus* 37B:35-40.
- *Bachmann C. 2002. Mechanisms of hyperammonemia. *Clin Chem Lab Med* 40(7):653-662.
- Bai G, Rama Rao KV, Murthy ChRK, et al. 2001. Ammonia induces the mitochondrial permeability transition in primary cultures of rat astrocytes. *J Neurosci Res* 66(5):981-991.
- *Ballal SG, Ali BA, Albar AA, et al. 1998. Bronchial asthma in two chemical fertilizer producing factories in eastern Saudi Arabia. *Int J Tuberc Lung Dis* 2(4):330-335.
- Baraldi M, Pinelli G, Ricci P, et al. 1984. Toxins in hepatic encephalopathy: The role of synergistic effect of ammonia, mercaptans and short chain fatty acids. *Arch Toxicol Suppl* 7:103-105.
- Barbieri P, Adami G, Predonzani S, et al. 1999. Survey of environmental complex systems: pattern recognition of physicochemical data describing coastal water quality in the Gulf of Trieste. *J Environ Monit* 3(2):210-216.
- *Barica J. 1990. Ammonia and nitrite contamination of Hamilton Harbour, Lake Ontario. *Water Pollut Res J Can* 25(3):359-386.
- *Barnes DG, Dourson M. 1988. Reference dose (RfD): Description and use in health risk assessments. *Regul Toxicol Pharmacol* 8:471-486.
- *Barrow CS, Steinhagen WH. 1980. NH₃ concentrations in the expired air of the rat: Importance to inhalation toxicology. *Toxicol Appl Pharmacol* 53:116-121.
- Barrow CS, Alarie Y, Stock M. 1978. Sensory irritation and incapacitation evoked by thermal decomposition products of polymers and comparisons with known sensory irritants. *Arch Environ Health* 33(2):79-88.
- Barry DAJ, Goorahoo D, Goss MJ. 1993. Estimation of nitrate concentrations in groundwater using a whole farm nitrogen budget. *J Environ Qual* 22(4):767-775.
- Barshaw ML, Wachtel RC, Cohen L, et al. 1986. Neurological outcome in premature infants with transient asymptomatic hyperammonemia. *J Pediatr* 108(2):271-275.

9. REFERENCES

- *Barzel US. 1975. The effect of chronic ammonium chloride ingestion on parathyroid hormone function. *Nephron* 14:339-346.
- *Barzel US, Jowsey J. 1969. The effects of chronic acid and alkali administration on bone turnover in adult rat. *Clin Sci* 36:517-524.
- Basile AS. 2002. Direct and indirect enhancement of GABAergic neurotransmission by ammonia: implications for the pathogenesis of hyperammonemic syndromes. *Neurochem Int* 41:115-122.
- *Bauer CF, Andren AW. 1985. Emissions of vapor-phase fluorine and ammonia from the Columbia coal-fired power plant. *Environ Sci Technol* 19:1099-1103.
- Baum MM, Kiyomiya ES, Kumar S, et al. 2001. Multicomponent remote sensing of vehicle exhaust by dispersive absorption spectroscopy. 2. Direct on road ammonia measurements. *Environ Sci Technol* 35(18):3735-3741.
- *Beare JD, Wilson RS, Marsh RJ. 1988. Ammonia burns of the eye: Old weapon in new hands. *Br Med J* 296:590.
- *Beauchamp EG, Kidd GE, Thurtell G. 1978. Ammonia volatilization from sewage sludge applied in the field. *J Environ Qual* 7:141-146.
- *Beauchamp EG, Kidd GE, Thurtell G. 1982. Ammonia volatilization from liquid dairy cattle manure in the field. *Can J Soil Sci* 62:11-19.
- Benjamin AM, Okamoto K, Quastel JH. 1978. Effects of ammonium ions on spontaneous action potentials and on contents of sodium, potassium, ammonium and chloride ions in brain *in vitro*. *J Neurochem* 30:131-143.
- *Benyajati S, Goldstein L. 1975. Renal glutaminase adaptation and ammonia excretion in infant rats. *Am J Physiol* 228:693-698.
- Berenzen N, Schulz R, Liess M. 2001. Effects of chronic ammonium and nitrite contamination on the macroinvertebrate community in running water microcosms. *Water Res* 35(14):3478-3482.
- *Berger GS. 1994. Epidemiology of endometriosis. In: Berger GS, ed. *Endometriosis: Advanced management and surgical techniques*. New York, NY: Springer-Verlag.
- Berger S, Bermes EW, Blanke RV, et al. Ammonia. 1970. In: Tietz NW, ed. *Fundamentals of clinical chemistry*. Philadelphia, PA: W.B. Saunders Company, 665-731.
- *Betterton EA. 1992. Henry's law constants of soluble and moderately soluble organic gases: effects on aqueous phase chemistry. In: Nriagu JO, ed. *Gaseous pollutants: Characterization and cycling*. Tuscon, AZ: John Wiley & Sons, Inc., 1-50.
- *Beyrouthy CA, Sommers LE, Nelson DW. 1988. Ammonia volatilization from surface-applied urea as affected by several phosphoroamide compounds. *Soil Sci Soc Am J* 52:1173-1178.
- Bilski J, Jaworek J, Cieszkowski M, et al. 1996. Exocrine pancreatic response to ammonia *in vivo* and *in vitro*. *Digestion* 57(4):219.

9. REFERENCES

- *Bishop RW, Belkin F, Gaffney R. 1986. Evaluation of a new ammonia sampling and analytical procedure. *Am Ind Hyg Assoc J* 47:135-137.
- Blade MC, Arola L, Alemany M. 1988. Rapid detoxification of infused ammonium by the anesthetized rat. *Biochem Int* 16(5):859-867.
- *Blei AT, Olafsson S, Therrien G, et al. 1994. Ammonia-induced brain edema and intracranial hypertension in rats after portacaval anastomosis. *Hepatology* 19(6):1437-1444.
- Blowey R, Packington A. 1994. Polioencephalomalacia associated with ingestion of ammonium sulphate. *Vet Rec* 134(24):636.
- *Bodansky A, Jaffe HL, Chandler JP. 1932. Serum phosphatase changes in calcium deficiency and in ammonium chloride osteoporosis. *Proc Soc Exp Biol Med* 29:871-873.
- *Bodega G, Suárez I, Boyano MC, et al. 1993. High ammonia diet: Its effect on the glial fibrillary acidic protein (GFAP). *Neurochem Res* 18(9):971-975.
- *Bodega G, Suárez I, Rubio M, et al. 1991. Heterogeneous astroglial response in the rat spinal cord to long-term portacaval shunt. An immunohistochemical study. *Glia* 4:400-407.
- *Bouwman AF, Lee DS, Asman WAH, et al. 1997. A global high-resolution emission inventory for ammonia. *Global Biogeochem Cycles* 11(4):561-587.
- *Bouwmeester RJ, Vlek PLG. 1981. Rate control of ammonia volatilization from rice paddies. *Atmos Environ* 15:131-140.
- *Boyano-Adanez MC, Bodega G, Barrios V, et al. 1996. Response of rat cerebral somatostatinergic system to a high ammonia diet. *Neurochem Res* 29(5):469-476.
- *Boyd EM, Seymour KGW. 1946. Ethylenediamine dihydrochloride or chlor-ethamine. II. Untoward and toxic reactions. *Exp Med Surg* 223-227.
- *Boyd EM, MacLachlan ML, Perry WF. 1944. Experimental ammonia gas poisoning in rabbits and cats. *J Ind Hyg Toxicol* 26:29-34.
- Braissant O, Henry H, Villard A, et al. 2002. Ammonium-induced impairment of axonal growth is prevented through glial creatine. *J Neurosci* 22(22):9810-9820.
- *Bremner JM. 1965. Inorganic forms of nitrogen. In: Black CA, Evans DD, Ensminger LE, et al., eds. *Agronomy. Methods of soil analysis. Part 2. Chemical and microbiological properties*. Madison, WI: American Society of Agronomy, Inc., 1179-1237.
- *Brimblecombe P, Dawson GA. 1984. Wet removal of highly soluble gases. *J Atmos Chem* 2:95-107.
- *Brinson SEJR, Cabrera ML, Tyson SC. 1994. Ammonia volatilization from surface-applied, fresh and composted poultry litter. *Plant Soil* 167(2):213-218.
- *BLR. 2002. Book of chemical lists on CD-Rom. Old Saybrook, CT: Business & Legal Reports, Inc.

9. REFERENCES

- *Broderson JR, Lindsey JR, Crawford JE. 1976. The role of environmental ammonia in respiratory mycoplasmosis of rats. *Am J Pathol* 85:115-130.
- Brooks KJ, Kauppinen RA, Williams SR, et al. 1989. Ammonia causes a drop in intracellular pH in metabolizing cortical brain slices: A phosphorus-31 and proton NMR study. *Neuroscience* 33(1):185-192.
- *Brown RH, Duda GD, Korkes S, et al. 1957. A colorimetric micromethod for determination of ammonia; the ammonia content of rat tissues and human plasma. *Arch Biochem Biophys* 66:301-309.
- *Brunke R, Alvo P, Schuepp P, et al. 1988. Effect of meteorological parameters on ammonia loss from manure in the field. *J Environ Qual* 17:431-436.
- *Brunner H, Thaler H, eds. 1981. *Hepatology: A festschrift for Hans Popper*. New York, NY: Raven Press, 212.
- *Budavari S, O'Neil MJ, Smith A, et al., eds. 1996. *The Merck index*, 12th ed. Whitehouse Station, NJ: Merck & Co. Inc., 87.
- *Buff R, Koller EA. 1974. Studies underlying the reflex hyperpnoea induced by inhalation of chemical irritants. *Respir Physiol* 21:371-383.
- Bugge M, Bengtsson F, Nobin A, et al. 1988. Serotonin metabolism in the rat brain following ammonia administration. In: Soeters PB, Wilson JHP, Meijer AJ, et al., eds. *Advances in ammonia metabolism and hepatic encephalopathy*. New York, NY: Excerpta Medica.
- Bugge M, Bengtsson F, Nobin A, et al. 1989. The effect of ammonia infusion on brain monoamine metabolism in portacaval-shunted rats. *Res Exp Med* 189(2):101-111.
- *Buijsman E, Maas HFM, Asman WAH. 1987. Anthropogenic NH₃ emissions in Europe. *Atmos Environ* 21(5):1009-1022.
- Burkhard LP, Jenson JJ. 1993. Identification of ammonia, chlorine, and diazinon as toxicants in a municipal effluent. *Arch Environ Contam Toxicol* 25:506-515.
- Burkitt DP. 1978. Workshop V - fiber and cancer. Summary and recommendations. *Am J Clin Nutr* 31:S213-S215.
- *Burns TR, Greenberg SD, Mace ML, et al. 1985. Ultrastructure of acute ammonia toxicity in the human lung. *Am J Forensic Med Pathol* 6:204-210.
- *Butterworth RF. 2002. Pathophysiology of hepatic encephalopathy: A new look at ammonia. *Metab Brain Dis* 17(4):221-227.
- Bytnerowicz A, Fenn ME. 1996. Nitrogen deposition in California forests: A review. *Environ Pollut* 92(2):127-146.
- *C & E News. 1987. Ammonia. *Chem Eng News*. March 2, 1987.
- *Cadle SH. 1985. Seasonal variations in nitric acid, nitrate, strong aerosol acidity, and ammonia in an urban area. *Atmos Environ* 19(1):181-188.

9. REFERENCES

Campbell CL, Dawes RK, Deolalkar S, et al. 1958. Effect of certain chemicals in water on the flavor of brewed coffee. *Food Res* 23:575-579.

Campbell SC. 1988. Severe ventilatory depression reversed with aromatic ammonia inhalation. *N Engl J Med* 319(8):1550.

*Caplin M. 1941. Ammonia-gas poisoning: Forty-seven cases in a London shelter. *Lancet* 241:95-96.

*Capuco AV. 1977. Ammonia: A modulator of 3T3 cell growth. *Diss Abstr Int B* 38(9):4085.

*Castell DO, Moore EW. 1971. Ammonia absorption from the human colon. *Gastroenterology* 60:33-42.

Chan H, Pannunzio M, Hazell AS, et al. 2001. Exposure to ammonia results in decreased glutamate transporter activity in cultured cerebellar granule cells. *J Neurochem* 78(Suppl 1):40.

Chapin RE, Gulati DK. 1997. Reproductive toxicology. Pesticide/fertilizer mix II (California). *Environ Health Perspect Suppl* 105(1):373-374.

Chou SF, Jacobs LW, Penner D. 1978. Absence of plant uptake and translocation of polybrominated biphenyls (PBBs). *Environ Health Perspect* 23:9-12.

*Choudat D, Goehen M, Korobaef M, et al. 1994. Respiratory symptoms and bronchial reactivity among pig and dairy farmers. *Scand J Work Environ Health* 20(1):48-54.

Chourasia HK. 2001. Efficacy of ammonia in detoxification of fumonisin contaminated corn. *Indian J Exp Biol* 39(5):493-495.

*Christesen HB. 1995. Prediction of complications following caustic ingestion in adults. *Clin Otolaryngol* 20(3):272-278.

*Clark EC, Nath KA, Hostetter MK, et al. 1990. Role of ammonia in tubulointerstitial injury. *Miner Electrolyte Metab* 16(5):315-321.

*Clemmesen JO, Larsen FS, Kondrup J, et al. 1999. Cerebral herniation in patients with acute liver failure is correlated with arterial ammonia concentration. *Hepatology* 29(3):648-653.

*Clewett HJ III, Andersen ME. 1985. Risk assessment extrapolations and physiological modeling. *Toxicol Ind Health* 1(4):111-131.

*Clinton SK, Bostwick DG, Olson LM, et al. 1988. Effects of ammonium acetate and sodium cholate on N-methyl-N'-nitro-N-nitrosoguanidine-induced colon carcinogenesis of rats. *Cancer Res* 48:3035-3039.

*Close LG, Catlin FI, Cohn AM. 1980. Acute and chronic effects of ammonia burns of the respiratory tract. *Arch Otolaryngol* 106:151-158.

CLPSD. 1987. Contract Laboratory Program (CLP) Statistical Database. Alexandria, VA: Viar and Company, Management Services Division, April 1987.

CMR. 1988. Ammonia. Chemical Market Reporter. October 3, 1998. 51-52.

9. REFERENCES

- *CMR. 1999. Chemical profile ammonia. Chemical Market Reporter. November 29, 1999.
- *Colborn T, Clement C, eds. 1992. Chemically-induced alterations in sexual and functional development: The wildlife human connection. Princeton, NJ: Princeton Scientific Publishing.
- *Cole TJ, Cotes JE, Johnson GR, et al. 1977. Ventilation, cardiac frequency and pattern of breathing during exercise in men exposed to *o*-chlorobenzylidene malonitrile (CS) and ammonia gas in low concentrations. *Q J Exp Physiol Cogn Med Sci.* 63:341-351.
- *Conn HO. 1972. Studies of the source and significance of blood ammonia IV. Early ammonia peaks after ingestion of ammonia salts. *Yale J Biol Med* 45:543-549.
- *Coon RA, Jones RA, Jenkins LJ Jr, et al. 1970. Animal inhalation studies on ammonia, ethylene, glycol, formaldehyde, dimethylamine and ethanol. *Toxicol Appl Pharmacol* 16:646-655.
- Córdoba J, Crespin J, Gottstein J, et al. 1999. Mild hypothermia modifies ammonia-induced brain edema in rats after portacaval anastomosis. *Gastroenterology* 116(3):686-693.
- *Córdoba J, Gottstein J, Blei AT. 1998. Chronic hyponatremia exacerbates ammonia-induced brain edema in rats after portacaval anastomosis. *J Hepatol* 29(4):589-594.
- *Cormier Y, Israel-Assayag E, Racine G, et al. 2000. Farming practices and the respiratory health risks of swine confinement buildings. *Eur Resp J* 15(3):560-565.
- *Corsi RL, Torres VM, Fredenberg S, et al. 2000. A screening assessment of non-point source ammonia emissions in Texas. Proceedings of the Air & Waste Management Association's, 93rd Annual Meeting & Exhibition: Salt Lake City, Utah, June 18-22, 2000. Pittsburgh, PA: Air and Waste Management Association, 4279-4292.
- *Coude FX, Grimber G. 1984. Inhibition of urea synthesis by pent-4-enoic acid: Potentiation by ammonia. *Biochem Biophys Res Commun* 118(1):47-52.
- *Couturier Y, Barbotin M, Bobin P, et al. 1971. [Apropos of three cases of toxic lung caused by vapors of ammonia and hydrogen sulfide.] *Bull Soc Med Afr Noire Lang Fr* 16:250-252. (French)
- *CPSC. 2001. Hazardous substances and articles; administration and enforcement regulations. Substances named in the Federal Caustic Poison Act. Consumer Product Safety Committee. Code of Federal Regulations. 16 CFR 1500.129(1). <http://frwebgate.access.gpo.gov/cgi>. May 02, 2002.
- *CRIS. 2003. Ammonia. Current Research Information Systems. <http://cris.csrees.usda.gov/>. July 01, 2004.
- *Crook B, Robertson JF, Travers Glass SA, et al. 1991. Airborne dust, ammonia, microorganisms, and antigens in pig confinement houses and the respiratory health of exposed farm workers. *Am Ind Hyg Assoc J* 52(7):271-279.
- *Crumpton WG, Isenhard TM. 1988. Diurnal patterns of ammonium and un-ionized ammonia in streams receiving secondary treatment effluent. *Bull Environ Contam Toxicol* 40(4):539-544.

9. REFERENCES

- *Crutzen PJ. 1983. Atmospheric interactions--homogeneous gas reactions of C, N, and S containing compounds. In: Bolin B, Cook RB, eds. The major biogeochemical cycles and their interactions. Chichester: John Wiley & Sons, 67-113.
- *Cuesta-Santos OA, Ortiz-Bulto PL, Gozalez MLG. 1998. Deposition and atmospheric nitrogen concentrations trends in Cuba. *Water Air Soil Pollut* 106(1-2):163-169.
- Cummings JH, Stephen AM, Branch WJ. 1981. Implications of dietary fiber breakdown in the human colon. *Banbury Rep* 7:71-81.
- *da Fonseca J, Brito MJ, Freitas J, et al. 1998. Acute colitis caused by caustic products. *Am J Gastroenterol* 93(12):2601-2602.
- *da Fonseca-Wollheim F. 1995. The influence of pH and various anions on the distribution of NH_4^+ in human blood. *Eur J Clin Chem Clin Biochem* 33(5):289-294.
- *Dalhamn T. 1963. Effect of ammonia alone and combined with carbon particles on ciliary activity in the rabbit trachea in vivo, with studies of the absorption capacity of the nasal cavity. *Int J Air Water Pollut* 7:531-539.
- Damm CJ, Lucas D, Sawyer RF, et al. 2001. Excimer laser fragmentation-fluorescence spectroscopy as a method for monitoring ammonium nitrate and ammonium sulfate particles. *Chemosphere* 42(5-7):655-661.
- *Daubert TE, Danner RP. 1989. Physical and thermodynamic properties of pure chemicals: Data compilation. Washington, DC: Taylor & Francis.
- *Davies BMA, Yudkin J. 1952. Studies in biochemical adaptation. The origin of urinary ammonia as indicated by the effect of chronic acidosis and alkalosis on some renal enzymes in the rat. *Biochem J* 52:407-412.
- *Dawson GA. 1977. Atmospheric ammonia from undisturbed land. *J Geophys Res* 82(21):3125-3133.
- *Dawson GA, Farmer JC. 1984. Highly soluble atmospheric trace gases in the Southwestern United States. I. Inorganic species: NH_3 , HNO_3 , SO_3 . *J Geophys Res* 89(D3):4779-4787.
- Debreczeni K, Berecz K. 1998. Monitoring of gaseous nitrogen losses from nitrogen fertilizers in model experiments. *Commun Soil Sci Plant Anal* 29(11-14):2207-2216.
- De Datta SK. 1995. Nitrogen transformations in wetland rice ecosystems. *Fert Res* 42(1-3):193-203.
- de Knecht RJ, Schalm SW, van der Rijt CC, et al. 1994. Extracellular brain glutamate during acute liver failure and during acute hyperammonemia simulating acute liver failure: An experimental study based on in vivo brain dialysis. *J Hepatol* 20(1):19-26.
- *de la Hoz RE, Schlueter DP, Rom WN. 1996. Chronic lung disease secondary to ammonia inhalation injury: A report on three cases. *Am J Ind Med* 29(2):209-214.
- *Demerec M, Bertani G, Flint J. 1951. A survey of chemicals for mutagenic action on *E. coli*. *Am Nat* 85:119-136.

9. REFERENCES

- *Denmead OT, Freney JR, Simpson JR. 1982. Atmospheric dispersion of ammonia during application of anhydrous ammonia fertilizer. *J Environ Qual* 11:568-572.
- *De Sousa RC, Harrington JT, Ricanati ES, et al. 1974. Renal regulation of acid-base equilibrium during chronic administration of mineral acid. *J Clin Invest* 53:465-476.
- DeSutter TM, Clay SA, Clay DE. 1998. Atrazine, alachlor, and total inorganic nitrogen concentrations of winter wind-eroded sediment samples. *J Environ Sci Health B* 33(6):683-691.
- *Diekman MA, Scheidt AB, Sutton AL, et al. 1993. Growth and reproductive performance, during exposure to ammonia, of gilts afflicted with pneumonia and atrophic rhinitis. *Am J Vet Res* 54(12):2128-2131.
- *Dimmock NA, Marshall GB. 1986. The determination of free ammonia in ambient air with diffusion/denuder tubes. *Anal Chim Acta* 185:159-169.
- *Dodd KT, Gross DR. 1980. Ammonia inhalation toxicity in cats: A study of acute and chronic respiratory dysfunction. *Arch Environ Health* 35:6-14.
- *DOI. 1985. Nitrogen (ammonia). Bureau of mines Bulletin 675. Washington, DC: Department of the Interior.
- *Doig PA, Willoughby RA. 1971. Response of swine to atmospheric ammonia and organic dust. *J Am Vet Med Assoc* 159:1353-1361.
- Dolinska M, Hilgier W, Albrecht J. 1996. Ammonia stimulates glutamine uptake to the cerebral non-synaptic mitochondria of the rat. *Neurosci Lett* 213(1):45-48.
- *Donham KJ. 1991. Association of environmental air contaminants with disease and productivity in swine. *Am J Vet Res* 52(10):1723-1730.
- *Donham KJ, Pependorf WJ. 1985. Ambient levels of selected gases inside swine confinement buildings. *Am Ind Hyg Assoc J* 46:658-661.
- *Donham KJ, Cumro D, Reynolds SJ, et al. 2000. Dose-response relationships between occupational aerosol exposures and cross-shift declines of lung function in poultry workers: recommendations for exposure limits. *J Occup Environ Med* 42(3):260-269.
- *Donham KJ, Knapp LW, Monson R, et al. 1982. Acute toxic exposure to gases from liquid manure. *J Occup Med* 24:142-145.
- *Donham KJ, Reynolds SJ, Whitten P, et al. 1995. Respiratory dysfunction in swine production facility workers: dose-response relationships of environmental exposures and pulmonary function. *Am J Ind Med* 27(3):405-418.
- Dow SW, Fettman MJ, Smith KR, et al. 1990. Effects of dietary acidification and potassium depletion on acid-base balance, mineral metabolism and renal function in adult cats. *J Nutr* 120(6):569-578.
- *Doyle GJ, Tuazon EC, Graham RA, et al. 1979. Simultaneous concentrations of ammonia and nitric acid in a polluted atmosphere and their equilibrium relationship to particulate ammonium nitrate. *Environ Sci Technol* 13:1416-1419.

9. REFERENCES

Doyle J, Watts S, Solley D, et al. 2001. Exceptionally high-rate nitrification in sequencing batch reactors treating high ammonia landfill leachate. *Water Sci Technol* 43(3):315-322.

*Drummond JG, Curtis SE, Simon J, et al. 1980. Effects of aerial ammonia on growth and health of young pigs. *J Anim Sci* 50(6):1085-1091.

*Duda GD, Handler P. 1958. Kinetics of ammonia metabolism in vivo. *J Biol Chem* 232:303-314.

Dueck TA, Van der Eerden LJ. 2000. Interactions between climate change and nitrogen deposition, with emphasis on ammonia. In: Singh SN, ed. *Trace gas emissions and plants*. Dordrecht, The Netherlands: Kluwer Academic Publishers, 291-307.

Duke JA. 1977. Phytotoxin tables. *CRC Crit Rev Toxicol* 5:189-237.

Duke-Elder S, MacFaul PA. 1972. In: Duke-Elder S, ed. *System of ophthalmology*. Injuries. Non-mechanical injuries. St. Louis, MO: The C.V. Mosby Company, 1079-1085.

Duncan K, Jennings E, Hettenbach S, et al. 1998. Nitrogen cycling and nitric oxide emissions in oil-impacted prairie soils. *Biorem J* 1(3):195-208.

*Eaton WC, Rickman EE, Jayanty RKM, et al. 1996. Method 301 evaluation of a technique for collection and measurement of ammonia in stationary source emissions. Measurement of toxic and related air pollutants: Proceedings of an international specialty conference, May 7-9, 1996, Research Triangle Park, North Carolina. Pittsburgh, PA: Air and Waste Management Association, 583-588.

Edwards PJ, Kochenderfer JN, Seegrist DW. 1991. Effects of forest fertilization on stream water chemistry in the Appalachians. *Water Resour Bull* 27(2):265-274.

Effler SW, Brooks CM, Auer MT, et al. 1990. Free ammonia and toxicity criteria in a polluted urban lake. *Res J Water Pollut Control Fed* 62(6):771-779.

*Effler SW, Gelda RK, Matthews CM. 2001. Implications of industrial loads for ammonia pollution in an urban lake. *Water Environ Res* 73(2):192-203.

*Egle JL. 1973. Retention of inhaled acetone and ammonia in the dog. *Am Ind Hyg Assoc J* 34:533-539.

*Englande AJ, Smith JK, English JN. 1978. Potable water quality of advanced wastewater treatment plant effluents. *Prog Water Technol* 10:17-39.

*EPA. 1979. Effects of endogenous ammonia on neutralization of inhaled sulfuric acid aerosols. Cininnatti, OH: U.S. Environmental Protection Agency, Health Effects Research Lab. EPA600179045. PB80147978.

*EPA. 1980. Source category survey: Ammonia manufacturing industry. Research Triangle Park, NC: U.S. Environmental Protection Agency, Office of Air Quality, EPA450380014.

*EPA. 1983. Methods for chemical analysis of water and wastes. Ammonia. U.S. Environmental Protection Agency. EPA600479020.

9. REFERENCES

*EPA. 1987a. Extremely hazardous substances (EHS) chemical profiles and emergency first aid guides. Ammonia. U.S. Environmental Protection Agency. <http://www.epa.gov/swercepp/ehs/ehslist.html>. July 06, 2004.

*EPA. 1987b. Toxic air pollutant/source crosswalk—a screening tool for locating possible sources emitting air pollutants. ResearchTriangle Park, NC: U.S. Environmental Protection Agency. Code of Federal Regulations. EPA4504487023A.

EPA. 1988. Analysis of clean water act effluent guidelines pollutants: Summary of the chemicals regulated by industrial point source category. Washington, DC: U.S. Environmental Protection Agency. 40 CFR Parts 400-475.

*EPA. 1990. Interim methods for development of inhalation reference concentrations. Washington, DC: U.S. Environmental Protection Agency, Office of Health and Environmental Assessment, Office of Research and Development, Environmental Criteria and Assessment Office. EPA600890066A.

*EPA. 1994. Methods for derivation of inhalation reference concentrations and application of inhalation dosimetry. Washington, DC: U.S. Environmental Protection Agency, Office of Research and Development. EPA600890066F.

*EPA. 1997a. Determination of ammonia in estuarine and coastal waters by gas segmented continuous flow colorimetric analysis. Method 349.0. U.S. Environmental Protection Agency.

*EPA. 1997b. Special report on environmental endocrine disruption: An effects assessment and analysis. Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. EPA630R96012.

*EPA. 2001a. Ammonia-N in water and biosolids by ion-selective electrode potentiometry with preliminary distillation. Method 1689. Washington, DC: U.S. Environmental Protection Agency. EPA821R01012.

*EPA. 2001b. Ammonia-N in water and biosolids by automated colorimetry with preliminary distillation. Method 1690. Washington, DC: U.S. Environmental Protection Agency. EPA821R01009.

*EPA. 2002a. Chemical accident prevention provisions. List of substances. U.S. Environmental Protection Agency. Code of Federal Regulations. 40 CFR 68.130. Table 1. <http://ecfr.access.gpo.gov/>. May 02, 2002.

*EPA. 2002b. Chemical accident prevention provisions. Table of toxic endpoints. U.S. Environmental Protection Agency. Code of Federal Regulations. 40 CFR 68. Appendix A. <http://ecfrback.access.gpo.gov/>. May 02, 2002.

*EPA. 2002c. Emergency planning and notification. The list of extremely hazardous substances and their threshold planning quantities. U.S. Environmental Protection Agency. Code of Federal Regulations. 40 CFR 355. Appendix A. <http://ecfrback.access.gpo.gov/>. May 02, 2002.

*EPA. 2002d. Superfund, emergency planning, and community right-to-know programs. Designation, reportable quantities, and notification. U.S. Environmental Protection Agency. Code of Federal Regulations. 40 CFR 302.4(a). <http://ecfrback.access.gpo.gov/>. May 02, 2002.

9. REFERENCES

- *EPA. 2002e. Tolerances and exemptions from tolerances for pesticide chemicals in food. Exemptions from the requirement of a tolerance. U.S. Environmental Protection Agency. Code of Federal Regulations. 40 CFR 180.1001(c) and (d). <http://ecfrback.access.gpo.gov/>. May 02, 2002.
- *EPA. 2002f. Tolerances and exemptions from tolerances for pesticide chemicals in food. Ammonia; exemption from the requirement of a tolerance. U.S. Environmental Protection Agency. Code of Federal Regulations. 40 CFR 180.1003. <http://ecfrback.access.gpo.gov/>. May 02, 2002.
- *EPA. 2002g. Toxic chemical release reporting: Community right-to-know. Chemicals and chemical categories to which this part applies. U.S. Environmental Protection Agency. Code of Federal Regulations. 40 CFR 372.65(a). <http://ecfr.access.gpo.gov/>. May 02, 2002.
- *EPA. 2002h. Water programs. Designation of hazardous substances. U.S. Environmental Protection Agency. Code of Federal Regulations. 40 CFR 116.4. Table A. <http://ecfrback.access.gpo.gov/>. May 02, 2002.
- *EPA. 2002i. Water programs. Determination of reportable quantities. U.S. Environmental Protection Agency. Code of Federal Regulations. 40 CFR 117.3. <http://ecfrback.access.gpo.gov/>. May 02, 2002.
- *EPA. 2002j. Effluent guidelines and standards. CFR Title 40: Protection of Environment. U.S. Environmental Protection Agency. <http://www.epa.gov/docs/epacfr40/chapt-I.info/subch-N.htm>. May 31, 2002.
- *EPA. 2004. Acute exposure guideline levels (AEGLs). Ammonia results. U.S. Environmental Protection Agency.
- *EPIWIN. 2000. Ammonia. <http://www.epa.gov/oppt/p2framework/docs/epiwin.htm>. July 1, 2004.
- Erismann J-W, Vermetten AWM, Asman WAH. 1988. Vertical distribution of gases and aerosols the behavior of ammonia and related components in the lower atmosphere. *Atmos Environ* 22(6):1153-1160.
- *Erxleben H, Simon J, Moskvina LN, et al. 2000. Automated procedures for the determination of ozone and ammonia contents in air by using the chromatomembrane method for gas-liquid extraction. *Fresenius J Anal Chem* 336(4):332-335.
- *Fangmeier A, Hadwiger-Fangmeier A, Van Der Eerden L, et al. 1994. Effects of atmospheric ammonia on vegetation: A review. *Environ Pollut* 86(1):43-82.
- *Farmer JC, Dawson GA. 1982. Condensation sampling of soluble atmospheric trace gases. *J Geophys Res* 87(C11):8931-8942.
- Farmer PM, Mulakkah T. 1990. The pathogenesis of hepatic encephalopathy. *Ann Clin Lab Sci* 20(2):91-97.
- *Fazekas IG. 1939. Experimental suprarenal hypertrophy induced by ammonia. *Endokrinologie* 21:315-337.
- FDA. 1973. GRAS (generally recognized as safe) food ingredients - ammonium ion. U.S. Food and Drug Administration. PB221235.

9. REFERENCES

- *FDA. 2001a. Direct food substances affirmed as generally recognized as safe. Ammonium chloride. U.S. Food and Drug Administration. Code of Federal Regulations. 21 CFR 184.1138. <http://frwebgate.access.gpo.gov/>. May 02, 2002.
- *FDA. 2001b. Direct food substances affirmed as generally recognized as safe. Ammonium hydroxide. U.S. Food and Drug Administration. Code of Federal Regulations. 21 CFR 184.1139. <http://frwebgate.access.gpo.gov/>. May 02, 2002.
- *FDA. 2001c. Direct food substances affirmed as generally recognized as safe. Ammonium sulfate. U.S. Food and Drug Administration. Code of Federal Regulations. 21 CFR 184.1143. <http://frwebgate.access.gpo.gov/>. May 02, 2002.
- *FDA. 2001d. Drug products containing certain active ingredients offered over-the-counter. U.S. Food and Drug Administration. Code of Federal Regulations. 21 CFR 310.545(a). <http://frwebgate.access.gpo.gov/>. May 02, 2002.
- *FDA. 2001e. Food additives permitted in feed and drinking water of animals. Anhydrous ammonia. U.S. Food and Drug Administration. Code of Federal Regulations. 21 CFR 573.180. <http://frwebgate.access.gpo.gov/>. May 02, 2002.
- *FDA. 2001f. Substances generally recognized as safe. Ammonium hydroxide. U.S. Food and Drug Administration. Code of Federal Regulations. 21 CFR 582.1139. <http://frwebgate.access.gpo.gov/>. May 02, 2002.
- *FDA. 2001g. Substances generally recognized as safe. Ammonium sulfate. U.S. Food and Drug Administration. Code of Federal Regulations. 21 CFR 582.1143. <http://frwebgate.access.gpo.gov/>. May 02, 2002.
- *FEDRIP. 2002. Federal research in Progress. Dialog Information Services, Inc.
- *FEDRIP. 2003. Federal research in Progress. Dialog Information Services, Inc.
- *Felipo V, Butterworth RF. 2002. Neurobiology of ammonia. *Prog Neurobiol* 67:259-279.
- *Felipo V, Grau E, Miñana MD, et al. 1993. Ammonium injection induces an *n*-methyl-d-aspartate receptor-mediated proteolysis of the microtubule-associated protein MAP-2. *J Neurochem* 60:1626-1630.
- *Felipo V, Hermenegildo C, Montoliu C, et al. 1998. Neurotoxicity of ammonia and glutamate: molecular mechanisms and prevention. *Neurotoxicology* 19(4-5):675-681.
- Felipo V, Miñana MD, Cabedo H, et al. 1994. L-Carnitine increases the affinity of glutamate for quisqualate receptors and prevents glutamate neurotoxicity. *Neurochem Res* 19(3):373-377.
- Felipo V, Monfort P, Fernandez-Marticorena I, et al. 2000. Activation of NMDA receptors in rat brain in vivo following acute ammonia intoxication. Characterization by in vivo brain microdialysis. *Eur J Neurosci* 12(Suppl 11):40.
- *Ferguson WS, Koch WC, Webster LB, et al. 1977. Human physiological response and adaption to ammonia. *J Occup Med* 19:319-326.

9. REFERENCES

- *Fernandez JM, Croom WJ, Johnson AD, et al. 1988. Subclinical ammonia toxicity in steers: Effects on blood metabolite and regulatory hormone concentrations. *J Anim Sci* 66(12):3259-3266.
- *Fernandez JM, Croom WJ, Tate LP, et al. 1990. Subclinical ammonia toxicity in steers: Effects on hepatic and portal-drained visceral flux of metabolites and regulatory hormones. *J Anim Sci* 68(6):1726-1742.
- *Ferrier B, Martin M, Baverel G. 1988. Valproate-induced stimulation of renal production and excretion in the rat. *J Clin Chem Clin Biochem* 26:65-67.
- Ferreira EB, Ceddia RB, Curi R, et al. 1998. Swimming-exercise increases the capacity of perfused rat liver to produce urea from ammonia and L-glutamine. *Res Commun Mol Pathol Pharmacol* 102(3):289-303.
- Flessner MF, Wall SM, Knepper MA. 1992. Ammonium and bicarbonate transport in rat outer medullary collecting ducts. *Am J Physiol* 262(1 Pt 2):F1-F7.
- *Flury KE, Dines DE, Rodarte JR, et al. 1983. Airway obstruction due to inhalation of ammonia. *Mayo Clin Proc* 58:389-393.
- *Fox DL. 1987. Air pollution. *Anal Chem* 59:280R-294R.
- Freedman LR, Beeson PB. 1961. Experimental pyelonephritis. VIII. The effect of acidifying agents on susceptibility to infection. *Yale J Biol Med* 33:318-332.
- *Fürst P, Josephson B, Maschio G, et al. 1969. Nitrogen balance after intravenous and oral administration of ammonium salts to man. *J Appl Phys* 26:13-22.
- *Gaafar H, Girgis R, Hussein M, et al. 1992. The effect of ammonia on the respiratory nasal mucosa of mice. A histological and histochemical study. *Acta Otolaryngol (Stockh)* 112(2):339-342.
- Gad HA, El-Nasr ES, El-Naasan A, et al. 1994. The effect of some environmental conditions on the pregnant does and its maternal behavior. *J Anim Sci* 72(Suppl 1):155.
- *Galbally IE. 1985. The emission of nitrogen to the remote atmosphere: Background paper. *NATO ASI Ser C* 159:27-53.
- Galbally IE, Roy CR. 1983. The fate of nitrogen compounds in the atmosphere. *Dev Plant Soil Sci* 9:265-284.
- Garcia MV, Lopez-Mediavilla C, Juanes de la Pena MC, et al. 2003. Tolerance of neonatal rat brain to acute hyperammonemia. *Brain Res* 973:31-38.
- *Gay WMB, Crane CW, Stone WD. 1969. The metabolism of ammonia in liver disease: A comparison of urinary data following oral and intravenous loading of [¹⁵N]ammonium lactate. *Clin Sci* 37:815-823.
- *George A, Bang RL, Lari AR, et al. 2000. Liquid ammonia injury. *Burns* 26(4):409-413.
- George JD, Fail PA, Grizzle TB, et al. 1993. Reproductive toxicity evaluation of a pesticide/fertilizer mixture administered in the drinking water to mice. *Toxicologist* 13(1):77.

9. REFERENCES

- *Georgii HW, Gravenhorst G. 1977. The ocean as source or sink of reactive trace-gases. *Pure Appl Geophys* 115:503-511.
- Gibson GE, Zimber A, Krook L, et al. 1971. Nucleic acids and brain and intestinal lesions in ammonia intoxicated mice [Abstract]. *Fed Proc* 30:578.
- *Gilbert GJ. 1988. Acute ammonia intoxication 37 years after ureterosigmoidostomy. *South Med J* 81(11):1443-1445.
- *Gilliam JW, Daniels RB, Lutz JF. 1974. Nitrogen content of shallow ground water in the North Carolina Coastal Plain. *J Environ Qual* 3(2):147-151.
- Giroux M, Bremont F, Salles JP, et al. 2002. Exhaled NH_3 and excreted NH_4^+ in children in unpolluted or urban environments. *Environ Int* 28:197-202.
- *Giwerzman A, Carlsen E, Keiding N, et al. 1993. Evidence for increasing incidence of abnormalities of the human testis: A review. *Environ Health Perspect Suppl* 101(2):65-71.
- Goldman AS, Yakovac WC. 1964. Salicylate intoxication and congenital anomalies. *Arch Environ Health* 8:648-656.
- *Gomzi M. 1999. Indoor air and respiratory health in preadolescent children. *Atmos Environ* 33(24-25):4081-4086.
- *Gomzi M, Šarić M. 1997. Respiratory impairment among children living in the vicinity of a fertilizer plant. *Int Arch Occup Environ Health* 70(5):314-320.
- *Goulding KWT, Bailey NJ, Bradbury NJ, et al. 1998. Nitrogen deposition and its contribution to nitrogen cycling and associated soil processes. *New Phytol* 139(1):49-58.
- *Graedel TE. 1978. Inorganic nitrogen compounds: Emission and detection. In: *Chemical compounds in the atmosphere*. New York, NY: Academic Press, 19.
- *Grant WM. 1974. Ammonia. In: *Toxicology of the eye*. 2nd edition. Springfield, IL: Charles C Thomas, 121-127.
- *Greenberg AE, Trussell RR, Clesceri LS, eds. 1985. Nitrogen (ammonia) standard methods for the examination of water and wastewater. Washington, DC: American Public Health Association, American Water Works Association, Water Pollution Control Federation, 374-391.
- Gregory JW, Beail N, Boyle NA, et al. 1989. Dietary treatment of hyperlysinemia. *Arch Dis Child* 64(5):716-720.
- *Gupta BN, Khanna RN, Data KK. 1979. Toxicological studies of ammonium sulfamate in rat after repeated oral administration. *Toxicology* 13:45-49.
- *Gustin P, Urbain B, Prouvost JF, et al. 1994. Effects of atmospheric ammonia on pulmonary hemodynamics and vascular permeability in pigs: Interaction with endotoxins. *Toxicol Appl Pharmacol* 125(1):17-26.

9. REFERENCES

*Guyton AC. 1981. The body fluids and kidneys. Textbook of medical physiology. Philadelphia: WB Saunders Company, 456-458, 889.

*Guzelian PS, Henry CJ, Olin SS, eds. 1992. Similarities and differences between children and adults: Implications for risk assessment. Washington, DC: International Life Sciences Institute Press.

Hagiwara A, Shibata M, Kurata Y, et al. 1983. Long-term toxicity and carcinogenicity test of ammonia-process caramel colouring given to B6C3F1 mice in the drinking-water. Food Cosmet Toxicol 21:701-706.

*Harada K, Murawaki Y, Hirayama C. 1985. Comparative studies of nicotinoxyhydroxamic acid and neomycin on ammonia and urea metabolism in rats. Res Commun Chem Pathol Pharmacol 49:309-312.

*Hargreaves JA. 1998. Nitrogen biogeochemistry of aquaculture ponds. Aquaculture 166(3-4):181-212.

*Harper LA, Sharpe RR. 1995. Nitrogen dynamics in irrigated corn: Soil-plant nitrogen and atmospheric ammonia transport. Agron J 87(4):669-675.

Harrison RM, Sturges WT, Kitto A-MN, et al. 1990. Kinetics of evaporation of ammonium chloride and ammonium nitrate aerosols. Atmos Environ 24(7):1883-1888.

*Harward CN, McClenny WA, Hoell JM, et al. 1982. Ambient ammonia measurements in coastal Southeastern Virginia. Atmos Environ 16(10):2497-2500.

Hatch DJ, Jarvis SC, Dollard GJ. 1990. Measurements of ammonia emission from grazed grassland. Environ Pollut 65(4):333-346.

*Hatton DV, Leach CS, Beaudet AL, et al. 1979. Collagen breakdown and ammonia inhalation. Arch Environ Health 34:83- 86.

*Hauser JR. 1984. Use of water hyacinth aquatic treatment systems for ammonia control and effluent polishing. J Water Pollut Control Fed 56(3):219-225.

Hawkins PA, DeJoseph MR, Hawkins RA. 1996. Eliminating metabolic abnormalities of portacaval shunting by restoring normal liver blood flow. Am J Physiol 270(6 PT 1):E1037-E1042.

Hawkins RA, Miller AL, Nielsen RC, et al. 1973. The acute action of ammonia on rat brain metabolism *in vivo*. Biochem J 134:1001-1008.

*HazDat. 2004. HazDat Database: ATSDR's Hazardous Substance Release and Health Effects Database. Atlanta, GA: Agency for Toxic Substances and Disease Registry. www.atsdr.cdc.gov/hazdat.html. July 02, 2004.

Hazell AS, Norenberg MD. 1996. Ammonia and manganese increase arginine uptake in cultured astrocytes. J Neurochem 66(Suppl 1):S69.

Hazell AS, Norenberg MD. 1998. Ammonia and manganese increase arginine uptake in cultured astrocytes. Neurochem Res 23(6):869-873.

9. REFERENCES

- *Heederik D, Brouwer R, Biersteker K, et al. 1991. Relationship of airborne endotoxin and bacteria levels in pig farms with the lung function and respiratory symptoms of farmers. *Int Arch Occup Environ Health* 62(8):595-601.
- *Heederik D, van Zwieten R, Brouwer R. 1990. Across-shift lung function changes among pig farmers. *Am J Ind Med* 17(1):57-58.
- *Hegg DA, Radke LF, Hobbs PV, et al. 1987. Nitrogen and sulfur emissions from the burning of forest products near large urban areas. *J Geophys Res* 92:701-714.
- *Hegg DA, Radke LF, Hobbs PV. 1988. Ammonia emissions from biomass burning. *Geophys Res Lett* 15(4):335-337.
- *Heifer U. 1971. [Casuistic contribution to acute lethal inhalation poisoning by ammonia.] *Lebensversicherungsmedizin* 22:60-62. (German)
- Heindel JJ, Chapin RE, Gulati DK, et al. 1994. Assessment of the reproductive and developmental toxicity of pesticide/fertilizer mixtures based on confirmed pesticide contamination in California and in Iowa groundwater. *Fundam Appl Toxicol* 22:605-621.
- Heindel JJ, George JD, Fail PA, et al. 1997. Reproductive toxicology. Pesticide/fertilizer mix III (Iowa). *Environ Health Perspect Suppl* 105(Suppl 1):375-376.
- Heindel JJ, Price CJ, George JD, et al. 1992. Developmental toxicity evaluation in rats of a pesticide/fertilizer mixture selected to mimic environmental exposure. *Teratology* 45(5):500.
- *Helmerts S, Top FH, Knapp LW. 1971. Ammonia injuries in agriculture. *J Iowa Med Soc* 61:271-280.
- *Henderson Y, Haggard HW. 1927. Group II. Irritant gases, their action, acute and chronic, and sequelae. Noxious gases and the principles of respiration influencing their action. New York: Chemical Catalog Company.
- *Henry RJ. 1964. Non-protein nitrogenous constituents. In: *Clinical chemistry principles and technics*. Los Angeles, CA: Harper & Row, 325-331.
- Herbst E, DeFrees DJ, Talbi D, et al. 1991. Calculations on the rate of the ion-molecule reaction between NH_3^+ and H_2 . *J Chem Phys* 94(12):7842-7849.
- Hermenegildo C, Marcaida G, Montoliu C, et al. 1996. NMDA receptor antagonists prevent acute ammonia toxicity in mice. *Neurochem Res* 21(10):1237-1244.
- Hermenegildo C, Monfort P, Felipe V. 2000. Activation of N-methyl-d-aspartate receptors in rat brain in vivo following acute ammonia intoxication: Characterization by in vivo brain microdialysis. *Hepatology* 31(3):709-715.
- *Herrera L, Kazemi H. 1980. Interaction between ammonia and CO_2 as ventilatory stimulants. *Bull Eur Physiopathol Respir* 16:459-468.
- *Hilado CJ, Casey CJ, Furst A. 1977. Effect of ammonia on swiss albino mice. *J Combust Toxicol* 4:385-388.

9. REFERENCES

- *Hilado CJ, Cumming HG, Machado AM, et al. 1978. Effect of individual gaseous toxicants on mice. *Proc West Pharmacol Soc* 21:159-160.
- *Hindfelt B, Plum F. 1975. L-methionine DL-sulphoximine and acute ammonia toxicity. *J Pharm Pharmacol* 27:456-458.
- Hindfelt B, Siesjo BK. 1971. Cerebral effects of acute ammonia intoxication II. The effect upon energy metabolism. *Scand J Clin Lab Invest* 28:365-374.
- *Hoel DG, Davis DL, Miller AB, et al. 1992. Trends in cancer mortality in 15 industrialized countries, 1969-1986. *J Natl Cancer Inst* 84(5):313-320.
- *Hoff JD, Nelson DW, Sutton AL. 1981. Ammonia volatilization from liquid swine manure applied to cropland. *J Environ Qual* 10(1):90-95.
- *Holness DL, Purdham JT, Nethercott JR. 1989. Acute and chronic respiratory effects of occupational exposure to ammonia. *Am Ind Hyg Assoc J* 50(12):646-650.
- *HSDB. 2003. Ammonia. Hazardous Substances Data Bank. National Library of Medicine. <http://toxnet.nlm.nih.gov>. June 27, 2004.
- Huddleston GM, Gillespie WB, Rodgers JH. 2000. Using constructed wetlands to treat biochemical oxygen demand and ammonia associated with a refinery effluent. *Ecotoxicol Environ Saf* 45(2):188-193.
- Hunt RD. 1977. Measurement of partial pressure of ammonia in breath and arterial blood: evidence for secretion of ammonia into expired gas by metabolism of monoamines in the lung. *Diss Abstr Int B* 37:3279.
- *Huizenga JR, Tangerman A, Gips CH. 1994. Determination of ammonia in biological fluids. *Ann Clin Biochem* 31(6):529-543.
- *Huizenga JR, Gips CH, Tangerman A. 1996. The contribution of various organs to ammonia formation: A review of factors determining the arterial ammonia concentration. *Ann Clin Biochem* 33:23-30.
- Hultberg B, Isaksson A, Bugge M. 1988. The effect of porta-caval shunt ammonia infusion and alcohol administration on rat plasma beta hexosaminidase. *Liver* 8(3):129-131.
- *Hutchinson GL, Viets FG. 1969. Nitrogen enrichment of surface water by absorption of ammonia volatilized from cattle feedlots. *Science* 166:514-515.
- *Hutchinson GL, Mosier AR, Andre CE. 1982. Ammonia and amine emissions from a large cattle feedlot. *J Environ Qual* 11:288-293.
- Hytonen M, Leino T, Sala E, et al. 1997. Nasal provocation test in the diagnostics of hairdressers' occupational rhinitis. *Acta Otolaryngol Suppl (Stockh)* 529:133-136.
- *IBT. 1974. Irritation threshold evaluation study with ammonia. Report to International Institute of Ammonia Refrigeration by Industrial Biotest Laboratories, Inc. 663-03161. (as cited in NIOSH 1974)

9. REFERENCES

- *Iles JF, Jack JJB. 1980. Ammonia: Assessment of its action on postsynaptic inhibition as a cause of convulsions. *Brain* 103:555-578.
- Ilies P, Mavinic DS. 2001. A pre- and post-denitrification system treating a very high ammonia landfill leachate: Effects of pH change on process performance. *Environ Technol* 22(3):289-300.
- *Industrial Bio-Test Laboratories, Inc. 1973. Irritation threshold evaluation study with ammonia. Report to international institute of ammonia refrigeration (IBT No. 663-03160). Northbrook, IL: Industrial Bio-Test Laboratories, Inc.
- *IRIS. 2004. Integrated Risk Information System. <http://www.epa.gov/iris>. May 02, 2002.
- *Irwin JG, Williams NL. 1988. Acid rain: Chemistry and transport. *Environ Pollut* 50:29-59.
- Itzhak Y, Norenberg MD. 1994. Attenuation of ammonia toxicity in mice by PK 11195 and pregnenolone sulfate. *Neurosci Lett* 182(2):251-254.
- *Iwaoka WT, Krone CA, Sullivan JJ, et al. 1981. Effect of pH and ammonium ions on mutagenic activity in cooked beef. *Cancer Lett* 12:335-341.
- *Izumi H, Karita K. 1993. Reflex vasodilatation in the cat lip elicited by stimulation of nasal mucosa by chemical irritants. *Am J Physiol* 265(4 Pt 2):R733-R738.
- Jaffe HL, Bodansky A, Blair JE. 1932. The effects of parathormone and ammonium chloride on the bones of rabbits. *J Exp Med* 55:695-701.
- *Janicki RH. 1970. Renal adaptation during chronic NH_4Cl acidosis in the rat: No role for hyperplasia. *Am J Physiol* 219:613-618.
- *Jarudi MD, Golden MD. 1973. Ammonia eye injuries. *J Iowa Med Soc* 63:260-263.
- *Jeffers LJ, Dubow RA, Zieve L, et al. 1988. Hepatic encephalopathy and orotic aciduria associated with hepatocellular carcinoma in a noncirrhotic liver. *Hepatology* 8(1):78-81.
- *Jin H, Yang X, Yu H, et al. 1999. Identification of ammonia and volatile phenols as primary toxicants in a coal gasification effluent. *Bull Environ Contam Toxicol* 63(3):399-406.
- *Jingsheng C, Xuemin G, Dawei H, et al. 2000. Nitrogen contamination in the Yangtze river system, China. *J Hazard Mater* 73(2):107-113.
- *Johnson CA, Sigg L, Zobrist J. 1987. Case studies on the chemical composition of fogwater: The influence of local gaseous emissions. *Atmos Environ* 21(11):2365-2374.
- Johnson L. 1975. The effects of anhydrous ammonia on the environment and its direct effect on humans. *J Environ Health* 37:462-465.
- *Jones JG, Simon BM, Horsley RW. 1982. Microbiological sources of ammonia in freshwater lake sediments. *J Gen Microbiol* 128:2823-2831.
- Jones RD, Schwab AP. 1993. Nitrate leaching and nitrite occurrence in a fine-textured soil. *Soil Sci* 155(4):272-282.

9. REFERENCES

- *Jones W, Morring K, Olenchock SA, et al. 1984. Environmental study of poultry confinement buildings. *Am Ind Hyg Assoc J* 45(11):760-766.
- *Kalandarov S, Bychkov VP, Frenkel ID, et al. 1984. [Effect of an increased ammonia content in an enclosed atmosphere on the adrenocortical system in man.] *Kosm Biol Aviakosm Med* 18:75-77. (Russian)
- Kamin H, Handler P. 1957. Amino acid and protein metabolism. *Annu Rev Biochem* 26:419-490.
- Kanamori K, Ross BD, Chung JC, et al. 1996. Severity of hyperammonemic encephalopathy correlates with brain ammonia level and saturation of glutamine synthetase in vivo. *J Neurochem* 67(4):1584-1594.
- Kapeghian JC, Jones AB, Waters IW. 1985. Effects of ammonia on selected hepatic microsomal enzyme activity in mice. *Bull Environ Contam Toxicol* 35:15-22.
- *Kapeghian JC, Mincer HH, Jones AL, et al. 1982. Acute inhalation toxicity of ammonia in mice. *Bull Environ Contam Toxicol* 29:371-378.
- *Kass I, Zamel N, Dobry CA, et al. 1972. Bronchiectasis following ammonia burns of the respiratory tract. *Chest* 62(3):282-285.
- *Kauppinen T. 1986. Occupational exposure to chemical agents in the plywood industry. *Ann Occup Hyg* 30(1):19-29.
- *Kawano S, Tsujii M, Fusamoto H, et al. 1991. Chronic effect of intragastric ammonia on gastric mucosal structures in rat. *Dig Dis Sci* 36(1):33-38.
- *Kelly TJ, Tanner RL, Newman L, et al. 1984. Trace gas and aerosol measurements at a remote site in the Northeast U.S. *Atmos Environ* 18(12):2565-2576.
- *Kemp MK, Dodds WK. 2002. The influence of ammonium, nitrate, and dissolved oxygen concentrations on uptake, nitrification, and denitrification rates associated with prairie stream substrata. *Limnol Oceanogr* 47(5):1380-1393.
- Kerr LA, McCoy CP, Boyle CR, et al. 1990. Effects of ammoniation of endophyte fungus-infested fescue hay on serum prolactin concentration and rectal temperature in beef cattle. *Am J Vet Res* 51(1):76-78.
- *Kerstein MD, Schaffzin DM, Hughes WB, et al. 2001. Acute management of exposure to liquid ammonia. *Mil Med* 166(10):913-914.
- Khera KS. 1991a. Chemically-induced alterations in maternal homeostasis and conceptual histology: their etiologic significance in rat fetal anomalies [Abstract]. *Teratology* 43(5):414.
- Khera KS. 1991b. Chemically induced alterations in maternal homeostasis and histology of conceptus: their etiologic significance in rat fetal anomalies. *Teratology* 44(3):259-297.
- *Kienzle E, Wilms-Eilers S. 1994. Struvite diet in cats: effect of ammonium chloride and carbonates on acid base balance of cats. *J Nutr* 124(Suppl 12):2652S-2659S.

9. REFERENCES

- *Kimura T, Budka H. 1986. Glial fibrillary acidic protein and S-100 protein in human hepatic encephalopathy: Immunocytochemical demonstration of dissociation of two glia associated proteins. *Acta Neuropathol* 70:17-21.
- *Kirchner M, Braeutigam S, Ferm M, et al. 1999. Field intercomparison of diffusive samplers for measuring ammonia. *J Environ Monitor* 1(3):259-265.
- *Klein J, Olson KR, McKinney HE. 1985. Caustic injury from household ammonia. *Am J Emerg Med* 3:320.
- *Kleinman MT, Hyde DM, Bufalino C, et al. 2003. Toxicity of chemical components of fine particles inhaled by aged rats: Effects of concentration. *J Air Waste Manage Assoc* 53:1080-1087.
- *Klendshoj NC, Rejent TA. 1966. Tissue levels of some poisoning agents less frequently encountered. *J Forensic Sci* 11(1):75-80.
- Kligerman AD, Chapin RE, Erexson GL, et al. 1993. Analyses of cytogenetic damage in rodents following exposure to simulated groundwater contaminated with pesticides and a fertilizer. *Mutat Res* 300(2):125-134.
- Knapka JJ, Smith KP, Judge FJ. 1977. Effect of crude fat and crude protein on reproduction and weanling growth in four strains of inbred mice. *J Nutr* 107:61-69.
- *Knapp KT, Durham JL, Ellestad TG. 1986. Pollutant sampler for measurements of atmospheric acidic dry deposition. *Environ Sci Tech* 20:633-637.
- *Koenig H, Koenig R. 1949. Production of acute pulmonary edema by ammonium salts. *Proc Soc Exp Biol Med* 70(3):375-380.
- Konsenko E, Felip V, Minana MD, et al. 1991. Ammonium ingestion prevents depletion of hepatic energy metabolites induced by acute ammonium intoxication. *Arch Biochem Biophys* 290(2):484-488.
- *Konsenko E, Kaminsky YG, Felipo V, et al. 1993. Chronic hyperammonemia prevents changes in brain energy and ammonia metabolites induced by acute ammonium intoxication. *Biochim Biophys Acta* 1180(3):321-326.
- *Kosenko E, Kaminisky Y, Grau E, et al. 1994. Brain ATP depletion induced by acute ammonia intoxication in rats is mediated by activation of the NMDA receptor and Na⁺, K(+) -ATPase. *J Neurochem* 63(6):2172-2178.
- Kosenko E, Kaminsky Y, Grau E, et al. 1995. Nitroarginine, an inhibitor of nitric oxide synthetase, attenuates ammonia toxicity and ammonia-induced alterations in brain metabolism. *Neurochem Res* 20(4):451-456.
- Kosenko E, Kaminisky Y, Lopata O, et al. 1999. Blocking NMDA receptors prevents the oxidative stress induced by acute ammonia intoxication. *Free Radic Biol Med* 22(11/12):1369-1374.
- Kosenko E, Llansola M, Montoliu C. 2003a. Glutamine synthetase activity and glutamine content in brain: modulation by NMDA receptors and nitric oxide. *Neurochem Int* 43:493-499.

9. REFERENCES

- Kosenko E, Venediktova N, Kaminsky Y, et al. 2003b. Sources of oxygen radicals in brain in acute ammonia intoxication in vivo. *Brain Res* 981:193-200.
- Koyuncuoglu H, Keyer M, Simsek S, et al. 1978. Ammonia intoxication: Changes of brain levels of putative neurotransmitters and related compounds and its relevance to hepatic coma. *Pharmacol Res Commun* 10(9):787-807.
- *Krajenbrink GJW, Ronen D, Van Duijvenbooden W, et al. 1988. Monitoring of recharge water quality under woodland. *J Hydrol* 98:83-102.
- *Kramer DA. 2000. Nitrogen. U.S. Geological survey minerals yearbook. <http://minerals.usgs.gov/minerals/pubs/commodity/nitrogen/480400.pdf>. June 05, 2001.
- *Kramer DA. 2002. Nitrogen (fixed)–ammonia. U.S. Geological survey, mineral commodity summaries. <http://minerals.usgs.gov/minerals/pubs/commodity/nitrogen/480302.pdf>. July 06, 2002.
- Kramer DA. 2003. Nitrogen. U.S. Geological survey minerals yearbook. <http://minerals.usgs.gov/minerals/pubs/commodity/nitrogen/nitromyb02.pdf>. July 19, 2003.
- Kramer DA. 2004. Nitrogen (fixed)–ammonia. U.S. Geological survey, mineral commodity summaries. <http://minerals.usgs.gov/minerals/pubs/commodity/nitrogen/nitromcs04.pdf>. June 15, 2004.
- *Kretzschmar HA, DeArmond SJ, Forno LS. 1985. Measurement of GFAP in hepatic encephalopathy by ELISA and transblots. *J Neuropathol Exp Neurol* 44:459-471.
- *Krishnan K, Andersen ME. 1994. Physiologically based pharmacokinetic modeling in toxicology. In: Hayes AW, ed. *Principles and methods of toxicology*. 3rd ed. New York, NY: Raven Press, Ltd., 149-188.
- *Krishnan K, Andersen ME, Clewell HJ III, et al. 1994. Physiologically based pharmacokinetic modeling of chemical mixtures. In: Yang RSH, ed. *Toxicology of chemical mixtures: Case studies, mechanisms, and novel approaches*. San Diego, CA: Academic Press, 399-437.
- *Kucey RMN. 1988. Ammonia loss following surface application of urea fertilizers to a calcareous soil. *Commun Soil Sci Plant Anal* 19(4):431-445.
- Kukkonen J, Savolainen AL, Valkama I, et al. 1993. Long-range transport of ammonia released in a major chemical accident at Ionava, Lithuania. *J Hazard Mater* 35(1):1-16.
- Kullman GJ, Thorne PS, Waldron F, et al. 1998. Organic dust exposures from work in dairy barns. *Am Ind Hyg Assoc J* 59:403-413.
- *Kumar S. 1985. An eulerian model for scavenging of pollutants by raindrops. *Atmos Environ* 19(5):769-778.
- *Kupprat I, Johnson RE, Hertig BA. 1976. Ammonia: A normal constituent of expired air during rest and exercise. *Fed Proc* 35:478.
- Kuta CC, Maickel RP, Borowitz JL. 1984. Modification of drug action by hyperammonemia. *J Pharmacol Exp Ther* 229:85-90.

9. REFERENCES

- *Lamar C. 1970. Ammonia toxicity in rats: Protection by α -methylglutamic acid. *Toxicol Appl Pharmacol* 17:795-803.
- *Landahl HD, Herrmann RG. 1950. Retention of vapors and gases in the human nose and lung. *Arch Ind Hyg Occup Med* 1:36-45.
- *Lane M, Gardner DK. 1994. Increase in postimplantation development of cultured mouse embryos by amino acids and induction of fetal retardation and exencephaly by ammonium ions. *J Reprod Fertil* 102(2):305-312.
- *Langland M. 1992. Atmospheric deposition of ammonia from open manure-storage lagoons in south-central Pennsylvania. *Environ Professional* 14:28-37.
- Larsen FS, Gottstein J, Blei AT. 2001. Cerebral hyperemia and nitric oxide synthase in rats with ammonia-induced brain edema. *J Hepatol* 34(4):548-554.
- *Larson T, Frank R, Covert D, et al. 1980. The chemical neutralization of inhaled sulfuric acid aerosol. *Am J Ind Med* 1:449-452.
- Last JA. 1989. Effects of inhaled acids on lung biochemistry. *Environ Health Perspect* 79:115-119.
- *Latenser BA, Lucktong TA. 2000. Anhydrous ammonia burns: Case presentation and literature review. *J Burn Care Rehab* 21(1 PT 1):40-42.
- *Lau N-C, Charlson RJ. 1977. On the discrepancy between background atmospheric ammonia gas measurements and the existence of acid sulfates as a dominant atmospheric aerosol. *Atmos Environ* 11:475-478.
- *LeBlanc JR, Madhavan S, Porter RE. 1978. Ammonia. In: Grayson M, Eckroth D, eds. *Kirk-Othmer encyclopedia of chemical technology*. 3rd ed. Vol. 2. New York, NY: John Wiley & Sons, Inc., 470-516.
- *Leduc D, Gris P, Lheureux P, et al. 1992. Acute and long term respiratory damage following inhalation of ammonia. *Thorax* 47(9):755-757.
- *Lee DS, Bouwman AF, Asman WAH, et al. 1997. Emissions of nitric oxide, nitrous oxide and ammonia from grasslands on a global scale. In: Jarvis SC, Pain BF, eds. *Gaseous nitrogen emissions from grasslands*. New York, NY: Cab International, 353-371.
- *Lee HS, Chan CC, Tan KT, et al. 1993. Burnisher's asthma-a case due to ammonia from silverware polishing. *Singapore Med J* 34(6):565-566.
- Lee JH, Kim WH, Cho H, et al. 1998. N-carbamoyl-L-glutamate plus L-arginine can protect ammonia intoxication in rats with reduced functional liver mass. *Biochem Biophys Res Commun* 248(2):391-394.
- Lee WM, Huang WM, Chen YY. 2001. Effect of relative humidity on mixed aerosols in atmosphere. *J Environ Sci Health Part A* 36(4):533-544.
- *Legters L, Morton JD, Nightingale TE, et al. 1981. Biological effects of short, high-level exposure to gases: Ammonia, carbon monoxide, sulfur dioxide and nitrogen oxides: Final summary report. Fort Detrick, MD: U.S. Army Medical Research and Development Command.

9. REFERENCES

- *Lehninger AL. 1975. Biochemistry: The molecular basis of cell structure and function. 2nd ed. New York: Worth Publishers, Inc., 579-585.
- Lemann J, Litzow JR, Lennon EJ. 1966. The effects of chronic acid loads in normal man: Further evidence for the participation of bone mineral in the defense against chronic metabolic acidosis. *J Clin Invest* 45(10):1608-1614.
- *Leonard JJ, Feddes JJR, McQuitty JB. 1984. Air quality in commercial broiler housing. *Can Agric Eng* 26(1):65-72.
- Leonard KM, Swanson GW. 2001. Comparison of operational design criteria for subsurface flow constructed wetlands for wastewater treatment. *Water Sci Technol* 43(11):301-307.
- *Leonardos G, Kendall D, Barnard N. 1969. Odor threshold determinations of 53 odorant chemicals. *J Air Pollut Control Assoc* 19:91-95.
- *Leung H-W. 1993. Physiologically-based pharmacokinetic modelling. In: Ballentine B, Marro T, Turner P, eds. General and applied toxicology. Vol. 1. New York, NY: Stockton Press, 153-164.
- *Levine JS, Augustsson TR, Hoell JM. 1980. The vertical distribution of tropospheric ammonia. *Geophys Res Lett* 7(5):317-320.
- *Levy DM, Divertie MB, Litzow TJ, et al. 1964. Ammonia burns of the face and respiratory tract. *JAMA* 190(10):95-98.
- *Liao CM, Bundy DS. 1995. Quantification of ventilation on distribution of gaseous pollutants emitted from stored swine manure. *J Environ Sci Health B* 30(6):859-893.
- Lide DR, ed. 1988. CRC handbook of chemistry and physics. 68th ed. Boca Raton, FL: CRC Press, Inc., 4-39.
- *Lide DR, ed. 1998. CRC handbook of chemistry and physics. 79th ed. Boca Raton, FL: CRC Press, Inc.
- *Liebhardt WC, Golt C, Tupin J. 1979. Nitrate and ammonium concentrations of ground water resulting from poultry manure applications. *J Environ Qual* 8(2):211-215.
- Lin S, Raabe W. 1985. Ammonia intoxication: Effects on cerebral cortex and spinal cord. *J Neurochem* 44:1252-1258.
- Lipsky RH, Vemuri MC, Silverman SJ. 1988. Changes in nuclear proteins of astrocytes as a result of acute ammonia or ethanol exposure. *Neurochem Int* 12(4):519-524.
- *Livingston, AL. 1978. Forage plant estrogens. *J Toxicol Environ Health* 4:301-324.
- *Llansola M, Erceg S, Hernandez-Viadel M. 2002. Prevention of ammonia and glutamate neurotoxicity by carnitine: molecular mechanisms. *Metab Brain Dis* 17(4):389-397.

9. REFERENCES

- *Lobasov M, Smirnov F. 1934. On the nature of the action of chemical agents on mutational process in *Drosophila melanogaster*. II. The effect of ammonia on the occurrence of lethal transgenations. *C R Seances Acad Sci Roum* 3:177-178.
- Lobley GE, Connell A, Lomax MA, et al. 1995. Hepatic detoxification of ammonia in the ovine liver: possible consequences for amino acid catabolism. *Br J Nutr* 73(5):667-685.
- *Lopez G, Dean BS, Krenzelok EP. 1988. Oral exposure to ammonia inhalants: A report of 8 cases. *Vet Hum Toxicol* 30:350.
- *Lotspeich WD. 1965. Renal hypertrophy in metabolic acidosis and its relation to ammonia excretion. *Am J Physiol* 208:1135-1142.
- Lu Y, Wassmann R, Neue HU, et al. 2000. Dissolved organic carbon and methane emissions from a rice paddy fertilized with ammonium and nitrate. *J Environ Qual* 29(6):1733-1740.
- *MacCarthy P, Klusman RW, Rice JW. 1987. Water analysis. *Anal Chem* 59:308R-337R.
- *MacEwen JD, Vernot EH. 1972. Toxic hazards research unit - annual technical report. Wright Patterson AFB, OH: Aerospace Medical Research Laboratory, AD755358. AMRLTR7262, 70-73.
- *MacEwen JD, Theodore J, Vernot EH. 1970. Human exposure to EEL concentrations of monomethylhydrazine. In: Proceedings 1st annual conference on environmental toxicology Wright-Patterson Air Force Base, OH: Aerospace Medical Research Laboratory. AMRLTR70102, 355-363.
- *Mahendrappa MK. 1982. Effects of SO₂ pollution on nitrogen transformation in some organic soils of northeastern New Brunswick: Ammonia volatilization. *Can J For Res* 12:458-462.
- *Malcolm SJ, Battersby NS, Stanley SO, et al. 1986. Organic degradation, sulphate reduction and ammonia production in the sediments of Loch Eil, Scotland. *Estuarine Coastal Shelf Sci* 23:689-706.
- *Manninen A. 1988. Analysis of airborne ammonia: Comparison of field methods. *Ann Occup Hyg* 32(3):399-404.
- *Manninen ATA, Savolainen H. 1989. Effect of short-term ammonia inhalation on selected amino acids in rat brain. *Pharmacol Toxicol* 64(3):244-246.
- Manninen A, Anttila S, Savolainen H. 1988. Rat metabolic adaptation to ammonia inhalation. *Proc Soc Exp Biol Med* 187(3):278-281.
- *Marcaggi P, Coles JA. 2001. Ammonium in nervous tissue: transport across cell membranes, fluxes from neurons to glial cells, and role in signalling. *Prog Neurobiol* 64:157-183.
- *Marcaida G, Felipo V, Hermenegildo C, et al. 1992. Acute ammonia toxicity is mediated by the NMDA type of glutamate receptors. *FEBS Lett* 296(1):67-68.
- Marconi AM, Battaglia FC, Meschia G, et al. 1989. A comparison of amino acid arteriovenous differences across the liver and placenta of the fetal lamb. *Am J Physiol* 257(6 Pt 1):E909-E915.
- Martinelle K, Haggstrom L. 1993. Mechanisms of ammonia and ammonium ion toxicity in animal cells: Transport across cell membranes. *J Biotechnol* 30(3):339-350.

9. REFERENCES

- *Maswoswe SM, Cyr DM, Griffith AD, et al. 1986. The effect of sodium benzoate on ammonia toxicity in rats. *Biochem Biophys Res Commun* 138(1):369-373.
- Matkowskyj KA, Marrero JA, Carroll RE, et al. 1999. Azoxymethane-induced fulminant hepatic failure in C57BL/6J mice: characterization of a new animal model. *Am J Physiol* 277(2 PT 1):G455-G462.
- Matsuoka M, Igisu H. 1993. Comparison of the effects of L-carnitine, D-carnitine and acetyl-L-carnitine on the neurotoxicity of ammonia. *Biochem Pharmacol* 46(1):159-164.
- Matsuoka M, Igisu H, Kohriyama K, et al. 1991. Suppression of neurotoxicity of ammonia by L-carnitine. *Brain Res* 567(2):328-331.
- *Matthews DA, Effler SW, Matthews CM. 2000. Ammonia and toxicity criteria in polluted Onondaga Lake, New York. *Water Environ Res* 72(6):731-741.
- Mautz WJ, Kleinman MT, Bhalla DK, et al. 2001. Respiratory tract responses to repeated inhalation of an oxidant and acid gas-particle air pollutant mixture. *Toxicol Sci* 61(2):331-341.
- *Mayan MH, Merilan CP. 1972. Effects of Ammonia inhalation on respiration rate of rabbits. *J Anim Sci* 34:448-452.
- Mayan MH, Merilan CP. 1976. Effects of ammonia inhalation on young cattle. *N Z Vet J* 24:221-224.
- *Mayr U, Butsch A, Schneider S. 1992. Validation of two in vitro test systems for estrogenic activities with zearalenone, phytoestrogens and cereal extracts. *Toxicology* 74:135-149.
- McBurney MI, Van Soest PJ, Jeraci JL. 1987. Colonic carcinogenesis: the microbial feast or famine mechanism. *Nutr Cancer* 10:23-28.
- McCandless DW. 1981. Metabolic turnover in the reticular activating system in ammonia induced coma. *Neurobehav Toxicol Teratol* 3:257-260.
- McCandless DW, Schenker S. 1981. Effect of acute ammonia intoxication on energy stores in the cerebral reticular activating system. *Exp Brain Res* 44:325-330.
- McCandless DW, Looney GA, Modak AT, et al. 1985. Cerebral acetylcholine and energy metabolism changes in acute ammonia intoxication in the lower primate *Tupaia glis*. *J Lab Clin Med* 106:183-186.
- McCubbin DR, Apelberg BJ, Roe S, et al. 2002. Livestock ammonia management and particulate-related health benefits. *Environ Sci Technol* 36(6):1141-1146.
- *McGuinness R. 1969. Ammonia in the eye. *Br Med J* 2:575.
- McKhann GM, Tower DB. 1961. Ammonia toxicity and cerebral oxidative metabolism. *Am J Physiol* 200:420-424.
- McKinney PE, Brent J, Kulig K. 1994. Acute zinc chloride ingestion in a child: Local and systemic effects. *Ann Emerg Med* 23(6):1383-1387.

9. REFERENCES

Megarbane B, Bruneel F, Bedos JP, et al. 2000. Ammonium chloride poisoning: a misunderstood cause of metabolic acidosis with normal anion gap. *Intensive Care Med* 26(12):1869.

*Mégraud F, Neman-Simha V, Brugmann D. 1992. Further evidence of the toxic effect of ammonia produced by *Helicobacter pylori* urease on human epithelial cells. *Infect Immun* 60(5):1858-1863.

*Melbostad E, Eduard W. 2001. Organic dust-related respiratory and eye irritation in Norwegian farmers. *Am J Ind Med* 39(2):209-217.

*Metges CC, Petzke KH, El-Khoury AE, et al. 1999. Incorporation of urea and ammonia nitrogen into ileal and fecal microbial proteins and plasma free amino acids in normal men and ileostomates. *Am J Clin Nutr* 70(6):1046-1058.

*Meybeck M. 1982. Carbon, nitrogen, and phosphorus transport by world rivers. *Am J Sci* 282:401-450.

*Meyerhoff ME, Robins RH. 1980. Disposable potentiometric ammonia gas sensors for estimation of ammonia in blood. *Anal Chem* 52(14):2383-2387.

Michaels RA. 1999. Emergency planning and the cute toxic potency of inhaled ammonia. *Environ Health Perspect* 107(8):617-627.

Michoudet C, Chauvin MF, Baverel G. 1994. Glutamine synthesis from glucose and ammonium chloride by guinea-pig kidney tubules. *Biochem J* 297:69-74.

*Millea TP, Kucan JO, Smoot EC. 1989. Anhydrous ammonia injuries. *J Burn Care Rehab* 10(5):448-453.

Miñana MD, Felipe V, Grisolia S. 1988. Protective effect of long term ammonium ingestion against acute ammonium intoxication. *Biochem Biophys Res Commun* 153(3):979-983.

*Miñana MD, Felipe V, Grisolia S. 1989a. Assembly and disassembly of brain tubulin is affected by high ammonia levels. *Neurochem Res* 14(3):235-238.

*Miñana MD, Felipe V, Quel A, et al. 1989b. Selective regional distribution of tubulin induced in cerebrum by hyperammonemia. *Neurochem Res* 14(12):1241-1243.

Miñana MD, Hermenegildo C, Llsansola M, et al. 1996. Carnitine and choline derivatives containing a trimethylamine group prevent ammonia toxicity in mice and glutamate toxicity in primary cultures of neurons. *J Pharmacol Exp Ther* 279(1):194-19.

*Miñana MD, Marcaida G, Grisolia S, et al. 1995. Prenatal exposure of rats to ammonia impairs NMDA receptor function and affords delayed protection against ammonia toxicity and glutamate neurotoxicity. *J Neuropathol Exp Neurol* 54(5):644-650.

Minelli A, Lyons S, Nolte C, et al. 2000. Ammonium triggers calcium elevation in cultured mouse microglial cells by initiating Ca^{2+} release from thapsigargin-sensitive intracellular stores. *Pflugers Arch* 439(3):370-377.

9. REFERENCES

- *Mishra S, Singh V, Jain A, et al. 2001. Simultaneous determination ammonia, aliphatic amines, aromatic amines and phenols at microgram 1-1 levels in environmental waters by solid-phase extraction of their benzoyl derivatives and gas chromatography-mass spectrometry. *Analyst* 126(10):1663-1668.
- *Mitz SV, Giesy JP. 1985. Sewage effluent biomonitoring. II. Biochemical indicators of ammonia exposure in channel catfish. *Ecotoxicol Environ Saf* 10:40-52.
- *Mohanachari V, Reddy KS, Indiri K. 1984. Metabolic fate of ammonia in the rat after ethanol loading. *Toxicol Lett* 20:225-228.
- *Moller D, Schieferdecker H. 1985. A relationship between agricultural NH_3 emissions and the atmospheric SO_2 content over industrial areas. *Atmos Environ* 19(5):695-700.
- Monfort P, Kosenko E, Erceg S. 2002. Molecular mechanism of acute ammonia toxicity: role of NMDA receptors. *Neurochem Int* 41:95-102.
- *Montague TJ, Macneil AR. 1980. Mass ammonia inhalation. *Chest* 77:496-498.
- *Morgan SE. 1997. Ammonia pipeline rupture: Risk assessment to cattle. *Vet Hum Toxicol* 39(3):159-161.
- *Mori S, Kaneko H, Mitsuma T, et al. 1998. Implications of gastric topical bioactive peptides in ammonia-induced acute gastric mucosal lesions in rats. *Scand J Gastroenterol* 33(4):386-393.
- Morimoto Y, Ukida M, Tsuji T. 1988. Dynamic relationship between urea and glutamine synthesis in the mechanism of ammonia detoxication: A tracer study using $^{15}\text{NH}_4\text{Cl}$ in fulminant hepatic failure rats. *Gastroenterol Jpn* 23(5):538-545.
- *Morris JC. 1978. Chemistry of aqueous chlorine in relation to water chlorination. In: Jolley RL, ed. *Water chlorination: Environmental impact and health effects*. Ann Arbor, MI: Ann Arbor Sci Pub, 21-36.
- *Morselli PL, Franco-Morselli R, Bossi L. 1980. Clinical pharmacokinetics in newborns and infants: Age-related differences and therapeutic implications. *Clin Pharmacokin* 5:485-527.
- *Mossberg SM, Ross G. 1967. Ammonia, movement in the small intestine: Preferential transport by the ileum. *J Clin Invest* 46(4):490-498.
- Mouille B, Morel E, Robert V, et al. 1999. Metabolic capacity for L-citrulline synthesis from ammonia in rat isolated colonocytes. *Biochim Biophys Acta* 1427(3):401-407.
- *Mulder JS, Van der Zalm HO. 1967. [Fatal case of ammonia poisoning.] *T Soc Genesck* 45:458. (German)
- *Munger JW. 1982. Chemistry of atmospheric precipitation in the North-Central United States. *Atmos Environ* 16(7):1633-1645.
- *Muntwyler E, Iacobellis M, Griffin GE. 1956. Kidney glutaminase and carbonic anhydrase activities and renal electrolyte excretion in rats. *Am J Physiol* 184:83-90.

9. REFERENCES

- *Murakami M, Asagoe K, Dekigai H, et al. 1995. Products of neutrophil metabolism increase ammonia-induced gastric mucosal damage. *Dig Dis Sci* 40(2):268-273.
- Murakami M, Saita H, Teramura S, et al. 1993. Gastric ammonia has a potent ulcerogenic action on the rat stomach. *Gastroenterology* 105(6):1710-1715.
- Nadelhoffer K, Downs M, Fry B, et al. 1999. Controls on N retention and exports in a forested watershed. *Environ Monit Assess* 55(1):187-210.
- Nagamine T, Saito S, Kaneko M, et al. 1995. Effect of biotin on ammonia intoxication in rats and mice. *J Gastroenterol* 30(3):351-355.
- *Nagy L, Kusstatscher S, Hauschka PV, et al. 1996. Role of cysteine proteases and protease inhibitors in gastric mucosal damage induced by ethanol or ammonia in the rat. *J Clin Invest* 98(4):1047-1054.
- Nakasaki K, Ohtaki A, Takano H. 2001. Effect of bulking agent on the reduction of NH₃ emissions during thermophilic composting of night-soil sludge. *Waste Manage Res* 19(4):301-307.
- Nakatsuka T, Fukikake M, Katoh M, et al. 1992. Amelioration of furosemide-induced fetal wavy ribs by co-administration of ammonium chloride in rats. *Teratology* 46(6):19B.
- Nakatsuka T, Fujikake N, Hasebe M, et al. 1993. Effects of sodium bicarbonate and ammonium chloride on the incidence of furosemide-induced fetal skeletal anomaly, wavy rib, in rats. *Teratology* 48(2):139-147.
- Nakazawa S, Quastel JH. 1968. Inhibitory effects of ammonium ions and some amino acids on stimulated brain respiration and cerebral amino acid transport. *Can J Biochem* 46:543-548.
- *NAS/NRC. 1989. Report of the oversight committee. In: *Biologic markers in reproductive toxicology*. Washington, DC: National Academy of Sciences, National Research Council, National Academy Press.
- NASA. 1967. Means of determining the maximum allowable concentration of toxic products of natural human metabolism. Washington, DC: National Aeronautics and Space Administration.
- *Nason GE, Pluth DJ, McGill WB. 1988. Volatilization and foliar recapture of ammonia following spring and fall application of nitrogen-15 urea to a douglas-fir ecosystem. *Soil Sci Soc Am J* 52:821-828.
- NATICH. 1988. NATICH data base report on state, local and EPA air toxics activities. Research Triangle Park, NC: National Air Toxics Information Clearinghouse, U.S. Environmental Protection Agency.
- Naumovski D, Pilovski G, Cibisev A, et al. 2001. Pulmonary injuries after exposure to irritative fumes of household cleaners. *Toxicol Lett* 123(Suppl 1):91-92.
- Navazio F, Gerritsen T, Wright GJ. 1961. Relationship of ammonia intoxication to convulsions and coma in rats. *J Neurochem* 8:146-151.
- NCI. 1975. In: Fraumeni JF, ed. *Persons at high risk of cancer: An approach to cancer etiology and control*. Bethesda, MD: National Cancer Institute.

9. REFERENCES

Nemitz E, Sutton MA, Gut A, et al. 2000. Sources and sinks of ammonia within an oilseed rape canopy. *Agric For Meteorol* 105(4):385-404.

Nemitz E, Sutton MA, Schjoerring JK, et al. 2000. Resistance modelling of ammonia exchange over oilseed rape. *Agric For Meteorol* 105(4):405-425.

*Neumann R, Mehlhorn G, Buchholz I, et al. 1987. [Experimental studies of the effect of chronic exposure of suckling pigs to aerogenous toxic gas with ammonia of varying concentrations.] *J Vet Med B34*:241-253. (German)

New Jersey Department of Health. 1996a. Ammonium fluoride. Trenton, NJ: New Jersey Department of Health, Right to Know Program. www.state.nj.us/health/eoh/rtkweb/0099.pdf. April 1996

New Jersey Department of Health. 1996b. Ammonium carbamate. Trenton, NJ: New Jersey Department of Health, Right to Know Program. www.state.nj.us/health/eoh/rtkweb/0091.pdf. January 1996

Niden AH. 1968. Effects of ammonia inhalation on the terminal airways. *Proc 11th Aspen Emphysema Conf* 11:41-44.

Nieto R, Calder AG, Anderson SE, et al. 1996. Method for the determination of $^{15}\text{NH}_3$ enrichment in biological samples by gas chromatography electron impact ionization mass spectrometry. *J Mass Spectrom* 31(3):289-294.

*NIOSH. 1974. Occupational exposure to ammonia: Criteria for a recommended standard. Cincinnati, OH: National Institute for Occupational Safety and Health. NIOSH 74-136.

NIOSH. 1982. Background information on ammonia. Cincinnati, OH: U.S. National Institute for Occupational Safety and Health. PB82109984.

NIOSH. 1984. Ammonia Method 6701. NIOSH manual of analytical methods 1. Cincinnati, OH: National Institute for Occupational Safety and Health, 6701-1 to 6701-4.

NIOSH. 1985. NIOSH pocket guide to chemical hazards. Washington, DC: National Institute for Occupational Safety and Health.

*NIOSH. 1987. Health hazard evaluation report. Cincinnati, OH: National Institute for Occupational Safety and Health. PB87232948.

*NIOSH. 1988. National Occupational Exposure Survey (NOES). Cincinnati, OH: National Institute for Occupational Safety Health, 93.

*NIOSH. 1994. Ammonia. NIOSH manual of analytical methods (NMAM), August 15, Fourth edition, 1994. National Institute for Occupational Safety and Health.

*NIOSH. 1996. Ammonia by ICNIOSH manual of analytical methods (NMAM), May 15, Fourth edition, 1996. National Institute for Occupational Safety and Health.

*NIOSH. 2002a. Ammonia. NIOSH pocket guide to chemical hazards. National Institute for Occupational Safety and Health. <http://www.cdc.gov/niosh/npg/npgd0028.html>. June 06, 2004.

9. REFERENCES

- *NIOSH. 2002b. Ammonia and ammonium chloride fume. NIOSH pocket guide to chemical hazards. National Institute for Occupational Safety and Health. [wysiwyg://92/http://www.cdc.gov/niosh/npg/npg.html](http://www.cdc.gov/niosh/npg/npg.html). May 02, 2002.
- *Noda K, Chikamori K. 1976. Effect of ammonia via prepyriform cortex on regulation of food intake in the rat. *Am J Physiol* 231:1263-1266.
- *NOES. 1989. Ammonia. National Occupational Exposure Survey. March 29, 1989, 19.
- Norenberg MD. 1996. Astrocytic-ammonia interactions in hepatic encephalopathy. *Semin Liver Dis* 16(3):245-253.
- *NRC. 1979. Transport and distribution of ammonia and the effect of pH. In: National Research Council, ed. Ammonia. Baltimore, MD: University Park Press.
- *NRC. 1993. Pesticides in the diets of infants and children. Washington, DC: National Academy Press, National Research Council.
- *NSF. 1999. Interim report: Environmental science and engineering for the 21st century. The role of the National Science Foundation. Arlington, VA: National Science Foundation. NSB 99-133.
- O'Connor JE, Costell M, Grisolia S. 1984. Prevention of ammonia toxicity by L-carnitine: Metabolic changes in the brain. *Neurochem Res* 9(4):563-570.
- *O'Connor JE, Costell M, Grisolia S. 1987. The potentiation of ammonia toxicity by sodium benzoate is prevented by L-Carnitine. *Biochem Biophys Res Commun* 145(2):817-824.
- O'Connor JE, Costell M, Grisolia S. 1989. Carbamyl glutamate prevents the potentiation of ammonia toxicity by sodium benzoate. *Eur J Pediatr* 148(6):540-542.
- *O'Connor JE, Guerri C, Grisolia S. 1982. Protective effect of ethanol on acute ammonia intoxication in mice. *Biochem Biophys Res Commun* 104(2):410-415.
- *O'Deen WA, Porter LK. 1986. Continuous flow system for collecting volatile ammonia and amines from senescing winter wheat. *Agron J* 78:746-749.
- OHM-TADS. 1988. Oil and Hazardous Materials Technical Assistance Data System. Washington, DC: Environmental Protection Agency, National Institute of Health.
- Ohtsuka Y, Griffith OW. 1991. L-carnitine protection in ammonia intoxication. Effect of aminocarnitine on carnitine-dependent metabolism and acute ammonia toxicity. *Biochem Pharmacol* 41(12):1957-1961.
- Okita M, Watanabe A, Tsuji T. 1988. Effect of branched-chain amino acid on ¹⁵N incorporation into liver and skeletal muscle proteins following [¹⁵N]-ammonium chloride administration to carbon tetrachloride-intoxicated rats. *J Nutr Sci Vitaminol* 34(1):85-96.
- *Olavarria JS, Artacho E, Sánchez F, et al. 1986. Synergistic effect of ammonia and potassium ions on pyruvate kinase from Ehrlich ascites tumor cells. *Enzyme* 35:34-41.

9. REFERENCES

- *Olivier JGJ, Bouwman AF, Van der Hoek KW, et al. 1998. Global air emission inventories for anthropogenic sources of NO_x, NH₃ and N₂O in 1990. *Environ Pollut* 102:135-148.
- Onay TT, Pohland FG. 2001. Nitrogen and sulfate attenuation in simulated landfill bioreactors. *Water Sci Technol* 44(2-3):367-372.
- OSHA. 1985. Permissible exposure limits (Table Z-1). Occupational Safety and Health Administration. Code of Federal Regulations. CFR 29(1910.1000):655-659.
- *OSHA. 1998. U. S. Department of Labor. Occupational Safety and Health Administration. Code of Federal Regulations. 29 CFR 1910.1000.
- *OSHA. 2001. Occupations in agriculture particularly hazardous for the employment of children below the age of 16. Occupational Safety and Health Administration. Code of Federal Regulations. 29 CFR 570.71. <http://frwebgate.access.gpo.gov/>. May 02, 2002.
- *OSHA. 2002a. Air contaminants. Occupational safety and health standards for shipyard employment. Occupational Safety and Health Administration. Code of Federal Regulations. 29 CFR 1915.1000. <http://www.osha.gov/>. May 02, 2002.
- *OSHA. 2002b. Blasting and the use of explosives. Safety and health regulations for construction. Occupational Safety and Health Administration. Code of Federal Regulations. 29 CFR 1926.914(e). <http://www.osha.gov/>. May 02, 2002.
- *OSHA. 2002c. Gases, vapors, fumes, dusts, and mists. Safety and health regulations for construction. Occupational Safety and Health Administration. Code of Federal Regulations. 29 CFR 1926.55. Appendix A. <http://www.osha.gov/>. May 02, 2002.
- *OSHA. 2002d. Limits for air contaminants. Occupational safety and health standards. Occupational Safety and Health Administration. Code of Federal Regulations. 29 CFR 1910.1000. Table Z-1. <http://www.osha.gov/>. May 02, 2002.
- *OSHA. 2002e. List of highly hazardous chemicals, toxics and reactives. Occupational safety and health standards. Occupational Safety and Health Administration. Code of Federal Regulations. 29 CFR 1910.119. Appendix A. <http://www.osha.gov/>. May 02, 2002.
- *OSHA. 2002f. List of highly hazardous chemicals, toxics and reactives. Safety and health regulations for construction. Occupational Safety and Health Administration. Code of Federal Regulations. 29 CFR 1926.64. <http://www.osha.gov/>. May 02, 2002.
- *OSHA. 2002g. Storage and handling of anhydrous ammonia. Occupational safety and health standards. Occupational Safety and Health Administration. Code of Federal Regulations. 29 CFR 1910.111. <http://www.osha.gov/>. May 02, 2002.
- Paik WK, Lawson D, Kim S. 1975. Effect of endocrine glands on the protection of rat from ammonia intoxication. *Life Sci* 15:1189-1196.
- Pal T, Pal A, Miller GH, et al. 1992. Passive dosimeter for monitoring ammonia vapor. *Anal Chim Acta* 263(1-2):175-178.

9. REFERENCES

- Paramasivam S, Alva AK, Fares A. 2000. Transformation and transport of nitrogen forms in a sandy entisol following a heavy loading of ammonium nitrate solution: Field measurements and model simulations. *J Soil Contam* 9(1):65-86.
- *Parris N. 1984. An improved fluorometric method for the determination of ammonia and volatile amines in meat tissue by high-performance liquid chromatography. *J Agric Food Chem* 32:829-831.
- *Parris N, Foglia TA. 1983. Simplified alcoholic extraction procedure for ammonia in meat tissue. *J Agric Food Chem* 31:887-889.
- *Parton WJ, Morgan JA, Altenhofen JM, et al. 1988. Ammonia volatilization from spring wheat plants. *Agron J* 80:419-425.
- Pascuzzi TA, Storrow AB. 1998. Mass casualties from acute inhalation of chloramine gas. *Mil Med* 163(2):102-104.
- Paul V. 2003. Inhibition of acute hyperammonemia-induced convulsions by systemically administered gamma aminobutyric acid in rats. *Pharmacol Biochem Behav* 74:523-528.
- *Payne WJ. 1981. Denitrification. New York, NY: John Wiley and Sons.
- Perry GF. 1995. Occupational medicine forum. *J Occup Environ Med* 37(10):1187-1188.
- Pinson DM, Schoeb TR, Lin SL, et al. 1988. Promotion of mycoplasma-pulmonis growth in rat tracheal organ cultures by ammonium chloride. *Lab Anim Sci* 38(2):143-147.
- *Pitts RF. 1971. The role of ammonia production and excretion in regulation of acid-base balance. *N Engl J Med* 284:32-38.
- *Plerson WR, Brachaczek WW. 1983. Emissions of ammonia and amines from vehicles on the road. *Environ Sci Technol* 17:757-760.
- *Pranitis DM, Meyerhoff ME. 1987. Continuous monitoring of ambient ammonia with a membrane-electrode-based detector. *Anal Chem* 59:2345-2350.
- *Price SK, Hughes JE, Morrison SC, et al. 1983. Fatal ammonia inhalation-A case report with autopsy findings. *S Afr Med J* 64:952-955.
- Prokop'eva AS, Yushkov GG. 1975. [Materials for a toxicological characteristic of the repeated effects of ammonia on the organs of animals after brief exposure.] *Gig Tr Prof Zabol* 9:54-55. (Russian)
- *Prokop'eva AS, Yushkov GG, Ubasheev IO. 1973. [Materials for a toxicological characteristic of the one-time effect of ammonia on the organisms of animals after brief exposures.] *Gig Tr Prof Zabol* 6:56-57. (Russian)
- *Prudhomme JC, Shusterman DJ, Blanc PD. 1998. Acute-onset persistent olfactory deficit resulting from multiple overexposures to ammonia vapor at work. *J Am Board Fam Pract* 11(1):66-69.
- Raabe W. 1989. Neurophysiology of ammonia intoxication. In: Butterworth RF, Pomier Layrargues G, eds. *Hepatic encephalopathy: Pathophysiology and treatment*. Clifton, NJ: Humana Press, 49-77.

9. REFERENCES

- *Raabe W. 1992. Ammonium ions abolish excitatory synaptic transmission between cerebellar neurons in primary dissociated tissue culture. *J Neurophysiol* 68:93-99.
- Raabe W, Gumnit RJ. 1975. Inhibition of cat motor cortex by ammonia. *J Neurophysiol* 38:347-355.
- Raabe W, Lin S. 1984. Ammonia, postsynaptic inhibition and CNS-energy state. *Brain Res* 303:67-76.
- *Rabkin R, Palathumpat M, Tsao T. 1993. Ammonium chloride alters renal tubular cell growth and protein turnover. *Lab Invest* 68(4):427-438.
- *Rajagopal R. 1978. Impact of land use on ground water quality in the Grand Traverse Bay Region of Michigan. *J Environ Qual* 7(1):93-98.
- Rama Rao KV, Jayakumar AR, Norenberg MD. 2003. Ammonia neurotoxicity: Role of the mitochondrial permeability transition. *Metab Brain Dis* 18(2):113-127.
- *Rapsomanikis S, Wake M, Kitto AMN, et al. 1988. Analysis of atmospheric ammonia and particulate ammonium by a sensitive fluorescence method. *Environ Sci Technol* 22:948-952.
- *Reynolds CM, Wolf DC. 1988. Effects of field methods and soil cover on estimating ammonia loss from nitrogen-15-urea. *Soil Sci Soc Am J* 52:706-712.
- *Reynolds SJ, Donham KJ, Whitten P, et al. 1996. Longitudinal evaluation of dose-response relationships for environmental exposures and pulmonary function in swine production workers. *Am J Ind Med* 29(1):33-40.
- *Richard D, Bouley G, Boudene C. 1978a. [Effects of continuous inhalation of ammonia in the rat and mouse.] *Bull Eur Physiopathol Respir* 14:573-582. (French)
- *Richard D, Jouany JM, Boudene C. 1978b. [Acute toxicity of ammonia gas in the rabbit by inhalation.] *C R Acad Hebd Seances Acad Sci Ser D* 287:375-378. (French)
- *Richards P, Brown CL, Houghton BJ, et al. 1975. The incorporation of ammonia nitrogen into albumin in man: the effects of diet, uremia and growth hormone. *Clin Nephrol* 3(5):172-179.
- *Robin ED, Travis DM, Bromberg PA, et al. 1959. Ammonia excretion by mammalian lung. *Science* 129:270-271.
- *Rogers HH, Anjea VP. 1980. Uptake of atmospheric ammonia by selected plant species. *Environ Exp Bot* 20:251-257.
- Ros M, Gantar A. 1998. Possibilities of reduction of recipient loading of tannery wastewater in Slovenia. *Water Sci Technol* 37(8):145-152.
- *Rosenbaum AM, Walner DL, Dunham ME, et al. 1998. Ammonia capsule ingestion causing upper aerodigestive tract injury. *Otolaryngol Head Neck Surg* 119(6):678-680.
- *Rosenfeld M. 1932. [Experimental modification of mitosis by ammonia.] *Arch Exp Zellforsch Besonders Gewebebeziehung* 14:1-13. (German)

9. REFERENCES

- *Rosswall T. 1981. The biogeochemical nitrogen cycle. In: Likens GE, ed. Some perspectives of the major biogeochemical cycles. New York, NY: John Wiley and Sons, 25-49.
- Rostron WM, Stuckey DC, Young AA. 2001. Nitrification of high strength ammonia wastewaters: comparative study of immobilisation media. *Water Res* 35(5):1169-1178.
- *Roy GS, Poricha RR. 1982. Ammonia nitrogen in fertiliser industry effluents - present status. *Proc No R&D (Fert Assoc India)*3:126-132.
- *RTECS. 1988. Registry of toxic effects of chemical substances. Cincinnati, OH: National Institute for Occupational Safety and Health.
- Rupp W, Sauer R, Hinz A. 1995. CO₂-laser sensor system for ammonia. *Proc SPIE Int Soc Opt Eng* 2506:162-166.
- *Russell AG, McCue KF, Cass GR. 1988. Mathematical modeling of the formation of nitrogen-containing air pollutants. 1. Evaluation of an Eulerian photochemical model. *Environ Sci Technol* 22:263-271.
- *Ryan JA, Keeney DR. 1975. Ammonia volatilization from surface-applied wastewater sludge. *J Water Pollut Control Fed* 47:386-393.
- *Ryden JC, Whitehead DC, Lockyer DR, et al. 1987. Ammonia emission from grassland and livestock production systems in the UK. *Environ Pollut* 48:173-184.
- Ryer-Powder JE. 1991. Health effects of ammonia. *Plant/Oper Prog* 10(4):228-232.
- Sadasivudu B, Murthy RK. 1978. Effects of ammonia on monoamine oxidase and enzymes of GABA metabolism in mouse brain. *Arch Int Physiol Biochim* 86:67-82.
- Sadasivudu B, Murthy CRK, Rao GN, et al. 1983. Studies on acetylcholinesterase and gamma-glutamyltranspeptidases in mouse brain in ammonia toxicity. *J Neurosci Res* 9:127-134.
- Sadasivudu B, Rao TI, Radhakrishna C. 1977. Acute metabolic effects of ammonia in mouse brain. *Neurochem Res* 2:639-655.
- Sadasivudu B, Rao TI, Radhakrishna M. 1979. Chronic metabolic effects of ammonia in mouse brain. *Arch Int Physiol Biochim* 87:871-885.
- *Salvatore F, Bocchinti V, Cimino F. 1963. Ammonia intoxication and its effects on brain ammonia levels. *Biochem Pharmacol* 12:1-6.
- Saran T, Hilgier W, Kocki T, et al. 1998. Acute ammonia treatment in vitro and in vivo inhibits the synthesis of a neuroprotectant kynurenic acid in rat cerebral cortical slices. *Brain Res* 787(2):348-350.
- Sato A, Gonmori K, Yoshioka N. 1999. A case of fatal intoxication with ammonium sulfate and a toxicological study using rabbits. *Forensic Sci Int* 101(2):141-149.
- *Saul RL, Archer MC. 1984. Oxidation of ammonia and hydroxylamine to nitrate in the rat and in vitro. *Carcinogenesis* 5(1):77-81.

9. REFERENCES

- *Sax NI, Lewis RJ, eds. 1987. Hawley's condensed chemical dictionary. New York, NY: Van Nostrand Reinhold, 1288.
- *Schaerdel AD, White WJ, Lang CM, et al. 1983. Localized and systemic effects of environmental ammonia in rats. *Lab Anim Sci* 33(1):40-45.
- Schenker S, Mendelson JH. 1964. Cerebral adenosine triphosphate in rats with ammonia-induced coma. *Am J Physiol* 206:1173-1176.
- Schenker S, Warren KS. 1962. Effect of temperature variation on toxicity and metabolism of ammonia in mice. *J Lab Clin Med* 60(2):291-301.
- Schenker S, McCandless DW, Brophy E, et al. 1967. Studies on the intracerebral toxicity of ammonia. *J Clin Invest* 46:838-848.
- *Schubiger G, Bachmann C, Barben P, et al. 1991. N-acetylglutamate synthetase deficiency: diagnosis, management and follow-up of a rare disorder of ammonia detoxication. *Eur J Pediatr* 150(5):353-356.
- *Scurkes JAAR, Maenen MMJ, Roelofs JGM. 1988. Chemical characteristics of precipitation in NH₃-affected areas. *Atmos Environ* 22(8):1689-1698.
- *Seegal BC. 1927. Chronic acidosis in rabbits and in dogs. *Arch Intern Med* 39:550-563.
- Seiler N. 1993. Is ammonia a pathogenetic factor in Alzheimer's disease? *Neurochem Res* 18(3):235-245.
- *Seiler N. 2002. Review: Ammonia and Alzheimer's disease. *Neurochem Int* 41:189-207.
- Seiler N, Sarhan S, Knoedgen B, et al. 1993. Enhanced endogenous ornithine concentrations protect against tonic seizures and coma in acute ammonia intoxication. *Pharmacol Toxicol* 72(2):116-123.
- *Seitzinger SP. 1987. Nitrogen biogeochemistry in an unpolluted estuary: The importance of benthic denitrification. *Mar Ecol Prog Ser* 41:177-186.
- *Sekizawa SI, Tsubone H. 1994. Nasal receptors responding to noxious chemical irritants. *Respir Physiol* 96(1):37-48.
- Semama DS, Huet F, Gouyon JB, et al. 1995. Use of peritoneal dialysis, continuous arteriovenous hemofiltration, and continuous arteriovenous hemodiafiltration for removal of ammonium chloride and glutamine in rabbits. *J Pediatr* 126:742-746.
- Seo Y, Murakami M. 1991. Monitoring of intracellular ammonium in perfused rat salivary gland by nitrogen-14 nuclear magnetic resonance spectroscopy. *Proc R Soc London, Ser B* 244(1311):191-196.
- Sharara FI, Seifer DB, Flaws JA. 1998. Environmental toxicants and female reproduction. *Fertil Steril* 70(4):613-622.
- Shibata M-A, Tamano S, Kurata Y, et al. 1989. Participation of urinary sodium potassium and L-ascorbic acid in the proliferative response of the bladder epithelium after the oral administration of various salts and-or ascorbic acid to rats. *Food Chem Toxicol* 27(6):403-413.

9. REFERENCES

- *Shimkin MB, de Lorimier AA, Mitchell JR, et al. 1954. Appearance of carcinoma following single exposure to a refrigeration ammonia-oil mixture. *Arch Ind Hyg Occup Med* 9:186-193.
- Shitara Y, Kato Y, Sugiyama Y. 1998. Effect of brefeldin A and lysosomotropic reagents on intracellular trafficking of epidermal growth factor and transferrin in Madin-Darby canine kidney epithelial cells. *J Controlled Release* 55:35-43.
- *Sine C, ed 1987. *Farm chemicals handbook*. Willoughby, OH: Meister Publishing Co., B16-B19.
- Silver SD, McGrath FP. 1948. A comparison of acute toxicities of ethylene imine and ammonia to mice. *J Ind Hyg Toxicol* 30:7-9.
- *Silverman L, Whittenberger JL, Muller J. 1949. Physiological response of man to ammonia in low concentrations. *J Ind Hyg Toxicol* 31:74-78.
- *Sjöblom E, Hojer J, Kulling PE, et al. 1999. A placebo-controlled experimental study of steroid inhalation therapy in ammonia-induced lung injury. *J Toxicol Clin Toxicol* 37(1):59-67.
- *Sloan CH, Morie GP. 1974. Determination of ammonia in tobacco and tobacco smoke with an ammonia electrode. *Anal Chim Acta* 69:243-247.
- *Slot GMJ. 1938. Electrocardiograms in 80 cases of angina of effort. *Lancet* 2:1356-1357.
- Smith LG, Busch RH, Buschbom RL, et al. 1989. Effects of sulfur dioxide or ammonium sulfate exposure, alone or combined, for 4 or 8 months on normal and elastase-impaired rats. *Environ Res* 49(1):60-78.
- *Snodgrass WJ, Ng PS. 1985. Biochemical models for the hypolimnetic oxygen depletion in lakes impacted by wastewater discharges 1. Alternative models. *Arch Hydrobiol Suppl* 72:81-109.
- Soar JB, Buttery PJ, Lewis D. 1973. Ammonia toxicity in sheep. *Proc Nutr Soc* 32:77A-78A.
- *Sobel RA, DeArmond SJ, Forno LS, et al. 1981. Glial fibrillary acidic protein in hepatic encephalopathy. *J Neuropathol Exp Neurol* 40:625-632.
- *Sobonya R. 1977. Fatal anhydrous ammonia inhalation. *Hum Pathol* 8:293-299.
- *Socolow RH. 1999. Nitrogen management and the future of food: Lessons from the management of energy and carbon. *Proc Natl Acad Sci USA* 96:6001-6008.
- Sorial GA, Smith FL, Suidan MT, et al. 2001. Removal of ammonia from contaminated air by trickle bed air biofilters. *J Air Waste Manage Assoc* 51(5):756-765.
- Sotiropoulos G, Kilagbhan T, Dougherty W, et al. 1998. Cold injury from pressurized liquid ammonia: a report of two cases. *J Emerg Med* 16(3):409-412.
- Spengler JD, Koutrakis P, Dockery DW, et al. 1996. Health effects of acid aerosols on North American children: Air pollution exposures. *Environ Health Perspect* 104(5):492-499.
- *Sprenger U, Bachmann K. 1987. Determination of ammonium in aerosols, cloud and rain water, and of gaseous ammonia in the troposphere. *Fresenius Z Anal Chem* 327:16.

9. REFERENCES

- *SRI. 1988. Directory of chemical producers USA. Menlo Park, CA: SRI International, 457-465.
- *SRI. 2000. Directory of chemical producers USA. Menlo Park, CA: SRI International, 457-463.
- *Stabenau JR, Warren KS, Rall DP. 1958. The role of pH gradient in the distribution of ammonia between blood and cerebro-spinal fluid, brain and muscle. *J Clin Invest* 38:373-383.
- Steppuhn H, Coxworth E, Kernan JA, et al. 1994. Response of *Kochia scoparia* to nitrogen fertilization on a saline soil. *Can J Soil Sci* 74(3):267-275.
- *Stombaugh DP, Teague HS, Roller WL. 1969. Effects of atmospheric ammonia on the pig. *J Anim Sci* 28:844-847.
- *Strogies M, Kallweit D. 1996. Nitrogen emissions in Germany and potential for their reduction. In: Sutton MA, Lee DS, Dollard GJ, et al., eds. *Atmospheric ammonia: Emission deposition and environmental impacts: Poster proceedings*. Midlothian, UK: Institute of Terrestrial Ecology, 53-56.
- *Stroud S. 1981. Ammonia inhalation-A case report. *Crit Care Nurse* 1:23-26.
- Stumpe JM, Vlek PLG. 1991. Acidification induced by different nitrogen sources in columns of selected tropical soils. *Soil Sci Soc Am J* 55(1):145-151.
- *Stupfel M, Romary F, Magnier M, et al. 1971. [Comparative acute toxicity of several atmospheric pollutants, in male and female mice: Automobile exhaust gases, nitrogen oxides, sulfur dioxide, ozone, ammonia and carbon dioxide.] *C R Seances Soc Biol Fil* 19:1869-1872. (French)
- *Suárez I, Bodega G, Arilla E, et al. 1992. Different response of astrocytes and Bergmann glial cells to portacaval shunt. An immunohistochemical study in the rat cerebellum. *Glia* 6:172-179.
- Suárez I, Bodega G, Fernandez B. 2002. Glutamine synthetase in brain: effect of ammonia. *Neurochem Int* 41:123-142.
- *Suh HH, Spengler JD, Koutrakis P. 1992. Personal exposures to acid aerosols and ammonia. *Environ Sci Technol* 26(12):2507-2517.
- *Summerskill WHJ, Wolpert E. 1978. Ammonia metabolism in the gut. *Am J Clin Nutr* 23:633-639.
- *Sunesson AL, Gullberg J, Blomquist G. 2001. Airborne chemical compounds on dairy farms. *J Environ Monitor* 3(2):210-216.
- Suzuki H, Yanaka A, Nakahara A, et al. 2000. The mechanism of gastric mucosal apoptosis induced by ammonia. *Gastroenterology* 118(Suppl 2):AGA A734.
- Swotinsky RB, Chase KH. 1990. Health effects of exposure to ammonia: Scant information. *Am J Ind Med* 17(4):515-521.
- Takagaki G, Berl S, Clarke DD, et al. 1961. Glutamic acid metabolism in brain and liver during infusion with ammonia labelled with nitrogen-15. *Nature* 189:326.

9. REFERENCES

- *Takahashi H, Koehler RC, Brusilow SW, et al. 1991. Inhibition of brain glutamine accumulation prevents cerebral edema in hyperammonemic rats. *Am J Physiol* 261:H825-H829.
- *Takeuchi K, Ohuchi T, Harada H, et al. 1995. Irritant and protective action of urea-urease ammonia in rat gastric mucosa. *Dig Dis Sci* 40(2):274-281.
- Tannenbaum SR, Young VR. 1980. Endogenous nitrite formation in man. *J Environ Pathol Toxicol* 3:357-368.
- *Tanner RL. 1982. An ambient experimental study of phase equilibrium in the atmospheric system: aerosol H^+ , NH_4^+ , SO_4^{2-} , $NO_3^-NH_3(g)$, $HNO_3(g)$. *Atmos Environ* 16(12):2935-2942.
- *Taplin GV, Chopra S, Yanda RL, et al. 1976. Radionuclidic lung-imaging procedures in the assessment of injury due to ammonia inhalation. *Chest* 69:582-586.
- *Targowski SP, Klucinski W, Babiker S, et al. 1984. Effect of ammonia on in vivo and in vitro immune response. *Infect Immun* 43(1):289-293.
- Tarr MA, Wang W, Bianchi TS, et al. 2001. Mechanisms of ammonia and amino acid photoproduction from aquatic humic and colloidal matter. *Water Res* 35(15):3688-3696.
- *Tepper A, Constock GW, Levine M. 1991. A longitudinal study of pulmonary function in fire fighters. *Am J Ind Med* 20(3):307-316.
- *Tepper JS, Weiss B, Wood RW. 1985. Alterations in behavior produced by inhaled ozone or ammonia. *Fundam Appl Toxicol* 5:1110-1118.
- Thomas RC. 1974. Intracellular pH of snail neurons measured with a new pH-sensitive glass micro-electrode. *J Physiol* 238:159-180.
- Thomson CJ, Marschner H, Roemheld V. 1993. Effect of nitrogen fertilizer form on pH of the bulk soil and rhizosphere and on the growth, phosphorus, and micronutrient uptake of bean. *J Plant Nutr* 16(3):493-506.
- Thornton FC, Bock BR, Tyler DD. 1996. Soil emissions of nitric oxide and nitrous oxide from injected anhydrous ammonium and urea. *J Environ Qual* 25:1378-1384.
- *Tian G, Cai Z, Cao J, et al. 2001. Factors affecting ammonia volatilisation from a rice-wheat rotation system. *Chemosphere* 42(2):123-129.
- *Tietz NW, ed. 1970. *Fundamentals of clinical chemistry*. Philadelphia, PA: W.B. Saunders Company.
- Tonkiss J, Trzcinska M, Galler JR, et al. 1998. Prenatal malnutrition-induced changes in blood pressure: dissociation of stress and nonstress responses using radiotelemetry. *Hypertension* 32(1):108-114.
- Topping DC, Visek WJ. 1976. Nitrogen intake and tumorigenesis in rats injected with 1,2-dimethylhydrazine. *J Nutr* 106:1583-1590.
- Torda C. 1952. Ammonium ion content and electrical activity of the brain during the preconvulsive and convulsive phases induced by various convulsants. *J Pharmacol Exp Ther* 197:197-203.

9. REFERENCES

Torres VE, Keith DS, Offord KP, et al. 1994. Renal ammonia in autosomal dominant polycystic kidney disease. *Kidney Int* 45(6):1745-1753.

*Toth B. 1972. Hydrazine, methylhydrazine and methylhydrazine sulfate carcinogenesis swiss mice. Failure of ammonium hydroxide to interfere in the development of tumors. *Int J Cancer* 9:109-118.

*TRI 01. 2003. Toxic Chemical Release Inventory. National Library of Medicine, National Toxicology Information Program, Bethesda, MD.

*Tsibulski V, Yatsenko-Khmelevskaya M, Milyaev V, et al. 1996. Ammonia emissions in Russia. In: Sutton MA, Lee DS, Dollard GJ, et al., eds. *Atmospheric ammonia: Emission deposition and environmental impacts: Poster proceedings*. Midlothian, UK: Institute of Terrestrial Ecology, 50-52.

Tsujii M, Kawano S, Tsuji S, et al. 1992a. Ammonia: A possible promotor in *Helicobacter pylori*-related gastric carcinogenesis. *Cancer Lett* 65(1):15-18.

*Tsujii M, Kawano S, Tsuji S, et al. 1992b. Mechanism of gastric mucosal damage induced by ammonia. *Gastroenterology* 102(6):1881-1888.

*Tsujii M, Kawano S, Tsuji S, et al. 1993. Cell kinetics of mucosal atrophy in rat stomach induced by long-term administration of ammonia. *Gastroenterology* 104(3):796-801.

*Tsujii M, Kawano S, Tsuji S, et al. 1995. Mechanism for ammonia-induced promotion of gastric carcinogenesis in rats. *Carcinogenesis* 16(3):563-566.

Turtureanu M. 1994. Ammonia production. In: Hodge CA, Popovici NN, eds. *Pollution control in fertilizer production*. New York, NY: Marcel Dekker, Inc, 35-50.

*Tuskes PM, Tilton MA, Graff RM. 1988. Ammonia exposures of blue-line printers in Houston, Texas. *Appl Ind Hyg* 3:155-157.

*Tyor MP, Owen EE, Berry JN, et al. 1960. The relative role of extremity, liver, and kidney as ammonia receivers and donors in patients with liver disease. *Gastroenterology* 39:420-424.

*Urbain B, Gustin P, Prouvost JF, et al. 1994. Quantitative assessment of aerial ammonia toxicity to the nasal mucosa by use of the nasal lavage method in pigs. *Am J Vet Res* 55(9):1335-1340.

*U.S. Army Corps of Engineers. 1980. *Wastewater treatment in cold regions by overland flow*. Washington, DC: U.S. Army Corps of Engineers.

*USC. 2002a. Assurances of availability of adequate supplies of chemicals necessary for treatment of water. United States Code. 42 USC 300j. <http://www4.law.cornell.edu/uscode/42/300j.html>. May 02, 2002.

*USC. 2002b. Definitions and special rules. United States Code. 26 USC 4662. <http://www4.law.cornell.edu/uscode/26/4662.html>. May 02, 2002.

*USC. 2002c. Definitions and special rules. United States Code. 26 USC 4672. <http://www4.law.cornell.edu/uscode/26/4672.html>. May 02, 2002.

9. REFERENCES

- *USC. 2002d. Imposition of tax. United States Code. 26 USC 4661. <http://www4.law.cornell.edu/uscode/26/4661.html>. May 02, 2002.
- *Utell MJ, Mariglio JA, Morrow PE, et al. 1989. Effects of inhaled acid aerosols on respiratory function: The role of endogenous ammonia. *J Aerosol Med* 2:141-147.
- *Uzvolgyi E, Bojan F. 1980. Possible in vivo formation of a carcinogenic substance from diethyl pyrocarbonate and ammonia. *J Cancer Res Clin Oncol* 97:205-207.
- *Uzvolgyi E, Bojan F. 1985. In vivo formation of a carcinogenic substance from diethyl pyrocarbonate in the presence of ammonia. *Arch Toxicol Suppl* 8:490-493.
- Valdes-Solis T, Marban G, Fuertes AB. 2001. Low-temperature SCR of NO_x with NH₃ over carbon-ceramic cellular monolith-supported manganese oxides. *Catal Today* 69(1-4):259-264.
- van Dongen U, Jetten MSM, van Loosdrecht MCM. 2001. The SHARON-Anammox process for treatment of ammonium rich wastewater. *Water Sci Technol* 44(1):153-160.
- *Van Hoesen SD, Dreibelbis WG. 1984. University of Minnesota-Duluth low-BTU gasifier workplace environment. In: Cowser KE, Jacobs VE, eds. *Proceedings of the 5th life science symposium: Synthetic fossil fuel technologies*. Boston, MA: Butterworth, 149-184.
- *Van Slyke DD, Phillips RA, Hamilton PB, et al. 1943. Glutamine as source material of urinary ammonia. *J Biol Chem* 150:481-482.
- *Verberk MM. 1977. Effects of ammonia in volunteers. *Int Arch Occup Environ Health* 39:73-81.
- *Verschueren K. 1983. *Handbook of environmental data on organic chemicals*. 2nd ed. New York, NY: Van Nostrand Reinhold Company, 194.
- Vernot EH, MacEwen JD, Haun CC, et al. 1977. Acute toxicity and skin corrosion data for some organic and inorganic compounds and aqueous solutions. *Toxicol Appl Pharmacol* 42:417-423.
- Visek WJ. 1984. Ammonia: Its effects on biological systems, metabolic hormones, and reproduction. *J Dairy Sci* 67(3):481-498.
- *Visek WJ, Kolodny GM, Gross PR. 1972. Ammonia effects in cultures of normal and transformed 3T3 cells. *J Cell Physiol* 1972:373-381.
- *Vitti TG, Vukmirovich R, Gaebler OH. 1964. Utilization of ammonia nitrogen, administered by intragastric, intraperitoneal, and subcutaneous routes: Effects of growth hormone. *Arch Biochem Biophys* 106:475-482.
- *Vogelzang PFJ, van der Gulden JWJ, Folgering H, et al. 2000. Longitudinal changes in bronchial responsiveness associated with swine confinement dust exposure. *Chest* 117(5):1488-1495.
- *Vogelzang PFJ, van der Gulden JWJ, Preller L, et al. 1997. Bronchial hyperresponsiveness and exposure in pig farmers. *Int Arch Occup Environ Health* 70(5):327-333.
- *Voisin C, Guerrin H, Furon D, et al. 1970. Sequelles fonctionnelles respiratoires des intoxications par l'ammoniac (a propos de 8 obserbations). *Le Poumon et le Coeur* 26:1079-1095.

9. REFERENCES

- Walker CO, Schienker S. 1970. Pathogenesis of hepatic encephalopathy- with special reference to the role of ammonia. *Am J Clin Nutr* 23(5):619-632.
- *Walton M. 1973. Industrial ammonia gassing. *Br J Ind Med* 30:78-86.
- *Wands RC. 1981. Alkaline materials. In: Clayton GD, Clayton FE, eds. *Patty's industrial hygiene and toxicology*, Vol. II, 4th ed. New York, NY: John Wiley & Sons, Inc., 3045-3069.
- *Ward K, Costello GP, Murray B. 1983. Acute and long-term pulmonary sequelae of acute ammonia inhalation. *Ir Med J* 76(6):279-281.
- Warland JS, Dias GM, Thurtell GW. 2001. A tunable diode laser system for ammonia flux measurements over multiple plots. *Environ Pollut* 114(2):215-221.
- Warren KS. 1958. The differential toxicity of ammonia salts. *J Clin Invest* 37:497-501.
- Warren KS, Nathan DG. 1958. The passage of ammonia across the blood-brain-barrier and its relation to blood pH. *J Clin Invest* 37:1724-1728.
- *Warren KS, Schenker S. 1964. Effect of inhibitor glutamine synthesis (methionine sulfoximine) on ammonia toxicity and metabolism. *J Lab Clin Med* 64(3):442-449.
- Wason S, Stephan M, Breide C. 1990. Ingestion of aromatic ammonia smelling salts capsules. *Am J Dis Child* 144(2):139-140.
- Weast RC, Astle MJ, Beyer WH, eds. 1983. *CRC handbook of chemistry and physics*. Boca Raton, FL: CRC Press, Inc., B-69, D-163, D-222-223.
- *Weast RC, Astle MJ, Beyer WH, eds. 1988. *CRC Handbook of chemistry and physics*. Boca Raton, Florida: CRC Press, Inc.
- *Weatherby JH. 1952. Chronic toxicity of ammonia fumes by inhalation. *Proc Soc Exp Biol Med* 81:300-301.
- *Weber FL, Veach GL. 1979. The importance of the small intestine in gut ammonium production in the fasting dog. *Gastroenterology* 77:235-240.
- *Weedon FR, Hartzell A, Setterstrom C. 1940. Toxicity of ammonia, chlorine, hydrogen cyanide, hydrogen sulphide, and sulphur dioxide gases. V. Animals. *Contrib Boyce Thompson Inst* 11:365-385.
- *Weir DC, Robertson AS, Jones S, et al. 1989. Occupational asthma due to soft corrosive soldering fluxes containing zinc chloride and ammonium chloride. *Thorax* 44(3):220-223.
- *Weiser JR, Mackenroth T. 1989. Acute inhalatory mass ammonia intoxication with fatal course. *Exp Pathol* 37(1-4):291-295.
- Weisskopf MG, Drew JM, Hanrahan LM, et al. 2003. Hazardous ammonia releases: Public health consequences and risk factors for evacuation and injury, United States, 1993-1998. *J Occup Environ Med* 45:197-204.

9. REFERENCES

- *West JR, Smith HW, Chasis H. 1948. Glomerular filtration rate, effective renal blood flow, and maximal tubular excretory capacity in infancy. *J Pediatr* 32:10-18.
- Westra HG, van Doorn JE, Tigchelaar RG. 2001. A method for the determination of volatile ammonia in air, using a nitrogen-cooled trap and fluorometric detection. *Anal Biochem* 296(2):225-231.
- *White ES. 1971. A case of near fatal ammonia gas poisoning. *J Occup Med* 13:543-550.
- *WHO. 1986. Ammonia. Environmental Health Criteria. Vol. 54. Geneva, Switzerland: World Health Organization.
- *WHO. 2002. Guidelines for drinking water. World Health Organization.
http://www.who.int/water_sanitation_health/GDWQ/Chemicals/ammonsum.htm. May 02, 2002.
- *Wibbenmeyer LA, Morgan LJ, Robinson BK, et al. 1999. Our chemical burn experience: Exposing the dangers of anhydrous ammonia. *J Burn Care Rehab* 20(3):226-231.
- *Wicherski B. 2000. Groundwater quality investigation and wellhead protection study. City of Fruitland, Idaho. Boise, Idaho: Idaho Department of Environmental Quality. Technical Services Division.
- Wieslander G, Norback D, Edling C. 1994. Occupational exposure to water based paint and symptoms from the skin and eyes. *Occup Environ Med* 51(3):181-186.
- *Wilkin DC, Flemal RC. 1980. Feasibility of water quality improvement in three Illinois rivers. *J Water Pollut Control Fed* 52(2):293-298.
- Williams PI, Gallagher MW, Choularton TW, et al. 2000. Aerosol development and interaction in an urban plume. *Aerosol Sci Technol* 32(2):120-126.
- Wilson RP, Muhrer ME, Bloomfield RA. 1968. Comparative ammonia toxicity. *Comp Biochem Physiol* 25:295-301.
- *Windholz M, Budavari S, Blumetti RF, et al., eds. 1983. The Merck index, 10th ed. Rahway, NJ: Merck & Co., Inc.
- *Withers J, Ten Berge W, Gordon J, et al. 1986. The lethal toxicity of ammonia: A report to the MHAP. *Northwestern Branch Papers* 1986 1:6.1-6.27.
- Wood CW, Marshall SB, Cabera ML. 2000. Improved method for field-scale measurement of ammonia volatilization. *Commun Soil Sci Plant Anal* 31(5-6):581-590.
- Wood RW. 1979. Behavioral evaluation of sensory irritation evoked by ammonia. *Toxicol Appl Pharmacol* 50:157-162.
- Worcel A, Erecinska M. 1962. Mechanism of inhibitory action of ammonia on the respiration of rat-liver mitochondria. *Biochim Biophys Acta* 65:27-33.
- Wrenn C. 2000. Real-time measurement of ammonia gas. *Occup Health Safe* 69(12):64-67.

9. REFERENCES

- Wu G, Pond WG, Ott T, et al. 1998. Maternal dietary protein deficiency decreases amino acid concentrations in fetal plasma and allantoic fluid of pigs. *J Nutr* 128(5):894-902.
- Wysmyk U, Oka SS, Saransaari P, et al. 1994. Long-term treatment with ammonia effects the content and release of taurine in cultured cerebellar astrocytes and granule neurons. *Neurochem Int* 24(4):317-322.
- *Yadav JS, Kaushik VK. 1997. Genotoxic effect of ammonia exposure on workers in a fertility factory. *Indian J Exp Biol* 35(5):487-492.
- *Yang GY, Tominack RL, Deng JF. 1987. An industrial mass ammonia exposure. *Vet Hum Toxicol* 29:476-477.
- Yeldandi AV, Tan X, Dwivedi RS, et al. 1990. Coexpression of glutamine synthetase and carbamoylphosphate synthase I genes in pancreatic hepatocytes of rat. *Proc Natl Acad Sci U S A* 87(3):881-885.
- *Yoo KP, Lee SH, Lee WH. 1986. Ionization and Henry's law constants for volatile, weak electrolyte water pollutants. *Korean J Chem Eng* 3(1):67-72.
- Zago C, Capodaglio G, Ceradini S, et al. 2000. Benthic fluxes of cadmium, lead, copper and nitrogen species in the northern Adriatic Sea in front of the River Po outflow, Italy. *Sci Total Environ* 246(2-3):121-137.
- Zange J, Gronczewski J, Jans AW. 1993. NH_4^+ metabolism and the intracellular pH in isolated perfused rat liver. *Biochem J* 293:667-673.
- Zeida JE, Barber E, Dosman JA, et al. 1994. Respiratory health status in swine producers relates to endotoxin exposure in the presence of low dust levels. *J Occup Med* 36(1):49-56.
- *Ziegler EE, Edwards BB, Jensen RL, et al. 1978. Absorption and retention of lead by infants. *Pediatr Res* 12:29-34.
- Zielinska M, Hilgier W, Law RO, et al. 1999. Effects of ammonia in vitro on endogenous taurine efflux and cell volume in rat cerebrocortical minislices: Influence of inhibitors of volume-sensitive amino acid transport. *Neuroscience* 91(2):631-638.
- Zielinska M, Law RO, Albrecht J. 2003. Excitotoxic mechanism of cell swelling in rat cerebral cortical slices treated acutely with ammonia. *Neurochem Int* 43:299-303.
- *Zieve L, Doiaki WM, Zieve FJ. 1974. Synergism between mercaptans and ammonia or fatty acids in the production of a coma: A possible role for mercaptans in the pathogenesis of hepatic coma. *J Lab Clin Med* 83:16-28.
- *Zimber A, Visek WJ. 1972a. Effect of urease injections on DNA synthesis in mice. *Am J Physiol* 233:1004-1008.
- *Zimber A, Visek WJ. 1972b. Effect of urease injections on ehrlich ascites tumor growth in mice. *Proc Soc Exp Biol Med* 139:43-49.

9. REFERENCES

*Zissu D. 1995. Histopathological changes in the respiratory tract of mice exposed to ten families of airborne chemicals. *J Appl Toxicol* 15:207-213.

10. GLOSSARY

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

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

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

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

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

Benchmark Dose (BMD)—Usually defined as the lower confidence limit on the dose that produces a specified magnitude of changes in a specified adverse response. For example, a BMD10 would be the dose at the 95% lower confidence limit on a 10% response, and the benchmark response (BMR) would be 10%. The BMD is determined by modeling the dose response curve in the region of the dose response relationship where biologically observable data are feasible.

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

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

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

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

Carcinogen—A chemical capable of inducing cancer.

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

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

10. GLOSSARY

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

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

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

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

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

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

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

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

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

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

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

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

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

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

10. GLOSSARY

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

Immunological Effects—Functional changes in the immune response.

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

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

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

In Vivo—Occurring within the living organism.

Lethal Concentration(Lo) (LC_{Lo})—The lowest concentration of a chemical in air that has been reported to have caused death in humans or animals.

Lethal Concentration(50) (LC₅₀)—A calculated concentration of a chemical in air to which exposure for a specific length of time is expected to cause death in 50% of a defined experimental animal population.

Lethal Dose(Lo) (LD_{Lo})—The lowest dose of a chemical introduced by a route other than inhalation that has been reported to have caused death in humans or animals.

Lethal Dose(50) (LD₅₀)—The dose of a chemical that has been calculated to cause death in 50% of a defined experimental animal population.

Lethal Time(50) (LT₅₀)—A calculated period of time within which a specific concentration of a chemical is expected to cause death in 50% of a defined experimental animal population.

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

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

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

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

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

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

10. GLOSSARY

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

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

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

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

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

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

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

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

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

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

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

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

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

10. GLOSSARY

Physiologically Based Pharmacokinetic (PBPK) Model—Comprised of a series of compartments representing organs or tissue groups with realistic weights and blood flows. These models require a variety of physiological information: tissue volumes, blood flow rates to tissues, cardiac output, alveolar ventilation rates, and possibly membrane permeabilities. The models also utilize biochemical information, such as air/blood partition coefficients, and metabolic parameters. PBPK models are also called biologically based tissue dosimetry models.

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

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

q₁*—The upper-bound estimate of the low-dose slope of the dose-response curve as determined by the multistage procedure. The q₁* can be used to calculate an estimate of carcinogenic potency, the incremental excess cancer risk per unit of exposure (usually µg/L for water, mg/kg/day for food, and µg/m³ for air).

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

Reference Concentration (RfC)—An estimate (with uncertainty spanning perhaps an order of magnitude) of a continuous inhalation exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious noncancer health effects during a lifetime. The inhalation reference concentration is for continuous inhalation exposures and is appropriately expressed in units of mg/m³ or ppm.

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

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

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

10. GLOSSARY

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

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

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

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

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

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

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

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

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

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

Toxic Dose(50) (TD50)—A calculated dose of a chemical, introduced by a route other than inhalation, which is expected to cause a specific toxic effect in 50% of a defined experimental animal population.

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

10. GLOSSARY

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

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

APPENDIX A. ATSDR MINIMAL RISK LEVELS AND WORKSHEETS

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

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

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

APPENDIX A

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

Proposed MRLs undergo a rigorous review process: Health Effects/MRL Workgroup reviews within the Division of Toxicology, expert panel peer reviews, and agency-wide MRL Workgroup reviews, with participation from other federal agencies and comments from the public. They are subject to change as new information becomes available concomitant with updating the toxicological profiles. Thus, MRLs in the most recent toxicological profiles supersede previously published levels. For additional information regarding MRLs, please contact the Division of Toxicology, Agency for Toxic Substances and Disease Registry, 1600 Clifton Road NE, Mailstop F-32, Atlanta, Georgia 30333.

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: Ammonia
CAS Number: 7664-41-7
Date: July 2004
Profile Status: Third Draft Post-Public
Route: ☒ Inhalation ☐ Oral
Duration: ☒ Acute ☐ Intermediate ☐ Chronic
Graph Key: 14
Species: Human

Minimal Risk Level: 1.7 ☐ mg/kg/day ☒ ppm

Reference: Verberk MM. 1977. Effects of ammonia in volunteers. Int Arch Occup Environ Health 39:73-81.

Experimental design: The study examined the effects of exposure to ammonia in a group of 16 volunteers. Eight of them (experts) knew the effects of ammonia from the literature, but had had no personal contact, whereas the remaining eight subjects (non-experts) were students from a non-science faculty and were not familiar with ammonia or experiments in laboratory situations. All members of a group were exposed on the same day to one of the concentrations tested (50, 80, 110, or 140 ppm). The testing was repeated with a 1-week interval. Immediately before and after exposure, vital capacity, forced expiratory volume, and forced inspiratory volume were measured. During exposure, each subject recorded subjective feelings every 15 minutes as no sensation (0), just perceptible (1), distinctly perceptible (2), nuisance (3), offensive (4), or unbearable (5). No statistical analysis was performed and there was no group exposed to air only. A few weeks after the experiments, the subjects were tested to measure (pre-existing) non-specific reactivity of the airways to exogenous stimuli.

Effects noted in study and corresponding doses: None of the participants was hypersusceptible to non-specific irritants. Results of the pulmonary function tests after exposure were not statistically significantly different from pre-exposure values. For the non-experts, there was a clear increase in the number of reported symptoms for smell, eye irritation, throat irritation, cough, and general discomfort as the exposure concentration increased. The latter was not as clear for the experts. The number of symptoms recorded with a score >3 (nuisance) for smell, eye irritation, nose, throat, and urge to cough for the 50, 80, 110, and 140 ppm exposure groups was 2, 2, 7, and 11, respectively, for the experts and 6, 12, 18, and 29, respectively, for the non-experts. It should also be mentioned that the subjective responses appeared more pronounced in the non-expert group than in the expert group.

Dose and end point used for MRL derivation: 50 ppm for mild irritation to the eyes, nose, and throat in humans exposed to ammonia gas for 2 hours.

Because the effects observed were local irritation effects, they were not time-dependent (but rather concentration-dependent), an adjustment to 24-hour exposure was not necessary.

☐ NOAEL ☒ LOAEL

APPENDIX A

Uncertainty Factors used in MRL derivation:

- ☒ 3 for use of a minimal LOAEL
- ☐ 10 for extrapolation from animals to humans
- ☒ 10 for human variability

Was a conversion used from ppm in food or water to a mg/body weight dose?

N/A

If an inhalation study in animals, list the conversion factors used in determining human equivalent dose:

N/A

Other additional studies or pertinent information which lend support to this MRL: Although the Verberk et al. (1977) study has limitations (no statistical analysis, subjective end points, no control group), it demonstrates that concentrations of 50 ppm ammonia can produce minimal discomfort in healthy members of the general population and therefore, should be avoided. Additional relevant information is provided by a study by Ferguson et al. (1977). In that study, a group of six healthy volunteers, not previously accustomed to working in an ammonia environment, were exposed 5 days/week to 25 ppm (2 hours/day), 50 ppm (4 hours/day), or 100 ppm (6 hours/day) of ammonia, or to 50 ppm of ammonia 6 hours/day for 6 weeks. End points monitored included subjective and objective measures of eye and throat irritation as well as pulse rate, respiration rate, pulmonary function (FVC, FEV), assessment of neurological function (reflex, balance, and coordination), and body weight. The exposure protocol consisted of a pre-exposure evaluation by a physician, 3 hours of exposure (this conflicts with exposure data on table 2 of the study and mentioned above), a mid-point physician's observation, lunch break, 3 additional hours of exposure, and a third physician's observation 30 minutes after exposure ceased. The conjunctiva and mucosa of the nose and throat were examined by a physician before and after each daily exposure and the degree of irritation noted was described as mild, moderate, or marked. Exposure to ammonia had no significant effect on the measures of respiratory function or in the neurological tests conducted. The results of the evaluations of irritation conducted by the physician showed no significant differences between the exposure groups, including the 0 ppm exposure group (pre-exposure). All subjects experienced some watering of the eyes and a sensation of dryness in the nose and throat and there was one observation of definite redness in the mucosa of the nose after a 6-hour exposure to 100 ppm during which time, there was an excursion to 200 ppm ammonia. No redness was observed in this subject the following morning. Throughout the study, the physician observed 6 cases of eye irritation, 20 of nose irritation, and 9 of throat irritation, and most cases appeared to have occurred the first week of the study during exposure to 50 ppm. It is difficult to determine in this study a NOAEL or LOAEL for irritation due to the different exposure durations experienced by the subjects, but it would appear that an exposure concentration of 100 ppm ammonia for 6 hours caused no significant changes in the vital functions measured and that 50 ppm can cause eye, nose, and throat irritation.

NIOSH (1974) reviewed 15 studies of case reports in which subjects were exposed to very high, but unquantified, concentrations of ammonia. The 15 reports provided a representative array of documented clinical findings including death, permanent eye lesions, and chronic respiratory symptoms, as well as acute lower and upper respiratory symptoms. The only quantitative information available was that a worker died 6 hours after estimated exposure to 10,000 ppm ammonia for an unspecified time (Mulder and Van der Zalm 1967). Studies with volunteers, also reviewed by NIOSH (1974), generally used concentrations of ammonia much higher than those in the studies by Verberk et al. (1977) or Ferguson et al. (1977) and/or exposure durations of only minutes. For example, exposure to a concentration of 500 ppm for 30 minutes caused respiratory irritation graded as severe by 2 out of 7 subjects (Silverman et al. 1949). Four out of 6 volunteers exposed to 50 ppm ammonia for 10 minutes graded the irritation as "moderate" and none described it as "discomforting" or "painful" (MacEwen et al. 1970). All of the

APPENDIX A

subjects rated the odor as “highly penetrating” at 50 ppm and 3 subjects gave the same rating to 30 ppm. IBT (1973) exposed 10 subjects to 32, 50, 72, and 134 ppm for 5 minutes and the frequency of positive findings was as follows: at 32 ppm, 1 subject complained of dryness of the nose; at 50 ppm, 2 subjects complained of dryness of the nose; at 72 ppm, 3 subjects experienced eye irritation, 2 had nasal irritation, and 3 had throat irritation; and at 134 ppm, 5 subjects had signs of lacrimation, 5 had eye irritation, 7 had nasal irritation, 8 had throat irritation, and 1 had chest irritation.

Collectively, the available information from studies in humans supports the 50 ppm exposure level from the Verberk et al. (1977) study as a minimal LOAEL for irritation in acute studies. In general, studies in animals have used higher exposure concentrations. For ammonia, a corrosive irritant gas that affects the portal of entry and produces irritation of the eyes and respiratory tract, use of human data should be preferred over animal studies.

Agency Contact (Chemical Manager): Nickolette Roney, MPH

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: Ammonia and Ammonium Compounds
CAS Number: 7664-41-7
Date: July 2004
Profile Status: Third Draft Post Public
Route: ☒ Inhalation ☐ Oral
Duration: ☐ Acute ☐ Intermediate ☒ Chronic
Graph Key: 47
Species: Humans

Minimal Risk Level: 0.1 ☐ mg/kg/day ☒ ppm

Reference: Holness DL, Purdham JT, Nethercott JR. 1989. Acute and chronic respiratory effects of occupational exposure to ammonia. Am Ind Hyg Assoc J 50:646-650.

Experimental design: The study evaluated sense of smell, prevalence of respiratory symptoms (cough, bronchitis, wheeze, dyspnea, and others), eye and throat irritation, and lung function parameters (FVC, FEV₁, FEV₁/FVC, FEF₅₀, and FEF₇₅) in humans exposed for an average of 12.2 years in a soda ash plant (Holness et al. 1989). The cohort consisted of 52 workers and 35 controls. The subjects were assessed on two workdays: on the first workday of their workweek and on the last workday of their workweek. Spirometry was performed at the beginning and end of each work shift, so that each worker had four tests done. To determine the exposure levels, exposed and control workers were sampled over one work shift; the average sample collection period was 8.4 hours. All of the participants in the study were males.

Effects noted in study and corresponding doses: Analysis of the results showed no significant differences in the prevalence of reported symptoms, but the exposed workers reported that exposure in the plant aggravated some of their reported symptoms (cough, wheeze, nasal complaints, eye irritation, and throat discomfort). Odor threshold was not affected by exposure to ammonia and there were no significant differences in baseline lung functions between exposed and control subjects. Analysis of each worker separately showed no significant relationship between the level of ammonia exposure and changes in lung function. Also, when the workers were divided into groups of individuals that were exposed to low (<6.25 ppm), medium (6.25–12.5 ppm), and high (>12.5 ppm) ammonia levels, no significant association was found between reporting of symptoms, decline in baseline function, or increasing decline in function over the work shift and exposure to ammonia. Furthermore, no association was evident between increasing years of exposure and decreasing lung function. However, the power of the indices of both level and length of exposure is low because only eight workers were in areas with relatively high ammonia exposure.

The MRL was calculated by adjusting the NOAEL of 9.2 ppm (the mean TWA exposure concentration) for continuous exposure (9.2 x 8/24 hours x 5/7 days) and dividing by an uncertainty factor of 10 for the protection of sensitive individuals. A modifying factor of 3 was used for the lack of reproductive and developmental studies.

Dose and end point used for MRL derivation: 9.2 ppm for no significant alterations in lung function in chronically exposed workers.

☒ NOAEL ☐ LOAEL

APPENDIX A

Uncertainty Factors used in MRL derivation:

- ☐ 10 for use of a LOAEL
- ☐ 10 for extrapolation from animals to humans
- ☒ 10 for human variability

Modifying Factors used in MRL derivation:

- ☒ 3 for lack of reproductive and developmental studies

Was a conversion used from ppm in food or water to a mg/body weight dose?

N/A.

If an inhalation study in animals, list the conversion factors used in determining human equivalent dose:

N/A.

Other additional studies or pertinent information which lend support to this MRL: Earlier studies summarized by NIOSH (1974) found that workers accustomed to 20 ppm ammonia did not complain of irritation symptoms, but showed slight redness in the conjunctiva. Those not accustomed had eye and respiratory discomfort and irritation. Another report stated that air levels below 5 ppm were associated with barely noticeable eye irritation. In yet an additional report, concentrations of 15–28 ppm in the work area produced slight eye irritation. More recent data reported respiratory effects associated with chronic-duration exposure to pollutants, including ammonia, in livestock confinement buildings and an increase in respiratory symptoms (such as bronchial reactivity/hyperresponsiveness, inflammation, cough, wheezing, or shortness of breath) and/or a decrease in lung function (such as forced expiratory volume in the first second [FEV_{1.0}], maximum expiratory flow rates [MEF₅₀ and MEF₇₅], and maximal mid-expiratory flow rate [MMEF]) in farmers exposed to ammonia levels of 2.3–20.7 ppm (Choudat et al. 1994; Cormier et al. 2000; Donham et al. 1995, 2000; Heederik et al. 1990; Reynolds et al. 1996; Vogelzang et al. 1997, 2000). The farmers were also exposed to other possible respiratory toxins, such as dust and endotoxins. A cross-sectional study of male workers at two fertilizer factories in Saudi Arabia showed a significant association between exposure to ammonia gas and respiratory symptoms and bronchial asthma (Ballal et al. 1998). No continuous exposure levels could be calculated for these workers because the number of days worked per week was not provided. There were no chronic-duration inhalation studies in animals.

Agency Contact (Chemical Manager): Nickolette Roney, MPH

APPENDIX B. USER'S GUIDE

Chapter 1

Public Health Statement

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

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

Chapter 2

Relevance to Public Health

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

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

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

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

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

Interpretation of Minimal Risk Levels

Where sufficient toxicologic information is available, ATSDR has derived MRLs for inhalation and oral routes of entry at each duration of exposure (acute, intermediate, and chronic). These MRLs are not

APPENDIX B

meant to support regulatory action, but to acquaint health professionals with exposure levels at which adverse health effects are not expected to occur in humans.

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

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

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

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

Chapter 3

Health Effects

Tables and Figures for Levels of Significant Exposure (LSE)

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

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

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

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

APPENDIX B

which was used to derive an intermediate exposure, inhalation MRL of 0.005 ppm (see footnote "b").

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

LEGEND

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

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

- (13) Exposure Period. The same exposure periods appear as in the LSE table. In this example, health effects observed within the acute and intermediate exposure periods are illustrated.
- (14) Health Effect. These are the categories of health effects for which reliable quantitative data exists. The same health effects appear in the LSE table.
- (15) Levels of Exposure. Concentrations or doses for each health effect in the LSE tables are graphically displayed in the LSE figures. Exposure concentration or dose is measured on the log scale "y" axis. Inhalation exposure is reported in mg/m³ or ppm and oral exposure is reported in mg/kg/day.
- (16) NOAEL. In this example, the open circle designated 18r identifies a NOAEL critical end point in the rat upon which an intermediate inhalation exposure MRL is based. The key number 18 corresponds to the entry in the LSE table. The dashed descending arrow indicates the extrapolation from the exposure level of 3 ppm (see entry 18 in the table) to the MRL of 0.005 ppm (see footnote "b" in the LSE table).
- (17) CEL. Key number 38m is one of three studies for which CELs were derived. The diamond symbol refers to a CEL for the test species-mouse. The number 38 corresponds to the entry in the LSE table.

APPENDIX B

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

SAMPLE

1 →

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

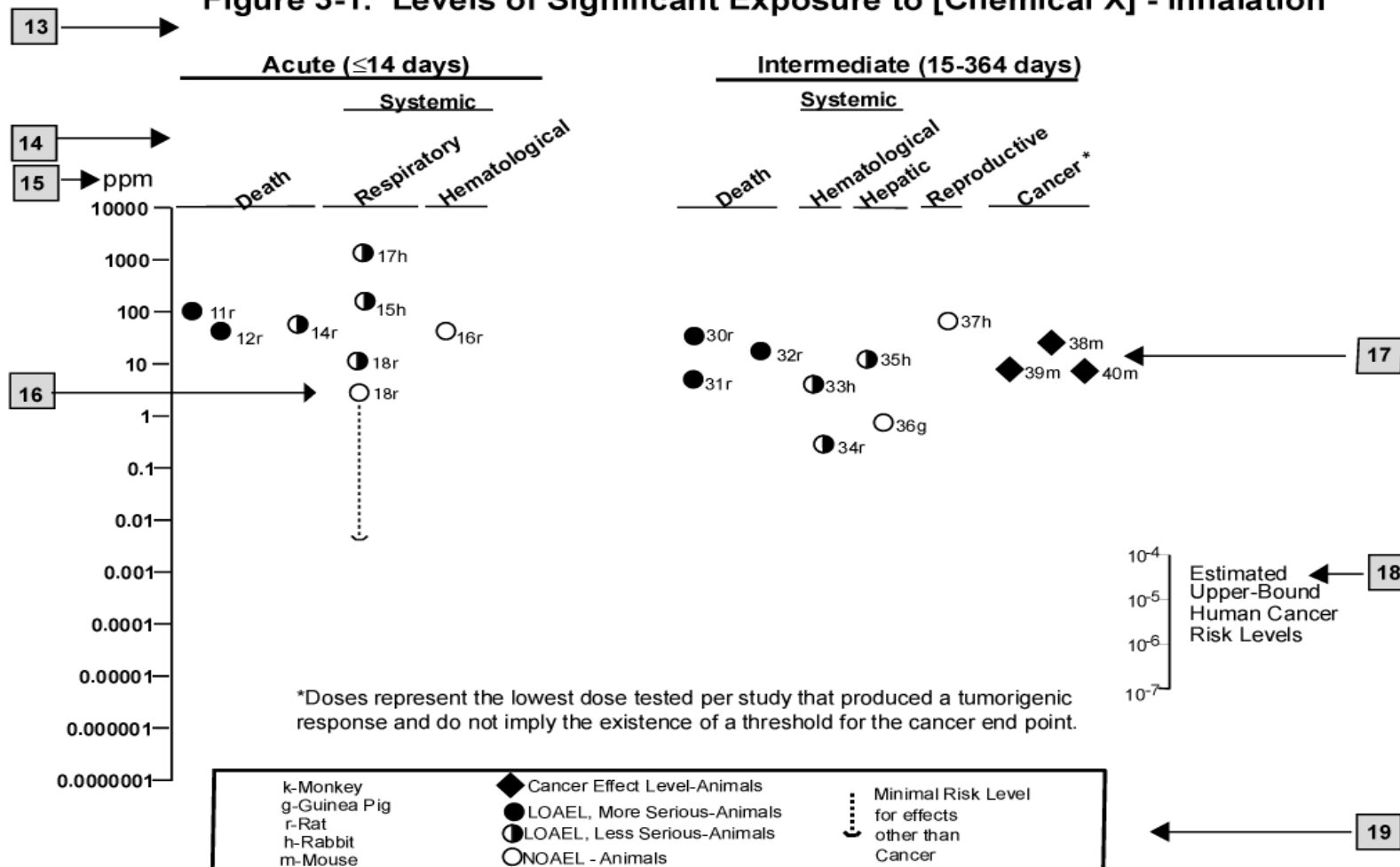
						LOAEL (effect)			
	Key to figure ^a	Species	Exposure frequency/ duration	System	NOAEL (ppm)	Less serious (ppm)	Serious (ppm)	Reference	
2	→	INTERMEDIATE EXPOSURE							
		5	6	7	8	9		10	
3	→	Systemic	↓	↓	↓	↓		↓	
4	→	18	Rat	13 wk 5 d/wk 6 hr/d	Resp	3 ^b	10 (hyperplasia)	Nitschke et al. 1981	
		CHRONIC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

12 →

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

SAMPLE

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



APPENDIX C. ACRONYMS, ABBREVIATIONS, AND SYMBOLS

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

APPENDIX C

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

APPENDIX C

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

APPENDIX C

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

APPENDIX C

$>$	greater than
$\geq$	greater than or equal to
$=$	equal to
$<$	less than
$\leq$	less than or equal to
$\%$	percent
α	alpha
β	beta
γ	gamma
δ	delta
μm	micrometer
μg	microgram
q_1^*	cancer slope factor
$-$	negative
$+$	positive
$(+)$	weakly positive result
$(-)$	weakly negative result

APPENDIX D. INDEX

absorbed dose	97
adenocarcinoma	49, 57
adrenal gland	21, 54, 67, 105
adsorption	14, 127, 135, 136
aerobic	137
anaerobic.....	126, 137
aspartate aminotransferase (see AST).....	68
AST (see aspartate aminotransferase).....	68
bioaccumulation	148
bioavailability	148
biomarker.....	96, 97, 98, 108, 109, 153, 160
blood cell count	53, 66
body weight effects.....	54, 67, 71
breast milk	7, 145
burns	4, 6, 7, 8, 15, 16, 25, 26, 48, 52, 55, 65, 66, 70, 71, 73, 75, 80, 90, 92, 95, 101, 102, 104, 107
cancer.....	6, 70, 94, 106
carcinogen.....	57, 70, 98
carcinogenic.....	6, 23, 24, 69, 98, 106, 108, 163
carcinogenicity	106
carcinoma	49, 56, 75
cardiac arrhythmia	52
cardiovascular	21, 52, 56, 57, 58, 71, 104
cardiovascular effects	52, 58, 71, 104
chromosomal aberrations.....	76, 106
death	6, 15, 17, 23, 25, 48, 58, 66, 73, 90, 102
deoxyribonucleic acid (see DNA).....	77
dermal effects	16, 17, 54, 73
DNA (see deoxyribonucleic acid).....	3, 15, 77, 78, 96
dyspnea	19, 47, 48, 49, 105, 163
endocrine	54, 67, 71, 92, 93
endocrine effects.....	54, 67
erythema	65, 74
fertilizer	2, 3, 4, 7, 13, 14, 46, 47, 76, 95, 105, 106, 108, 118, 122, 126, 127, 131, 132, 133, 139, 140, 141, 143, 144, 145, 162, 163, 168
fetus	7, 56, 93, 106
gastrointestinal effects	52, 65, 71
general population	14, 18, 70, 96, 106, 127, 143, 146
genotoxic	23, 76
genotoxicity	106
groundwater.....	95, 128, 131, 136, 137, 142
half-life	14, 96, 126, 127, 137
hematological effects.....	53, 66
hepatic effects.....	53, 66
hydrolysis	133
hydroxyl radical.....	126, 137
hyperammonemia	15, 17, 68, 90, 93, 95, 98, 99
immune system.....	55, 75, 107
immunological.....	23, 55, 67, 75
immunological effects	67, 75
irritation	6, 8, 16, 18, 19, 26, 46, 47, 79, 95, 97, 99, 102, 104, 105, 163
K _{ow}	115
LD ₅₀	55

APPENDIX D

livestock.....	5, 8, 13, 16, 46, 74, 105, 126, 131, 132, 133, 139, 140, 141, 147, 151
manure	2, 5, 13, 14, 131, 136, 140, 142, 143, 144, 145, 151
metabolic acidosis.....	21, 57, 58, 66, 104
micronuclei	76, 106
milk.....	150, 151
muscarinic receptor.....	90
musculoskeletal effects.....	53, 66
neoplastic.....	49, 57
neurobehavioral	93
neurochemical.....	107
neurotransmitter.....	95, 107
nitrate.....	2, 14, 83, 113, 122, 126, 127, 136, 137, 138, 142, 159, 166, 167, 168, 169
nitrite	14, 126, 127, 159
nitrogen cycle	117, 123, 131, 137, 148
nitrogen fixation	117, 123, 126, 134
ocular effects	17, 54, 58, 71, 74
pharmacodynamic.....	87
pharmacokinetic.....	87, 88, 89, 90, 94
placenta.....	7
poultry	5, 14, 127, 131, 133, 142, 144, 150, 151
pulmonary function.....	16, 18, 19, 26, 46, 47, 102, 105, 163
rate constant.....	137
renal effects	15, 21, 53, 66, 73, 104, 105
retention.....	79, 81
solubility	16, 73, 90, 101, 113
toxicokinetic	23, 109
urea	15, 17, 21, 57, 78, 79, 80, 81, 82, 83, 86, 87, 90, 91, 95, 97, 99, 101, 109, 111, 122, 131, 133, 144, 155
vapor phase.....	159
volatility.....	113
volatilization	126, 132, 135, 136, 137, 138, 140, 144, 156, 159
weanling	58, 65, 66, 67

Safety Assessment of Ammonia and Ammonium Hydroxide as Used in Cosmetics

Status: Final Report
Release Date: February 15, 2018
Panel Date: December 4-5, 2017

The 2017 Cosmetic Ingredient Review Expert Panel members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Ronald A. Hill, Ph.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; James G. Marks, Jr., M.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The CIR Executive Director is Bart Heldreth, Ph.D. This report was prepared by Wilbur Johnson, Jr., M.S., Senior Scientific Analyst.

ABSTRACT: The Cosmetic Ingredient Review (CIR) Expert Panel (Panel) reviewed the safety of Ammonia and Ammonium Hydroxide, which function as pH adjusters in cosmetic products. Ammonium Hydroxide is also reported to function as a denaturant. The Panel reviewed data relevant to the safety of these ingredients and concluded that Ammonia and Ammonium Hydroxide are safe as used in hair dyes and colors and safe in cosmetics applied directly to the skin in the present practices of use and concentration described in the safety assessment, when formulated to be non-irritating.

INTRODUCTION

The safety of Ammonia and Ammonium Hydroxide in cosmetics is reviewed in this Cosmetic Ingredient Review (CIR) safety assessment. According to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI Dictionary), both ingredients are reported to function as pH adjusters in cosmetic products.¹ Additionally, Ammonia is reported to function as an external analgesic and fragrance ingredient, and Ammonium Hydroxide is reported to function as a denaturant in cosmetic products. External analgesic and fragrance are functions the CIR Expert Panel (Panel) may exclude from their purview and, therefore, they will not evaluate safety in relation to those uses.

This safety assessment includes relevant published and unpublished data for each endpoint that is evaluated. Published data are identified by conducting an exhaustive search of the world's literature. A list of the typical search engines and websites used, sources explored, and endpoints that CIR evaluates is available on the CIR website (<http://www.cir-safety.org/supplementaldoc/preliminary-search-engines-and-websites>; <http://www.cir-safety.org/supplementaldoc/cir-report-format-outline>). Unpublished data are provided by the cosmetics industry, as well as by other interested parties.

An Environmental Protection Agency (EPA) toxicological review that covers gaseous Ammonia (NH₃) and Ammonia dissolved in water (Ammonium Hydroxide, NH₄OH) was published in 2016.² It should be noted that portions of the EPA review are adapted from the toxicological profile for Ammonia that was developed by the Agency for Toxic Substances and Disease Registry (ATSDR). This CIR safety assessment also includes toxicity data on Ammonia/Ammonium Hydroxide that have become available since the ATSDR and EPA documents were published.

In addition to the ATSDR and EPA reports on Ammonia, an expert assessment of the effects on human health and the environment posed by Ammonia, prepared by a 14-member task group, is available.³ This assessment was published under the joint sponsorship of the United Nations Environment Program, the International Labor Organization, and the World Health Organization.

Furthermore, in addition to the safety test data on Ammonia and Ammonium Hydroxide that are included in this safety assessment, the Panel addressed the use of chemicals for read-across, and determined that information reported for the following chemicals is appropriate for read-across: data on "ammonium ion" (reproductive and developmental toxicity, genotoxicity, and carcinogenicity data; counter ion not identified) that are included in the ATSDR toxicological profile for Ammonia; diammonium phosphate (repeated dose (short-term) oral toxicity and reproductive and developmental toxicity data) that are included in the CIR Final Report on Phosphoric Acid and Its Salts and in the European Chemicals Agency (ECHA) registration dossier on Ammonia; and data on ammonium chloride (genotoxicity data [micronucleus test]) and ammonium sulfate (oral carcinogenicity and chronic oral toxicity data) that are included also included in the ECHA dossier (Table 1).^{4,5,6}

CHEMISTRY

Definition and General Characterization

Ammonia, ammonia gas, anhydrous ammonia, and liquid ammonia are terms that are often used interchangeably to refer to the ingredient, Ammonia, in either its liquid (compressed) or gaseous state.⁷ Ammonia dissolved in water is referred to as aqueous ammonia, ammonia solution, and the cosmetic ingredient name, Ammonium Hydroxide. In an aqueous formulation, these two ingredients will each consist of at least some of the other, dependent on the effective pH of the formulation.

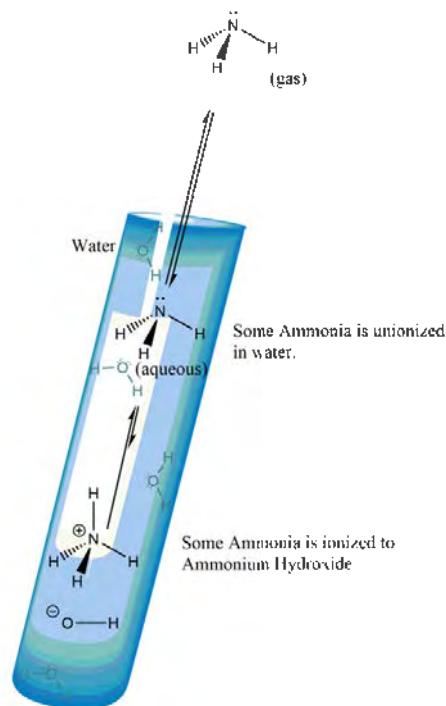


Figure 1. The gas and aqueous solution equilibria of Ammonia and Ammonium Hydroxide

Referring to Figure 1, some ammonia is actually ionized to ammonium ion, with hydroxide anion being formed in an equal amount (stoichiometrically) in pure water to produce Ammonium Hydroxide.

Ammonium Hydroxide is formed simply by the solution of Ammonia in water. Regardless of whether the ingredient is named Ammonia or Ammonium Hydroxide, if the formulation or test article contains water, both are present in equilibrium. At or near neutral pH, more than 99% is in the form of ammonium ion, and less than 1% is Ammonia. In more alkaline (i.e. higher pH) solutions, the Ammonia concentration can be significantly higher (e.g., at pH 9.25 the ratio of Ammonia to Ammonium Hydroxide is about 1:1; $\text{pK}_b \sim 4.8$ at room temperature). Accordingly, the ratio of dissolved molecular Ammonia versus the ions of Ammonium Hydroxide is mainly dependent on the pH of the formulation. Saturation in water, at room temperature and atmospheric pressure, is approximately 34%.⁸

Since the functions of fragrance and external analgesic may be excluded from the purview of the CIR Expert Panel, the only function of Ammonia under review herein is pH adjuster. The term "pH" is defined as the negative $\log_{10}$ of the concentration of hydrogen ions (protons), existing as solvated forms, including hydronium ion and higher solvates in water. Above pH 7, the concentration of hydroxyl anion (OH^-) becomes greater than hydronium, increasing as pH becomes more alkaline. Accordingly, pH adjusters function in aqueous formulations and this safety assessment evaluates Ammonia and Ammonium Hydroxide in that context. (Ammonium Hydroxide is also reported functions as a denaturant.)

The definitions, structures, and functions in cosmetics of these ingredients are presented in Table 2.

Chemical and Physical Properties

Ammonia is a small nitrogenous compound with a molecular weight of 17 that is a gas at standard temperature and pressure.⁹ It is a weak base that exists in protic equilibrium with Ammonium Hydroxide, as shown in Figure 1. Solvation of ammonia in water results in an equilibrium between dissolved ammonia and ammonium, the latter being formed by the abstraction of a proton from one equivalent of water molecules to produce a stoichiometric amount of hydroxyl anions (i.e., an acid-base reaction in essentially instantaneous equilibrium when in free aqueous solution). Additional chemical properties are presented in Table 3.^{9,10,11,12}

Method of Manufacture

Ammonia can be formed from water gas and producer gas via the Haber-Bosch process (i.e., under high temperature and pressure, hydrogen and nitrogen are combined to produce Ammonia).⁸

Ammonium Hydroxide can be produced by passing Ammonia gas into water.¹³

Composition

According to the *Food Chemicals Codex*, Ammonium Hydroxide contains not less than 27% and not more than 30% by weight NH_3 .¹⁴ The monograph on strong Ammonia solution in the *United States Pharmacopoeia* states that this is a solution of NH_3 , containing not less than 27% and not more than 31 % (w/w) NH_3 .¹⁵

Impurities

According to the *Food Chemicals Codex*, the acceptance criteria for Ammonium Hydroxide include: lead (not more than 0.5 mg/kg), nonvolatile residue (not more than 0.02%), and readily oxidizable substances (pink color does not completely disappear within 10 minutes).¹⁴ Similarly, according to the *United States Pharmacopoeia*, the limitations on strong Ammonia solution include: heavy metals (0.0013% limit), nonvolatile residue (not more than 5 mg of residue remains [0.05%]), and readily oxidizable substances (pink color does not completely disappear within 10 minutes).¹⁵

USE

Cosmetic

The safety of Ammonia and Ammonium Hydroxide is evaluated based on data received from the U.S. Food and Drug Administration (FDA) and the cosmetics industry on the expected use of this ingredient in cosmetics. Use frequencies of individual ingredients in cosmetics are collected from manufacturers and reported by cosmetic product category in FDA's Voluntary Cosmetic Registration Program (VCRP) database.¹⁶ Use concentration data are submitted by the cosmetics industry in response to surveys, conducted by the Personal Care Products Council (Council), of maximum reported use concentrations by product category.¹⁷

According to 2017 VCRP data, Ammonia is being used in 599 cosmetic products (mostly rinse-off products) and Ammonium Hydroxide is being used in 1354 cosmetic products (mostly rinse-off products) (Table 4).¹⁶ Most of the uses of these 2 ingredients are in hair coloring products (582/599 (97.2%) uses for Ammonia and 1104/1354 (81.5%) uses for Ammonium Hydroxide). The results of a concentration of use survey provided by the Council in 2017 indicate that the highest maximum cosmetic use concentration of Ammonia is 4.6 % (in hair dyes and colors) and that the highest maximum cosmetic use concentration of Ammonium Hydroxide is 12.5% (in hair dyes and colors) (Table 4).¹⁷ Regarding use concentrations in leave-on products, the highest maximum cosmetic use concentrations are 0.73% (Ammonia - in tonics, dressings, and other hair grooming aids) and 1.5% (Ammonium Hydroxide - in face and neck products [not spray]).

Cosmetic products containing Ammonia or Ammonium Hydroxide may be applied to the skin and hair or, incidentally, may come in contact with the eyes (e.g., at maximum use concentrations up to 0.58% (Ammonium Hydroxide in eye brow pencils) and mucous membranes (e.g., at maximum use concentrations up to 0.0012% Ammonium Hydroxide in bath soaps and detergents). Products containing Ammonia or Ammonium Hydroxide may be applied as frequently as several times per day and may come in contact with the skin or hair for variable periods following application. Daily or occasional use may extend over many years.

The denaturant use of Ammonium Hydroxide is relevant to cosmetic use. The formula for SD alcohol 36 is: "To every 100 gallons of strong ammonia solution, N.F.: 17.5 pounds of caustic soda, liquid grade, containing 50 percent sodium hydroxide by weight; or 12.0 pounds of caustic soda, liquid grade, containing 73 percent sodium hydroxide by weight." (27CFR21.63) This denatured alcohol is permitted for use in shampoos and soap and bath preparations.

Ammonia (CAS No. 7664-41-7) and Ammonium Hydroxide (CAS No. 1336-21-6) are on the European Union's list of substances that cosmetics must not contain, except when subject to the following restriction: maximum concentration in ready for use preparation (6% (as NH_3)).¹⁸ Furthermore, the following phrase appears in the wording of "conditions of use and warnings" category: above 2%: contains Ammonia.

Non-Cosmetic

Ammonia is a chemical with diverse uses, such as fertilizer and as a refrigerant.¹⁹ Ammonia is also used in production of dyes, plastics, synthetic fibers, pesticides, explosives, refrigerants, and pharmaceuticals, and in the purification of water.⁷ 27CFR21.63 also permits (in addition to the cosmetic uses above) the use of Ammonium Hydroxide as a denaturant in household cleaners.

Ammonium Hydroxide is affirmed as generally recognized as safe (GRAS) as a direct human food ingredient. [21CFR184.1139] Ammonium Hydroxide must meet the specifications of the *Food Chemicals Codex* (see Impurities section), and, in accordance with these specifications, the ingredient is used in food with no limitation other than current good manufacturing practice. Concerning animals, anhydrous Ammonia is a food additive permitted in feed and drinking water, and it is used or intended for use as a source of non-protein nitrogen in cattle feed. [21CFR573.180]

In Australia, Ammonia and Ammonium Hydroxide are listed in the *Poisons Standard*, the standard for the uniform scheduling of medicines and poisons (SUSMP) in schedules 5 and 6.²⁰ Under schedule 5, Ammonia and Ammonium Hydroxide are permitted in preparations containing $\leq 5\%$ Ammonia, with the following exceptions: in preparations for human internal therapeutic use; in preparations for inhalation when absorbed in an inert solid material; or in preparations containing $\leq 0.5\%$ free Ammonia. Schedule 5 chemicals are defined as substances with a low potential for causing harm, the extent of which can be reduced through the use of appropriate packaging with simple warnings and safety directions on the label; schedule 5 chemicals are labeled with "Caution". Schedule 6 chemicals are classified as poisons with a moderate potential for harm.

Ammonia, as an intravenously-injected prescription drug, is included on the list of FDA-approved drug products.²¹ Ammonia solution has been classified as an over-the-counter (OTC) drug active ingredient as a skin protectant and external analgesic, and the same is true for Ammonium Hydroxide as a skin protectant. However, FDA has determined that there are inadequate data to establish general recognition of the safety and effectiveness of these ingredients for the specified uses.²²

TOXICOKINETIC STUDIES

Because of the equilibrium nature of these two ingredients, the studies that follow will simply recite "Ammonia" for most cases, regardless of whether Ammonia or Ammonium Hydroxide was reported.

Absorption, Distribution, Metabolism, and Excretion

Ammonia is the principal by-product of amino acid metabolism, and the liver is indicated as the central organ of Ammonia metabolism.²³ It is generated, in vivo, from the breakdown of nitrogenous substances in the gut and from the use of glutamine as a metabolic fuel in the small intestine, and is taken up by the liver where it is detoxified by conversion to urea and, to a lesser extent, glutamine.^{24,25} The main source of in vivo Ammonia generation occurs in the intestines, from lysis of blood-borne urea and also from protein digestion/deamination by urease-positive bacteria and microbial deaminase.^{26,27} A large amount of metabolically-generated Ammonia is absorbed into the blood and, via the portal vein, is transported to the liver where it is detoxified.^{26,28,29} The normal concentration of Ammonia in the portal blood varies from 300 to 600 μM . But, in the blood leaving the liver the concentration is reduced to 20–60 μM . The liver occupies a central position in the regulation of Ammonia levels in the organism.^{30,31} According to another source, the normal range for blood serum levels is 10–40 $\mu\text{mol/L}$.³²

The substrates from which Ammonia may be formed in the gut comprise derivatives of ingested nitrogenous material, epithelial and bacterial debris, and compounds secreted from the circulation to the mucosal cells and lumen (e.g., certain peptides, amino acids, and smaller diffusible substances such as urea).³³ Both the gut and kidneys generate substantial amounts of Ammonia from the deamidation of glutamine.²³ The glutamine-glutamate cycle in the body works in conjunction with the glucose-alanine cycle to shuttle Ammonia from peripheral to visceral organs.

Renal regulation of acid-base balance involves the formation and excretion of Ammonia to buffer hydrogen ions that are excreted in the urine. Approximately two-thirds of urinary Ammonia is derived from the amide nitrogen of glutamine, a reaction that is catalyzed by the glutaminase enzyme in renal tubular cells.²³

The first step in the degradation of most amino acids is the removal of an α -amino residue, and an amino residue is transferred to α -ketoglutaric acid to produce glutamate.³⁴ Glutamate dehydrogenase converts glutamate to α -ketoglutarate and Ammonia. To prevent a toxic buildup of Ammonia, it is converted to glutamine and alanine in a number of tissues for transportation to the liver. Ammonia is then converted to urea via the urea cycle in the liver, and urea is excreted in the urine. The urea cycle, a cycle of biochemical reactions that produces urea from Ammonia, is the major pathway for Ammonia detoxification in terrestrial mammals.³⁵ In the liver, the urea cycle is essential to the conversion of excess nitrogen from Ammonia and aspartate into urea.³⁶ When the supply of Ammonia in mammals exceeds the capacity for its detoxification, the excretion of orotic acid in the urine increases.³⁵ Orotic acid (from the urea cycle) is an intermediate product in the biosynthesis of pyrimidines and purines.

There is evidence that Ammonia can cross the blood-brain barrier (BBB), mostly through ion transporters rather than by passive diffusion of gaseous Ammonia.^{26,37}

Animal

Inhalation

Brain glutamine levels have been shown to increase in rats that inhaled 25 or 300 ppm Ammonia vapor for 6 hours/day for 5 days, which is likely a result of Ammonia metabolism by the astrocytic glutamate-glutamine cycle.^{38,39}

Continuous exposure of rats for 24 h to concentrations up to 32 ppm Ammonia resulted in significant increase in blood Ammonia levels.⁴⁰ Exposures to 310 - 1157 ppm led to significantly increased blood concentrations of Ammonia within 8 h of exposure initiation, but blood Ammonia returned to pre-exposure values within 12 hours of continuous exposure and did not change over the remainder of the 24-hour exposure period.

Parenteral

Following the administration of [¹³N]Ammonia to rats (via either the carotid artery or cerebrospinal fluid), most metabolized labelled nitrogen was in glutamine (amide), and little was in glutamate (plus aspartate).⁴¹

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

Dermal

Acute dermal toxicity studies on Ammonia were not found in the published literature, nor were these data submitted.

Acute oral toxicity studies summarized below are presented in Table 5, and acute inhalation studies are summarized below and presented in Table 6.

Oral

In a study in which groups of 6 rats were dosed orally with Ammonium Hydroxide at concentrations ranging from 0.03% to 1%, the minimum concentration at which gastric lesions were observed was 0.03%; the ulcer index increased in a concentration-dependent manner.⁴² An acute oral LD₅₀ of 350 mg/kg for Ammonia in rats (groups of 10) has been reported,^{6,43,44} and the oral administration of 1 % or 3% (w/w as Ammonium Hydroxide) to groups of 4 to 8 rats by gavage has produced severe hemorrhagic lesions of the stomach.⁴⁵

Inhalation

In a 10-minute exposure study involving mice exposed to Ammonia at concentrations ranging from 8723 to 12,870 ppm, an LC₅₀ of 10,096 ppm was reported.^{24,46} An LC₅₀ of 10,150 ppm was reported in another 10-minute exposure study in which mice were exposed to Ammonia at concentrations ranging from 8770 to 12,940 ppm.^{47,46,48} In mice exposed to Ammonia (100-800 ppm) for 30 minutes, an RD₅₀ (exposure concentration that produced a 50% reduction in respiratory rate) of 303 ppm was reported.^{24,49,48} The following effects were observed in mice that were exposed to Ammonia at a concentration of 21,400 ppm for 30 minutes: eye irritation, dyspnea, histopathological changes in the lungs (alveolar disruption and loss of septal continuity), coma, and death.^{24,50} The following results were observed in mice that were exposed to Ammonia for 1 h at various concentrations: nasal/eye irritation, liver congestion, and LC₅₀ = 4837 ppm (concentration range: 3600 to 5720 ppm);^{24,51,48} mild to moderate pneumonitis, hepatic lesions, and LC₅₀ = 4230 ppm (concentration range: 1190 to 4860 ppm);^{24,52,48} eye irritation and alveolar disruption (at 4840 ppm);^{24,53} and liver necrosis (at 3440 ppm).⁵²

In acute inhalation toxicity studies involving rats, LC₅₀s ranging from 7068 ppm⁵⁴ to 40,300 ppm⁵⁵ and RD₅₀s of 1396 ppm and 1299 ppm^{24,56} have been reported. Exposure durations included 10 minutes (14,170 to 54,289 ppm; LC₅₀ = 40,300 ppm),⁵⁵ 20 minutes (14,170 to 54,289 ppm; LC₅₀ = 28,595 ppm),⁵⁵ 40 minutes (14,170 to 54,289 ppm; LC₅₀ = 20,300 ppm),⁵⁵ 1 h (14,170 to 54,289 ppm; LC₅₀ = 16,600 ppm),⁵⁵ 1 h (3028-14,044 ppm; LC₅₀ = 17,633 ppm),⁵⁴ and 4 h (3028-14,044 ppm; LC₅₀ = 7068 ppm).⁵⁴ Signs of upper respiratory tract irritation were observed within the following concentration ranges: 14,170 to 54,289 ppm;⁵⁵ 9000 to 35,000 ppm (LC₅₀ = 23,672 ppm);⁵⁷ 9,000 to 23,000 ppm;⁵⁸ 3028 to 14,044 ppm;⁵⁴ and 1436, 4307, and 6814 ppm.⁵⁹ No effects were observed at a concentration of 144 ppm.⁶⁰ Increased production of respiratory tract fluid was observed in rabbits after a 1-h exposure to Ammonia at the following concentrations: 5200 to 12,800 ppm;²⁴ average concentration of 10,360 ppm;²⁴ and at 3.5 and 8.7 ppm.⁶¹ A significant decrease in respiratory rate was observed in rabbits exposed to Ammonia (50 and 100 ppm) for 2.5 to 3 h.⁶² Congestion of the respiratory tract/lungs was reported in cats exposed to 1000 ppm Ammonia for 10 minutes^{63,64} and, for 1 h, to 5200 ppm to 12,800 ppm Ammonia,^{47,65} an average concentration of 10,360 ppm,^{47,65} and 3.5 and 8.7 ppm.⁶⁶ Gross pathological findings in the

respiratory tract after the 10-minute exposure included varying degrees of congestion, hemorrhage, edema, interstitial emphysema, and lung collapse.

It has been noted that acute exposure data have demonstrated that injury to respiratory tissues is primarily due to Ammonia's alkaline (i.e., caustic) properties, resulting from the formation of hydroxide when Ammonia reacts with water.² Furthermore, Ammonia readily dissolves in the moisture on mucous membranes, forming Ammonium Hydroxide, which causes liquefactive necrosis of the tissues.

Short-Term Toxicity Studies

Animal

Dermal

Short-term dermal toxicity data on Ammonium Hydroxide or Ammonia were not found in the published literature, nor were these data submitted.

Short-term oral and inhalation toxicity studies are summarized below and presented in Table 7.

Oral

Mucosal atrophy in the stomach antrum and enlargement of the proliferative zone in the mucosa of the stomach antrum and body were observed in rats that received 0.01% Ammonium Hydroxide (in drinking water) for 8 weeks.⁶⁷ A no-observed-adverse effect-level (NOAEL) of 250 mg/kg/day for general toxicity and a lowest-observed-adverse effect-level (LOAEL) of 750 mg/kg/day for general toxicity were reported for diammonium phosphate (identified as an appropriate read across material for short-term oral toxicity and included herein to support the overall weight of evidence) in rats dosed orally (by gavage) for 5 weeks.^{6,48}

Inhalation

Rhinitis and pathological lesions with metaplasia and necrosis were observed in mice exposed to Ammonia for 14 days at a concentration of 711 ppm, but not at concentrations of 78 and 271 ppm.^{24,68} Histopathologic findings (nasal cavity) for mice exposed to Ammonia (303 ppm) repeatedly for 5 days included slight squamous metaplasia, ulceration, necrosis, and moderate inflammatory changes.^{24,69} Lung congestion and edema were observed in mice exposed to Ammonia (20 ppm) for 42 days.^{2,70} A low incidence of carcinoma of the nasal mucosa was observed in mice exposed to Ammonia (12% solution) for 8 weeks, and these results are summarized in more detail in the Carcinogenicity section.^{2,71} Rats were exposed repeatedly to Ammonia at concentrations ranging from 10 or 150 ppm (for 75 days)^{2,48,72} to 1306 ppm (for 42 days (5 days/week and 8 h/day)).⁷³ The higher concentration was tolerated for 42 days in rats, and increased thickness of the nasal epithelium was observed at 150 ppm, but not 10 ppm. Nasal irritation and inflammation of the upper respiratory tract were observed in rats exposed to 500 ppm Ammonia for 3 weeks; reactions had cleared by week 8.^{48,74}

When rats, rabbits, guinea pigs, monkeys, and dogs were exposed to Ammonia at a concentration of ~ 223 ppm or ~ 1105 ppm for 6 weeks (5 days/week and 8 h/day), the following effects were observed: focal pneumonitis in 1 of 3 monkeys at 223 ppm; nonspecific lung inflammation in guinea pigs and rats, but not other species at 1105 ppm; and mild to moderate dyspnea in rabbits and dogs during week 1 only at 1105 ppm.^{2,40,73} Upper respiratory effects (e.g., dyspnea and nasal lesions, irritation, and inflammation) were observed over most of the range of concentrations tested (145 ppm (5-week exposure)^{2,75} to 1306 ppm (42-day exposure (5 days/week and 8 h/day))⁷³ in short-term inhalation toxicity studies on Ammonia involving mice, rats, guinea pigs, pigs, rabbits or dogs. At lower Ammonia concentrations, there were no treatment-related effects in rats (at 50 or 90 ppm (continuous exposure for 50 days))^{48,76} and there was no increase in the incidence of respiratory diseases in pigs exposed to Ammonia (37 ppm or ~ 14.2 ppm, inhalable dust exposure) for 5 weeks.^{2,77} In other studies, nearly 64% lethality was reported for rats exposed to Ammonia (653 ppm) for 25 days (continuous exposure)⁹ and 50 of 51 rats exposed to Ammonia (650 ppm) were dead by day 65 of continuous exposure.^{46,73} In a study in which pigs were exposed to Ammonia (5 to 100 ppm) for 6 days, nasal irritation was observed at concentrations down to 25 ppm.⁷⁸

Subchronic Toxicity Studies

Neither subchronic dermal nor oral toxicity data on Ammonium Hydroxide or Ammonia were found in the published literature, nor were these data submitted.

Inhalation

Subchronic inhalation toxicity studies on Ammonia and Ammonium Hydroxide are summarized below and presented in Table 7.

Fatty changes of liver plate cells were seen in rats following continuous exposure to Ammonia (642 ppm) for 90 days.⁹ The following results were reported for guinea pigs exposed to ~170 ppm Ammonia for 18 weeks: mild congestion of the liver, spleen, and kidneys; degenerative changes in the adrenal glands; hemosiderosis in the spleen; and cloudy swelling in proximal kidney tubules.^{47,81} Damaged tracheal mucosae were observed in rats exposed repeatedly to Ammonia (100 ppm) for 12 weeks.^{47,82} Mild leukocytosis was noted in rats after exposure to 143 ppm, but not 43 ppm, Ammonia repeatedly (25- or 60-minute exposures every 48 h) for 3 months.⁴⁸

A low incidence of mortalities (13 of 515 rats and 4 of 15 guinea pigs) was observed in rats and guinea pigs exposed continuously to 671 ppm Ammonia for 90 days. However, there were no mortalities or treatment-related histopathological findings in rats, guinea pigs, rabbits, monkeys, or dogs exposed continuously to ~57.43 ppm Ammonium Hydroxide for 114 days.^{48,73}

Chronic Toxicity Studies

Animal

Dermal

Chronic dermal toxicity data on Ammonium Hydroxide/Ammonia were not found in the published literature, nor were these data submitted.

Oral

Enlarged adrenal glands were observed in rabbits that received 124 mg ammonium/kg/day as (as Ammonium Hydroxide) by gavage in water for 17 months.⁸³

Ammonium Sulfate (read-across for Ammonia)

Limited chronic oral toxicity data were available for Ammonia. However, ammonium sulfate was identified as an appropriate read-across surrogate. The chronic oral toxicity of ammonium sulfate was evaluated using groups of 10 Fischer 344/DuCrj rats (males and females). Ammonium sulfate was administered in the diet daily at concentrations of 0%, 0.1%, 0.6%, and 3% for 52 weeks. None of the animals died, and there were no macroscopic findings. There was a significant increase in kidney and/or liver weights in males and females of the 3% dietary group, but there were no effects on survival rate, body weights, or hematological, serum biochemical, or histopathological parameters at any concentration. Several non-neoplastic lesions, such as bile duct proliferation in the liver and focal myocarditis in the heart were observed in the 3% dietary group, but also in animals of the control group, and the difference in results was not statistically significant when the 2 groups were compared. The NOAEL for ammonium sulfate was estimated to be 0.6% in both sexes, which is equivalent to 256 and 284 mg/kg/day in males and females, respectively.^{6,84} Neoplastic lesions (classified as unrelated to ammonium sulfate in the diet) reported in a carcinogenicity study in the same report are included in Table 9.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Developmental/reproductive toxicity studies are summarized below and presented in Table 8.

A relationship between the duration of exposure and the incidence of exencephaly (concentration-related increase) was observed in an *in vitro* study in which mouse embryos were cultured with Ammonia (38 to 300 $\mu\text{mol/l}$) for up to 93 h.⁸⁹ In a developmental toxicity study involving pregnant rats exposed to Ammonia in the diet (4293 mg/kg/day; as the ammonium ion [read-across for Ammonia]) from gestation day 1 through day 21 of lactation, body weights of offspring were reduced by 25% (males) and 16% (females).^{9,90} Neither reproductive nor developmental toxicity was reported in a study in which female pigs were exposed (inhalation exposure) to ~7 ppm or ~35 ppm Ammonia from 6 weeks prior to breeding until day 30 of gestation.⁹¹ In an oral reproductive and developmental toxicity study on diammonium phosphate (identified as an appropriate read across material for developmental and reproductive toxicity and included herein to support the overall weight of evidence) involving rats in which embryos were treated *in vitro* and then implanted, an NOAEL of 1500 mg/kg/day (highest dose tested) was reported.^{6,44} The only histological findings relating to maternal toxicity in this study were the inflammatory/degenerative changes in all treatment groups (diammonium phosphate at 250, 750, and 1500 mg/kg/day),

which were considered likely to have been the result of an irritant effect. No statistically significant differences in the number of live fetuses, fetal length, or fetus-to-corpus luteum ratio were observed after gilts were exposed (inhalation) to Ammonia (7 or 35 ppm) from 6 weeks prior to breeding until day 30 of gestation.⁹¹

GENOTOXICITY STUDIES

In Vitro

Ammonia was non-genotoxic when tested at concentrations up to 25,000 ppm (with and without metabolic activation) in the following bacterial strains: *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, TA 1537, TA1538, and *Escherichia coli* strain WP2 uvr A.^{6,48,44}

Ammonia was non-genotoxic to *E. coli* strain Sd-4-73 in an in vitro assay without metabolic activation.⁴⁴

In Vivo

Blood samples from 22 workers who had been exposed to Ammonia (concentrations unknown) in a fertilizer factory were compared with samples obtained from 42 unexposed controls. Results (compared to controls) were as follows: increased frequency of chromosomal aberrations (CAs), sister chromatid exchanges (SCEs), and mitotic index, with increasing duration of exposure. Smokers had higher SCE and CA values than non-smokers and alcoholics had more CAs and SCEs than non-alcoholics.⁹²

Ammonia and Ammonium Chloride (read-across for Ammonia)

An increased frequency of micronuclei (compared to controls) was observed in Swiss albino mice that received single intraperitoneal (i.p.) doses of Ammonia (12, 25, or 50 mg/kg). The maximum number of micronucleated polychromatic erythrocytes was associated with mice that received the highest dose (50 mg/kg), and there was clear correlation of dose-yield effects. In another micronucleus test, groups of 10 (5 males, 5 females) ddY mice received single i.p. doses of 62.5, 125, 250 and 500 mg/kg ammonium chloride (identified as an appropriate read across material for in vivo genotoxicity and included to support the overall weight of evidence) or i.p. doses of 31.3, 62.5, 125, and 250 mg/kg ammonium chloride (4 injections within 24 h). Femoral bone marrow cells were examined for polychromatic erythrocytes, and there was no evidence of genotoxicity at the doses of ammonium chloride that were administered.^{6,92}

CARCINOGENICITY STUDIES

Carcinogenicity and tumor promotion studies are summarized below and presented in Table 9.

There was no evidence of a tumorigenic effect in Kid: CFLP mice treated by gavage with ammonia dissolved in water alone at a dose of 42 mg Ammonia /kg/day for 4 weeks or with diethyl pyrocarbonate alone, but 9 of 16 mice treated with a combination of ammonium ion and diethyl pyrocarbonate developed lung tumors.⁹³ Following the oral dosing of mice (Swiss and C3H) with Ammonia (in drinking water, 193 mg/kg/day) for 2 years, there was no evidence of carcinogenicity and no effect on the spontaneous development of adenocarcinoma of the breast (associated with C3H mouse strain).^{44,48,94} Ammonium sulfate was classified as non-carcinogenic in rats in a study involving dietary concentrations up to 3% daily for 104 weeks.⁸⁴ Neoplastic lesions were observed in this study, but were deemed not treatment-related because of the spontaneous occurrence of these lesions in the rat strain (F344/DuCrj) that was tested. Malignant pheochromocytoma of the adrenal gland and pituitary adenomas were observed in F344/DuCrj rats fed 3% ammonium sulfate, but not 0.1% or 0.6%, in the diet for 52 weeks.⁶ After pregnant HSL mice were exposed (by gavage) to ammonium ion + diethyl pyrocarbonate during pregnancy and lactation, there was no evidence of lung tumors in mice treated during pregnancy or in the suckling mice of dams treated during lactation.⁹⁵ When 10 mice were exposed (vapor inhalation) to 12% Ammonia solution repeatedly for 8 weeks, carcinoma of the nasal mucosa was observed in 2 mice.⁷¹

When 10 mice were exposed (vapor inhalation) to 12% Ammonia solution repeatedly for 8 weeks, carcinoma of the nasal mucosa was observed in 2 mice.⁷¹

Tumor Promotion

A statistically significant increase in the incidence of gastric cancer (70%) was observed in rats dosed orally with the initiator *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) and 0.01% Ammonia, when compared to dosing with MNNG alone.⁹⁶ In another study, the size, depth, and metastasis of MNNG-initiated tumors was enhanced in rats dosed orally with Ammonia (~42 mg/kg/day).⁹⁷

OTHER RELEVANT STUDIES

Neurotoxicity

Ammonia is most toxic in the brain, and chronic hyperammonemia (metabolic disturbance) may lead to brain damage, especially in children.²³ During normal body function, approximately 10% of arterial Ammonia is extracted by the brain. Neurotoxicity is observed only when circulating levels of Ammonia are elevated. It has been reported that hyperammonemia is associated with neuronal cell loss and cerebral atrophy that lead to mental retardation and cerebral palsy in pediatric patients.⁹⁸ These toxic effects are specific to the developing brain, as neuronal damage is not observed in the brain of adult patients with hyperammonemia due to liver failure.

According to another source, many neurologic disorders are related to congenital or acquired hyperammonemia. Evidence obtained with the use of experimental hyperammonemia models suggests that acute neurotoxic effects of Ammonia are mediated by over activation of ionotropic glutamate receptors, mainly the *N*-methyl-D-aspartate (NMDA) receptors, and, to a lesser degree, the kainic acid (KA)/ α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors.⁹⁹ Results from other studies suggest that glutamine is also a mediator of Ammonia neurotoxicity.^{100,101}

Toxic levels of Ammonia and alterations in pH, electrolyte disturbances, and membrane potential depolarization are thought to lead to neurological dysfunction, primarily by causing cellular swelling accompanied by brain edema and metabolic dysfunction.^{26,102} Studies have suggested that Ammonia is likely to be particularly toxic to astrocytes, as they are the only cells that possess the enzyme glutamine synthetase (which is responsible for detoxifying Ammonia in the brain through condensation with glutamate).^{103,104}

In *in vitro* studies, it has been demonstrated that acute intoxication with large doses of Ammonia leads to excessive activation of NMDA receptors.^{105,106,107,108} Furthermore, excessive activation of NMDA receptors leads to neuronal degeneration and death, and is responsible for most of the neuronal damage that is found in brain ischemia.¹⁰⁵

Brain Pathology

The excessive formation of Ammonia within the brains of Alzheimer's disease patients and its release into the periphery has been demonstrated.^{109,110} Furthermore, a higher expression of AMP-deaminase in the brains of Alzheimer's disease patients has been observed, and this finding indicates the existence of a pathologically elevated source of Ammonia within the brain of Alzheimer's disease patients.^{109,111}

Cytotoxicity

Lymphocytes separated from peripheral bovine (Holstein-Friesian cows) blood were incubated for 2 h in control medium and test medium with various concentrations of Ammonia (w/v as Ammonium Hydroxide; 0.01 mg/dl, 0.1 mg/dl, 1 mg/dl, and 10 mg/dl).¹¹² Viability of the lymphocytes, measured by trypan blue exclusion test, was significantly reduced after 2 h of incubation. At a concentration of 0.01 mg/ml, lymphocyte viability was significantly reduced after 24 h and 48 h of incubation. In another experiment, in which lymphocytes were preincubated with Ammonia (w/v as Ammonium Hydroxide; 10 mg/dl) and then washed and resuspended in the fresh medium with Ammonia, the number of viable cells was reduced to 51% $\pm$ 8 at 24 h, 40% $\pm$ 7 at 48 h, and to 39% $\pm$ 6 at 72 h of incubation.

Effect on Mitosis

The ability of Ammonia to affect the mitogenic response of bovine lymphocytes to phytohemagglutinin (PHA) or concanavalin A (Con A) was examined.¹¹² Lymphocytes from 10 Holstein-Friesian cows were incubated with various concentrations of PHA and Ammonia. Lymphocytes from 6 cows were incubated with Con A and Ammonia. Mitogenic reactivity was measured by the incorporation of methyl-³H-thymidine into the DNA of lymphocytes. Ammonia at concentrations of 0.01 mg/dl (w/v as Ammonium Hydroxide) significantly ($P < 0.01$) suppressed PHA (optimal dose = 0.5 μ g/ml) stimulation of lymphocytes from only 1 animal. Other concentrations of Ammonia, at 0.1 mg/dl, 1 mg/dl, and 10 mg/dl (w/v as Ammonium Hydroxide), significantly ($P < 0.01$) reduced the response to PHA of lymphocytes from 5 cows, 9 cows, and from all animals, respectively. These concentrations significantly reduced Con A (optimal dose = 0.5 μ g/ml) stimulation of lymphocytes from 1 animal, 5 animals, and all animals, respectively. A significant suppression ($P < 0.01$) of blastogenesis of lymphocytes from 1 cow by 0.01 mg/dl, 6 by 0.1 mg/dl, 14 by 1.0 mg/dl, and from 16 cows by 10.0 mg/dl was observed. The mitogenic response of lymphocytes was reduced when lymphocytes were preincubated with Ammonia for a duration as short as 1 h.

Generation of Free Radicals

Elevated concentrations of Ammonia have been shown to generate free radicals in rats and rat cell cultures (liver and brain),^{113,114} leading to excessive production of nitric oxide (NO) by stimulating the citrulline-NO cycle.¹¹⁵

Immunological Effects

A delayed-type hypersensitivity test was used to evaluate cell-mediated immunity in groups of 8 Hartley guinea pigs.¹¹⁶ The animals were vaccinated with *Mycobacterium bovis* bacillus Calmette-Guérin (BCG) and exposed to Ammonia (< 15 ppm, 50 ppm, or 90 ppm (6.75 µg of Ammonia per deciliter of air)) for 3 weeks. Exposure to Ammonia was followed by intradermal challenge with a purified protein derivative of tuberculin. Dermal lesion size was reduced in animals that were exposed to Ammonia at a concentration of 90 ppm (mean diameter of dermal lesion = 8.7 mm, statistically significantly different from control ($p < 0.05$)). Results were not statistically significant in the 2 other exposure groups. Also, blood and bronchial lymphocytes were harvested from guinea pigs exposed to Ammonia, and the cells were then stimulated with the mitogens PHA or Con A. Reduced T-cell proliferation was observed; however, bactericidal activity in alveolar macrophages isolated was not affected. In an *in vitro* experiment in which lymphocytes and macrophages were isolated from unexposed Hartley guinea pigs and then treated with Ammonia, reduced proliferation and bactericidal capacity were observed only at concentrations that reduced viability. Specifically, the response of blood lymphocytes to Ammonia at 1.0 mg/dl and 10 mg/dl, but not ≤ 0.1 mg/dl, significantly reduced ($p < 0.01$) the response to phytohemagglutinin stimulation. The exposure of alveolar macrophages to Ammonia at 1 mg/dl and 5 mg/dl, but not 0.01 mg/dl or 0.1 mg/dl, caused significant inhibition ($p < 0.01$) of bactericidal activity. These results were indicative of nonspecific effects of Ammonia-induced immunosuppression. The authors noted that the data in this study indicate that T cells may be the target of Ammonia exposure, in that specific macrophage effects were not observed.

Neurological Effects

Acute inhalation exposure to low levels of Ammonia (100 or 300 ppm) for 6 h continuously has been shown to depress free-access wheel running behavior in rodents.¹¹⁷

DERMAL IRRITATION AND SENSITIZATION STUDIES

Irritation

Dermal irritation studies are summarized in Table 10.

An undiluted Ammonia solution (as 30% Ammonium Hydroxide) was classified as a corrosive material after topical application to the stratum corneum surface in reconstructed human skin cultures *in vitro*.¹¹⁸ At histologic examination of the cultures, epidermal necrosis was observed. The minimum concentration of Ammonia that caused an inflammatory reaction when applied (single application) to the skin of rats and mice (6 per species) was > 25% (rats) and = 25% (mice).¹¹⁹ In a skin irritation study in which groups of 4 rats, guinea pigs, and mice were injected intradermally with Ammonia (0.01 ml), the minimum concentration that caused a positive reaction was 0.05% in rats, mice, and guinea pigs.¹¹⁹ Ammonia (20% as Ammonium Hydroxide) was corrosive to the skin of rabbits.^{20,44} In another study involving rabbits, 12% aqueous Ammonia was corrosive to the skin, whereas 10% was not.⁶

In clinical testing, the application of a saturated aqueous solution of Ammonia to the skin of 16 subjects resulted in blister formation and skin irritation.¹²⁰ In a study involving 110 subjects, Ammonia (1:1 aqueous solution) was applied to the skin and minimal blistering time (MBT) served as an indicator of cutaneous irritability.¹²¹ The inflammatory reaction observed was considered slight, and MBT ranged from 3 to 57 minutes. Results from another study in which 50% Ammonium Hydroxide solution (0.5 ml of freshly prepared 1:1 aqueous solution of Ammonium Hydroxide) was applied to the skin indicated that the time required to produce a full blister was greatly prolonged in the aged, when compared to young adults.¹²²

Sensitization

Skin sensitization data on Ammonia were not found in the published literature, nor were these data submitted.

OCULAR IRRITATION STUDIES

Ocular irritation studies are summarized in Table 11.

Ammonia was classified as a severe ocular irritant in the *in vitro* ⁵¹Cr-release assay involving human corneal endothelial cell cultures.¹²³ In rabbits, Ammonia (as Ammonium Hydroxide) at 1 mg was classified as an ocular irritant.⁴⁴ At a concentration of 28.5%, Ammonia induced corneal opacity in rabbits.^{9,124} In a study involving groups of 6 rabbits, Ammonium Hydroxide caused conjunctivitis at concentrations of 1% to 10%, but not 0.3%; the 10% concentration also caused corneal opacities within 1 h of instillation.¹²⁵ Conjunctivitis and corneal damage were also observed in a study involving 3 rabbits, whereby 3% Ammonia, 100 µl was instilled into the eyes.^{126 9,44,123,124,125,126}

In a study involving rats, there was no evidence of ocular irritation following exposure to Ammonia at vapor concentrations ranging from 15 to 1157 ppm.^{24,40}

MUCOUS MEMBRANE IRRITATION STUDIES

The stomachs of male Sprague-Dawley rats were exposed (mounted in an ex vivo gastric chamber) to 2 ml of Ammonia (15-60 mmol/l, in saline) for 15 minutes (for microscopic study) or for 60 minutes (for macroscopic study), and exposure was followed by examination for mucosal lesions. Microscopic damage to the gastric mucosa was observed.¹²⁷

CLINICAL STUDIES

Case Reports

A 68-yr-old male patient, employed for 18 years, was exposed frequently to anhydrous Ammonia leaks from a microfilm processor camera while on the job. He was diagnosed with interstitial lung disease and severe restrictive lung disease due to Ammonia inhalation. Marked diffuse interstitial fibrosis throughout the lung was observed.¹²⁸

A male custodian had used Ammonia (28% Ammonium Hydroxide solution; which he dilutes in water) to clean office floors daily for 19 years.¹²⁹ He experienced regular episodes of upper airway irritation, coughing, and eye irritation when mixing the chemical in water. An evaluation of the patient revealed a negative rheumatoid factor and positive antinuclear antibody at a 1:320 dilution. The gallium lung scan was normal, but pulmonary function testing indicated a moderate restrictive defect and a formal exercise study indicated ventilator restriction upon attainment of maximum oxygen consumption. The results of a transbronchial lung biopsy with fiberoptic bronchoscopy revealed interstitial fibrosis with chronic inflammation. Granulomata were not present and cultures for tuberculosis and fungal infection were negative. A decrease in the diameter of the hypopharynx, secondary to hypertrophy of the soft tissues in the hypopharynx, was also observed. The opacification of the optic lens capsule, bronchiectasis, and fibrous obliteration of the small airways observed were described as chronic lesions that follow acute exposure to Ammonia.

Other Clinical Reports

Clinical reports relating to inhalation exposure are summarized in Table 12.

In various clinical reports, individuals were exposed to Ammonia at concentrations ranging from 25 ppm to 700 ppm. The periods of exposure ranged from 5 minutes to 6 weeks (5 days per week [2-6 h/day]). Nose, throat, and eye irritation were observed.^{9,47,53,79,130,131,132,133}

EPIDEMIOLOGICAL STUDIES

Non-Cancer Endpoints

A retrospective study was performed to assess the association between petrochemical exposure and spontaneous abortion. Study participants included 2853 non-smoking women who had been pregnant at least once, 96 of whom had been exposed to Ammonia (actual exposure levels unknown). Exposure during the pre-conception period and the first trimester of pregnancy was calculated based on information on perceived Ammonia exposure. Exposure during the first, second, and third trimesters was recorded separately for each pregnancy. Data analyses did not indicate any effect on spontaneous abortion (Odds ratio: 1.2; 95% confidence interval (CI): 0.5-2.60).⁶

OCCUPATIONAL EXPOSURE

Airway Inflammation

Airway inflammation due to Ammonia exposure was evaluated in female Palestinian hairdressers in Hebron who had worked in this profession for 12 ± 7 years.¹³⁴ Study participants included 33 non-smoking hairdressers (from 13 salons total) and 35 non-smoking control subjects. The hairdressers worked in relatively small-sized salons with scarce ventilation, lacked respiratory protective equipment, and were exposed to Ammonia concentrations ranging from 3 to 61 mg/m³ (geometric mean = 7.3 mg/m³; geometric standard deviation = 3). The longest and shortest durations of Ammonia measurement in a salon were 45 minutes and 305 minutes, respectively. Peak exposure to Ammonia was evaluated by obtaining the number and duration of peaks above 10.5 mg/m³ for each measurement session in a salon. The percentage of total sampling time when Ammonia levels exceeded 10.5 mg/m³ was very high in 3 salons (percentages of 92%, 99.6%, and

75%, respectively). Both groups completed a questionnaire relating to respiratory symptoms during the past 12 months, and lung function and exhaled nitric oxide tests were performed. Blood and sputum samples were collected from all participants. When compared to unexposed controls, hairdressers were found to have a higher level of sputum neutrophil count (i.e., sign of neutrophilic airway inflammation; absolute numbers of neutrophils per mg sputum: 376 (for hairdressers) versus 182 (for controls)). The hairdressers also had significantly elevated exhaled nitric oxide levels and blood C reactive protein levels when compared to control subjects (controlled for age and body mass index).

Inhalation - Risk Assessment

A minimal risk level (MRL) of 1.7 ppm has been derived for “acute-duration” inhalation exposure (14 days or less) to Ammonia. The study involved 16 subjects exposed to Ammonia (50 ppm, 80 ppm, 110 ppm, or 140 ppm). The MRL is based on a LOAEL of 50 ppm for mild irritation to the eyes (6 subjects), nose (20 subjects), and throat (9 subjects) in humans exposed to Ammonia as a gas for 2 hours. The 1.7 ppm MRL was calculated $(50 \text{ ppm} \div 30 [\text{uncertainty factor}] = 1.7; \text{uncertainty factor} = 10 [\text{to protect sensitive individuals}] \times 3 [\text{for use of a minimal LOAEL}] = 30)$.⁷⁹ It should be noted that the Occupational Safety and Health Administration (OSHA) has established an 8-hour time weighted average exposure limit of 50 ppm (35 mg/m³) for Ammonia in the workplace.⁸⁰ Exposure to Ammonia shall not exceed the 50 ppm limit in any 8-h work shift of a 40-h work week.

Chronic occupational exposure (about 12.2 years) to low levels of airborne Ammonia (9.2 ppm) had no significant effect on pulmonary function or odor sensitivity in a group of workers at a soda ash factory, compared to a control group from the same factory that was not exposed to Ammonia.⁸⁵ The ATSDR derived a chronic inhalation MRL of 0.1 ppm for Ammonia from this study.⁹ An MRL is an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse non-cancer health effects over a specified duration of exposure. MRLs are based on non-cancer health effects only and are not based on a consideration of cancer effects. Derivation of the MRL is described below.

An MRL of 0.1 ppm has been derived for chronic-duration inhalation exposure (365 days or more) to Ammonia. The MRL is based on a NOAEL of 9.2 ppm for sense of smell, prevalence of respiratory symptoms (cough, bronchitis, wheeze, dyspnea, and others), eye and throat irritation, and lung function parameters (forced vital capacity (FVC), forced expiratory volume at end of 1 second of forced expiration (FEV1), FEV1/FVC, forced expiratory flow at 50% of FVC (FEF50), and FEF at 75% of FVC (FEF75)) in humans exposed for an average of 12.2 years in a soda ash plant; no LOAEL was determined.⁸⁵ The cohort consisted of 52 workers and 35 controls (all males). The subjects were assessed on the first and last workday of their workweek. Spirometry was performed at the beginning and end of each work shift, so that each worker had four tests done. To determine the exposure levels, exposed and control workers were sampled (breathing zone air sample) over one work shift; the average sample collection period was 8.4 hours. Air samples were collected on sulfuric acid-treated silica gel adsorption tubes (tube holder attached to the collar).

Analysis of the results showed no significant differences in the prevalence of reported symptoms, but the exposed workers reported that exposure in the plant aggravated some of their reported symptoms (cough, wheeze, nasal complaints, eye irritation, and throat discomfort). There were no significant differences in baseline lung functions between exposed and control subjects. Analysis of each worker separately showed no significant relationship between the level of Ammonia exposure and changes in lung function. Also, when the workers were divided into groups of individuals that were exposed to low (< 6.25 ppm), medium (6.25 – 12.5 ppm), and high (> 12.5 ppm) Ammonia levels, no significant association was found between reporting of symptoms, decline in baseline function, or increasing decline in function over the work shift and exposure to Ammonia. Furthermore, no association was evident between increasing years of exposure and decreasing lung function. However, the power of the indices of both level and length of exposure is low because only eight workers were in areas with relatively high Ammonia exposure. The MRL was calculated by adjusting the mean time-weighted average (TWA) exposure concentration of 9.2 ppm for continuous exposure (8/24 hours x 5/7 days) and dividing by an uncertainty factor of 10 to protect all of the sensitive individuals. A modifying factor of 3 was added for the lack of reproductive and developmental studies.⁹

Based on occupational epidemiology studies, the EPA calculated a chronic inhalation reference concentration (RfC) of 0.5 mg/m³.² The critical effects in these studies were decreased lung function and respiratory symptoms.^{86,85,87,88} The RfC is an estimate (with uncertainty ~ one order of magnitude) of a continuous inhalation exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime.

SUMMARY

The safety of Ammonia and Ammonium Hydroxide as used in cosmetics is reviewed in this safety assessment. According to the *Dictionary*, both ingredients function as pH adjusters in cosmetic products. Additionally, Ammonia

functions as an external analgesic and fragrance ingredient and Ammonium Hydroxide functions as a denaturant in cosmetic products. Functioning as an external analgesic is not a cosmetic use and, therefore, the Panel did not evaluate safety in relation to that use in cosmetics. Additionally, the function of fragrance may be excluded from the purview of the Panel.

According to 2017 VCRP data, Ammonia is being used in 599 cosmetic products (mostly rinse-off products) and Ammonium Hydroxide is being used in 1354 cosmetic products (mostly rinse-off products). These uses of both ingredients are mostly in hair coloring products. The results of a concentration of use survey provided by the Council in 2017 indicate that the highest maximum cosmetic use concentration of Ammonia is 4.6% (in rinse off products (hair dyes and colors)) and the highest maximum cosmetic use concentration of Ammonium Hydroxide is 12.5% (in rinse off products (hair dyes and colors)). Regarding use concentrations in leave-on products, the highest maximum cosmetic use concentrations are 0.73% (Ammonia - in tonics, dressings, and other hair grooming aids) and 1.5% (Ammonium Hydroxide - in face and neck products (not spray)).

These two ingredients are indistinguishable from each other in aqueous formulation. Since the only cosmetic function of Ammonia applicable to this safety assessment is pH adjuster (which by default means aqueous formulations only) and Ammonium Hydroxide does not exist outside of water, regardless of which ingredient is added the final formulations will contain an equilibrium of molecular Ammonia and the ions of Ammonium Hydroxide in water. Thus, whether toxicity data is reported for Ammonia or Ammonium Hydroxide, it is applicable to both (as the test articles would have had this same equilibrium).

A large amount of metabolically-generated Ammonia is absorbed into the blood and is detoxified by the liver. The urea cycle (in liver), a cycle of biochemical reactions that produces urea from Ammonia, is the major pathway for Ammonia detoxification in terrestrial mammals. The normal range for Ammonia blood serum levels has been reported as 10-40 $\mu\text{mol/L}$.

An acute oral LD_{50} of 350 mg/kg has been reported in a study involving rats dosed orally with Ammonia dissolved in water. Gastric lesions in rats have been observed after oral dosing (gavage) with 0.03% to 1% Ammonia and 1% and 3% Ammonium Hydroxide. The increase in gastric lesions observed was both concentration- and pH-dependent.

It has been noted that acute exposure data have demonstrated that injury to respiratory tissues is primarily due to Ammonia's alkaline (i.e., caustic) properties from the formation of hydroxide when it reacts with water. In acute inhalation toxicity studies, Ammonia was tested at concentrations ranging from 3.5 ppm (cats and rabbits, 1 h exposure) to 54,289 ppm (rats, 10-minute exposure). Exposure to the highest concentration resulted in hemorrhagic lungs, and increased respiratory fluid output was noted at the lowest concentration. In 10-minute exposure studies involving mice, LC_{50} values ranging from 8723 ppm to 10,150 ppm have been reported. In mice exposed to Ammonia (100 - 800 ppm) for 30 minutes, an RD_{50} of 303 ppm was reported. Within the range of concentrations tested (3440 ppm to 12,940 ppm) in 1-h exposure studies involving mice, the following effects have been observed: hepatic lesions, congestion, and necrosis; eye irritation; dyspnea; pneumonitis and atelectasis; histopathological changes in the lung (alveolar disruption and loss of septal continuity), and, in some cases, coma and death.

Exposure durations ranged from 10 minutes (14,170-55,289 ppm) to 1-4 h (3028-5053 ppm) in acute inhalation toxicity studies involving rats. For the 10-minute exposure, LC_{50} values were ~ 22,885 ppm (males) and ~31,430 ppm (females) (at highest exposure concentration) and ~14,141 ppm (males) and ~19,769 ppm (females) (at lowest exposure concentration). For the 1 h and 4 h exposures, the LC_{50} s were ~17,633 ppm and ~7068 ppm, respectively, and corneal opacity and signs of typical upper respiratory tract irritation were observed.

In short-term oral toxicity studies involving rats, doses of ~ 42 mg/kg/day for 8 weeks resulted in mucosal atrophy in the stomach antrum, and doses up to 1500 mg/kg/day for 35 days resulted in treatment-related changes in body weight, hematological findings, clinical biochemistry findings, and non-neoplastic histopathological findings.

Ammonia was evaluated at concentrations ranging from 0.6 ppm to 1306 ppm in short-term inhalation toxicity studies. The results of these studies indicate histopathological changes of respiratory tissues in several animal species (lung inflammation in guinea pigs and rats; focal or interstitial pneumonitis in monkeys, dogs, rabbits, and guinea pigs; pulmonary congestion in mice; thickening of nasal epithelium in rats and pigs; nasal inflammation or lesions in rats and mice) across different dosing regimens. In general, responses in respiratory tissues increased with increasing Ammonia exposure concentration.

Fatty changes of liver plate cells were seen in rats following continuous exposure to Ammonia (642 ppm) for 90 days. Mild congestion/degenerative changes in internal organs were reported for guinea pigs exposed to ~ 170 ppm Ammonia for 18 weeks. Damaged tracheal mucosae were observed in rats exposed repeatedly to Ammonia (100 ppm) for 12 weeks. Mild leukocytosis was noted in rats after exposure to 143 ppm, but not 43 ppm of Ammonia repeatedly for 3 months.

(25- or 60-minute exposures every 48 h). A low incidence of mortalities (13 of 515 rats and 4 of 15 guinea pigs) was observed in mice and guinea pigs exposed continuously to 671 ppm Ammonia (reported as Ammonium Hydroxide) for 90 days. However, there were no mortalities or treatment-related histopathological findings in rats, guinea pigs, rabbits, monkeys, or dogs exposed continuously to ~57.43 ppm for 114 days.

Enlarged adrenal glands were observed in rabbits that received 124 mg /kg/day Ammonia (as Ammonium Hydroxide) by gavage in water for 17 months.

In a developmental toxicity study involving pregnant rats exposed to Ammonia in the diet (4293 mg/kg/day; as the ammonium ion [read-across for Ammonia]) from gestation day 1 through day 21 of lactation, body weights of male and female offspring were reduced. Neither reproductive nor developmental toxicity were reported in a study in which female pigs were exposed (inhalation exposure) to ~7 ppm or ~35 ppm Ammonia from 6 weeks prior to breeding until day 30 of gestation. In an oral reproductive and developmental toxicity study on diammonium phosphate (identified as an appropriate read across material for developmental and reproductive toxicity and included herein to support the overall weight of evidence) involving rats in which embryos were treated *in vitro* and then implanted, a NOAEL of 1500 mg/kg/day (highest dose tested) was reported.

In the Ames test with and without metabolic activation, Ammonia was non-genotoxic in *S. typhimurium* strains and in *E. coli* strain WP2 uvr A. Without metabolic activation, it was non-genotoxic to *E. coli* strain Sd-4-73. An increased frequency of micronuclei (compared to controls) was observed in Swiss albino mice that received single i.p. doses of Ammonia (12, 25, or 50 mg/kg). The maximum number of micronucleated polychromatic erythrocytes was associated with mice that received the highest dose (50 mg/kg), and there was clear correlation of dose-yield effects. In another micronucleus test, groups of 10 (5 males, 5 females) ddY mice received single i.p. doses of 62.5, 125, 250 and 500 mg/kg ammonium chloride (read-across for Ammonia, or i.p. doses of 31.3, 62.5, 125, and 250 mg/kg ammonium chloride (4 injections within 24 h). Ammonium chloride was not genotoxic.⁶

Increased frequencies of chromosomal aberrations, sister chromatid exchanges, and mitotic index, with increasing duration of exposure were reported for workers who had been exposed to Ammonia in a fertilizer factory.

Ammonia (whether reported as Ammonia or Ammonium Hydroxide) was not carcinogenic in Swiss and C3H mice dosed orally. A statistically significant increase in the incidence of gastric cancer (70%) was observed in rats dosed orally with MNNG and 0.01% Ammonia, when compared to dosing with MNNG alone. In another study, the size, depth, and metastasis of MNNG-initiated tumors were enhanced in rats dosed orally with Ammonia (~42 mg/kg/day). There was no evidence of a tumorigenic effect in Kid: CFLP mice treated by gavage with ammonia dissolved in water alone at a dose of 42mg/kg/day of Ammonia for 4 weeks or with diethyl pyrocarbonate alone, but 9 of 16 mice treated with a combination of ammonium and pyrocarbonate developed lung tumors. In inbred HSL mice treated by gavage with ammonium ion + diethyl pyrocarbonate during pregnancy and lactation, lung tumors were not observed in mice treated during pregnancy or in the suckling mice of dams treated during lactation. Ammonium sulfate was classified as non-carcinogenic in rats in a study involving dietary concentrations up to 3% daily for 104 weeks. Neoplastic lesions were observed in this study, but were deemed not treatment-related because of the spontaneous occurrence of these lesions in the rat strain (F344/DuCrj) that was tested.

It has been reported that hyperammonemia (a metabolic disturbance characterized by an excess of Ammonia in the blood) is associated with neuronal cell loss and cerebral atrophy that lead to mental retardation and cerebral palsy in pediatric patients.

At a concentration of 0.01 mg/ml Ammonia, bovine lymphocyte viability was significantly reduced after 24 h and 48 h of incubation. In another study, the mitogenic response of lymphocytes was reduced after preincubation with Ammonia.

Guinea pigs exposed to 90 ppm Ammonia for 3 weeks developed a significant decrease in the cell-mediated immune response to challenge with a derivative of tuberculin.

No overt symptoms of neurological disorders were reported in guinea pigs or monkeys that were exposed to up to 1105 ppm Ammonia for 6 weeks.

The minimum concentration of Ammonia that caused an inflammatory reaction when applied (single application) to the skin of rats and mice (6 per species) was > 25% (rats) and = 25% (mice). In a skin irritation study in which groups of 4 rats, guinea pigs, and mice were injected intradermally with Ammonia (0.01 ml), the minimum concentration that caused a positive reaction was 0.05% in rats, mice, and guinea pigs. Ammonia (reported as Ammonium Hydroxide; 20% and 12%) was corrosive to the skin of rabbits, whereas the 10% concentration was not.

The application of a saturated aqueous solution of Ammonia (reported as Ammonium Hydroxide) to the skin of 16 subjects resulted in blister formation and skin irritation. In a study involving 110 subjects, Ammonia (reported as Ammonium Hydroxide; 1:1 aqueous solution) was applied to the skin and the inflammatory reaction observed was considered slight.

In rabbits, Ammonia (1 mg of Ammonium Hydroxide) was classified as an ocular irritant and 28.5% Ammonia (reported as Ammonium Hydroxide) induced corneal opacity. Additionally, Ammonia caused conjunctivitis in rabbits at concentrations of 1% to 10% (reported as Ammonium Hydroxide), but not 0.3%.

Microscopic damage to the gastric mucosa was observed in the stomachs of male rats exposed (ex vivo) to Ammonia (up to 60 mmol/l of Ammonium Hydroxide) for 15 minutes.

In various clinical reports, ocular, nasal, and throat irritation were observed in human subjects exposed to Ammonia in the 25 ppm to 700 ppm concentration range.

A retrospective study was performed to assess the association between petrochemical exposure and spontaneous abortion. Study participants included 2853 non-smoking women who had been pregnant at least once, 96 of whom had been exposed to unknown Ammonia concentrations. Data analyses did not indicate any effect on spontaneous abortion.

Results from an occupational exposure study indicated that female Palestinian hairdressers exposed to high levels of Ammonia in the workplace had higher neutrophilic airway inflammation when compared to a non-exposed control group.

DISCUSSION

The Panel noted that Ammonia and Ammonium Hydroxide, well-known skin irritants, are indistinguishable from each other in aqueous formulation. Furthermore, since the only cosmetic function of Ammonia applicable to this safety assessment is pH adjuster (which by default means aqueous formulations only) and Ammonium Hydroxide does not exist outside of water, regardless of which ingredient is added, the final formulations will contain an equilibrium of molecular Ammonia and the ions of Ammonium Hydroxide in water. Thus, whether toxicity data are reported for Ammonia or Ammonium Hydroxide, these data are applicable to both (as the test articles would have had this same equilibrium).

The Panel addressed the use of chemicals for read-across, and determined that information reported for the following chemicals is appropriate for read-across to Ammonia and Ammonium Hydroxide: data on “ammonium ion” (reproductive and developmental toxicity, genotoxicity, and carcinogenicity data; counter-ion not identified) that are included in the ATSDR toxicological profile for Ammonia; diammonium phosphate (repeated dose (short-term) oral toxicity and reproductive and developmental toxicity data) that are included in the CIR Final Report on Phosphoric Acid and Its Salts and in an ECHA registration dossier on Ammonia; and data on ammonium chloride (genotoxicity data [micronucleus test]) and ammonium sulfate (oral carcinogenicity and chronic oral toxicity data) that are included also included in the ECHA dossier. Ammonium Hydroxide, diammonium phosphate, ammonium chloride, and ammonium sulfate are all low-formula-weight, inorganic ammonium salts. The Panel stated that, because the distribution and metabolism of the ammonium exposure from these salts should be essentially identical, information on diammonium phosphate, ammonium chloride, and ammonium sulfate is useful for evaluating the safety of Ammonia and Ammonium Hydroxide. The use of these chemicals for read-across is presented in Table 1.

Skin sensitization data are absent from the safety assessment. However, the Panel noted that these ingredients are corrosive, but there are no concerns relating to the sensitization potential of Ammonia or Ammonium Hydroxide. Therefore, the Panel determined that cosmetic products containing these ingredients should be formulated to be non-irritating, and that skin contact should be avoided to minimize irritation.

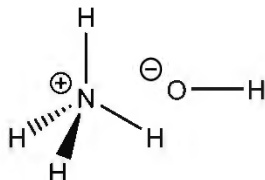
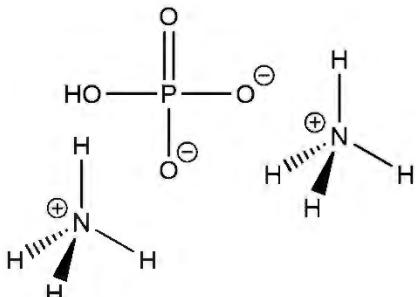
Lastly, the Panel recognized that there are reports of safety issues relating to chronic ingredient exposure experienced by hairdressers, but acknowledged that evaluation of occupational safety is not within the purview of the Panel. However, the Panel reviewed one such report in which hairdressers had signs of airway inflammation after exposure to Ammonia at concentrations up to 61 mg/m³ in relatively small-sized salons with scarce ventilation. The Panel questioned the validity of this study in terms of any association between Ammonia inhalation exposure and airway inflammation, having noted that many chemicals are present in the salon environment.

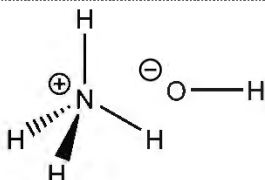
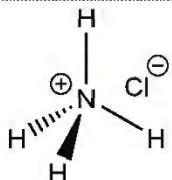
CONCLUSION

The CIR Expert Panel concluded that Ammonia and Ammonium Hydroxide are safe as used in hair dyes and colors and safe in cosmetics applied directly to the skin in the present practices of use and concentration described in the safety assessment when formulated to be non-irritating.

TABLES

Table 1. Read-Across Justifications

Target Material		Read-Across Material
Name	Ammonia/Ammonium Hydroxide	diammonium phosphate
CAS No(s).	7664-41-7; 8007-57-6; 1336-21-6	7783-28-0
Structure		
read-across endpoints	• short-term toxicity – oral • reproductive & developmental	
justification	chemical properties, physical properties and metabolism are expected to be essentially identical for these two ammonium salts	
Examples:		
Formula weight (Da)	35.05	132.03
log K _{ow} (estimated) ^{II}	-4.37 – 0.28 (dissolved NH ₃)	-2.85

Ammonia/Ammonium Hydroxide		ammonium chloride
CAS No(s).	7664-41-7; 8007-57-6; 1336-21-6	1448438-95-6; 12125-02-9
Structure		
read-across endpoints	• genotoxicity; <i>in vitro</i>	
justification	chemical properties, physical properties and metabolism are expected to be essentially identical for these two ammonium salts	
Examples:		
Formula weight (Da)	35.05	53.49
log K _{ow} (estimated) ^{II}	-4.37 – 0.28 (dissolved NH ₃)	-4.37

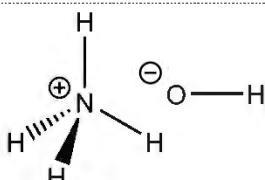
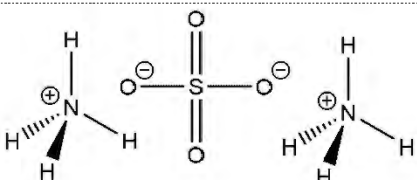
Ammonia/Ammonium Hydroxide		ammonium sulfate
CAS No(s).	7664-41-7; 8007-57-6; 1336-21-6	7783-20-2
Structure		
read-across endpoints	• chronic toxicity; oral • carcinogenicity; oral	
justification	chemical properties, physical properties and metabolism are expected to be essentially identical for these two ammonium salts	
Examples:		
Formula weight (Da)	35.05	132.13
log K _{ow} (estimated) ^{II}	-4.37 – 0.28 (dissolved NH ₃)	0.48

Table 2. Definition, Idealized Structures, and Functions of the Ingredients in this Safety Assessment. ^(1; CIR Staff)

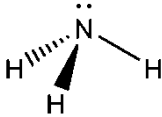
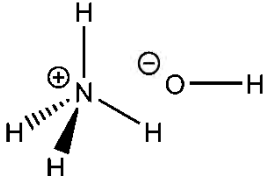
Ingredient CAS No.	Definition & Idealized Structures	Function
Ammonia 7664-41-7 8007-57-6	Ammonia is an inorganic gas that conforms to the formula:  (See also Ammonium Hydroxide)	External Analgesics; Fragrance Ingredients; pH Adjusters
Ammonium Hydroxide 1336-21-6	Ammonium Hydroxide is an inorganic base that conforms to the formula:  [In reality however, the solid, anhydrous salt does not exist. Instead, Ammonium Hydroxide is only present as an aqueous ion pair, the result of protic equilibration upon dissolution (not dissociation of a solid salt), in equilibrium with dissolved ammonia]	Denaturants; pH Adjusters

Table 3. Physical and Chemical Properties of Ammonia and Ammonium Hydroxide

Property	Value	Reference
Ammonia		
physical form and/or color	Gas at room temperature; colorless	9
molecular weight (Daltons (Da))	17.03	9
water solubility (% w/w at 20°C)	33.1	9
Other solubility (%w/w at 25°C)	10 (absolute ethanol); 16 (methanol); soluble in chloroform and ether	9
density (g/L)	0.7710 (gas);	9
density (g/L at -33.5°C and 1 atm)	0.6818 (liquid); 0.7 (liquid)	9,10
vapor density (air = 1)	0.5967	9
specific gravity (g/L at 25°C)	0.747	9
melting point (°C)	-77.7	9,10
boiling point (°C)	-33.35	9,10
autoignition temperature (°C)	650	9
vapor pressure (atm at 20°C)	8.5	9
log K _{ow} (estimated)	0.23	9
Ammonium Hydroxide		
Physical form	Colorless liquid	12
density (g/L at 20°C)	0.89801(28% aqueous)	9
Formula weight (Da)	35.05	10
melting point (°C)	-58 (25% aqueous)	12
boiling point (°C)	38 (25% aqueous)	12
vapor pressure (kPa at 20°C)	48 (25% aqueous)	12
log K _{ow} (estimated)	-4.37	11

Table 4. Frequency and Concentration of Use According to Duration and Type of Exposure.^{16,17}

	Ammonia		Ammonium Hydroxide	
	# of Uses	Conc. (%)	# of Uses	Conc. (%)
Totals/Conc. Range	599	0.00002-4.6	1354	0.00028-12.5
Duration of Use				
<i>Leave-On</i>	7	0.00002-0.73	163	0.003-1.5
<i>Rinse off</i>	592	0.00015-4.6	1191	0.00028-12.5
<i>Diluted for (bath) Use</i>	NR	NR	NR	NR
Exposure Type				
<i>Eye Area</i>	1	NR	42	0.022-0.58
<i>Incidental Ingestion</i>	NR	NR	NR	NR
<i>Incidental Inhalation- Sprays</i>	3***	0.73*	6*	0.29-1.3*
<i>Incidental Inhalation- Powders</i>	3***	0.00002-0.14**	NR	0.45-1.5**
<i>Dermal Contact</i>	6	0.00002-0.14	159	0.0012-1.7
<i>Deodorant (underarm)</i>	NR	NR	NR	NR
<i>Hair - Non-Coloring</i>	10	0.00006-1.4	72	0.00028-3.6
<i>Hair-Coloring</i>	582	2.8-4.6	1104	2.5-12.5
<i>Nail</i>	1	0.00008-0.00075	3	0.003-1.2
<i>Mucous Membrane</i>	NR	NR	1	NR
<i>Baby Products</i>	NR	NR	NR	NR

NR = Not Reported; Totals = Rinse-off + Leave-on + Diluted for Bath Product Uses.

*It is possible that these products may be sprays, but it is not specified whether the reported uses are sprays.

**It is possible that these products may be powders, but it is not specified whether the reported uses are powders.

***Not specified whether a powder or spray, so this information is captured for both categories of incidental inhalation.

Note: Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure type uses may not equal the sum total uses.

Table 5. Acute Oral Toxicity Studies

Ingredient	Animals	Protocol	Results
Ammonia (0.03, 0.1, 0.3, 0.5, or 1%)	Male Wistar rats (groups of 6)	Administered by oral gavage	Minimal concentration at which gastric lesions observed was 0.03%. Over the range of concentrations administered, there was an increase in gastric juice pH and the ulcer index in an Ammonia concentration-dependent manner (mean pH: 4.38 to 8.45). ⁴²
Ammonia (dissolved in water)	Male Wistar rats (groups of 10)	Administered by gavage according to Organization for Economic Co-operation and Development (OECD) Guideline 401. Dosing followed by 14-day observation period	LD ₅₀ (calculated) = 350 mg/kg. ^{6,43,44}
Ammonium Hydroxide (1% or 3%)	Male Sprague-Dawley rats (groups of 4 to 8)	Administered by gavage	Severe hemorrhagic lesions produced in a concentration-related manner. The lesion scores at 1% and 3% concentrations were 26.6 ± 9.3 mm ² and 97.7 ± 8.3 mm ² , respectively. The pH of 3% solution was 11.5. When this pH was decreased up to 7.0, by neutralizing with 0.1 N hydrochloric acid, the ulcerogenic activity of Ammonium Hydroxide was significantly mitigated at pH 10 and completely disappeared at pH 9. ⁴⁵

Table 6. Acute Inhalation Toxicity

Ingredient	Animals/Protocol	Results
Ammonia (21,400 ppm)	Mice. 30-minute exposure	Signs and symptoms included eye irritation (blinking and scratching), dyspnea, frothing, convulsions, excitation/escape behavior, coma, and death. Histopathology of the lungs of mice that died showed alveolar disruption and loss of septal continuity. ^{24,50}
Ammonia (8770-12,940 ppm)	Mice (groups of 20). 10-minute exposure	LC ₅₀ = 10,150 ppm. ^{47,46,48}
Ammonia (8723-12,870 ppm)	Mice (groups of 20). 10-minute exposure	At 8,723 ppm, 25% of the animals died. At 12,870 ppm, 80% of the animals died. LC ₅₀ = 10,096 ppm. ^{24,46}
Ammonia (3600-5720 ppm)	Mice. 1-h exposure	Nasal and eye irritation, followed by labored breathing, in all groups. Gross examination of surviving mice showed mild congestion of the liver at the intermediate (4550 ppm) and high (5720 ppm) concentrations. LC ₅₀ = 4837 ppm (95% CI = 4409–5305 ppm). ^{24,51,48}
Ammonia (1190-4860 ppm)	ICR male mice (groups of 12). 1-h exposure	In animals that survived 14-day observation period, pathologic lesions included mild-to-moderate pneumonitis (dose-related severity), focal atelectasis in the lungs (4,860 ppm), and degenerative hepatic lesions (dose-related severity, 3,440–4,860 ppm). LC ₅₀ = 4,230 ppm. ^{24,52,48}
Ammonia (4840 ppm)	Mice. 1-h exposure	Signs and symptoms included eye irritation (blinking and scratching), dyspnea, frothing, convulsions, excitation/escape behavior, coma, and death. Histopathology of the lungs of mice that died showed alveolar disruption and loss of septal continuity. ^{24,53}
Ammonia (3440 ppm)	Mice (groups of 12). 1-h exposure	Liver necrosis. ⁵²
Ammonia (92 mg/m ³ [~132 ppm] to 1243 mg/m ³ [~1785 ppm])	SPF mice of the OF1-ICO strain. Nose-only exposure for 45 minutes	Mice appeared more susceptible to ammonia in presence of dry air (RD ₅₀ (exposure concentration producing a 50% decrease in respiratory rate) = 582 [407 ppm] and 732 mg/m ³ [547 ppm] in dry and wet air, respectively). ^{24,56}
Ammonia (100-800 ppm)	Male Swiss-Webster mice (groups of 4). 30-minute exposure	RD ₅₀ = 303 ppm (95% confidence limits = 188–490 ppm). ^{24,49,48}
Ammonia (9870 mg/m ³ air [14,170 ppm] to 37,820 mg/m ³ air [54,289 ppm])	SPF-bred Wistar rats (5 males, 5 females/group). Exposures: 20,950 to 37,820 mg/m ³ air (10 minutes); 18,290 to 23,200 mg/m ³ air (20 minutes); 12,620 to 16,840 mg/m ³ air (40 minutes), and 9870 to 13,240 mg/m ³ air (60 minutes)	LC ₅₀ (10-minute exposure) = 28,130 mg/m ³ air (40,300 ppm); LC ₅₀ (20-minute exposure) = 19,960 mg/m ³ air (28,595 ppm); LC ₅₀ (40-minute exposure) = 14,170 mg/m ³ air (20,300 ppm); and LC ₅₀ (60-minute exposure) = 11,590 mg/m ³ air (16,600 ppm). Hemorrhagic lungs in animals that died. ⁵⁵

Table 6. Acute Inhalation Toxicity

Ingredient	Animals/Protocol	Results
Ammonia (9000-35,000 ppm)	Male Sprague-Dawley rats: 4 groups of 6 (9,000 to 26,000 ppm), 1 group of 8 (30,000 ppm), and 1 group of 4 (35,000 ppm). Exposure for 20 minutes in head-only exposure system	Lung edema increased in all groups. Dose-dependent increases in ocular irritation, lacrimation, and labored breathing. LC_{50} (determined by probit analysis) = 23,672 ppm. ⁵⁷
Ammonia (9000 to 23,000 ppm)	Groups of 6 male Sprague-Dawley rats. Exposure for 20 minutes in head-only exposure system for 20 minutes	Peak inspiratory and expiratory flow decreased after exposure to 20,000 and 23,000 ppm. Weight loss, and increased total blood cell counts (white blood cells, neutrophils, and platelets) after exposure to 20,000 ppm. Morphological changes at histopathologic examination of lungs and trachea: alveolar, bronchial, and tracheal edema; epithelial necrosis, and exudate at 20,000 ppm. ⁵⁸
Ammonia (3028-14,044 ppm)	Male and female SPF-bred Wistar rats (Hsd Cpb:WU strain; 5 males, 5 females). Nose-only exposure to 9,222-14,044 ppm for 1 h and 3,028-5,053 ppm for 4 h.	Signs typical of upper respiratory tract irritation. No gross abnormalities in any organ or nasal passages were found at necropsy of surviving rats (2 weeks post-exposure). Rats that died had corneal opacity, collapsed lungs, nasal discharge, reddened larynx, and tracheal epithelial desquamation. LC_{50} (1-h exposure) = 12,303 mg/m ³ [~17,633 ppm]. LC_{50} (4-h exposure) = 4,923 mg/m ³ [~7068 ppm]. ⁵⁴
Ammonia (6210-9840 ppm)	Groups of 10 male CFE rats. 1-h exposure	Signs of eye and nasal irritation observed immediately, followed by labored breathing and gasping. Surviving animals exposed to the low concentration weighed less than controls on day 14, and gross examination showed mottling of the liver and fatty changes at the two highest concentrations. LC_{50} = 7338 ppm (95% CI = 6822–7893 ppm). ^{24,51,48}
Ammonia (431, 1436, and 4307 ppm)	Rats. Inhalation exposure for 5, 15, 30, or 60 minutes	Decrease in static muscular tension and other sublethal effects. ⁴⁸
Ammonia (1436, 4307, and 6814 ppm)	White rats (number not stated). Inhalation exposure for 5, 15, 30, or 60 minutes	Dyspnea, irritation of respiratory tract and eyes, cyanosis of extremities, and increased excitability. ⁴⁸
Ammonia (92 mg/m ³ [~132 ppm] to 1243 mg/m ³ [~1785 ppm])	Groups of 4 male specific pathogen free (SPF) Wistar rats of the Hsd Cpb:WU (SPF) strain. Nose-only exposure for 45 minutes	RD_{50} = 972 and 905 mg/m ³ (corresponding to ~1396 and ~1299 ppm, respectively) in rats in dry and wet air, respectively. ^{24,56}
Ammonia (144 ppm)	Rats (number not stated). Inhalation exposure for 5, 10, 15, 30, or 60 minutes	No effects. ⁴⁸
Ammonia (5,200-12,800 ppm)	Rabbits. 1-h exposure	Average survival: 18 h (gassed after cannulation), 33 h (gassed before cannulation). 2- to 3-fold increase in production of respiratory tract fluid. No change in water content of lungs. Increased blood hemoglobin. Increased plasma lipids. ²⁴

Table 6. Acute Inhalation Toxicity

Ingredient	Animals/Protocol	Results
Ammonia (10,360 ppm, average)	Rabbits. 1-h exposure	Congestion of respiratory tract tissues. ²⁴
Ammonia (50 ppm and 100 ppm)	16 New Zealand White rabbits. Inhalation Exposure for 2.5 h to 3 h	Significant decrease in rate of respiration. ⁴⁸
Ammonia (3.5 ppm and 8.7 ppm)	54 rabbits. Exposure for 1 h	Increased respiratory tract fluid output by 2- to 3-fold. No appreciable effect on water content of respiratory tract tissues. Transient decrease in blood hemoglobin. Lipemia also observed. ⁴⁸
Ammonia (5,200-12,800 ppm)	Cats. 1-h exposure	Average survival: 18 h (gassed after cannulation), 33 h (gassed before cannulation). 2- to 3-fold increase in production of respiratory tract fluid. No change in water content of lungs. Increased blood hemoglobin. Increased plasma lipids. ^{47,65}
Ammonia (10,360 ppm, average)	Cats. 1-h exposure	Congestion of respiratory tract tissues. ^{47,65}
Ammonia (1,000 ppm)	20 cats. 10-minute exposure	Biphasic course of respiratory pathology. Effects at 24 h post-exposure included severe dyspnea, anorexia, and dehydration; rhonchi and coarse rales evident upon auscultation. Gross pathology revealed varying degrees of congestion, hemorrhage, edema, interstitial emphysema, and collapse of the lungs at all time points. Pulmonary resistance increased throughout the study. ^{48,64}
Ammonia (3.5 ppm and 8.7 ppm)	18 cats. Exposure for 1 h	Increased respiratory tract fluid output by 2- to 3-fold. No appreciable effect on water content of respiratory tract tissues. Transient decrease in blood hemoglobin. ⁴⁸

Table 7. Short-Term and Subchronic Toxicity Studies

Ingredient	Animals	Protocol	Results
Short-term Oral Studies			
Ammonia (0.01% in drinking water)	Rats (groups of 36)	5 groups initially received tap water : group 1 (for 7 weeks and 4 days), group 2 (7 weeks), group 3 (6 weeks), group 4 (4 weeks), and group 5 (0 water before Ammonia dosing). Groups then received Ammonia at dose of ~ 42 mg/kg/day for 8 weeks.	No mucosal lesions at macroscopic or microscopic examination. Mucosal atrophy in stomach antrum and enlargement of proliferative zone in antral and body mucosa. ⁶⁷
diammonium phosphate (read-across for Ammonia, 17.9% N and 46.86% P ₂ O ₅ equivalent)	Groups of Crj: CD(SD) rats (5 males, 5 female/group)	Administered by gavage daily (doses of 0, 250, 750, and 1500 mg/kg/day, 7 days/week) for 35 days	Clinical signs were not observed, and none of the animals died. However, there were treatment-related changes in body weight, hematological findings, clinical biochemistry findings, and non-neoplastic histopathological findings. Histological examination of stomachs revealed some submucosal inflammation at all doses, but this change was not dose-dependent and was not statistically significant at the low dose. NOAEL for general toxicity of diammonium phosphate = 250 mg/kg/day; LOAEL for general toxicity of diammonium phosphate = 750 mg/kg/day. ^{6,48}
Short-term Inhalation Studies			
Ammonia (~1306 ppm)	Rats	5 days/week (8 h/day)	Exposure tolerated for 42 days. ⁷³
Ammonia (~223 ppm or ~1105 ppm)	Sprague-Dawley and Long-Evans rats (males and females, groups of 15); Male New Zealand albino rabbits (groups of 3); Princeton-derived guinea pigs (males and females, groups of 15); Male squirrel monkeys (<i>Saimiri sciureus</i> , groups of 3); Beagle dogs (groups of 2)	Exposure 5 days per week (8 h/day) for 6 weeks	Lung effects: Gross necropsies normal. Focal pneumonitis in 1 of 3 monkeys at 223 ppm. Nonspecific lung inflammation in guinea pigs and rats, but not in other species at 1105 ppm. Upper respiratory tract effects: mild to moderate dyspnea in rabbits and dogs exposed to 1105 ppm during week 1 only; no indication of irritation after week 1. Nasal turbinates not examined for gross or histopathologic changes. ^{2,40,73}
Ammonia (1086 ppm)	Rats, squirrel monkeys, and guinea pigs (number per species not stated)	Inhalation exposure 5 days per week (8 h/day) for 6 weeks	No fatty changes of liver plate cells. No pathological changes in kidney. ⁹
Ammonia (653 ppm)	Rats (number not stated)	Continuous inhalation exposure for 25 days	Nearly 64% lethality. ⁹
Ammonia (~653 ppm)	Sprague-Dawley or Long-Evans rats (males and females, 15 to 51/group)	Inhalation exposure for 65 days	Lung effects: Focal or diffuse interstitial pneumonitis in all animals. Upper respiratory tract effects: Dyspnea and nasal irritation/discharge. ^{2,73}
Ammonia (650 ppm; Ct [product of concentration and exposure time (ppm-h)] = 390,000 and 1,014,000)	51 rats	Continuously for 65 days	32 of 51 rats dead by day 25 (390,000 ppm-h); 50 of 51 rats dead by day 65 (1,014,000 ppm-h). ^{47,73}

Table 7. Short-Term and Subchronic Toxicity Studies

Ingredient	Animals	Protocol	Results
Ammonia (500 ppm)	27 male rats	Continuous inhalation exposure for up to 8 weeks	After 3 weeks, nasal irritation and inflammation of upper respiratory tract, but no effects observed in bronchioles and alveoli. No lesions observed at 8 weeks. ^{48,74}
Ammonia (250 ppm)	F344 rats (6/sex/group)	Exposure in inhalation chamber for 35 days	Increased thickness of nasal epithelium (3 to 4 times) and nasal lesions at 150 ppm. ^{2,72}
Ammonia (221 ppm; Ct [ppm-h] = 53,040)	Rats, guinea pigs, rabbits, squirrel monkeys, and beagle dogs (number per species not stated)	5 days per week (8 h per day) for 6 weeks	No effect. ^{47,73}
Ammonia (10 or 150 ppm)	Sherman rats (5/sex/group)	Inhalation exposure from bedding for 75 days	Increased thickness of nasal epithelium (3 to 4 times) and nasal lesions at 150 ppm. ^{2,48,72}
Ammonia (50 or 90 ppm)	Male Wistar rats (8-14 per group)	Inhalation exposure continuously for 50 days	None of the animals died and there were no treatment-related effects. ^{48,76}
Ammonia (12% solution)	50 male White albino mice	Vapor exposure 6 days per week (15 minutes/day) for 4, 5, 6, 7, or 8 weeks	Nasal mucosa adversely affected. Histological changes progressed from weeks 4–8 from crowding of cells forming crypts and irregular arrangements to epithelial hyperplasia, patches of squamous metaplasia, loss of cilia, and dysplasia of the nasal epithelium. One animal that had loss of polarity of the epithelium, hyperchromatism, and mitotic figures with an intact basement membrane also had a carcinoma <i>in situ</i> in one nostril. At week 8, one mouse had an invasive adenocarcinoma of the nasal mucosa. Histochemical results were also abnormal. ^{2,71}
Ammonia (78 ppm, 271 ppm, and 711 ppm)	Groups of 10 male Swiss mice	Exposure for 4, 9, or 14 days (6 h/day)	No clinical signs of toxicity were noted for mice exposed to ammonia. Rhinitis and pathologic lesions with metaplasia and necrosis were seen only in the respiratory epithelium of the nasal cavity of mice inhaling 711 ppm, the severity of the lesions increased with duration of exposure, ranging from moderate on day 4, severe on day 9, to very severe on day 14. No lesions were seen in the controls or in mice inhaling the 78 ppm. No effects were seen at 271 ppm, even after 9 days of exposure. ^{24,68}
Ammonia (303 ppm)	Groups of 16 to 24 male Swiss Webster mice	Exposure for 5 days (6 h/day)	Histopathological findings, which were confined to the respiratory epithelium of the nasal cavity, included minimal exfoliation, erosion, ulceration, and necrosis; moderate inflammatory changes; and slight squamous metaplasia. ^{24,69}

Table 7. Short-Term and Subchronic Toxicity Studies

Ingredient	Animals	Protocol	Results
Ammonia (20 ppm)	Swiss albino mice (males and females, groups of 4)	Exposure for 7, 14, 21, 28, or 42 days	Lung congestion, edema, and hemorrhage observed after 42 days. ^{2,70}
Ammonia (170 ppm; Ct [ppm-h] =30,600 to 91,800)	12 male Guinea pigs	5 days per week (6 h per day) for 6 weeks	No histopathologic changes. ^{47,81}
Ammonia (50 ppm)	Guinea pigs (males and females, groups of 6)	Exposure for 42 days	Lung congestion, edema, and hemorrhage. ^{2,70}
Ammonia (20 ppm)	Guinea pigs (males and females, groups of 2)	Exposure for 7, 14, 21, 28, or 42 days	Lung congestion, edema, and hemorrhage after 42 days. ^{2,70}
Ammonia (100 ppm [average range = 20 to 203 ppm; Ct [ppm-h] =100,800) alone and with corn starch dust	Yorkshire-Landrace pigs (groups of 6)	Continuously for 6 weeks	Tracheal damage (thickened tracheal epithelium [50 to 100% increase] and goblet cells reduced) at end of week 2 in animals exposed to 100 ppm (33,600 ppm-h) without dust. Changes more prominent by week 6. Conjunctival irritation more severe in pigs exposed to ammonia and corn starch dust, persisting for 2 weeks. ^{2, 47,135}
Ammonia (10 ppm and 50 to 150 ppm; Ct [ppm-h] = 42,000 to 126,000)	Duroc Pigs (groups of 36)	Continuously for 5 weeks	Excessive nasal, lacrimal and mouth secretions at 50, 100, and 150 ppm; more pronounced at 100 and 150 ppm, gradually diminishing over 1-2 weeks. No histopathologic changes in nasal turbinates or lung. ^{6,47,75}
Ammonia (12, 61, 103, or 145 ppm)	Duroc pigs (males and females, groups of 9)	Exposure for 5 weeks	Excessive nasal, lacrimal, and mouth secretions, and increased frequency of cough at 103 and 145 ppm. ^{2,75}
Ammonia (5 ppm [range = 0 to 7 ppm] to 100 ppm [range = 90 to 112 ppm])	Belgian Landrace pigs (groups of 7)	Nasal lavage technique. 6-day exposure in chamber	No-observed-effect value for Ammonia-induced somatic growth inhibition < 25 ppm. Nasal irritation down to 25 ppm. Conjunctival irritation observed in 4 pigs exposed to 100 ppm. Lethargy in groups exposed to 25, 50 and 100 ppm for 2 to 3 days after placement in chamber. ⁷⁸
Ammonia (0.6, 10, 18.8, or 37 ppm)	Pigs (different breeds, groups of 24)	Inhalable dust exposure for 5 weeks	No increase in incidence of respiratory diseases. ^{2, 77}
Ammonia (~1.8, ~3.9, ~7.3, or ~14.2 ppm)	Pigs (different breeds, groups of 24)	Inhalable dust exposure for 5 weeks	No increase in incidence of respiratory diseases. ^{2, 77}
Subchronic Inhalation Studies			
Ammonia (642 ppm)	Rats (number not stated)	Continuous exposure for 90 days	Fatty changes of liver plate cells. ⁹
Ammonia (43 ppm or 143 ppm)	White rats (number not stated)	Inhalation exposure for 3 months (25- or 60-minute exposures every 48 h)	Mild leukocytosis after exposure to 143 ppm. No adverse effects after exposure to 43 ppm. ⁴⁸
Ammonia (100 ppm)	Rats	Inhalation exposure 5 days per week (5 h/day) for 12 weeks	Damaged tracheal mucosae. ^{47,82}

Table 7. Short-Term and Subchronic Toxicity Studies

Ingredient	Animals	Protocol	Results
Ammonia (~170 ppm)	12 male guinea pigs (additional 6 were controls)	Inhalation exposure 5 days per week (6 h/day) for 18 weeks	No significant findings after 6 and 12 weeks of exposure. Results at 18 weeks were: relatively mild congestion of the liver, spleen, and kidneys; degenerative changes in adrenal glands; hemosiderosis in spleen (indicative of hepatotoxicity); and cloudy swelling in epithelium of proximal kidney tubules, with albumin precipitation in lumen. ^{47,81}
Ammonium Hydroxide (671 ppm)	515 rats and 15 guinea pigs	Inhalation exposure continuously for 90 days	13 rats and 4 guinea pigs died. ⁴⁸
Ammonium Hydroxide (~57.43 ppm)	15 Sprague-Dawley/Long-Evans rats (males and females), 15 Princeton-derived guinea pigs (males and females), 3 male New Zealand albino rabbits, 3 male squirrel monkeys, and 2 purebred male beagle dogs	Inhalation exposure continuously for 114 days	No mortalities or signs of toxicity. Necropsy observations were normal and there were no treatment-related histopathological findings. ⁷³

Table 8. Developmental and Reproductive Toxicity Studies

Ingredient	Animals/Embryos	Protocol	Results
In Vitro/In Vivo Study			
Ammonium ion (read-across for Ammonia, 38 to 300 $\mu\text{mol/l}$)	Mouse embryos (conceived in vivo)	Embryos cultured in modified mouse tubal fluid medium (mMTF) or mMTF supplemented with 300 $\mu\text{mol/L}$ ammonium ion for 48, 69, or 93 h before being transferred to pseudo-pregnant mouse dams	Examination on gestational day 15 showed apparent relationship between the duration of exposure and the incidence of exencephaly. Increased incidence of exencephaly with increased ammonium concentration (38–300 $\mu\text{mol/L}$) and decreased percentage of implantation sites with increased ammonium concentration. ⁸⁹
Oral Studies			
Ammonium ion (read-across for Ammonia)	Pregnant rats	Feeding with ammonium ion in the diet (4293 mg ammonium/kg/day) from gestation day 1 through day 21 of lactation	Growth of rats exposed during pregnancy and lactation was significantly lower than in controls until approximately day 60. Body weights of offspring reduced by 25% (males) and 16% (females). ^{9,90}
diammonium phosphate (read-across for Ammonia, 17.9% NH_4 and 46.86% P_2O_5 equivalent)	Groups of Crj: CD(SD) rats (5 males, 10 females [reproductive subgroup])	Administered by gavage daily (doses of 0, 250, 750, and 1500 mg/kg/day) for, at most, 28 days (males) and 53 days (females).	No treatment-related deaths and no signs of overt clinical toxicity. Body weight gain was reduced during the first week of gestation (82% of control) in females dosed with 1500 mg/kg/day, but returned to control levels for remainder of study. Mating performance and fertility were unaffected by treatment, and parental treatment had no apparent effect on the offspring to day 4 of age. NOAEL for reproductive and developmental toxicity = 1500 mg/kg/day; LOAEL not identified. ^{6,44}
Inhalation Study			
Ammonia (7 ppm or 35 ppm)	Groups of 40 gilts (Yorkshire x Hampshire x Chester White)	Exposure for 6 weeks (7 ppm or 35 ppm). Exposure to ~7 ppm or ~35 ppm from 6 weeks prior to breeding until day 30 of gestation	No statistically significant differences in ovarian or uterine weights after 6 weeks of exposure. After exposure from 6 weeks prior to breeding until day 30 of gestation, no statistically significant differences in age at puberty, number of live fetuses, fetal length, or fetus-to-corpus luteum ratio compared to pigs exposed to only about 7 ppm. No unexposed controls were included in this study. ⁹¹

Table 9. Carcinogenicity and Tumor Promotion Studies

Ingredient	Animals	Protocol	Results
Oral Studies			
Ammonia (dissolved in water)	Kid: CFLP mice	Development of lung tumors can be observed in Kid: CFLP mice treated intragastrically with diethyl pyrocarbonate and ammonia. The lung tumors may result from a carcinogenic substance, supposedly urethane, formed in vivo from diethyl pyrocarbonate in the presence of ammonia. Animals treated with 200 mg/kg diethyl pyrocarbonate and or 42 mg/kg Ammonia dissolved in water (8.4 mg/ml) by stomach tube twice per week for 4 weeks. Necropsy at 20 weeks after first treatment. Number of tumors counted under dissecting microscope.	No evidence of a tumorigenic effect was found in mice treated by gavage with ammonia dissolved in water alone at a dose of 42 mg Ammonia/kg/day for 4 weeks or with diethyl pyrocarbonate alone, but 9 of 16 mice treated with a combination of ammonium and pyrocarbonate developed lung tumors. ⁹³
Ammonium Hydroxide	Swiss and C3H mice	Exposure of mice to 193 mg ammonium/kg/day, as Ammonium Hydroxide (in drinking water), for 2 years	No carcinogenic effects, and did not affect spontaneous development of breast cancer (adenocarcinoma), which is common to C3H female mice. ^{44, 48, 94}
Ammonium ion (read-across for Ammonia, and diethyl pyrocarbonate)	Groups of 16 inbred HSL mice	Exposure (by gavage) during pregnancy and lactation	No lung tumors on the surfaces of the lungs of mice treated during pregnancy or the suckling mice of dams treated during lactation. ⁹⁵
Ammonium Sulfate (read-across for Ammonia)	Groups of 100 F344/DuCrj rats (50 males and 50 females per group)	Dietary concentrations of 0%, 1.5%, and 3% daily for 104 weeks	<u>Survival rates</u> : males - 88% (controls), 78% (1.5% group), and 76% (3% group); females - , 76% (controls), 80% (1.5% group), and 80% (3% group) <u>gross finding</u> : massive, nodular or focal lesions suggestive of neoplastic change <u>neoplastic lesions</u> (not treatment-related; occur spontaneously in rats of this strain): C-cell adenomas/adenocarcinomas in the thyroids, fibroadenomas/adenomas/adenocarcinomas in mammary glands, adenomas/adenocarcinomas in pituitary glands, interstitial cell tumors in testes, and endometrial stromal polyps in uteri <u>non-neoplastic lesions</u> : the incidence of chronic nephropathy was statistically significantly increased in low-dose males No evidence of long-term carcinogenic activity. ⁸⁴

Table 9. Carcinogenicity and Tumor Promotion Studies

Ingredient	Animals	Protocol	Results
Ammonium Sulfate (read-across for Ammonia)	Groups of 10 F344/DuCrj rats (male and female)	Dietary concentrations of 0%, 0.1%, 0.6%, and 3% for 52 weeks	Neoplastic lesions reported included malignant pheochromocytoma of the adrenal gland in males of the 3% dietary group, 2 adenomas in the anterior pituitary of females of the 3% dietary group, and uterine endometrial stromal polyp in a female control rat. ⁶
Ammonia (12% solution)	10 male mice	Inhalation Study Vapor exposure 6 days per week (15 minutes/day) for 4, 5, 6, 7, or 8 weeks	Histological changes progressed (weeks 4 to 8) from crowding of cells forming crypts and irregular arrangements to epithelial hyperplasia, patches of squamous metaplasia, loss of cilia, and dysplasia of the nasal epithelium. One mouse had a carcinoma <i>in situ</i> in 1 nostril. At week 8, 1 mouse with invasive adenocarcinoma of the nasal mucosa. Authors noted that prolonged exposure to Ammonia may interfere with normal protective reflexes of the respiratory nasal mucosa, resulting in the accumulation of particulate matter initiating or promoting a neoplastic process. ⁷¹
Ammonia (dissolved in water)	Rats	Tumor Promotion Rats pretreated with the initiator <i>N</i> -methyl- <i>N'</i> -nitro- <i>N</i> -nitrosoguanidine (MNNG) in drinking water for 4 weeks, prior to receiving 0.01% Ammonia solution in drinking water for 24 weeks	Statistically significantly greater incidence of gastric cancer (70% of rats) and number of tumors per tumor-bearing rat (2.1) than rats that received only MNNG and tap water (31% and 1.3 tumors/rat). ^{48,96}
Ammonia	Rats	Rats pretreated with MNNG prior to dosing with Ammonia (~ 42 mg/kg/day)	The size, depth, and metastasis of the MNNG-initiated tumors enhanced in rats dosed with Ammonia. ⁹⁷

Table 10. Dermal Irritation Studies

Ingredient	Animals/Subjects/Cells	Protocol	Results
Skin Irritation Studies			
<u>In Vitro Studies</u>			
Undiluted Ammonium Hydroxide (30% active material in neat substance)	Reconstructed human skin cultures	Test substance applied topically to stratum corneum surface of cultures. Skin culture damage or cytotoxicity measured as decreased 3-[4,5-dimethylthiazol-2-yl] 2,5-diphenyltetrazolium bromide (MTT) vital dye metabolism. In time-course experiments, the time (in minutes) of test material exposure eliciting a 50% reduction of MTT metabolism (i.e., t_{50} value) was calculated.	Histologic examination of the cultures indicated gradations of epidermal necrosis quantitated using a specially designed grading scale, which correlated well with the corrosivity of treatment chemicals and cytotoxicity measurements. Ammonium Hydroxide (30% active in neat substance) was classified as corrosive (t_{50} = 0.90 minutes). ¹¹⁸

Table 10. Dermal Irritation Studies

Ingredient	Animals/Subjects/Cells	Protocol	Results
<u>Animal Studies</u>			
Ammonia	Wistar rats (3 males, 3 females) and ddY mice (3 males, 3 females)	Test solutions (1 ml/kg or 1 g/kg) applied once, unoccluded, to shaved skin of the back. Area of application was 3 x 4 cm for rats and 1 x 2 cm for mice. Distilled water control. Test sites observed for inflammatory reactions for 1 week after application.	Minimum concentration of Ammonia that caused a positive reaction was >25% (minimum amount = >250 mg/kg) in rats and 25% (minimum amount = 250 mg/kg) in mice. ¹¹⁹
Ammonia	Wistar rats (4), Hartley guinea pigs (4), and ddY mice (4)	Injected intradermally with test solutions (0.01 ml) at 4 spots on shaved dorsal skin. Saline served as the control. The test sites were evaluated for skin irritation for up to 1 week after application.	The minimum concentration that resulted in a positive reaction was 0.05% in rats (minimum amount = 25 µg/kg), mice (minimum amount = 250 µg/kg), and guinea pigs (minimum amount = 12.5 µg/kg). ¹¹⁹
Ammonium Hydroxide (10% and 20%)	Groups of 3 New Zealand Albino rabbits	Each concentration (0.5 ml) applied to the skin (2 replicates at each dose)	Results positive for skin corrosion at 20% concentration. Negative results at 10% concentration. ^{20,44}
Ammonium Hydroxide (10% and 12% aqueous)	Female Albino New Zealand White rabbits (groups of 3)	Each solution (0.1 ml) applied, under an occlusive patch (1" x 1"), to the skin for 4 h. There were 3 rabbits per dose, with 2 replicates per rabbit at each concentration.	The 12% solution was corrosive to the skin, but the 10% solution was not. ⁶
<u>Human Studies</u>			
Ammonium Hydroxide (saturated aqueous solution)	16 subjects (10 men, 6 women)	Applied (via a chamber) to middle of ventral aspect of forearm	Formation of a well-defined, sub-epidermal blister (positive reaction) observed within a few minutes of chamber application; skin irritation observed in all subjects. ¹²⁰
Ammonium Hydroxide (1:1aqueous solution)	110 subjects	Test substance (0.5 ml) placed in 8 mm well drilled in acrylic plastic block (3 x 3 x 1 cm) that was strapped to the skin. Block (used to measure minimal blistering time (MBT, indicator of cutaneous irritability, defined as total exposure in well that results in a single bulla, occupying the total area of contact)).	MBT ranged from 3 to 57 minutes. Inflammatory reaction considered slight; healing was rapid and without scarring. Intensity of the dermatitis provoked by a 24-h exposure to sodium lauryl sulfate was strongly correlated with the MBT. ¹²¹
Ammonium Hydroxide solution (50% solution)	Young adults and older adults (number not stated)	Acrylic plastic block with 14 mm well loosely strapped to skin. Well was then filled with 0.5 ml of freshly prepared 1:1 aqueous solution of Ammonium Hydroxide. Site was examined at 30-minute intervals, and blistering response was measured.	Mild discomfort during procedure. The initial response, characterized by the appearance of tiny follicular vesicles, occurred more quickly in older adults. The time required to produce a full blister was greatly prolonged in the aged. ¹²²

Table 11. Ocular Irritation Studies

Ingredient	Animals/Cells	Test Protocol	Results
<u>In Vitro</u>			
Ammonium Hydroxide	Human corneal endothelial cell cultures	⁵¹ Cr-release assay. Performed by loading the cells with isotope, incubating the cells with Ammonium Hydroxide, and measuring the isotope that was recovered in the medium.	Severe ocular irritant (ED ₅₀ = 3.9 x 10 ⁻³ M). ¹²³
<u>Animal</u>			
Ammonia (15, 32, 310, or 1157 ppm vapor concentrations)	Rats (CrI:COBS CD(SD) strain)	In phase 1 of study, groups of 8 rats exposed for 24 h. In phase 2, groups of 14 rats exposed for 3 or 7 days.	No clinical signs or evidence of irritation to the eyes or mucous membranes. No histologic differences in tracheal or lung sections between control and experimental groups. ^{24,40}
Ammonium Hydroxide	Rabbits (number not stated)	Instillation of test substance (1 mg) followed by ocular rinsing	Ocular irritant. ⁴⁴
Ammonium Hydroxide (28.5%)	Rabbits (number not stated)	Brief exposures (2 seconds). Volume instilled not stated	Corneal opacity. ^{9,124}
Ammonium Hydroxide (0.3%, 1%, 2.5%, and 10%)	New Zealand albino rabbits (groups of 6)	Draize test. Test substance (0.1 ml) instilled into the eye. In 1 group, eyes rinsed after instillation	Conjunctivitis (at 1% to 10%, but not at 0.3%). Ammonium Hydroxide (10%) produced pannus in 5/6 unwashed rabbit eyes and 2.5% produced pannus in 1/6 unwashed and 6/6 washed eyes. Ammonium Hydroxide at 1% produced pannus in 3/6 washed eyes. Keratoconus was produced by 10% Ammonium Hydroxide in 4/6 unwashed eyes and 2/6 washed eyes and 2.5% produced keratoconus in 2/6 unwashed eyes. Ammonium Hydroxide (10%) caused corneal opacities within 1 h of instillation. ¹²⁵
Ammonium Hydroxide (prepared with 3% Ammonia)	3 New Zealand White Albino Rabbits	Draize test. Test substance (100 µl) instilled into eye	Conjunctivitis (score = 3 at 96 h; mean maximum Draize score = 3), chemosis (score = 3 at 96 h; mean maximum score = 4), iritis (score = 1; mean maximum Draize score = 2), corneal opacity (score = 4; mean maximum Draize score = 4), and mean surface of corneal damage (70% corneal damage; mean maximum Draize value = 100%). Risk of serious damage to the eyes. ¹²⁶

Table 12. Other Clinical Reports

Ingredient	Number of Subjects	Protocol	Results
Inhalation Exposure			
Ammonia (700 ppm)	Number of subjects not available	Not available	Eye irritation. ¹³⁰
Ammonia (500 ppm)	Number of subjects not available	30-minute exposure	Variable lacrimation. ¹³⁰
Ammonia (500 ppm)	Number of subjects not available	30-minute exposure	Increased blood pressure and pulse rate. ¹³⁰
Ammonia (500 pm)	Number of subjects not available	30-minute exposure	Nasal and throat irritation, increased minute volume, and cyclic pattern of hyperpnea. ¹³⁰
Ammonia (500 ppm)	7 men	30-minute exposure	Increase in ventilation minute volume of 50-250%, accompanied by cyclic increase in respiratory rate. Irritation of the nose and throat. No significant change in nitrogen or urea in blood and urine. No significant change in serum nonprotein nitrogen. ¹³¹
Ammonia (500 ppm)	7 subjects	30-minute exposure via face mask	Ventilation minute volume increased 50 to 250% over pre-exposure values. Respiratory minute volumes fell below pre-exposure levels at termination of exposure. ^{47,131}
Ammonia (101 to 335 ppm)	Number of subjects not available	20-minute exposure	Decrease in exercise ventilation minute volume at 151-335 ppm, related either to a decrease in respiratory rate (at 151 ppm) or tidal volume (at 205 and 335 ppm); no significant effects at 101 ppm. ^{47,132}
Ammonia (50 to 140 ppm)	16 subjects	2-h exposure. Testing repeated after a 1-week interval.	110 ppm tolerable for all subjects. 140 ppm intolerable at 1 h (4 subjects) and at 2 h (4 subjects). No significant increase in vital capacity, forced expiratory volume at end of 1 second of forced expiration (FEV ₁), or forced inspiratory volume inhaled at end of 1 st second of forced inspiration (FIV ₁). Lowest-observed-adverse-effect level (LOAEL) of 50 ppm for mild irritation to the eyes (6 subjects), nose (20 subjects), and throat (9 subjects). LOAEL divided by uncertainty factor of 30 (10 to protect sensitive individuals and 3 for the use of a minimal LOAEL) ⁷⁹
Ammonia (135 ppm)	6 subjects	5-minute exposure	Chest irritation in 1 of 6 subjects. ¹³⁰
Ammonia (135 ppm)	Number of subjects not available	5-minute exposure	Nose and throat irritation. ¹³⁰
Ammonia (135 ppm)	Number of subjects not available	5-minute exposure	Eye irritation with lacrimation. ¹³⁰

Table 12. Other Clinical Reports

Ingredient	Number of Subjects	Protocol	Results
Ammonia (25, 50, and 100 ppm)	6 subjects	Exposure: 5 days per week (2 to 6 h per day) for 6 weeks	Mild to moderate irritation of the eyes, nose and throat: 16/54 (30%) of observations on 6 subjects in week 2; 12/90 (13%) in week 3; 2/60 (3%) in week 4; 0/78 in week 5; and 5/78 (6%) in week 6. No apparent effects on pulse, respiration rate, blood pressure, FVC, or FEV ₁ . ¹³³
Ammonia (25-100 ppm)	Not available	Exposure to varying concentrations for varying periods (2-6 h) 5 days/week for 6 weeks	Decreasing signs of irritation of the mucous membranes of the eyes, nose and throat over the 6-week observation period were reported, and there was no evidence of adverse health effects. ^{47,133}
Ammonia (72 ppm)	Number of subjects not available	5-minute exposure	Eye irritation with lacrimation. ¹³⁰
Ammonia (50 ppm)	Number of subjects not available	5-minute exposure	Eye irritation with lacrimation. ¹³⁰
Ammonia (50 ppm)	Number of subjects not available	120-minute exposure	Eye irritation. ¹³⁰
Ammonia (50 ppm)	Number of subjects not available	120-minute exposure	Nose and throat irritation. Urge to cough. ¹³⁰
Ammonia (30 and 50 ppm)	6 subjects	10-minute exposure	Barely perceptible irritant effects (nose and eye) in 2 of 6 subjects (30 ppm). Faint to moderate irritation (nose and eye) in 5 of 6 subjects (50 ppm). ⁵³
Ammonia (30 ppm and 50 ppm)	6 subjects	10-minute exposure	Moderate irritation of nose and eyes at 50 ppm (4 of 6 subjects), but not at 30 ppm. ⁹
Ammonia (32 ppm)	Number of subjects not available	5-minute exposure	Eye irritation with lacrimation. ¹³⁰

REFERENCES

1. Nikitakis, J. and Lange B. International Cosmetic Ingredient Dictionary and Handbook Online Version (wINCI). <http://webdictionary.personalcarecouncil.org/jsp/Home.jsp>. Washington, DC. Last Updated 2017. Date Accessed 3-6-2017.
2. United States Environmental Protection Agency (EPA). Toxicological review of ammonia noncancer inhalation. https://cfpub.epa.gov/ncea/iris/iris_documents/documents/toxreviews/0422tr.pdf. Last Updated 2016.
3. World Health Organization (WHO). Ammonia - published under the joint sponsorship of the United Nations Environment Program, the International Labor Organization, and the World Health Organization. Geneva: World Health Organization, 1986.
4. Cosmetic Ingredient Review. Safety Assessment of Phosphoric Acid and Its Salts as Used in Cosmetics. Final Report. Available for review at the Cosmetic Ingredient Review, 1620 L Street, NW, Suite 1200, Washington, D.C. 20036-4702. 2016. Washington, D.C.:
5. Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological profile for ammonia (Update). *U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry, Division of Toxicology/Toxicology Information Branch., 1600 Clifton Road NE., E-29., Atlanta., GA 30333., USA, Sep. 2004.* xix., 223p.
6. European Chemicals Agency (ECHA). Registration, Evaluation, and Authorization of Chemicals (REACH) Dossier. Anhydrous Ammonia. <https://echa.europa.eu/registration-dossier/-/registered-dossier/15557>. Last Updated 2017. Date Accessed 6-8-2017.
7. Welch, A. Exposing the dangers of anhydrous ammonia. http://journals.lww.com/tnpi/Citation/2006/11000/Exposing_the_Dangers_of_Anhydrous_Ammonia.8.aspx. Last Updated 2006. Date Accessed 5-17-2017.
8. O'Neil, M. J. The Merck Index. An Encyclopedia of Chemicals, Drugs, and Biologicals. 15th Edition *ed.* Cambridge, UK: Royal Society of Chemistry, 2013.
9. Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological profile for ammonia. <https://www.atsdr.cdc.gov/toxprofiles/tp126.pdf>. Last Updated 2004.
10. Scifinder. Chemical Abstracts Service: Columbus, OH. CAS Registry Numbers 7664-41-7 and 1336-21-6. Substance Identifier. <http://www.cas.org/products/scifinder>. Last Updated 2017. Date Accessed 6-20-2017.
11. United States Environmental Protection Agency (EPA). Estimation Programs Interface Suite™ for Microsoft® Windows, Calculations based on KOWWIN v1.68.4.10. 2017. Washington, D.C.: EPA.
12. National Center for Biotechnology Information. Pubchem Compound Database. Compound summary for CID 14923. Ammonium Hydroxide. https://pubchem.ncbi.nlm.nih.gov/compound/ammonium_hydroxide#section=Top. Last Updated 2017. Date Accessed 10-18-2017.
13. United States Food and Drug Administration (FDA). Listing of specific substances affirmed as GRAS. Ammonium hydroxide. 21 CFR 184.1139. <https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm>. Last Updated 2016. Date Accessed 6-8-2017.
14. United States Pharmacopeial Convention. Food Chemicals Codex. Tenth *ed.* Rockville, MD: The United States Pharmacopeial Convention, 2016.
15. The United States Pharmacopeial Convention. The United States Pharmacopeia (USP). Rockville, MD: The United States Pharmacopeial Convention, 2009.
16. United States Food and Drug Administration (FDA). Information supplied to FDA by industry as part of the VCRP FDA database. 2017. Washington, D.C.: FDA.
17. Personal Care Products Council. Concentration of use by FDA product category: Ammonia and Ammonium Hydroxide. Unpublished data submitted by the Personal Care Products Council on 2-2-2017. 2017.
18. European Commission. CosIng database; following Cosmetic Regulation No. 1223/2009. <http://ec.europa.eu/growth/tools-databases/cosing/>. Last Updated 2017. Date Accessed 6-8-2017.

19. Bhattacharya, S. K. Hom G. G. Fernandez C. and Hom L. G. Ocular effects of exposure to industrial chemicals: Clinical management and proteomic approaches to damage assessment. *Cutaneous and Ocular Toxicology*. 2007;26(3):203-225.
20. National Industrial Chemicals Notification and Assessment Scheme (NICNAS). Human health tier II assessment for ammonia and ammonium hydroxide. https://www.nicnas.gov.au/chemical-information/imap-assessments/imap-group-assessment-report?assessment_id=1180. Last Updated 2013. Date Accessed 6-8-2017.
21. United States Food and Drug Administration (FDA). Drugs@FDA: FDA Approved Drug Products. Ammonia. <https://www.accessdata.fda.gov/scripts/cder/daf/index.cfm?event=browseByLetter.page&productLetter=A>. Last Updated 2017. Date Accessed 6-11-2017.
22. United States Food and Drug Administration (FDA). Drug products containing certain active ingredients offered over-the-counter (OTC) for certain uses. <https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm>. Last Updated 2016. Date Accessed 6-11-2017.
23. Souba, W. W. Review. Interorgan ammonia metabolism in health and disease: A surgeon's view. *Journal of Parenteral and Enteral Nutrition*. 1987;11(6):569-579.
24. Cavender, F. and Milner G. Exposure to ammonia. Salem, H. and Katz S. A. In: *Inhalation Toxicology*. 3rd ed. Boca Raton: CRC Press; 2015:257-293.
25. Cooper, A. J. L. Ammonia metabolism in normal and portacaval-shunted rats. *Advances in Experimental Medicine and Biology*. 1990;272:23-46.
26. Dasarathy, S. Mookerjee R. P. Rackayova V. Thrane V. R. Vairappan B. Ott P. and Rose C. F. Ammonia toxicity: from head to toe? *Metab.Brain Dis*. 2017;32(2):529-538.
27. Jones, E. A. Smallwood R. A. Craigie A. and Rosenoer V. M. The enterohepatic circulation of urea nitrogen. *Clin.Sci*. 1969;37:825-836.
28. Cooper, J. L. A. and Plum F. Biochemistry and physiology of brain ammonia. *Physiol.Rev*. 1987;67:440-519.
29. Brusilow, S. W. Koehler R. C. Traystman R. J. and Cooper A. J. L. Astrocyte glutamine synthetase: importance in hyperammonemic syndromes and potential target for therapy. *NeuroRx*. 2010;7:452-470.
30. Oja, S. S. Saransaari P. Korpi E. R. Neurotoxicity of ammonia. *Neurochem.Res*. 2017;42:713-720.
31. Walker, V. Ammonia metabolism and hyperammonemic disorders. *Adv.Clin.Chem*. 2014;67:73-150.
32. Odigwe, C. C. Khatiwada B. Holbrook C. Ekeh I. S. Uzoka C. Ikwu I. and Upadhyay B. Noncirrhotic hyperammonemia causing relapsing altered mental status. *Proc (Bayl.Univ.Med.Cent.)*. 2017;28(4):472-474.
33. Summerskill, V. H. J. and Wolpert E. Ammonia metabolism in the gut. *The American Journal of Clinical Nutrition*. 2017;23(5):633-639.
34. Katayama, K. Ammonia metabolism and hepatic encephalopathy. *Hepatology Research*. 2004;30S:S71-S78.
35. Visek, W. J. Ammonia metabolism, urea cycle capacity and their biochemical assessment. *Nutrition Reviews*. 1979;37(9):273-282.
36. Sandesh, C. S. Nagamani and Erez A. A metabolic link between the urea cycle and cancer cell proliferation. DOI: 10.1080/23723556.2015. 1127314. *Molecular & Cellular Oncology*. 2016;3(2):e1127314
37. Sorensen, M. Update on cerebral uptake of blood ammonia. *Metab.Brain Dis*. 2013;28:155-159.
38. Manninen, A. T. A. and Savolainen H. Effect of short-term ammonia inhalation on selected amino acids in rat brain. *Pharmacol.Toxicol*. 1989;64(3):244-246.
39. Manninen, A. Anttila S. and Savolainen H. Rat metabolic adaptation to ammonia inhalation. *Proc.Soc.Exp.Biol.Med*. 1988;187(3):278-281.
40. Schaerdel, A. D. White W. J. Lang C. M. et al. Localized and systemic effects of environmental ammonia in rats. *Lab Anim.Sci*. 1983;33(1):40-45.

41. Cooper, A. J. L. and Lai J. C. K. Cerebral ammonia metabolism in normal and hyperammonemic rats. *Neurochemical Pathology*. 1987;6:67-95.
42. Mori, S. Kaneko H. Mitsuma T. et al. Implications of gastric topical bioactive peptides in ammonia-induced acute gastric mucosal lesions in rats. *Scand.J.Gastroenterol*. 1998;33(4):386-393.
43. Ruden, C. and Hansson S. O. How accurate are the European Union's classifications of chemical substances. *Toxicology Letters*. 2003;144:159-172.
44. Organization for Economic Co-operation and Development (OECD). Final Assessment Report. SIDS Dossier on Ammonium Hydroxide. SIDS Ammonia Zip: SIDS_Dossier_Ammonia_1336216.
http://webnet.oecd.org/HPV/UI/SIDS_Details.aspx?key=d5ae737b-77d7-4d61-8687-4df45f52cace&idx=0. Last Updated 2007.
45. Takeuchi, K. Ohuchi T. Harada H. et al. Irritant and protective action of urea-urease ammonia in rat gastric mucosa. *Dig.Dis.Sci*. 1995;40(2):274-281.
46. Silver, S. D. and McGrath, FP. A Comparison of Acute Toxicities of Ethylene Imine and Ammonia to Mice. *Journal of Industrial Hygiene and Toxicology*. 1948;30(1):7-9.
47. Legters, L. Biological effects of short, high-level exposure to gases: Ammonia. Contract No. DAMD17-79-C-9086. 1980. pp.1-87. Fort Detrick, Frederick, Maryland: U.S. Army Medical Research and Development Command.
48. Organization for Economic Co-operation and Development (OECD). SIDS Dossier. CAS number 76645-41-7. Ammonia, anhydrous. <http://webnet.oecd.org/hpv/ui/Search.aspx>. Last Updated 2007.
49. Barrow, C. S. Alarie Y. and Stock M. F. Sensory irritation and incapacitation evoked by thermal decomposition products of polymers and comparisons with known sensory irritants. *Arch.Environ.Health*. 1978;33:79-88.
50. Hilaldo, C. J. Casey C. J. and Furst A. Effect of ammonia on Swiss albino mice. *J.Combust.Toxicol*. 1977;4:385-388.
51. MacEwen, J. D. and Vernot, EH. Toxic Hazards Research Unit Annual Technical Report. *Aerospace Medical Research Laboratory, Air Force.Systems Command., Wright.-Patterson.Air Force.Base., Ohio., Report No.AMRL.-TR.-72.-62., NTIS AD755.-358., 162.pages., 37.references*. 1972;
52. Kapeghian, J. C. Mincer H. H. Hones A. B. et al. Acute inhalation toxicity of ammonia in mice. *Bull.Environ.Contam.Toxicol*. 1982;29:371-378.
53. MacEwen, J. D., Theodore, J, and Vernot, EH. Human Exposure to EEL Concentrations of Monomethylhydrazine. *Aerospace Medical Research Laboratory, Aerospace Division., Air Force.Systems Command., Wright.-Patterson.Air Force.Base., Ohio., Report No.AMRL.-TR.-70.-102., (Proceedings of the First.Annual Conference.on Environmental Toxicology, 1970)*. 1970;(Proceedings of the First Annual Conference on Environmental Toxicology):355-363.
54. Pauluhn, J. Acute inhalation toxicity of ammonia: Revisiting the importance of RD50 and LCT01/50 relationships for setting emergency response guideline values. *Regulatory Toxicology and Pharmacology*. 2013;66:315-325.
55. Appelman, L. M., Ten Berge, WF, and Reuzel, PG. Acute inhalation toxicity study of ammonia in rats with variable exposure periods. *Am Ind Hyg Assoc J*. 1982;43(9):662-665.
56. Li, W. L. and Pauluhn J. Comparative assessment of sensory irritation in rats and mice nose-only exposed to dry and humidified atmospheres. *Toxicology*. 2010;276:135-142.
57. Perkins, M. W. Wong B. Tressler J. Coggins A. Rodriguez A. Devorak J. and Sciuto A. M. Assessment of inhaled ammonia-induced lung injury in rats. *Inhal.Toxicol*. 2016;28(2):71-79.
58. Perkins, M. W. Wong B. Tressler J. Rodriguez A. Sherman K. Andres J. Devorak J. Wilkins W. L. and Sciuto A. M. Adverse respiratory effects in rats following inhalation exposure to ammonia: respiratory dynamics and histopathology. *Inhalation Toxicology*. 2017;29(1):32-41.
59. Organization for Economic Co-operation and Development (OECD). SIDS Dossier. CAS number 76645-41-7. Ammonia, anhydrous. <http://webnet.oecd.org/hpv/ui/Search.aspx>. Last Updated 2007.
60. Organization for Economic Co-operation and Development (OECD). SIDS Dossier. CAS number 76645-41-7. Ammonia, anhydrous. <http://webnet.oecd.org/hpv/ui/Search.aspx>. Last Updated 2007.

61. Organization for Economic Co-operation and Development (OECD). SIDS Dossier. CAS number 76645-41-7. Ammonia, anhydrous. <http://webnet.oecd.org/hpv/ui/Search.aspx>. Last Updated 2007.
62. Organization for Economic Co-operation and Development (OECD). SIDS Dossier. CAS number 76645-41-7. Ammonia, anhydrous. <http://webnet.oecd.org/hpv/ui/Search.aspx>. Last Updated 2007.
63. Organization for Economic Co-operation and Development (OECD). SIDS Dossier. CAS number 76645-41-7. Ammonia, anhydrous. <http://webnet.oecd.org/hpv/ui/Search.aspx>. Last Updated 2007.
64. Dodd, K. T. and Gross D. R. Ammonia inhalation toxicity in cats: A study of acute and chronic respiratory dysfunction. *Arch.Environ.Health*. 1980;35:6-14.
65. Boyd, E. M., MacLachland, ML, and Perry, WF. Experimental Ammonia Gas Poisoning in Rabbits and Cats. *Journal of Industrial Hygiene and Toxicology*. 1944;26(1):29-34.
66. Organization for Economic Co-operation and Development (OECD). SIDS Dossier. CAS number 76645-41-7. Ammonia, anhydrous. <http://webnet.oecd.org/hpv/ui/Search.aspx>. Last Updated 2007.
67. Tsujii, M. Kawano S. Tsuji S. et al. Cell kinetics of mucosal atrophy in rat stomach induced by long-term administration of ammonia. *Gastroenterology*. 1993;104(3):796-801.
68. Zissu, D. Histopathological Changes in the Respiratory Tract of Mice Exposed to Ten Families of Airborne Chemicals. *Journal of Applied Toxicology*. 1995;15(3):207-213.
69. Buckley, L. A. Jiang X. Z. James R. A. Morgan K. T. and Barrow C. S. Respiratory tract lesions induced by sensory irritants at the median respiratory rate decrease concentration. *Toxicol.Pharmacol*. 1984;74:417-429.
70. Anderson, D. P. Beard C. W. and Hanson R. P. The adverse effects of ammonia on chickens including resistance to infection with Newcastle disease virus. *Avian.Dis*. 1964;8:369-379.
71. Gaafar, H. Girgis R. and Hussein M. et al. The effect of ammonia on the respiratory nasal mucosa of mice. A histological and histochemical study. *Acta Otolaryngol (Stockh)*. 1992;112(2):339-342.
72. Broderson, J. R. Lindsey J. R. and Crawford J. E. The role of environmental ammonia in respiratory mycoplasmosis of rats. *Am.J.Pathol*. 1976;85:115-130.
73. Coon, R. A. Jones R. a. Jenkins L. T. Jr. and Siegel J. Animal inhalation studies on ammonia, ethylene glycol, formaldehyde, dimethylamine, and ethanol. *Toxicol.Appl.Pharmacol*. 1970;16:646-655.
74. Richard, D. Bouley G. and Boudene C. Effects of continuous inhalation of ammonia in the rat and mouse (French). In: Agency for Toxic Substances and Disease Registry (ATSDR). 2004. Toxicological profile for ammonia. <https://www.atsdr.cdc.gov/toxprofiles/tp126.pdf>. Last Updated 2004. Date Accessed 5-23-0017.
75. Stombaugh, D. P., Teague, HS, and Roller, WL. Effects of Atmospheric Ammonia on the Pig. *Journal of Animal.Science*. 1969;20:844-847.
76. Stolpe, J. and Sedlag R. Die Einzel- und Komplexwirkung von Ammoniak und Schwefelwasserstoff in der Luft auf kleine Versuchstiere (Ratten) bei unterschiedlichen Umweltbedingungen. *Ach.Exper.Vet.Med*. 1976;30:533-539.
77. Done, S. H. Chennells D. J. Gresham A. C. Williamson S. Hunt B. Taylor L. L. Bland V. et al. Clinical and pathological responses of weaned pigs to atmospheric ammonia and dust. *Vet.Rec*. 2005;157:71-80.
78. Urbain, B. and Gustin P. Prouvost J. F. and Ansay M. Quantitative assessment of aerial ammonia toxicity to the nasal mucosa by use of the nasal lavage method in pigs. *Am.J.Vet.Res*. 1994;55(9):1335-1340.
79. Verberk, M. M. Effects of ammonia in volunteers. *Int.Arch.Occup.Environ.Health*. 1977;39:73-81.
80. Occupational Safety and Health Administration (OSHA). Air contaminants. 29 CFR:1910.1000. https://www.ecfr.gov/cgi-bin/text-idx?SID=c5407149c832a3a7892a2e80712a59ba&mc=true&node=se29.6.1910_11000&rgn=div8. Last Updated 2017. Date Accessed 6-21-2017.
81. Weatherby, J. H. Chronic toxicity of ammonia fumes by inhalation. *Proc.Soc.Exp.Biol.Med*. 1952;81:300-301.

82. Dalhamn, T. and Reid I. Ciliary activity and histologic observations in the trachea after exposure to ammonia and carbon particles. Davies, C. N. In: *Inhaled particles and vapors II*. Elmsford, NY: Pergamon Publishing Company; 1967:299-306.
83. Fazekas, I. G. Experimental suprarenal hypertrophy induced by ammonia. *Endokrinologie*. 1939;21:315-337.
84. Ota, Y. Hasumura M. Okamura M. et al. Chronic toxicity and carcinogenicity of dietary administered ammonium sulfate in F344 rats. *Fd.Chem.Toxicol*. 2006;44:17-27.
85. Holness, D. L., Purdham, JT, and Nethercott, JR. Acute and Chronic Respiratory Effects of Occupational Exposure to Ammonia. *American Industrial Hygiene Association Journal*. 1989;50(12):646-650.
86. Curtis, S. E., Anderson, CR, Simon, J, Jensen, AH, Day, DL, and Kelley, KW. Effects Of Aerial Ammonia, Hydrogen Sulfide And Swine-House Dust On Rate Of Gain And Respiratory-Tract Structure In Swine. *Journal of Animal.Science*. 1975;41(3):735-739.
87. Ballal, S. G. Ali B. A. Albafr A. A. Ahmed H. O. and Al-Hasan A. Y. **Bronchial asthma in two chemical fertilizer producing factories in eastern Saudi Arabia**. *Tuberc.Lung.Dis*. 1998;2:330-335.
88. Ali, B. A. Ahmed H. O. Ballal S. G. and Albar A. A. Pulmonary function of workers exposed to ammonia: A study in Eastern Province of Saudi Arabia. *Int.J.Occup.Environ.Health*. 2001;7:19-22.
89. Lane, M. and Gardner D. K. Increase in postimplantation development of cultured mouse embryo by amino acids and induction of fetal retardation and exencephaly by ammonium ions. *J.Reprod.Fertil*. 1994;102(2):305-312.
90. Minana, M. D. Marcaida G. Grisolia S. et al. Prenatal exposure of rats to ammonia impairs NMDA receptor function and affords delayed protection against ammonia toxicity and glutamate neurotoxicity. *J.Neuropathol.Exp.Neurol*. 1995;54(5):644-650.
91. Diekman, M. A. Scheidt A. B. Sutton A. L. et al. Growth and reproductive performance, during exposure to ammonia, of gilts afflicted with pneumonia and atrophic rhinitis. *Am.J.Vet.Res*. 1993;54(12):2128-2131.
92. Yadav, J. S. and Kaushik V. K. Genotoxic effect of ammonia exposure on workers in a fertilizer factory. *Indian J.Exp.Biol*. 1997;35(5):487-492.
93. Uzvolgyi, E. and Bojan F. Possible in vivo formation of a carcinogenic substance from diethyl pyrocarbonate and ammonia. *J.Cancer Res.Clin.Oncol*. 1980;(97):205-207.
94. Toth, B. Hydrazine, methylhydrazine and methylhydrazine sulfate carcinogenesis in swiss mice. Failure of ammonium hydroxide to interfere in the development of tumors. *Int.J.Cancer*. 1972;9:109-118.
95. Uzvolgyi, E. and Bojan F. In vivo formation of a carcinogenic substance from diethyl pyrocarbonate in the presence of ammonia. *Arch.Toxicol.Suppl*. 1985;8:490-493.
96. Tsujii, M. Kawano S. Tsuji S. et al. Ammonia: A possible promoter in Helicobacter pylori related gastric carcinogenesis. *Cancer Lett*. 1992;65(1):15-18.
97. Tsujii, M. Kawano S. Tsuji S. et al. Mechanism for ammonia-induced promotion of gastric carcinogenesis in rats. *Carcinogenesis*. 1995;16(3):563-566.
98. Cagnon, L. and Braissant O. Hyperammonemia-induced toxicity for the developing central nervous system. *Brain Research Reviews*. 2007;56:183-197.
99. Albrecht, J. Mini-Review. Roles of neuroactive amino acids in ammonia neurotoxicity. *Journal of Neuroscience Research*. 1998;51:133-138.
100. Albrecht, J. Zelinska M. and Norenberg. Glutamine as a mediator of ammonia neurotoxicity: A critical appraisal. *Biochemical Pharmacology*. 2010;(doi:10.1016/j.bcp.2010.07.024)
101. Cooper, A. J. Role of glutamine in cerebral nitrogen metabolism and ammonia neurotoxicity. *Ment.Retard.Dev.Disabil.Res.Rev*. 2001;7:280-286.
102. Bosoi, C. R. Zwingmann C. Marin H. Parent-Robitaille C. Huynh J. Tremblay M. and Rose C. F. Increased brain lactate is central to the development of brain edema in rats with chronic liver disease. *J.Hepatol*. 2014;60:554-560.

103. Martinez-Hernandez, A. Bell K. P. and Norenberg. Glutamine synthetase: glial localization in brain. *Science*. 1977;195:1356-1358.
104. Hertz, L. and Zielke H. R. Astrocytic control of glutamatergic activity: astrocytes as stars of the show. *Trends Neurosci*. 2004;27:735-743.
105. Monfort, P. Montoliu C. Hermenegildo C. Munoz M. D. and Felipe V. Differential effects of acute and chronic hyperammonemia on signal transduction pathways associated with NMDA receptors. *Neurochemistry International*. 2000;37:249-253.
106. Marcaida, G. Felipe V. Hermenegildo C. Minana M. D. and Grisolia S. Acute ammonia toxicity is mediated by the NMDA type of glutamate receptors. *Federation of European Biochemical Society Letters*. 1992;296:67-68.
107. Hermenegildo, C. Marcaida G. Montoliu C. Grisolia S. Minana M. D. and Felipe V. NMDA receptor antagonists prevent acute ammonia toxicity in mice. *Neurochemical Research*. 1996;21:1237-1244.
108. Monfort, P. Kosenko E. Erceg S. Canales J. J. and Felipe V. Molecular mechanisms of acute ammonia toxicity: Role of NMDA receptors. *Neurochemistry International*. 2002;41:95-102.
109. Seiler, N. Review. Ammonia and Alzheimer's disease. *Neurochemistry International*. 2002;41:189-207.
110. Hoyer, S. Henneberg N. Knapp S. Lannert H. and Martin E. Brain glucose metabolism is controlled by amplification and desensitization of the neuronal insulin receptor. *Ann.N.Y.Acad.Sci*. 1996;777:374-379.
111. Sims, B. Powers R. E. Sabina R. L. and Theibert A. B. Elevated adenosine monophosphate deaminase activity in Alzheimer's disease brain. *Neurobiol.Aging*. 1998;19:385-391.
112. Targowski, S. P. Klucinski W. and Jaworek D. Effect of ammonia on viability and blastogenesis of bovine lymphocytes. *Veterinary Immunology and Immunopathology*. 1984;5:297-310.
113. Kosenko, E. Kaminsky Y. Kaminsky A. Valencia M. Lee L. Hermenegildo C. and Felipe V. Superoxide production and antioxidant enzymes in ammonia intoxication in rats. *Free Radic.Res*. 1997;27:637-644.
114. Murthy, C. R. Rama Rao K. V. Bai G. and Norenberg. Ammonia induced production of free radicals in primary cultures of rat astrocytes. *J.Neurosci.Res*. 2001;66:282-288.
115. Zielinska, M. Ruszkiewicz J. Hilgier W. Fresko I. and Albrecht J. Hyperammonemia increases the expression and activity of the glutamine/arginine transporter y + LAT2 in rat cerebral cortex: implications for the nitric oxide/cGMP pathway. *Neurochem.Int*. 2011;58:190-195.
116. Targowski, S. P. Klucinski W. Babiker S. et al. Effect of ammonia on in vivo and in vitro immune response. *Infect.Immun*. 1984;43(1):289-293.
117. Tepper, J. S. Weiss B. and Wood R. W. Alterations in behavior produced by inhaled ozone or ammonia. *Fundam.Appl.Toxicol*. 1985;5:1110-1118.
118. Perkins, M. A. Osborne R. and Johnson G. R. Development of an in vitro method for skin corrosion testing. *Fundamental and Applied Toxicology*. 1996;31:9-18.
119. Sekizawa, J. Yasuhara K. Suyama Y. Yamanaka S. Tobe M. and Nishimura M. A simple method for screening assessment of skin and eye irritation. *The Journal of Toxicological Sciences*. 1994;19:25-35.
120. Hamami, I. and Marks R. Structural determinants of the response of the skin to chemical irritants. *Contact Dermatitis*. 1988;18:71-75.
121. Frosch, P. J. and Kligman A. M. Rapid blister formation in human skin with ammonium hydroxide. *British Journal of Dermatology*. 1977;96:461-473.
122. Grove, G. L. Duncan S. and Kligman A. M. Effect of aging on the blistering of human skin with ammonium hydroxide. *British Journal of Dermatology*. 1982;107:393-400.
123. Goldberg, A. M. Product Safety Evaluation. In: *Alternative Methods in Toxicology*. Vol. 1. New York: Mary Ann Liebert, Inc.; 1983:

124. Grant, W. M. Toxicology of the eye. 2nd ed. Springfield, IL: Charles C. Thomas, 1974.
125. Murphy, J. C. Osterberg R. E. Seabaugh V. M. and Bierbower G. W. Ocular irritancy responses to various pHs of acids and bases with and without irrigation. *Toxicology*. 1982;23:281-291.
126. Jacobs, G. A. OECD eye irritation tests on 2 alkalis. *Journal of the American College of Toxicology*. 1992;11(6):727
127. Murakami, M. Saita H. Teramura S. Dekigai H. Asagoe K. Kusaka S. and Kita T. Gastric ammonia has a potent ulcerogenic action on the rat stomach. *Gastroenterology*. 1993;105:1710-1715.
128. Brautbar, N. Wu M. and Richter E. D. Chronic ammonia inhalation and interstitial pulmonary fibrosis: A case report and review of the literature. *Archives of Environmental Health*. 2003;58(9):592-596.
129. Kollef, M. H. Chronic ammonium hydroxide exposure. *Annals of Internal Medicine*. 1987;107(1):118
130. Michaels, R. A. Emergency planning and the acute toxic potency of inhaled ammonia. *Environmental Health Perspectives*. 1999;107(8):617-627.
131. Silverman, L. Whittenberger J. L. and Muller J. Physiological response of man to ammonia in low concentrations. *J.Ind.Hyg.Toxicol*. 1949;31(2):74-78.
132. Cole, T. J. Cotes J. E. Johnson G. R. Martin H. Reed J. W. and Saunders M. J. Ventilation, cardiac frequency and pattern of breathing during exercise in men exposed to o-chlorobenzylidene malonitrile (CS) and ammonia gas in low concentrations. *J.Exp.Physiol*. 1977;64:341-351.
133. Ferguson, W. S. Koch W. C. Webster L. B. and Gould J. R. Human physiological response and adaptation to ammonia. *J.Occup.Med*. 1977;19(5):319-326.
134. Nemer, M. Sikkeland L. I. B. Kasem M. Kristensen P. Nijem K. Bjertness E. Skare O. Bakke B. Kongerud J. and Skogstad M. Airway inflammation and ammonia exposure among female Palestinian hairdressers: a cross-sectional study. *Occup.Environ.Med*. 2015;72:428-434.
135. Doig, P. A. and Willoughby R. A. Response of swine to atmospheric ammonia and organic dust. *J.Am.Vet.Med.Assoc*. 1971;159(11):1353-1361.

SCIENTIFIC OPINION

Scientific Opinion on Flavouring Group Evaluation 46, Revision 1 (FGE.46Rev1):

Ammonia and three ammonium salts from chemical group 30¹

EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
(CEF)^{2,3}

European Food Safety Authority (EFSA), Parma, Italy

KEYWORDS

Ammonia, ammonia salts, flavourings, safety.

SUMMARY

The Scientific Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (the Panel) was asked to provide scientific advice to the Commission on the implications for human health of chemically defined flavouring substances used in or on foodstuffs in the Member States. In particular, the Panel was requested to evaluate four flavouring substances in the Flavouring Group Evaluation 46, Revision 1 (FGE.46Rev1), using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000. These four flavouring substances belong to chemical group 30, Annex I of the Commission Regulation (EC) No 1565/2000.

The present Flavouring Group Evaluation deals with ammonia [FL-no: 16.009], and three ammonia salts (diammonium sulphide [FL-no: 16.002], ammonium chloride [FL-no: 16.048] and ammonium hydrogen sulphide [FL-no: 16.059]).

The flavouring substances cannot exist as geometrical or optical isomers.

1 On request from the Commission, Question No EFSA-Q-2010-01133 adopted on 25 November 2010.

2 Panel members Arturo Anadon, Mona-Lise Binderup, Wilfried Bursch, Laurence Castle, Riccardo Crebelli, Karl-Heinz Engel, Roland Franz, Nathalie Gontard, Thomas Haertle, Trine Husøy, Klaus-Dieter Jany, Catherine Leclercq, Jean Claude Lhuguenot, Wim Mennes, Maria Rosaria Milana, Karla Pfaff, Kjetil Svensson, Fidel Toldra, Rosemary Waring, Detlef Wölflé. CEF-Unit@efsa.europa.eu

3 Acknowledgement: The Panel wishes to thank the members of the Working Groups on Flavourings for the preparation of this Opinion: Ulla Beckman Sundh, Vibe Beltoft, Wilfried Bursch, Angelo Carere, Karl-Heinz Engel, Henrik Frandsen, Rainer Gürtler, Frances Hill, Trine Husøy, John Christian Larsen, Pia Lund, Wim Mennes, Gerard Mulder, Karin Nørby, Gerard Pascal, Iona Pratt, Gerrit Speijers, Harriet Wallin and EFSA's staff member Kim Rygaard Nielsen for the support provided to this EFSA scientific output.

Suggested citation: EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF); Scientific Opinion on Flavouring Group Evaluation 46, Revision 1 (FGE.46Rev1):

Ammonia and three ammonium salts from chemical group 30. EFSA Journal 2011; 9(2):1925. [35 pp.]. doi:10.2903/j.efsa.2011.1925. Available online: www.efsa.europa.eu

Two of the flavouring substances are classified into structural class I and two are classified into structural class III according to the decision tree approach presented by Cramer et al. (1978).

The flavouring substance ammonia in the present group has been reported to occur naturally in a wide range of food items up to very high amounts. Hydrogen sulphide is also reported to occur naturally in a wide range of food items.

In its evaluation, the Panel as a default used the “Maximised Survey-derived Daily Intake” (MSDI) approach to estimate the *per capita* intakes of the flavouring substances in Europe. However, when the Panel examined the information provided by the European Flavouring Industry on the use levels in various foods, it appeared obvious that the MSDI approach in a number of cases would grossly underestimate the intake by regular consumers of products flavoured at the use level reported by the Industry, especially in those cases where the annual production values were reported to be small. In consequence, the Panel had reservations about the data on use and use levels provided and the intake estimates obtained by the MSDI approach.

In the absence of more precise information that would enable the Panel to make a more realistic estimate of the intakes of the flavouring substances, the Panel has decided also to perform an estimate of the daily intakes per person using a “modified Theoretical Added Maximum Daily Intake” (mTAMDI) approach based on the normal use levels reported by Industry. In those cases where the mTAMDI approach indicated that the intake of a flavouring substance might exceed its corresponding threshold of concern, the Panel decided not to carry out a formal safety assessment using the Procedure. In these cases the Panel requires more precise data on use and use levels.

According to the default MSDI approach, the two flavouring substances [FL-no: 16.009 and 16.048] belonging to structural class I have estimated intakes in Europe of 34 and 140 microgram/*capita*/day, respectively, which are below the threshold of concern for structural class I substances (1800 microgram/person/day). The two substances belonging to structural class III have estimated intake in Europe of 62 and 5.6 microgram/*capita*/day, respectively, which is below the threshold of concern for structural class III substances (90 microgram/person/day).

Although the genotoxicity data for the flavouring substances in this group are limited, the available data on genotoxicity do not preclude an evaluation of the candidate substances through the Procedure. For the candidate substance ammonium chloride [FL-no: 16.048] there is a well-performed carcinogenicity study available, which indicates that the substance does not induce tumours.

Ammonia is a substance that is readily absorbed in the gut. It is produced endogenously in amounts that far exceed those that are to be ingested as flavourings. The three ammonium salts are expected to give rise to ammonium ion and chloride or hydrogen sulphide. Ammonia is expected to be transported by the portal circulation to the liver and metabolised to urea by the Krebs urea cycle and subsequently excreted by the kidneys. Hydrogen sulphide is a substance that is produced endogenously. The major pathway for sulphide metabolism is oxidation to sulphate and excretion by the kidney. The major oxidation product of sulphide is thiosulphate which is then converted to sulphate. The primary location for these reactions is the liver. All four substances are accordingly expected to be metabolised to innocuous substances at the anticipated levels of intake as flavouring substances.

It was noted that where toxicity data were available they were consistent with the conclusions in the present flavouring group evaluation using the Procedure.

On the basis of the default MSDI approach the Panel concluded that the flavouring substances would not give rise to safety concerns at the estimated levels of intake arising from their use as flavouring substances.

When the estimated intakes were based on the mTAMDI approach the values for the two substances from structural class I, ammonia and ammonium chloride [FL-no: 16.009 and 16.048], are 110000 microgram/person/day and 220000 microgram/person/day, respectively. These values are above the

threshold of concern for structural class I of 1800 microgram/person/day. For one of the substances from structural class III, ammonium hydrogen sulphide [FL-no: 16.059], the mTAMDI value is 220 microgram/person/day. This value is above the threshold for structural class III of 90 microgram/person/day. For the other substance from structural class III no data are available on use and use levels.

Thus, intake estimates based on the mTAMDI approach exceed the threshold of concern for the three flavouring substances in this flavouring group, and more reliable exposure data are requested for all four substances. On the basis of such additional data, these flavouring substances should be reconsidered using the Procedure. Subsequently, additional data might become necessary.

In order to determine whether this evaluation could be applied to the materials of commerce, it is necessary to consider the available specifications. Specifications including complete purity criteria for the materials of commerce have been provided for the four flavouring substances.

Identity tests is missing for one of the flavouring substances, ammonium hydrogen sulphide [FL-no: 16.059]. Thus, the final evaluation of the materials of commerce cannot be performed for this substance, pending further information. The remaining three flavouring substances, ammonia [FL-no: 16.009], ammonium chloride [FL-no: 16.048] and diammonium sulfide [FL-no: 16.002] would present no safety concern at the levels of intakes estimated on the basis of the MSDI approach.

TABLE OF CONTENTS

Keywords	1
Summary	1
Table of contents	4
Background	5
History of the Evaluation	5
Terms of Reference	5
Assessment	6
1. Presentation of the Substances in Flavouring Group Evaluation 46, Revision 1	6
1.1. Description.....	6
1.2. Stereoisomers.....	6
1.3. Natural Occurrence in Food.....	6
2. Specifications.....	7
3. Intake Data.....	7
3.1. Estimated Daily <i>per Capita</i> Intake (MSDI Approach)	7
3.2. Intake Estimated on the Basis of the Modified TAMDI (mTAMDI)	8
4. Absorption, Distribution, Metabolism and Elimination	9
5. Application of the Procedure for the Safety Evaluation of Flavouring Substances	10
6. Comparison of the Intake Estimations Based on the MSDI Approach and the mTAMDI Approach	11
7. Considerations of Combined Intakes from Use as Flavouring Substances	11
8. Toxicity.....	11
8.1. Acute Toxicity	11
8.2. Subacute, Subchronic, Chronic and Carcinogenicity Studies.....	12
8.3. Developmental / Reproductive Toxicity Studies	13
8.4. Genotoxicity Studies.....	13
9. Conclusions	14
Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 46, revision 1	16
Table 2: Summary of Safety Evaluation Applying the Procedure (Based on Intakes Calculated by the MSDI Approach).....	17
Annex I: Procedure for the Safety Evaluation.....	18
Annex II: Use Levels / mTAMDI	20
Annex III: Metabolism	23
Annex IV: Toxicity	26
References	30
Abbreviations	35

BACKGROUND

Regulation (EC) No 2232/96 of the European Parliament and the Council (EC, 1996a) lays down a Procedure for the establishment of a list of flavouring substances the use of which will be authorised to the exclusion of all other substances in the EU. In application of that Regulation, a Register of flavouring substances used in or on foodstuffs in the Member States was adopted by Commission Decision 1999/217/EC (EC, 1999a), as last amended by Commission Decision 2009/163/EC (EC, 2009a). Each flavouring substance is attributed a FLAVIS-number (FL-number) and all substances are divided into 34 chemical groups. Substances within a group should have some metabolic and biological behaviour in common.

Substances which are listed in the Register are to be evaluated according to the evaluation programme laid down in Commission Regulation (EC) No 1565/2000 (EC, 2000a), which is broadly based on the Opinion of the Scientific Committee on Food (SCF, 1999a). For the submission of data by the manufacturer, deadlines have been established by Commission Regulation (EC) No 622/2002 (EC, 2002b).

The FGE is revised to include substances for which data were submitted after the deadline as laid down in Commission Regulation (EC) No 622/2002 and to take into account additional information that has been made available since the previous Opinion on this FGE.

The Revision also includes newly notified substances belonging to the same chemical groups evaluated in this FGE.

After the completion of the evaluation programme the Union List of flavouring substances for use in or on foods in the EU shall be adopted (Article 5 (1) of Regulation (EC) No 2232/96) (EC, 1996a).

HISTORY OF THE EVALUATION

FGE	Opinion adopted by EFSA	Link	No. of candidate substances
FGE.46	March 2008	http://www.efsa.europa.eu/en/scdocs/scdoc/955.htm	3
FGE.46Rev1	November 2010		4

The present Revision of FGE.46, FGE.46Rev1, includes the assessment of one additional candidate substance, diammonium sulfide [FL-no: 16.002]. No toxicity and/or metabolism data were provided for this substance (Flavour Industry, 2009p). A literature search for this substance did not provide any further data on toxicity or metabolism.

Furthermore, additional information on the specifications requested in FGE.46 have become available for two substances. These data have been included in the present FGE as well.

TERMS OF REFERENCE

The European Food Safety Authority (EFSA) is requested to carry out a risk assessment on flavouring substances in the Register prior to their authorisation and inclusion in a Union List according to Commission Regulation (EC) No 1565/2000 (EC, 2000a). In addition, the Commission requested EFSA to evaluate newly notified flavouring substances, where possible, before finalising the evaluation programme.

ASSESSMENT

1. Presentation of the Substances in Flavouring Group Evaluation 46, Revision 1

1.1. Description

The present Flavouring Group Evaluation 46, Revision 1 (FGE.46, Rev1), using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000 (EC, 2000a) (The Procedure – shown in schematic form in Annex I), deals with ammonia and three ammonium salts. These four flavouring substances (candidate substances) belong to chemical group 30, Annex I of Commission Regulation (EC) No 1565/2000 (EC, 2000a). The four candidate substances, their chemical Register names, FLAVIS- (FL-), Chemical Abstract Service- (CAS-), Council of Europe- (CoE-) and Flavor and Extract Manufacturers Association- (FEMA-) numbers, structure and specifications, are listed in Table 1. This group of candidate substances includes diammonium sulphide [FL-no: 16.002], ammonia [FL-no: 16.009], ammonium chloride [FL-no: 16.048] and ammonium hydrogen sulfide [FL-no: 16.059].

The Joint FAO/WHO Expert Committee on Food Additives (the JECFA) evaluated ammonium carbonate and ammonium hydrogen carbonate in 1982 (JECFA, 1982a). Because toxicological studies were limited for these compounds, data on related ammonium salts (primarily ammonium chloride) and carbonate salts (primarily sodium and potassium carbonate and hydrogen carbonate (bicarbonate)) were also summarised and discussed. An acceptable daily intake (ADI) in mg/kg body weight (bw) was not deemed necessary: “ADI not specified”.

The SCF made a recommendation on ammonium chloride in liquorice products in an Opinion expressed on 11 October 1991 (SCF, 1992), in which the SCF concluded that it is not possible to allocate an acceptable daily intake (ADI) to ammonium chloride as no data are available to establish a no adverse effect level for the ingestion of ammonium chloride.

The outcome of the safety evaluation is summarised in table 2.

1.2. Stereoisomers

The candidate substances cannot exist as geometrical or optical isomers.

1.3. Natural Occurrence in Food

Ammonia [FL-no: 16.009] and hydrogen sulphide (from ammonium hydrogen sulfide [FL-no: 16.059] and diammonium sulphide [FL-no: 16.002]) have been reported to occur naturally in apple, banana, barley, beef, beer, beetroot, butter, cabbage, carrot, cauliflower, caviar, celery, cheese, chicken, coffee, sweet corn, egg, fish (fatty, lean), fruit juice, hop oil, kale, lettuce, malt, milk, mushroom, onion, pork, potato chips, radish, rhubarb, rice, rutabaga, shrimps, soybean, squid, black tea, tomato, wasabi and wine (TNO, 2000; TNO, 2010).

Quantitative data on the natural occurrence in food reported include (TNO, 2000; TNO, 2010):

- Ammonia [FL-no: 16.009]/ammonium ion: 235 mg/kg in fresh apple, 8130 mg/kg in barley, 8800 mg/kg in beetroot, up to 11060 mg/kg in cooked cabbage, 3970 mg/kg in carrot, 6376 mg/kg in cauliflower, 19600 mg/kg in celery leaves and/or stalks, 164400 mg/kg in tilsit cheese, 0.2 mg/kg in fried chicken, up to 820 mg/kg in coffee, 10030 mg/kg in sweet corn, up to 9 mg/kg in egg, up to 2928 mg/kg in fish (fatty, salted), 10660 mg/kg in hop oil, 15260 mg/kg in kale, 10260 mg/kg in lettuce, 1192 mg/kg in malt, 8450 mg/kg in radish (raw), 6340 mg/kg in rhubarb, 400 mg/kg in black tea, up to 40 mg/kg in red wine and up to 69 mg/kg in white wine.
H₂S/HS⁻ ion: up to 0.004 mg/kg in beer, up to 0.002 mg/kg in butter, up to 0.8 mg/kg in cheese, up to 1.7 mg/kg in chicken, up to 0.2 mg/kg in sweet corn, up to 1.5 mg/kg in egg, trace amount in

grapefruit juice, up to 0.0005 mg/kg in milk, 0.01 mg/kg in mushroom, up to 0.03 mg/kg in orange juice, trace amount in soybean and up to 0.1 mg/kg in strawberry.

According to TNO, the diammonium sulphide [FL-no: 16.002] has not been reported in any food items (TNO, 2010).

2. Specifications

Purity criteria for the four substances have been provided by the Flavour Industry (EFFA, 2006g; Flavour Industry, 2009p) (Table 1).

Judged against the requirements in Annex II of Commission Regulation (EC) No 1565/2000 (EC, 2000), the purity criteria for one of the candidate substances are deficient in one of the parameters as the identification test is missing. Otherwise the specifications are adequate for all four candidate substances (see Section 1.2 and Table 1).

3. Intake Data

Annual production volumes of the flavouring substances as surveyed by the Industry can be used to calculate the “Maximised Survey-derived Daily Intake” (MSDI) by assuming that the production figure only represents 60 % of the use in food due to underreporting and that 10 % of the total EU population are consumers (SCF, 1999a).

However, the Panel noted that due to year-to-year variability in production volumes, to uncertainties in the underreporting correction factor and to uncertainties in the percentage of consumers, the reliability of intake estimates on the basis of the MSDI approach is difficult to assess.

The Panel also noted that in contrast to the generally low *per capita* intake figures estimated on the basis of this MSDI approach, in some cases the regular consumption of products flavoured at use levels reported by the Flavour Industry in the submissions would result in much higher intakes. In such cases, the human exposure thresholds below which exposures are not considered to present a safety concern might be exceeded.

Considering that the MSDI model may underestimate the intake of flavouring substances by certain groups of consumers, the SCF recommended also taking into account the results of other intake assessments (SCF, 1999a).

One of the alternatives is the “Theoretical Added Maximum Daily Intake” (TAMDI) approach, which is calculated on the basis of standard portions and upper use levels (SCF, 1995) for flavourable beverages and foods in general, with exceptional levels for particular foods. This method is regarded as a conservative estimate of the actual intake by most consumers because it is based on the assumption that the consumer regularly eats and drinks several food products containing the same flavouring substance at the upper use level.

One option to modify the TAMDI approach is to base the calculation on normal rather than upper use levels of the flavouring substances. This modified approach is less conservative (e.g., it may underestimate the intake of consumers being loyal to products flavoured at the maximum use levels reported) (EC, 2000a). However, it is considered as a suitable tool to screen and prioritise the flavouring substances according to the need for refined intake data (EFSA, 2004a).

3.1. Estimated Daily *per Capita* Intake (MSDI Approach)

The intake estimation is based on the Maximised Survey-derived Daily Intake (MSDI) approach, which involves the acquisition of data on the amounts used in food as flavourings (SCF, 1999a). These

data are derived from surveys on annual production volumes in Europe. These surveys were conducted in 1995 by the International Organization of the Flavour Industry, in which flavour manufacturers reported the total amount of each flavouring substance incorporated into food sold in the EU during the previous year (IOFI, 1995). The intake approach does not consider the possible natural occurrence in food.

Average *per capita* intake (MSDI) is estimated on the assumption that the amount added to food is consumed by 10 % of the population⁴ (Eurostat, 1998). This is derived for candidate substances from estimates of annual volume of production provided by Industry and incorporates a correction factor of 0.6 to allow for incomplete reporting (60 %) in the Industry surveys (SCF, 1999a).

In the present Flavouring Group Evaluation, Revision 1 (FGE.46Rev1) the total annual production volume of the four candidate substances for use as flavouring substances in Europe was reported to be approximately 2000 kg (EFFA, 2006g; Flavour Industry, 2009p). The daily *per capita* intakes for the candidate substances are 62 microgram (diammonium sulphide, [FL-no: 16.002]), 140 microgram (ammonium chloride, [FL-no: 16.048]), 34 microgram (ammonia, [FL-no: 16.009]) and 5.6 microgram (ammonium hydrogen sulphide, [FL-no: 16.059]) (Table 2).

3.2. Intake Estimated on the Basis of the Modified TAMDI (mTAMDI)

The method for calculation of modified Theoretical Added Maximum Daily Intake (mTAMDI) values is based on the approach used by SCF up to 1995 (SCF, 1995).

The assumption is that a person may consume a certain amount of flavourable foods and beverages per day.

For the present evaluation of the four candidate substances, information on food categories and normal and maximum use levels^{5,6,7} were submitted by the Flavour Industry (EFFA, 2006g; EFFA, 2007a) only for three of the four candidate substances. The four candidate substances are used in flavoured food products divided into the food categories, outlined in Annex III of the Commission Regulation (EC) No 1565/2000 (EC, 2000a), as shown in Table 3.1. For the present calculation of mTAMDI, the reported normal use levels were used. In case different use levels were reported for different food categories the highest reported normal use level was used.

For diammonium sulphide, [FL-no: 16.002] no use and use levels have been reported by Industry.

⁴ EU figure 375 millions. This figure relates to EU population at the time for which production data are available, and is consistent (comparable) with evaluations conducted prior to the enlargement of the EU. No production data are available for the enlarged EU.

⁵ "Normal use" is defined as the average of reported usages and "maximum use" is defined as the 95th percentile of reported usages (EFFA, 2002i).

⁶ The normal and maximum use levels in different food categories (EC, 2000) have been extrapolated from figures derived from 12 model flavouring substances (EFFA, 2004e).

⁷ The use levels from food category 5 "Confectionery" have been inserted as default values for food category 14.2 "Alcoholic beverages" for substances for which no data have been given for food category 14.2 (EFFA, 2007a).

Table 3.1 Use of Candidate Substances, for which Industry has Provided Data on Food Categories and Normal and Maximum Use Levels (three of the four Candidate Substances)

Food category	Description	Flavourings used
01.0	Dairy products, excluding products of category 2	All three
02.0	Fats and oils, and fat emulsions (type water-in-oil)	Only [FL-no: 16.059]
03.0	Edible ices, including sherbet and sorbet	Only [FL-no: 16.059]
04.1	Processed fruits	Only [FL-no: 16.059]
04.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	None
05.0	Confectionery	All three
06.0	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	Only [FL-no: 16.059]
07.0	Bakery wares	Only [FL-no: 16.059]
08.0	Meat and meat products, including poultry and game	All three
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	All three
10.0	Eggs and egg products	None
11.0	Sweeteners, including honey	None
12.0	Salts, spices, soups, sauces, salads, protein products etc.	All three
13.0	Foodstuffs intended for particular nutritional uses	Only [FL-no: 16.059]
14.1	Non-alcoholic ("soft") beverages, excl. dairy products	Only [FL-no: 16.059]
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts	All three
15.0	Ready-to-eat savouries	Only [FL-no: 16.059]
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 1 – 15	All three

According to the Flavour Industry the normal use levels for the three candidate substances, for which use levels have been provided, are in the range of 0.1 - 1000 mg/kg food, and the maximum use levels are in the range of 1 - 3000 mg/kg (EFFA, 2002i; EFFA, 2006g; EFFA, 2007a).

The mTAMDI values for the two candidate substances, ammonia and ammonium chloride [FL-no: 16.009 and 16.048] from structural class I are 110000 and 220000 microgram/person/day, respectively. For the third candidate substance, ammonium hydrogen sulfide [FL-no: 16.059] from structural class III (see Section 5) the mTAMDI is 220 microgram/person/day. No use levels have been provided for [FL-no: 16.002].

For detailed information on use levels and intake estimations based on the mTAMDI approach, see Section 6 and Annex II.

4. Absorption, Distribution, Metabolism and Elimination

Ammonia and ammonium ion are present in aqueous environment at different ratios depending on the pH.

Ammonia is produced endogenously in all mammalian species. In the gut ammonia is produced by bacterial degradation of nucleic and amino acids from ingested food. The estimated production of ammonia in the human intestine may range from 10 mg/day in the duodenum to 3 g/day in the colon. Ammonia is readily absorbed from the gastro-intestinal tract, after which it enters the portal circulation and is transformed to urea in the liver via the urea cycle, and is subsequently excreted as urea via the kidneys. Ammonium chloride is easily absorbed.

Ammonium hydrogen sulphide and diammonium sulphide are expected to give rise to ammonium ion and hydrogen sulphide in the stomach and to be rapidly absorbed.

Hydrogen sulphide is also endogenously produced. The main source is from metabolism of sulphur-containing amino acids in the intestinal tract; other sources are bacterial reduction of inorganic sulphate and sulphite in the large intestine and fermentation of sulphur-containing amino acids.

Sulphide concentrations in whole blood ranged from 10 – 100 micromol/l (corresponding to 320 – 3200 microgram/l) in six healthy adults (Richardson et al., 2000). The major pathway for sulphide metabolism is oxidation to sulphate and excretion by the kidneys. The primary location for these reactions is the liver. There is an intestinal enzymatic detoxification system for endogenously produced sulphide that may take care of the low amounts of hydrogen sulphide that may be ingested as flavouring substance.

All four candidate substances may therefore be predicted to be metabolised to innocuous products at the estimated levels of exposure as flavouring substances, according to the MSDI approach.

A more detailed discussion of the metabolism of the candidate substances in this evaluation is provided in Annex III.

5. Application of the Procedure for the Safety Evaluation of Flavouring Substances

The application of the Procedure is based on intakes estimated on the basis of the MSDI approach. Where the mTAMDI approach indicates that the intake of a flavouring substance might exceed its corresponding threshold of concern, a formal safety assessment is not carried out using the Procedure. In these cases the Panel requires more precise data on use and use levels. For comparison of the intake estimations based on the MSDI approach and the mTAMDI approach, see Section 6.

For the safety evaluation of the four candidate substances from chemical group 30 the Procedure as outlined in Annex I was applied, based on the MSDI approach. The stepwise evaluations of the four substances are summarised in Table 2.

Step 1

The candidate substances are classified according to the decision tree approach by Cramer et al. (Cramer et al, 1978). Two substances are classified into structural class I [FL-no: 16.009 and 16.048] and two into structural class III [FL-no: 16.002 and 16.059].

Step 2

On the basis of the metabolism information the two candidate substances, ammonia and ammonium chloride [FL-no: 16.009 and 16.048] in structural class I may be predicted to be metabolised to innocuous products, and their evaluation will proceed via the A-side of the Procedure scheme.

The two substances belonging to structural class III, diammonium sulphide [FL-no: 16.002] and ammonium hydrogen sulphide [FL-no: 16.059] may also be predicted to be metabolised to innocuous products, and accordingly their evaluation proceeds via the A-side of the Procedure scheme.

Step A3

The two candidate substances [FL-no: 16.009 and 16.048] assigned to structural class I have estimated European daily *per capita* intakes (MSDI) of 34 and 140 microgram, respectively (Table 6.1). These intakes are below the threshold of concern of 1800 microgram/person/day for structural class I.

The two candidate substances [FL-no: 16.002 and 16.059] assigned to structural class III have estimated daily *per capita* intake (MSDI) of 62 and 5.6 microgram, respectively (Table 6.1). These intakes are below the threshold of concern of 90 microgram/person/day for structural class III.

Based on the results of the safety evaluation sequence of the Procedure, these four candidate substances proceeding via the A-side of the Procedure scheme do not pose a safety concern when used as flavouring substances, based on the MSDI approach.

6. Comparison of the Intake Estimations Based on the MSDI Approach and the mTAMDI Approach

The estimated intakes for the two candidate substances [FL-no: 16.009 and 16.048] in structural class I based on the mTAMDI, are 110000 and 220000 microgram/person/day, respectively. For these two substances the mTAMDI is above the threshold of concern of 1800 microgram/person/day. For comparison of the intake estimates based on the MSDI approach and the mTAMDI approach see Table 6.1.

The estimated intake of the third substance [FL-no: 16.059] assigned to structural class III, based on the mTAMDI, is 220 microgram/person/day, which is above the threshold of concern for structural class III substances of 90 microgram/person/day. For comparison of the MSDI- and mTAMDI-values, see Table 6.1.

The estimated intake of [FL-no: 16.002] assigned to structural class III, cannot be calculated as Industry has not provided information on use and use levels.

For all the candidate substances further information is required. This would include more reliable intake data and then, if required, additional toxicological data.

Table 6.1 Estimated intakes based on the MSDI approach and the mTAMDI approach

FL-no	EU Register name	MSDI (µg/capita/day)	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
16.009	Ammonia	34	110000	Class I	1800
16.048	Ammonium chloride	140	220000	Class I	1800
16.002	Diammonium sulfide	62		Class III	90
16.059	Ammonium hydrogen sulphide	5.6	220	Class III	90

7. Considerations of Combined Intakes from Use as Flavouring Substances

The total estimated combined daily *per capita* intake of structurally related flavourings is estimated by summing the MSDI for individual substances.

The combined intake of the two candidate substances [FL-no: 16.009 and 16.048] from structural class I is approximately 170 microgram/capita/day, which is below the threshold of concern of 1800 microgram/person/day for structural class I substances.

The combined intake of the two candidate substances [FL-no: 16.002 and 16.059] from structural class III is approximately 70 microgram/capita/day, which is below the threshold of concern of 90 microgram/person/day for structural class III substances.

8. Toxicity

8.1. Acute Toxicity

An oral LD₅₀ of 1650 mg/kg body weight (bw) has been reported for the candidate substance ammonium chloride [FL-no: 16.048] in the rat (IUCLID Dataset, 2000d).

The acute toxicity data are summarised in Annex IV, Table IV.1.

8.2. Subacute, Subchronic, Chronic and Carcinogenicity Studies

Studies on only one candidate substance, ammonium chloride [FL-no: 16.048], have been submitted. Ammonium chloride has not been tested *per se*, but has been used to produce metabolic acidosis, and the aim of most studies has been to study the effect of acidosis on different parameters.

In studies designed to examine the effects of feeding an acidogenic diet to rats, ammonium chloride [FL-no: 16.048] was fed to rats for periods of 4 weeks, 13 weeks (approx. 1.6-3.7 g/kg bw/day) or 18 months (approx. 0.5-1.4 g/kg bw/day). A carcinogenicity study of 30 months (approx. 0.5-1.2 g/kg bw/day) was also conducted. All animals fed NH_4Cl had reduced urinary pH. Clinical condition and death rate were not affected by treatment. Growth retardation was noted in the high treatment group, and in females of the low treatment group. Urinary calcium and phosphorus excretion was increased in treatment groups, but there was no indication that bone minerals were involved. Kidney weights were increased in all ammonium chloride-treated animals. Hypertrophy of the adrenal zona glomerulosa occurred, due to chronic stimulation of the adrenal cortex by treatment-induced acidosis. No treatment-related changes in any specific tumour type were noted among treated animals. It was not possible to derive a No Observed Adverse Effect Level (NOAEL) from these studies. The 30 month carcinogenicity study gave no indication that the feeding of ammonium chloride to rats at dose levels of approximately 0.5-1.2 g/kg bw/day would induce tumours (Lina & Kuijpers, 2004).

Dietary levels of 3 g/kg bw per day ammonium chloride [FL-no: 16.048] for 6 days gave rise to renal hypertrophy in rats (Thompson & Halliburton, 1966). The same effect occurred when ammonium chloride [FL-no: 16.048] was given *ad libitum* in drinking water for 7 days, and approximately 1 g/kg bw/day was ingested. No increase occurred in uptake of tritiated thymidine in the kidney, implying that no cell division or DNA synthesis occurred (Lotspeich, 1965).

Rats receiving 1.5 % (corresponding to approximately 1.5 g/kg bw) ammonium chloride [FL-no: 16.048]/kg bw/day in drinking water for 330 days showed significant loss of bone tissue. Changes were independent of dietary Ca, and not associated with blood Ca or P. Rats that received 2 % of the same compound in drinking water for 6 months showed significant reduction in bone density, which was accentuated by a low-Ca-diet (Barzel, 1969; Barzel & Jowsey, 1969).

Although the available data do not allow the identification of a NOAEL, high dose levels were used to study effects of ammonium chloride in several animal species (1-4 g/kg bw). The results support the conclusion of the endogenous production of ammonia.

No studies have been submitted on the candidate substances ammonium hydrogen sulphide [FL-no: 16.059] and diammonium sulphide [FL-no: 16.002] or on structurally related flavouring substances. However, it is structurally related to hydrogen sulphide, which is considered below.

The mechanism of toxicity of hydrogen sulphide is the inhibition of cytochrome oxidase and through this, the inhibition of the electron transport system.

In a U.S. Environmental Protection Agency's toxicological review on hydrogen sulphide (EPA, 2003) a 90-day study on rats is described. This study by Anderson was reported in 1987 and is not publicly available. According to the description of the study, hydrogen sulphide (presumably as solution, vehicle not known) was given by gavage to groups of 20 rats daily for 89 days in doses of 0, 1, 3.5 or 7 mg/kg bw per day. For preparation of hydrogen sulphide solution, hydrogen sulphide was purged through deoxygenated, deionised water. The solution was stored in sealed vials and fresh vials were used at each administration. Blood samples were collected at days 27-31 of treatment and at necropsy. Animals were subjected to full necropsy and extensive microscopical examination. Mortality was 50 % in high-dose males compared to 5 % in control males, cause of death in the high dose group was, however, not determined. No deaths were observed in high-dose females. Compound-related neuromuscular and behavioral signs were observed in high-dose male and female animals. There were no treatment-related effects in hematology or clinical chemistry nor in body weights. Ophthalmological findings were normal. The most common findings at necropsy were red mottled

lungs. No gross lesions could be attributed to compound administration and all microscopic findings were considered incidental to compound administration. However, the information indicates procedural problems with the dosing of the animals; a 75-100 % incidence of pneumonia was reported in vehicle control and treatment groups whereas 0 % incidence of pneumonia was reported for the non-gavaged control group. According to the EPA evaluation, the lack of quality assurance precludes the use of this study for derivation of a NOEL (EPA, 2003).

In WHO drinking water guidelines (WHO, 1996) the oral dose of sodium sulphide fatal to humans (60 kg) has been estimated at 10-15 g. For drinking water no health guideline is proposed. The organoleptic threshold for hydrogen sulphide in drinking water is < 0.05 mg/l, and it is stated that the quantity of sulphide ingested from drinking water containing sulphide at this concentration is well below the amount that the body can detoxify.

Conclusions on toxicity

From the available toxicity data it is not possible to derive NOAELs for the candidate substances. However, ammonia and hydrogen sulphide are substances that are formed endogenously in the body at levels much higher than what can be formed from use of the candidate substances at the estimated levels of exposure (6 – 140 microgram/ *capita*/ day based on the MSDI approach) and for which the body has intrinsic detoxification systems. Data on metabolism and kinetics (see Section 4 and Annex III) along with the available information on toxicity give clear indication that the amounts of the candidate substances that may be ingested from the use as flavourings, according to the MSDI approach, do not raise concern with respect to toxicity.

Repeated dose toxicity data are summarised in Annex IV, Table IV.2.

8.3. Developmental / Reproductive Toxicity Studies

No studies on developmental or reproductive toxicity have been submitted for any of the candidate substances. Developmental toxicity studies in rats exposed to hydrogen sulphide via the inhalational route throughout gestation showed no adverse effects in the embryo or foetus, nor in offspring during postnatal development, at exposures up to 80 ppm (for review, see (US ATSDR, 2006)) For ammonia (actually ammonium ion) an oral developmental toxicity study has been reported (for review, see (US ATSDR, 2004)), but the effect (reduced body weight gain in off-spring) was observed at such high dose levels (> 4000 mg/kg bw/day) that the effects in the offspring were most likely the result of maternal toxicity.

8.4. Genotoxicity Studies

Ammonia gas at concentrations of 500-25000 ppm has been reported to give a negative result in a bacterial mutagenicity assay using *Salmonella typhimurium* sp. TA98, TA100, TA1535, TA1537 and TA1538 in the presence and absence of metabolic activation (Shimoi et al., 1985). Tests using *Escherichia Coli* WRP uvrA were also negative (Shimoi et al., 1985).

There are very limited *in vivo* mammalian data on the effects of ammonia. A mouse micronucleus test (single intraperitoneal dose of ammonium at 12, 25 or 50 mg/kg bw) reported dose dependent increases in the frequencies of micronuclei when compared to controls (Yadav & Kaushik, 1997). Few details were given and no conclusions can be drawn. Slight mutagenic activity has been reported in *Drosophila* following exposure to ammonia gas, but only at very toxic levels (US ATSDR, 2004).

Ammonium chloride at concentrations up to 10 mg/plate has been reported to give negative results in a bacterial mutation assay using *S. typhimurium* strains TA92, TA94, TA98, TA100, TA1535 and TA1537 (Ishidate et al., 1984; Ishidate et al., 1988). Ammonium chloride in the absence of metabolic activation caused an increase in the incidence of cells with structural chromosomal aberrations (including gaps) in Chinese hamster fibroblasts, which the authors suggested were due to osmotic

effects rather than a direct interaction with DNA (Ishidate et al., 1984; Ishidate et al., 1988). *In vivo*, ammonium chloride did not induce an increase in the incidence of micronucleated polychromatic erythrocytes obtained from the bone marrow of male ddY mice, 24 hours after one intraperitoneal injection of 62.5-500 mg/kg bw or four intraperitoneal injections of 31.3-250 mg/kg bw (Hayashi et al., 1988).

Ammonium sulphate has also been reported to give negative results in bacterial mutagenicity tests but to enhance the frequency of chromosome type aberrations (UNEP, 2004). A similar effect has however been observed with other salts (magnesium chloride, calcium chloride and sodium chloride), and is considered to be linked to osmotic effects rather than being indicative of a clastogenic effect of ammonium sulphate or the ammonium ion.

No genotoxicity studies are available on ammonium hydrogen sulphide. No mutagenicity was observed with hydrogen sulfide gas in Ames assays using *S. typhimurium* TA97, TA98, and TA100 strains in the presence and absence of metabolic activation (US ATSDR, 2006).

Conclusion on genotoxicity

Overall, the genotoxicity data available do not preclude an evaluation of the four substances through the Procedure.

Genotoxicity data are summaries in Annex IV, Table IV.4 and Table IV.5.

9. Conclusions

The present Flavouring Group Evaluation deals with ammonia [FL-no: 16.009], and three ammonia salts (diammonium sulphide [FL-no: 16.002], ammonium chloride [FL-no: 16.048] and ammonium hydrogen sulphide [FL-no: 16.059]).

The flavouring substances cannot exist as geometrical or optical isomers.

Two of the flavouring substances are classified into structural class I and two are classified into structural class III according to the decision tree approach presented by Cramer et al. (1978).

The flavouring substance ammonia in the present group has been reported to occur naturally in a wide range of food items up to very high amounts. Hydrogen sulphide is also reported to occur naturally in a wide range of food items.

In its evaluation, the Panel as a default used the Maximised Survey-derived Daily Intake (MSDI) approach to estimate the *per capita* intakes of the flavouring substances in Europe. However, when the Panel examined the information provided by the European Flavour Industry on the use levels in various foods, it appeared obvious that the MSDI approach in a number of cases would grossly underestimate the intake by regular consumers of products flavoured at the use level reported by the Industry, especially in those cases where the annual production values were reported to be small. In consequence, the Panel had reservations about the data on use and use levels provided and the intake estimates obtained by the MSDI approach.

In the absence of more precise information that would enable the Panel to make a more realistic estimate of the intakes of the flavouring substances, the Panel has decided also to perform an estimate of the daily intakes per person using a modified Theoretical Added Maximum Daily Intake (mTAMDI) approach based on the normal use levels reported by Industry. In those cases where the mTAMDI approach indicated that the intake of a flavouring substance might exceed its corresponding threshold of concern, the Panel decided not to carry out a formal safety assessment using the Procedure. In these cases the Panel requires more precise data on use and use levels.

According to the default MSDI approach, the two flavouring substances [FL-no: 16.009 and 16.048] belonging to structural class I have estimated intakes in Europe of 34 and 140 microgram/*capita*/day, respectively, which are below the threshold of concern for structural class I substances (1800 microgram/person/day). The two substances belonging to structural class III have estimated intake in Europe of 62 and 5.6 microgram/*capita*/day, respectively, which is below the threshold of concern for structural class III substances (90 microgram/person/day).

Although the genotoxicity data for the flavouring substances in this group are limited, the available data on genotoxicity do not preclude an evaluation of the candidate substances through the Procedure. For the candidate substance ammonium chloride [FL-no: 16.048] there is a well-performed carcinogenicity study available, which indicates that the substance does not induce tumours.

Ammonia is a substance that is readily absorbed in the gut. It is produced endogenously in amounts that far exceed those that are to be ingested as flavourings. The three ammonium salts are expected to give rise to ammonium ion and chloride or hydrogen sulphide. Ammonia is expected to be transported by the portal circulation to the liver and metabolised to urea by the Krebs urea cycle and subsequently excreted by the kidneys. Hydrogen sulphide is a substance that is produced endogenously. The major pathway for sulphide metabolism is oxidation to sulphate and excretion by the kidney. The major oxidation product of sulphide is thiosulphate which is then converted to sulphate. The primary location for these reactions is the liver. All four substances are accordingly expected to be metabolised to innocuous substances at the anticipated levels of intake as flavouring substances.

It was noted that where toxicity data were available they were consistent with the conclusions in the present flavouring group evaluation using the Procedure.

On the basis of the default MSDI approach the Panel concluded that the flavouring substances would not give rise to safety concerns at the estimated levels of intake arising from their use as flavouring substances.

When the estimated intakes were based on the mTAMDI approach the values for the two substances from structural class I, ammonia and ammonium chloride [FL-no: 16.009 and 16.048], are 110000 microgram/person/day and 220000 microgram/person/day, respectively. These values are above the threshold of concern for structural class I of 1800 microgram/person/day. For one of the substances from structural class III, ammonium hydrogen sulphide [FL-no: 16.059], the mTAMDI value is 220 microgram/person/day. This value is above the threshold for structural class III of 90 microgram/person/day. For the other substance from structural class III no data are available on use and use levels.

Thus, intake estimates based on the mTAMDI approach exceed the threshold of concern for the three flavouring substances in this flavouring group, and more reliable exposure data are requested for all four. On the basis of such additional data, these flavouring substances should be reconsidered using the Procedure. Subsequently, additional data might become necessary.

In order to determine whether this evaluation could be applied to the materials of commerce, it is necessary to consider the available specifications. Specifications including complete purity criteria for the materials of commerce have been provided for the four flavouring substances.

Identity test is missing for one of the flavouring substances, ammonium hydrogen sulphide [FL-no: 16.059]. Thus, the final evaluation of the materials of commerce cannot be performed for this substance, pending further information. The remaining three flavouring substances, ammonia [FL-no: 16.009], ammonium chloride [FL-no: 16.048] and diammonium sulfide [FL-no: 16.002] would present no safety concern at the levels of intakes estimated on the basis of the MSDI approach.

TABLE 1: SPECIFICATION SUMMARY OF THE SUBSTANCES IN THE FLAVOURING GROUP EVALUATION 46, REVISION 1

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 46, Revision 1

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
16.002	Diammonium sulfide	(NH ₄) ₂ S	2053 482 12135-76-1	Liquid H ₈ N ₂ S 68.15	Soluble Freely soluble	40 IR 95 %	1.378-1.398 0.995-0.999	
16.009	Ammonia	NH ₃	739 7664-41-7	Gas H ₃ N 17.03	Soluble Freely soluble	n.a. n.a. 95 %	n.a. n.a.	Physical form: Gas. ID test not applicable.
16.048	Ammonium chloride	NH ₄ ⁺ , Cl ⁻	12125-02-9	Solid H ₄ NCl 53.49	Very soluble Freely soluble	decomposes 99 %	n.a. n.a.	ID test not applicable.
16.059	Ammonium hydrogen sulphide	NH ₄ ⁺ , HS ⁻	2053 482 12124-99-1	Liquid H ₅ NS 51.11	Soluble Freely soluble	40 95 %	1.378-1.398 0.995-0.999	ID 6)

1) Solubility in water, if not otherwise stated.

2) Solubility in 95 % ethanol, if not otherwise stated.

3) At 1013.25 hPa, if not otherwise stated.

4) At 20°C, if not otherwise stated.

5) At 25°C, if not otherwise stated.

6) ID: Missing identification test.

TABLE 2: SUMMARY OF SAFETY EVALUATION APPLYING THE PROCEDURE (BASED ON INTAKES CALCULATED BY THE MSDI APPROACH)

Table 2: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

FL-no	EU Register name	Structural formula	MSDI 1) ($\mu\text{g/capita/day}$)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
16.009	Ammonia	NH_3	34	Class I A3: Intake below threshold	4)	7)	
16.048	Ammonium chloride	$\text{NH}_4^+, \text{Cl}^-$	140	Class I A3: Intake below threshold	4)	7)	
16.002	Diammonium sulfide	$(\text{NH}_4)_2\text{S}$	62	Class III A3: Intake below threshold	4)	7)	
16.059	Ammonium hydrogen sulphide	$\text{NH}_4^+, \text{HS}^-$	5.6	Class III A3: Intake below threshold	4)	6)	

1) EU MSDI: Amount added to food as flavour in (kg / year) $\times 10\text{E}9 / (0.1 \times \text{population in Europe} (= 375 \times 10\text{E}6) \times 0.6 \times 365) = \mu\text{g/capita/day}$.

2) Thresholds of concern: Class I = 1800, Class II = 540, Class III = 90 $\mu\text{g/person/day}$.

3) Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.

4) No safety concern based on intake calculated by the MSDI approach of the named compound.

5) Data must be available on the substance or closely related substances to perform a safety evaluation.

6) No safety concern at estimated level of intake of the material of commerce meeting the specification of Table 1 (based on intake calculated by the MSDI approach).

7) Tentatively regarded as presenting no safety concern (based on intake calculated by the MSDI approach) pending further information on the purity of the material of commerce and/or information on stereoisomerism.

8) No conclusion can be drawn due to lack of information on the purity of the material of commerce.

ANNEX I: PROCEDURE FOR THE SAFETY EVALUATION

The approach for a safety evaluation of chemically defined flavouring substances as referred to in Commission Regulation (EC) No 1565/2000 (EC, 2000a), named the "Procedure", is shown in schematic form in Figure I.1. The Procedure is based on the Opinion of the Scientific Committee on Food expressed on 2 December 1999 (SCF, 1999a), which is derived from the evaluation Procedure developed by the Joint FAO/WHO Expert Committee on Food Additives at its 44th, 46th and 49th meetings (JECFA, 1995; JECFA, 1996a; JECFA, 1997a; JECFA, 1999b).

The Procedure is a stepwise approach that integrates information on intake from current uses, structure-activity relationships, metabolism and, when needed, toxicity. One of the key elements in the Procedure is the subdivision of flavourings into three structural classes (I, II, III) for which thresholds of concern (human exposure thresholds) have been specified. Exposures below these thresholds are not considered to present a safety concern.

Class I contains flavourings that have simple chemical structures and efficient modes of metabolism, which would suggest a low order of oral toxicity. Class II contains flavourings that have structural features that are less innocuous, but are not suggestive of toxicity. Class III comprises flavourings that have structural features that permit no strong initial presumption of safety, or may even suggest significant toxicity (Cramer et al., 1978). The thresholds of concern for these structural classes of 1800, 540 or 90 microgram/person/day, respectively, are derived from a large database containing data on subchronic and chronic animal studies (JECFA, 1996a).

In Step 1 of the Procedure, the flavourings are assigned to one of the structural classes. The further steps address the following questions:

- can the flavourings be predicted to be metabolised to innocuous products⁸ (Step 2)?
- do their exposures exceed the threshold of concern for the structural class (Step A3 and B3)?
- are the flavourings or their metabolites endogenous⁹ (Step A4)?
- does a NOAEL exist on the flavourings or on structurally related substances (Step A5 and B4)?

In addition to the data provided for the flavouring substances to be evaluated (candidate substances), toxicological background information available for compounds structurally related to the candidate substances is considered (supporting substances), in order to assure that these data are consistent with the results obtained after application of the Procedure.

The Procedure is not to be applied to flavourings with existing unresolved problems of toxicity. Therefore, the right is reserved to use alternative approaches if data on specific flavourings warranted such actions.

⁸ "Innocuous metabolic products": Products that are known or readily predicted to be harmless to humans at the estimated intakes of the flavouring agent" (JECFA, 1997a).

⁹ "Endogenous substances": Intermediary metabolites normally present in human tissues and fluids, whether free or conjugated; hormones and other substances with biochemical or physiological regulatory functions are not included (JECFA, 1997a).

Procedure for Safety Evaluation of Chemically Defined Flavouring Substances

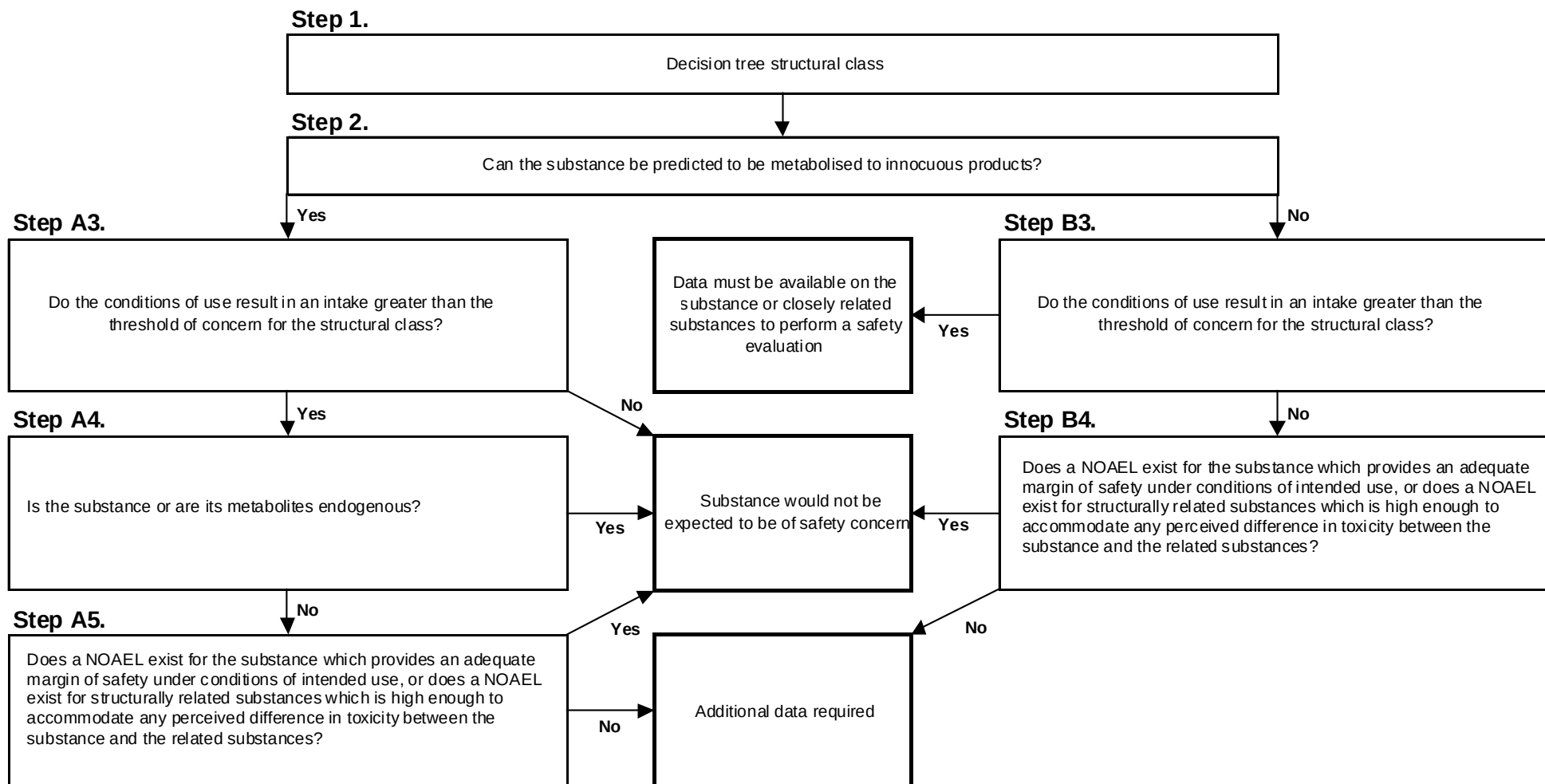


Figure I.1 Procedure for Safety Evaluation of Chemically Defined Flavouring Substances

ANNEX II: USE LEVELS / mTAMDI

II.1 Normal and Maximum Use Levels

For each of the 18 Food categories (Table II.1.1) in which the candidate substances are used, Flavour Industry reports a “normal use level” and a “maximum use level” (EC, 2000a). According to the Industry the “normal use” is defined as the average of reported usages and “maximum use” is defined as the 95th percentile of reported usages (EFFA, 2002i). The normal and maximum use levels in different food categories have been extrapolated from figures derived from 12 model flavouring substances (EFFA, 2004e).

Table II.1.1 Food categories according to Commission Regulation (EC) No 1565/2000 (EC, 2000a)

Food category	Description
01.0	Dairy products, excluding products of category 02.0
02.0	Fats and oils, and fat emulsions (type water-in-oil)
03.0	Edible ices, including sherbet and sorbet
04.1	Processed fruit
04.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds
05.0	Confectionery
06.0	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery
07.0	Bakery wares
08.0	Meat and meat products, including poultry and game
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms
10.0	Eggs and egg products
11.0	Sweeteners, including honey
12.0	Salts, spices, soups, sauces, salads, protein products, etc.
13.0	Foodstuffs intended for particular nutritional uses
14.1	Non-alcoholic (“soft”) beverages, excl. dairy products
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts
15.0	Ready-to-eat savouries
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 01.0 - 15.0

The “normal and maximum use levels” are provided by Industry for three of the four candidate substances in the present flavouring group (Table II.1.2).

Table II.1.2 Normal and Maximum use levels (mg/kg) for the candidate substances in FGE.46, Revision 1 (EFFA, 2006g; EFFA, 2007a).

FL-no	Food Categories																	
	Normal use levels (mg/kg)																	
	Maximum use levels (mg/kg)																	
	01.0	02.0	03.0	04.1	04.2	05.0	06.0	07.0	08.0	09.0	10.0	11.0	12.0	13.0	14.1	14.2	15.0	16.0
16.009	500	0	0	0	-	500	0	0	500	500	-	-	500	0	0	500	500	500
	1500	0	0	0	-	1500	0	0	1500	1500	-	-	1500	0	0	1500	1500	1500
16.048	1000	0	0	0	-	1000	0	0	1000	1000	-	-	1000	0	0	1000	1000	1000
	3000	0	0	0	-	3000	0	0	3000	3000	-	-	3000	0	0	3000	3000	3000
16.059	0,4	0,2	0,4	0,3	-	0,4	0,2	0,4	0,1	0,1	-	-	0,2	1	0,2	0,4	-	0,2
	2	1	2	1,5	-	2	1	2	0,4	0,4	-	-	1	5	1	2	-	1

II.2 mTAMDI Calculations

The method for calculation of modified Theoretical Added Maximum Daily Intake (mTAMDI) values is based on the approach used by SCF up to 1995 (SCF, 1995). The assumption is that a person may consume the amount of flavourable foods and beverages listed in Table II.2.1. These consumption estimates are then multiplied by the reported use levels in the different food categories and summed up.

Table II.2.1 Estimated amount of flavourable foods, beverages, and exceptions assumed to be consumed per person per day (SCF, 1995)

Class of product category	Intake estimate (g/day)
Beverages (non-alcoholic)	324.0
Foods	133.4
Exception a: Candy, confectionery	27.0
Exception b: Condiments, seasonings	20.0
Exception c: Alcoholic beverages	20.0
Exception d: Soups, savouries	20.0
Exception e: Others, e.g. chewing gum	e.g. 2.0 (chewing gum)

The mTAMDI calculations are based on the normal use levels reported by Industry. The seven food categories used in the SCF TAMDI approach (SCF, 1995) correspond to the 18 food categories as outlined in Commission Regulation (EC) No 1565/2000 (EC, 2000a) and reported by the Flavour Industry in the following way (see Table II.2.2):

- Beverages (SCF, 1995) correspond to food category 14.1 (EC, 2000a)
- Foods (SCF, 1995) correspond to the food categories 1, 2, 3, 4.1, 4.2, 6, 7, 8, 9, 10, 13, and/or 16 (EC, 2000a)
- Exception a (SCF, 1995) corresponds to food category 5 and 11 (EC, 2000a)
- Exception b (SCF, 1995) corresponds to food category 15 (EC, 2000a)
- Exception c (SCF, 1995) corresponds to food category 14.2 (EC, 2000a)
- Exception d (SCF, 1995) corresponds to food category 12 (EC, 2000a)
- Exception e (SCF, 1995) corresponds to others, e.g. chewing gum.

Table II.2.2 Distribution of the 18 food categories listed in Commission Regulation (EC) No 1565/2000 (EC, 2000a) into the seven SCF food categories used for TAMDI calculation (SCF, 1995)

Food categories according to Commission Regulation 1565/2000		Distribution of the seven SCF food categories		
Key	Food category	Food	Beverages	Exceptions
01.0	Dairy products, excluding products of category 02.0	Food		
02.0	Fats and oils, and fat emulsions (type water-in-oil)	Food		
03.0	Edible ices, including sherbet and sorbet	Food		
04.1	Processed fruit	Food		
04.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	Food		
05.0	Confectionery			Exception a
06.0	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	Food		
07.0	Bakery wares	Food		
08.0	Meat and meat products, including poultry and game	Food		
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	Food		
10.0	Eggs and egg products	Food		
11.0	Sweeteners, including honey			Exception a
12.0	Salts, spices, soups, sauces, salads, protein products, etc.			Exception d
13.0	Foodstuffs intended for particular nutritional uses	Food		
14.1	Non-alcoholic ("soft") beverages, excl. dairy products		Beverages	
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts			Exception c
15.0	Ready-to-eat savouries			Exception b
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be	Food		

Table II.2.2 Distribution of the 18 food categories listed in Commission Regulation (EC) No 1565/2000 (EC, 2000a) into the seven SCF food categories used for TAMDI calculation (SCF, 1995)

Food categories according to Commission Regulation 1565/2000	Distribution of the seven SCF food categories
placed in categories 01.0 - 15.0	

The mTAMDI values (see Table II.2.3) are presented for each of the three flavouring substances in the present Flavouring Group Evaluation 46, Revision 1, for which Industry has provided use and use levels (EFFA, 2006g; EFFA, 2007a). The mTAMDI values are only given for highest reported normal use.

Table II.2.3 Estimated intakes based on the mTAMDI approach

FL-no	EU Register name	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
16.009	Ammonia	110000	Class I	1800
16.048	Ammonium chloride	220000	Class I	1800
16.002	Diammonium sulfide		Class III	90
16.059	Ammonium hydrogen sulphide	220	Class III	90

ANNEX III: METABOLISM

III.1. Absorption, Distribution and Elimination

The candidate substance ammonia [FL-no: 16.009] is produced endogenously in all mammalian species and is a metabolite from the degradation of purines and the deamination of amino acids. In the gut ammonia is formed by bacterial degradation of amino acids and nucleic acids from ingested food. The estimated production of ammonia in the human intestine may range from 10 mg/day in the duodenum to about 3 g/day in the colon. Almost all ammonia formed is absorbed. Ammonia uptake increases with higher pH of luminal contents (Castell & Moore, 1971). After absorption ammonia enters the portal circulation, and in healthy humans ammonium ions are rapidly transformed to urea in the liver, so only small amounts of ammonium reaches the systemic circulation (Summerskill & Wolpert, 1970).

For ammonium salts the anion may play an important role in determining what effects will occur. Ammonium chloride has been used in many animal studies to induce metabolic acidosis, due to the formation of hydrogen chloride. Acidosis may occur with other ammonium salts, but the degree of acidosis is determined by the ability of the kidneys to excrete the specific anion. Therefore, it is not appropriate to extrapolate the findings from one ammonium salt to another (De Sousa et al., 1974).

Ammonium chloride [FL-no: 16.048] is easily absorbed. Ammonium hydrogen sulphide [FL-no: 16.059] will produce ammonia and hydrogen sulphide after absorption. Hydrogen sulphide and soluble sulphides are rapidly absorbed following ingestion. Soluble sulphides occur as hydrogen sulphide in body fluids; in terms of their systemic effects, no distinctions are recognised between them and hydrogen sulphide (U.S. National Research Council, 1979).

Ammonia is normally present in all tissues. The distribution is pH dependent, as NH_3 diffuses more readily than NH_4^+ . The lower the pH is in a compartment the higher the amount of ammonia may be (U.S. National Research Council, 1979). Although most ingested ammonia that is absorbed in the intestine is transformed to urea by the liver, some nitrogen from ammonia has been reported to be incorporated into tissue proteins (Vitti et al., 1964; Richards et al., 1968; Fürst et al., 1970).

The majority of ingested ammonia is excreted as urea in the urine (Haworth et al., 1978; Nelson & Cox, 2000c). During metabolic acidosis the concentrations of renal glutaminase, which catalyses the release of ammonia in the kidney tubular epithelium, increase proportionally to ammonium ion excretion (Davies & Yudkin, 1952; Muntwyler et al., 1956; Kamin & Handler, 1957). Ammonia may be excreted through expired air, which most likely is due to synthesis of ammonia from salivary urea by oral microflora (Biswas & Kleinberg, 1971; Hunt, 1977; Larson et al., 1977; Barrow & Steinhagen, 1980). Mammals may excrete ammonia directly into the urine; glutaminase catalyses the release of ammonia in the kidney tubular epithelium where it serves as an acceptor of H^+ and regulates the acid-base balance (White et al., 1973). Less than 1 % of the total amount of ammonia produced in the human intestinal tract is excreted in the faeces (Summerskill & Wolpert, 1970).

In humans (20 male and female volunteers orally administered 9 mg/kg bw ammonium chloride), a transient increase in arterial blood ammonia concentrations occurred in about 50 % of the subjects. At 15 minutes concentrations peaked (mean of 1.4 mg NH_3/l) and by 30 minutes concentrations returned to fasting levels (mean of 1.05 mg NH_3/l). In subjects with liver cirrhosis (50 males), fasting levels were already elevated (mean of 1.56 mg NH_3/l) and increased to much higher peak concentrations (mean of 3.7 mg NH_3/l) at 15 minutes than in volunteers without known liver disease. The return to fasting levels was slow and indicated impaired hepatic urea synthesis (Conn, 1972).

Sulphides are found in a number of foodstuffs and beverages. In cooked foods sulphides may be formed from sulphur-containing amino acids. In skimmed milk the level may be 0.8 mg/l. Hydrogen sulphide in cooked meat may range from 0.3 mg/kg in beef to 0.4 in lamb. Levels of hydrogen sulphide are higher in anaerobically packaged meat (Kraft et al., 1956).

Hydrogen sulphide is endogenously produced; the main source is from metabolism of sulphur-containing amino acids in the intestinal tract. Other sources are bacterial reduction of inorganic sulphate and sulphite in the large intestine and fermentation of sulphur-containing amino acids (Richardson et al., 2000). At pH 6 hydrogen sulphide occurs mainly as the acid, whereas at pH 7.5 a large proportion is in the anionic form, mainly as monohydrogen sulphide. At pH 10 sulphide is present in appreciable concentrations (Roediger et al., 1997). Six healthy human subjects showed blood sulphide concentrations ranging from 10 to 100 micromol/l. Whole blood sulphide did not change significantly when increasing amounts of protein from meat were fed in the diet; however, faecal sulphide showed a significant increase from 160 to 750 nmol/g in four subjects fed diets containing 60 or 420 g meat (Richardson et al., 2000). It has been shown that the proximal small intestine extracts sulphate from the circulation against the concentration gradient indicating the existence of a sulphur pump (Roediger et al., 1997).

III.2. Metabolism

In the liver the majority of excess nitrogen, either in the form of ammonia absorbed from the intestinal tract or in the form of glutamine or alanine transported from peripheral tissues, is transformed to urea in the Krebs urea cycle. The formation of urea occurs only in the liver.

Ammonia that is produced in peripheral tissues is not transported as such to the liver but is converted to glutamine or alanine. Blood levels of ammonia are kept low, since the compound is toxic and high levels may impair brain function. Most tissues convert ammonia to glutamine, using glutamine synthetase. Muscle tissue, however, uses the glucose-alanine cycle in which glycolysis produces pyruvate, which produces alanine and alpha-ketoglutarate following transamination with glutamate. In the liver alanine loses its nitrogen yielding ammonia and pyruvate. Glutamine is hydrolysed in the liver by glutaminase to release ammonium for urea synthesis.

Whatever its source, ammonium that is generated in the liver mitochondria is immediately used together with carbon dioxide (as carbonate ion) produced by mitochondrial respiration, to form carbamoyl phosphate, in an ATP-dependent reaction. The carbamoyl phosphate, which is an activated carbamoyl group donor, then enters the urea cycle. In the cycle, urea is produced from the guanidino group of arginine by the hydrolytic enzyme arginase. The other product is ornithine. The amino nitrogen of catabolised amino acids or from ammonia is used to convert ornithine back to arginine.

The cycle has four enzymatic steps:

- 1) Carbamoyl phosphate donates its carbamoyl group to ornithine to form citrulline, with the release of inorganic phosphor.
- 2) The second amino group is introduced from aspartate by a condensation reaction between the amino group of aspartate and the carbonyl group of citrulline forming argininosuccinate. Aspartate is generated in mitochondria by transamination.
- 3) The argininosuccinate is then reversibly cleaved to form free arginine and fumarate.
- 4) Arginase then cleaves arginine to yield urea and ornithine. Urea is then released into the cytosolic pool of metabolites (Fürst et al., 1969; Pitts, 1971; Haworth et al., 1978; Nelson & Cox, 2000c).

Metabolism of hydrogen sulphide may follow three different pathways. The major pathway is oxidation via thiosulphate to sulphate and subsequent excretion by the kidney. The primary location for these reactions is the liver (Bartholomew et al., 1980). The other options are methylation to methanethiol and dimethyl sulphide or reaction with disulphide- or metallo-containing proteins. In the colon, sulphate-reducing bacteria reduce sulphate to hydrogen sulphide. Protective mechanisms against injury by hydrogen sulphide may be pH regulation and enzymes of detoxification e.g. thiol methyl transferases, which methylate sulphides to methyl mercaptans. Thiol methyl transferase activity in man is high in the colon, but does not exceed the activity in the liver (Roediger et al., 1997). Although it is shown that methylation is a method of detoxification of hydrogen sulphide produced in the intestine, the extent to which the toxicity of exogenous hydrogen sulphide is detoxified by methylation is not known.

The reaction with essential proteins is the reaction that causes the toxicity of hydrogen sulphide after inhalation. The mechanism of toxicity of hydrogen sulphide is the inhibition of cytochrome oxidase and, through this, the inhibition of the electron transport system (Weisiger et al., 1979; Tabacova, 1986). However, hydrogen sulphide may also react with other metalloproteins and this may in some instances represent a detoxification pathway (Beauchamp et al., 1984).

ANNEX IV: TOXICITY

Oral acute toxicity data are available for one candidate substance of the present Flavouring Group Evaluation from chemical group 30.

TABLE IV.1: ACUTE TOXICITY

Chemical Name [FL-no]	Species	Sex	Route	LD ₅₀ (mg/kg bw)	Reference	Comments
Ammonium chloride [FL-no: 16.048]	Guinea pig	NR	Gavage 0.9 – 1.2 g/kg bw	NR	(Koenig & Koenig, 1949)	The dose-range gave rise to pulmonary oedema. No study details given. Study not considered relevant.
	Albino rat, guinea pig, rabbit	NR	Gavage 0.05, 0.25, 1.0 g/kg bw	NR	(Boyd & Seymour, 1946)	No untoward effects were noted. No study details given. Study not considered relevant.
	Rat	NR	Oral	1650	(IUCIID Dataset, 2000d)	No study details given.

NR: Not reported

Subacute / subchronic / chronic / carcinogenic toxicity data are available for one candidate substance of the present Flavouring Group Evaluation from chemical group 30.

TABLE IV.2: SUBACUTE / SUBCHRONIC / CHRONIC / CARCINOGENICITY STUDIES

Chemical Name [FL-no]	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg bw/day)	Reference	Comments
Ammonium chloride [FL-no: 16.048]	Rabbit (2 kg) 9 in total	Gavage in drinking water	2 %, 30-50 ml per day (approx. 300-500 mg/kg bw/day)	4 weeks	-	(Joblin & Meeker, 1936)	Study was designed to assess the effect of abnormal diets on atherosclerosis. Study not considered relevant.
	Not given	Oral in diet	3 % (approx. 3 g/kg bw/day)	6 days	-	(Thompson & Halliburton, 1966)	The study assessed mechanisms of renal hypertrophy. No details given. Study not considered relevant.
	Mongrel dogs/male 1 per group	Tablet	0, 25, 46, 91, 170, 220 mg/kg bw/day	7 days	-	(Short & Hammond, 1964)	The study assessed amount of ammonium chloride needed to acidify urine without appreciable systemic acidosis. Study not considered relevant.
	Holtzman rats/female	Drinking water	0.28 M (approx. 1 g/kg bw/day)	7 days	-	(Lotspeich, 1965)	The study assessed mechanisms of renal hypertrophy. Study not considered relevant.
	Mongrel dogs/male 1 control 5 in dosage group	Capsule	0 g, 6 g per day	7 days	-	(Pollak et al., 1965)	The study aim was to investigate enzymatic activity in the kidney in metabolic acidosis. No other parameters were studied. Study not considered relevant.
	Sprague-Dawley rats 6 per group	Drinking water	0.28 M 1.2 M; 0.1 ml/10 g bw twice daily	7 days	-	(Janicki, 1970)	The study assessed mechanisms of renal hypertrophy. No other parameters were studied. Study not considered relevant.
		Gavage	0.6 M; 5 ml twice daily				
	Sprague-Dawley rats/male	Drinking water	0 %, 2 %, 2 % + low-Ca-diet	6 months	-	(Barzel, 1969)	The study assessed the effect of chronic ammonium chloride ingestion on bone formation. No other parameters were studied.
	Sprague-Dawley rats/male	Drinking water	0 %, 1.5 % (approx 1.5 g/kg bw) 1.5 % + low-Ca-diet	330 days	-	(Barzel & Jowsey, 1969)	The study assessed the effect of chronic ammonium chloride ingestion on bone formation. No other parameters were studied.
Wistar rats 10 male, 10 female/group	Oral in diet		0 %, 2 %, 4 % (2 % = 40.9 mmol/bw/day=2188 mg/kg bw/day M; 41.9=2242 F; 4 % = 80.2 mmol/bw/day=4291 mg/kg bw/day M 77.9=4168 F)	4 weeks	-	(Lina & Kuijpers, 2004)	The study was designed to assess effects of acidogenic diet. Well performed study. No NOAEL may be derived.
			0 %, 2.1 %, 4 % (2.1 % = 29.8 mmol/ bw/day=1594 mg/kg bw/day M; 33.6=1798 F; 4 % = 57.0 mmol/ bw/day=3050)	13 weeks	-	(Lina & Kuijpers, 2004)	The study was designed to assess effects of acidogenic diet. Well performed study. No NOAEL may be derived.

TABLE IV.2: SUBACUTE / SUBCHRONIC / CHRONIC / CARCINOGENICITY STUDIES

Chemical Name [FL-no]	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg bw/day)	Reference	Comments
			mg/kg bw/day M 69.1=3697 F), 0 % + 3 %KCl				
	Wistar rat 15 male, 15 female/group	Oral in diet	0 %, 1 %, 2.1 % (1 % = 9.0 mmol/bw/day=482 mg/kg bw/day M, 11.4=610 F; 2.1 % = 19.0 mmol/ bw/day=1017 mg/kg bw/day M, 25.6=1370 F), 0 % + 3 % KCl	18 months	-	(Lina & Kuijpers, 2004)	The study was designed to assess effects of acidogenic diet. Well performed study. No NOAEL may be derived.
	Wistar rat 50 male, 50 female/group	Oral in diet	0 %, 1 %, 2.1 % (1 % = 8.5 mmol/bw/day=455 mg/kg bw/day M, 10.3=551 F; 2.1 % = 18.8 mmol/bw/day=1006 mg/kg bw/day M 22.5=1204 F), 0 % + 3 % KCl	30 months	-	(Lina & Kuijpers, 2004)	Carcinogenicity study. The study was designed to assess effects of acidogenic diet. Well performed study. No NOAEL may be derived. There is no indication that NH ₄ Cl induces tumours.

No developmental and reproductive toxicity data are available for the four candidate substances of the present Flavouring Group Evaluation from chemical group 30.

TABLE IV.3: DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

In vitro mutagenicity/genotoxicity data are available for two candidate substances of the present Flavouring Group Evaluation from chemical group 30.

TABLE IV.4: GENOTOXICITY (*IN VITRO*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
Ammonia [16.009]	Ames assay	<i>S. typhimurium</i> TA98; TA100 TA1535, TA1537, TA1538 and <i>E. Coli</i> WRP uvrA	500-25000 ppm	Negative (±S9)	(Shimizu et al., 1985)	Valid study although results for ammonia not given in paper, reported as negative. Ammonium fluoride and ammonium peroxodisulfate tested in same study with negative results.
Ammonium chloride [16.048]	Ames assay	<i>S. typhimurium</i> TA92, TA94, TA98, TA100, TA1535, and TA1537	Up to 10 mg/plate	Negative (±S9)	(Ishidate et al., 1984) (Ishidate et al., 1988)	Valid study.
	Chromosome aberration	Chinese hamster fibroblasts	-	Positive (-S9)	(Ishidate et al., 1984) (Ishidate et al., 1988)	Valid study. Positive result attributed to osmotic effects.

In vivo mutagenicity/genotoxicity data are available for two candidate substances of the present Flavouring Group Evaluation from chemical group 30.

TABLE IV.5: GENOTOXICITY (*IN VIVO*)

Chemical Name	Test System	Test Object	Route	Dose	Result	Reference	Comments
Ammonia [16.009]	Micronucleus test	Mice	intraperitoneal	12, 25 or 50 mg/kg	Positive	(Yadav & Kaushik, 1997)	Study not reliable.
Ammonium chloride [16.048]	Micronucleus test	Mice	intraperitoneal	31.3-250 mg/kg bw x 4 or single doses of 62.5-500 mg/kg bw	Negative	(Hayashi et al., 1988)	Valid study.

REFERENCES

- Barrow, C.S., Steinhagen, W.H., 1980. NH₃ concentrations in the expired air of the rat: Importance to inhalation toxicology. *Toxicol. Appl. Pharmacol.* 53, 116-121.
- Bartholomew, T.C., Powell, G.M., Dodgson, K.S., Curtis C.G., 1980. Oxidation of sodium sulphide by rat liver, lungs and kidney. *Biochem. Pharmacol.* 29(18), 2431-7.
- Barzel, U.S., Jowsey, J., 1969. The effects of chronic acid and alkali administration on bone turnover in adult rats. *Clin. Sci.* 36, 517-524.
- Barzel, U.S., 1969. Effect of excessive acid feeding on bone. *Calc. Tiss. Res.* 4(2), 94-100.
- Beauchamp, R.O., Jr., Bus, J.S., Popp, J.A., Boreiko, C.J., Andjekovich, D.A., 1984. A critical review of the literature on hydrogen sulfide toxicity. *Crit. JRev. Toxicol.* 13(1), 25-97.
- Biswas, S.D., Kleinberg, I., 1971. Effect of urea concentration on its utilisation, on the pH and the formation of ammonia and carbon dioxide in a human salivary sediment system. *Arch. Oral Biol.* 16, 759-780.
- Boyd, E.M., Seymour, K.G.W., 1946. Ethylenediamine dihydrochloride or "chlorethamine". II. Untoward and toxic reactions. *Exp. Med. Surg.* 223-227.
- Castell D.O., Moore E.W., 1971. Ammonia absorption from the human colon. *Gastroenterology.* 60(1), 33-42.
- Conn, H.O., 1972. Studies of the source and significance of blood ammonia. IV. Early ammonia peaks after ingestion of ammonium salts. *Yale J. Biol. Med.* 45, 543-549.
- Cramer, G.M., Ford, R.A., Hall, R.L., 1978. Estimation of toxic hazard - a decision tree approach. *Food Cosmet. Toxicol.* 16(3), 255-276.
- Davies, B.,M.,A., Yudkin, J., 1952. Studies in biochemical adaptation. The origin of urinary ammonia as indicated by the effect of chronic acidosis and alkalosis on some renal enzymes in the rat. *Biochem. J.* 52, 407-412.
- De Sousa, R.C., Harrington, J.T., Ricanati, E.S., et al., 1974. Renal regulation of acid-base equilibrium during chronic administration of mineral acid. *J Clin Invest.* 53, 465.
- EC, 1996a. Regulation No 2232/96 of the European Parliament and of the Council of 28 October 1996. *Official Journal of the European Communities* 23.11.1996, L 299, 1-4.
- EC, 1999a. Commission Decision 1999/217/EC of 23 February 1999 adopting a register of flavouring substances used in or on foodstuffs. *Official Journal of the European Communities* 27.3.1999, L 84, 1-137.
- EC, 2000a. Commission Regulation No 1565/2000 of 18 July 2000 laying down the measures necessary for the adoption of an evaluation programme in application of Regulation (EC) No 2232/96. *Official Journal of the European Communities* 19.7.2000, L 180, 8-16.
- EC, 2002b. Commission Regulation No 622/2002 of 11 April 2002 establishing deadlines for the submission of information for the evaluation of chemically defined flavouring substances used in or on foodstuffs. *Official Journal of the European Communities* 12.4.2002, L 95, 10-11.

- EC, 2009a. Commission Decision 2009/163/EC of 26 February 2009 amending Decision 1999/217/EC as regards the Register of flavouring substances used in or on foodstuffs. Official Journal of the European Union 27.2.2009, L 55, 41.
- EFFA, 2002i. Letter from EFFA to Dr. Joern Gry, Danish Veterinary and Food Administration. Dated 31 October 2002. Re.: Second group of questions. FLAVIS/8.26.
- EFFA, 2004e. Intake - Collection and collation of usage data for flavouring substances. Letter from Dan Dils, EFFA to Torben Hallas-Møller, EFSA. May 31, 2004.
- EFFA 2006g. Submission 2006-30. Flavouring group evaluation of 3 flavouring substances (candidate chemicals) of chemical group 30 (annex I of 1565/2000/EC) ammonia and its salts used as flavouring substances. Unpublished report submitted by EFFA to FLAVIS Secretariat. FLAVIS/8.XX.
- EFFA, 2007a. E-mail from Jan Demyttenaere, EFFA to Flavis Secretariat, National Foodinstitute, Technical University of Denmark. Dated 8 February 2007. RE: FLAVIS submissions - use levels for Category 14.2 - Alcoholic beverages FLAVIS/8.70.
- EFSA, 2004a. Minutes of the 7th Plenary meeting of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food, Held in Brussels on 12-13 July 2004. Brussels, 28 September 2004. [Online]. Available: http://www.efsa.europa.eu/cs/BlobServer/Event_Meeting/afc_minutes_07_en1.pdf?ssbinary=true
- EPA, 2003. Toxicological review of hydrogen sulfide. In Support of Summary Information on the Integrated Risk Information System (IRIS). Washington DC, June 2003.
- Eurostat, 1998. Total population. Cited in Eurostat, 2004. The EU population, Total population. [Online]. Available: http://epp.eurostat.ec.europa.eu/portal/page?_pageid=1090,30070682,1090_33076576&_dad=portal&_schema=PORTAL, Population and social conditions, Population, Demography, Main demographic indicators, Total population. December 2008.
- Flavour Industry, 2009p. Unpublished information submitted by Flavour Industry to DG SANCO and forwarded to EFSA. A-46rev1
- Fürst, P., Josephson, B., Maschio, G., and Vinnars, E., 1969. Nitrogen balance after intravenous and oral administration of ammonium salts to man. *J. Appl. Physiol.* 26(1), 13-22.
- Fürst, P., Jonsson, A., Josephson, B., Vinnars, E., 1970. Distribution in muscle and liver vein protein of ¹⁵N administered as ammonium acidosis to man. *J. Appl. Physiol.* 29(3), 307-312.
- Haworth, S., Koo, S., Lawlor, T., 1978. Salmonella/mammalian-microsome plate incorporation mutagenesis assay of compound #T0332.01 (MRI #78). EG_G Mason Research Institute, Rockville, MD. Unpublished report to the Flavor and Extract Manufacturers Association (FEMA). Submitted to WHO by the Flavor and Extract Manufacturers Association of the United States, Washington, DC, USA.
- Hayashi, M., Kishi, M., Sofuni, T., Ishidate Jr., M., 1988. Micronucleus tests in mice on 39 food additives and eight miscellaneous chemicals. *Food Chem. Toxicol.* 26(6), 487-500.
- Hunt, R.D., 1977. Measurement of partial pressure of ammonia in breath and arterial blood. Evidence for secretion of ammonia into expired gas by metabolism of monoamines in the lung. *Diss. Abst. Int. Ser. B.* 37, 3279.
- IOFI, 1995. European inquiry on volume of use. IOFI, International Organization of the Flavor Industry, 1995.

- Ishidate, Jr. M., Sofuni, T., Yoshikawa, K., Hayashi, M., Nohmi, T., Sawada, M., Matsuoka, A., 1984. Primary mutagenicity screening of food additives currently used in Japan. *Food Chem. Toxicol.* 22(8), 623-636.
- Ishidate Jr., M., Harnois MC, Sofuni T., 1988. A comparative analysis of data on the clastogenicity of 951 chemical substances tested in mammalian cell cultures. *Mutat Res.* 195: 151-213.
- IUCLID Dataset, 2000d. European Commission - European Chemicals Bureau. Substance ID: 12125-02-9, EINECS Name ammonium chloride. Section 1 - 7.
- Janicki, R.H., 1970. Renal adaptation during chronic NH_4Cl acidosis in the rat: No role for hyperplasia. *Am. J. Physiol* 210(3), 613-618.
- JECFA, 1982a. 26. Report: Twenty-sixth Meeting of the Joint FAO/WHO Expert Committee on Food Additives; Report: WHO Technical Report Series, no. 683.
- JECFA, 1995. Evaluation of certain food additives and contaminants. Forty-fourth Meeting of the Joint FAO/WHO Expert Committee on Food Additives. 14-23 February 1995. WHO Technical Report Series, no. 859. Geneva.
- JECFA, 1996a. Toxicological evaluation of certain food additives. The forty-fourth meeting of the Joint FAO/WHO Expert Committee on Food Additives and contaminants. WHO Food Additives Series: 35. IPCS, WHO, Geneva.
- JECFA, 1997a. Evaluation of certain food additives and contaminants. Forty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, 6-15 February 1996. WHO Technical Report Series, no. 868. Geneva.
- JECFA, 1999b. Evaluation of certain food additives and contaminants. Forty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives. Rome, 17-26 June 1997. WHO Technical Report Series, no. 884. Geneva.
- Joblin, J.W., Meeker, D.R., 1936. Further investigations on experimental atherosclerosis. *Arch. Pathol.* 22, 293-300.
- Kamin, H., Handler, P., 1957. Amino acid and protein metabolism. *Annu. Rev. Biochem.* 26, 419-490.
- Koenig, H., Koenig, R., 1949. Production of acute pulmonary edema by ammonium salts. *Proc. Soc. Exp. Biol. Med.* 70, 375-380.
- Kraft, A.A., Brant, A.W., Ayres, J.C., 1956. Detection of hydrogen sulfide in packaged meats and in brokenout shell eggs. *Food Technol.* 10, 443-444.
- Larson, T.V., Covert, D.S., Frank, R., Charlson, R.J., 1977. Ammonia in the human airways: Neutralization of inspired acid sulphate aerosols. *Science.* 197, 161-163.
- Lina, B.A.R., Kuijpers, M.H.M., 2004. Toxicity and carcinogenicity of acidogenic or alkalogenic diets in rats; effects of feeding NH_4Cl , KHCO_3 or KCl . *Food Chem. Toxicol.* 42, 135-153.
- Lotspeich, W.D., 1965. Renal hypertrophy in metabolic acidosis and its relation to ammonia excretion. *Am. J. Physiol.* 208(6), 1135-1142.
- Muntwyler, E., Iacobellis, M., Griffin, G., 1956. Kidney glutaminase and carbonic anhydrase activities and renal electrolyte excretion in rats. *Am. J. Physiol.* 184, 83-90.

- Nelson, D.L., Cox, M.M., 2000c. Lehninger Principles of Biochemistry. Chapter 18: Amino Acid Oxidation and the Production of Urea. Chapter 22: Biosynthesis of Amino Acids, Nucleotides, and Related Molecules. Worth Publishers, Inc., New York, pp. 623-658, 818-868.
- Pitts, R.F., 1971. The role of ammonia production and excretion in regulation of acid-base balance. *New Engl. J. Med.* 284, 32-38.
- Pollak, V.E., Mattenheimer, H., Debruin, H., Weinman, K.J., 1965. Experimental metabolic acidosis: The enzymic basis of ammonia production by the dog kidney. *J. Clin. Invest.* 44, 169-181.
- Richards, P., Houghton, B.J., Metcalfe-Gibson, A., Ward, E.E., Wrong, O., 1968. Incorporation of orally administered ammonia into tissue proteins in man; the influence of diet and uraemia. In: *Nutrition and renal disease*, Edinburgh, E., and S. Livingstone, pp. 93-98.
- Richardson, C.J., Magee, E.A.M., Cummings, J.H., 2000. A new method for the determination of sulphide in gastrointestinal contents and whole blood by microdistillation and ion chromatography. *Clin. Chim. Acta.* 293, 115-125.
- Roediger, W.E.W., Moore, J., Babidge, W., 1997. Colonic sulfide in pathogenesis and treatment of ulcerative colitis. *Dig. Dis. Sci.* 42, 1571-1579.
- SCF, 1992. Scientific Committee for Food. Food science and techniques. Reports of the scientific committee for food. 29th series. Second report on extration solvents. Opinion expressed on June, 21, 1991. Report no. 14482, Office for the official publications of the European Communities, Luxembourg.
- SCF, 1995. Scientific Committee for Food. First annual report on chemically defined flavouring substances. May 1995, 2nd draft prepared by the SCF Working Group on Flavouring Substances (Submitted by the SCF Secretariat, 17 May 1995). CS/FLAV/FL/140-Rev2. Annex 6 to Document III/5611/95, European Commission, Directorate-General III, Industry.
- SCF, 1999a. Opinion on a programme for the evaluation of flavouring substances (expressed on 2 December 1999). Scientific Committee on Food. SCF/CS/FLAV/TASK/11 Final 6/12/1999. Annex I the minutes of the 119th Plenary meeting. European Commission, Health & Consumer Protection Directorate-General.
- Shimizu, H., Suzuki, Y., Takemura, N., Goto, S., Matsushita, H., 1985. The results of microbial mutation test for forty-three industrial chemicals. *Jap. J. Ind. Health* 27, 400-419.
- Shimoi, K., Nakamura, Y., Noro, T., Tomita, I., Fukushima, S., Inoue, T., Kada, T., 1985. Methyl cinnamate derivatives enhance UV-induced mutagenesis due to the inhibition of DNA excision repair in *Escherichia coli* B/r. *Mutat. Res.* 146, 15-22.
- Short, E.C., Hammond, P.B., 1964. Ammonium chloride as a urinary acidifier in the dog. *J. Am. Vet. Med. Assoc.* 144(8), 864-867.
- Summerskill, W.H.J., Wolpert, E., 1970. Ammonia metabolism in the gut. *Am. J. Clin. Nutr.* 23, 633-639.
- Tabacova, S., 1986. Maternal exposure to environmental chemicals. *Neurotoxicology*, 7(2), 421.
- Thompson, R.Y., Halliburton, I.W., 1966. Effect of diet on the composition of the kidney. *Biochem. J.* 99(3), 44.
- TNO, 2000. Volatile Compounds in Food - VCF Database. TNO Nutrition and Food Research Institute. Boelens Aroma Chemical Information Service BACIS, Zeist, The Netherlands.

- TNO, 2010. Volatile Compounds in Food - VCF Database. TNO Nutrition and Food Research Institute. Boelens Aroma Chemical Information Service BACIS, Zeist, The Netherlands.
- U.S. National Research Council, 1979. Hydrogen sulphide. Committee on Medical and Biologic Effects of Environmental Pollutants, Subcommittee on Hydrogen Sulphide. University Park Press, Baltimore, MD.
- UNEP, 2004. SIDS Initial Assessment Report for ammonium sulfate. UNEP Publications <http://www.chem.unep.ch/irptc/sids/OECDSEIDS/7783202.pdf>
- US ATSDR, 2004. Toxicological profile for ammonia. U.S. Department of Health and Human Services, Agency for Toxic Substances and Disease Registry.
- US ATSDR, 2006. Toxicological profile for hydrogen sulfide. U.S. Department of Health and Human Services, Agency for Toxic Substances and Disease Registry.
- Vitti, T.G., Vukmirovich, R., Gaebler, O.H., 1964. Utilization of ammonia nitrogen, administered by intragastric, intraperitoneal and subcutaneous routes: Effects of growth hormone. Arch. Biochem. Biophys. 106, 475-482.
- Weisiger, R.A., Pinkus, L.M., Jakoby, W.B., 1979. Potential role of thiomethyltransferase in the detoxification of intestinal hydrogen sulphide and methanethiol. Gastroenterology, 76, 1269.
- White, A., Handler, P., Smith E.L., 1973. Renal function and the composition of urine. In: Principles of biochemistry, 5th ed., New York, McGraw Hill Book Co., p. 604 and 928.
- WHO, 1996. Guidelines for drinking-water quality. 2nd Ed. vol. 2. Health criteria and other supporting information. World Health Organization, International Programme on Chemical Safety. Geneva.
- Yadav, J., S., Kaushik, V., K., 1997. Genotoxic effect of ammonia exposure on workers in a fertilizer factory. Indian J. Exp. Biol. 35, 487-92.

ABBREVIATIONS

ADI	Acceptable Daily Intake
ATP	Adenosine TriPhosphate
BW	Body Weight
CAS	Chemical Abstract Service
CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids Chemical Abstract Service
CHO	Chinese hamster ovary (cells)
CoE	Council of Europe
DNA	Deoxyribonucleic acid
EC	European Commission
EFFA	European Flavour and Fragrance Association
EFSA	The European Food Safety Authority
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FEMA	Flavor and Extract Manufacturers Association
FGE	Flavouring Group Evaluation
FLAVIS (FL)	Flavour Information System (database)
ID	Identity
IOFI	International Organization of the Flavour Industry
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
LD ₅₀	Lethal Dose, 50 %; Median lethal dose
MS	Mass spectrometry
MSDI	Maximised Survey-derived Daily Intake
mTAMDI	Modified Theoretical Added Maximum Daily Intake
No	Number
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
SCF	Scientific Committee on Food
TAMDI	Theoretical Added Maximum Daily Intake
UDS	Unscheduled DNA Synthesis
WHO	World Health Organisation

Provisional Peer Reviewed Toxicity Values for

Ammonia
(CASRN 7664-41-7)

Superfund Health Risk Technical Support Center
National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, OH 45268

Acronyms and Abbreviations

bw	body weight
cc	cubic centimeters
CD	Caesarean Delivered
CERCLA	Comprehensive Environmental Response, Compensation and Liability Act of 1980
CNS	central nervous system
cu.m	cubic meter
DWEL	Drinking Water Equivalent Level
FEL	frank-effect level
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
g	grams
GI	gastrointestinal
HEC	human equivalent concentration
Hgb	hemoglobin
i.m.	intramuscular
i.p.	intraperitoneal
i.v.	intravenous
IRIS	Integrated Risk Information System
IUR	inhalation unit risk
kg	kilogram
L	liter
LEL	lowest-effect level
LOAEL	lowest-observed-adverse-effect level
LOAEL(ADJ)	LOAEL adjusted to continuous exposure duration
LOAEL(HEC)	LOAEL adjusted for dosimetric differences across species to a human
m	meter
MCL	maximum contaminant level
MCLG	maximum contaminant level goal
MF	modifying factor
mg	milligram
mg/kg	milligrams per kilogram
mg/L	milligrams per liter
MRL	minimal risk level

MTD	maximum tolerated dose
MTL	median threshold limit
NAAQS	National Ambient Air Quality Standards
NOAEL	no-observed-adverse-effect level
NOAEL(ADJ)	NOAEL adjusted to continuous exposure duration
NOAEL(HEC)	NOAEL adjusted for dosimetric differences across species to a human
NOEL	no-observed-effect level
OSF	oral slope factor
p-IUR	provisional inhalation unit risk
p-OSF	provisional oral slope factor
p-RfC	provisional inhalation reference concentration
p-RfD	provisional oral reference dose
PBPK	physiologically based pharmacokinetic
ppb	parts per billion
ppm	parts per million
PPRTV	Provisional Peer Reviewed Toxicity Value
RBC	red blood cell(s)
RCRA	Resource Conservation and Recovery Act
RDDR	Regional deposited dose ratio (for the indicated lung region)
REL	relative exposure level
RfC	inhalation reference concentration
RfD	oral reference dose
RGDR	Regional gas dose ratio (for the indicated lung region)
s.c.	subcutaneous
SCE	sister chromatid exchange
SDWA	Safe Drinking Water Act
sq.cm.	square centimeters
TSCA	Toxic Substances Control Act
UF	uncertainty factor
µg	microgram
µmol	micromoles
VOC	volatile organic compound

PROVISIONAL PEER REVIEWED TOXICITY VALUES FOR AMMONIA (CASRN 7664-41-7)

Background

On December 5, 2003, the U.S. Environmental Protection Agency's (EPA's) Office of Superfund Remediation and Technology Innovation (OSRTI) revised its hierarchy of human health toxicity values for Superfund risk assessments, establishing the following three tiers as the new hierarchy:

1. EPA's Integrated Risk Information System (IRIS).
2. Provisional Peer-Reviewed Toxicity Values (PPRTV) used in EPA's Superfund Program.
3. Other (peer-reviewed) toxicity values, including:
 - ▶ Minimal Risk Levels produced by the Agency for Toxic Substances and Disease Registry (ATSDR),
 - ▶ California Environmental Protection Agency (CalEPA) values, and
 - ▶ EPA Health Effects Assessment Summary Table (HEAST) values.

A PPRTV is defined as a toxicity value derived for use in the Superfund Program when such a value is not available in EPA's Integrated Risk Information System (IRIS). PPRTVs are developed according to a Standard Operating Procedure (SOP) and are derived after a review of the relevant scientific literature using the same methods, sources of data, and Agency guidance for value derivation generally used by the EPA IRIS Program. All provisional toxicity values receive internal review by two EPA scientists and external peer review by three independently selected scientific experts. PPRTVs differ from IRIS values in that PPRTVs do not receive the multi-program consensus review provided for IRIS values. This is because IRIS values are generally intended to be used in all EPA programs, while PPRTVs are developed specifically for the Superfund Program.

Because science and available information evolve, PPRTVs are initially derived with a three-year life-cycle. However, EPA Regions (or the EPA HQ Superfund Program) sometimes request that a frequently used PPRTV be reassessed. Once an IRIS value for a specific chemical becomes available for Agency review, the analogous PPRTV for that same chemical is retired. It should also be noted that some PPRTV manuscripts conclude that a PPRTV cannot be derived based on inadequate data.

Disclaimers

Users of this document should first check to see if any IRIS values exist for the chemical of concern before proceeding to use a PPRTV. If no IRIS value is available, staff in the regional Superfund and RCRA program offices are advised to carefully review the information provided in this document to ensure that the PPRTVs used are appropriate for the types of exposures and circumstances at the Superfund site or RCRA facility in question. PPRTVs are periodically updated; therefore, users should ensure that the values contained in the PPRTV are current at the time of use.

It is important to remember that a provisional value alone tells very little about the adverse effects of a chemical or the quality of evidence on which the value is based. Therefore, users are strongly encouraged to read the entire PPRTV manuscript and understand the strengths and limitations of the derived provisional values. PPRTVs are developed by the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center for OSRTI. Other EPA programs or external parties who may choose of their own initiative to use these PPRTVs are advised that Superfund resources will not generally be used to respond to challenges of PPRTVs used in a context outside of the Superfund Program.

Questions Regarding PPRTVs

Questions regarding the contents of the PPRTVs and their appropriate use (e.g., on chemicals not covered, or whether chemicals have pending IRIS toxicity values) may be directed to the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center (513-569-7300), or OSRTI.

INTRODUCTION

The HEAST (U.S. EPA, 1997) lists subchronic and chronic oral reference doses (RfDs) of 34 mg/L for ammonia. A comment in the HEAST indicates that 34 mg/L is a concentration in drinking water that is specifically related to the organoleptic (taste) threshold and that a safe concentration for ammonia may be higher than 34 mg/L, but the data are inadequate to assess the safe level. The source document for derivation of the HEAST subchronic and chronic oral RfD values is the Health Effects Assessment (HEA) for Ammonia (U.S. EPA, 1987). The HEAST subchronic and chronic RfD values are based on a determination of the organoleptic (taste) threshold of ammonia in redistilled water by Campbell et al. (1958). The value selected for the HEAST subchronic and chronic RfDs was supported by the closely similar value of 35 mg/L identified as the taste threshold for ammonia in a World Health Organization Environmental Health Criteria (EHC) document (WHO, 1986) and as the ambient water quality criterion to

protect human health derived by U.S. EPA (1981). No oral assessment is included on IRIS (U.S. EPA, 2003) or the Drinking Water Standards and Health Advisories list (U.S. EPA, 2002). No relevant documents other than the HEA and the AWQC document were included in the CARA list (U.S. EPA, 1991, 1994a).

The HEAST includes a value of $1\text{E-}1\text{ mg/m}^3$ for the subchronic inhalation RfC. The HEAST subchronic RfC used the same data and is the same as the RfC ($1\text{E-}1\text{ mg/m}^3$) reported in IRIS (U.S. EPA, 2003). The IRIS chronic RfC value was derived from a free-standing NOAEL of 6.4 mg/m^3 (9.2 ppm) identified for lack of evidence of decreased pulmonary function or changes in subjective symptomatology in an occupational study of workers exposed to ammonia in a soda ash (sodium carbonate) facility (Holness et al., 1989). A LOAEL was not identified in the study. The NOAEL was adjusted for intermittent exposure to a value of 2.3 mg/m^3 and divided by a composite uncertainty factor (UF) of 30. The composite UF included a factor of 10 for protection of sensitive individuals and a factor of 3 for database deficiencies, including lack of chronic data, proximity of the occupational $\text{NOAEL}_{\text{HEC}}$ to a $\text{LOAEL}_{\text{HEC}}$ observed in a subchronic inhalation study in rats (Broderson et al., 1976), and lack of data on reproductive or developmental toxicity. The RfD/RfC Workgroup verified the RfC on February 21, 1991. The HEA had previously derived subchronic and chronic inhalation RfDs of 0.36 mg/m^3 by dividing the ammonia air odor threshold of 3.6 mg/m^3 (Carson et al., 1981) by an uncertainty factor of 10 to obtain an estimate of the lower bound limit for odor detection.

The public review draft of the ATSDR Toxicological Profile on ammonia (ATSDR, 2002) derived an intermediate oral minimal risk level (MRL) value of 0.3 mg/kg-day based on a duration-adjusted NOAEL of 39.5 mg/kg-day for weight loss in rats exposed to ammonium sulfamate in drinking water for 90 days (Gupta et al., 1979) and an uncertainty factor of 100 (10 for extrapolation from rats to humans and 10 to protect sensitive individuals). ATSDR (2002) also derived a chronic inhalation MRL of 0.3 ppm ($200\text{ }\mu\text{g/m}^3$) based on a duration-adjusted NOAEL of 3.1 ppm in the Holness et al. (1989) study and an uncertainty factor of 10 for human variability. The State of California (OEHHA, 2002) has derived a chronic inhalation reference exposure level of $200\text{ }\mu\text{g/m}^3$ (0.3 ppm) for ammonia. This value ($200\text{ }\mu\text{g/m}^3$) is based on the occupational study of Holness et al. (1989) with a duration adjusted NOAEL of 2 mg/m^3 and an uncertainty factor of 10 for intraspecies variability. The OEHHA (2002) used the same methodology as ATSDR. ACGIH (2001) lists a TLV-TWA of 25 ppm (17 mg/m^3) and a STEL of 35 ppm (24 mg/m^3) for ammonia. These values are intended to minimize the potential for acute ocular and respiratory tract irritation. NIOSH (2002) lists values of 25 ppm (18 mg/m^3) and 35 ppm (27 mg/m^3) for the REL-TWA and REL-ST, respectively. OSHA (2002) lists a value of 50 ppm (35 mg/m^3) for the PEL-TWA.

Ammonia is not included in the HEAST (U.S. EPA, 1997) cancer table. IRIS (U.S. EPA, 2003) and the Drinking Water Standards and Health Advisories list (U.S. EPA, 2002) do not provide a carcinogenicity assessment for ammonia. IARC (2002) has not evaluated the

carcinogenicity of ammonia. NTP (2002) does not list ammonia among the chemicals it considers to be known human carcinogens or reasonably anticipated to be human carcinogens.

Literature searches to identify studies relevant to the derivation of provisional toxicity values for ammonia were conducted for the period 1988 through September 18, 2002. Databases searched included: TOXLINE, MEDLINE, TSCATS, RTECS, CCRIS, DART, EMIC/EMICBACK, HSDB, GENETOX and CANCERLIT. Additional literature searches were conducted through May 2004 by NCEA-Cincinnati using TOXLINE, MEDLINE, Chemical and Biological Abstract databases and no relevant information was found.

REVIEW OF PERTINENT DATA

Human Studies

Holness et al. (1989) studied workers exposed to ammonia in a sodium carbonate production plant. Fifty-two of the 64 available workers agreed to participate in the study. The control group consisted of 31 office and stores workers employed at the plant who were without previous exposure to ammonia. Information was collected on age, height, work history, smoking history, respiratory symptoms, and skin and eye complaints. Respiratory questions were based on an American Thoracic Society questionnaire. Sense of smell was evaluated at the beginning and end of the work week. Pulmonary function tests were performed at the beginning and end of each work shift on two test days. The parameters measured were forced vital capacity (FVC), forced expiratory volume in one second (FEV₁); and forced expiratory flow rate at 50% and 75% of the vital capacity (FEF₅₀ and FEF₇₅). Mean time-weighted average (TWA) exposures to ammonia were determined by personal air sampling over one shift following NIOSH recommendations. The average sampling time was 8.4 hours. The mean age of the exposed workers was 38.9 ± 11.7 years and the average duration of exposure was 12.2 ± 8.9 years. Only weight differed significantly when demographics for the exposed and control workers were compared. Time-weighted average airborne concentrations of ammonia were 9.2 ± 1.4 ppm (6.4 mg/m^3) and 0.3 ± 0.1 ppm (0.2 mg/m^3) for the exposed and control groups, respectively. Although no significant difference was evident between exposed and control groups in reporting of respiratory symptoms, workers reported that exposure at the plant aggravated specific symptoms including coughing, wheezing, nasal complaints, eye irritation, throat discomfort, and skin problems. No significant differences were evident between the exposed and control groups in reporting of respiratory symptoms, sense of smell, baseline lung function, or change in lung function at the beginning and end of a work week. No significant relationships between level or length of ammonia exposure and lung function results were demonstrated. The NOAEL in this study was 9.2 ppm (6.4 mg/m^3), based on lack of evidence for decreased pulmonary function or changes in subjective assessments of respiratory symptoms. A LOAEL was not identified in this study.

Ferguson et al. (1977) exposed healthy human volunteers (2/concentration) employed in an alkali plant to ammonia concentration of 25 ppm, 50 ppm, or 100 ppm, 5 days/week for six weeks. Conclusions of the study were actually based on 5 weeks of exposure as a result of technical difficulties during the first week of the study. Toxicity was assessed by subjective and objective indications of eye and respiratory tract irritation, pulse rate, respiration rate, pulmonary function (FVC, FEV), physical examination, and the ability to perform routine tasks. Exposure to ammonia did not result in abnormalities of the chest, heart, vital organs, neurological response, task performance or significant weight changes as assessed during weekly medical examinations. Transient irritation of the throat was observed at exposures of 50 ppm (4 hours/day).

More recently published occupational studies were examined to identify data potentially suitable for calculation of a subchronic RfC. Ballal et al. (1998) reported the results of a cross-sectional study of male workers employed in two fertilizer plants in Saudi Arabia. Exposure to ammonia concentrations of 25 ppm and above were significantly associated with respiratory symptoms including wheezing, cough, phlegm, dyspnea, and asthma. Ali et al. (2001) examined the pulmonary function of workers (gender not specified) in an ammonia-producing factory in Saudi Arabia. Cumulative exposure of greater than 50 mg/m³-years was associated with significantly reduced FEV₁ and FVC. Symptomatic workers (i.e., those reporting cough, phlegm, wheeze, and/or dyspnea) showed significantly reduced FEV₁ and FEV₁/FVC ratio when compared to asymptomatic workers. Neither study adequately reported details of worker exposure, such as the number of hours worked per week, and were not further considered for derivation of reference doses.

A number of studies have examined the relationship between inhalation exposure to pollutants (including ammonia) in livestock confinement buildings and occurrence of respiratory symptoms and/or changes in pulmonary function in workers (Heerderik et al., 1990; Choudat et al., 1994; Donham et al., 1995, 2000; Reynolds et al., 1996; Vogelzang et al. 1997, 2000; Cormier et al., 2000). Exposure to ammonia concentrations of 2.3 to 20.7 ppm was associated with symptoms of bronchial reactivity, inflammation, cough, wheezing, or shortness of breath and decrements in pulmonary function as measured by FEV₁, maximum expiratory flow rate, and maximal mid-expiratory flow rate. These data are of limited use for derivation of a subchronic toxicity reference value for ammonia because workers were concurrently exposed to other potential respiratory toxicants such as dusts, endotoxins, and nitrogen dioxide.

The carcinogenic potential of ammonia via the inhalation route has not been assessed in humans.

The experimental database for human oral exposure to ammonia consists of acute and short-term studies of exposure to ammonium chloride. No subchronic or chronic duration oral exposure studies were located in the literature examined. The availability of studies on ammonium chloride is a result of its use for experimental induction of hyperchloremic metabolic

acidosis. Few of the available studies have been designed or conducted to specifically assess the toxicity of ammonia or ammonium ion in response to oral dosing.

U.S. EPA (1981) reviewed fifteen existing short-term studies of ammonium chloride in humans. Administration of ammonium chloride to all age groups produced metabolic acidosis, with increased susceptibility observed in infants. The results of these studies indicate that metabolic acidosis, impaired glucose tolerance, and reduced tissue sensitivity to insulin may result from doses of ammonium chloride greater than or equal to 100 mg/kg-day (31.8 mg ammonia/kg-day, as estimated by U.S. EPA, 1981). Although frank toxicity was not reported, U.S. EPA (1981) expressed concern for potential bone demineralization as a result of impaired acid-base balance.

In the longest duration human study found, Lemann et al. (1966) investigated the electrolyte balance of five men who were given doses of ammonium chloride to induce metabolic acidosis. Each individual served as his own control. Following baseline observations, each subject was given a small initial dose which was progressively increased over a period of six to nine days, after which the dose remained constant until administration of ammonium chloride was discontinued after day 18. U.S. EPA (1987) reported total doses of 733 mEq (approximately 93 mg/kg-day) for the initial loading period and 2771 mEq (approximately 177 mg/kg-day) for the remainder of the experiment. During ammonium chloride loading, net fixed acid production was increased by an average of 3425 mEq. Progressive acid retention was initially accompanied by a progressive decrease in serum bicarbonate concentration. Serum bicarbonate levels dropped as acid was retained during the first nine days of ammonium chloride dosing, stabilized at a reduced level by about day 12, and rose slightly between days 13 to 18, but did not return to baseline levels until after treatment with ammonium chloride was discontinued. Calcium and phosphorus balances became negative as a result of urinary losses, suggesting to the study authors that slow dissolution of bone mineral was occurring to provide additional buffering capacity. The LOAEL in this study was the initial dose of 93 mg/kg-day.

The carcinogenic potential of ammonia via the oral route has not been assessed in adequately designed epidemiological studies.

Animal Studies

Broderson et al. (1976) continuously exposed F344 rats (6 rats/sex/dose) to ammonia concentrations of 25, 50, 150, or 250 ppm for seven days prior to inoculation with *Mycoplasma pulmonaris* and for 28 to 42 days following inoculation. These exposures were conducted using purified ammonia from a commercial source. In addition, one treatment group was exposed to ammonia produced from a natural source (soiled bedding) for 30 days following inoculation. Each treatment group had a corresponding control group that was inoculated with *M. pulmonaris* and exposed only to background levels of ammonia. Additional groups were exposed to

background or high levels of ammonia (trace and 250 ppm, respectively) without *M. pulmonaris* inoculation. Toxicity was assessed by observation of clinical signs and histopathological examination of nasal passages, middle ear, trachea, lungs, liver, kidney, adrenal, pancreas, testicle, spleen, mediastinal nodes, and thymus. Clinical signs were similar in control and exposed groups during the pre-inoculation exposure period. Signs of murine respiratory mycoplasmosis (MRM) were observed in all groups approximately 10 days after inoculation. All levels of ammonia from bedding or the commercial source increased the severity of the rhinitis, otitis media, tracheitis, and pneumonia characteristic of MRM. The prevalence and extent of gross atelectasis and consolidation were greater in rats exposed to high ammonia concentrations (i.e., ammonia concentrations greater than background) and the prevalence of microscopic respiratory lesions was also greater. The prevalence of gross and microscopic lung lesions differed significantly from controls when data from all high exposure groups were summed and compared with pooled control data. Regression analysis indicated a positive relationship between ammonia concentration and prevalence of gross or microscopic lesions. Exposure of uninoculated rats to ammonia resulted in lesions that were unlike those of MRM and which were restricted to the nasal passages. A LOAEL of 25 ppm (17.4 mg/m³) was identified in this study.

Schoeb et al. (1982) inoculated pathogen-free F344 rats with *M. pulmonis* and exposed groups to trace or 100 ppm (70 mg/m³) concentrations of ammonia for up to 28 days. Growth of *M. pulmonis* was greater in ammonia-exposed rats than in controls and serum immunoglobulin response to the inoculum was also greater in the exposed population. Results of an experiment conducted in rats with cannulated tracheas demonstrated that the nasal passages absorbed virtually all ammonia at administered concentrations of 500 ppm (348 mg/m³) or below.

Coon et al. (1970) continuously exposed male and female Sprague-Dawley and Long Evans rats for a minimum of 90 days to ammonia concentrations of 0, 40, 127, 262, 455, or 470 mg/m³. A LOAEL of 262 mg/m³ was identified on the basis of nasal discharge in 25% of the rats and nonspecific degenerative and circulatory changes in the lungs and kidneys. The upper respiratory tract was not examined for microscopic lesions. In another series of experiments, Coon et al. (1970) exposed rats, guinea pigs, rabbits, dogs and monkeys to ammonia concentrations of 0, 155, or 770 mg/m³ for 8 hours/day, 5 days/week for a total of 30 exposures. This study identified a LOAEL of 770 mg/m³ for lung inflammation in rats and guinea pigs and ocular and nasal irritation in dogs and rabbits. The upper respiratory tract was not examined for presence of lesions.

Anderson et al. (1964) conducted a series of experiments that included continuous exposure of guinea pigs and Swiss albino mice to 20 ppm (13.9 mg/m³) ammonia for up to six weeks and exposure of Leghorn chickens for up to 12 weeks. A separate group of guinea pigs was exposed to 50 ppm (35 mg/m³) ammonia for six weeks. Although no effects were observed after exposure to 20 ppm for four weeks, gross lesions including edema, congestion, and hemorrhage were observed in the lungs of all three species after six weeks. Grossly enlarged and

congested spleens, congested livers and lungs, and pulmonary edema were observed in guinea pigs exposed to 50 ppm ammonia for six weeks.

Weatherby (1952) exposed guinea pigs to 0 or 170 ppm (118 mg/m³) 6 hours/day, 5 days/week for up to 18 weeks. No adverse effects were observed in animals exposed for 6 to 12 weeks. Mild changes were observed in the spleen, kidney suprarenal glands, and liver at 18 weeks. No effects on the lungs were observed. The upper respiratory tract was not examined for lesions.

No effects on ovarian or uterine weights were observed in pigs exposed by inhalation to approximately 5 or 35 ppm ammonia for 6 weeks (Diekman et al., 1993). Continuous exposure of female pigs to approximately 35 ppm ammonia from 6 weeks prior to breeding through gestation day 30 did not significantly affect age to puberty, number of live fetuses, fetus-to-corpus luteum ratio, or fetal length when compared to females exposed to 7 ppm ammonia for the same duration (Diekman et al., 1993).

Limited subchronic and chronic toxicity data are available for ammonia. In an early study, Seegal (1927) administered ammonium chloride doses of 0 or 372 mg/kg-day to rabbits by gavage for 36 days and observed episodes of severe metabolic acidosis and epithelial degeneration in the renal tubules. Similar effects plus softening of the teeth, skull, and ribs were observed at a dose of 234 mg/kg-day given by gavage for 11 months.

Freedman and Beeson (1961) exposed 12 adult male Sprague-Dawley rats to 1.6% ammonium chloride in the drinking water for periods of up to three weeks to evaluate effects on the kidney. Six control animals were provided with tap water. An additional group of 10 rats was given drinking water containing 1% ammonium chloride for an additional 2.5 months to assess subchronic effects. No abnormalities were detected by urinalysis, gross pathology or histologic examination. Physiological adaptation to metabolic acidosis was indicated by increased glutaminase activity per gram of kidney with duration of treatment. No data on water consumption or body weight of the test animals were provided. Assuming that the rats weighed 250 grams and consumed 25 mL of drinking water per day, U.S. EPA (1987) estimated a time-weighted average dose of approximately 360 mg/kg-day.

Gupta et al. (1979) conducted a subchronic exposure study in adult female and weanling male and female ITRC rats (20/sex/age/dose). The test animals were treated with ammonium sulfamate (NH₄SO₃NH₂) at doses of 0, 100, 250, or 500 mg/kg-day, 6 days per week for 30, 60, or 90 days. The ammonium sulfamate was given as a 10% solution, but the study report did not clearly indicate whether the dose was administered by gavage. Food and water consumption, appearance, behavior, and body weight were monitored during the study. Hematological parameters and organ weights were measured at interim and terminal sacrifices and tissue samples were collected for histopathological examination. Food and water consumption were

decreased in male and female weanlings at the 500 mg/kg-day dose relative to the controls. No compound-related clinical signs of toxicity were observed in dosed rats. Body weight of adult females receiving 500 mg/kg-day was significantly reduced at 60 day (9%) and 90 days (16%) when compared to the control group. No significant differences were noted in hematological parameters, organ weights, or histopathology. Although the study authors indicated that ammonium sulfamate would be expected (on the basis of its structure) to cause metabolic acidosis, this prediction does not appear to have been confirmed experimentally. These data identify NOAEL and LOAEL values of 250 and 500 mg/kg-day as ammonium sulfamate, respectively. The effective dose of ammonia at each of these dose levels is uncertain, because under certain conditions the sulfamate ion is hydrolyzed to bisulfate ion and ammonia (U.S. EPA, 1981, 1987). Assuming no hydrolysis of the sulfamate ion, these doses correspond to 37.3 and 74.8 mg/kg-day of ammonia, respectively.

Bodega et al. (1993) fed diets containing 0 or 20% ammonium acetate to pathogen-free female Wistar rats (5 rats/group) for 3, 7, 15, 45, or 90 days to assess effects on glial fibrillary acidic protein (GFAP) in the spinal cord. The ammonium acetate in the diet was supplemented by addition of 5 mM ammonium acetate to the drinking water. The total exposure from the combined food and water was not provided by the author. Exposure to ammonium acetate had no effect on behavior, water consumption, or spinal GFAP levels of the test animals. Body weight gain was significantly reduced in dosed animals at all time points. Body weight gain in animals exposed to ammonia for 90 days was 69% of the control value.

Fazekas (1939, 1954a,b) conducted studies in rabbits that ranged from 3 to 17 months in duration. The administration of various ammonium salts (carbonate, chloride, sulfate, hydrophosphate, acetate, or lactate) or ammonium hydroxide resulted in enlargement of the parathyroids. Similar results were obtained with a variety of other chemicals (sodium dihydrophosphate, sodium ammonium phosphate, calcium chloride, hydrochloric acid, acetic acid, lactic acid) (Fazekas, 1954a). The chemicals were given for three week periods separated by one week intervals. The administered dose of ammonium salts in this study is unclear, but based on descriptions in secondary sources is likely to be less than or equal to 0.4 mg/kg-day. In a related study, similar treatment of rabbits with ammonium chloride or ammonium sulfate resulted in fluctuations in serum calcium and phosphorus levels (Fazekas, 1954b). Rabbits given gavage doses of 100 mg/kg by gavage on alternate days and then daily for 17 months developed enlarged adrenal glands. An initial fall in blood pressure of 20 to 30 mm Hg was followed by a gradual rise to levels 10 to 30 mm Hg after several months of treatment.

In a chronic study, Barzel and Jowsey (1969) exposed male Sprague-Dawley rats to 1.5% ammonium chloride in the drinking water for 330 days. The effects of ammonium on animals receiving a nutritionally complete diet included decreased bone content of fat-free solid and calcium; decreased body weight and body fat; and decreased blood pH and plasma carbon dioxide. Barzel (1975) reported effects on bone (decreased density, ash weight, and calcium

content), but no effects on growth, in intact and ovariectomized female rats exposed to 1.5% ammonium chloride in the drinking water for 300 days. U.S. EPA (1981) estimated an average daily dose of 1500 mg/kg-day for both studies.

The carcinogenic potential of ammonia has been investigated in an oral bioassay conducted in mice. Toth et al. (1972) exposed male and female Swiss mice (49-50/sex/dose) to 0.1, 0.2, or 0.3% ammonium hydroxide in the drinking water for their lifetime. U.S. EPA (1987) estimated an average daily dose of 565 mg/kg-day at the highest concentration. Male and female C3H mice (40/sex) were exposed to 0.1% ammonium hydroxide in the drinking water for their lifetime. This concentration corresponded to average daily doses of approximately 270 mg/kg-day, respectively, as calculated by U.S. EPA (1987). While data for a control group are reported in the publication, it is not clear whether this group was run concurrently with the ammonia treatment groups. The mice were examined and weighed at weekly intervals. Moribund animals were humanely sacrificed. Complete necropsies were performed on all animals and the liver, kidney, spleen, lung, and organs with gross lesions were processed for histopathological examination. No evidence for carcinogenicity was observed in males or females of either strain.

Two studies have examined the interaction of ammonia with other compounds in the induction of tumors. Uzvölgyi and Bojan (1980) investigated the interaction of ammonia with diethyl pyrocarbonate (DEPC) in induction of lung tumors in CFPL mice (a urethane-sensitive strain). Mice given gavage doses of either ammonia or DEPC alone did not develop lung tumors, whereas development of lung tumors was observed in mice dosed with both ammonia and DEPC. Induction of tumors in the sensitive CFPL strain may have resulted from formation of urethane *in vivo* from ammonia and DEPC (Uzvölgyi and Bojan, 1985). Tsujii et al. (1995) studied the effect of ammonia on tumor development in male Sprague-Dawley rats pretreated with N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) in the drinking water for 24 weeks and subsequently exposed to drinking water containing 0 or 0.01% ammonia for an additional 24 weeks. Exposure to ammonia significantly increased the incidence, multiplicity, size, and depth of tumors in the glandular stomach and stimulated cell proliferation in the gastric mucosa.

Reproductive and developmental toxicity data on ammonia from animal studies are limited. Treatment of virgin female rabbits with oral doses of various ammonium salts (carbonate, chloride, hydrophosphate, or sulfate) or ammonium hydroxide was associated with enlargement of the ovaries, follicle maturation, and formation of corpora lutea (Fazekas, 1949). Enlargement of the uterus, hypertrophy of the teats, and secretion of milk were also reported in treated rabbits. However, several aspects of this study are poorly documented, including the use of controls and exact method of dose administration.

Minaña et al. (1995) examined the effect of prenatal exposure to 20% ammonium acetate in the diet on NMDA receptor function in Wistar rats. As judged from graphically presented data, offspring of dams treated from day 1 of pregnancy through lactation had body weights at

birth that were comparable to the control group. The body weight of weanlings maintained on a diet containing 20% ammonium acetate was reduced by approximately 27% and 26% in males and females, respectively, at 120 days of age when compared to animals maintained on the control diet. Rats exposed to ammonia during pregnancy and lactation and fed an unsupplemented diet at weaning had a lower growth rate than the controls until day 60, indicating persistent effects of prenatal and lactational exposure to ammonia. Prenatal exposure to ammonia reduced binding of [^3H]MK-801 to NMDA receptors in primary cultures of cerebellar neurons by approximately 60%. No data were provided for feed intake in this study; therefore, an average daily dose can not be reliably estimated.

Other Studies

Information on the toxicokinetic properties of ammonia have been reviewed and summarized in ATSDR (2002). Inhalation exposure studies in humans show that ammonia dissolves in the mucous of the upper respiratory tract. At low levels of exposure, most inhaled ammonia is retained in the upper respiratory system. As the ammonia concentration increases, the capacity of the upper respiratory system is saturated and a larger percentage is absorbed. Development of nasal and pharyngeal irritation, but not tracheal irritation, following exposure is consistent with retention of inhaled ammonia in the upper respiratory tract. Animal data provide supporting evidence for high nasal retention. Quantitative differences in the amount of ammonia in inhaled and exhaled air suggest that small amounts are absorbed across the nasopharyngeal membranes into the systemic circulation. Limited systemic absorption is also inferred from lack of change in blood nitrogen and urinary-ammonia compounds following exposure. The available evidence suggests that ammonium absorbed via inhalation would be distributed to all body compartments by the blood. Ammonium reaching the tissues would be used in protein synthesis or as a buffer, with excess levels reduced by urinary excretion or conversion in the liver to glutamine and urea. Absorbed ammonia is excreted by the kidneys as urea and urinary ammonium compounds. Bioaccumulation to toxic levels is not expected to occur from chronic inhalation exposure based on the low levels of absorption and existence of multiple effective mechanisms for detoxification and excretion.

Human data indicate that ingested ammonium compounds are readily absorbed. The absorbed ammonium ion is transported via the hepatic portal vein to the liver, where most is metabolized to urea in healthy individuals. Data from animals and humans suggest that little of the ingested compound reaches the systemic circulation as ammonia or ammonium ion. Ingested ammonium compounds are excreted primarily in the urine as urea. Small amounts may be excreted in the sweat or in exhaled air.

Genotoxicity data are available from studies in humans, mice, *Escherichia coli*, *Drosophila melanogaster*, and cultured chick fibroblast cells. Yadav and Kaushik (1997) conducted cytogenetic assays on blood samples collected from 22 workers exposed to ammonia

gas (ambient level = 0.09 mg/m³) during production of nitrogen fertilizers and 42 unexposed staff employed at the same facility. The exposed workers did not show clinical symptoms of ammonia toxicity. The mitotic index, total number of chromosome aberrations (CA), and frequency of sister chromatid exchange (SCE) were significantly increased in the exposed workers as compared to their matched controls. The frequency of CA and SCE increased with the duration of exposure. Concurrent exposure to other compounds such as nitrogen dioxide was not addressed in the study report. The frequency of micronuclei was significantly increased in Swiss albino mice treated with intraperitoneal doses of ammonia ranging from 12.5 to 50 mg/kg as compared to controls (Yadav and Kaushik, 1997). Positive results were obtained for reverse mutation in *E. coli*, but only at levels of ammonia that were cytotoxic (Demerec et al., 1951). Negative results were reported for ammonium sulfate in *Salmonella typhimurium* and *Saccharomyces* (Litton Bionetics, 1975). Positive results were observed for chromosomal aberrations in chick fibroblasts treated with buffered ammonium chloride (Rosenfeld, 1932). Reduced cell division and inhibition of DNA repair were observed in mouse fibroblasts treated with ammonia and/or ammonium chloride (Visek et al., 1972; Capuco, 1977). Lobasov and Smirnov (1934) reported slightly mutagenic activity in *D. melanogaster*. Auerbach and Robson (1947) obtained doubtful, probably negative, results for sex-linked recessive mutations in *D. melanogaster* and reported negative results for dominant lethality.

DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC ORAL RfD VALUES FOR AMMONIA

Adequate toxicity data for derivation of provisional subchronic or chronic RfD values are not available. The human experimental database consists primarily of older acute and short-term studies in which ammonium chloride was used to induce metabolic acidosis. The longest duration of exposure among these studies was 18 days. The animal database includes one study (Gupta et al., 1979) that was reasonably well-documented, evaluated appropriate endpoints, and included a histopathological evaluation of potential target tissues. This study was not used to derive p-RfD values for two reasons. First, the test article was ammonium sulfamate, which is hydrolyzed under certain conditions to bisulfate ion and ammonia. It is not known whether hydrolysis occurred when the compound was administered to rats; thus, the actual dose of ammonia/ammonium ion administered to the test animals is uncertain. Second, comparison of the data from this study to results from human studies suggests that health effects may occur in humans at lower concentrations of ammonium salts. Gupta et al. (1979) identified a NOAEL equivalent to 37.3 mg ammonia/kg-day and a LOAEL equivalent to 74.8 mg ammonia/kg-day (assuming no hydrolysis of the sulfamate ion; the actual dose may differ). This LOAEL is higher than the 31.8 mg/kg-day level of concern identified for humans by U.S. EPA (1981) for potential bone demineralization. Route-to-route extrapolation is not feasible for derivation of oral reference values because the toxicokinetic properties of ammonia differ significantly for the oral and inhalation pathways. This evaluation of data adequacy is consistent with previous

assessments conducted by U.S. EPA (1981, 1987), which did not use the existing toxicity data for derivation of reference doses.

Because adequate data are lacking for oral exposure to ammonia, previous determinations of toxicity reference values (U.S. EPA, 1981, 1987, 1997) have used organoleptic (taste) data to estimate acceptable ammonium levels in drinking water at 34-35 mg/L. However, organoleptic (taste) data are not reliable predictors of either toxicity or intake. Furthermore, WHO (1986) has identified several limitations of the "triangle test" methodology used to derive the organoleptic (taste) threshold for ammonia: 1) the definition of the threshold is somewhat arbitrary; 2) McBride & Laing (1979) have reported significant positional bias in using the triangle test to determine taste threshold; and 3) the triangle test is not intended to mimic environmental exposures in which the taste thresholds could be substantially higher. Due to the high uncertainty associated with use of the organoleptic (taste) data for ammonia, no oral subchronic or chronic p-RfD is derived.

DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC INHALATION RfC VALUES FOR AMMONIA

A **chronic RfC of 1E-1 mg/m³** is listed for ammonia on IRIS (U.S. EPA, 2003) based on lack of evidence of decreased pulmonary function in human workers exposed to an estimated concentration of 6.4 mg/m³ for an average of 12.2 years (Holness et al., 1989). The presence of a chronic RfC on IRIS precludes derivation of a provisional chronic RfC for this chemical.

The occupational study of Holness et al. (1989) and the subchronic study conducted in rats by Broderick et al. (1976) were also considered to be an appropriate basis for derivation of a provisional subchronic RfC. Holness et al. (1989) identified a NOAEL of 9.2 ppm (6.4 mg/m³) for apparent lack of effect on pulmonary function or changes in subjective assessments of symptoms in workers exposed to ammonia for a mean duration of 12.2 years while employed at a sodium carbonate production plant. A LOAEL was not identified in this study. The NOAEL_{HEC} was calculated using the default dosimetric adjustment for human data (U.S. EPA, 1994b), as follows:

$$\text{NOAEL}_{\text{ADJ}} = 6.4 \text{ mg/m}^3 \times 5\text{days}/7\text{days} = 4.6 \text{ mg/m}^3$$

$$\begin{aligned} \text{NOAEL}_{\text{HEC}} &= \text{NOAEL}_{\text{ADJ}} \times (\text{VE}_{\text{ho}}/\text{VE}_{\text{h}}) \\ &= 4.6 \text{ mg/m}^3 \times (10 \text{ m}^3/20 \text{ m}^3) \\ &= 2.3 \text{ mg/m}^3 \end{aligned}$$

where,

VE_{ho} = human occupational default minute volume (10 m³/8 hours; U.S. EPA, 1994b)

VE_h = human ambient default minute volume (20 m³/24 hours; U.S. EPA, 1994b)

A $LOAEL_{HEC}$ was calculated from the rat data of Broderick et al. (1976) for comparison with the human $NOAEL_{HEC}$. These researchers identified a $LOAEL$ of 17.4 mg/m³, the lowest concentration tested, for increased severity of rhinitis and pneumonia (with respiratory lesions) in F344 rats inoculated with *M. pulmonis* and continuously exposed to ammonia. The $LOAEL_{HEC}$ is calculated using the procedure for a respiratory effect of a category 1 gas in the extrathoracic region (U.S. EPA, 1994b) as follows:

$$LOAEL_{ADJ} = LOAEL_{OBSERVED} = 17.4 \text{ mg/m}^3 \text{ (continuous exposure)}$$

$$LOAEL_{HEC} = LOAEL_{ADJ} \times RDGR_{ET}$$

$$\begin{aligned} RDGR_{ET} &= (V_E / SA_{ET})_A / (V_E / SA_{ET})_H \\ &= (0.14 \text{ m}^3/\text{day} / 15 \text{ cm}^2) / (20 \text{ m}^3/\text{day} / 200 \text{ cm}^2) = 0.093 \end{aligned}$$

$$\begin{aligned} LOAEL_{HEC} &= 17.4 \text{ mg/m}^3 \times 0.093 \\ &= 1.62 \text{ mg/m}^3 \approx 1.6 \text{ mg/m}^3 \end{aligned}$$

where:

$RDGR_{ET}$ = regional gas deposition ratio in the extrathoracic region

V_E = ventilation rate (m³/day)

SA_{ET} = surface area of extrathoracic region (cm²)

A, H = subscripts denoting laboratory animal and human, respectively

$(V_E)_A$ = 0.14 m³/day (subchronic, female F344 rats; U.S. EPA, 1988)

$(V_E)_H$ = 20 m³/day (U.S. EPA, 1988)

$(SA_{ET})_A$ = 15 cm² (U.S. EPA, 1994b)

$(SA_{ET})_H$ = 200 cm² (U.S. EPA, 1994b)

A **subchronic p-RfC of 0.1 mg/m³ (1E-1 mg/m³)** is derived by applying a composite uncertainty factor of 30 to the human $NOAEL$ of 2.3 mg/m³ (Holness et al., 1989). The composite UF includes a factor of 10 to protect sensitive individuals and a factor of 3 for proximity of the animal $LOAEL$ to the human $NOAEL$ and database limitations, including lack of adequate reproductive and developmental toxicity studies. The UF is applied to the human $NOAEL_{HEC}$ of 2.3 mg/m³, as follows:

$$\begin{aligned} \text{subchronic p-RfC} &= NOAEL_{HEC} / UF \\ &= 2.3 \text{ mg/m}^3 / 30 \\ &= 0.1 \text{ mg/m}^3 \text{ or } 1\text{E-1 mg/m}^3 \end{aligned}$$

The animal LOAEL of 1.62 mg/m³ is in close proximity to the human NOAEL of 2.3 mg/m³; however, the extrathoracic effects observed in the animal study were mild and reversible. Furthermore, the animal LOAEL_{HEC} of 1.6 mg/m³ gives a sixteen-fold comparative ceiling to the p-sRfC of 0.1 mg/m³. This adds confidence to the human NOAEL. Thus, the human NOAEL is considered for the derivation of this subchronic p-RfC. Thus the subchronic p-RfC, based on pharmacokinetics, remained the same as the RfC.

Confidence in the principal study is medium because the study was conducted in humans (but the sample size was relatively small), data were collected on males only, and a LOAEL was not identified. Although complaints of exacerbated upper respiratory symptoms were recorded in the principal study and support the extrathoracic region as the critical region for effects, an objective assessment of the workers' nasal epithelium was not performed. However, the observation of mild extrathoracic effects in animals at a HEC similar to the NOAEL support the human findings.

Confidence in the database is medium. The developmental, reproductive, and chronic toxicity of ammonia have not been tested, but toxicokinetic data suggest that ammonia is absorbed by the nasal passages at concentrations comparable to the NOAEL_{HEC} and systemic distribution is unlikely (U.S. EPA, 2003). Medium confidence in the subchronic p-RfC follows.

DERIVATION OF A PROVISIONAL CARCINOGENICITY ASSESSMENT FOR AMMONIA

Human data on the carcinogenic effects of ammonia or ammonia compounds are not available. Among animals, no evidence for carcinogenicity was observed in two strains of mice administered ammonium hydroxide in drinking water for two years or in a urethane-sensitive strain of mice administered ammonia in water by gavage for 4 weeks. There is some indication that ammonia contributes to the development of cancer when coadministered with DEPC (via formation of urethane) or MNNG (via stimulation of cell proliferation in the gastric mucosa). Limited genotoxicity testing of ammonia has produced mixed results. Under the proposed guidelines (U.S. EPA, 1999), the data for carcinogenicity of ammonia *are inadequate for an assessment of human carcinogenic potential*.

Derivation of quantitative estimates of cancer risk for ammonia is precluded by the absence of data indicating a carcinogenic effect for this chemical.

REFERENCES

- ACGIH (American Conference of Governmental Industrial Hygienists). 2001. 2001 Threshold Limit Values for Chemical Substances and Physical Agents and Biological Exposure Indices. ACGIH, Cincinnati, OH.
- Ali, B.A., H.O. Ahmed, S.G. Ballal et al. 2001. Pulmonary function of workers exposed to ammonia: a study in the Eastern Province of Saudi Arabia. *Int. J. Occup. Environ. Health.* 7(1): 19-22.
- Anderson, D.P., C.W. Beard and R.P. Hanson. 1964. The adverse effects of ammonia chickens including resistance to infection with Newcastle disease virus. *Avian Dis.* 8: 369-379. (Cited in U.S. EPA, 1981)
- ATSDR (Agency for Toxic Substances and Disease Registry). 2002. Toxicological Profile on Ammonia. Draft for Public Comment. U.S. Department of Health and Human Services, Public Health Service. Atlanta, GA. Online. <http://www.atsdr.cdc.gov/toxpro2.html>
- Auerbach, C. and J.M. Robson. 1947. Tests of chemical substances for mutagenic action. *Proc. Roy. Soc. Edinburgh. Sec. B Biol.* 62B: 284. (Cited in U.S. EPA, 1981; ATSDR, 2002)
- Ballal, S.G., B.A. Ali, A.A. Albar et al. 1998. Bronchial asthma in two chemical fertilizer producing plants in Eastern Saudi Arabia. *Int. J. Tuberc. Lung Dis.* 2(4): 330-335.
- Barzel, U.S. 1975. The effect of chronic ammonium chloride ingestion on parathyroid hormone function. *Nephron.* 14: 339-346.
- Barzel, U.S. and J. Jowsey. 1969. The effects of chronic acid and alkali administration on bone turnover in the adult. *Clin. Sci.* 36: 517-524.
- Bodega, G., I. Suárez, M.C. Boyano et al. 1993. High ammonia diet: its effect on the glial fibrillary acidic protein (GFAP). *Neurochem. Res.* 18(9): 971-975.
- Broderson, J.R., J.R. Lindsey and J.E. Crawford. 1976. The role of environmental ammonia in respiratory mycoplasmosis of rats. *Am. J. Pathol.* 85: 115-130.
- Carson, B.L., C.M. Beall, H.V. Ellis, III et al. 1981. Ammonia Health Effects. Prepared by Midwest Research Institute for Office of Mobile Source Air Pollution Control, Emission Control Technology Division, U.S. EPA, Ann Arbor, MI. EPA 460/3-81-027. (Cited in U.S. EPA, 1987)

Campbell, C.L., R.K. Dawes, S. Deolalkar and M.C. Merritt. 1958. Effect of certain chemicals in water on the flavor of brewed coffee. *Food Res.* 25: 575-579.

Capuco, V. 1977. Ammonia: a modulator of 3T3 cell growth. *Diss. Abstr. Int. B.* 38(9): 4085. (Cited in ATSDR, 2002)

Choudat, D., M. Goehen, M. Korobaeff et al. 1994. Respiratory symptoms and bronchial reactivity among pig and dairy farmers. *Scand. J. Work. Environ. Health.* 20(1): 48-54. (Cited in ATSDR, 2002)

Coon, R.A., R.A. Jones, L.J. Jenkins, Jr. et al. 1970. Animal inhalation studies on ammonia, ethylene, glycol, formaldehyde, dimethylamine and ethanol. *Toxicol. Appl. Pharmacol.* 16: 646-655.

Cormier, Y., E. Israel-Assayag, G. Racine et al. 2000. Farming practices and the respiratory health risks of swine confinement buildings. *Eur. Resp. J.* 15(3): 560-565.

Demerec, M., G. Bertani and J. Flint. 1951. A survey of chemicals for mutagenic action on *E. coli*. *Am. Nat.* 85: 119-136.

Diekman M.A., A.B. Scheidt, A.L. Sutton et al. 1993. Growth and reproduction performance, during exposure to ammonia, of gilts afflicted with pneumonia and atrophic rhinitis. *Am. J. Vet. Res.* 54(12): 2128-2131. (Cited in ATSDR, 2002)

Donham, K.J., S.J. Reynolds, P. Whitten et al. 1995. Respiratory dysfunction in swine production facility workers: dose-response relationships of environmental exposures and pulmonary functions. *Am. J. Ind. Med.* 27(3): 405-418. (Cited in ATSDR, 2002)

Donham, K.J., D. Cumro, S.J. Reynolds et al. 2000. Dose-response relationships between occupational aerosol exposures and cross-shift declines of lung function in poultry workers: recommendations for exposure limits. *J. Occup. Environ. Med.* 42(3): 260-269. (Cited in ATSDR, 2002)

Fazekas, I.G. 1939. Experimental suprarenal hypertrophy induced by ammonia. *Endokrinologie.* 21: 315-337. (Ger.; Cited in U.S. EPA, 1981)

Fazekas, I.G. 1949. Experimental data on influencing ovarian function by simple compounds. *Orvosi Hetilap.* 90: 777-781. (Ger.; Cited in U.S. EPA, 1981)

Fazekas, I.G. 1954a. Enlargement of the parathyroid by treatment with simple acidotic compounds. *Virchow's Arch. Pathol. Anat. u. Physiol.* 324: 531-542. (Ger.; Cited in U.S. EPA, 1981)

Fazekas, I.G. 1954b. The influence of acidotic compounds on the parathyroid function (serum Ca and P). *Endokrinologie.* 32: 45-57. (Ger.; Cited in U.S. EPA, 1981)

Ferguson, W.S., W.C. Koch, L.B. Webster et al. 1977. Human physiological response and adaptation to ammonia. *J. Occup. Med.* 19: 319-326.

Freedman, L.R. and P.B. Beeson. 1961. Experimental pyelonephritis. VIII. The effect of acidifying agents on susceptibility to infection. *Yale J. Biol. Med.* 33: 318-332.

Gupta, B.N., R.N. Kanna and K.K. Data. 1979. Toxicological studies of ammonium sulfamate in rat after repeated oral administration. *Toxicology.* 13: 45-49.

Heerderik, D., R. Brouwer, K. Biersteker et al. 1990. Relationship of airborne endotoxin and bacteria levels in pig farms with the lung function and respiratory symptoms of farmers. *Int. Arch. Occup. Environ. Health.* 62(8): 595-601. (Cited in ATSDR, 2002)

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: 646-650.

IARC (International Agency for Research on Cancer). 2002. Summaries and Evaluations Search. Online. <http://www.inchem.org/pages/iarc.html>

Lemann, J., J.R. Litzow and E.J. Lennon. 1966. The effects of chronic acid loads in normal man: further evidence for the participation of bone mineral in the defense against chronic metabolic acidosis. *J. Clin. Invest.* 45(10): 1608-1614.

Lobasov, M.E. and F. Smirnov. 1934. On the nature of the action of chemical agents on mutational process in *Drosophila Melanogaster*. II. The effect of ammonia on the occurrence of lethal transgenations. *C.R. Seances Acad. Sci. Roum.* 3: 177-178.

Litton Bionetics. 1975. Mutagenic evaluation of compound FDA 73-42 ammonium sulfate, granular, food grade. Prepared for U.S. Food and Drug Administration. NTIS PB-245 506. (Cited in U.S. EPA, 1981)

McBride, R.L. and D.G. Laing. 1979. Threshold determination by triangle testing: Effects of judgmental procedure, positional bias and incidental training. *Chem. Senses Flavor.* 4(4): 319-328. (Cited in WHO, 1986)

Minaña, M.D., V. Felipo, S. Grisolia et al. 1995. Prenatal exposure of rats to ammonia impair NMDA receptor function and affords delayed protection against ammonia toxicity and glutamate neurotoxicity. *J. Neuropathol. Exp. Neurol.* 54(5): 644-650.

NIOSH (National Institute for Occupational Safety and Health). 2002. Online NIOSH Pocket Guide to Hazardous Chemicals. Index of Chemical Abstract Numbers (7664-41-7). Online. <http://www.cdc.gov/niosh/npg/npgd0066.html>

NTP (National Toxicology Program). 2002. Management Status Report. Online. http://ntp-server.niehs.nih.gov/cgi/iH_Indexes/ALL_SRCH/iH_ALL_SRCH_Frames.html

OEHHA (Office of Environmental Health Hazard Assessment). 2002. Chronic Toxicity Summary: Ammonia. California Environmental Protection Agency. Online. http://www.oehha.org/air/chronic_rels/pdf/7664417.pdf

OSHA (Occupational Safety and Health Administration). 2002. OSHA Standard 1910.1000 Table Z-1. Part Z, Toxic and Hazardous Substances. Online. http://www.osha-slc.gov/OshStd_data/1910_1000_TABLE_Z-1.html

Reynolds, S.J., K.J. Donham, P. Whitten et al. 1996. Longitudinal evaluation of dose-response relationships for environmental exposures and pulmonary function in swine production workers. *Am. J. Ind. Med.* 29(1): 33-40.

Rosenfeld, M. 1932. Experimental modification of mitosis by ammonia. *Arch. Exp. Zellforsch* Besonder Gewebebezt. 14: 1-13. (Cited in ATSDR, 2002)

Schoeb, T.R., M.K. Davidson and J.R. Lindsey. 1982. Intracage ammonia promotes growth of *Mycoplasma pulmonis* in the respiratory tract of rats. *Infect. Immun.* 38: 212-217.

Seegal, B.C. 1927. Chronic acidosis in rabbits and dogs, with relation to kidney pathologic change. *Arch. Intern. Med.* 39(4): 550-563. (Cited in U.S. EPA, 1981)

Toth, B. 1972. Hydrazine, methylhydrazine and methylhydrazine sulfate carcinogenesis in Swiss mice. Failure of ammonium hydroxide to interfere in the development of tumors. *Int. J. Cancer.* 9: 109-118.

Tsujii, M., S. Kawano, S. Tsuji et al. 1995. Mechanism for ammonia-induced promotion of gastric carcinogenesis in rats. *Carcinogenesis.* 16(3): 563-566.

U.S. EPA. 1981. Ambient Water Quality Criterion for the Protection of Human Health: Ammonia. Prepared by the Environmental Criteria and Assessment Office, Cincinnati, OH for the Office of Water Regulations and Standards, Washington, DC.

U.S. EPA. 1987. Health Effects Assessment for Ammonia. Prepared by the Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH for the Office of Solid Waste and Emergency Response, Washington, DC.

U.S. EPA. 1988. Recommendations For and Documentation of Biological Values of Use in Risk Assessment. Prepared by the Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH for the Office of Solid Waste and Emergency Response, Washington, DC.

U.S. EPA. 1991. Chemical Assessments and Related Activities (CARA). Office of Health and Environmental Assessment, Washington, DC. April.

U.S. EPA. 1994a. Chemical Assessments and Related Activities (CARA). Office of Health and Environmental Assessment, Washington, DC. December.

U.S. EPA. 1994b. Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry. Office of Research and Development, Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Washington, DC. October, 1994. EPA/600/8-90/066F.

U.S. EPA. 1997. Health Effects Assessment Summary Tables (HEAST). FY-1997 Update. Prepared by the Office of Research and Development, National Center for Environmental Assessment, Cincinnati, OH for the Office of Emergency and Remedial Response, Washington, DC. July, 1997. EPA-540-R-97-036. NTIS PB97-921199.

U.S. EPA. 1999. Proposed Guidelines for Cancer Risk Assessment. Risk Assessment Forum, National Center for Environmental Assessment, Office of Research and Development, Washington, DC. July 1999.

U.S. EPA. 2002. 2002 Edition of the Drinking Water Standards and Health Advisories. Office of Water, Washington, DC. Summer, 2002. EPA 822-R-02-038. Online. <http://www.epa.gov/waterscience/drinking/standards/dwstandards.pdf>

U.S. EPA. 2003. Integrated Risk Information System (IRIS). Office of Research and Development. National Center for Environmental Assessment, Washington, DC. Online. <http://www.epa.gov/iris/>

- Uzvölgyi E. and F. Bojan. 1980. Possible in vivo formation of a carcinogenic substance from diethyl pyrocarbonate and ammonia. *J. Cancer Res. Clin. Oncol.* 97: 205-207.
- Uzvölgyi E. and F. Bojan. 1985. In vivo formation of a carcinogenic substance from diethyl pyrocarbonate in the presence of ammonia. *Arch. Toxicol. Suppl.* 8: 490-493.
- Visek, W.J., G.M. Kolodny and P.R. Gross. 1972. Ammonia effects in cultures of normal and transformed 3T3 cells. *J. Cell Physiol.* 1972: 373-381.
- Vogelzang, P.F.J., J.W.J. van der Gulden, H. Folgering et al. 2000. Longitudinal changes in bronchial responsiveness associated with swine confinement dust exposure. *Chest.* 117(5): 1488-1495.
- Vogelzang, P.F.J., J.W.J. van der Gulden, L. Preller et al. 1997. Bronchial hyperresponsiveness and exposure in pig farmers. *Int. Arch. Occup. Environ. Health.* 70(5): 327-333.
- Weatherby, J.H. 1952. Chronic toxicity of ammonia fumes by inhalation. *Proc. Soc. Exp. Biol. Med.* 81: 300-301. (Cited in U.S. EPA, 1981)
- WHO (World Health Organization). 1986. Environmental Health Criteria 54. Ammonia. WHO, Geneva. Online. <http://www.inchem.org/pages/ehc.html>
- Yadav J.S. and V.K. Kushik. 1997. Genotoxic effect of ammonia exposure on workers in a fertilizer factory. *Indian J. Exp. Biol.* 35(5): 487-492.



EPA/635/R-16/163Fa
www.epa.gov/iris

Toxicological Review of Ammonia Noncancer Inhalation

[CASRN 7664-41-7]

September 2016

Integrated Risk Information System
National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Washington, DC

DISCLAIMER

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

CONTENTS

AUTHORS CONTRIBUTORS REVIEWERS.....	vi
PREFACE	ix
PREAMBLE TO IRIS TOXICOLOGICAL REVIEWS.....	xii
EXECUTIVE SUMMARY	xx
LITERATURE SEARCH STRATEGY STUDY SELECTION AND EVALUATION	xxvi
1. HAZARD IDENTIFICATION	1-1
1.1. OVERVIEW OF CHEMICAL PROPERTIES AND TOXICOKINETICS	1-1
1.1.1. Chemical Properties	1-1
1.1.2. Toxicokinetics	1-2
1.2. SYNTHESIS OF EVIDENCE	1-3
1.2.1. Respiratory Effects	1-3
1.2.2. Immune System Effects.....	1-20
1.2.3. Other Systemic Effects	1-25
1.3. SUMMARY AND EVALUATION	1-33
1.3.1. Weight of Evidence for Effects Other than Cancer	1-33
1.3.2. Susceptible Populations and Lifestages	1-34
2. DOSE-RESPONSE ANALYSIS	2-1
2.1. INHALATION REFERENCE CONCENTRATION FOR EFFECTS OTHER THAN CANCER	2-1
2.1.1. Identification of Studies and Effects for Dose-Response Analysis	2-1
2.1.2. Methods of Analysis.....	2-5
2.1.3. Derivation of the Reference Concentration.....	2-7
2.1.4. Uncertainties in the Derivation of the Reference Concentration	2-9
2.1.5. Confidence Statement	2-12
2.1.6. Previous IRIS Assessment.....	2-12
REFERENCES	R-1

TABLES

Table ES-1. Summary of reference concentration (RfC) derivation.....	xxii
Table LS-1. Inclusion/exclusion criteria for inhalation health effect/toxicity studies (pre-peer review)	xxix
Table LS-2. Summary of epidemiology database	xxxviii
Table LS-3. Considerations and relevant experimental information for evaluation of experimental animal studies.....	xxxix
Table LS-4. Summary of experimental animal database.....	xlii
Table 1-1. Chemical and physical properties of ammonia.....	1-1
Table 1-2. Evidence pertaining to respiratory effects in humans following inhalation exposure in industrial settings	1-8
Table 1-3. Evidence pertaining to respiratory effect in humans following inhalation exposure in cleaning settings	1-12
Table 1-4. Evidence pertaining to respiratory effects in animals	1-16
Table 1-5. Evidence pertaining to immune system effects in animals	1-22
Table 1-6. Evidence pertaining to other systemic effects in animals	1-27
Table 2-1. Frequency distribution of ammonia exposure from Holness et al. (1989).....	2-6

FIGURES

Figure LS-1. Summary of literature search and screening process for ammonia.	xxviii
Figure 1-1. Exposure-response array of respiratory effects following inhalation exposure to ammonia.....	1-18
Figure 1-2. Exposure-response array of immune system effects following inhalation exposure to ammonia.....	1-24
Figure 1-3. Exposure-response array of systemic effects following inhalation exposure to ammonia.....	1-31

ABBREVIATIONS

ADME	absorption, distribution, metabolism, excretion	MRM	murine respiratory mycoplasmosis
AEGL	Acute Exposure Guideline Level	NCEA	National Center for Environmental Assessment
ACGIH	American Conference of Governmental Industrial Hygienists	NH ₃	ammonia
ALP	alkaline phosphatase	NH ₄ ⁺	ammonium ion
ATSDR	Agency for Toxic Substances and Disease Registry	NIOSH	National Institute for Occupational Safety and Health
BCG	bacillus Calmette-Guérin	NOAEL	no-observed-adverse-effect level
BMCL	95% lower bound on the benchmark concentration	NRC	National Research Council
BUN	blood urea nitrogen	ORD	EPA's Office of Research and Development
CAAC	Chemical Assessment Advisory Committee	PEF	peak expiratory flow
CAC	cumulative ammonia concentration	PEFR	peak expiratory flow rate
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act	PM	particulate matter
CFU	colony forming unit	POD	point of departure
CI	confidence interval	PPD	purified protein derivative
DAP	diammonium phosphate	RfC	reference concentration
EPA	Environmental Protection Agency	RfD	reference dose
FEF	forced expiratory flow	RTECS	Registry of Toxic Effects of Chemical Substances
FEV ₁	forced expiratory volume in 1 second	SAB	Science Advisory Board
FEV ₁ %	ratio of FEV ₁ to FVC (FEV ₁ /FVC)	TLV	Threshold Limit Value
FVC	forced vital capacity	TSCATS	Toxic Substance Control Act Test Submission Database
HERO	Health and Environmental Research Online	TWA	time-weighted average
HPV	high production volume	UF	uncertainty factor
HPVIS	high production volume information system	UF _A	interspecies uncertainty factor
IgE	immunoglobulin E	UF _H	intraspecies uncertainty factor
IgG	immunoglobulin G	UF _L	LOAEL to NOAEL uncertainty factor
IOM	Institute of Medicine	UF _S	subchronic-to-chronic uncertainty factor
IRIS	Integrated Risk Information System	UF _D	database deficiencies uncertainty factor
LC ₅₀	50% lethal concentration	VEh	human occupational default minute volume
LD ₅₀	50% lethal dose	VEho	human ambient default minute volume
LOAEL	lowest-observed-adverse-effect level	WOS	Web of Science
MLE	maximum likelihood estimate		

AUTHORS | CONTRIBUTORS | REVIEWERS

Assessment Team

Audrey Galizia, Dr. PH (Assessment Manager)	U.S. EPA/ORD/NCEA
James Ball, Ph.D. (no longer with EPA)	
Glinda Cooper, Ph.D. (no longer with EPA)	
Louis D'Amico, Ph.D.	
Keith Salazar, Ph.D.	
Christopher Sheth, Ph.D. (no longer with EPA)	
Christopher Brinkerhoff, Ph.D.	ORISE Postdoctoral Fellow at the U.S. EPA Washington, DC (currently with the Office of Chemical Safety and Pollution Prevention)

Contributors

Ted Berner, MS	U.S. EPA/ORD/NCEA
Vincent Coglianò, Ph.D.	
Jason Fritz, Ph.D.	
Martin Gehlhaus, MPH (currently with EPA Region 3)	
Samantha Jones, Ph.D.	
John Stanek, Ph.D.	
Jamie Strong, Ph.D. (currently with the Office of Water)	

Production Team

Maureen Johnson	U.S. EPA/ORD/NCEA
Ellen F. Lorang, MA (Retired)	
Vicki Soto	
Dahnish Shams	

Contractor Support

Amber Bacom, MS	SRC, Inc., Syracuse, NY
Fernando Lladòs, Ph.D.	
Julie Stickney, Ph.D.	

Executive Direction

Kenneth Olden, Ph.D., Sc.D., L.H.D. (Center Director - Retired)	U.S. EPA/ORD/NCEA
Lynn Flowers, Ph.D., DABT (Associate Director for Health, currently with the Office of Science Policy)	
John Vandenberg, Ph.D. (HHRA National Program Director)	
Vincent Coglianò, Ph.D. (IRIS Program Director)	
Gina Perovich, M.S. (IRIS Program Deputy Director)	
Samantha Jones, Ph.D. (IRIS Associate Director for Science)	
Susan Rieth, MPH (Branch Chief)	

Internal Reviewers

Marian Rutigliano, DO, MSEV	U.S. EPA/ORD/NCEA
Amanda S. Persad, Ph.D.	
Paul Reinhart, Ph.D.	
John Whalan, BS	

Reviewers

This assessment was provided for review to scientists in EPA's Program and Regional Offices. Comments were submitted by:

Office of the Administrator/Office of Children's Health Protection
Office of Air and Radiation/Office of Air Quality and Planning Standards
Office of Air and Radiation/Office of Transportation and Air Quality
Office of Policy
Office of Water
Region 2, New York City

This assessment was provided for review to other federal agencies and the Executive Office of the President (EOP). A summary and EPA's disposition of major comments from the other federal agencies and EOP is available on the IRIS website. Comments were submitted by:

Department of Agriculture/Food Safety and Inspection Service
DHHS/Agency for Toxic Substances and Disease Registry
Executive Office of the President/Council on Environmental Quality

This assessment was released for public comment on June 8, 2012 and comments were due on August 7, 2012. The public comments are available on Regulations.gov. A summary and EPA's disposition of the comments from the public is included in the revised external review draft assessment on the IRIS website. Comments were received from the following entities:

The American Chemistry Council	Washington, DC
The Fertilizer Institute	Washington, DC

This assessment was peer reviewed by independent, expert scientists external to EPA convened by EPA's Science Advisory Board (SAB), the Chemical Assessment Advisory Committee Augmented for the IRIS Ammonia Assessment. A peer review meeting was held on July 14 to 16, 2014. The report of the SAB's review of EPA's Draft Toxicological Review of Ammonia, dated August 6, 2015, is available on the IRIS website. A summary and EPA's disposition of the comments received from the SAB is included in Appendix D.

Dr. Michael Dourson (chair), Toxicology Excellence for Risk Assessment, Cincinnati, OH
Dr. Daniel Acosta, National Center for Toxicological Research, U. S. Food and Drug Administration, Jefferson, AR
Dr. Henry Anderson, Wisconsin Division of Public Health, Madison, WI
Dr. Scott Bartell, Program in Public Health, University of California – Irvine, Irvine, CA
Dr. Arthur Cooper, Department of Biochemistry and Molecular Biology, New York Medical College, Valhalla, NY
Dr. David Eastmond, Department of Cell Biology and Neuroscience and Environmental Toxicology Graduate Program, University of California, Riverside, CA
Dr. William Michael Foster, Pulmonary and Critical Care Medicine, Duke University Medical Center, Durham, NC

Toxicological Review of Ammonia

Dr. Russ Hauser, Department of Environmental Health, Harvard T. H. Chan School of Public Health, Harvard University, Boston, MA

Dr. Abby A. Li, Health Science Practice, Exponent Incorporated, San Francisco, CA

Dr. Maria Morandi, Independent Consultant, Houston, TX

Dr. Victoria Persky, Epidemiology & Biostatistics Program, School of Public Health, University of Illinois at Chicago, Chicago, IL

Dr. Kenneth Ramos, Precision Health Sciences and Professor of Medicine, Arizona Health Sciences Center, Tucson, AZ

Dr. Alan H. Stern, New Jersey Department of Environmental Protection, Trenton, NJ, and Department of Environmental and Occupational Health, Rutgers University School of Public Health, NJ

Dr. I. David Weiner, Division of Nephrology, Hypertension & Renal Transplantation, University of Florida, Gainesville, FL

Designated Federal Officer: Dr. Suhair Shallal

The post-external review draft of the assessment was provided for review to scientists in EPA's Program and Regional Offices, and to other federal agencies and the Executive Office of the President (EOP). A summary and EPA's disposition of major comments from the other federal agencies and EOP is available on the IRIS website. Comments were submitted by:

Office of the Administrator/Office of Children's Health Protection

Office of Land and Emergency Management

Executive Office of the President/Office of Management and Budget

PREFACE

This Toxicological Review critically reviews the publicly available studies on ammonia in order to identify its adverse health effects and to characterize exposure-response relationships. The assessment covers gaseous ammonia (NH₃) and ammonia dissolved in water (ammonium hydroxide, NH₄OH). It was prepared under the auspices of the Environmental Protection Agency's (EPA's) Integrated Risk Information System (IRIS) program.

Ammonia and ammonium hydroxide are listed as hazardous substances under the Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (CERCLA). Ammonia is subject to reporting requirements for the Toxics Release Inventory under the Emergency Planning and Community Right-to-Know Act of 1986 and to emergency planning requirements under section 112(r) of the Clean Air Act.

This assessment updates a previous IRIS assessment of ammonia that was developed in 1991. The previous assessment included only an inhalation reference concentration (RfC) for effects other than cancer. This assessment provides an updated review of information on all noncancer health effects by the inhalation route only.

This assessment was conducted in accordance with EPA guidance; relevant EPA guidance documents can be found on the IRIS website (<http://www.epa.gov/iris/>). The findings of this assessment and related documents produced during its development are also available on the IRIS website (<http://www.epa.gov/iris/>). Appendices for other health toxicity values, details of the literature search strategy and study selection and evaluation, supporting information for hazard identification and dose response, and other information are provided as Supplemental Information to this assessment (see Appendices A to C).

Portions of this Toxicological Review were adapted from the Toxicological Profile for Ammonia developed by the Agency for Toxic Substances and Disease Registry ([ATSDR, 2004](#)) under a Memorandum of Understanding that encourages interagency collaboration, sharing of scientific information, and more efficient use of resources.

The IRIS program released this assessment for public comment and peer review in June 2012, as it was beginning to implement systematic review. The approach to implementation is to use procedures and tools available at the time, without holding assessments until new methods become available. Accordingly, the IRIS program edited this assessment to increase transparency and clarity and to use more tables and figures. It conducted literature searches and evaluated studies using tools and documentation standards then available. Problem formulation materials and protocol development began with assessments started in 2015, after this assessment was well into peer review. This assessment addresses peer review comments and retains the structure of the peer review draft, to maintain fidelity with what the peer reviewers saw. Implementation of

systematic review is a process of continuous improvement subject to periodic review by the Chemical Assessment Advisory Committee of the EPA's Science Advisory Board. This assessment represents a step in the evolution of the IRIS program.

Assessments by Other National and International Health Agencies

Toxicity information on ammonia has been evaluated by ATSDR, the National Research Council (NRC), the National Institute for Occupational Safety and Health (NIOSH), and the Food and Drug Administration. The results of these assessments are presented in Appendix A of the Supplemental Information. It is important to recognize that these assessments may have been prepared for different purposes and may utilize different methods, and that newer studies may be included in the IRIS assessment.

Overview of Uses, Sources, and Environmental Exposure

About 80% of commercially produced ammonia is used in agricultural fertilizers. Ammonia is also used as a corrosion inhibitor, in water purification, as a household cleaner, as an antimicrobial agent in food products, as a refrigerant, as a stabilizer in the rubber industry, in the pulp and paper and metallurgy industries, as a source of hydrogen in the hydrogenation of fats and oils, and as a chemical intermediate in the production of pharmaceuticals, explosives, and other chemicals. Ammonia is also used to reduce nitrogen oxide emissions from combustion sources such as industrial and municipal boilers, power generators, and diesel engines ([HSDB, 2012](#); [Johnson et al., 2009](#); [Eggeman, 2001](#)).

Major sources of ammonia gas include leaks and spills during commercial synthesis, production, storage, processing, or transporting of ammonia; refrigeration equipment failure; decaying manure from livestock; application of fertilizers; sewage or wastewater effluent; burning of coal, wood, or other natural products; volcanic eruptions; forest fires; and the decomposition of nitrogenous compounds. Ammonia from agricultural and other sources, along with sulfate and nitrate salts, is an important contributor to fine inorganic particulate matter (PM_{2.5}) mass (e.g., see [Paulot and Jacob \(2014\)](#)). This literature on airborne particulate matter is reviewed and evaluated in EPA's Integrated Science Assessment for Particulate Matter ([U.S. EPA, 2009b](#)).

Environmental exposures to ammonia in the air vary widely. Average ambient concentrations of ammonia in the United States range from 0.28 to 15 µg/m³, as measured in 2012 by the National Atmospheric Deposition Program's Ammonia Monitoring Network ([AMoN, 2012](#)). Indoor residential ammonia concentrations can vary widely; one survey reported ammonia concentrations in homes in Connecticut and southwest and central Virginia ranging from 0.09 to 166 µg/m³, depending on the season, use of air conditioning, type of heating, and other factors ([Leaderer et al., 1999](#)).

Ammonia is found naturally in the environment and is a component of the global nitrogen cycle; it is essential to many biological processes. Nitrogen-fixing bacteria convert atmospheric nitrogen to ammonia that is available for uptake into plants. Organic nitrogen released from biota

can be converted to ammonia. Ammonia in water and soil can be converted to nitrite and nitrate through the process of nitrification. Ammonia is also endogenously produced in humans and other mammals, where it is an essential metabolite used in nucleic acid and protein synthesis, is necessary for maintaining acid-base balance, and is an integral part of nitrogen homeostasis ([Nelson and Cox, 2008](#); [Socolow, 1999](#); [Rosswall, 1981](#)).

Scope of this Assessment

This assessment presents an evaluation of the noncancer health effects of ammonia by the inhalation route of exposure. To address peer-review recommendations to expand the scope of the oral toxicity literature to include ammonium salts and to allow expeditious completion of the assessment of inhaled ammonia, ingested ammonia, including consideration of ammonium salts, will be the focus of a separate assessment. Because carcinogenicity studies of ammonia have been performed by the oral route of exposure only, the cancer assessment will be moved into the separate oral assessment.

For additional information about this assessment or for general questions regarding IRIS, please contact EPA's IRIS Hotline at 202-566-1676 (phone), 202-566-1749 (fax), or hotline.iris@epa.gov.

PREAMBLE TO IRIS TOXICOLOGICAL REVIEWS

The Preamble summarizes the objectives and scope of the IRIS program, general principles and systematic review procedures used in developing IRIS assessments, and the overall development process and document structure.

1. Objectives and Scope of the IRIS Program

Soon after EPA was established in 1970, it was at the forefront of developing risk assessment as a science and applying it in support of actions to protect human health and the environment. EPA's IRIS program¹ contributes to this endeavor by reviewing epidemiologic and experimental studies of chemicals in the environment to identify adverse health effects and characterize exposure-response relationships. Health agencies worldwide use IRIS assessments, which are also a scientific resource for researchers and the public.

IRIS assessments cover the hazard identification and dose-response steps of risk assessment. Exposure assessment and risk characterization are outside the scope of IRIS assessments, as are political, economic, and technical aspects of risk management. An IRIS assessment may cover one chemical, a group of structurally or toxicologically related chemicals, or a chemical mixture. Exceptions outside the scope of the IRIS program are radionuclides, chemicals used only as pesticides, and the "criteria air pollutants" (particulate matter, ground-level ozone, carbon monoxide, sulfur oxides, nitrogen oxides, and lead).

Enhancements to the IRIS program are improving its science, transparency, and productivity. To improve the science, the IRIS program is adapting and implementing principles of systematic review (i.e., using

explicit methods to identify, evaluate, and synthesize study findings). To increase transparency, the IRIS program discusses key science issues with the scientific community and the public as it begins an assessment. External peer review, independently managed and in public, improves both science and transparency. Increased productivity requires that assessments be concise, focused on EPA's needs, and completed without undue delay.

IRIS assessments follow EPA guidance² and standardized practices of systematic review. This Preamble summarizes and does not change IRIS operating procedures or EPA guidance.

Periodically, the IRIS program asks for nomination of agents for future assessment or reassessment. Selection depends on EPA's priorities, relevance to public health, and availability of pertinent studies. The IRIS multiyear agenda³ lists upcoming assessments. The IRIS program may also assess other agents in anticipation of public health needs.

2. Planning an Assessment: Scoping, Problem Formulation, and Protocols

Early attention to planning ensures that IRIS assessments meet their objectives and properly frame science issues.

Scoping refers to the first step of planning, where the IRIS program consults with EPA's program and regional offices to ascertain their needs. Scoping specifies the agents an

¹IRIS program website: <http://www.epa.gov/iris/>.

²EPA guidance documents: <http://www.epa.gov/iris/basic-information-about-integrated-risk-information-system#guidance/>.

³IRIS multiyear agenda: <https://www.epa.gov/iris/iris-agenda>.

assessment will address, routes and durations of exposure, susceptible populations and lifestages, and other topics of interest.

Problem formulation refers to the science issues an assessment will address and includes input from the scientific community and the public. A preliminary literature survey, beginning with secondary sources (e.g., assessments by national and international health agencies and comprehensive review articles), identifies potential health outcomes and science issues. It also identifies related chemicals (e.g., toxicologically active metabolites and compounds that metabolize to the chemical of interest).

Each IRIS assessment comprises multiple systematic reviews for multiple health outcomes. It also evaluates hypothesized mechanistic pathways and characterizes exposure-response relationships. An assessment may focus on important health outcomes and analyses rather than expand beyond what is necessary to meet its objectives.

Protocols refer to the systematic review procedures planned for use in an assessment. They include strategies for literature searches, criteria for study inclusion or exclusion, considerations for evaluating study methods and quality, and approaches to extracting data. Protocols may evolve as an assessment progresses and new agent-specific insights and issues emerge.

3. Identifying and Selecting Pertinent Studies

IRIS assessments conduct systematic literature searches with criteria for inclusion and exclusion. The objective is to retrieve the pertinent primary studies (i.e., studies with original data on health outcomes or their mechanisms). *PECO statements* (Populations, Exposures, Comparisons, Outcomes) govern the literature searches and screening criteria. “Populations” and animal species generally have no restrictions. “Exposures” refers to the agent

and related chemicals identified during scoping and problem formulation and may consider route, duration, or timing of exposure. “Comparisons” means studies that allow comparison of effects across different levels of exposure. “Outcomes” may become more specific (e.g., from “toxicity” to “developmental toxicity” to “hypospadias”) as an assessment progresses.

For studies of absorption, distribution, metabolism, and elimination, the first objective is to create an inventory of pertinent studies. Subsequent sorting and analysis facilitates characterization and quantification of these processes.

Studies on mechanistic events can be numerous and diverse. Here, too, the objective is to create an inventory of studies for later sorting to support analyses of related data. The inventory also facilitates generation and evaluation of hypothesized mechanistic pathways.

The IRIS program posts initial protocols for literature searches on its website and adds search results to EPA’s HERO database.⁴ Then the IRIS program takes extra steps to ensure identification of pertinent studies: by encouraging the scientific community and the public to identify additional studies and ongoing research; by searching for data submitted under the Toxic Substances Control Act or the Federal Insecticide, Fungicide, and Rodenticide Act; and by considering late-breaking studies that would impact the credibility of the conclusions, even during the review process.⁵

4. Evaluating Study Methods and Quality

IRIS assessments evaluate study methods and quality, using uniform approaches for each group of similar studies. The objective is that subsequent syntheses can weigh study results on their merits. Key concerns are potential *bias* (factors that affect the magnitude or direction of an effect) and *insensitivity* (factors that limit the ability of a study to detect a true effect).

⁴Health and Environmental Research Online: <https://hero.epa.gov/hero/>.

⁵IRIS “stopping rules”: https://www.epa.gov/sites/production/files/2014-06/documents/iris_stoppingrules.pdf.

For human and animal studies, the evaluation of study methods and quality considers study design, exposure measures, outcome measures, data analysis, selective reporting, and study sensitivity. For human studies, this evaluation also considers selection of participant and referent groups and potential confounding. Emphasis is on discerning bias that could substantively change an effect estimate, considering also the expected direction of the bias. Low sensitivity is a bias towards the null.

Study-evaluation considerations are specific to each study design, health effect, and agent. Subject-matter experts evaluate each group of studies to identify characteristics that bear on the informativeness of the results. For carcinogenicity, neurotoxicity, reproductive toxicity, and developmental toxicity, there is EPA guidance for study evaluation ([U.S. EPA, 2005a, 1998, 1996, 1991](#)). As subject-matter experts examine a group of studies, additional agent-specific knowledge or methodologic concerns may emerge and a second pass become necessary.

Assessments use evidence tables to summarize the design and results of pertinent studies. If tables become too numerous or unwieldy, they may focus on effects that are more important or studies that are more informative.

The IRIS program posts initial protocols for study evaluation on its website, then considers public input as it completes this step.

5. Integrating the Evidence of Causation for Each Health Outcome

Synthesis within lines of evidence. For each health outcome, IRIS assessments synthesize the human evidence and the animal evidence, augmenting each with informative subsets of mechanistic data. Each synthesis considers aspects of an association that may suggest causation: consistency, exposure-response relationship, strength of association, temporal relationship, biological plausibility, coherence, and “natural experiments” in humans ([U.S. EPA, 1994](#), §2.1.3) ([U.S. EPA, 2005a](#), §2.5).

Each synthesis seeks to reconcile ostensible inconsistencies between studies, taking into

account differences in study methods and quality. This leads to a distinction between *conflicting evidence* (unexplained positive and negative results in similarly exposed human populations or in similar animal models) and *differing results* (mixed results attributable to differences between human populations, animal models, or exposure conditions) ([U.S. EPA, 2005a](#), §2.5).

Each synthesis of human evidence explores alternative explanations (e.g., chance, bias, or confounding) and determines whether they may satisfactorily explain the results. Each synthesis of animal evidence explores the potential for analogous results in humans. Coherent results across multiple species increase confidence that the animal results are relevant to humans.

Mechanistic data are useful to augment the human or animal evidence with information on precursor events, to evaluate the human relevance of animal results, or to identify susceptible populations and lifestages. An agent may operate through multiple mechanistic pathways, even if one hypothesis dominates the literature ([U.S. EPA, 2005a](#), §2.4.3.3).

Integration across lines of evidence. For each health outcome, IRIS assessments integrate the human, animal, and mechanistic evidence to answer the question: *What is the nature of the association between exposure to the agent and the health outcome?*

For cancer, EPA includes a standardized hazard descriptor in characterizing the strength of the evidence of causation. The objective is to promote clarity and consistency of conclusions across assessments ([U.S. EPA, 2005a](#), §2.5).

Carcinogenic to humans: convincing epidemiologic evidence of a causal association; or strong human evidence of cancer or its key precursors, extensive animal evidence, identification of mode-of-action and its key precursors in animals, and strong evidence that they are anticipated in humans.

Likely to be carcinogenic to humans: evidence that demonstrates a potential hazard to humans. Examples include a plausible association in humans with supporting experimental evidence, multiple positive

results in animals, a rare animal response, or a positive study strengthened by other lines of evidence.

Suggestive evidence of carcinogenic potential: evidence that raises a concern for humans. Examples include a positive result in the only study, or a single positive result in an extensive database.

Inadequate information to assess carcinogenic potential: no other descriptors apply. Examples include little or no pertinent information, *conflicting evidence*, or negative results not sufficiently robust for *not likely*.

Not likely to be carcinogenic to humans: robust evidence to conclude that there is no basis for concern. Examples include no effects in well-conducted studies in both sexes of multiple animal species, extensive evidence showing that effects in animals arise through modes-of-action that do not operate in humans, or convincing evidence that effects are not likely by a particular exposure route or below a defined dose.

If there is credible evidence of carcinogenicity, there is an evaluation of mutagenicity, because this influences the approach to dose–response assessment and subsequent application of adjustment factors for exposures early in life ([U.S. EPA, 2005a](#), §3.3.1, §3.5), ([U.S. EPA, 2005b](#), §5).

6. Selecting Studies for Derivation of Toxicity Values

The purpose of toxicity values (slope factors, unit risks, reference doses, reference concentrations; see section 7) is to estimate exposure levels likely to be without appreciable risk of adverse health effects. EPA uses these values to support its actions to protect human health.

The health outcomes considered for derivation of toxicity values may depend on the hazard descriptors. For example, IRIS assessments generally derive cancer values for agents that are *carcinogenic* or *likely to be carcinogenic*, and sometimes for agents with *suggestive evidence* ([U.S. EPA, 2005a](#), §3).

Derivation of toxicity values begins with a new evaluation of studies, as some studies used qualitatively for hazard identification may not be useful quantitatively for exposure–response assessment. Quantitative analyses require quantitative measures of exposure and response. An assessment weighs the merits of the human and animal studies, of various animal models, and of different routes and durations of exposure ([U.S. EPA, 1994](#), §2.1). Study selection is not reducible to a formula, and each assessment explains its approach.

Other biological determinants of study quality include appropriate measures of exposure and response, investigation of early effects that precede overt toxicity, and appropriate reporting of related effects (e.g., combining effects that comprise a syndrome, or benign and malignant tumors in a specific tissue).

Statistical determinants of study quality include multiple levels of exposure (to characterize the shape of the exposure–response curve) and adequate exposure range and sample sizes (to minimize extrapolation and maximize precision) ([U.S. EPA, 2012](#), §2.1).

Studies of low sensitivity may be less useful if they fail to detect a true effect or yield toxicity values with wide confidence limits.

7. Deriving Toxicity Values

General approach. EPA guidance describes a two-step approach to dose–response assessment: analysis in the range of observation, then extrapolation to lower levels. Each toxicity value pertains to a route (e.g., oral, inhalation, dermal) and duration or timing of exposure (e.g., chronic, subchronic, gestational) ([U.S. EPA, 2002](#), §4).

IRIS assessments derive a candidate value from each suitable data set. Consideration of candidate values yields a toxicity value for each organ or system. Consideration of the organ/system-specific values results in the selection of an overall toxicity value to cover all health outcomes. The organ/system-specific values are useful for subsequent cumulative risk assessments that consider the combined effect of

multiple agents acting at a common anatomical site.

Analysis in the range of observation. Within the observed range, the preferred approach is modeling to incorporate a wide range of data. Toxicokinetic modeling has become increasingly common for its ability to support target-dose estimation, cross-species adjustment, or exposure-route conversion. If data are too limited to support toxicokinetic modeling, there are standardized approaches to estimate daily exposures and scale them from animals to humans (U.S. EPA, 1994, §3), (U.S. EPA, 2005a, §3.1), (U.S. EPA, 2011, 2006).

For human studies, an assessment may develop exposure–response models that reflect the structure of the available data (U.S. EPA, 2005a, §3.2.1). For animal studies, EPA has developed a set of empirical (“curve-fitting”) models⁶ that can fit typical data sets (U.S. EPA, 2005a, §3.2.2). Such modeling yields a *point of departure*, defined as a dose near the lower end of the observed range, without significant extrapolation to lower levels (e.g., the estimated dose associated with an extra risk of 10% for animal data or 1% for human data, or their 95% lower confidence limits) (U.S. EPA, 2005a, §3.2.4), (U.S. EPA, 2012, §2.2.1).

When justified by the scope of the assessment, toxicodynamic (“biologically based”) modeling is possible if data are sufficient to ascertain the key events of a mode-of-action and to estimate their parameters. Analysis of model uncertainty can determine the range of lower doses where data support further use of the model (U.S. EPA, 2005a, §3.2.2, §3.3.2).

For a group of agents that act at a common site or through common mechanisms, an assessment may derive relative potency factors based on relative toxicity, rates of absorption or metabolism, quantitative structure–activity relationships, or receptor-binding characteristics (U.S. EPA, 2005a, §3.2.6).

Extrapolation: slope factors and unit risks. An *oral slope factor* or an *inhalation unit risk* facilitates subsequent estimation of human cancer risks. Extrapolation proceeds linearly (i.e., risk proportional to dose) from the point of

departure to the levels of interest. This is appropriate for agents with direct mutagenic activity. It is also the default if there is no established mode-of-action (U.S. EPA, 2005a, §3.3.1, §3.3.3).

Differences in susceptibility may warrant derivation of multiple slope factors or unit risks. For early-life exposure to carcinogens with a mutagenic mode-of-action, EPA has developed default *age-dependent adjustment factors* for agents without chemical-specific susceptibility data (U.S. EPA, 2005a, §3.5), (U.S. EPA, 2005b, §5).

If data are sufficient to ascertain the mode-of-action and to conclude that it is not linear at low levels, extrapolation may use the reference-value approach (U.S. EPA, 2005a, §3.3.4).

Extrapolation: reference values. An *oral reference dose* or an *inhalation reference concentration* is an estimate of human exposure (including in susceptible populations) likely to be without appreciable risk of adverse health effects over a lifetime (U.S. EPA, 2002, §4.2). Reference values generally cover effects other than cancer. They are also appropriate for carcinogens with a nonlinear mode-of-action.

Calculation of reference values involves dividing the point of departure by a set of *uncertainty factors* (each typically 1, 3, or 10, unless there are adequate chemical-specific data) to account for different sources of uncertainty and variability (U.S. EPA, 2002, §4.4.5), (U.S. EPA, 2014).

Human variation: An uncertainty factor covers susceptible populations and lifestages that may respond at lower levels, unless the data originate from a susceptible study population.

Animal-to-human extrapolation: For reference values based on animal results, an uncertainty factor reflects cross-species differences, which may cause humans to respond at lower levels.

Subchronic-to-chronic exposure: For chronic reference values based on subchronic studies, an uncertainty factor reflects the likelihood that a lower level over a longer duration may induce a similar response. This

⁶Benchmark Dose Software: <http://www.epa.gov/bmds/>.

factor may not be necessary for reference values of shorter duration.

Adverse-effect level to no-observed-adverse-effect level: For reference values based on a lowest-observed-adverse-effect level, an uncertainty factor reflects a level judged to have no observable adverse effects.

Database deficiencies: If there is concern that future studies may identify a more sensitive effect, target organ, population, or lifestage, a *database uncertainty factor* reflects the nature of the database deficiency.

8. Process for Developing and Peer-Reviewing IRIS Assessments

The IRIS process (revised in 2009 and enhanced in 2013) involves extensive public engagement and multiple levels of scientific review and comment. IRIS program scientists consider all comments. Materials released, comments received from outside EPA, and disposition of major comments (steps 3, 4, and 6 below) become part of the public record.

Step 1: Draft development. As outlined in section 2 of this Preamble, IRIS program scientists specify the scope of an assessment and formulate science issues for discussion with the scientific community and the public. Next, they release initial protocols for the systematic review procedures planned for use in the assessment. IRIS program scientists then develop a first draft, using structured approaches to identify pertinent studies, evaluate study methods and quality, integrate the evidence of causation for each health outcome, select studies for derivation of toxicity values, and derive toxicity values, as outlined in Preamble sections 3–7.

Step 2: Agency review. Health scientists across EPA review the draft assessment.

Step 3: Interagency science consultation. Other federal agencies and the Executive Office of the President review the draft assessment.

Step 4: Public comment, followed by external peer review. The public reviews the draft

assessment. IRIS program scientists release a revised draft for independent external peer review. The peer reviewers consider whether the draft assessment assembled and evaluated the evidence according to EPA guidance and whether the evidence justifies the conclusions.

Step 5: Revise assessment. IRIS program scientists revise the assessment to address the comments from the peer review.

Step 6: Final agency review and interagency science discussion. The IRIS program discusses the revised assessment with EPA's program and regional offices and with other federal agencies and the Executive Office of the President.

Step 7: Post final assessment. The IRIS program posts the completed assessment and a summary on its website.

9. General Structure of IRIS Assessments

Main text. IRIS assessments generally comprise two major sections: (1) Hazard Identification and (2) Dose–Response Assessment. Section 1.1 briefly reviews chemical properties and toxicokinetics to describe the disposition of the agent in the body. This section identifies related chemicals and summarizes their health outcomes, citing authoritative reviews. If an assessment covers a chemical mixture, this section discusses environmental processes that alter the mixtures humans encounter and compares them to mixtures studied experimentally.

Section 1.2 includes a subsection for each major health outcome. Each subsection discusses the respective literature searches and study considerations, as outlined in Preamble sections 3 and 4, unless covered in the front matter. Each subsection concludes with evidence synthesis and integration, as outlined in Preamble section 5.

Section 1.3 links health hazard information to dose–response analyses for each health outcome. One subsection identifies susceptible populations and lifestages, as observed in human

or animal studies or inferred from mechanistic data. These may warrant further analysis to quantify differences in susceptibility. Another subsection identifies biological considerations for selecting health outcomes, studies, or data sets, as outlined in Preamble section 6.

Section 2 includes a subsection for each toxicity value. Each subsection discusses study selection, methods of analysis, and derivation of a toxicity value, as outlined in Preamble sections 6 and 7.

Front matter. The Executive Summary provides information historically included in IRIS summaries on the IRIS program website. Its structure reflects the needs and expectations of EPA's program and regional offices.

A section on systematic review methods summarizes key elements of the protocols, including methods to identify and evaluate pertinent studies. The final protocols appear as an appendix.

The Preface specifies the scope of an assessment and its relation to prior assessments. It discusses issues that arose during assessment development and emerging areas of concern.

This Preamble summarizes general procedures for assessments begun after the date below. The Preface identifies assessment-specific approaches that differ from these general procedures.

August 2016

References

- [U.S. EPA](#). (1991). Guidelines for developmental toxicity risk assessment (pp. 1-83). (EPA/600/FR-91/001). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. <http://cfpub.epa.gov/ncea/cfm/recorddisplay.cfm?deid=23162>
- [U.S. EPA](#). (1994). Methods for derivation of inhalation reference concentrations and application of inhalation dosimetry [EPA Report] (pp. 1-409). (EPA/600/8-90/066F). Research Triangle Park, NC: U.S. Environmental Protection Agency, Office of Research and Development, Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office. <https://cfpub.epa.gov/ncea/risk/recorddisplay.cfm?deid=71993&CFID=51174829&CFTOKEN=25006317>
- [U.S. EPA](#). (1996). Guidelines for reproductive toxicity risk assessment (pp. 1-143). (EPA/630/R-96/009). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum.
- [U.S. EPA](#). (1998). Guidelines for neurotoxicity risk assessment. Fed Reg 63: 26926-26954.
- [U.S. EPA](#). (2002). A review of the reference dose and reference concentration processes (pp. 1-192). (EPA/630/P-02/002F). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. <http://www.epa.gov/osa/review-reference-dose-and-reference-concentration-processes>
- [U.S. EPA](#). (2005a). Guidelines for carcinogen risk assessment [EPA Report] (pp. 1-166). (EPA/630/P-03/001F). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. <http://www2.epa.gov/osa/guidelines-carcinogen-risk-assessment>
- [U.S. EPA](#). (2005b). Supplemental guidance for assessing susceptibility from early-life exposure to carcinogens (pp. 1-125). (EPA/630/R-03/003F). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum.
- [U.S. EPA](#). (2006). Approaches for the application of physiologically based pharmacokinetic (PBPK) models and supporting data in risk assessment (Final Report) [EPA Report] (pp. 1-123). (EPA/600/R-05/043F). Washington, DC: U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment. <http://cfpub.epa.gov/ncea/cfm/recorddisplay.cfm?deid=157668>
- [U.S. EPA](#). (2011). Recommended use of body weight 3/4 as the default method in derivation of the oral reference dose (pp. 1-50). (EPA/100/R11/0001). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum, Office of the Science Advisor. <https://www.epa.gov/risk/recommended-use-body-weight-34-default-method-derivation-oral-reference-dose>
- [U.S. EPA](#). (2012). Benchmark dose technical guidance (pp. 1-99). (EPA/100/R-12/001). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum.
- [U.S. EPA](#). (2014). Guidance for applying quantitative data to develop data-derived extrapolation factors for interspecies and intraspecies extrapolation. (EPA/100/R-14/002F). Washington, DC: Risk Assessment Forum, Office of the Science Advisor. <http://www.epa.gov/raf/DDEF/pdf/ddef-final.pdf>

EXECUTIVE SUMMARY

Occurrence and Health Effects

Ammonia occurs naturally in air, soil, and water. Ammonia is also produced by humans and other animals as part of normal biological processes.

Ammonia is used as an agricultural fertilizer and in many cleaning products. Exposure to ammonia occurs primarily through breathing air containing ammonia gas, and may also occur via diet, drinking water, or direct skin contact. Measured concentrations of ammonia range from 0.28 to 15 $\mu\text{g}/\text{m}^3$ in ambient outdoor air and from 0.09 to 166 $\mu\text{g}/\text{m}^3$ in indoor air.

Health effects of inhaled ammonia observed at levels exceeding naturally-occurring concentrations are generally limited to the respiratory tract, the site of direct contact with ammonia. Short-term inhalation exposure to high levels of ammonia in humans can cause irritation and serious burns in the mouth, lungs, and eyes. Chronic exposure to airborne ammonia can increase the risk of respiratory irritation, cough, wheezing, tightness in the chest, and impaired lung function in humans. Studies in experimental animals similarly indicate that breathing ammonia at sufficiently high concentrations can result in effects on the respiratory system. Animal studies also suggest that exposure to high levels of ammonia in air may adversely affect other organs, such as the liver, kidney, and spleen.

This assessment presents an evaluation of the noncancer health effects of ammonia by the inhalation route of exposure.

Chemical Properties

Ammonia (NH_3) is a colorless alkaline gas with a pungent odor. In solution, ammonia exists as ammonium hydroxide, a weak base that is only partially ionized in water according to the following equilibrium ([ATSDR, 2004](#)): $\text{NH}_3 + \text{H}_2\text{O} \rightleftharpoons \text{NH}_4^+ + \text{OH}^-$. A decrease in pH results in an increase in the concentration of ammonium ion (NH_4^+) and a decrease in the concentration of the un-ionized form (NH_3). At physiological pH (7.4), this equilibrium favors the formation of NH_4^+ .

Toxicokinetics

Inhaled ammonia is almost completely retained in the upper respiratory tract. Ammonia produced endogenously in the intestines through the use of amino acids as an energy source and by bacterial degradation of nitrogenous compounds from ingested food is largely absorbed. At physiological pH, 98.3% of ammonia is present in the blood as the ammonium ion (NH_4^+). Given its importance in amino acid metabolism, the urea cycle, and acid-base balance, ammonia is homeostatically regulated to remain at low concentrations in the blood. Ammonia is present in fetal circulation and in human breast milk as a source of nonprotein nitrogen. Ammonia production

occurs endogenously by catabolism of amino acids by glutamate dehydrogenase or glutaminase primarily in the liver, renal cortex, and intestines, but also in the brain and heart. Ammonia is metabolized to glutamine via glutamine synthetase in the glutamine cycle or incorporated into urea as part of the urea cycle. The principal means of excretion of ammonia is as urinary urea; lesser amounts are eliminated in the feces, through sweat production, and in expired air.

Effects Other Than Cancer Observed Following Inhalation Exposure

Respiratory effects have been identified as a human health hazard following inhalation exposure to ammonia. This hazard determination is based on findings from multiple epidemiology studies in human populations exposed to ammonia in different settings (workers in industrial, cleaning, and agricultural settings, volunteers exposed for up to 6 hours under controlled conditions, and case reports) and animals (short-term and subchronic studies in several species and across different exposure regimes).

Cross-sectional occupational studies involving chronic exposure to ammonia in industrial settings provide evidence of an increased prevalence of respiratory symptoms ([Rahman et al., 2007](#); [Ballal et al., 1998](#)) and decreased lung function ([Rahman et al., 2007](#); [Ali et al., 2001](#); [Ballal et al., 1998](#); [Bhat and Ramaswamy, 1993](#)). Other studies of exposure to ammonia when used as a disinfectant or cleaning product provide evidence of asthma, asthma symptoms, and impaired pulmonary function, using a variety of study designs ([Casas et al., 2013](#); [Arif and Delclos, 2012](#); [Dumas et al., 2012](#); [Lemiere et al., 2012](#); [Vizcaya et al., 2011](#); [Zock et al., 2007](#); [Medina-Ramón et al., 2006](#); [Medina-Ramón et al., 2005](#)). Further evidence of respiratory effects of ammonia is seen in studies of pulmonary function in an agricultural setting, specifically in studies that accounted for effects of co-exposures to other agents such as endotoxin and dust ([Donham et al., 2000](#); [Reynolds et al., 1996](#); [Donham et al., 1995](#); [Preller et al., 1995](#); [Heederik et al., 1990](#)) and in one study that did not control for co-exposures ([Loftus et al., 2015](#)). Despite the variation in population characteristics, level and pattern of exposure, and potential confounders across these three settings of epidemiology studies, respiratory effects were consistently observed in these studies. Further, but more limited, support for the respiratory system as a target of ammonia toxicity comes from controlled human exposure studies of ammonia inhalation and case reports of injury in humans with inhalation exposure to ammonia. Additionally, respiratory effects were observed in several animal species following short-term and subchronic inhalation exposures to ammonia.

Overall, there are suggestions in experimental animals that ammonia exposure may be associated with effects on organs distal from the portal of entry, but there is inadequate information to draw conclusions about the liver, kidney, spleen, or heart as sensitive targets of ammonia toxicity.

Inhalation Reference Concentration (RfC) for Effects Other Than Cancer

Table ES-1. Summary of reference concentration (RfC) derivation

Critical effect	Point of departure ^a	UF	Chronic RfC
Decreased lung function and respiratory symptoms Occupational epidemiology studies Holness et al. (1989) , supported by Rahman et al. (2007) , Ballal et al. (1998) , and Ali et al. (2001)	NOAEL _{ADJ} : 4.9 mg/m ³	10	0.5 mg/m ³

^aAn estimate of the 95% lower confidence bound of the mean exposure concentration in the high-exposure group of the [Holness et al. \(1989\)](#) study was used as the NOAEL. Because the study involved workplace exposure conditions, the NOAEL of 13.6 mg/m³ was adjusted for continuous exposure based on the ratio of VE_{ho} (human occupational default minute volume of 10 m³ breathed during an 8-hour workday) to VE_h (human ambient default minute volume of 20 m³ breathed during the entire day) and an exposure of 5 days out of 7 days.

NOAEL = no-observed-adverse-effect level; UF = uncertainty factor

The study of ammonia exposure in workers in a soda ash plant by [Holness et al. \(1989\)](#), with support from three studies in urea fertilizer plants by [Rahman et al. \(2007\)](#), [Ballal et al. \(1998\)](#), and [Ali et al. \(2001\)](#), was identified as the principal study for RfC derivation. Respiratory effects, characterized as increased respiratory symptoms based on self-report (including cough, wheezing, and other asthma-related symptoms) and decreased lung function in workers exposed to ammonia, were selected as the critical effect. [Rahman et al. \(2007\)](#) observed an increased prevalence of respiratory symptoms and decreased lung function in workers exposed in a plant with a mean ammonia concentration of 18.5 mg/m³, but not in workers in a second plant exposed to a mean concentration of 4.9 mg/m³. [Ballal et al. \(1998\)](#) observed an increased prevalence of respiratory symptoms among workers in one factory with exposures ranging from 2 to 27.1 mg/m³,⁷ but no increase in another factory with exposures ranging from 0.02 to 7 mg/m³. A companion study by [Ali et al. \(2001\)](#) also observed decreased lung function among workers exposed to higher cumulative ammonia levels (>50 mg/m³-years), with an approximate 5–7% decrease in FVC% predicted and FEV₁% predicted (see definition of spirometry measures in Section 1.2.1). [Holness et al. \(1989\)](#), who investigated a plant with exposures generally lower than other studies, found no differences in the prevalence of respiratory symptoms or lung function between workers (mean exposure 6.5 mg/m³) and the control group, and no differences when stratified by exposure level (highest exposure group, >8.8 mg/m³).

⁷This concentration range does not include exposures in the urea store (number of employees = 6; range of ammonia concentrations = 90–130.4 mg/m³) because employees in this area were required to wear full protective clothing, thus minimizing potential exposure.

These four studies addressed smoking by a variety of methods (e.g., adjustment for smoking, exclusion of smokers, stratification of the results by smoking status). Two of the studies—[Rahman et al. \(2007\)](#) and [Holness et al. \(1989\)](#)—addressed other potential confounders as appropriate. In particular, a high level of control of exposures in the facility studied by [Holness et al. \(1989\)](#) was reported, suggesting a low potential for co-exposures. As discussed in more detail in the Literature Search Strategy | Study Selection and Evaluation section, confounding by other workplace exposures, although a potential concern, was unlikely to be a major limitation of these studies.

Considerations in selecting the principal study for RfC derivation include the higher confidence placed in the measures of ammonia exposure in [Holness et al. \(1989\)](#), evaluation of both respiratory symptoms and lung function parameters in this study, and the fact that the estimate of the no-observed-adverse-effect level (NOAEL) for respiratory effects of 13.6 mg/m³ from [Holness et al. \(1989\)](#) was the highest of the studies with adequate exposure-response information. The synthesis of findings from the full body of evidence demonstrates that there is a relationship between ammonia exposure and respiratory effects. Although [Holness et al. \(1989\)](#) do not report associations between ammonia exposure and respiratory effects, it is included in the body of epidemiologic studies of industrial settings because it is informative of the levels above which ammonia causes effects. Other epidemiology studies include those with higher workplace ammonia concentrations associated with respiratory effects (i.e., higher concentrations relative to those reported by [Holness et al. \(1989\)](#)) and for which lowest-observed-adverse-effect levels (LOAELs) could be identified. The [Holness et al. \(1989\)](#) study is identified as the principal study for RfC derivation based on the quality of the exposure data and other factors, as stated above.

In summary, the study of ammonia exposure in workers in a soda ash plant by [Holness et al. \(1989\)](#) was identified as the principal study for RfC derivation, with support from [Rahman et al. \(2007\)](#), [Ballal et al. \(1998\)](#), and [Ali et al. \(2001\)](#), and respiratory effects were identified as the critical effect. The NOAEL, represented by an estimate of the 95% lower confidence bound of the mean exposure concentration in the high-exposure group from the [Holness et al. \(1989\)](#) study, or 13.6 mg/m³, was used as the point of departure (POD) for RfC derivation. The NOAEL adjusted to continuous exposure (NOAEL_{ADJ}) was 4.9 mg/m³.

An RfC of 0.5 (rounded) mg/m³ was calculated by dividing the POD (adjusted for continuous exposure, i.e., NOAEL_{ADJ}) by a composite uncertainty factor (UF) of 10 to account for potentially susceptible individuals in the absence of data evaluating variability of response to inhaled ammonia in the human population.

Confidence in the Chronic Inhalation RfC

Study – medium

Database – medium

RfC – medium

Consistent with Environmental Protection Agency (EPA) *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* ([U.S. EPA, 1994](#)), the overall confidence in the RfC is medium and reflects medium confidence in the principal study (adequate design, conduct, and reporting of the principal study; limited by small sample size and identification of a NOAEL only) and medium confidence in the database, which includes occupational, cleaner, agricultural, and human exposure studies and studies in animals that are mostly of subchronic duration. There are no studies of developmental toxicity, and studies of reproductive and other systemic endpoints are limited; however, the likelihood of reproductive, developmental, and other systemic effects at the RfC is considered small because it is well documented that ammonia is endogenously produced in humans and animals, and any changes in blood ammonia levels at the POD would be small relative to normal blood ammonia levels. Further, EPA is not aware of any mechanisms by which ammonia can exert effects at the point of contact (i.e., respiratory system) that could directly or indirectly impact tissues or organs distal to the point of contact.

Susceptible Populations and Lifestages

Studies of the toxicity of ammonia in children that would support an evaluation of childhood susceptibility are limited. [Casas et al. \(2013\)](#) and [Loftus et al. \(2015\)](#) reported evidence of an association between ammonia exposure and decrements in lung function in children; however, these studies did not report information that would allow a comparison of children and adults.

A limited number of studies provides inconsistent evidence of greater respiratory sensitivity to ammonia exposure in asthmatics ([Loftus et al., 2015](#); [Petrova et al., 2008](#); [Sigurdarson et al., 2004](#); [Preller et al., 1995](#)). [Loftus et al. \(2015\)](#) reported no increase in asthma symptoms and medication use in asthmatic children living near animal feeding operations; however, ammonia exposure was associated with lower FEV₁.

Hyperammonemia is a condition of elevated levels of circulating ammonia that can occur in individuals with severe diseases of the liver or kidney or with hereditary urea [CO(NH₂)₂] cycle disorders. These elevated ammonia levels can predispose an individual to encephalopathy due to the ability of ammonia to cross the blood-brain barrier; these effects are especially marked in newborn infants. Thus, individuals with disease conditions that lead to hyperammonemia may be

more susceptible to the effects of ammonia from external sources, but there are no studies that specifically support this susceptibility.

Key Issues Addressed in This Assessment

Comparison of Exhaled Ammonia to the RfC

Ammonia is generated endogenously in multiple organs and plays central roles in nitrogen balance and acid-base homeostasis ([Weiner et al., 2014](#); [Weiner and Verlander, 2013](#)). Given its important metabolic role, free ammonia is homeostatically regulated to remain at low concentrations in blood ([Souba, 1987](#)). Elimination of ammonia occurs primarily in urine and exhaled breath. Consideration was given to the presence of ammonia in exhaled air because the range of ammonia concentrations in exhaled breath (0.009–2 mg/m³) overlaps the ammonia RfC (0.5 mg/m³).

In general, higher and more variable ammonia concentrations (0.03–2 mg/m³) are reported in human breath exhaled from the mouth or oral cavity ([Schmidt et al., 2013](#); [Smith et al., 2008](#); [Španěl et al., 2007a, b](#); [Turner et al., 2006](#); [Diskin et al., 2003](#); [Smith et al., 1999](#); [Norwood et al., 1992](#); [Larson et al., 1977](#)). Ammonia concentrations measured in breath derived from oral breathing largely reflect the production of ammonia via bacterial degradation of food protein in the oral cavity or gastrointestinal tract, and can be influenced by diet, oral hygiene, age, and saliva pH. In contrast, concentrations of ammonia in breath exhaled from the nose and trachea of humans (0.0092–0.1 mg/m³) are lower than those in air exhaled from the mouth ([Schmidt et al., 2013](#); [Smith et al., 2008](#); [Larson et al., 1977](#)), and are generally lower than the RfC by a factor of five or more. Concentrations in breath exhaled from the nose appear to better represent levels at the alveolar interface of the lung and are more relevant to understanding systemic levels of ammonia than breath exhaled from the mouth ([Schmidt et al., 2013](#); [Smith et al., 2008](#)); however, concentrations in breath from neither the mouth nor the nose can be used to predict blood ammonia concentration or previous exposure to environmental (ambient) concentrations of ammonia (see Appendix C, Section C.1.4).

Regardless of the source of expired ammonia (mouth or nose), the level of ammonia in breath, even at concentrations that exceed the RfC, does not necessarily raise questions about the appropriateness of the RfC. The exhalation of ammonia is a clearance mechanism for a product of metabolism that is otherwise toxic in the body at sufficiently high concentrations. Thus, ammonia concentrations in exhaled breath may be higher than inhaled concentrations. However, the presence of ammonia in exhaled breath is not considered an uncertainty in the RfC.

LITERATURE SEARCH STRATEGY | STUDY SELECTION AND EVALUATION

Literature Search and Screening Strategy

The literature search for ammonia was conducted in six online scientific databases, including PubMed, Toxline, the Toxic Substances Control Act Test Submissions (TSCATS) database, Web of Science (WOS), Health and Environmental Research Online (HERO)⁸, and Toxcenter. The initial search was performed in March 2012 (PubMed, Toxline, TSCATS, HERO, and Toxcenter) and literature search updates were conducted in March 2013 (PubMed, Toxline, TSCATS, HERO, and WOS) and September 2015 (PubMed, Toxline, TSCATS, and WOS). Toxcenter is a database in which titles may be viewed for free after a fee-based search, but full citations and abstracts are purchased. The use of Toxcenter was discontinued in 2013. No unique relevant hits were returned in the 2013 update search of HERO; therefore, this search was not repeated in 2015. The detailed search approach, including the query strings, is presented in Appendix B, Table B-1. This search of online databases identified approximately ~28,000 unique citations (after electronically eliminating duplicates).

The core computerized database searches were supplemented by a review of citations in other national and international health agency documents (see Table B-2). The [ATSDR \(2004\)](#) *Toxicological Profile of Ammonia*⁹ was used to identify toxicokinetic studies for ammonia. A search of online chemical assessment-related websites was performed in 2012 and 2015; links to the websites that were searched are provided in Table B-2. An additional focused search strategy was also employed to obtain studies of cleaning and hospital workers to address a new area of research identified during the 2013 literature search update. This strategy involved a manual reference list review of several seminal studies published in 2012 (see Appendix B, Table B-2). In addition,

⁸HERO is a database of scientific studies and other references used to develop Environmental Protection Agency (EPA) assessments aimed at understanding the health and environmental effects of pollutants and chemicals. It is developed and managed in EPA's Office of Research and Development (ORD) by the National Center for Environmental Assessment (NCEA). The database includes more than 1.6 million scientific references, including articles from the peer-reviewed literature. New studies are added continuously to HERO. For each IRIS assessment, a HERO project page is created that stores all citations identified from that chemical-specific literature search. These citations may be organized using various tags to indicate if the citations are used in the assessment and how they are categorized.

⁹Portions of this Toxicological Review were developed under a Memorandum of Understanding with the Agency for Toxic Substances and Disease Registry (ATSDR) and were adapted from the [ATSDR \(2004\)](#) *Toxicological Profile for Ammonia* and the references cited in that document, as part of a collaborative effort in the development of human health toxicological assessments for the purposes of making more efficient use of available resources and sharing scientific information.

electronic forward searches were conducted in WOS in 2013 and 2015, using a methods paper describing the development of a job exposure matrix focusing on asthma as a health outcome ([Kennedy et al., 2000](#)). The disposition of studies obtained from the manual backward and electronic forward searches is presented in Table B-3.

In Federal Register notices announcing annual Integrated Risk Information System (IRIS) agendas and on the IRIS website, EPA encouraged the public to submit information on IRIS chemicals throughout the assessment development process, and specifically requested that the public submit additional data to support development of the ammonia assessment on December 21, 2007 and November 2, 2009 ([U.S. EPA, 2009a, 2007](#)). No public submissions were received in response to these calls for data.

Figure LS-1 depicts a summary of the literature search and screening process and the number of references included or excluded at each step. In 2012, the initial literature search was conducted in core computerized databases. These citations were electronically screened in an EndNote database using a set of terms intended to prioritize “on-topic” references for title and abstract review. The electronic screening process created two broad categories: one of all citations that contain (in title, abstract, or keywords) at least one inclusion term related to health outcomes, epidemiological or toxicological study design, absorption/distribution/metabolism/excretion (ADME) or toxicokinetics, or mechanistic information (see Appendix B, Table B-4), and one that did not contain any of the terms. Some of the electronic inclusion terms listed in Table B-4 are generic (i.e., not chemical specific) and are intended to capture health effect studies of any type. Other terms are specific to ammonia and are based on previous knowledge of health effects and possible mechanisms of toxicity summarized in other health agency review documents (see Appendix A). Citations that did not contain at least one inclusion term in Table B-4 (i.e., excluded by the electronic screening) were subjected to a quality control check to verify that relevant references were not missed. Specifically, a random sample (approximately 10%) of the electronically excluded citations were subjected to title and abstract review by a toxicologist to confirm that the electronic screening process produced acceptable results (i.e., no relevant citations were inadvertently missed). Relevant items were added to the HERO project page for ammonia and retrieved for full-text review. The results from the updated literature searches performed in March 2013 and September 2015 were not screened electronically in EndNote. All titles and abstracts obtained from these search updates were reviewed manually by a toxicologist.

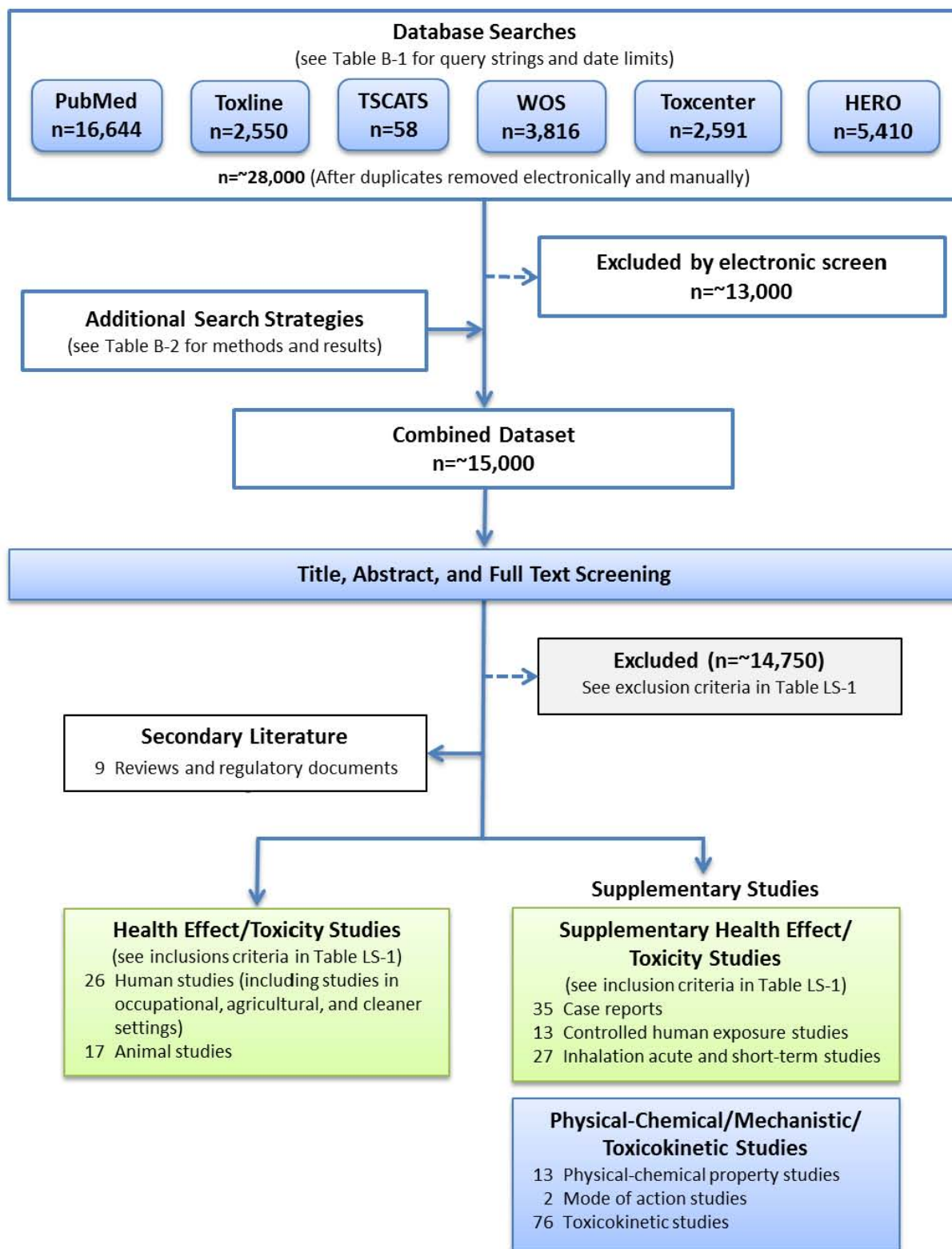


Figure LS-1. Summary of literature search and screening process for ammonia.

Manual screening of titles/abstracts and full text was accomplished using a set of inclusion and exclusion criteria to identify sources of primary human health effects data and sources of primary data that supplement the assessment of ammonia health effects (i.e., bottom boxes in Figure LS-1). The inclusion/exclusion criteria that were used prior to peer review are presented in Table LS-1. Manual screening of the post-peer review literature search update (i.e., September 2015) was performed using more stringent inclusion and exclusion criteria to capture studies that would impact the credibility of the assessment's conclusions consistent with EPA's IRIS Stopping Rules (http://www.epa.gov/sites/production/files/2014-06/documents/iris_stoppingrules.pdf). For ammonia, those references identified in the post-peer review literature search that were considered for inclusion in hazard identification were in vivo animal toxicity and epidemiology studies. No additional in vivo animal toxicity studies of ammonia were identified in the post-peer review search. The disposition of epidemiology studies obtained from the post-peer review literature search update (i.e., September 2015) is provided in Table B-5.

Specific inclusion/exclusion criteria were not applied in identifying sources of mechanistic and toxicokinetic data. Because ammonia is produced endogenously and serum ammonia levels are measured in certain disease states, the toxicokinetics literature is large and complex; relevant toxicokinetic studies for ammonia were initially identified using the [ATSDR \(2004\) Toxicological Profile of Ammonia](#) and supplemented by more recent studies identified in literature search updates. The number of mechanistic studies identified for ammonia was not large, and therefore, all mechanistic studies were included.

Table LS-1. Inclusion/exclusion criteria for inhalation health effect/toxicity studies (pre-peer review)^a

	Inclusion criteria	Exclusion criteria
Population	• Humans, including occupational workers, livestock workers and those in close proximity to agricultural operations, hospital workers/cleaners and volunteers • Standard mammalian animal models, including rat, mouse, hamster, rabbit, guinea pig, monkey, dog • Pigs	• Ecological species/ecosystem effects • Nonmammalian species • Agricultural species/livestock (except pigs)
Exposure	• Exposure is to ammonia by the inhalation route (any duration) • Exposure is measured as a concentration in air • Exposure is in vivo	• Not chemical specific (i.e., not ammonia-specific) • Animal studies: exposure is to a mixture only • Human studies: exposure is inferred but not measured (e.g., some cleaning and hospital worker studies) • Exposure by oral, dermal, injection or instillation routes • Studies of quaternary ammonia

	Inclusion criteria	Exclusion criteria
Outcome	One or more of the following health effect endpoints is evaluated: effects on the cardiovascular, dermal/integumentary, endocrine, gastrointestinal, immune, musculoskeletal, nervous, reproductive, respiratory, hepatic, or renal (urinary) systems; effects on the eyes, survival, growth, or development	- No health outcome evaluated - Pathogenic effects of <i>Helicobacter pylori</i> infection
Other		- Review article or abstract only (i.e., no primary data) - Environmental fate and transport of ammonia - Analytical methods for measuring ammonia in environmental media, and use in sample preparations and assays - Study of physical-chemical properties - Study of in vitro or in vivo toxicokinetics - Study of in vitro or in vivo mechanistic endpoints - Other studies not on topic and not captured by other exclusion criteria

^aReviews and regulatory documents were retained as Secondary Literature. Studies that provided primary information on the physical-chemical properties, mode of action, or toxicokinetics of ammonia were also retained, but were not screened as sources of health effect/toxicity information for ammonia.

The results of the pre- and post-peer review literature screening are described below and graphically in Figure LS-1:

- 43 references (including 26 human studies and 17 animal studies) were identified as studies with health effects data and were considered for data extraction to evidence tables and exposure-response arrays.
- Supplementary health effect/toxicity studies included 35 case reports, 13 acute-duration controlled human exposure studies, and 27 acute or short-term animal studies. Information from these studies was not extracted into evidence tables; however, these studies were considered as supplementary studies for assessing ammonia health effects.
- 91 studies were identified as physical-chemical, mode-of-action, or toxicokinetic studies, including 13 studies of physical-chemical properties, 2 studies providing mode-of-action information, and 76 toxicokinetic studies. Information from these studies was not extracted into evidence tables; however, these studies were considered as supplementary studies for assessing ammonia health effects (e.g., consideration of toxicokinetic information in assessing the health effects literature).

- Nine reviews or regulatory/health assessment documents were identified as secondary literature. These references were retained as additional resources in developing the Toxicological Review.
- More than 27,000 references were identified as not pertinent to an evaluation of the inhalation health effects of ammonia. Approximately 13,000 were excluded by electronic screening (see Table B-4) and approximately 14,750 were excluded by manual screening (see Table LS-1 for exclusion criteria).

Study Selection and Evaluation

Selection of studies for inclusion in the Toxicological Review was based on consideration of the extent to which the study was informative and relevant to the assessment as well as on general study quality considerations. In general, the relevance and scientific quality of the available studies were evaluated as outlined in the Preamble and in EPA guidance (i.e., *A Review of the Reference Dose and Reference Concentration Processes* ([U.S. EPA, 2002](#)) and *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhaled Dosimetry* ([U.S. EPA, 1994](#))). The scientific considerations used to evaluate and select studies and the relevance of these studies to the assessment are described in the section below.

Considerations for Evaluation of Epidemiology Studies

Case reports are often anecdotal and describe unusual or extreme exposure situations, providing little information that would be useful for characterizing chronic health hazards. Ammonia case studies were only briefly reviewed; representative citations from the collection of case reports are provided as supplemental information in Appendix C, Section C.2.4. Similarly, acute controlled human exposure studies would not be useful for characterizing chronic health effects; these studies were therefore briefly reviewed and are provided as supplemental information in Appendix C, Section C.2.3.

Epidemiology studies of chronic exposure to ammonia have primarily focused on industrial worker populations, workers exposed to ammonia as a cleaning or disinfectant product, and those exposed in an agricultural setting. There is considerable variation in population characteristics, level and pattern of exposure, and potential confounders across the three categories of studies. Evaluations of the observational epidemiology studies of industrial worker populations and workers exposed to ammonia as a cleaning or disinfectant product identified in Figure LS-1 (i.e., the studies considered most informative for evaluating ammonia toxicity from chronic exposure) are provided in Appendix B (Tables B-6 to B-8). The process used to evaluate these studies addressed aspects relating to the selection of study participants, exposure parameters, outcome measurement, confounding, and statistical analysis. As discussed below, studies of populations exposed in agricultural settings were considered to be supporting material because of the variety of potential co-exposures in these studies (including dust, endotoxin, mold, and disinfectant products). The process for evaluating studies in an agricultural setting considered the same five aspects (selection

of study participants, exposure parameters, outcome measurement, confounding, and statistical analysis); however, specific study evaluation tables were not provided in Appendix B for this set of studies.

For study evaluation purposes, EPA differentiated between “major” limitations, defined as biases or deficiencies that could materially affect the interpretation of the study, and “minor” limitations, defined as limitations that are not likely to be severe or to have a substantive impact on the results. These categories are similar to the “serious risk of bias” and “moderate risk of bias” categories, respectively, described by [Stearne et al. \(2014\)](#) in the Cochrane Collaborative Assessment Tool for non-randomized studies of clinical interventions. Identification of major limitations in the epidemiology studies of populations exposed in industrial, cleaning, and agricultural settings is included in the broader evaluation of study quality below. Uninformative studies are also noted.

Studies of Industrial Settings

Selection of study participants

All of the studies were cross-sectional analyses in occupational settings. The workers were healthy enough to remain in the work area for a considerable time; with one exception, mean duration ranged from 52 months to 16 years. One study ([Bhat and Ramaswamy, 1993](#)) grouped workers into those exposed for up to 10 years and those with more than 10 years of exposure; a minimum exposure duration was not provided. As an inherent property of occupational studies, these designs may result in a “healthy worker” bias. In addition, the workers in these studies are not representative of the general population, as they do not include children and only one study of ammonia exposure in hair salons included women ([Nemer et al., 2015](#)). These aspects of the study design may result in an underestimate of the risk of health effects of ammonia exposure, as the worker population may not exhibit health effects (such as decreased lung function or increased prevalence of respiratory symptoms) to the same degree that would be seen in the general population under the same conditions. In addition to the “healthy worker” effect, the [Nemer et al. \(2015\)](#) study exhibited a potential selection bias in the controls due to differences in recruitment (self-selected based on interest) or workload.

Exposure parameters

Exposure methods differ across these occupational studies, which makes comparison of ammonia measurements among the studies difficult. Spectrophotometric absorption measures of area samples ([Ali et al., 2001](#); [Ballal et al., 1998](#)) are not directly comparable to direct-reading diffusion methods ([Rahman et al., 2007](#)) or electrochemical sensor methods ([Nemer et al., 2015](#)) used to analyze personal samples. Nor are they comparable to the National Institute for Occupational Safety and Health (NIOSH)-recommended protocol for personal sampling and analysis of airborne contaminants ([Holness et al., 1989](#)). In the study by [Rahman et al. \(2007\)](#), exposure

concentrations were determined by both the Dräger tube and Dräger PAC III methods. The Dräger tube method yielded concentrations of ammonia in the two plants studied that were approximately 4-fold higher than the concentrations obtained by the Dräger PAC III method; a strong correlation between measurements by the two methods was reported. [Rahman et al. \(2007\)](#) stated that their measurements indicated only relative differences in exposures between workers and production areas, and did not identify one analytical measure as the more valid of the two. Based on communication with technical support at Dräger Safety Inc. ([Bacom and Yanosky, 2010](#)), EPA considered the PAC III instrument to be a more sensitive monitoring technology than the Dräger tubes. Ammonia concentrations based on the PAC III method were also in line with concentrations reported in other studies. Therefore, exposure levels based on PAC III air measurements of ammonia were used in the current health assessment to characterize the exposure-response relationship in the [Rahman et al. \(2007\)](#) study.

In the [Abdel Hamid and El-Gazzar \(1996\)](#) study, no direct measurement of ammonia exposure was made; blood urea was used as a surrogate measure of ammonia exposure. The correlation of blood urea with ammonia is not reported by the authors. EPA considered this a major limitation of this study, based on other data indicating no correlation between ammonia levels in air and serum urea levels in a study of six groups of workers with varying types of exposure ([Giroux and Ferrières, 1998](#)). No exposure measurements of ammonia were used in the study by [Bhat and Ramaswamy \(1993\)](#). EPA considered the lack of exposure measure in this study to be a major limitation. In the [Nemer et al. \(2015\)](#) study, the measurement device had limited specificity for measuring ammonia relative to other gases and could therefore have produced false positive results in the presence of other gases. In addition, few exposure measurements were made in the [Nemer et al. \(2015\)](#) study. EPA considered the limited specificity for measuring ammonia, the limited number of exposure measurements, and the possible misclassification of exposure in the [Nemer et al. \(2015\)](#) study to be major limitations.

Outcome measurement

Assessment of respiratory symptoms in [Rahman et al. \(2007\)](#), [Ballal et al. \(1998\)](#), [Holness et al. \(1989\)](#), and [Nemer et al. \(2015\)](#) was based on four different questionnaires; each of these, however, is a standardized, validated questionnaire. Self-reporting of types and severity of respiratory symptoms could be biased by the knowledge of exposure, for example, in studies comparing factory workers to office workers. EPA evaluated this non-blinded outcome assessment as a potential bias. In each of these studies, comparisons were made across exposure categories among the exposed; EPA concluded that the non-blinded outcome assessment as a potential bias is unlikely in these types of comparisons. One study also compared exposed to nonexposed, and observed little differences in symptom prevalence between these groups ([Holness et al., 1989](#)). Thus, EPA concluded that the non-blinded outcome assessment was not a major bias in this analysis either. Assessment of lung function was performed by standard spirometry protocols in five

studies ([Nemer et al., 2015](#); [Rahman et al., 2007](#); [Ali et al., 2001](#); [Bhat and Ramaswamy, 1993](#); [Holness et al., 1989](#)). EPA did not consider any of these procedures for assessing lung function to be a source of bias.

Confounding

Co-exposures to other ambient chemicals in urea fertilizer factories included inorganic gases (nitrogen dioxide and sulfur dioxide) and dust. In one of these studies ([Rahman et al., 2007](#)), nitrogen dioxide was measured concurrently with ammonia and found to be below detection limits for all areas (urea plant, ammonia plant, and administration area). The other urea fertilizer studies ([Ali et al., 2001](#); [Ballal et al., 1998](#); [Abdel Hamid and El-Gazzar, 1996](#)) did not describe potential co-exposures. [It appears from the exposure measurements that the plant in [Ali et al. \(2001\)](#) is “Factory A” in [Ballal et al. \(1998\)](#).] In the fertilizer plant in [Bhat and Ramaswamy \(1993\)](#), co-exposures were not discussed, but the workers were grouped based on different parts of the plant (ammonia, urea, and diammonium phosphate); effects observed with respect to lung function tests were similar in magnitude, albeit slightly stronger, in the ammonia plant workers compared with the urea plant workers. One study was conducted in a soda ash production plant ([Holness et al., 1989](#)). No measurements of co-exposures were described in this study, but the authors noted the high level of control of exposures (resulting in low ammonia levels) in this facility. Because of the lack of demonstration of co-exposures correlated with ammonia levels in these studies, and lack of demonstration of stronger associations between potential co-exposures and respiratory outcomes, EPA concluded that confounding by other workplace exposures, although a potential concern, was unlikely to be a major limitation for the urea plant and soda ash plant studies. However, in a study of ammonia exposure among hairdressers ([Nemer et al., 2015](#)), co-exposures to other workplace contaminants (such as persulfates and paraphenylenediamine) were not measured or controlled for in the analysis; therefore, possible confounding is considered to be a limitation in this study.

The analyses of respiratory symptoms and lung function may also be confounded by smoking. In six studies, analyses accounted for smoking as follows: the analysis included either an adjustment for smoking ([Rahman et al., 2007](#); [Holness et al., 1989](#)), the exclusion of smokers ([Nemer et al., 2015](#); [Bhat and Ramaswamy, 1993](#)), or stratification of the results by smoking status ([Ali et al., 2001](#); [Ballal et al., 1998](#)). Thus, EPA did not consider potential confounding by smoking to be a major limitation of these studies.

Ammonia is present in both tobacco and cigarette smoke ([Callicutt et al., 2006](#)). Typical concentrations of ammonia in commercial U.S. tobacco blends range from 0.02 to 0.4% ([Seeman and Carchman, 2008](#)). Thus, there is some potential for additional exposure to ammonia associated with use of ammonia-containing tobacco products and/or inhalation of tobacco smoke. This finding reinforces the importance of controlling for smoking in the analyses of the respiratory symptoms and lung function. EPA did not consider potential confounding by smoking of ammonia-containing tobacco or by inhaling tobacco smoke to be a major limitation of these studies because smoking as a

potential confounder was adequately addressed in the studies that examined effects on the respiratory system.

Information on smoking habits and use of alcohol (an exposure potentially affecting liver function tests) was not documented in the study of liver function by [Abdel Hamid and El-Gazzar \(1996\)](#). The lack of information and potential failure to control for these confounders is considered a major limitation.

Statistical analysis

EPA considered the statistical analysis in the epidemiological studies ([Nemer et al., 2015](#); [Rahman et al., 2007](#); [Ali et al., 2001](#); [Ballal et al., 1998](#); [Abdel Hamid and El-Gazzar, 1996](#); [Bhat and Ramaswamy, 1993](#); [Holness et al., 1989](#)) to be adequate and appropriate. Although the type of statistical testing was not specified in [Abdel Hamid and El-Gazzar \(1996\)](#), the results were presented in sufficient detail to allow interpretation of the data and analysis. Sample size, an important consideration with respect to statistical power, was also considered. EPA noted the small number of exposed workers and low levels of exposure in the study by [Holness et al. \(1989\)](#) as limitations that could result in “false negative” results (i.e., a test result indicating a lack of association, whereas a positive association between exposure and a health effect exists).

Identification of uninformative studies

The study by [Abdel Hamid and El-Gazzar \(1996\)](#) was determined to have major limitations. Air concentrations of ammonia were not directly measured, and the use of blood urea has not been established as a reliable surrogate of ammonia exposure. Further, the lack of information on smoking and alcohol use, factors that could affect liver function, in a study intended to examine the association between liver function and ammonia exposure, was considered a significant flaw. Therefore, [Abdel Hamid and El-Gazzar \(1996\)](#) was not further considered in this assessment.

Major limitations were also identified in the [Nemer et al. \(2015\)](#) study: potential selection bias in the control group due to differences in recruitment (self-selected based on interest in the study) or workload; limited specificity of the analytical method used to measure ammonia (i.e., potential for false positives from other gases); and failure to control for confounders. In addition, the study used small sample sizes and only a single measurement of ammonia for each location (which may not have been representative of workplace exposures). Therefore, the [Nemer et al. \(2015\)](#) study was deemed to be uninformative and was not further considered in this assessment.

Studies of Health Care and Cleaning Settings

Selection of study participants

EPA evaluated the studies that examined exposure to ammonia when used as a cleaning or disinfectant product. EPA noted the potential for the “healthy worker” bias arising from movement out of jobs by affected individuals in most of these studies ([Le Moual et al., 2008](#)). This issue was

less of a concern in the study by [Zock et al. \(2007\)](#), which was conducted in a general (non-occupational) population sample, focusing on cleaning activities in the home. In a birth cohort that evaluated the association between exposure to cleaning products and children's respiratory health ([Casas et al., 2013](#)), 35% of the recruited population was excluded because information on the use of cleaning products and/or respiratory tests was not available, representing a potential study limitation. However, the authors of this study noted that the children included were not different from those excluded regarding most study characteristics (sex, atopy, asthma, parental asthma, and parental smoking).

Exposure parameters

None of these studies used a direct measure of ammonia exposure in the analysis, precluding interpretation of the results in relation to an absolute level of exposure. The limited data available concerning exposure levels in cleaning scenarios found median exposures of 0.6–5.4 ppm (0.4–3.8 mg/m³), with peaks exceeding 50 ppm (35 mg/m³), in a small study (n = 9) using personal samples during a domestic cleaning session ([Medina-Ramón et al., 2005](#)). Although an absolute level of exposure is not available, the relative ranking of exposure used in these studies does allow examination of risk by relative levels of exposure.

Key considerations regarding the validity of the exposure measures are the specificity of the classification and the extent to which classification could be influenced by knowledge of the disease or symptoms under study. Methodological research has reported underestimation of self-reported exposure to specific products by health care workers, and differential reporting by disease status (i.e., asthma) for self-reported use of cleaning products in patient care, but not in instrument cleaning or building materials ([Donnay et al., 2011](#); [Delclos et al., 2009](#); [Kennedy et al., 2000](#)). Two of these studies used an exposure assessment protocol that incorporated an independent, expert review, blinded to disease status ([Dumas et al., 2012](#); [Lemiere et al., 2012](#)); one study collected exposure information using a 2-week daily diary ([Medina-Ramón et al., 2006](#)) and one study developed a composite exposure score based on an interviewer-led questionnaire concerned with the frequency of use and number of products used ([Casas et al., 2013](#)). EPA considered these two studies to be the strongest of the exposure protocols used within this set of studies.

Outcome measures

Six of the studies in this set used standard protocols for the assessment of respiratory symptoms in epidemiological studies ([Casas et al., 2013](#); [Arif and Delclos, 2012](#); [Dumas et al., 2012](#); [Vizcaya et al., 2011](#); [Zock et al., 2007](#); [Medina-Ramón et al., 2005](#)), and one study included a clinical assessment protocol designed specifically for the assessment of occupational asthma ([Lemiere et al., 2012](#)). Details of the specific questions were provided, and EPA did not consider any of these methods to be a limitation in terms of specificity of the outcome. The study by [Medina-Ramón et al. \(2006\)](#) collected information on daily respiratory symptoms in a 2-week diary, and also trained the

participants to measure peak expiratory flow 3 times daily. A potential limitation in the [Casas et al. \(2013\)](#) study was the lack of information about the reliability of the pulmonary function measures.

Confounding

All of these studies addressed the potential for smoking to act as a confounder in the analysis. Two of the studies reported relatively weak correlations between ammonia and other products assessed ([Zock et al., 2007](#); [Medina-Ramón et al., 2005](#)) and one study reported stronger associations with ammonia than with bleach ([Dumas et al., 2012](#)). Based on this information, EPA did not consider potential confounding to be a major limitation of this set of studies.

Statistical analysis

EPA considered the statistical analysis in this set of studies to be appropriate. One study, however, was limited in terms of the level of detail provided pertaining to the results for ammonia from multivariate models ([Medina-Ramón et al., 2005](#)).

Studies of Agricultural Settings

Selection of study participants

EPA evaluated a set of studies conducted among livestock farmers and one study of asthmatic children in close proximity to animal feeding operations ([Loftus et al., 2015](#)). As with the other occupational studies discussed above, the selection of sensitive individuals out of the workforce (“healthy worker bias”) would be a potential bias in cross-sectional studies of livestock farmers.

Exposure parameters

Among the studies examining pulmonary function, one study collected 24-hour air sampling from 14 ammonia monitoring devices located outside the home of a subset of the participants every 6 days for at least 3 months during the air monitoring period ([Loftus et al., 2015](#)), two studies used area-based exposure sampling in animal confinement buildings ([Monsó et al., 2004](#); [Zejda et al., 1994](#)), one study used area samples taken in conjunction with specific tasks and calculated a personal exposure measure taking into account duration spent in specific locations and tasks ([Heederik et al., 1990](#)), four studies collected personal samples over a work shift ([Donham et al., 2000](#); [Reynolds et al., 1996](#); [Preller et al., 1995](#)) or an unspecified time period ([Donham et al., 1995](#)), and two studies used colorimetric tubes, which are generally less precise, to measure ammonia exposure ([Monsó et al., 2004](#); [Zejda et al., 1994](#)). EPA considered the use of the area-based samples without consideration of exposure duration to be limitations of the studies by [Zejda et al. \(1994\)](#) and [Monsó et al. \(2004\)](#).

Outcome measures

All of the studies reported using a standard spirometric technique; one study ([Loftus et al., 2015](#)) used twice daily home lung function measurements taken by the test subject; four studies compared two measures per individual (i.e., pre- and post-shift) ([Monsó et al., 2004](#); [Donham et al., 2000](#); [Reynolds et al., 1996](#); [Heederik et al., 1990](#)); and two studies used a single pulmonary function measure, adjusted for height, age, and smoking variables ([Preller et al., 1995](#); [Zeida et al., 1994](#)). EPA did not consider any of these outcome measures to be limitations in these studies, although the self-administered spirometry testing in the [Loftus et al. \(2015\)](#) study is a potential limitation.

Confounding

Six of these studies addressed confounding in some way. Four studies controlled for co-exposures (e.g., endotoxin, dust, disinfectants) ([Melbostad and Eduard, 2001](#); [Reynolds et al., 1996](#); [Donham et al., 1995](#); [Preller et al., 1995](#)), one study noted only weak correlations (i.e., Spearman $r < 0.20$) between ammonia and dust or endotoxin ([Donham et al., 2000](#)), and one study observed associations with ammonia but not with endotoxin or dust measures ([Heederik et al., 1990](#)). Three studies did not address confounding ([Loftus et al., 2015](#); [Monsó et al., 2004](#); [Zeida et al., 1994](#)).

Based on these considerations, EPA considered the studies by [Reynolds et al. \(1996\)](#), [Preller et al. \(1995\)](#), [Donham et al. \(2000\)](#), [Donham et al. \(1995\)](#), and [Heederik et al. \(1990\)](#) to be the methodologically strongest studies of this set.

Based on the evaluation of the epidemiology studies of ammonia in terms of selection of study participants, exposure parameters, outcome measurement, confounding, and statistical analysis, the studies listed in Table LS-2 were selected for data extraction into evidence tables in Chapter 1.

Table LS-2. Summary of epidemiology database

Study setting	Reference
Industrial	Rahman et al. (2007) Ali et al. (2001) Ballal et al. (1998) Bhat and Ramaswamy (1993) Holness et al. (1989)
Cleaning	Casas et al. (2013) Arif and Delclos (2012) Dumas et al. (2012) Lemiere et al. (2012) Vizcaya et al. (2011) Zock et al. (2007) Medina-Ramón et al. (2006) Medina-Ramón et al. (2005)

Study setting	Reference
Agricultural	Loftus et al. (2015) Monsó et al. (2004) Melbostad and Eduard (2001) Donham et al. (2000) Reynolds et al. (1996) Donham et al. (1995) Preller et al. (1995) Choudat et al. (1994) Zeida et al. (1994) Crook et al. (1991) Heederik et al. (1990)

Considerations for Evaluation of Animal Studies

Repeat-exposure toxicity studies of ammonia in experimental animals were evaluated using the study quality considerations outlined in the Preamble and discussed in various EPA guidance documents ([U.S. EPA, 2005a](#), [2002](#), [1994](#)), including consideration of aspects of design, conduct, or reporting that could affect the interpretation of results, overall contribution to the synthesis of evidence, and determination of hazard potential. The objective was to identify the stronger, more informative studies based on a uniform evaluation of quality characteristics across studies of similar design.

Additionally, a number of general questions, presented in Table LS-3, were considered in evaluating the animal studies. Much of the key information for conducting this evaluation can be determined based on study methods and how the study results were reported.

Table LS-3. Considerations and relevant experimental information for evaluation of experimental animal studies

Methodological feature	Considerations (relevant information extracted into evidence tables)
Test animal	Suitability of the species, strain, sex, and source of the test animals
Experimental design	Suitability of animal age/lifestage at exposure and endpoint testing; periodicity and duration of exposure (e.g., hrs/d, d/wk); timing of endpoint evaluations; sample size and experimental unit (e.g., animals, dams, litters)
Exposure	Characterization of test article source, composition, purity, and stability; suitability of the control (e.g., vehicle control); documentation of exposure techniques (e.g., chamber type); verification of exposure levels (e.g., consideration of homogeneity, stability, analytical methods)
Endpoint evaluation	Suitability of specific methods for assessing the endpoint(s) of interest
Results presentation	Data presentation for endpoint(s) of interest (including measures of variability) and for other relevant endpoints needed for results interpretation (e.g., decrements in body weight in relation to organ weight)

Information relevant to study evaluation is reported in evidence tables and was considered in the synthesis of evidence. Discussions of study strengths and limitations (that ultimately supported preferences for the studies and data relied upon) were included in the text where relevant. The general findings of this evaluation are presented in the remainder of this section. Study evaluation considerations that are outcome specific are discussed in the relevant hazard section in Section 1.2.

Test animal

The ammonia database consists of toxicology studies conducted in rats (F344, Sprague-Dawley, Long-Evans, Sherman, Wistar), mice (OF1, Swiss albino), New Zealand White rabbits, guinea pigs (Princeton-derived, Hartley), beagle dogs, squirrel monkeys, and pigs (several strains). The species and strains of animals used are consistent with those typically used in laboratory studies, and all were considered relevant to assessing the potential human health effects of ammonia. The species, strain, and sex of the animals used in the experimental studies were recorded in the evidence tables. The [Anderson et al. \(1964\)](#) and [Weatherby \(1952\)](#) guinea pig studies provided no information on the strain of the test animal; this is considered a minor limitation of these studies.

Experimental design

General aspects of study design and experimental design were evaluated to determine if they were appropriate for evaluation of specific endpoints. Key features of the experimental design, including the periodicity and duration of exposure and sample sizes, were summarized in the evidence tables in Chapter 1.

A single exposure group was used in a number of the general toxicity studies ([Gaafar et al., 1992](#); [Broderson et al., 1976](#); [Doig and Willoughby, 1971](#); [Anderson et al., 1964](#); [Weatherby, 1952](#)), and in about half of the studies that examined immune endpoints ([Hamilton et al., 1999](#); [Hamilton et al., 1998](#); [Schoeb et al., 1982](#); [Richard et al., 1978a](#)). Use of a single exposure group limits the extent to which conclusions about a dose-response relationship can be drawn.

Sample size was not a basis for excluding a study from consideration, as studies with small numbers of animals can still inform the consistency of effects observed for a specific endpoint. Nevertheless, the following studies with small sample sizes were considered relatively less informative: [Anderson et al. \(1964\)](#) studies in the mouse (four animals/exposure interval) and guinea pigs (two animals/exposure interval); the [Weatherby \(1952\)](#) study in guinea pigs (two control and four exposed animals/exposure interval); and the [Coon et al. \(1970\)](#) studies in the rabbit (three animals/group), monkey (three animals/group), and dog (two animals/group).

Exposure

Because inhalation toxicity studies can be technically difficult to perform, particular attention was paid to each study's exposure methods and documentation for assurance that the

animals were properly exposed to gaseous ammonia. Exposure evaluation focused on those studies that reported effects on the respiratory system. Of the studies evaluated for exposure quality, six provided information on generation method, analytical method used to measure ammonia concentrations, analytical chamber concentrations, and chamber type; exposure characterization for these studies was considered robust ([Done et al., 2005](#); [Diekman et al., 1993](#); [Broderson et al., 1976](#); [Doig and Willoughby, 1971](#); [Coon et al., 1970](#); [Stombaugh et al., 1969](#)). Studies by [Anderson et al. \(1964\)](#) and [Curtis et al. \(1975\)](#) failed to report analytical chamber concentrations, but exposures were otherwise considered to be adequately characterized. Exposure characterization in two studies ([Gaafar et al., 1992](#); [Weatherby, 1952](#)) was considered poor because the studies failed to report analytical chamber concentrations, analytical method, and type of inhalation chamber used. One of these two studies ([Gaafar et al., 1992](#)) also failed to describe how gaseous ammonia was generated from a 12% “ammonia solution.”

Endpoint evaluation

Respiratory system and other noncancer effects were largely evaluated based on clinical signs (in the case of respiratory system effects) and histopathologic examination. All studies identified the tissues taken for histopathologic examination; however, the extent to which histopathologic methods were described varied across studies. Because histopathology is considered a relatively routine measure, limited reporting of methodologic details was not considered a significant study deficiency.

Essentially all studies examined tissues from the lung and approximately half of the studies examined upper respiratory tissues. This is a concern because the highest exposure would have been to the upper respiratory tract due to the fact that ammonia is both water soluble and highly reactive. [Gaafar et al. \(1992\)](#) examined only the nasal mucosa. Tissues from other organs remote from the point of entry were inconsistently examined. [Coon et al. \(1970\)](#) examined sections from the heart, lung, liver, kidney, and spleen from all surviving monkeys, dogs, and rabbits, but from approximately half of the surviving guinea pigs and rats only; this incomplete histopathological investigation of guinea pigs and rats is considered a limitation. [Anderson et al. \(1964\)](#) examined only the liver and spleen from exposed mice and guinea pigs. [Broderson et al. \(1976\)](#) examined sections from the liver, kidney, adrenal gland, pancreas, testicle, spleen, mediastinal nodes, and thymus. [Curtis et al. \(1975\)](#) noted that “visceral organs” were taken at necropsy for subsequent histopathologic examination, but provided no further details. [Weatherby \(1952\)](#) examined the heart, liver, stomach, small intestines, spleen, kidney, and suprarenal gland, but only reported limited incidence and severity information for the exposed and control guinea pigs. The extent of histopathological examination of the tissues was taken into consideration in evaluating animal findings.

Methodological considerations related to immune-specific endpoints are discussed in Section 1.2.2.

Results presentation

The majority of studies reported only limited qualitative results. With the exception of [Broderon et al. \(1976\)](#), none provided information on the incidence of histopathologic lesions.

In summary, relatively few repeat-dose toxicity studies of inhaled ammonia in experimental animals are available. The majority of these studies come from the older toxicological literature and were generally limited in terms of study design (e.g., small group sizes), documentation of methods, and reporting of results. Nevertheless, no study was considered sufficiently flawed as to be uninformative. Therefore, all in vivo animal toxicity studies, as listed in Table LS-4, were considered in hazard identification and data extraction to evidence tables.

Table LS-4. Summary of experimental animal database

Reference and study description (duration, route, species/strain)
Done et al. (2005) : 5-wk inhalation study in pigs (several breeds) Andreassen et al. (2000) : 63-d inhalation study in Landrace X large white pigs Hamilton et al. (1999) : 4-wk inhalation study in large white pigs Hamilton et al. (1998) : 14-d inhalation study in large white pigs Diekmann et al. (1993) : 6-wk inhalation study in crossbred gilts (female pigs) Gaafar et al. (1992) : 8-wk inhalation study in white albino mice Gustin et al. (1994) : 6-d inhalation study in pigs Manninen and Savolainen (1989) : 5-d inhalation study in Wistar rats ^a Manninen et al. (1988) : 15-d inhalation study in Wistar rats ^a Neumann et al. (1987a) : 35-d inhalation study in unweaned piglets Targowski et al. (1984) : 3-wk inhalation study in Hartley guinea pigs Schaerdel et al. (1983) : 24-hr inhalation study in CrI:COBS CD(SD) rats ^a Schoeb et al. (1982) : 35-d study in F344 rats Richard et al. (1978a) : 7-d study in OF1 mice Broderon et al. (1976) : 35–75-d inhalation studies in Sherman rats and F344 rats Curtis et al. (1975) : 109-d inhalation study in crossbred pigs Doig and Willoughby (1971) : 6-wk inhalation study in Yorkshire-Landrace pigs Coon et al. (1970) : 42–90-d inhalation studies in Sprague-Dawley and Long-Evans rats, New Zealand albino rabbits, Princeton-derived guinea pigs, squirrel monkeys, and beagle dogs Stombaugh et al. (1969) : 5-wk inhalation study in Duroc pigs Anderson et al. (1964) : 7–42-d inhalation studies in Swiss albino mice and guinea pigs (strain not specified) Weatherby (1952) : 6–18-wk inhalation study in guinea pigs (strain not provided)

^aThese studies were not identified as health effect/toxicity studies in Figure LS-1, but were included in Table 1-6 (evidence pertaining to other system effects in animals) as studies that provided useful quantitative information on the biochemical/metabolic effects of ammonia.

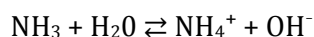
The references considered and cited in this document, including bibliographic information and abstracts, can be found on the HERO website (<http://hero.epa.gov/ammonia>).

1. HAZARD IDENTIFICATION

1.1. OVERVIEW OF CHEMICAL PROPERTIES AND TOXICOKINETICS

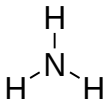
1.1.1. Chemical Properties

Ammonia (NH₃) is a colorless alkaline gas with a pungent odor. Ammonia is very soluble in water ([NRC, 2008](#)); in solution, it exists as ammonium hydroxide. Ammonium hydroxide is a weak base that is partially ionized in water according to the following equilibrium ([ATSDR, 2004](#)):



Ammonium hydroxide ionizes with a dissociation constant of 1.77×10^{-5} at 25°C that increases slightly with increasing temperature ([Read, 1982](#)). A decrease in pH results in an increase in the concentration of ammonium ion (NH₄⁺ or protonated form), a decrease in the concentration of the un-ionized form (NH₃), and an increase in solubility of ammonia in water. At pH 9.25, half of the ammonia will be ionized (NH₄⁺) and half will be un-ionized (NH₃). At pH values of 8.25 and 7.25, 90% and 99%, respectively, of ammonia will be ionized (NH₄⁺) ([ATSDR, 2004](#)). Thus, at physiological pH (7.4), the equilibrium between NH₃ and NH₄⁺ favors the formation of NH₄⁺. Chemical and physical properties of ammonia are listed in Table 1-1.

Table 1-1. Chemical and physical properties of ammonia

Parameter	Value	Reference
Chemical name	Ammonia ^a	
Synonym(s)	AM-Fol; anhydrous ammonia; ammonia gas; Nitro-sil; R 717; Spirit of hartshorn	NLM (2012)
Structure		NLM (2012)
Chemical formula	NH ₃	NLM (2012)
CASRN	7664-41-7a	NLM (2012)
Molecular weight	17.031	Lide (2008), pp. 4.46-4.48, 8.40
Form	Colorless gas; corrosive	O'Neil et al. (2006)
Melting point	-77.73°C	Lide (2008), pp. 4.46-4.48, 8.40
Boiling point	-33.33°C	Lide (2008), pp. 4.46-4.48, 8.40

Parameter	Value	Reference
Odor threshold	53 ppm (37 mg/m ³) 2.6 ppm (2 mg/m ³)	O'Neil et al. (2006) Smeets et al. (2007)
Density	0.7714 g/L at 25°C	O'Neil et al. (2006)
Vapor density	0.5967 (air = 1)	O'Neil et al. (2006)
pKa (ammonium ion)	9.25	Lide (2008), pp. 4.46-4.48, 8.40
Solubility: Water Organic solvents	4.82 × 10 ⁵ mg/L at 24°C Soluble in ethanol, chloroform, and ether	Lange and Dean (1985), pp. 10-3, 10-23; Lide (2008), pp. 4.46-4.48, 8.40; O'Neil et al. (2006)
Vapor pressure	7.51 × 10 ³ mm Hg at 25°C	AIChE (1999)
Henry's law constant	1.61 × 10 ⁻⁵ atm·m ³ /mol at 25°C	Betterton (1992)
Conversion factors ppm to mg/m ³ mg/m ³ to ppm	1 ppm = 0.707 mg/m ³ 1 mg/m ³ = 1.414 ppm	Verschuere (2001)

^aAmmonia dissolved in water is sometimes referred to as ammonium hydroxide (CASRN 1336-21-6). Ammonium hydroxide does not exist outside of solution.

1.1.2. Toxicokinetics

Ammonia is absorbed by the inhalation route of exposure. Most inhaled ammonia is retained in the upper respiratory tract and is subsequently eliminated in expired air. Ammonia (as NH₄⁺) is produced endogenously in the human intestines through the use of amino acids as an energy source (glutamine deamination) and by bacterial degradation of nitrogenous compounds from ingested food. At physiological pH, 98.3% of ammonia is present in the blood as the ammonium ion (NH₄⁺). Given its importance in amino acid metabolism, the urea cycle, and acid-base balance, ammonia is homeostatically regulated to remain at low concentrations in the blood. Ammonia is normally present in circulation at all lifestages (with fetal blood ammonia concentrations higher than maternal concentrations) and in human breast milk as one of the sources of nonprotein nitrogen. Ammonia is produced endogenously by catabolism of amino acids by glutamate dehydrogenase or glutaminase primarily in the liver, renal cortex, and intestines, but also in the brain and heart. Ammonia is metabolized to glutamine via glutamine synthetase in the glutamine cycle or incorporated into urea as part of the urea cycle. The liver removes an amount of ammonia from circulation equal to the amount added by the intestines at metabolic steady state, such that the gut does not contribute significantly to systemic ammonia release under normal conditions. Renal elimination via the kidney is a major contributor to ammonia homeostasis; however, the kidneys are themselves a source of systemic ammonia. The principal means of excretion of ammonia is as urinary urea; lesser amounts are eliminated in the feces, through sweat production, and in expired air. A more detailed summary of ammonia toxicokinetics is provided in Appendix C, Section C.1.

1.2. SYNTHESIS OF EVIDENCE

Section 1.2 provides a synthesis and evaluation of the literature on the health effects of inhaled ammonia in humans and experimental animals organized by organ/system. Evidence for ammonia health effects is also summarized both in organ/system-specific evidence tables, which present key study design information and results, and in exposure-response arrays. More detailed study design information and results are provided in individual study summaries in Appendix C in the Supplemental Information.

1.2.1. Respiratory Effects

The respiratory system is the primary target of toxicity of inhaled ammonia in humans and experimental animals. Five cross-sectional occupational epidemiology studies in industrial settings ([Rahman et al., 2007](#); [Ali et al., 2001](#); [Ballal et al., 1998](#); [Bhat and Ramaswamy, 1993](#); [Holness et al., 1989](#)) examined the association between inhaled ammonia and prevalence of respiratory symptoms or changes in lung function (Table 1-2). Another set of studies examined pulmonary function or asthma symptoms in relation to ammonia exposure in health care workers and domestic cleaners ([Arif and Delclos, 2012](#); [Dumas et al., 2012](#); [Lemiere et al., 2012](#); [Vizcaya et al., 2011](#); [Zock et al., 2007](#); [Medina-Ramón et al., 2006](#); [Medina-Ramón et al., 2005](#)) (Table 1-3). The association between ammonia exposure and respiratory effects indicated by these studies is also informed by studies of pulmonary function in individuals in agricultural settings and subchronic inhalation toxicity studies in various experimental animal species (Table 1-4). The evidence of respiratory effects in humans and experimental animals exposed to ammonia is summarized in an exposure-response array in Figure 1-1 at the end of this section.

Respiratory Symptoms

Respiratory symptoms (including cough, wheezing, and other asthma-related symptoms) were reported in two cross-sectional studies of industrial worker populations exposed to ammonia at levels greater than or equal to approximately 18 mg/m³ ([Rahman et al., 2007](#); [Ballal et al., 1998](#)) (Table 1-2). One of these studies also examined frequency of respiratory symptoms by cumulative ammonia concentration (CAC, mg/m³-years) and observed significantly higher relative risks (2.4–5.3) with a higher CAC (>50 mg/m³-years) compared to those with a lower CAC (≤50 mg/m³-years) ([Ballal et al., 1998](#)). In three studies examining lower exposure settings ([Rahman et al., 2007](#); [Ballal et al., 1998](#); [Holness et al., 1989](#)) (Table 1-2), no differences were observed in the prevalence of respiratory symptoms between ammonia-exposed workers and controls. Ammonia concentrations reported in these lower exposure settings included a mean ammonia concentration of <6.5 mg/m³ and a high-exposure group defined as >8.8 mg/m³ in [Holness et al. \(1989\)](#), an exposure range of 0.2–7 mg/m³ in “Factory B” of [Ballal et al. \(1998\)](#), and a

mean concentration of 4.9 mg/m³ in [Rahman et al. \(2007\)](#). The primary limitation noted in all of these studies was the potential under-ascertainment of effects inherent in the study of a long-term worker population (i.e., “healthy worker” effect) (see Literature Search Strategy | Study Selection and Evaluation section and Table B-6 in the Supplemental Information). Confounding by other workplace exposures, although a potential concern, was unlikely to be a major limitation affecting the interpretation of the pattern of results seen in these studies, given the lack of nitrogen dioxide measurements above the detection limit in one study ([Rahman et al., 2007](#)) and the high level of control of exposures in another study ([Holness et al., 1989](#)).

Studies of health care workers or hospital workers ([Arif and Delclos, 2012](#); [Dumas et al., 2012](#)) (Table 1-3) provide evidence that exposure to ammonia as a cleaning or disinfectant product is associated with increased risk of asthma or asthma symptoms. Use of ammonia as a cleaning product in other settings has also been associated with asthma and respiratory symptoms ([Casas et al., 2013](#); [Vizcaya et al., 2011](#); [Zock et al., 2007](#); [Medina-Ramón et al., 2005](#)) (Table 1-3). Occupational exposure to ammonia was associated with work-exacerbated asthma (compared to nonwork-related asthma) in a study at two occupational asthma specialty clinics by [Lemiere et al. \(2012\)](#) (Table 1-3). Six studies, from Europe, Canada, and the United States, observed elevated odds ratios, generally between 1.5 and 2.0, with varying degrees of precision. These studies were conducted using a variety of designs, including a prospective study ([Zock et al., 2007](#)) and two nested case-control studies ([Medina-Ramón et al., 2006](#); [Medina-Ramón et al., 2005](#)). Criteria used to define current asthma or asthma symptoms were generally well defined and based on validated methods. A major limitation of this collection of studies is the lack of direct measures of ammonia exposure. Two of the studies included expert assessment of exposure (blinded to case status); expert assessment improves reliance on self-reported exposure ([Dumas et al., 2012](#); [Lemiere et al., 2012](#)). Confounding by other cleaning products is an unlikely explanation for these results, as two of the studies noted only weak correlations between ammonia and other product use ([Zock et al., 2007](#); [Medina-Ramón et al., 2005](#)), and another study observed stronger associations with ammonia than with bleach ([Dumas et al., 2012](#)). All of the studies addressed smoking as a potential confounder.

Studies in populations exposed in agricultural settings, including swine and dairy farmers, that analyzed for prevalence of respiratory symptoms (including cough, phlegm, wheezing, chest tightness, and eye, nasal, and throat irritation) in relation to ammonia exposure provided generally negative results ([Loftus et al., 2015](#); [Melbostad and Eduard, 2001](#); [Preller et al., 1995](#); [Zeida et al., 1994](#)) (Appendix C, Table C-7). Two other studies that measured ammonia, but did not present an analysis in relation to variability in ammonia levels, reported an increased prevalence of respiratory symptoms in pig farmers exposed to ammonia from animal waste ([Choudat et al., 1994](#); [Crook et al., 1991](#)) (Appendix C, Table C-8). With the exception of the [Loftus et al. \(2015\)](#) study, all studies involving exposure in agricultural settings documented exposures to compounds in

addition to ammonia, such as airborne dust, endotoxin, mold, and disinfectants; [Loftus et al. \(2015\)](#) did not analyze for other contaminants.

Reports of irritation and hyperventilation in volunteers acutely exposed to ammonia at concentrations ranging from 11 to 354 mg/m³ ammonia for durations up to 4 hours under controlled exposure conditions ([Petrova et al., 2008](#); [Smeets et al., 2007](#); [Ihrig et al., 2006](#); [Verberk, 1977](#); [Silverman et al., 1949](#)) provide support for ammonia as a respiratory irritant (Appendix C, Section C.2.3 and Table C-9). Two controlled-exposure studies provide some evidence of habituation to eye, nose, and throat irritation in volunteers after repeated ammonia exposure. Following exposure to ammonia at concentrations ranging from 7 to 35 mg/m³ for 4 hours/day on 5 consecutive days, [Ihrig et al. \(2006\)](#) reported higher mean intensities for irritative, olfactory, and respiratory symptoms in male volunteers unfamiliar with ammonia when compared to male chemical company workers exposed to ammonia vapor for several years in a urea department. Differences were statistically significant only for olfactory symptoms; however, the sample size was small. In a more limited study with only four male volunteers each exposed to 18, 35, or 71 mg/m³ ammonia (exposure to each concentration was for 1 week, 2–6 hours/day, 5 days/week), fewer occurrences of irritation occurred upon the second weekly exposure to the same concentration ([Ferguson et al., 1977](#)).

Numerous case reports document the acute respiratory effects of inhaled ammonia, ranging from mild symptoms (including nasal and throat irritation and perceived tightness in the throat) to moderate effects (including pharyngitis, tachycardia, dyspnea, rapid and shallow breathing, cyanosis, transient bronchospasm, and rhonchi in the lungs) to severe effects (including burns of the nasal passages, soft palate, posterior pharyngeal wall, and larynx; upper airway obstruction; bronchospasm; persistent, productive cough; bilateral diffuse rales and rhonchi; mucous production; pulmonary edema; marked hypoxemia; and necrosis of the lung) (Appendix C, Section C.2.3).

Experimental studies in laboratory animals also provide consistent evidence that repeated exposure to ammonia can affect the respiratory system (Table 1-4 and Appendix C, Section C.3). The majority of available animal studies did not look at measures of respiratory irritation, in contrast to the majority of human studies, but rather examined histopathological changes of respiratory tract tissues. Histopathological changes in the nasal passages were observed in Sherman rats after 75 days of exposure to 106 mg/m³ ammonia and in F344 rats after 35 days of exposure to 177 mg/m³ ammonia, with respiratory and nasal epithelium thicknesses increased 3–4 times that of normal ([Broderson et al., 1976](#)). Thickening of nasal and tracheal epithelium (50–100%) was also observed in pigs exposed to 71 mg/m³ ammonia continuously for 1–6 weeks ([Doig and Willoughby, 1971](#)). Nonspecific inflammatory changes (not further described) were reported in the lungs of Sprague-Dawley and Long-Evans rats and guinea pigs intermittently exposed to 770 mg/m³ ammonia for 6 weeks; continuous exposure to 455 and 470 mg/m³ ammonia increased mortality in rats ([Coon et al., 1970](#)). Focal or diffuse interstitial pneumonitis

was observed in all Princeton-derived guinea pigs, New Zealand White rabbits, beagle dogs, and squirrel monkeys exposed to 470 mg/m³ ammonia ([Coon et al., 1970](#)). Additionally, under these exposure conditions, dogs exhibited nasal discharge and other signs of irritation (marked eye irritation, heavy lacrimation). Nasal discharge was observed in 25% of rats exposed to 262 mg/m³ ammonia for 90 days ([Coon et al., 1970](#)).

At lower concentrations, approximately 50 mg/m³ and below, the majority of studies of inhaled ammonia did not identify respiratory effects in laboratory animals exposed to ammonia. No increase in the incidence of respiratory or other diseases common to young pigs was observed after continuous exposure to ammonia and inhalable dust at concentrations representative of those found in commercial pig farms (≤ 26 mg/m³ ammonia) for 5 weeks ([Done et al., 2005](#)). No gross or histopathological changes in the turbinates, trachea, or lungs of pigs were observed after continuous exposure to 35 or 53 mg/m³ ammonia for up to 109 days ([Curtis et al., 1975](#)). No signs of toxicity in rats or dogs were observed after continuous exposure to 40 mg/m³ ammonia for 114 days or after intermittent exposure (8 hours/day) to 155 mg/m³ ammonia for 6 weeks ([Coon et al., 1970](#)). Only one study reported respiratory effects at concentrations < 50 mg/m³ (i.e., lung congestion, edema, and hemorrhage in guinea pigs and mice exposed to 14 mg/m³ ammonia for up to 42 days; [Anderson et al. \(1964\)](#)), but confidence in the findings from this study is limited by inadequate reporting and the small numbers of animals tested.

Lung Function

Decreased lung function in ammonia-exposed workers has been reported in three of the four studies examining this outcome measure ([Rahman et al., 2007](#); [Ali et al., 2001](#); [Bhat and Ramaswamy, 1993](#)); the exception is the study by [Holness et al. \(1989\)](#) in which no significant changes in lung function were observed in workers exposed to ammonia in an industrial setting with relatively low ammonia exposure levels (Table 1-2). These effects were observed in short-term scenarios (i.e., cross-work shift changes in lung function) in fertilizer factory workers (mean ammonia concentration of 18.5 mg/m³) compared with administrative staff controls ([Rahman et al., 2007](#)), and in longer-term scenarios, in workers with a cumulative exposure of > 50 mg/m³-years when compared with workers with a lower cumulative exposure of ≤ 50 mg/m³-years (with an approximate 5–7% decrease in forced vital capacity [FVC]% predicted and forced expiratory volume in 1 second [FEV₁]% predicted)¹⁰ ([Ali et al., 2001](#)). There were no decrements in the percent of predicted lung function values when comparing the total exposed group to a control group of office workers in the latter study, in the relatively low exposure scenario examined in [Holness et al. \(1989\)](#) (mean ammonia concentration of 6.5 mg/m³ and high-exposure group defined

¹⁰ FVC = total amount of air exhaled during a maximal forced expiratory effort; FEV₁ = volume of air exhaled in the first second of forced expiration; FEV₁% = ratio of FEV₁ to FVC; when expressed as % predicted, indicates that value for the subject is divided by the average value in the population for any person of similar age, sex, and body composition.

as $>8.8 \text{ mg/m}^3$), or in the low-exposure group (mean ammonia concentration of 4.9 mg/m^3) in [Rahman et al. \(2007\)](#). Another study of ammonia plant fertilizer workers reported statistically significant decreases in FEV_1 and peak expiratory flow rate (PEFR/minute) in workers compared to controls ([Bhat and Ramaswamy, 1993](#)); however, measurements of ammonia levels were not included in this study. As discussed previously in the summary of respiratory symptoms studies, the primary limitation within this set of studies is the potential under-ascertainment of effects in these studies of long-term worker populations.

One of the studies of domestic cleaning workers described in Table 1-3 included a measure of pulmonary function ([Medina-Ramón et al., 2006](#)). Ammonia use was associated with a decrease in peak expiratory flow (PEF) (-9.4 ; 95% confidence interval [CI] $-17, -2.3$). A limitation of this study was the use of lung function measurements conducted by the participant; the reliability of this procedure has not been established. In a study by [Casas et al. \(2013\)](#) on the effects of cleaning product use on the respiratory health of children, ammonia exposure was associated with decreased lung function (FEV_1 : -28 [95% CI $-131, 76$]) (Table 1-3).

Impaired respiratory function (e.g., decreased FEV_1 and/or FVC) in an agricultural setting was associated with ammonia exposure in six of the eight studies that included pulmonary function measures ([Loftus et al., 2015](#); [Monsó et al., 2004](#); [Donham et al., 2000](#); [Reynolds et al., 1996](#); [Donham et al., 1995](#); [Preller et al., 1995](#); [Zeida et al., 1994](#); [Heederik et al., 1990](#)) (Appendix C, Table C-7). In general, the U.S. Environmental Protection Agency (EPA) considered these eight studies to be the strongest with respect to methodology, based on considerations of exposure assessment as well as assessment of potential confounding (see Literature Search Strategy | Study Selection and Evaluation section).

Changes in lung function following acute exposure to ammonia have been observed in some, but not all, controlled human exposure studies conducted in volunteers (Appendix C, Section C.2.3 and Table C-9). [Cole et al. \(1977\)](#) reported reduced lung function as measured by reduced expiratory minute volume and changes in exercise tidal volume in volunteers exposed for a half-day in a chamber at ammonia concentrations $\geq 106 \text{ mg/m}^3$, but not at 71 mg/m^3 . Bronchoconstriction was reported in volunteers exposed to ammonia through a mouthpiece for 10 inhaled breaths of ammonia gas at a concentration of 60 mg/m^3 ([Douglas and Coe, 1987](#)); however, there were no bronchial symptoms reported in volunteers exposed to ammonia in an exposure chamber at concentrations of up to 35 mg/m^3 for 10 minutes ([MacEwen et al., 1970](#)). Similarly, no changes in bronchial responsiveness or lung function (as measured by FVC and FEV_1) were reported in healthy volunteers exposed to ammonia at concentrations up to 18 mg/m^3 for 1.5 hours during exercise ([Sundblad et al., 2004](#)). There were no changes in lung function as measured by FEV_1 in 25 healthy volunteers and 15 mild/moderate persistent asthmatic volunteers exposed to ammonia concentrations up to 354 mg/m^3 ammonia for up to 2.5 hours ([Petrova et al., 2008](#)), or in 6 healthy volunteers and 8 mildly asthmatic volunteers exposed to $11\text{--}18 \text{ mg/m}^3$ ammonia for 30-minute sessions ([Sigurdarson et al., 2004](#)).

Lung function effects following ammonia exposure were not evaluated in the available animal studies.

Table 1-2. Evidence pertaining to respiratory effects in humans following inhalation exposure in industrial settings

Study reference and design	Results				
Respiratory symptoms					
Rahman et al. (2007) (Bangladesh) Urea fertilizer factory worker (all men); 24 ammonia plant workers, 64 urea plant workers, and 25 controls (staff from administration building). Mean employment duration: 16 yrs Exposure: Personal samples (two methods^a; correlation = 0.80) Low-exposure group (ammonia plant), mean: 6.9 ppm (4.9 mg/m³); range: 2.8–11.1 ppm (2–8 mg/m³) High-exposure group (urea plant), mean: 26.1 ppm (18.5 mg/m³); range: 13.4–43.5 ppm (9–31 mg/m³) Outcome: Respiratory symptoms (five-point scale for severity over last shift), based on Optimal Symptom Score Questionnaire	Percentage of workers reporting symptoms (<i>p</i> -value):				
		Controls (<i>n</i> = 25)	Low exposed (<i>n</i> = 24) (<i>p</i> -value)*	High exposed (<i>n</i> = 64) (<i>p</i> -value) [‡]	(<i>p</i> -value) [†]
	Cough	8	17 (0.42)	28 (0.05)	(0.41)
	Chest tightness	8	17 (0.42)	33 (0.02)	(0.19)
	Stuffy nose	4	12 (0.35)	16 (0.17)	(1.0)
	Runny nose	4	4 (1.0)	16 (0.17)	(0.28)
	Sneeze	8	0 (0.49)	22 (0.22)	(0.01)
	* <i>p</i> -value for ammonia plant compared to control				
	[‡] <i>p</i> -value for urea plant compared to control				
	[†] <i>p</i> -value for urea plant compared to ammonia plant				
Ballal et al. (1998) (Saudi Arabia) Urea fertilizer factory workers (two factories) (all men); 161 exposed workers and 355 unexposed controls^b. Mean employment duration: 51.8 mo (exposed workers) and 73.1 mo (controls) Exposure: Area monitors (3 sets in each work section taken at least 3 mo apart, mean 16 measures per set). Factory A (high-exposure factory): 2–130^c mg/m³ (mid-point = 66 mg/m³); geometric mean <18 mg/m³, except for urea packaging and store areas (geometric means = 18.6 and 115 mg/m³, respectively) Factory B (low-exposure factory): 0.02–7 mg/m³; geometric mean <18 mg/m³ Cumulative exposure calculated based on exposure and duration; dichotomized to high and low at 50 mg/m³-yrs Outcome: Respiratory symptoms based on British Medical Research Council questionnaire	Relative risk (95% CI), compared with controls				
			Factory B* (0.02–7 mg/m ³ ; <i>n</i> = 77)	Factory A* (2–27.1 mg/m ³ ; <i>n</i> = 78) ^c	
	Cough		No cases	2.0 (0.38, 10.4)	
	Phlegm		No cases	2.0 (0.38, 10.4)	
	Wheezing		0.97 (0.21, 4.5)	3.4 (1.2, 9.5)	
	Dyspnea		0.45 (0.11, 1.9)	1.8 (0.81, 4.2)	
	*Factory-specific analyses stratified by smoking status; results presented here are for nonsmokers. Similar patterns seen in other smoking categories.				
	Relative risk (95% CI), compared with lower exposure setting (≤18 mg/m ³ [<i>n</i> = 138] or ≤50 mg/m ³ -years [<i>n</i> = 130])				
			>18 mg/m ³ (<i>n</i> = 17)	Cumulative >50 mg/m ³ -years (<i>n</i> = 30)	
	Cough		3.5 (1.8, 6.6)	2.8 (1.6, 5.0)	
Phlegm		3.8 (2.0, 7.1)	3.0 (1.7, 5.5)		

Study reference and design	Results			
	Wheezing	5.0 (2.4, 10.6)	5.2 (2.9, 9.5)	
	Dyspnea	4.6 (2.4, 8.8)	2.6 (1.3, 5.4)	
	Asthma	4.3 (2.1, 9.0)	2.4 (1.1, 5.4)	
	Chronic bronchitis	2.3 (0.31, 17)	5.3 (1.7, 16)	
	Approximate 1.3–1.5 relative risk ($p < 0.05$) per unit increase in ammonia concentration for cough, phlegm, wheezing, and asthma, adjusting for duration of work, cumulative exposure, smoking, and age.			
Holness et al. (1989) (Canada) Soda ash plant workers (all men); 58 exposed workers and 31 controls (from stores and office areas of plant) ^c . Average exposure: 12.2 yrs Exposure: Personal samples, one work-shift per person, mean 8.4 hrs Low: <6.25 ppm (<4.4 mg/m ³); n = 34 Medium: 6.25–12.5 ppm (4.4–8.8 mg/m ³); n = 12 High: >12.5 ppm (>8.8 mg/m ³); n = 12 All exposed workers (mean): 6.5 mg/m ³ Outcome: Respiratory symptoms based on American Thoracic Society questionnaire	Percentage of workers reporting symptoms (%):			
	Control (n = 31)	Exposed (n = 58)	p -value	
	Cough	10	16	0.53
	Sputum	16	22	0.98
	Bronchitis	19	22	0.69
	Wheeze	10	10	0.91
	Chest tightness	6	3	0.62
	Dyspnea (shortness of breath)	13	7	0.05
	Chest pain	6	2	0.16
	Rhinitis (nasal complaints)	19	10	0.12
	Throat irritation	3	7	0.53
	No increased risk seen in analyses stratified by exposure group.			
Lung function				
Rahman et al. (2007) (Bangladesh) Urea fertilizer factory workers (all men); 24 ammonia plant workers, 64 urea plant workers, and 25 controls (staff from administration building). Mean employment duration: 16 yrs Exposure: Personal samples (2 methods ^a ; correlation = 0.80) Low-exposure group (ammonia plant), mean: 6.9 ppm (4.9 mg/m ³); range: 2.8–11.1 ppm (2–8 mg/m ³)		Pre-shift	Post-shift	p -value
	Ammonia plant (low-exposure group, 4.9 mg/m ³); n = 24 ammonia plant workers			
	FVC	3.308	3.332	0.67
	FEV ₁	2.627	2.705	0.24
	PEFR	8.081	8.313	0.22
	Urea plant (high-exposure group, 18.5 mg/m ³); n = 64 urea plant workers			
	FVC	3.362	3.258	0.01

Study reference and design	Results				
High-exposure group (urea plant), mean: 26.1 ppm (18.5 mg/m ³); range: 13.4–43.5 ppm (9–31 mg/m ³) Outcome: Lung function (standard spirometry)	FEV ₁	2.701	2.646	0.05	
	PEFR	7.805	7.810	0.97	
	<i>p</i> -value reflects the comparison of pre- and post-shift values.				
	Multiple regression model (data from 23 ammonia and urea plant workers with concurrent measurements of ammonia exposure and lung function): Concentration of ammonia and exposure duration (yrs of employment as proxy for duration) were significantly correlated with percentage cross-shirt decrease in FEV ₁ % (ΔFEV ₁ %). Each year of work in a production section was associated with a decrease in ΔFEV ₁ % of 0.6%. [Limitation of analysis: failure to explore the age parameter; age and years of work were highly correlated (Pearson correlation coefficient 0.97).]				
Ali et al. (2001) (Saudi Arabia) Urea fertilizer factory workers (all men)—(additional study of “Factory A” in Ballal et al. (1998)); 73 exposed workers and 348 unexposed controls. Mean employment duration: not reported Exposure: 4-hr measurements. Cumulative exposure calculated based on exposure and duration; dichotomized to high and low at 50 mg/m ³ -yrs Outcome: Lung function (standard spirometry; morning measurement)		≤50 mg/m ³ -yr (n = 45)	>50 mg/m ³ -yr (n = 28)	<i>p</i> -value	
	FVC ₁ % predicted	100.7	93.4	0.006	
	FVC% predicted	105.6	100.2	0.03	
	FEV ₁ /FVC%	84.7	83.4	NS	
	NS = not significant (<i>p</i> -values not provided by study authors)				
Bhat and Ramaswamy (1993) (India) Fertilizer chemical plant workers; 30 diammonium phosphate (DAP) plant workers, 30 urea plant workers, 31 ammonia plant workers, and 68 controls (people with comparable body surface area chosen from the same socio-economic status and sex as exposed workers) Exposure: Measurements not reported; duration dichotomized as ≤10 and >10 yrs Outcome: Lung function (standard spirometry)		Controls (n = 68)	DAP plant (n = 30)	Urea plant (n = 30)	Ammonia plant (n = 31)
	FVC	3.4 ± 0.21	2.5 ± 0.06*	3.3 ± 0.11	3.2 ± 0.07
	FEV ₁	2.8 ± 0.10	2.1 ± 0.08*	2.7 ± 0.10	2.5 ± 0.1*
	PEFR	383 ± 7.6	228 ± 18*	307 ± 19*	314 ± 20*
	* <i>p</i> < 0.05				
Holness et al. (1989) (Canada) Soda ash plant workers (all men); 58 exposed workers and 31 controls (from stores and office areas of plant) ^d . Average exposure: 12.2 yrs Exposure: Personal samples, one work-shift per person, mean 8.4 hrs		Control (n = 31)	Exposed (n = 58)	<i>p</i> -value*	
	Lung function (% predicted values) [†] :				
	FVC	98.6 ± 11.3	96.8 ± 11.0	0.094	
	FEV ₁	95.1 ± 12.5	94.1 ± 12.9	0.35	

Study reference and design	Results			
Low: <6.25 ppm (<4.4 mg/m ³); n = 34 Medium: 6.25–12.5 ppm (4.4–8.8 mg/m ³); n = 12 High: >12.5 ppm (>8.8 mg/m ³); n = 12 All exposed workers (mean): 6.5 mg/m ³ Outcome: Lung function (standard spirometry; beginning and end of shift, at least 2 test days per worker)	FEV ₁ /FVC	96.5 ± 6.1	97.1 ± 7.1	0.48
	Change in lung function over work shift:			
	FVC day 1	–0.9	–0.8	0.99
	day 2	+0.1	–0.0	0.84
	FEV ₁ day 1	–0.2	–0.2	0.94
	day 2	+0.5	+0.7	0.86
	* <i>p</i> -value for difference between exposed and control workers calculated by using actual baseline values and correcting for age, height, and pack-years smoked determined by multiple regression analysis. ‡Percentage of the subject's predicted value (% predicted) has been widely adopted as follows: % predicted = recorded value × 100/predicted value); this value is now calculated on automated spirometers based on sex, race, age, and height.			

^aExposure concentrations were determined by both the Dräger tube and Dräger PAC III methods. Using the Dräger tube method, concentrations of ammonia in the ammonia and urea plants were 17.7 and 88.1 mg/m³, respectively; using the Dräger PAC III method, ammonia concentrations were 4.9 and 18.5 mg/m³, respectively ([Rahman et al. \(2007\)](#)). The study authors observed that their measurements indicated only relative differences in exposures between workers and production areas, and that the validity of the exposure measures could not be evaluated based on their results. Based on communication with technical support at Dräger Safety Inc. (telephone conversations and e-mails dated June 22, 2010, from Michael Yanosky, Dräger Safety Inc., Technical Support Detection Products to Amber Bacom, SRC, Inc., contractor to the National Center for Environmental Assessment [NCEA], Office of Research and Development [ORD], EPA), EPA considered the PAC III instrument to be a more sensitive monitoring technology than the Dräger tubes. Therefore, higher confidence is attributed to the PAC III air measurements of ammonia for the [Rahman et al. \(2007\)](#) study.

^bThe process of fertilizer production involved synthesis of ammonia from natural gas, followed by reaction of the ammonia and carbon dioxide to form ammonium carbamate, which was then converted to urea.

^cThe ammonia concentration range in Factory A is better represented as 2–27.1 mg/m³. This range excludes the employees in the urea store (n = 6; range of ammonia concentrations = 90–130.4 mg/m³) who were required to wear full protective clothing, thus minimizing potential exposure. Number of workers in Factory A excluding urea store workers = 78.

^dAt this plant, ammonia, carbon dioxide, and water were the reactants used to form ammonium bicarbonate, which in turn was reacted with salt to produce sodium bicarbonate and subsequently processed to form sodium carbonate. Ammonia and carbon dioxide were recovered in the process and reused.

Table 1-3. Evidence pertaining to respiratory effect in humans following inhalation exposure in cleaning settings

Study reference and design	Results
Asthma or asthma symptoms	
Dumas et al. (2012) (France) Hybrid design, hospital workers, drawn from population-based case-control study; 179 hospital workers (136 women), 545 other workers (333 women). Exposure: Asthma-specific job exposure matrix plus expert review (blinded), ever exposed, 18 specific products, based on all jobs held at least 3 mo; ammonia prevalence 23% in female hospital workers Outcome: Current asthma: Asthma attack, respiratory symptoms or asthma treatment in the last 12 mo (based on standardized questionnaire)	Odds ratio (95% CI), current asthma Women: 3.05 (1.19, 7.82) Men: no associations with any specific products (prevalence low) Adjusted for age and smoking, and accounting for familial dependence (due to sampling of cases and first-degree relatives)
Arif and Delclos (2012) (United States, Texas) Population survey of 3,650 health care workers (physicians, nurses, respiratory therapists, occupational therapists), (total n = 5,600, response rate 66%) Exposure: Structured questionnaire—frequency of use of products for longest job held; ever contact with list of 28 products; ammonia prevalence 23% Outcome: Structured questionnaire • Work-related asthma symptoms: wheezing/whistling at work or shortness of breath at works that gets better away from work or worse at work • Work-exacerbated asthma: onset before began work • Occupational asthma: onset after began work	Odds ratio (95% CI) [n cases] Work-related asthma symptoms [n = 132] 2.45 (1.28, 4.69) Work-exacerbated asthma [n = 41] 1.58 (0.56, 4.43) Occupational asthma [n = 33] 1.86 (0.49, 7.13) Adjusted for age, sex, race/ethnicity, body mass index, seniority, atopy, and smoking status
Lemiere et al. (2012) (Quebec, Canada) Case-control study, workers seen at two tertiary care centers specializing in occupational asthma. Asthma (defined below) based on reversible airflow limitation or airway hyper-responsiveness tests; referent group = non-work-related asthma seen at same clinics but symptoms did not worsen at work (n = 33). Exposure: Structured interview focusing on last/current job, combined with expert review (blinded); ammonia prevalence 19/153 = 12% Outcome: Diagnoses made based on reference tests • Occupational asthma if specific inhalation challenge test was positive • Work-exacerbated asthma if specific inhalation test was negative but symptoms worsened at work	Odds ratio (95% CI) [n cases] Work exacerbation [n = 53] 8.4 (1.1, 371.7) Occupational asthma [n = 67] 3.7 (0.4, 173.4) Age, smoking, and occupational exposure to heat, cold, humidity, dryness, and physical strain assessed as confounders. [Wide CIs reflect sparseness in referent group, with only 1 of the 33 classified as exposed to ammonia.]

Study reference and design	Results
Vizcaya et al. (2011) (Spain) Survey of cleaning service workers (n = 917) from 37 businesses (19% response rate to questionnaire distributed through the employers); 761 current cleaners, 86 former cleaners, 70 never cleaners; referent group = never cleaners and current cleaners who had not used any of the specified cleaning products in the past year (n = 161) Exposure: Structured questionnaire, use of cleaning tasks and 12 products; ammonia prevalence 66% Outcome: Structured questionnaire • Current asthma: in past 12 mo, woken by an attack of shortness of breath, had an attack of asthma, or currently taking any asthma medications (including inhalers, aerosols, or tablets) • Asthma score: Sum of “yes” answers to five symptoms in last 12 mo (wheeze with breathlessness, woken up with chest tightness, attack of shortness of breath at rest, attack of shortness of breath after exercise, woken by attack of shortness of breath)	Odds ratio (95% CI) (among current cleaners) [n] Current asthma 1.4 (0.6, 3.2) [81] Wheeze without having a cold 2.1 (0.9, 4.7) [83] Chronic cough 1.6 (0.8, 3.3) [95] Asthma score 1.6 (1.0, 2.5) [mean 0.59, SD 1.12] Adjusted for age, country of birth (Spanish versus non-Spanish), sex, and smoking status
Zock et al. (2007) (Europe, 22 sites) Longitudinal study, n = 3,503, 9-yr follow-up of European Community Respiratory Health Survey, population-based sample, ages 20–44 yrs. Excluded 764 individuals with asthma at baseline; limited to individuals reporting doing the cleaning or washing in their home. Exposure: Structured interview at follow-up; frequency of use of 15 products Outcome: Structured interview at follow-up • New onset (since baseline survey) current asthma, defined by asthma attack or nocturnal shortness of breath in the past 12 mo or current use of medication for asthma • Current wheeze defined as wheezing or whistling in the chest in last 12 mo when not having a cold • New onset physician-diagnosed asthma, asthma defined as above with confirmation by a physician and information on age or date of first attack	Odds ratio (95% CI) [n] Current asthma 1.4 (0.87, 2.23) [199] Current wheeze 1.3 (0.81, 2.13) [226] Physician-diagnosed asthma 0.92 (0.33, 2.59) [71] Adjusted for sex, age, smoking, employment in a cleaning job during follow-up, and study center; heterogeneity by center also assessed. Correlations among products were generally weak (Spearman rho < 0.3).

Study reference and design	Results		
Medina-Ramón et al. (2005) (Spain) Nested case-control, cleaning workers; case (n = 40; 74% participation rate) based on asthma and/or bronchitis at both assessments. Controls (n = 155, 69% participation rate)—no history of respiratory symptoms in preceding year and no asthma at either assessment. Exposure: Structured interview; frequency of use of 22 products; ammonia prevalence 16% undiluted, 56% diluted Outcome: Asthma: asthma attack or being woken by attack or shortness of breath in past 12 mo; Chronic bronchitis: regular cough or regular bringing up phlegm for at least 3 mo each year	Odds ratio (95% CI) (unadjusted), ≥12 compared with <12 times per year Undiluted 3.1 (1.2, 8.0) Diluted 0.8 (0.4, 1.7)		
Fraction of exhaled nitric oxide (FeNO) and pulmonary function			
Casas et al. (2013) (Spain) Population based cross sectional birth cohort study; n = 432 infants enrolled; n = 295 total number of individuals recruited that completed the 10-yr follow up visit and the cleaning products questionnaire and performed the FeNO and/or lung function test; 35% of recruited population were excluded because information on use of cleaning products and/or respiratory tests was not available; only 46 individuals reported use of ammonia Exposure: Interviewer-led questionnaire; frequency of use of 10 different cleaning products (bleach, ammonia, polishes or waxes, acids, solvents, furniture sprays, glass cleaning sprays, degreasing sprays, air freshening sprays, and air freshening plug- ins); exposure score developed based on frequency of use and number of products used Outcome: Questionnaires on wheezing asthma, treatment and allergies were administered by mother from birth to age 10; at age 10–13 yrs, FeNO and lung function tests were carried out	<u>Adjusted* associations of FeNO, FVC and FEV₁[‡] with weekly use of ammonia (n = 46; 16%)</u>		
	FeNO [†] ppb	FVC mL	FEV ₁ mL
	GM ratio (95% CI)	β (95% CI)	β (95% CI)
	0.86 (0.66, 1.12)	3 (–127, 133)	–28 (–131, 76)
[*] Adjusted for sex, age, asthma medication, season of respiratory measurement, maternal education, and parental smoking; FVC and FEV ₁ models were additionally adjusted for height and weight. [‡] Change in FeNO, FVC and FEV ₁ per interquartile range increase of the score (interquartile range = 6.5 d of product use per wk). [†] FeNO (fraction of exhaled nitric oxide) is used to characterize asthma or other conditions associated with airway inflammation; it is measured in a breath test.			

Study reference and design	Results		
Medina-Ramón et al. (2006) (Spain) Panel study, sample selected from participants in nested case-control study by Medina-Ramón et al. (2005). Current asthma symptoms or chronic bronchitis in 2000–2001 survey; n = 51 of 80 (64%); 8 excluded for possible recording errors, outliers, learning effects Exposure: Daily diary of use of products Outcome: Respiratory symptoms based on 2-wk daily diary (seven symptoms, five-point intensity scale); summed score for upper respiratory symptoms (blocked nose, throat irritation, watery eyes) and lower respiratory symptoms (chest tightness, wheezing, shortness of breath, and cough); PEF measured with mini-Wright peak flow meter (with training and written instructions); measured morning, lunchtime, night (three measurements each; highest recorded)		Diluted and undiluted	Diluted only
		Odds ratio (95% CI)	
	Upper respiratory symptoms	1.8 (0.7, 4.9)	1.3 (0.3, 5.0)
	Lower respiratory symptoms	1.6 (0.6, 4.4)	3.0 (1.0, 9.1)
		Beta (95% CI)	
	PEF at night	–9.4 (–17, –2.3)	–10.3 (–18, –2.7)
	PEF, following morning	–1.2 (–8.5, 6.2)	–2.9 (–11, 6.2)
Adjusted for respiratory infection, use of maintenance medication, and age; daily number of cigarettes smoked, years of employment in domestic cleaning, and/or weekly working hours in domestic cleaning also assessed as potential confounders			

GM= geometric mean; SD = standard deviation.

Table 1-4. Evidence pertaining to respiratory effects in animals

Study reference and design	Results
Effects on the lungs	
Coon et al. (1970) Sprague-Dawley and Long-Evans rat; male and female; 15/group New Zealand albino rabbit; male; 3/group Princeton-derived guinea pig; male and female; 15/group Squirrel monkey (<i>Saimiri sciureus</i>); male; 3/group Beagle dog; male; 2/group 0, 155, or 770 mg/m ³ for 8 hrs/d, 5 d/wk for 6 wks	Gross necropsies were normal; focal pneumonitis in one of three monkeys at 155 mg/m ³ . Nonspecific lung inflammation observed in guinea pigs and rats, but not in other species, at 770 mg/m ³ . ^a
Coon et al. (1970) New Zealand albino rabbit; male; 3/group Princeton-derived guinea pig; male and female; 15/group Squirrel monkey (<i>S. sciureus</i>); male; 3/group Beagle dog; male; 2/group 0 or 40 mg/m ³ for 114 d or 470 mg/m ³ for 90 d	At 470 mg/m ³ , focal or diffuse interstitial pneumonitis in all animals. Calcification of bronchial epithelium observed in several animals. Hemorrhagic lung lesion in one of two dogs; moderate lung congestion in two of three rabbits. ^a (This exposure was lethal to ~25% of the guinea pigs.)
Coon et al. (1970) Sprague-Dawley or Long-Evans rat; male and female; 15–51/group 0 or 40 mg/m ³ for 114 d, 127, 262, or 470 mg/m ³ for 90 d, or 455 mg/m ³ for 65 d	Focal or diffuse interstitial pneumonitis in all animals, and calcification of bronchial epithelium observed in several animals at 470 mg/m ³ , an exposure that was lethal to most of the rats. ^a
Anderson et al. (1964) Swiss albino mouse; male and female; 4/exposure interval 0 or 20 ppm (0 or 14 mg/m ³) for 7, 14, 21, 28, or 42 d	Lung congestion, edema, and hemorrhage observed at 14 mg/m ³ after 42 d. ^a
Anderson et al. (1964) Guinea pig (strain not specified); male and female; 2/exposure interval at 20 ppm, 6/exposure interval at 50 ppm 0 or 20 ppm (0 or 14 mg/m ³) for 7, 14, 21, 28, or 42 d or 50 ppm (35 mg/m ³) for 42 d	Lung congestion, edema, and hemorrhage observed at 14 and 35 mg/m ³ after 42 d. ^a
Done et al. (2005) Pig (several breeds); sex not specified; 24/group 0, 0.6, 10, 18.8, or 37 ppm (0, 0.4, 7, 13.3, or 26 mg/m ³) and 1.2, 2.7, 5.1, or 9.9 mg/m ³ inhalable dust for 5 wks (Exposure to ammonia and inhalable dust at concentrations commonly found at pig farms)	No increase in the incidence of respiratory or other diseases.
Curtis et al. (1975) Pig (crossbred); sex not specified; 4–8/group 0, 50, or 75 ppm (0, 35, or 53 mg/m ³ for 109 d)	Turbinates, trachea, and lungs of all pigs were classified as normal.

Study reference and design	Results
Effects on the upper respiratory tract	
Coon et al. (1970) Sprague-Dawley and Long-Evans rat; male and female; 15/group New Zealand albino rabbit; male; 3/group Princeton-derived guinea pig; male and female; 15/group Squirrel monkey (<i>S. sciureus</i>); male; 3/group Beagle dog; male; 2/group 0, 155, or 770 mg/m ³ for 8 hrs/d, 5 d/wk for 6 wks	Dyspnea in rabbits and dogs exposed to 770 mg/m ³ during wk 1 only; no indication of irritation after wk 1; nasal tissues not examined for gross or histopathologic changes.
Brodereson et al. (1976)^b Sherman rat; 5/sex/group 10 or 150 ppm (7 or 106 mg/m ³) from bedding for 75 d	↑ thickness of the nasal epithelium (3–4 times) and nasal lesions at 106 mg/m ³ . ^a
Brodereson et al. (1976)^b F344 rat; 6/sex/group 0 or 250 ppm (0 or 177 mg/m ³) in an inhalation chamber for 35 d	↑ thickness of the nasal epithelium (3–4 times) and nasal lesions at 177 mg/m ³ . ^a
Coon et al. (1970) Sprague-Dawley or Long-Evans rat; male and female; 15–51/group 0 or 40 mg/m ³ for 114 d, 127, 262, or 470 mg/m ³ for 90 d, or 455 mg/m ³ for 65 d	Nasal discharge at 262 mg/m ³ (25% of rats). Dyspnea and nasal irritation/discharge in all animals at 455 and 470 mg/m ³ , an exposure that was lethal to the majority of the rats. ^a
Gaafar et al. (1992) White albino mouse; male; 50 Ammonia vapor of 0 or 12% ammonia solution for 15 min/d, 6 d/wk, for 8 wks	Histological changes in the nasal mucosa. ^a
Doig and Willoughby (1971) Yorkshire-Landrace pig; sex not specified; 6/group 0 or 100 ppm (0 or 71 mg/m ³) for 6 wks	↑ thickness of nasal and tracheal epithelium (50–100% increase). ^a
Stombaugh et al. (1969) Duroc pig; both sexes; 9/group 12, 61, 103, 145 ppm (8, 43, 73, or 103 mg/m ³) for 5 wks	Excessive nasal, lacrimal, and mouth secretions and ↑ frequency of cough at 73 and 103 mg/m ³ . ^a
Coon et al. (1970) Beagle dog; male; 2/group 0 or 40 mg/m ³ for 114 d or 470 mg/m ³ for 90 d	Nasal discharge at 470 mg/m ³ . ^a

^aIncidence data not provided.

^bThe [Brodereson et al. \(1976\)](#) paper includes a number of experiments in rats designed to examine whether ammonia at concentrations commonly encountered in laboratory cage environments plays a role in the pathogenesis of murine respiratory mycoplasmosis caused by the bacterium, *Mycoplasma pulmonis*. The experiments conducted without co-exposure to *M. pulmonis* are summarized in this table; the results of experiments involving co-exposure to *M. pulmonis* are discussed in Section 1.2.2, Immune System Effects.

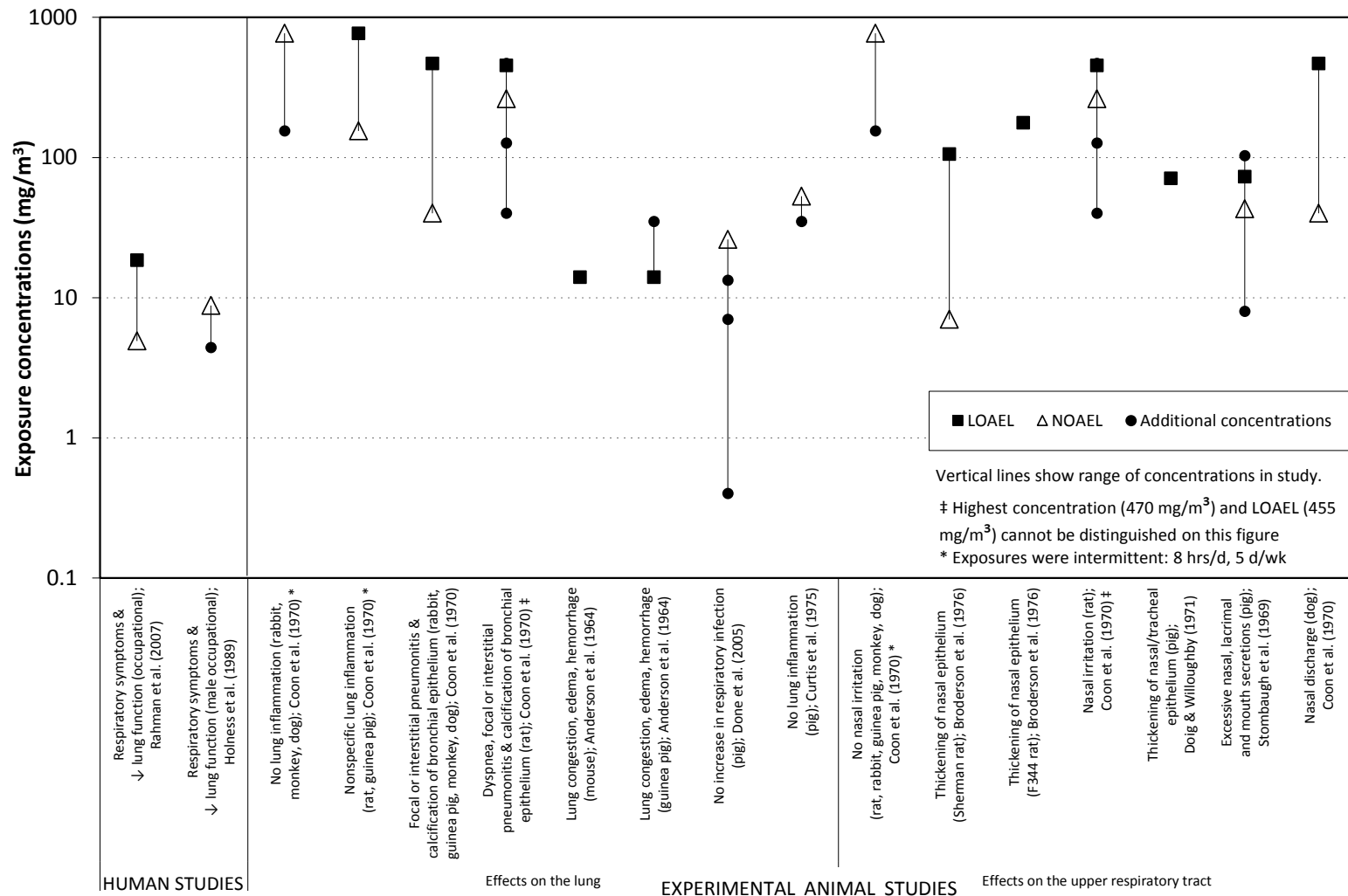


Figure 1-1. Exposure-response array of respiratory effects following inhalation exposure to ammonia.

Mode-of-Action Analysis—Respiratory Effects

Data on the potential mode of action for respiratory effects associated with chronic exposure to ammonia are limited. However, acute exposure data demonstrate that injury to respiratory tissues is primarily due to ammonia's alkaline (i.e., caustic) properties from the formation of hydroxide ion when it comes in contact with water and is solubilized. Ammonia readily dissolves in the moisture on the mucous membranes, forming ammonium hydroxide, which causes liquefactive necrosis of the tissues. Specifically, ammonia directly denatures tissue proteins and causes saponification of cell membrane lipids, which leads to cell disruption and death (necrosis). In addition, the cellular breakdown of proteins results in an inflammatory response, which further damages the surrounding tissues ([Amshel et al., 2000](#); [Millea et al., 1989](#); [Jarudi and Golden, 1973](#)).

Summary of Respiratory Effects

Evidence for respiratory toxicity associated with exposure to ammonia comes from studies in humans and animals. Multiple occupational studies involving chronic exposure to ammonia in industrial settings provide evidence of an increased prevalence of respiratory symptoms ([Rahman et al., 2007](#); [Ballal et al., 1998](#)) and decreased lung function ([Rahman et al., 2007](#); [Ali et al., 2001](#); [Bhat and Ramaswamy, 1993](#)) (Table 1-2 and Appendix C, Section C.2.1). An increase in respiratory effects was reported both with higher workplace ammonia concentrations ([Rahman et al., 2007](#); [Ballal et al., 1998](#)) and with greater cumulative ammonia concentration (expressed in mg/m³-years) ([Ali et al., 2001](#); [Ballal et al., 1998](#)). Evidence of respiratory effects is provided by studies of asthma, asthma symptoms, and pulmonary function in workers and others exposed to cleaning agents containing ammonia, in a variety of study designs and populations ([Casas et al., 2013](#); [Arif and Delclos, 2012](#); [Dumas et al., 2012](#); [Lemiere et al., 2012](#); [Vizcaya et al., 2011](#); [Zock et al., 2007](#); [Medina-Ramón et al., 2006](#); [Medina-Ramón et al., 2005](#)) (Table 1-3). Additional evidence of respiratory effects of ammonia is seen in studies of pulmonary function in an agricultural setting, specifically in livestock farmer studies that accounted for effects of co-exposures to other agents such as endotoxin and dust ([Donham et al., 2000](#); [Reynolds et al., 1996](#); [Donham et al., 1995](#); [Preller et al., 1995](#); [Heederik et al., 1990](#)), and in one study of asthmatic children who lived near animal feeding operations that did not control for co-exposures ([Loftus et al., 2015](#)) (Appendix C, Table C-7). The livestock farmer studies, however, do not provide evidence of associations between ammonia and respiratory symptoms. Controlled human exposure studies of ammonia inhalation and case reports of injury in humans with inhalation exposure to ammonia provide additional support for the respiratory system as a target of ammonia toxicity when inhaled (Appendix C, Section C.2.3). Overall, the consistency of findings across three categories of epidemiological studies (industrial, cleaner, and agricultural settings) that differed in population characteristics, level and pattern of exposure, and potential confounders, and support from studies of acute

exposures, adds strength to the evidence for an association between respiratory effects and ammonia exposure.

Evidence from animal studies supports an association between inhaled ammonia and respiratory effects. Short-term and subchronic animal studies show histopathological changes of respiratory tissues in several animal species (lung inflammation in guinea pigs and rats; focal or interstitial pneumonitis in monkeys, dogs, rabbits, and guinea pigs; pulmonary congestion in mice; thickening of nasal epithelium in rats and pigs; nasal inflammation or lesions in rats and mice) across different dosing regimens ([Gaafar et al., 1992](#); [Broderick et al., 1976](#); [Doig and Willoughby, 1971](#); [Coon et al., 1970](#); [Anderson et al., 1964](#)) (Table 1-4 and Appendix C, Section C.3). In general, responses in respiratory tissues increased with increasing ammonia exposure concentration. Based on evidence of respiratory effects in multiple human and animal studies (including epidemiological studies in different settings and populations), respiratory system effects are identified as a hazard associated with inhalation exposure to ammonia.

1.2.2. Immune System Effects

A limited number of studies have evaluated the immunotoxicity of ammonia in human populations and in experimental animal models. Immunological function was evaluated in two independent investigations of livestock farmers exposed to ammonia via inhalation. Immunoglobulin G- (IgG) and E-specific (IgE) antibodies for pig skin and urine ([Crook et al., 1991](#)), elevated neutrophils from nasal washes, and increased white blood cell counts ([Cormier et al., 2000](#)) were reported. These data on immunological function are suggestive of immunostimulatory effects; however, the test subjects were also exposed to a number of other respirable agents in addition to ammonia, such as endotoxin, bacteria, fungi, and mold that are known to stimulate immune responses. Data in humans following exposure to ammonia only are not available.

Animal studies that examined ammonia immunotoxicity were conducted using short-term inhalation exposures and were measured by three general types of immune assays: host resistance, T cell proliferation, and delayed-type hypersensitivity. Immunotoxicity studies of ammonia using measures of host resistance provide the most relevant data for assessing immune function since they directly measure the ability of the immune system to control microorganism growth. Other available studies of ammonia employed assays that evaluated immune function. Changes in immune cell populations without corresponding functional data are considered to be the least predictive, and studies that looked only at these endpoints ([Gustin et al., 1994](#); [Neumann et al., 1987b](#)) were considered less informative and were not further considered in evaluating the immune system effects of ammonia.

Several host resistance studies utilized lung pathogens to assess bacterial clearance following ammonia exposure; however, these studies were not designed to discriminate between direct immunosuppression associated with ammonia exposure or immune effects secondary to damage to the protective mucosal epithelium of the respiratory tract. The available studies also do

not correlate increased bacterial colonization with reduced immune function. Lung lesions, both gross and microscopic, were positively correlated with ammonia concentration in F344 rats continuously exposed to ammonia in an inhalation chamber for 7 days prior to inoculation with 10^8 colony forming units [CFU] of *Mycoplasma pulmonis* followed by up to 42 days of ammonia exposure post inoculation ([Broderon et al., 1976](#)). (Inoculation with the respiratory pathogen *M. pulmonis* causes murine respiratory mycoplasmosis [MRM] characterized by lung lesions.) The incidence of lung lesions was significantly increased at ammonia concentrations ≥ 35 mg/m³, suggesting that ammonia exposure decreased bacterial clearance resulting in the development of *M. pulmonis*-induced MRM. However, increasing ammonia concentration was not associated with increased CFU of *M. pulmonis* isolated from the respiratory tract. The high number of inoculating CFU could have overwhelmed the innate immune response and elicited a maximal response that could not be further increased in immunocompromised animals.

Conversely, significantly increased CFU of *M. pulmonis* bacteria isolated in the trachea, nasal passages, lungs, and larynx were observed in F344 rats continuously exposed to 71 mg/m³ ammonia for 7 days prior to *M. pulmonis* (10^4 – 10^6 CFU) inoculation and continued for 28 days post inoculation ([Schoeb et al., 1982](#)). This increase in bacterial colonization indicates a reduction in bacterial clearance following exposure to ammonia. Lesions were not assessed in this study.

OF1 mice exposed to 354 mg/m³ ammonia for 7 days prior to inoculation with a 50% lethal dose (LD₅₀) of *Pasteurella multocida* exhibited significantly increased mortality compared to controls (86% versus 50%, respectively); however, an 8-hour exposure was insufficient to affect mortality ([Richard et al., 1978a](#)). The authors suggested that the irritating action of ammonia destroyed the tracheobronchial mucosa and caused inflammatory lesions, thereby increasing sensitivity to respiratory infection with prolonged ammonia exposure.

Pig studies support the findings observed in the rodent studies that ammonia exposure increases the colonization of respiratory pathogens. [Andreasen et al. \(2000\)](#) demonstrated that 63 days of ammonia exposure increased the number of bacterial-positive nasal swabs following inoculation with *P. multocida* and *Mycoplasma hyopneumoniae*; however, the effect was not dose responsive and did not result in an increase in lung lesions. Additional data obtained from pigs suggest that ammonia exposure eliminates the commensal flora of the nasal cavities, which allows for increased colonization of *P. multocida*; however, this effect abates following cessation of ammonia exposure ([Hamilton et al., 1999](#); [Hamilton et al., 1998](#)).

Suppressed cell-mediated immunity and decreased T cell proliferation was observed following ammonia exposure. Using a delayed-type hypersensitivity test to evaluate cell-mediated immunity, Hartley guinea pigs were vaccinated with *Mycobacterium bovis* bacillus Calmette-Guérin (BCG) and exposed to ammonia followed by intradermal challenge with a purified protein derivative (PPD). Dermal lesion size was reduced in animals exposed to 64 mg/m³ ammonia, indicating immunosuppression ([Targowski et al., 1984](#)). Blood and bronchial lymphocytes harvested from naïve guinea pigs treated with the same 3-week ammonia exposure and stimulated

with phytohaemagglutinin or concanavalin A demonstrated reduced T cell proliferation ([Targowski et al., 1984](#)). Bactericidal activity in alveolar macrophages isolated from ammonia-exposed guinea pigs was not affected. Lymphocytes and macrophages isolated from unexposed guinea pigs and treated with ammonia in vitro showed reduced proliferation and bactericidal capacity only at concentrations that reduced viability, indicating nonspecific effects of ammonia-induced immunosuppression ([Targowski et al., 1984](#)). These data suggest that T cells may be the target of ammonia exposure since specific macrophage effects were not observed.

The evidence of immune system effects in experimental animals exposed to ammonia is summarized in Table 1-5 and is presented graphically as an exposure-response array in Figure 1-2.

Table 1-5. Evidence pertaining to immune system effects in animals

Study reference and design	Results
Host resistance	
Brodereson et al. (1976) F344 rat; male and female; 11–12/sex/group ≤5 (control), 25, 50, 100, or 250 ppm (≤3.5 [control], 18, 35, 71, or 177 mg/m ³), 7 d (continuous exposure) pre-inoculation/28–42 d post-inoculation with <i>M. pulmonis</i>	% of animals with gross lung lesions: 16, 46, 66*, 33, and 83% No effect on CFU
Schoeb et al. (1982) F344 rat; 5–15/group (sex unknown) <2 or 100 ppm (<1.4 [control] or 71 mg/m ³), 7 d (continuous exposure) pre-inoculation/28 d post-inoculation with <i>M. pulmonis</i>	↑ bacterial colonization (as a result of reduced bacterial clearance).
Richard et al. (1978a) OF1 mouse; male; 99/group 0 or 500 ppm (0 or 354 mg/m ³), 8 hrs or 7 d (continuous exposure), prior to infection with <i>P. multocida</i>	% Mortality: 50 and 86%*
Andreasen et al. (2000) Landrace X large white pigs; 10/group (sex unknown) <5 (control), 50, or 100 ppm (3.5, 35, or 71 mg/m ³), 63 d (continuous exposure) inoculated with <i>M. hyopneumoniae</i> on day 9 and <i>P. multocida</i> on d 28, 42, and 56	% of animals with positive day 49 nasal swab: 24, 100*, and 90%*
Hamilton et al. (1998) Large white pigs; 4–7/group (sex unknown) 0 or 20 ppm (0 or 14 mg/m ³), 14 d (continuous exposure), inoculated with <i>P. multocida</i> on d 0	↑ bacterial colonization
Hamilton et al. (1999) Large white pigs; 5/group (sex unknown) 0 or 50 ppm (0 or 35 mg/m ³), 1 wk pre-inoculation with <i>P. multocida</i> , 3 wks post-inoculation	↑ bacterial colonization <i>Bacteria isolated from nasal cavities:</i> 3.18 and 4.30* CFU

Study reference and design	Results
<i>T cell proliferation</i>	
Targowski et al. (1984) Hartley guinea pig; 8/group (sex unknown) <15, 50, or 90 ppm (<11 [control], 35, or 64 mg/m ³), 3 wks (continuous exposure)	↓ proliferation in blood and bronchial T cells
<i>Delayed-type hypersensitivity</i>	
Targowski et al. (1984) Hartley guinea pig, BCG immunized; 8/group (sex unknown) <15, 50, or 90 ppm (<11 [control], 35, or 64 mg/m ³), 3 wks (continuous exposure) followed by PPD challenge	Mean diameter of dermal lesion (mm): 12, 12.6, and 8.7*

*Statistically significantly different from the control ($p < 0.05$).

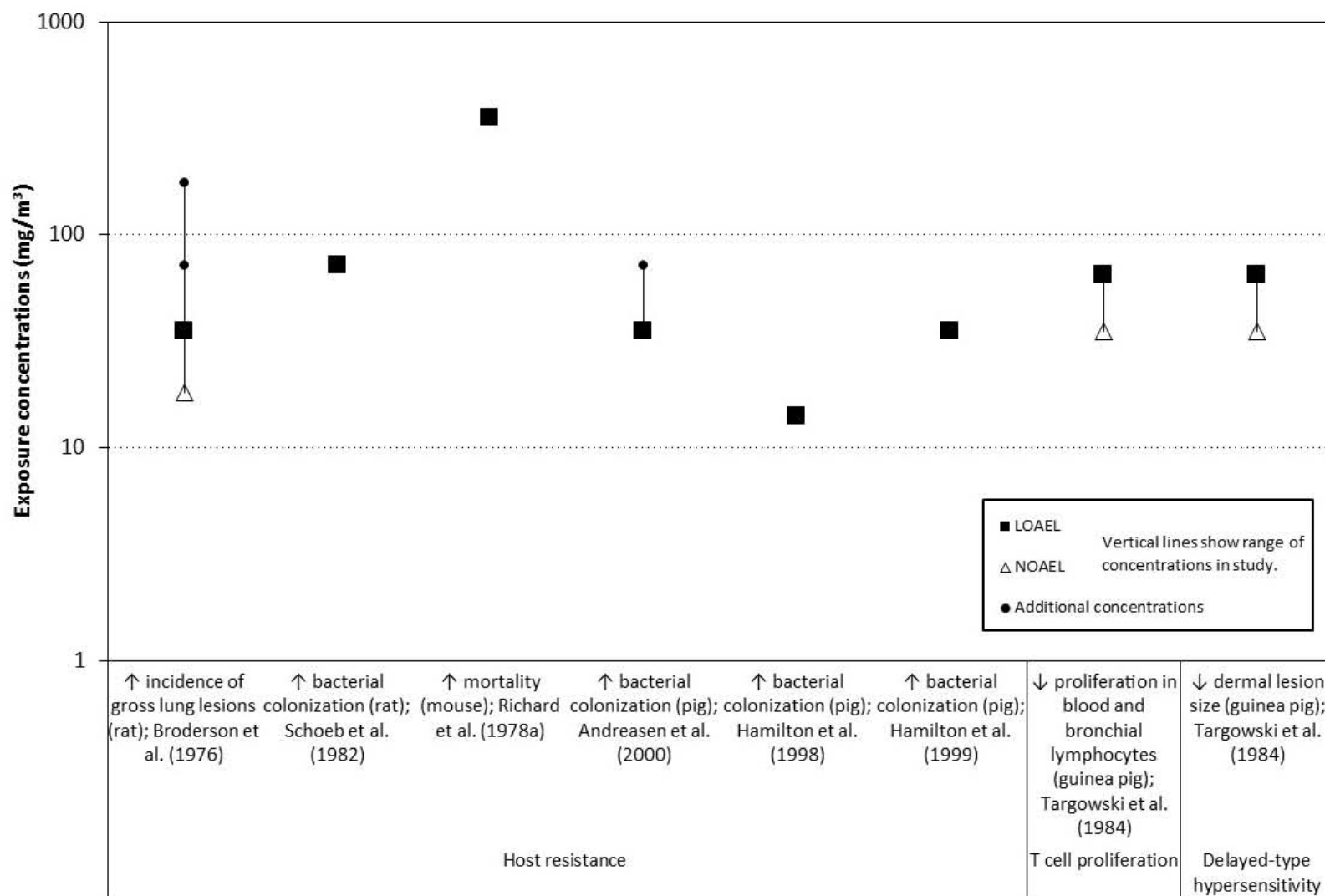


Figure 1-2. Exposure-response array of immune system effects following inhalation exposure to ammonia.

Summary of Immune System Effects

The evidence for ammonia immunotoxicity is based on epidemiological and animal studies. Available epidemiological studies that addressed immunological function are confounded by exposures to a number of other respirable agents that have been demonstrated to be immunostimulatory. Single-exposure human studies of ammonia evaluating immune endpoints are not available. Therefore, human studies are not particularly informative for evaluating whether ammonia has immunotoxic properties.

Animal studies provide consistent evidence of elevated bacterial growth following ammonia exposure. This is supported by observations of lung lesions ([Broderick et al., 1976](#)), elevated CFU ([Schoeb et al., 1982](#)), and increased mortality ([Richard et al., 1978a](#)) in rats or mice exposed to ammonia; however, the findings from the [Broderick et al. \(1976\)](#) study (which described the percent of animals with gross lesions) were not dose-responsive, and the other studies used single concentrations of ammonia and therefore did not provide information on dose-response. A single study suggested that T cells are inhibited by ammonia ([Targowski et al., 1984](#)), but the data were not dose responsive.

Overall, there are suggestions that ammonia exposure may be associated with immunotoxicity, but it is unclear if elevated bacterial colonization is the result of damage to the protective mucosal epithelium of the respiratory tract or the result of suppressed immunity. Therefore, there is inadequate information to draw a conclusions about the immune system as a potential hazard of ammonia exposure.

1.2.3. Other Systemic Effects

The majority of information suggests that ammonia induces effects in and around the portal of entry. As discussed below, there is limited evidence from experimental animals that ammonia can produce effects on organs distal from the portal of entry, including the liver, kidney, spleen, and heart.

Evidence of liver toxicity in animals comes from observations of histopathological alterations in the liver. Histopathologic changes described as “fatty changes of the liver plate cells” were reported at an exposure concentration of 470 mg/m³ ammonia in rats, guinea pigs, rabbits, dogs, and monkeys following the same subchronic inhalation exposure regimens ([Coon et al., 1970](#)); this concentration was lethal to approximately 25% of exposed guinea pigs and the majority of exposed rats. Congestion of the liver was reported in guinea pigs following inhalation exposure to 35 mg/m³ for 42 days and 120 mg/m³ 18 weeks ([Anderson et al., 1964](#); [Weatherby, 1952](#)); no liver effects were observed in similarly exposed mice at 14 mg/m³ ([Anderson et al., 1964](#)).

Experimental animal studies provide some evidence that inhaled ammonia can affect the kidney and spleen. Alterations in the kidneys (calcification and proliferation of tubular epithelium) were reported in rats, rabbits, guinea pigs, monkeys, and dogs exposed to 470 mg/m³, an ammonia

concentration that was lethal to rats and guinea pigs ([Coon et al., 1970](#)). “Congestion” of the kidneys and spleen was reported in four guinea pigs exposed to 120 mg/m³ ammonia for 18 weeks (but not 6 or 12 weeks) ([Weatherby, 1952](#)). Enlarged and “congested” spleens were reported in guinea pigs exposed to 35 mg/m³ ammonia for 6 weeks ([Anderson et al., 1964](#)). None of these studies provided incidence of histopathologic lesions.

Myocardial fibrosis was observed in monkeys, dogs, rabbits, guinea pigs, and rats following subchronic inhalation exposure to 470 mg/m³ ammonia, a concentration lethal to exposed guinea pigs and rats; no changes were observed at lower concentrations ([Coon et al., 1970](#)). At the same concentration, ocular irritation (characterized as heavy lacrimation, erythema, discharge, and ocular opacity of the cornea) was also reported by [Coon et al. \(1970\)](#) in small numbers of dogs and rabbits, but was not observed in similarly exposed monkeys or rats.

“Early degenerative changes” in the adrenal gland were reported in four guinea pigs exposed to 120 mg/m³ ammonia by inhalation for 18 weeks, but not in guinea pigs exposed for 6 or 12 weeks ([Weatherby, 1952](#)). With the exception of [Broderon et al. \(1976\)](#), no other investigators examined effects on the adrenal gland following exposure to inhaled ammonia, and [Broderon et al. \(1976\)](#) did not describe effects on nonrespiratory tissues. These limited findings are insufficient to draw conclusions about possible effects of ammonia on the adrenal gland.

As discussed above, [Coon et al. \(1970\)](#) reported effects on the liver, kidney, and heart following continuous exposure to 470 mg/m³; however, no histopathological changes were observed in rats, guinea pigs, rabbits, dogs, or monkeys when these animals were repeatedly, but not continuously, exposed to ammonia even at high concentrations (e.g., 770 mg/m³ for 8 hours/day, 5 days/week; Table 1-6). These findings suggest that animals can recover from intermittent exposure to elevated ammonia levels ([Coon et al., 1970](#)), although the evidence to support this observation is limited.

Additionally, there is limited evidence of biochemical or metabolic effects of acute or short-term ammonia exposure. Evidence of slight acidosis, as indicated by a decrease in blood pH, was reported in rats exposed to 18 or 212 mg/m³ ammonia for 5 days; the study authors stated that differences in pH leveled off at 10 and 15 days ([Manninen et al., 1988](#)). In another study, blood pH in rats was not affected by exposure to ammonia at concentrations up to 818 mg/m³ for up to 24 hours ([Schaerdel et al., 1983](#)).

Encephalopathy related to ammonia may occur in humans following disruption of the body’s normal homeostatic regulation of the glutamine and urea cycles (e.g., due to severe liver disease resulting in elevated ammonia levels in blood) ([Miñana et al., 1995](#); [Souba, 1987](#)). Acute inhalation exposure studies have identified alterations in amino acid levels and neurotransmitter metabolism (including glutamine concentrations) in the brain of rats and mice ([Manninen and Savolainen, 1989](#); [Manninen et al., 1988](#); [Sadasivudu et al., 1979](#); [Sadasivudu and Radha Krishna Murthy, 1978](#)). It has been suggested that glutamate and γ-amino butyric acid play a role in

ammonia-induced neurotoxicity ([Jones, 2002](#)). There is no evidence, however, that ammonia is neurotoxic in humans or animals following chronic inhalation exposure.

In the only study of the reproductive and developmental toxicity of ammonia, no changes in reproductive or developmental endpoints were found between two groups of female pigs (crossbred gilts) exposed to ammonia via inhalation for 6 weeks at mean concentrations of 5 or 25 mg/m³ and then mated ([Diekman et al., 1993](#)). A control group without ammonia exposure was not evaluated. Age at puberty did not differ significantly between the two groups. Gilts exposed to 25 mg/m³ ammonia weighed 7% less ($p < 0.05$) at puberty than those exposed to 5 mg/m³; however, body weights of the two groups were similar at gestation day 30. Conception rates in the mated females were similar between the two groups (94.1 versus 100% in 5- versus 25-mg/m³ groups). At sacrifice on day 30 of gestation, there were no significant differences between the two exposed groups in body weights of the pregnant gilts, number of corpora lutea, number of live fetuses, or weight and length of the fetuses. The strength of the findings from this study are limited by the absence of a control group with no ammonia exposure and possible confounding by exposures to bacterial and mycoplasma pathogens.

The evidence of systemic toxicity in experimental animals exposed to ammonia is summarized in Table 1-6 and displayed graphically as an exposure-response array in Figure 1-3.

Table 1-6. Evidence pertaining to other systemic effects in animals

Study reference and design	Results
Liver effects	
Coon et al. (1970) Sprague-Dawley and Long-Evans rat; male and female; 15/group New Zealand albino rabbit; male; 3/group Princeton-derived guinea pig; male and female; 15/group Squirrel monkey (<i>S. sciureus</i>); male; 3/group Beagle dog; male; 2/group 0, 155, or 770 mg/m ³ for 8 hrs/d, 5 d/wk for 6 wks	No histopathologic changes observed.
Coon et al. (1970) New Zealand albino rabbit; male; 3/group Princeton-derived guinea pig; male and female; 15/group Squirrel monkey (<i>S. sciureus</i>); male; 3/group Beagle dog; male; 2/group 0 or 40 mg/m ³ for 114 d or 470 mg/m ³ for 90 d	“Fatty changes of the liver plate cells” in several animals of each species at 470 mg/m ³ . ^a
Coon et al. (1970) Sprague-Dawley or Long-Evans rat; male and female; 15–51/group 0 or 40 mg/m ³ for 114 d, 127, 262, or 470 mg/m ³ for 90 d, or 455 mg/m ³ for 65 d	“Fatty changes of the liver plate cells” in several rats at 470 mg/m ³ , an exposure that was lethal to the majority of the rats. ^a

Study reference and design	Results
<u>Anderson et al. (1964)</u> Swiss albino mouse; male and female; 4/exposure interval 0 or 20 ppm (0 or 14 mg/m ³) for 7, 14, 21, 28, or 42 d	No visible signs of liver toxicity.
<u>Weatherby (1952)</u> Guinea pig (strain not specified); male; 2 control and 4 exposed/exposure interval 0 or 170 ppm (0 or 120 mg/m ³) for 6 hrs/d, 5 d/wk for 6, 12, or 18 wks	Congestion of the liver at 18 wks, not reported at earlier times. ^a
<u>Anderson et al. (1964)</u> Guinea pig (strain not specified); male and female; 2/exposure interval at 20 ppm, 6/exposure interval at 50 ppm 0 or 20 ppm (0 or 14 mg/m ³) for 7, 14, 21, 28, or 42 d or 50 ppm (35 mg/m ³) for 42 d	Congestion of the liver at 35 mg/m ³ for 42 d. ^a
Adrenal gland effects	
<u>Weatherby (1952)</u> Guinea pig (strain not specified); male; 2 control and 4 exposed/exposure interval 0 and 170 ppm (0 and 120 mg/m ³) 6 hrs/d, 5 d/wk for 6, 12, or 18 wks	“Early” degenerative changes in the adrenal gland (swelling of cells, degeneration of the cytoplasm with loss of normal granular structure) at 18 wks, not observed at earlier times. ^a
Kidney and spleen effects	
<u>Coon et al. (1970)</u> Sprague-Dawley and Long-Evans rat; male and female; 15/group New Zealand albino rabbit; male; 3/group Princeton-derived guinea pig; male and female; 15/group Squirrel monkey (<i>S. sciureus</i>); male; 3/group Beagle dog; male; 2/group 0, 155, or 770 mg/m ³ for 8 hrs/d, 5 d/wk for 6 wks	No histopathologic changes reported.
<u>Coon et al. (1970)</u> New Zealand albino rabbit; male; 3/group Princeton-derived guinea pig; male and female; 15/group Squirrel monkey (<i>S. sciureus</i>); male; 3/group Beagle dog; male; 2/group 0 or 40 mg/m ³ for 114 d or 470 mg/m ³ for 90 d	Calcification and proliferation of renal tubular epithelium at 470 mg/m ³ . ^a (This exposure was lethal to ~25% of guinea pigs.)
<u>Coon et al. (1970)</u> Sprague-Dawley or Long-Evans rat; male and female; 15–51/group 0 or 40 mg/m ³ for 114 d, 127, 262, or 470 mg/m ³ for 90 d, or 455 mg/m ³ for 65 d	Calcification and proliferation of renal tubular epithelium at 470 mg/m ³ , an exposure that was lethal to the majority of the rats. ^a
<u>Anderson et al. (1964)</u> Swiss albino mouse; male and female; 4/exposure interval 0 or 20 ppm (0 or 14 mg/m ³) for 7, 14, 21, 28, or 42 d	No visible signs of toxicity.

Study reference and design	Results
Weatherby (1952) Guinea pig (strain not specified); male; 2 control and 4 exposed/exposure interval 0 or 170 ppm (0 or 120 mg/m ³) 6 hrs/d, 5 d/wk for 6, 12, or 18 wks	Congestion of the spleen and kidneys. ^a
Anderson et al. (1964) Guinea pig (strain not specified); male and female; 2/exposure interval at 20 ppm, 6/exposure interval at 50 ppm 0 or 20 ppm (0 or 14 mg/m ³) for 7, 14, 21, 28, or 42 d or 50 ppm (35 mg/m ³) for 42 d	Enlarged and congested spleens at 35 mg/m ³ . ^a
Myocardial effects	
Coon et al. (1970) Sprague-Dawley and Long-Evans rat; male and female; 15/group New Zealand albino rabbit; male; 3/group Princeton-derived guinea pig; male and female; 15/group Squirrel monkey (<i>S. sciureus</i>); male; 3/group Beagle dog; male; 2/group 0, 155, or 770 mg/m ³ for 8 hrs/d, 5 d/wk for 6 wks	No histopathologic changes reported.
Coon et al. (1970) New Zealand albino rabbit; male; 3/group Princeton-derived guinea pig; male and female; 15/group Squirrel monkey (<i>S. sciureus</i>); male; 3/group Beagle dog; male; 2/group 0 or 40 mg/m ³ for 114 d or 470 mg/m ³ for 90 d	Myocardial fibrosis at 470 mg/m ³ . ^a (This exposure was lethal to ~25% of guinea pigs.)
Coon et al. (1970) Sprague-Dawley or Long-Evans rat; male and female; 15–51/group 0 or 40 mg/m ³ for 114 d, 127, 262, or 470 mg/m ³ for 90 d, or 455 mg/m ³ for 65 d	Myocardial fibrosis at 470 mg/m ³ , an exposure that was lethal to the majority of the rats. ^a
Ocular effects	
Coon et al. (1970) Princeton-derived guinea pig; male and female; 15/group Squirrel monkey (<i>S. sciureus</i>); male; 3/group 0 or 40 mg/m ³ for 114 d or 470 mg/m ³ for 90 d	No ocular irritation reported.
Coon et al. (1970) Sprague-Dawley and Long-Evans rat; male and female; 15/group New Zealand albino rabbit; male; 3/group Princeton-derived guinea pig; male and female; 15/group Squirrel monkey (<i>S. sciureus</i>); male; 3/group Beagle dog; male; 2/group 0, 155, or 770 mg/m ³ for 8 hrs/d, 5 d/wk for 6 wks	No ocular irritation reported.

Study reference and design	Results
Coon et al. (1970) Sprague-Dawley and Long-Evans rat; male and female; 15–51/group 0 or 40 mg/m ³ for 114 d, 127, 262, or 470 mg/m ³ for 90 d, or 455 mg/m ³ for 65 d	No ocular irritation reported.
Coon et al. (1970) New Zealand albino rabbit; male; 3/group 0 or 40 mg/m ³ for 114 d or 470 mg/m ³ for 90 d	Erythema, discharge, and ocular opacity over ¼–½ of cornea at 470 mg/m ³ . ^a
Coon et al. (1970) Beagle dog; male; 2/group 0 or 40 mg/m ³ for 114 d or 470 mg/m ³ for 90 d	Heavy lacrimation at 470 mg/m ³ . ^a
Blood pH changes	
Manninen et al. (1988) Wistar rat; female; 5/group 0, 25 or 300 ppm (0, 18, or 212 mg/m ³) 6 hrs/d for 5, 10 or 15 d	↓ blood pH at 5 days; pH differences “leveled off at later time points (data not shown)”. <i>Blood pH (day 5): 7.43, 7.34*, 7.36*</i>
Schaerdel et al. (1983) Crl:COBS CD(SD) rat; male; 8/group (blood oxygen partial pressure [pO ₂] based on n = 5) 15, 32, 310, or 1,157 ppm (11, 23, 219, or 818 mg/m ³) for 0 (control), 8, 12, or 24 hrs	↑ blood pO ₂ at 11 and 23 mg/m ³ at 8-, 12-, and 24-hr time points; no change at higher concentrations; no change in blood pH. <i>Percent change in pO₂ from time 0 (at 24 hours of exposure^b): 20*, 17*, 1, –2%</i>
Amino acid levels and neurotransmitter metabolism in the brain	
Manninen and Savolainen (1989) Wistar rat; female; 5/group 0, 25, or 300 ppm (0, 18, or 212 mg/m ³) 6 hrs/d for 5 d	% change compared to control: ^c Brain glutamine: 42*, 40*%
Manninen et al. (1988) Wistar rat; female; 5/group 0, 25, or 300 ppm (0, 18, or 212 mg/m ³) 6 hrs/d for 5, 10, or 15 d	% change compared to control at 212 mg/m ³ : ^c Blood glutamine (5, 10, 15 d): 44*, 13, 14% Brain glutamine (5, 10, 15 d): 40*, 4, 2%
Reproductive and developmental effects	
Diekman et al. (1993) Crossbred gilt (female pig); 4.5 mo old; 40/group 7 ppm (5 mg/m ³), range 4–12 ppm (3–8.5 mg/m ³) or 35 ppm (25 mg/m ³), range 26–45 (18–32 mg/m ³) for 6 wks ^d	No change in any of the reproductive or developmental parameters measured (age at puberty, conception rates, body weight of pregnant gilts, number of corpora lutea, number of live fetuses, and weight or length of fetuses).

^aIncidence data not provided.

^bMeasurements at time zero were used as a control; the study did not include an unexposed control group.

^cPercent change compared to control calculated as: (treated value – control value)/control value × 100.

^dA control group was not included. Prior to exposure to ammonia, pigs were also exposed naturally in conventional grower units to *M. hyopneumoniae* and *P. multocida*, which cause pneumonia and atrophic rhinitis, respectively.

*Statistically significantly different from the control (*p* < 0.05).

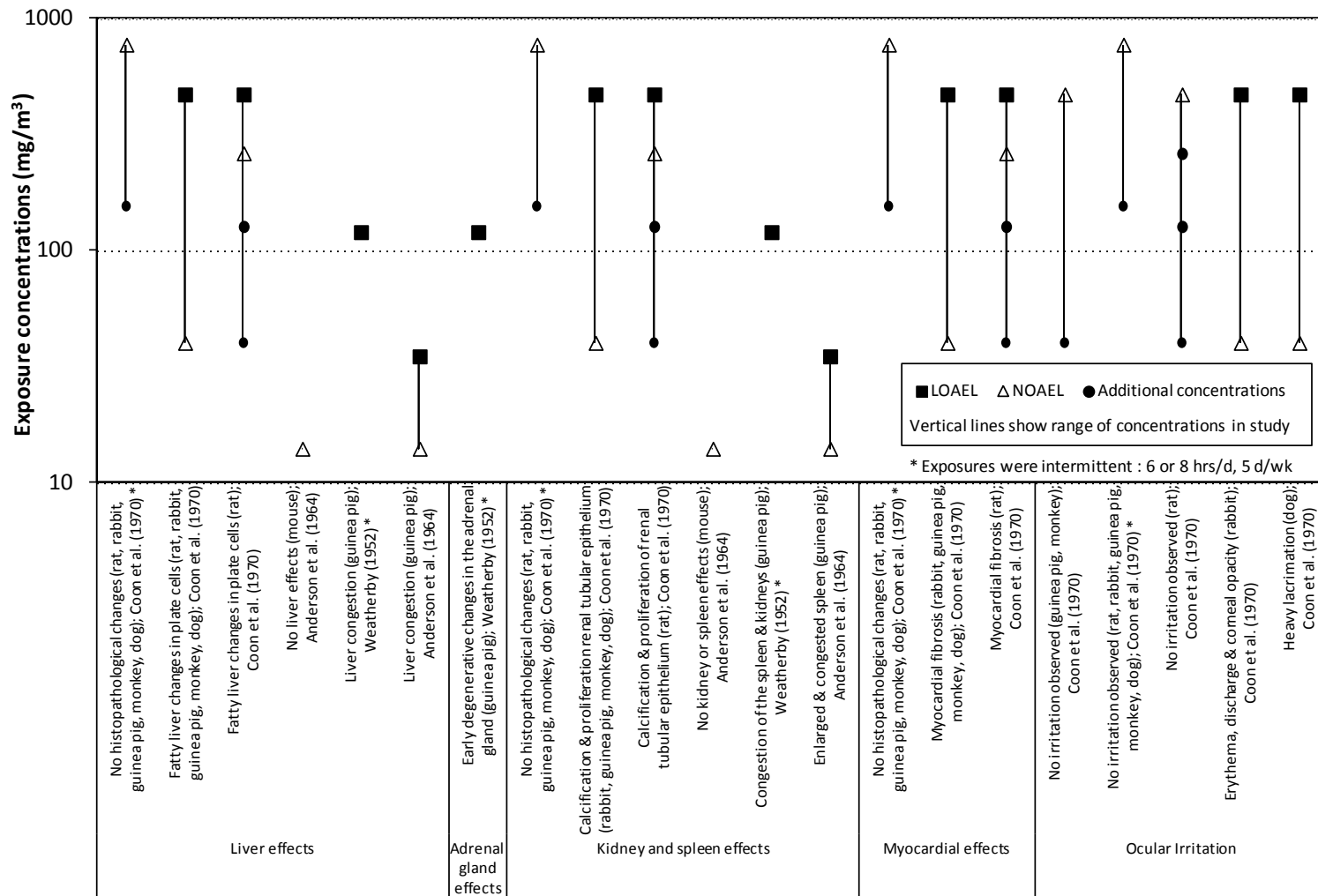


Figure 1-3. Exposure-response array of systemic effects following inhalation exposure to ammonia.

Summary of Other Systemic Effects

Effects of ammonia exposure on organs distal from the portal of entry (systemic effects) are based on evidence in animals. Effects on various organs, including liver, kidney, spleen, and heart, were observed in several studies that examined responses to ammonia exposure in a number of laboratory animal species. While effects on many of these organs were observed in multiple species, including monkey, dog, rabbit, guinea pig, and rat, effects were not consistent across exposure protocols. Evidence of ocular irritation in experimental animals was inconsistently observed, and then only at high ammonia concentrations (470 mg/m³).

Studies of ammonia toxicity that examined other systemic effects were all published in the older toxicological literature. Three subchronic inhalation studies were published between 1952 and 1970 ([Coon et al., 1970](#); [Anderson et al., 1964](#); [Weatherby, 1952](#)). In general, the information from these studies is limited by small group sizes, minimal characterization of reported histopathological changes (e.g., “congestion,” “enlarged,” “fatty liver”), insufficiently detailed reporting of study results, and incomplete, if any, incidence data. In addition, [Weatherby \(1952\)](#), [Anderson et al. \(1964\)](#), and some of the experiments reported by [Coon et al. \(1970\)](#) used only one ammonia concentration in addition to the control, so no dose-response information is available from the majority of experimental studies to inform the evidence for systemic effects of ammonia. Finally, exposure characterization in [Weatherby \(1952\)](#) was considered poor.

Overall, there are suggestions in experimental animals that ammonia exposure may be associated with effects on organs distal from the portal of entry, but there is inadequate information to draw conclusions about the liver, kidney, spleen, or heart as sensitive targets of ammonia toxicity.

Given the inadequacies of the available toxicology literature for other systemic effects, the potential toxicity of inhaled ammonia at sites distal from the respiratory system was evaluated by considering ammonia levels normally present in blood. As discussed in more detail in Appendix C, Section C.1.2, ammonia is produced endogenously in all human and animal tissues during all lifestages, including prenatal. In adults, the normal range of ammonia in venous blood is 0.1–0.8 µg/mL. Concentrations in fetal circulation are higher than maternal blood concentrations; two studies reported that mean umbilical concentrations of ammonia in venous blood at delivery were 50% to 3-fold higher than mean concentrations in maternal blood, with umbilical concentrations ranging from approximately 0.5 to 5 µg/mL ([Jóźwik et al., 2005](#); [DeSanto et al., 1993](#)). Human fetal umbilical blood levels of ammonia at birth were not influenced by gestational age based on deliveries ranging from gestation week 25 to 43 ([DeSanto et al., 1993](#)).

At external concentrations that do not measurably change normal (baseline) levels of ammonia, the likelihood is low that exposures would pose a hazard for systemic effects. In rats, exposure to ammonia concentrations ≤18 mg/m³ did not produce a statistically significant change in blood or brain ammonia concentrations ([Manninen et al., 1988](#); [Schaerdel et al., 1983](#)). Higher

external ammonia concentrations (≥ 212 mg/m³) were associated with elevated blood ammonia levels, but even at these relatively high concentrations, experimental findings in rats indicate that compensation readily occurs ([Manninen et al., 1988](#)). In a 24-hour exposure duration study, blood ammonia concentrations at 12 hours of exposure to ≥ 219 mg/m³ ammonia in air were lower than at 8 hours; in a second 15-day exposure duration study, blood ammonia concentrations that were elevated on day 5 of exposure to 212 mg/m³ ammonia in air were not significantly different from control values on days 10 and 15 of exposure ([Schaerdel et al., 1983](#)). See Appendix C, Section C.1.3, Metabolism/Endogenous Production of Ammonia, for a more detailed summary of the available literature that describes the relationship between environmental ammonia concentrations and blood ammonia levels. Therefore, the available experimental data suggest that any changes in blood ammonia at external concentrations ≤ 18 mg/m³ would be small relative to levels normally present in blood. The potential for systemic effects (i.e., on tissues/organs distal from the respiratory system), including reproductive and developmental effects, at these concentrations cannot be ruled out, but the likelihood of such effects is considered small.

Because the health effects literature identified the respiratory system as the primary target of ammonia toxicity, EPA also considered the possibility that point-of-contact effects could translate into effects on tissues or organs distal from the respiratory system. EPA is not aware of any mechanisms by which point-of-contact effects could directly or indirectly impact distal tissues or organs.

1.3. SUMMARY AND EVALUATION

1.3.1. Weight of Evidence for Effects Other than Cancer

The respiratory system is the primary and most sensitive target of inhaled ammonia toxicity in humans and experimental animals. Evidence for respiratory system toxicity in humans comes from cross-sectional occupational studies in industrial settings that reported changes in lung function and an increased prevalence of respiratory symptoms. The findings of respiratory effects in workers exposed to ammonia as a disinfectant or cleaning product (primarily studies of asthma or asthma symptoms), studies in agricultural settings (primarily lung function studies), controlled human exposure studies, and case reports of injury following acute exposure provide additional evidence that the respiratory system is a target of inhaled ammonia. Short-term and subchronic animal studies show respiratory effects in several animal species across different dose regimens. Thus, the weight of evidence of observed respiratory effects observed across multiple human and animal studies identifies respiratory system effects as a hazard from ammonia exposure.

Evidence for an association between inhaled ammonia exposure and effects on other organ systems distal from the portal of entry is less compelling than for the respiratory system. Overall, there are suggestions in experimental animals that ammonia exposure may be associated with effects on the liver, kidney, spleen, or heart, but the available information is inadequate to draw

conclusions. The two epidemiological studies that addressed immunological function are confounded by exposures to a number of other respirable agents that have been demonstrated to be immunostimulatory and provide little support for ammonia immunotoxicity. Animal studies provide consistent evidence of elevated bacterial growth following ammonia exposure. It is unclear, however, whether elevated bacterial colonization is the result of suppressed immunity or damage to the barrier provided by the mucosal epithelium of the respiratory tract. Overall, the weight of evidence does not support the immune system as a target of ammonia toxicity.

Studies of the potential reproductive or developmental toxicity of ammonia in humans are not available. Reproductive effects were not associated with inhaled ammonia in the only animal study that examined the reproductive effects of ammonia (i.e., a limited-design inhalation study in the pig). As discussed in Section 1.1.2, ammonia is produced endogenously in human and animal tissues during all lifestages, including prenatal. Although the potential for effects on reproduction and the developing fetus cannot be ruled out at external concentrations that do not alter normal blood or tissue ammonia levels, there is no evidence that raises concerns for the developing fetus or reproduction or to other distal tissues/organs.

1.3.2. Susceptible Populations and Lifestages

Studies of the toxicity of ammonia in children or young animals that would support an evaluation of childhood susceptibility are limited. [Casas et al. \(2013\)](#) found evidence of airway inflammation (as indicated by increased exhaled nitric oxide) and decreased lung function in school-age children exposed to cleaning products. No studies were identified that would support the comparison of response to ammonia at different lifestages.

Infants are exposed to ammonia in human breast milk. Ammonia in human breast milk is present as one of the sources of nonprotein nitrogen, with approximately 1% of the nitrogen derived from nonprotein nitrogen sources coming from ammonia ([Atkinson et al., 1980](#)). No evidence was identified that inhaled ammonia would influence the ammonia nitrogen content of breast milk.

Because the respiratory system is a target of ammonia toxicity, individuals with respiratory disease (e.g., asthmatics) might be expected to be a susceptible population. [Loftus et al. \(2015\)](#) reported no increase in asthma symptoms and medication use in asthmatic children living near animal feeding operations; however, ammonia exposure was associated with lower FEV₁. Controlled human exposure studies that examined both healthy adult volunteers and volunteers with asthma ([Petrova et al., 2008](#); [Sigurdarson et al., 2004](#)) did not demonstrate greater respiratory sensitivity in asthmatics than healthy volunteers after acute exposure to ammonia. Under longer-term exposure conditions, however, as seen among livestock farmers, one study observed associations between ammonia exposure and decreased lung function among workers with chronic respiratory symptoms, but not among the asymptomatic workers ([Preller et al., 1995](#)). Additional

research focusing on the question of susceptibility and variability in response to ammonia exposure in these populations is needed.

Individuals with disease conditions that lead to hyperammonemia, a condition of elevated levels of circulating ammonia, may be more susceptible to the effects of ammonia from external sources. Hyperammonemia can occur in individuals with severe diseases of the liver (e.g., cirrhosis) or kidney, organs that biotransform and excrete ammonia, urea cycle disorders, and other conditions such as fatty acid oxidation defects and Reye syndrome ([Bürki et al., 2015](#); [Auron and Brophy, 2012](#); [Romero-Gómez et al., 2004](#); [Córdoba et al., 1998](#); [Davies et al., 1997](#); [Schubiger et al., 1991](#); [Gilbert, 1988](#); [Jeffers et al., 1988](#); [Souba, 1987](#)). Elevated ammonia levels can predispose an individual to encephalopathy as a result of the ability of ammonia to cross the blood-brain barrier and subsequent disturbances in amino acid synthesis and alterations in neurotransmission systems. Neonates and infants are particularly susceptible to the neurological effects of elevated levels of ammonia; hyperammonemia can cause irreparable damage to the developing brain ([Auron and Brophy, 2012](#); [Miñana et al., 1995](#); [Souba, 1987](#)). While patients with hyperammonemia could plausibly be considered a susceptible population, there are no studies that specifically support this hypothesized susceptibility.

2. DOSE-RESPONSE ANALYSIS

2.1. INHALATION REFERENCE CONCENTRATION FOR EFFECTS OTHER THAN CANCER

The reference concentration (RfC; expressed in units of mg/m³) is defined as an estimate (with uncertainty spanning perhaps an order of magnitude) of a continuous inhalation exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. It can be derived from a no-observed-adverse-effect level (NOAEL), lowest-observed-adverse-effect level (LOAEL), or the 95% lower bound on the benchmark concentration (BMCL), with uncertainty factors (UFs) generally applied to these points of departure (PODs) to reflect limitations of the data used.

2.1.1. Identification of Studies and Effects for Dose-Response Analysis

As discussed in Section 1.2, the respiratory system is the primary and most sensitive target of inhaled ammonia in humans and experimental animals, and respiratory effects have been identified as a hazard following inhalation exposure to ammonia. The experimental toxicology literature for ammonia provides evidence that inhaled ammonia may be associated with toxicity to target organs other than the respiratory system, including the liver, kidney, spleen, heart, and immune system. Effects in these other (nonrespiratory) target organs were not considered as the basis for RfC derivation because the evidence for these associations is weak relative to that for respiratory effects.

Respiratory effects, characterized as increased prevalence of respiratory symptoms or decreased lung function, have been observed in worker populations exposed to ammonia concentrations ≥ 18.5 mg/m³ ([Rahman et al., 2007](#); [Ali et al., 2001](#); [Ballal et al., 1998](#)). Decrements in lung function parameters and increased prevalence of respiratory symptoms, such as wheezing, chest tightness, and cough/phlegm, have been identified as adverse respiratory health effects by the American Thoracic Society ([ATS, 2000](#)) and are similarly noted as adverse in the Environmental Protection Agency (EPA) *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* ([U.S. EPA, 1994](#)). At the population level, [ATS \(2000\)](#) stated that “any detectable level of permanent pulmonary function loss attributable to air pollution exposure should be considered as adverse” and that:

“It should be emphasized that a small but significant reduction in a population mean FEV₁ or FEV_{0.75} is probably medically significant, as such a difference may indicate an increase in the number of persons with respiratory impairment in the population. In other words, a small part of the population may manifest a marked

change that is medically significant to them, but when diluted with the rest of the population the change appears to be small ([ATS, 2000](#)).”

Thus, even a small change in the average (mean) of a distribution of pulmonary function parameters is considered adverse for purposes of deriving an RfC.

In general, human data are preferred over animal data for deriving reference values because these data are more relevant for assessing human health effects than animal studies and avoid the uncertainty associated with interspecies extrapolation when animal data serve as the basis for the RfC. In the case of ammonia, the available occupational studies provide adequate data for the quantitative analysis of health outcomes considered relevant to potential general population exposures. Respiratory effects have also been observed in animals, but at ammonia concentrations higher than those associated with respiratory effects in humans and in studies involving exposure durations (up to 114 days) shorter than those in occupational studies (Section 1.2.1). Therefore, data on respiratory effects in humans were used for the derivation of the RfC and respiratory effects in animals were not further considered.

Of the available human data, associations between ammonia exposure and respiratory effects have been examined in epidemiology studies of industrial worker populations (Table 1-2), in studies of ammonia exposure in a cleaning setting (Table 1-3), and in studies of populations in agricultural settings. Studies using ammonia as a cleaning product provide evidence of an association between ammonia exposure and an increased risk of asthma; however, these studies did not measure ammonia concentrations and are thus not useful for dose-response analysis. Studies in agricultural settings also support an association between ammonia exposure and decreased pulmonary function; however, because of co-exposures to other agents (including dust, endotoxin, mold, and disinfectant products) and the availability of studies with fewer co-exposures, studies in agricultural settings were considered to be supportive of the association between ammonia exposure and respiratory effects, but were not carried forward for dose-response analysis. In addition, several controlled-exposure studies in volunteers evaluated the effects of ammonia on irritation and lung function following acute exposures. These human exposure studies have several methodological strengths compared to epidemiological studies of worker populations, including well-characterized exposures and resistance to confounding; however, the short exposure durations used in these studies (i.e., 15 seconds to 6 hours) make them inappropriate for evaluating the effects of chronic exposure to ammonia.

Of the available studies of ammonia exposure in industrial settings, four cross-sectional epidemiology studies of industrial worker populations—three studies in urea fertilizer plants by [Rahman et al. \(2007\)](#), [Ballal et al. \(1998\)](#), and [Ali et al. \(2001\)](#), and a study in a soda ash plant by [Holness et al. \(1989\)](#)—provide information useful for examining the relationship between chronic ammonia exposure and increased prevalence of respiratory symptoms and/or decreased lung function. [Bhat and Ramaswamy \(1993\)](#) evaluated lung function in ammonia plant workers, but did

not measure ammonia concentrations in workplace air. Therefore, this study was not considered useful for RfC derivation.

In general, these four cross-sectional occupational studies provide a coherent set of estimated NOAELs and effect levels, and are considered candidate principal studies for RfC derivation. A brief description of these studies and the contribution of each to the understanding of the dose-response relationship between ammonia exposure and respiratory effects follow. More study details are provided in the Supplemental Information, Section C.2.1 and in Table 1-2, and evaluation of the strengths and limitations are more fully considered in the Literature Search Strategy | Study Selection and Evaluation section.

- [Rahman et al. \(2007\)](#) observed an increased prevalence of respiratory symptoms (coughing, chest tightness) in urea fertilizer plant workers (mean employment duration: 16 years) exposed to a mean ammonia concentration of 18.5 mg/m³ (range: 9–31 mg/m³), but not in workers in a second plant exposed to a mean ammonia concentration of 4.9 mg/m³ (range: 2–8 mg/m³). Decrements in lung function (forced vital capacity [FVC] and forced expiratory volume in 1 second [FEV₁]) between pre- and post-shift in the high-exposure group (2–3%) were statistically significant. Exposure was measured by personal samples using two different analytical methods.
- [Ballal et al. \(1998\)](#) observed an increased prevalence of respiratory symptoms (cough, phlegm, wheezing, and dyspnea) among urea fertilizer factory workers (mean employment duration: 4.3 years) in one factory (Factory A) with ammonia exposures ranging from 2 to 27.1 mg/m³,¹¹ but no increase in symptoms in another factory (Factory B) with exposures ranging from 0.02 to 7 mg/m³. Lung function was not measured.
- A companion study by [Ali et al. \(2001\)](#) examined lung function among workers in Factory A from [Ballal et al. \(1998\)](#); respiratory symptoms were not evaluated. Workers with cumulative exposure >50 mg/m³-years had significantly lower lung function values (declines of 5–7% in FVC% predicted and FEV₁% predicted) than workers with cumulative exposure <50 mg/m³-years. In this and the [Ballal et al. \(1998\)](#) study, exposure was measured by air monitors.
- [Holness et al. \(1989\)](#) found no differences in the prevalence of respiratory symptoms or lung function between soda ash plant workers (mean exposure: 6.5 mg/m³; mean exposure duration: 12.2 years) and the control group, and also no differences in respiratory symptoms or lung function when workers were stratified by ammonia exposure level (lowest exposure group, <4.4 mg/m³; middle exposure group, 4.4–8.8 mg/m³; highest exposure group, >8.8 mg/m³). Exposure was measured by personal samples. EPA identified the concentration range for the high-exposure group (i.e., >8.8 mg/m³) as the NOAEL from this study. The authors stated that 3 of the 12 workers in the high-exposure group were exposed to concentrations >17.7 mg/m³; therefore, the majority of workers in

¹¹This concentration range does not include exposures in the urea store (number of employees = 6; range of ammonia concentrations = 90–130.4 mg/m³) because employees in this area were required to wear full protective clothing, thus minimizing potential exposure.

the high-exposure group (9 of 12) would have been exposed to ammonia concentrations in the range of 8.8–17.7 mg/m³.

In selecting the principal study for RfC derivation, consideration was given to exposure measures, assessment of outcomes, potential for co-exposures, and the value of the NOAEL. Of the four candidate principal studies, higher confidence was associated with the exposure measures from [Holness et al. \(1989\)](#). Both [Holness et al. \(1989\)](#) and [Rahman et al. \(2007\)](#) collected personal air samples, but confidence in the analytical method used by [Holness et al. \(1989\)](#) is higher than that used by [Rahman et al. \(2007\)](#). [Rahman et al. \(2007\)](#) used two analytical methods for measuring ammonia concentrations in workplace air (i.e., Dräger PAC III and Dräger tube); concentrations measured by the two methods differed by 4–5-fold, indicating some uncertainty across the two measurement methods, although ammonia concentrations measured by the two methods were strongly correlated (correlation coefficient of 0.8). In contrast, the [Holness et al. \(1989\)](#) study used an established analytical method for measuring exposure to ammonia recommended by the National Institute for Occupational Safety and Health (NIOSH) that involved the collection of air samples on acid-treated silica gel absorption tubes. [Ballal et al. \(1998\)](#) used area monitors rather than personal air sampling methods; the latter method provides a better estimate of an individual's exposure.

As discussed in the Literature Search Strategy | Study Selection and Evaluation section, assessment of respiratory symptoms in all studies that measured this outcome was based on self-reporting by questionnaire, and assessment of lung function was performed using standard spirometry protocols. While considered unlikely, nonblinded outcome assessments of respiratory symptoms could introduce bias. Therefore, both [Holness et al. \(1989\)](#) and [Rahman et al. \(2007\)](#), the two studies of industrial populations that examined both respiratory symptoms and lung function, provide stronger evidence of respiratory effects than studies that evaluated symptom data only (notably [Ballal et al. \(1998\)](#)).

Also as discussed in the Literature Search Strategy | Study Selection and Evaluation section, confounding by other workplace exposures is a potential concern, although not likely to be a major limitation of the studies considered for dose-response analysis. Only [Rahman et al. \(2007\)](#) measured another workplace chemical (nitrogen dioxide; below detection limits); other studies did not describe potential co-exposures. Therefore, a more rigorous examination of the potential for confounding by co-exposure to other workplace chemicals could not be performed. [Holness et al. \(1989\)](#) noted the high level of control of exposures in the facility used in their study, resulting in low ammonia levels.

Three of the four occupational studies supported the identification of a NOAEL (or, more correctly, an exposure range not associated with an increase in respiratory effects). [Rahman et al. \(2007\)](#) did not observe a change in respiratory effects in workers exposed to a mean ammonia concentration of 4.9 mg/m³ (range: 2–8 mg/m³). [Holness et al. \(1989\)](#) found no differences in respiratory effects in soda ash plant workers when compared to a control group or when workers

were stratified by exposure level (low, medium, and high); the concentration range for the high-exposure group (i.e., $>8.8 \text{ mg/m}^3$) was identified as the NOAEL. [Ballal et al. \(1998\)](#) reported no increase in respiratory symptoms in a factory with exposures ranging from 0.02 to 7 mg/m^3 . Because [Ali et al. \(2001\)](#), the companion study to [Ballal et al. \(1998\)](#), evaluated only workers in a second factory with higher exposures, study findings did not support the identification of an estimated NOAEL.

In light of the above considerations, overall confidence in the [Holness et al. \(1989\)](#) study as the principal study for RfC derivation was higher than other candidate studies in terms of: measurement of ammonia exposure, evaluation of both respiratory symptoms and lung function parameters, smaller potential for co-exposures to other workplace chemicals, and the fact that the estimated NOAEL for respiratory effects of $\geq 8.8 \text{ mg/m}^3$ was the highest of the NOAELs estimated from the candidate principal studies. The [Holness et al. \(1989\)](#) study does not demonstrate a relationship between ammonia exposure and respiratory effects. The relationship between ammonia exposure and respiratory effects is based on the body of evidence, and the [Holness et al. \(1989\)](#) study is identified as the principal study for derivation of the RfC for the reasons given above.

In summary, the occupational study of ammonia exposure in workers in a soda ash plant by [Holness et al. \(1989\)](#) was identified as the principal study for RfC derivation, with support from [Rahman et al. \(2007\)](#), [Ballal et al. \(1998\)](#), and [Ali et al. \(2001\)](#), and respiratory effects were identified as the critical effect.

2.1.2. Methods of Analysis

A NOAEL of 13.6 mg/m^3 , or an estimate of the lower confidence bound of the mean exposure concentration in the high-exposure group of the [Holness et al. \(1989\)](#) study, was used as the POD for RfC derivation. The POD for respiratory effects was based on the NOAEL representing the high-exposure group in [Holness et al. \(1989\)](#). The individual subject data from this study were no longer available (call from S. Rieth, EPA, to C. Clayton, administrative assistant to Dr. Holness, St. Michael's Hospital, Center for Research Expertise in Occupational Health, Toronto, Canada, February 11, 2015), so that the mean exposure in the high-exposure group could not be calculated precisely based on the data. Therefore, the mean was estimated assuming that the data in the study followed a skewed probability distribution, specifically the lognormal distribution. The frequency distribution provided in [Holness et al. \(1989\)](#) (see Table 2-1) was used to estimate the parameters (log-scale mean and standard deviation) of the lognormal distribution that best fit the data.

Table 2-1. Frequency distribution of ammonia exposure from [Holness et al. \(1989\)](#)

Exposure group	Interval of exposures (mg/m ³)	Interval of exposures (ppm)	Number of exposed workers
Low	0–4.4	0–6.25	34
Medium	4.4–8.8	6.25–12.5	12
High ^a	8.8–17.7	12.5–25	9
	>17.7	>25	3

^aEPA divided the high-exposure group into two subgroups based on the statement in [Holness et al. \(1989\)](#): “Three workers were exposed to TWA concentrations of ammonia in excess of 25 ppm, the current exposure guideline.”

TWA = time-weighted average

Lognormal parameter estimates were obtained by applying the maximum likelihood method to this frequency distribution. Using the estimated distribution defined by these parameter estimates, the estimated mean exposure in the high-exposure group and 95% lower confidence bound on this mean were calculated as follows (see Appendix C, Section C.4 for detailed documentation of this calculation):

mean exposure estimate (high-exposure group) = 17.9 mg/m³

95% lower confidence bound on this mean (high-exposure group) = 13.6 mg/m³

The lower confidence bound of 13.6 mg/m³ was used as the POD for respiratory effects.

Because the RfC assumes continuous human exposure over a lifetime, the POD was adjusted to account for the noncontinuous exposure associated with occupational exposure (i.e., 8-hour workday and 5-day workweek). Cross-shift data for FVC and FEV₁ from the [Rahman et al. \(2007\)](#) study provide some evidence of an immediate effect of ammonia exposure on lung function¹², which could argue against adjustment from noncontinuous to continuous exposure; however, [Rahman et al. \(2007\)](#) also reported that duration of exposure (using years of employment as a proxy for exposure duration) was significantly associated with percentage cross-shift decrease in FEV₁%. In addition, [Ballal et al. \(1998\)](#) found a significant correlation between respiratory symptoms (cough, phlegm, and wheezing) and duration of service (a proxy for exposure duration). In the absence of clear evidence that respiratory effects in occupationally-exposed populations are an acute response, and given evidence for contributions of exposure duration (cumulative exposure) to the

¹²[Rahman et al. \(2007\)](#) reported that mean pre-shift FVC and FEV₁ values in ammonia and urea plants workers were similar, suggesting similar lung function in low- and high-exposure workers upon arrival at work. Cross-shift changes in FVC and FEV₁ were statistically significant decreased in the urea plant (more highly-exposed) workers only.

respiratory effects of ammonia, the standard adjustment to continuous exposure was applied. The duration-adjusted POD was calculated as follows:

$$\begin{aligned}\text{NOAEL}_{\text{ADJ}} &= \text{NOAEL} \times \text{VEho}/\text{VEh} \times 5 \text{ days}/7 \text{ days} \\ &= 13.6 \text{ mg/m}^3 \times 10 \text{ m}^3/20 \text{ m}^3 \times 5 \text{ days}/7 \text{ days} \\ &= 4.9 \text{ mg/m}^3 \text{ or } 5 \text{ mg/m}^3 \text{ (rounded)}\end{aligned}$$

Where:

VEho = human occupational default minute volume (10 m³ breathed during an 8-hour workday) ([U.S. EPA, 1994](#)). This inhalation rate corresponds to more current inhalation rates for light to moderate activity levels from [U.S. EPA \(2009c\)](#), as cited in [U.S. EPA \(2011a\)](#). An occupational inhalation rate of 10.8 m³ for an 8-hour workday, similar to the default value from [U.S. EPA \(1994\)](#), can be derived as an average of activity-specific inhalation rates for males, in age groups of 21–60 years, for combined light and moderate activity from Table 6-17 of [U.S. EPA \(2011a\)](#). The average inhalation rate of 1.3 m³/hour (0.022 m³/minute) can be multiplied by 8 hours to obtain an inhalation rate of 10.8 m³/8-hour workday.

VEh = human ambient default minute volume (20 m³ breathed during the entire day) ([U.S. EPA, 1994](#)). This value is consistent with the average of the daily average inhalation rates for males, in age groups of 21–60 years, of 20.2 m³/day, from [U.S. EPA \(2009c\)](#), as summarized in Table 6-14 of [U.S. EPA \(2011a\)](#).

2.1.3. Derivation of the Reference Concentration

Consistent with EPA's *A Review of the Reference Dose and Reference Concentration Processes* ([U.S. EPA, 2002; Section 4.4.5](#)), also described in the Preamble, five possible areas of uncertainty and variability were considered when deriving the RfC. A **composite UF of 10** was applied to the selected duration-adjusted POD of 4.9 mg/m³ to derive the RfC of 0.5 mg/m³. An explanation of the five possible areas of uncertainty and variability follows:

- An intraspecies uncertainty factor, UF_H, of 10 was applied to account for potentially susceptible individuals in the absence of data evaluating variability of response to inhaled ammonia in the human population;
- An interspecies uncertainty factor, UF_A, of 1 was applied to account for uncertainty in extrapolating from laboratory animals to humans because the POD was based on human data from an occupational study;
- A subchronic to chronic uncertainty factor, UF_S, of 1 was applied because the occupational exposure period in the principal study ([Holness et al., 1989](#)), defined as the mean number of years at the present job for exposed workers, of approximately 12 years was considered to be of chronic duration;
- An uncertainty factor for extrapolation from a LOAEL to a NOAEL, UF_L, of 1 was applied because a NOAEL was used as the POD; and

- A database uncertainty factor, UF_D , of 1 was applied to account for deficiencies in the database. As discussed in Section 1.2, available epidemiological studies include studies of workers exposed in industrial settings, in agriculture, or through use of cleaning products. There are also controlled human exposure studies involving short-duration exposure to ammonia vapors and many case reports of acute exposures to high concentrations. Available animal studies include subchronic studies that investigated respiratory and systemic effects in rats, guinea pigs, and pigs. There are also several immunotoxicity studies and one limited reproductive toxicity study in young female pigs. The database lacks developmental and multigenerational reproductive toxicity studies. The EPA's review of RfD and RfC processes ([U.S. EPA, 2002](#)) states that "the size of the database factor to be applied will depend on other information in the database and on how much impact the missing data may have on determining the toxicity of a chemical and, consequently, the POD." [U.S. EPA \(2002\)](#) also states,

"If data from the available toxicology studies raise suspicions of developmental toxicity and signal the need for developmental data on specific organ systems (e.g., detailed nervous system, immune system, carcinogenesis, or endocrine system), then the database factor should take into account whether or not these data are available and used in the assessment and their potential to affect the POD . . ."

Although the database lacks developmental and multigeneration reproductive toxicity studies, these studies are not expected to impact the determination of ammonia toxicity at the POD. Accordingly, a database UF to account for the lack of these studies is not considered necessary. This determination is based on the observation that ammonia is produced endogenously in all human and animal tissues during all lifestages, including the prenatal period. Ammonia produced within the uteroplacenta is released into the fetal and maternal circulations (see Appendix C, Section C.1.2). As discussed in Section 1.2.3, concentrations in fetal circulation are higher than maternal blood concentrations, by as much as 50% to 3-fold ([Jóźwik et al., 2005](#); [DeSanto et al., 1993](#)). Further, human fetal umbilical blood levels of ammonia at birth were not influenced by gestational age based on deliveries ranging from gestation week 25 to 43 ([DeSanto et al., 1993](#)). This evidence provides some assurance that endogenous ammonia concentrations in the fetus are similar to other lifestages, and that baseline ammonia concentrations would not be associated with developmental toxicity. Additionally, evidence in animals ([Manninen et al., 1988](#); [Schaerdel et al., 1983](#)) suggests that exposures to ammonia at concentrations up to 18 mg/m³ do not produce a statistically significant change in blood or brain ammonia concentrations (see Section 1.2.3 and Appendix C, Section C.1.3). Accordingly, any changes in blood ammonia concentrations at the duration-adjusted POD (3.1 mg/m³) would be small relative to levels normally present in blood. The likelihood of developmental or reproductive effects at these concentrations, while they cannot be ruled out, is considered small.

The RfC for ammonia was calculated as follows:

$$\begin{aligned}\text{RfC} &= \text{NOAEL}_{\text{ADJ}} \div \text{UF} \\ &= 4.9 \text{ mg/m}^3 \div 10 \\ &= 0.49 \text{ mg/m}^3 \text{ or } \mathbf{0.5 \text{ mg/m}^3 \text{ (rounded to one significant figure)}}\end{aligned}$$

2.1.4. Uncertainties in the Derivation of the Reference Concentration

As presented earlier in this section and in the Preamble, EPA standard practices and RfC guidance ([U.S. EPA, 2002, 1995, 1994](#)) were followed in applying a UF approach to a POD (from a NOAEL) to derive the RfC. Specific uncertainties were accounted for by the application of UFs (i.e., in the case of the ammonia RfC, a factor to address the absence of data to evaluate the variability in response to inhaled ammonia in the human population). The following discussion identifies additional uncertainties associated with the quantification of the RfC for ammonia.

Use of a NOAEL as a POD

Data sets that support benchmark dose modeling are generally preferred for reference value derivation because the shape of the dose-response curve can be taken into account in establishing the POD. For the ammonia RfC, no decreases in lung function or increases in the prevalence of respiratory symptoms were observed in the worker population studied by [Holness et al. \(1989\)](#) (i.e., the principal study used to derive the RfC, and as such, the data from this study did not support dose-response modeling). Rather, a NOAEL from the [Holness et al. \(1989\)](#) study was used to estimate the POD. The availability of dose-response data from a study of ammonia, especially in humans, would increase the confidence in the estimation of the POD.

Comparison of Exhaled Ammonia to the RfC

Ammonia is generated endogenously in multiple organs, including the liver, kidneys, intestines, brain, and skeletal muscle, as a product of amino acid catabolism. Ammonia plays central roles in nitrogen balance and acid-base homeostasis ([Weiner et al., 2014](#); [Weiner and Verlander, 2013](#)). Given its important metabolic role, free ammonia is homeostatically regulated to remain at low concentrations in blood ([Souba, 1987](#)). Elimination of ammonia occurs primarily in urine and exhaled breath. (See Appendix C, Section C.1.3 for additional information on production and regulation of endogenous ammonia.)

Further consideration was given to the presence of ammonia in exhaled air because the range of ammonia concentrations in exhaled breath overlaps the ammonia RfC. Specifically, ammonia has been measured in exhaled breath at concentrations ranging from 0.009 to 2 mg/m³ (see Appendix C, Table C-1), a range that exceeds the RfC of 0.5 mg/m³. This section reviews information related to the exhalation of ammonia that provides context for this comparison.

In general, higher and more variable ammonia concentrations are reported in human breath exhaled from the mouth or oral cavity. Investigators reported concentrations ranging from 0.03 to 2 mg/m³, with the majority of concentrations ≥ 0.2 mg/m³ ([Schmidt et al., 2013](#); [Smith et al., 2008](#); [Španěl et al., 2007a, b](#); [Turner et al., 2006](#); [Diskin et al., 2003](#); [Smith et al., 1999](#); [Norwood et al.,](#)

[1992](#); [Larson et al., 1977](#)). Ammonia concentrations measured in breath derived from oral breathing largely reflect the production of ammonia via bacterial degradation of food protein in the oral cavity or gastrointestinal tract ([Turner et al., 2006](#); [Smith et al., 1999](#); [Vollmuth and Schlesinger, 1984](#)). Ammonia concentrations from exhaled breath can be influenced by factors such as diet, oral hygiene, and age ([Solga et al., 2013](#); [Španěl et al., 2007a, b](#); [Turner et al., 2006](#); [Diskin et al., 2003](#); [Norwood et al., 1992](#)). [Schmidt et al. \(2013\)](#) reported that ammonia concentrations in breath from the mouth strongly depended on saliva pH.

Concentrations of ammonia in breath exhaled from the nose and trachea of humans (0.0092–0.1 mg/m³) are lower than those in air exhaled from the mouth ([Schmidt et al., 2013](#); [Smith et al., 2008](#); [Larson et al., 1977](#)). Whereas the upper end of the range of ammonia concentrations in mouth breath exceeds the RfC of 0.5 mg/m³, concentrations from the nose and trachea are generally lower than the ammonia RfC by a factor of five or more. Ammonia concentrations in breath exhaled from the nose appear to better represent levels at the alveolar interface of the lung and are thought to be more relevant to understanding systemic levels of ammonia than breath exhaled from the mouth ([Schmidt et al., 2013](#); [Smith et al., 2008](#)). Nevertheless, the relationship between nose ammonia concentrations and systemic levels is complicated by the possibility that nose ammonia concentrations are still influenced by the oral cavity (e.g., in individuals with the soft palate incompletely closed) and tracheobronchial fluids that, like saliva, can influence the airway concentration of ammonia. Further, measurements of exhaled ammonia reported in the literature were generally not conducted in ammonia-free environments, and the ammonia in inhaled air may thus account for some of the ammonia measured in exhaled air (e.g., see [Španěl et al. \(2013\)](#)).

Thus, ammonia concentrations in exhaled breath, and particularly those exhaled through the mouth, are not correlated with blood ammonia; factors identified as influencing exhaled ammonia concentrations include bacterial populations in the oral cavity, salivary pH, diet, oral hygiene, and age (see Appendix C, Section C.1.4). Concentration in breath cannot be used to predict blood ammonia concentration or previous exposure to environmental (ambient) concentrations of ammonia.

Regardless, the level of ammonia in breath, even at concentrations that exceed the RfC, does not necessarily raise questions about the appropriateness of the RfC. The exhalation of ammonia is a clearance mechanism for a product of metabolism that is otherwise toxic in the body at sufficiently high concentrations. Ammonia concentrations in exhaled breath may be higher than inhaled concentrations, particularly when compared to exhaled air from the mouth or oral cavity. However, the fact that humans may exhale ammonia at concentrations higher than 0.5 mg/m³ (i.e., the RfC) is not considered an uncertainty in the RfC.

Consideration of Tolerance and the Healthy Worker Effect on Selection of the POD

As discussed in Section 1.2.1, two controlled-exposure studies provide some evidence of habituation to eye, nose, and throat irritation in volunteers after repeated ammonia exposure. Following exposure to ammonia at concentrations ranging from 7 to 35 mg/m³ for 4 hours/day on 5 consecutive days, [Ihrig et al. \(2006\)](#) reported higher mean intensities for irritative, olfactory, and respiratory symptoms in male volunteers unfamiliar with ammonia when compared to male chemical company workers exposed to ammonia vapor for several years in a urea department; differences were statistically significant only for olfactory symptoms. In a more limited study with only four male volunteers each exposed to 18, 35, or 71 mg/m³ ammonia (exposure to each concentration was for 1 week, 2–6 hours/day, 5 days/week; individuals were exposed to each concentration twice), fewer occurrences of irritation were reported during week 2 than during week 1 at the same exposure concentration ([Ferguson et al., 1977](#)). However, in the same [Ferguson et al. \(1977\)](#) study, the occurrences of irritation in two individuals exposed to 50 ppm for 6 hours/day, 5 days/week for 6 weeks were variable from week to week and did not show any clear trend. The study by [Ihrig et al. \(2006\)](#), and to a lesser extent the study by [Ferguson et al. \(1977\)](#), provide some evidence of decreased irritation following repeated exposure; the results of [Ihrig et al. \(2006\)](#) may also be influenced by attrition out of the workforce of those most affected by the irritation symptoms. These studies raise the possibility that repeated exposure could lead to the development of tolerance to ammonia (i.e., to decreased sensory responsiveness). It is possible, therefore, that industrially-exposed populations considered in deriving the RfC for ammonia (i.e., [Holness et al. \(1989\)](#), [Rahman et al. \(2007\)](#), [Ballal et al. \(1998\)](#), and [Ali et al. \(2001\)](#)) may have developed some degree of tolerance to ammonia, and may underpredict responses to ammonia that would be observed in the general population. The magnitude of tolerance, if any, cannot be estimated from the available studies.

In addition, as discussed in the Literature Search Strategy | Study Selection and Evaluation section, the workers in the cross-sectional occupational studies used to derive the RfC were healthy enough to remain in the plant for a considerable time; mean employment duration ranged from 52 months to 18 years. In general, studies in these populations may result in a “healthy worker survivor” bias and in an underestimate of the risk of health effects of ammonia exposure, as a healthy worker population may not exhibit health effects (such as decreased lung function or increased prevalence of respiratory symptoms) to the same degree that would be seen in the general population under the same conditions.

Therefore, there is potential for tolerance development in populations exposed occupationally to ammonia and “healthy worker” bias, both of which may result in underestimation of the general population response. However, the evidence is limited and not conclusive, and thus, does not warrant increasing the intraspecies uncertainty factor.

2.1.5. Confidence Statement

A confidence level of high, medium, or low is assigned to the study used to derive the RfC, the overall database, and the RfC itself, as described in Section 4.3.9.2 of EPA's *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* ([U.S. EPA, 1994](#)). Confidence in the principal study ([Holness et al., 1989](#)) is medium. The design, conduct, and reporting of this occupational exposure study were adequate, but the study was limited by a small sample size and by the fact that workplace ammonia concentrations to which the study population was exposed were below those associated with ammonia-related effects (i.e., only a NOAEL was identified). However, the results from the principal study are supported by the results from other cross-sectional studies of workers in industrial settings, studies of ammonia exposure in a cleaning setting, studies in agricultural settings, multiple studies of acute ammonia exposure in volunteers, and available inhalation data from animals.

Confidence in the database is medium. The ammonia database includes a number of epidemiological studies of workers exposed in different occupational settings, and multiple repeat-exposure experimental animal studies, the majority from the older toxicological literature. The inhalation ammonia database includes one limited study of reproductive and developmental toxicity in pigs that did not examine a complete set of reproductive or developmental endpoints. Normally, confidence in a database lacking reproductive and developmental toxicity studies is considered to be lower due to the uncertainty surrounding the use of any one or several studies to adequately address all potential endpoints following chemical exposure at critical lifestages. Unless a comprehensive array of endpoints is addressed by the database, there is uncertainty as to whether the critical effect chosen for RfC derivation is the most sensitive or appropriate. However, the likelihood of reproductive, developmental, and other systemic effects at the RfC is considered small because it is well documented that ammonia is endogenously produced in humans and animals, and any changes in blood ammonia levels at the POD would be small relative to normal blood ammonia levels. Further, EPA is not aware of any mechanisms by which effects at the point of contact (i.e., respiratory system) could directly or indirectly impact tissues or organs distal to the point of contact. Thus, confidence in the database, in the absence of these types of studies, is medium.

Reflecting medium confidence in the principal study and medium confidence in the database, the overall confidence in the RfC is medium.

2.1.6. Previous IRIS Assessment

The previous Integrated Risk Information System (IRIS) assessment for ammonia (posted to the database in 1991) presented an RfC of 0.1 mg/m³ based on co-principal studies—the occupational exposure study of workers in a soda ash plant by [Holness et al. \(1989\)](#) and the subchronic study by [Broderson et al. \(1976\)](#) that examined the effects of ammonia exposure in F344 rats inoculated on day 7 of the study with the bacterium, *Mycoplasma pulmonis*. The NOAEL

of 6.4 mg/m³ (estimated as the mean concentration of the entire exposed group) from the [Holness et al. \(1989\)](#) study (duration adjusted: NOAEL_{ADJ} = 2.3 mg/m³) was used as the POD.¹³

The previous RfC was derived by dividing the exposure-adjusted POD of 2.3 mg/m³ (from a NOAEL of 6.4 mg/m³) by a composite UF of 30: 10 to account for the protection of sensitive individuals and 3 for database deficiencies to account for the lack of chronic data, the proximity of the LOAEL from the subchronic inhalation study in the rat ([Broderson et al., 1976](#)) to the NOAEL, and the lack of reproductive and developmental toxicity studies. A UF_D of 3 (rather than 10) was applied because studies in rats ([Schaerdel et al., 1983](#)) showed no increase in blood ammonia levels at an inhalation exposure up to 32 ppm (22.6 mg/m³) and only minimal increases at 300–1,000 ppm (212–707 mg/m³), suggesting that no significant distribution is likely to occur at the human equivalent concentration.

¹³In this document, the lower confidence bound of the estimated mean exposure concentration in the high-exposure group from the [Holness et al. \(1989\)](#) study (13.6 mg/m³, adjusted for continuous exposure to 4.9 mg/m³) was identified as the POD because workers in this high-exposure group, as well as those in the two lower-exposure groups, showed no statistically significant increase in the prevalence of respiratory symptoms or decreases in lung function.

REFERENCES

Multiple references published in the same year by the same author(s) have been assigned a letter (e.g., 1986a, 1986b) based on author(s), year, and title. Those same letters have been retained for the appendices.

- [Abdel Hamid, HA; El-Gazzar, RM.](#) (1996). Effect of occupational exposure to ammonia on enzymatic activities of catalase and mono amine oxidase. *J Egypt Public Health Assoc* 71: 465-475.
- [AIChE](#) (American Institute of Chemical Engineers). (1999). Ammonia H3N. In TE Daubert; RP Danner; HM Sibul; CC Stebbins (Eds.), *Physical and thermodynamic properties of pure chemicals: Evaluated process design data*. Philadelphia, PA: Taylor & Francis.
- [Ali, BA; Ahmed, HO; Ballal, SG; Albar, AA.](#) (2001). Pulmonary function of workers exposed to ammonia: A study in the Eastern Province of Saudi Arabia. *Int J Occup Environ Health* 7: 19-22. <http://dx.doi.org/10.1179/oeh.2001.7.1.19>
- [AMoN](#) (Ammonia Monitoring Network). (2012). Ammonia Monitoring Network. Champaign, IL: National Atmospheric Deposition Program (NADP). <http://nadp.sws.uiuc.edu/amon/>
- [Amshel, CE; Fealk, MH; Phillips, BJ; Caruso, DM.](#) (2000). Anhydrous ammonia burns case report and review of the literature [Review]. *Burns* 26: 493-497.
- [Anderson, DP; Beard, CW; Hanson, RP.](#) (1964). The adverse effects of ammonia on chickens including resistance to infection with Newcastle disease virus. *Avian Dis* 8: 369-379. <http://dx.doi.org/10.2307/1587967>
- [Andreasen, M; Baekbo, P; Nielsen, JP.](#) (2000). Lack of effect of aerial ammonia on atrophic rhinitis and pneumonia induced by *Mycoplasma hyopneumoniae* and toxigenic *Pasteurella multocida*. *J Vet Med B* 47: 161-171. <http://dx.doi.org/10.1046/j.1439-0450.2000.00335.x>
- [Arif, AA; Delclos, GL.](#) (2012). Association between cleaning-related chemicals and work-related asthma and asthma symptoms among healthcare professionals. *Occup Environ Med* 69: 35-40. <http://dx.doi.org/10.1136/oem.2011.064865>
- [Atkinson, SA; Anderson, GH; Bryan, MH.](#) (1980). Human milk: Comparison of the nitrogen composition in milk from mothers of premature and full-term infants. *Am J Clin Nutr* 33: 811-815.
- [ATS](#) (American Thoracic Society). (2000). What constitutes an adverse health effect of air pollution? *Am J Respir Crit Care Med* 161: 665-673. <http://dx.doi.org/10.1164/ajrccm.161.2.ats4-00>
- [ATSDR](#) (Agency for Toxic Substances and Disease Registry). (2004). Toxicological profile for ammonia [ATSDR Tox Profile]. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service. <http://www.atsdr.cdc.gov/toxprofiles/tp.asp?id=11&tid=2>
- [Auron, A; Brophy, PD.](#) (2012). Hyperammonemia in review: Pathophysiology, diagnosis, and treatment [Review]. *Pediatr Nephrol* 27: 207-222. <http://dx.doi.org/10.1007/s00467-011-1838-5>
- [Bacom, A; Yanosky, M.](#) (2010). E-mails dated June 22, 2010, from Michael Yanosky, Dräger Safety Inc., Technical Support Detection Products to Amber Bacom, SRC, Inc., contractor to NCEA, ORD, U.S. EPA. Available online
- [Ballal, SG; Ali, BA; Albar, AA; Ahmed, HO; Al-Hasan, AY.](#) (1998). Bronchial asthma in two chemical fertilizer producing factories in eastern Saudi Arabia. *Int J Tuberc Lung Dis* 2: 330-335.

- Betterton, EA. (1992). Henry's law constants of soluble and moderately soluble organic gases: Effects in aqueous phase chemistry. In JO Nriagu (Ed.), Gaseous pollutants : Characterization and cycling (pp. 1-50). New York, NY: Wiley.
<http://www.wiley.com/WileyCDA/WileyTitle/productCd-0471548987.html>
- Bhat, MR; Ramaswamy, C. (1993). Effect of ammonia, urea and diammonium phosphate (DAP) on lung functions in fertilizer plant workers. *Indian J Physiol Pharmacol* 37: 221-224.
- Broderson, JR; Lindsey, JR; Crawford, JE. (1976). The role of environmental ammonia in respiratory mycoplasmosis of rats. *Am J Pathol* 85: 115-130.
- Bürki, R; Mohebbi, N; Bettoni, C; Wang, X; Serra, AL; Wagner, CA. (2015). Impaired expression of key molecules of ammoniogenesis underlies renal acidosis in a rat model of chronic kidney disease. *Nephrol Dial Transplant* 30: 770-781. <http://dx.doi.org/10.1093/ndt/gfu384>
- Callicutt, CH; Cox, RH; Hsu, F; Kinser, RD; Laffoon, SW; Lee, PN; Podraza, KF; Sanders, EB; Seeman, JL. (2006). The role of ammonia in the transfer of nicotine from tobacco to mainstream smoke. *Regul Toxicol Pharmacol* 46: 1-17. <http://dx.doi.org/10.1016/j.yrtph.2006.05.008>
- Casas, L; Zock, JP; Torrent, M; García-Esteban, R; Gracia-Lavedan, E; Hyvärinen, A; Sunyer, J. (2013). Use of household cleaning products, exhaled nitric oxide and lung function in children [Letter]. *Eur Respir J* 42: 1415-1418. <http://dx.doi.org/10.1183/09031936.00066313>
- Choudat, D; Goehen, M; Korobaef, M; Boulet, A; Dewitte, JD; Martin, MH. (1994). Respiratory symptoms and bronchial reactivity among pig and dairy farmers. *Scand J Work Environ Health* 20: 48-54. <http://dx.doi.org/10.5271/sjweh.1429>
- Cole, TJ; Cotes, JE; Johnson, GR; HDeV, M; Reed, JW; Saunders, MJ. (1977). Ventilation, cardiac frequency and pattern of breathing during exercise in men exposed to O-chlorobenzylidene malononitrile (CS) and ammonia gas in low concentrations. *Q J Exp Physiol Cogn Med Sci* 62: 341-351. <http://dx.doi.org/10.1113/expphysiol.1977.sp002406>
- Coon, RA; Jones, RA; Jenkins, LJ, Jr; Siegel, J. (1970). Animal inhalation studies on ammonia, ethylene glycol, formaldehyde, dimethylamine, and ethanol. *Toxicol Appl Pharmacol* 16: 646-655. [http://dx.doi.org/10.1016/0041-008X\(70\)90069-4](http://dx.doi.org/10.1016/0041-008X(70)90069-4)
- Córdoba, J; Gottstein, J; Blei, AT. (1998). Chronic hyponatremia exacerbates ammonia-induced brain edema in rats after portacaval anastomosis. *J Hepatol* 29: 589-594. [http://dx.doi.org/10.1016/S0168-8278\(98\)80154-2](http://dx.doi.org/10.1016/S0168-8278(98)80154-2)
- Cormier, Y; Israël-Assayag, E; Racine, G; Duchaine, C. (2000). Farming practices and the respiratory health risks of swine confinement buildings. *Eur Respir J* 15: 560-565.
- Crook, B; Robertson, JF; Glass, SA; Botheroyd, EM; Lacey, J; Topping, MD. (1991). Airborne dust, ammonia, microorganisms, and antigens in pig confinement houses and the respiratory health of exposed farm workers. *Am Ind Hyg Assoc J* 52: 271-279. <http://dx.doi.org/10.1080/15298669191364721>
- Curtis, SE; Anderson, CR; Simon, J; Jensen, AH; Day, DL; Kelley, KW. (1975). Effects of aerial ammonia, hydrogen sulfide and swine-house dust on rate of gain and respiratory-tract structure in swine. *J Anim Sci* 41: 735-739. <http://dx.doi.org/10.2134/jas1975.413735x>
- Davies, S; Spanel, P; Smith, D. (1997). Quantitative analysis of ammonia on the breath of patients in end-stage renal failure. *Kidney Int* 52: 223-228. <http://dx.doi.org/10.1038/ki.1997.324>
- Delclos, GL; Gimeno, D; Arif, AA; Benavides, FG; Zock, J, -P. (2009). Occupational exposures and asthma in health-care workers: Comparison of self-reports with a workplace-specific job exposure matrix. *Am J Epidemiol* 169: 581-587. <http://dx.doi.org/10.1093/aje/kwn387>
- DeSanto, JT; Nagomi, W; Liechty, EA; Lemons, JA. (1993). Blood ammonia concentration in cord blood during pregnancy. *Early Hum Dev* 33: 1-8. [http://dx.doi.org/10.1016/0378-3782\(93\)90168-T](http://dx.doi.org/10.1016/0378-3782(93)90168-T)

- [Diekman, MA; Scheidt, AB; Sutton, AL; Green, ML; Clapper, JA; Kelly, DT; Van Alstine, WG.](#) (1993). Growth and reproductive performance, during exposure to ammonia, of gilts afflicted with pneumonia and atrophic rhinitis. *Am J Vet Res* 54: 2128-2131.
- [Diskin, AM; Španěl, P; Smith, D.](#) (2003). Time variation of ammonia, acetone, isoprene and ethanol in breath: A quantitative SIFT-MS study over 30 days. *Physiol Meas* 24: 107-119.
<http://dx.doi.org/10.1088/0967-3334/24/1/308>
- [Doig, PA; Willoughby, RA.](#) (1971). Response of swine to atmospheric ammonia and organic dust. *J Am Vet Med Assoc* 159: 1353-1361.
- [Done, SH; Chennells, DJ; Gresham, AC; Williamson, S; Hunt, B; Taylor, LL; Bland, V; Jones, P; Armstrong, D; White, RP; Demmers, TG; Teer, N; Wathes, CM.](#) (2005). Clinical and pathological responses of weaned pigs to atmospheric ammonia and dust. *Vet Rec* 157: 71-80.
- [Donham, KJ; Cumro, D; Reynolds, SJ; Merchant, JA.](#) (2000). Dose-response relationships between occupational aerosol exposures and cross-shift declines of lung function in poultry workers: Recommendations for exposure limits. *J Occup Environ Med* 42: 260-269.
- [Donham, KJ; Reynolds, SJ; Whitten, P; Merchant, JA; Burmeister, L; Pependorf, WJ.](#) (1995). Respiratory dysfunction in swine production facility workers: Dose-response relationships of environmental exposures and pulmonary function. *Am J Ind Med* 27: 405-418.
<http://dx.doi.org/10.1002/ajim.4700270309>
- [Donnay, C; Denis, M, -A; Magis, R; Fevotte, J; Massin, N; Dumas, O; Pin, I; Choudat, D; Kauffmann, F; Le Moual, N.](#) (2011). Under-estimation of self-reported occupational exposure by questionnaire in hospital workers. *Occup Environ Med* 68: 611-617.
<http://dx.doi.org/10.1136/oem.2010.061671>
- [Douglas, RB; Coe, JE.](#) (1987). The relative sensitivity of the human eye and lung to irritant gases. *Ann Occup Hyg* 31: 265-267. <http://dx.doi.org/10.1093/annhyg/31.2.265>
- [Dumas, O; Donnay, C; Heederik, DJ; Héry, M; Choudat, D; Kauffmann, F; Le Moual, N.](#) (2012). Occupational exposure to cleaning products and asthma in hospital workers. *Occup Environ Med* 69: 883-889. <http://dx.doi.org/10.1136/oemed-2012-100826>
- [Eggeman, T.](#) (2001). Ammonia. In Kirk-Othmer encyclopedia of chemical technology. New York, NY: John Wiley & Sons, Inc.
- [Ferguson, WS; Koch, WC; Webster, LB; Gould, JR.](#) (1977). Human physiological response and adaption to ammonia. *J Occup Med* 19: 319-326.
- [Gaafar, H; Girgis, R; Hussein, M; el-Nemr, F.](#) (1992). The effect of ammonia on the respiratory nasal mucosa of mice. A histological and histochemical study. *Acta Otolaryngol* 112: 339-342.
<http://dx.doi.org/10.1080/00016489.1992.11665429>
- [Gilbert, GJ.](#) (1988). Acute ammonia intoxication 37 years after ureterosigmoidostomy. *South Med J* 81: 1443-1445.
- [Giroux, M; Ferrières, J.](#) (1998). Serum nitrates and creatinine in workers exposed to atmospheric nitrogen oxides and ammonia. *Sci Total Environ* 217: 265-269.
[http://dx.doi.org/10.1016/S0048-9697\(98\)00190-9](http://dx.doi.org/10.1016/S0048-9697(98)00190-9)
- [Gustin, P; Urbain, B; Prouvost, J, -F; Ansay, M.](#) (1994). Effects of atmospheric ammonia on pulmonary hemodynamics and vascular permeability in pigs: Interaction with endotoxins. *Toxicol Appl Pharmacol* 125: 17-26. <http://dx.doi.org/10.1006/taap.1994.1044>
- [Hamilton, TD; Roe, JM; Hayes, CM; Jones, P; Pearson, GR; Webster, AJ.](#) (1999). Contributory and exacerbating roles of gaseous ammonia and organic dust in the etiology of atrophic rhinitis. *Clin Diagn Lab Immunol* 6: 199-203.
- [Hamilton, TD; Roe, JM; Hayes, CM; Webster, AJ.](#) (1998). Effects of ammonia inhalation and acetic acid pretreatment on colonization kinetics of toxigenic *Pasteurella multocida* within upper respiratory tracts of swine. *J Clin Microbiol* 36: 1260-1265.

- Heederik, D; van Zwieten, R; Brouwer, R. (1990). Across-shift lung function changes among pig farmers. *Am J Ind Med* 17: 57-58. <http://dx.doi.org/10.1002/ajim.4700170110>
- Holness, DL; Purdham, JT; Nethercott, JR. (1989). Acute and chronic respiratory effects of occupational exposure to ammonia. *AIHA J* 50: 646-650. <http://dx.doi.org/10.1080/15298668991375308>
- HSDB (Hazardous Substances Data Bank). (2012). Ammonia. Washington, DC: National Library of Medicine.
- Ihrig, A; Hoffmann, J; Triebig, G. (2006). Examination of the influence of personal traits and habituation on the reporting of complaints at experimental exposure to ammonia. *Int Arch Occup Environ Health* 79: 332-338. <http://dx.doi.org/10.1007/s00420-005-0042-y>
- Jarudi, NI; Golden, B. (1973). Ammonia eye injuries. *J Iowa Med Soc* 63: 260-263.
- Jeffers, LJ; Dubow, RA; Zieve, L; Reddy, KR; Livingstone, AS; Neimark, S; Viamonte, M; Schiff, ER. (1988). Hepatic encephalopathy and orotic aciduria associated with hepatocellular carcinoma in a noncirrhotic liver. *Hepatology* 8: 78-81. <http://dx.doi.org/10.1002/hep.1840080116>
- Johnson, DR; Bedick, CR; Clark, NN; McKain, DL, Jr. (2009). Design and testing of an independently controlled urea SCR retrofit system for the reduction of NO_x emissions from marine diesels. *Environ Sci Technol* 43: 3959-3963. <http://dx.doi.org/10.1021/es900269p>
- Jones, EA. (2002). Ammonia, the GABA neurotransmitter system, and hepatic encephalopathy [Review]. *Metab Brain Dis* 17: 275-281. <http://dx.doi.org/10.1023/A:1021949616422>
- Jóźwik, M; Jóźwik, M; Pietrzycki, B; Chojnowski, M; Teng, C; Jóźwik, M; Battaglia, FC. (2005). Maternal and fetal blood ammonia concentrations in normal term human pregnancies. *Biol Neonate* 87: 38-43. <http://dx.doi.org/10.1159/000081702>
- Kennedy, SM; Le Moual, N; Choudat, D; Kauffmann, F. (2000). Development of an asthma specific job exposure matrix and its application in the epidemiological study of genetics and environment in asthma (EGEA). *Occup Environ Med* 57: 635-641. <http://dx.doi.org/10.1136/oem.57.9.635>
- Lange, NA; Dean, JA. (1985). Lange's handbook of chemistry (13th ed.). New York, NY: McGraw-Hill.
- Larson, TV; Covert, DS; Frank, R; Charlson, RJ. (1977). Ammonia in the human airways: Neutralization of inspired acid sulfate aerosols. *Science* 197: 161-163. <http://dx.doi.org/10.1126/science.877545>
- Le Moual, N; Kauffmann, F; Eisen, EA; Kennedy, SM. (2008). The healthy worker effect in asthma: Work may cause asthma, but asthma may also influence work [Review]. *Am J Respir Crit Care Med* 177: 4-10. <http://dx.doi.org/10.1164/rccm.200703-415PP>
- Leaderer, BP; Naeher, L; Jankun, T; Balenger, K; Holford, TR; Toth, C; Sullivan, J; Wolfson, JM; Koutrakis, P. (1999). Indoor, outdoor, and regional summer and winter concentrations of PM₁₀, PM₂₅, SO₄²⁻, H⁺, NH₄⁺, NO₃⁻, NH₃, and nitrous acid in homes with and without kerosene space heaters. *Environ Health Perspect* 107: 223-231.
- Lemiere, C; Bégin, D; Camus, M; Forget, A; Boulet, L, -P; Gérin, M. (2012). Occupational risk factors associated with work-exacerbated asthma in Quebec. *Occup Environ Med* 69: 901-907. <http://dx.doi.org/10.1136/oemed-2012-100663>
- Lide, DR. (2008). CRC handbook of chemistry and physics: A ready-reference book of chemical and physical data. In DR Lide (Ed.), (89th ed.). Boca Raton, FL: CRC Press.
- Loftus, C; Yost, M; Sampson, P; Torres, E; Arias, G; Breckwich Vasquez, V; Hartin, K; Armstrong, J; Tchong-French, M; Vedal, S; Bhatti, P; Karr, C. (2015). Ambient ammonia exposures in an agricultural community and pediatric asthma morbidity. *Epidemiology* 26: 794-801. <http://dx.doi.org/10.1097/EDE.0000000000000368>

- MacEwen, JD; Theodore, J; Vernot, EH. (1970). Human exposure to EEL concentrations of monomethylhydrazine. In Proceedings of the first annual conference on environmental toxicology. Wright-Patterson Air Force Base, OH: Aerospace Medical Research Laboratory.
- Manninen, A; Anttila, S; Savolainen, H. (1988). Rat metabolic adaptation to ammonia inhalation. *Proc Soc Exp Biol Med* 187: 278-281. <http://dx.doi.org/10.3181/00379727-187-42664>
- Manninen, ATA; Savolainen, H. (1989). Effect of short-term ammonia inhalation on selected amino acids in rat brain. *Pharmacol Toxicol* 64: 244-246. <http://dx.doi.org/10.1111/j.1600-0773.1989.tb00639.x>
- Medina-Ramón, M; Zock, JP; Kogevinas, M; Sunyer, J; Basagaña, X; Schwartz, J; Burge, PS; Moore, V; Antó, JM. (2006). Short-term respiratory effects of cleaning exposures in female domestic cleaners. *Eur Respir J* 27: 1196-1203. <http://dx.doi.org/10.1183/09031936.06.00085405>
- Medina-Ramón, M; Zock, JP; Kogevinas, M; Sunyer, J; Torralba, Y; Borrell, A; Burgos, F; Antó, JM. (2005). Asthma, chronic bronchitis, and exposure to irritant agents in occupational domestic cleaning: A nested case-control study. *Occup Environ Med* 62: 598-606. <http://dx.doi.org/10.1136/oem.2004.017640>
- Melbostad, E; Eduard, W. (2001). Organic dust-related respiratory and eye irritation in Norwegian farmers. *Am J Ind Med* 39: 209-217. [http://dx.doi.org/10.1002/1097-0274\(200102\)39:2<209::AID-AJIM1008>3.0.CO;2-5](http://dx.doi.org/10.1002/1097-0274(200102)39:2<209::AID-AJIM1008>3.0.CO;2-5)
- Millea, TP; Kucan, JO; Smoot, EC, III. (1989). Anhydrous ammonia injuries. *Journal of Burn Care and Rehabilitation* 10: 448-453.
- Miñana, M, -D; Marcaida, G; Grisolia, S; Felipo, V. (1995). Prenatal exposure of rats to ammonia impairs NMDA receptor function and affords delayed protection against ammonia toxicity and glutamate neurotoxicity. *J Neuropathol Exp Neurol* 54: 644-650. <http://dx.doi.org/10.1097/00005072-199509000-00005>
- Monsó, E; Riu, E; Radon, K; Magarolas, R; Danuser, B; Iversen, M; Morera, J; Nowak, D. (2004). Chronic obstructive pulmonary disease in never-smoking animal farmers working inside confinement buildings. *Am J Ind Med* 46: 357-362. <http://dx.doi.org/10.1002/ajim.20077>
- Nelson, DL; Cox, MM. (2008). Amino acid oxidation and the production of urea. In DL Nelson; AL Lehninger; MM Cox (Eds.), *Lehninger principles of biochemistry* (5th ed., pp. 656-689). New York, NY: W.H. Freeman & Co.
- Nemer, M; Sikkeland, LIB; Kasem, M; Kristensen, P; Nijem, K; Bjertness, E; Skare, Ø; Bakke, B; Kongerud, J; Skogstad, M. (2015). Airway inflammation and ammonia exposure among female Palestinian hairdressers: A cross-sectional study. *Occup Environ Med* 72: 428-434. <http://dx.doi.org/10.1136/oemed-2014-102437>
- Neumann, R; Mehlhorn, G; Buchholz, I; Johannsen, U; Schimmel, D. (1987a). [Experimental studies of effect of chronic aerogenous toxic gas exposure of unweaned piglets with ammonia of various concentrations: 2. Reaction of cellular and humoral infection defense mechanisms of ammonia exposed unweaned piglets under conditions of experimental pasteurella-multocida infection with and without thermo-motor stress]. *J Vet Med B* 34: 241-253.
- Neumann, R; Mehlhorn, G; Leonhardt, W; Kastner, P; Willig, R; Schimmel, D; Johannsen, U. (1987b). [Experimental studies on the effect on the effects of chronic airborne pollutant gas burden of Saugferkels with ammonia different concentrations]. *J Vet Med B* 34: 183-196. <http://dx.doi.org/10.1111/j.1439-0450.1987.tb00386.x>
- NLM (National Institutes of Health, National Library of Medicine). (2012). ChemIDplus: Ammonia. Bethesda, MD: National Library of Medicine. <http://chem.sis.nlm.nih.gov/chemidplus/name/startswith/amonia>
- Norwood, DM; Wainman, T; Lioy, PJ; Waldman, JM. (1992). Breath ammonia depletion and its relevance to acidic aerosol exposure studies. *Arch Environ Health* 47: 309-313. <http://dx.doi.org/10.1080/00039896.1992.9938367>

- NRC (National Research Council). (2008). Acute exposure guideline levels for selected airborne chemicals: Volume 6. Washington, DC: The National Academies Press.
http://www.nap.edu/catalog.php?record_id=12018
- O'Neil, MJ; Heckelman, PE; Koch, CB; Roman, KI. (2006). Ammonia. In *The Merck Index: An Encyclopedia of Chemicals, Drugs, and Biologicals* (14th ed.). Whitehouse Station, NJ: Merck & Co., Inc.
- Paulot, F; Jacob, DJ. (2014). Hidden cost of U.S. agricultural exports: Particulate matter from ammonia emissions. *Environ Sci Technol* 48: 903-908.
<http://dx.doi.org/10.1021/es4034793>
- Petrova, M; Diamond, J; Schuster, B; Dalton, P. (2008). Evaluation of trigeminal sensitivity to ammonia in asthmatics and healthy human volunteers. *Inhal Toxicol* 20: 1085-1092.
<http://dx.doi.org/10.1080/08958370802120396>
- Preller, L; Heederik, D; Boleij, JSM; Vogelzang, PFJ; Tielen, MJM. (1995). Lung function and chronic respiratory symptoms of pig farmers: Focus on exposure to endotoxins and ammonia and use of disinfectants. *Occup Environ Med* 52: 654-660.
<http://dx.doi.org/10.1136/oem.52.10.654>
- Rahman, MH; Bråtveit, M; Moen, BE. (2007). Exposure to ammonia and acute respiratory effects in a urea fertilizer factory. *Int J Occup Environ Health* 13: 153-159.
<http://dx.doi.org/10.1179/oeh.2007.13.2.153>
- Read, AJ. (1982). Ionization constants of aqueous ammonia from 25 to 250C and to 2000 bar. *Journal of Solution Chemistry* 11: 649-664. <http://dx.doi.org/10.1007/bf00650397>
- Reynolds, SJ; Donham, KJ; Whitten, P; Merchant, JA; Burmeister, LF; Popendorf, WJ. (1996). Longitudinal evaluation of dose-response relationships for environmental exposures and pulmonary function in swine production workers. *Am J Ind Med* 29: 33-40.
[http://dx.doi.org/10.1002/\(SICI\)1097-0274\(199601\)29:1<33::AID-AJIM5>3.0.CO;2-#](http://dx.doi.org/10.1002/(SICI)1097-0274(199601)29:1<33::AID-AJIM5>3.0.CO;2-#)
- Richard, D; Bouley, G; Boudène, C. (1978a). [Effects of ammonia gas continuously inhaled by rats and mice]. *Bull Europ Physiol Resp* 14: 573-582.
- Richard, D; Jouany, JM; Boudène, C. (1978b). [Acute inhalation toxicity of ammonia in rabbits]. *C R Acad Sci Hebd Seances Acad Sci D* 287: 375-378.
- Romero-Gómez, M; Ramos-Guerrero, R; Grande, L; de Terán, LC; Corpas, R; Camacho, I; Bautista, JD. (2004). Intestinal glutaminase activity is increased in liver cirrhosis and correlates with minimal hepatic encephalopathy. *J Hepatol* 41: 49-54.
<http://dx.doi.org/10.1016/j.jhep.2004.03.021>
- Rosswall, T. (1981). The biogeochemical nitrogen cycle. In *GE Likens (Ed.), Some perspectives of the major biogeochemical cycles* (pp. 25-49). Chichester, England: John Wiley & Sons.
http://dgc.stanford.edu/SCOPE/SCOPE_17/SCOPE_17.html
- Sadasivudu, B; Radha Krishna Murthy, C. (1978). Effects of ammonia on monoamine oxidase and enzymes of GABA metabolism in mouse brain. *Arch Physiol Biochem* 86: 67-82.
- Sadasivudu, B; Rao, TI; Murthy, CR. (1979). Chronic metabolic effects of ammonia in mouse brain. *Arch Physiol Biochem* 87: 871-885.
- Schaerdel, AD; White, WJ; Lang, CM; Dvorchik, BH; Bohner, K. (1983). Localized and systemic effects of environmental ammonia in rats. *Lab Anim Sci* 33: 40-45.
- Schmidt, FM; Vaittinen, O; Metsälä, M; Lehto, M; Forsblom, C; Groop, PH; Halonen, L. (2013). Ammonia in breath and emitted from skin. *J Breath Res* 7: 017109.
<http://dx.doi.org/10.1088/1752-7155/7/1/017109>
- Schoeb, TR; Davidson, MK; Lindsey, JR. (1982). Intracage ammonia promotes growth of *Mycoplasma pulmonis* in the respiratory tract of rats. *Infect Immun* 38: 212-217.

- [Schubiger, G; Bachmann, C; Barben, P; Colombo, JP; Tönz, O; Schüpbach, D.](#) (1991). N-acetylglutamate synthetase deficiency: Diagnosis, management and follow-up of a rare disorder of ammonia detoxication. *Eur J Pediatr* 150: 353-356.
- [Seeman, JI; Carchman, RA.](#) (2008). The possible role of ammonia toxicity on the exposure, deposition, retention, and the bioavailability of nicotine during smoking [Review]. *Food Chem Toxicol* 46: 1863-1881. <http://dx.doi.org/10.1016/j.fct.2008.02.021>
- [Sigurdarson, ST; O'Shaughnessy, PT; Watt, JA; Kline, JN.](#) (2004). Experimental human exposure to inhaled grain dust and ammonia: Towards a model of concentrated animal feeding operations. *Am J Ind Med* 46: 345-348. <http://dx.doi.org/10.1002/ajim.20055>
- [Silverman, L; Whittenberger, JL; Muller, J.](#) (1949). Physiological response of man to ammonia in low concentrations. *J Ind Hyg Toxicol* 31: 74-78.
- [Smeets, MA; Bulsing, PJ; van Rooden, S; Steinmann, R; de Ru, JA; Ogink, NW; van Thriel, C; Dalton, PH.](#) (2007). Odor and irritation thresholds for ammonia: A comparison between static and dynamic olfactometry. *Chem Senses* 32: 11-20. <http://dx.doi.org/10.1093/chemse/bjl031>
- [Smith, D; Spanel, P; Davies, S.](#) (1999). Trace gases in breath of healthy volunteers when fasting and after a protein-calorie meal: A preliminary study. *J Appl Physiol* 87: 1584-1588.
- [Smith, D; Wang, T; Pysanenko, A; Spanel, P.](#) (2008). A selected ion flow tube mass spectrometry study of ammonia in mouth- and nose-exhaled breath and in the oral cavity. *Rapid Commun Mass Spectrom* 22: 783-789. <http://dx.doi.org/10.1002/rcm.3434>
- [Socolow, RH.](#) (1999). Nitrogen management and the future of food: Lessons from the management of energy and carbon. 96: 6001-6008.
- [Solga, SF; Mudalel, M; Spacek, LA; Lewicki, R; Tittel, F; Loccioni, C; Russo, A; Risby, TH.](#) (2013). Factors influencing breath ammonia determination. *J Breath Res* 7: 037101. <http://dx.doi.org/10.1088/1752-7155/7/3/037101>
- [Souba, WW.](#) (1987). Interorgan ammonia metabolism in health and disease: A surgeon's view [Review]. *JPEN J Parenter Enteral Nutr* 11: 569-579. <http://dx.doi.org/10.1177/0148607187011006569>
- [Španěl, P; Dryahina, K; Smith, D.](#) (2007a). Acetone, ammonia and hydrogen cyanide in exhaled breath of several volunteers aged 4-83 years. *J Breath Res* 1: 011001. <http://dx.doi.org/10.1088/1752-7155/1/1/011001>
- [Španěl, P; Dryahina, K; Smith, D.](#) (2007b). The concentration distributions of some metabolites in the exhaled breath of young adults. *J Breath Res* 1: 026001. <http://dx.doi.org/10.1088/1752-7155/1/2/026001>
- [Španěl, P; Dryahina, K; Smith, D.](#) (2013). A quantitative study of the influence of inhaled compounds on their concentrations in exhaled breath. *J Breath Res* 7: 017106. <http://dx.doi.org/10.1088/1752-7155/7/1/017106>
- [Stearne, JAC; Higgins, JPT; Reaves, BC.](#) (2014). A Cochrane risk of bias assessment tool: For non-randomized studies of interventions (ACROBAT-NRSI) (Version 1.0.0). Retrieved from <http://www.riskofbias.info>
- [Stombaugh, DP; Teague, HS; Roller, WL.](#) (1969). Effects of atmospheric ammonia on the pig. *J Anim Sci* 28: 844-847.
- [Sundblad, B, -M; Larsson, BM; Acevedo, F; Ernstgård, L; Johanson, G; Larsson, K; Palmberg, L.](#) (2004). Acute respiratory effects of exposure to ammonia on healthy persons. *Scand J Work Environ Health* 30: 313-321. <http://dx.doi.org/10.5271/sjweh.800>
- [Targowski, SP; Klucinski, W; Babiker, S; Nonnecke, BJ.](#) (1984). Effect of ammonia on in vivo and in vitro immune responses. *Infect Immun* 43: 289-293.
- [Turner, C; Spanel, P; Smith, D.](#) (2006). A longitudinal study of ammonia, acetone and propanol in the exhaled breath of 30 subjects using selected ion flow tube mass spectrometry, SIFT-MS. *Physiol Meas* 27: 321-337. <http://dx.doi.org/10.1088/0967-3334/27/4/001>

- [U.S. EPA](#) (U.S. Environmental Protection Agency). (1991). Guidelines for developmental toxicity risk assessment (pp. 1-83). (EPA/600/FR-91/001). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum.
<http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=23162>
- [U.S. EPA](#) (U.S. Environmental Protection Agency). (1994). Methods for derivation of inhalation reference concentrations and application of inhalation dosimetry [EPA Report] (pp. 1-409). (EPA/600/8-90/066F). Research Triangle Park, NC: U.S. Environmental Protection Agency, Office of Research and Development, Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office.
<https://cfpub.epa.gov/ncea/risk/recordisplay.cfm?deid=71993&CFID=51174829&CFTOKEN=25006317>
- [U.S. EPA](#) (U.S. Environmental Protection Agency). (1995). The use of the benchmark dose approach in health risk assessment. (EPA/630/R-94/007). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum.
<http://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=30004WBL.txt>
- [U.S. EPA](#) (U.S. Environmental Protection Agency). (1996). Guidelines for reproductive toxicity risk assessment (pp. 1-143). (EPA/630/R-96/009). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum.
- [U.S. EPA](#) (U.S. Environmental Protection Agency). (1998). Guidelines for neurotoxicity risk assessment [EPA Report] (pp. 1-89). (EPA/630/R-95/001F). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum.
<http://www.epa.gov/risk/guidelines-neurotoxicity-risk-assessment>
- [U.S. EPA](#) (U.S. Environmental Protection Agency). (2002). A review of the reference dose and reference concentration processes (pp. 1-192). (EPA/630/P-02/002F). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum.
<http://www.epa.gov/osa/review-reference-dose-and-reference-concentration-processes>
- [U.S. EPA](#) (U.S. Environmental Protection Agency). (2005a). Guidelines for carcinogen risk assessment [EPA Report] (pp. 1-166). (EPA/630/P-03/001F). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum.
<http://www2.epa.gov/osa/guidelines-carcinogen-risk-assessment>
- [U.S. EPA](#) (U.S. Environmental Protection Agency). (2005b). Supplemental guidance for assessing susceptibility from early-life exposure to carcinogens (pp. 1-125). (EPA/630/R-03/003F). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum.
- [U.S. EPA](#) (U.S. Environmental Protection Agency). (2006). Approaches for the application of physiologically based pharmacokinetic (PBPK) models and supporting data in risk assessment (Final Report) [EPA Report] (pp. 1-123). (EPA/600/R-05/043F). Washington, DC: U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment.
<http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=157668>
- [U.S. EPA](#) (U.S. Environmental Protection Agency). (2007). Integrated Risk Information System (IRIS); announcement of 2008 program. Fed Reg 72: 72715-72719.
- [U.S. EPA](#) (U.S. Environmental Protection Agency). (2009a). Integrated Risk Information System (IRIS); announcement of availability of literature searches for IRIS assessments [FRL89741; Docket ID No. EPAHQORD20070664]. Fed Reg 74: 56611-56612.
- [U.S. EPA](#) (U.S. Environmental Protection Agency). (2009b). Integrated science assessment for particulate matter [EPA Report]. (EPA/600/R-08/139F). Research Triangle Park, NC: U.S. Environmental Protection Agency, National Center for Environmental Assessment.
<http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=216546>

- U.S. EPA (U.S. Environmental Protection Agency). (2009c). Metabolically derived human ventilation rates: A revised approach based upon oxygen consumption rates (final report) [EPA Report]. (EPA/600/R-06/129F). Washington, DC.
<http://cfpub.epa.gov/ncea/cfm/recorddisplay.cfm?deid=202543>
- U.S. EPA (U.S. Environmental Protection Agency). (2011a). Exposure factors handbook: 2011 edition (final) [EPA Report]. (EPA/600/R-090/052F). Washington, DC: U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment. <http://cfpub.epa.gov/ncea/cfm/recorddisplay.cfm?deid=236252>
- U.S. EPA (U.S. Environmental Protection Agency). (2011b). Recommended use of body weight 3/4 as the default method in derivation of the oral reference dose (pp. 1-50). (EPA/100/R11/0001). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum, Office of the Science Advisor.
<https://www.epa.gov/risk/recommended-use-body-weight-34-default-method-derivation-oral-reference-dose>
- U.S. EPA (U.S. Environmental Protection Agency). (2012). Benchmark dose technical guidance (pp. 1-99). (EPA/100/R-12/001). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum.
- U.S. EPA (U.S. Environmental Protection Agency). (2014). Guidance for applying quantitative data to develop data-derived extrapolation factors for interspecies and intraspecies extrapolation [EPA Report]. (EPA/100/R-14/002F). Washington, DC: Risk Assessment Forum, Office of the Science Advisor. <https://www.epa.gov/risk/guidance-applying-quantitative-data-develop-data-derived-extrapolation-factors-interspecies-and>
- Verberk, MM. (1977). Effects of ammonia in volunteers. *Int Arch Occup Environ Health* 39: 73-81.
<http://dx.doi.org/10.1007/BF00380887>
- Verschueren, K. (2001). Ammonia. In *Handbook of Environmental Data on Organic Chemicals* (4th ed.). New York, NY: John Wiley & Sons.
- Vizcaya, D; Mirabelli, MC; Antó, J.-M; Orriols, R; Burgos, F; Arjona, L; Zock, J. -P. (2011). A workforce-based study of occupational exposures and asthma symptoms in cleaning workers. *Occup Environ Med* 68: 914-919. <http://dx.doi.org/10.1136/oem.2010.063271>
- Vollmuth, TA; Schlesinger, RB. (1984). Measurement of respiratory tract ammonia in the rabbit and implications to sulfuric acid inhalation studies. *Fundam Appl Toxicol* 4: 455-464.
[http://dx.doi.org/10.1016/0272-0590\(84\)90203-3](http://dx.doi.org/10.1016/0272-0590(84)90203-3)
- Weatherby, JH. (1952). Chronic toxicity of ammonia fumes by inhalation. *Proc Soc Exp Biol Med* 81: 300-301. <http://dx.doi.org/10.3181/00379727-81-19857>
- Weiner, ID; Mitch, WE; Sands, JM. (2014). Urea and ammonia metabolism and the control of renal nitrogen excretion. *Clin J Am Soc Nephrol* 10: 1444-1458.
<http://dx.doi.org/10.2215/CJN.10311013>
- Weiner, ID; Verlander, JW. (2013). Renal ammonia metabolism and transport [Review]. *Compr Physiol* 3: 201-220. <http://dx.doi.org/10.1002/cphy.c120010>
- Zeida, JE; Barber, E; Dosman, JA; Olenchok, SA; McDuffie, HH; Rhodes, C; Hurst, T. (1994). Respiratory health status in swine producers relates to endotoxin exposure in the presence of low dust levels. *J Occup Med* 36: 49-56.
- Zock, J. -P; Plana, E; Jarvis, D; Antó, JM; Kromhout, H; Kennedy, SM; Künzli, N; Villani, S; Olivieri, M; Torén, K; Radon, K; Sunyer, J; Dahlman-Hoglund, A; Norbäck, D; Kogevinas, M. (2007). The use of household cleaning sprays and adult asthma: An international longitudinal study. *Am J Respir Crit Care Med* 176: 735-741. <http://dx.doi.org/10.1164/rccm.200612-1793OC>

Ammonia

MAK value (1996)	20 ml/m³ (ppm) $\cong$ 14 mg/m³
Peak limitation (1986)	Category I
Absorption through the skin	–
Sensitization	–
Carcinogenicity	–
Prenatal toxicity (1986)	Pregnancy risk group C
Germ cell mutagenicity	–
BAT value	–
CAS number	7664-41-7

The toxicology of ammonia was reviewed in Volume 6 of the present series; this chapter is a supplement to the earlier review.

Ammonia is an irritant gas with severe local effects. Systemic-toxic effects are of less importance for occupational health. The MAK value for ammonia was established in 1963 at 50 ml/m³ and confirmed in 1973. The main reasons for this were the fact that no effects occurred in man at exposure concentrations of about 50 ml/m³ and the existence of animal experiments which came to the same conclusion (see Volume 6).

Evaluation of more recent documentation in 1986 showed that in man irritation of the eyes, nose and throat can also occur in isolated cases after exposure to 50 ml/m³ or even lower concentrations. Persons exposed to ammonia for the first time were particularly sensitive. On the other hand, habituation occurred both in man and in animal experiments after repeated or long-term exposure. After habituation, long-term exposure to concentrations as high as 100 ml/m³ could be tolerated without problems. As a result of the exposure situation usual at the workplace, which presupposes habituation of the affected workers, the MAK value of 50 ml/m³ was retained (see Volume 6).

Since then the database for effects known in man under workplace conditions has hardly improved (see also HSE 1993).

In 58 workers of a soda factory who were exposed to ammonia concentrations of 9.2 ± 1.4 ml/m³ (exposure peaks of about 25 ml/m³), lung function parameters (FVC, FEV₁, FEF₅₀, FEF₇₅)¹ and sense of smell (perception of pyridine) were unchanged relative to those of a control collective who were not exposed. The exposure period was 12.2 ± 8.9 years. Also comparison of the lung function parameters at the beginning and end of the

¹ FVC forced vital capacity; FEV₁ forced expiratory volume in one second
FEF₅₀ forced expiratory flow at 50 % FVC; FEF₇₅ forced expiratory flow at 75 % FVC

work week revealed no differences. The American Thoracic Society questionnaire was used to define the symptoms. There was no increase in the incidence of symptoms in the workers exposed to ammonia (Holness *et al.* 1989).

In an insufficiently documented experiment with exposure to ammonia concentrations of 8 and 12 mg/m³ (12 and 17 ml/m³) in the air of the room used for the experiment, 25 volunteers had to complete complex choice reaction tasks. The volunteers were trainees and 17–18 years old; it is not described whether they were exposed to ammonia at the workplace. The number of errors increased with the ammonia concentration and at 12 mg/m³ corresponded more or less with the number which also occurred during exposure to 90 db (A) noise. The volunteers estimated the disturbing effect of exposure also to be roughly the same as that at 90 db (A). The difference from results obtained under control conditions was statistically significant ($p < 0.05$) in each case (Bauch and Junghans 1986).

Manifesto (MAK value/classification)

There is evidence from animal experiments and experience in man that irritation of the mucous membranes can occur after exposure to ammonia concentrations of 50 ml/m³. Persons exposed for the first time are particularly sensitive. Despite the incomplete documentation of the experiments, the results for increased errors in choice reaction tests and for the disturbance experienced during tasks support the theory that ammonia concentrations below 50 ml/m³ cause disturbing irritation. It has been shown that long-term exposure of workers to 10 ml/m³ has no effects. The MAK value for ammonia was therefore lowered to 20 ml/m³; this also takes into account the uncertainty resulting from our lack of knowledge about habituation and its mechanisms. Peak limitation according to category I and classification in Pregnancy risk group C have been retained.

There are no studies available which would justify designation with an “H” for substances which can be absorbed by the skin or an “S” for substances with sensitizing potential.

References

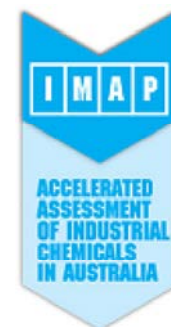
- Bauch P, Junghans R (1986) Zu Einflüssen von Lärm, Chlor und Ammoniak auf die Leistung bei Tätigkeiten mit hohen Aufmerksamkeitsanforderungen. Leistungsverhalten bei Einzelwirkung von Lärm, Chlor und Ammoniak. *Z Gesamte Hyg* 32: 33–36
- Holness DL, Purdham JT, Nethercott JR (1989) Acute and chronic respiratory effects of occupational exposure to ammonia. *Am Ind Hyg Assoc J* 50: 646–650
- HSE (Health and Safety Executive) (1993) *Ammonia*. Criteria document for an occupational exposure limit, HSE Books, Suffolk

completed 28.03.1996

Ammonia and Ammonium hydroxide: Human health tier II assessment

04 July 2014

- Chemicals in this assessment
- Preface
- Grouping Rationale
- Import, Manufacture and Use
- Restrictions
- Existing Worker Health and Safety Controls
- Health Hazard Information
- Risk Characterisation
- NICNAS Recommendation
- References



Chemicals in this assessment

Chemical Name in the Inventory	CAS Number
Ammonium hydroxide	1336-21-6
Ammonia	7664-41-7

Preface

This assessment was carried out by staff of the National Industrial Chemicals Notification and Assessment Scheme (NICNAS) using the Inventory Multi-tiered Assessment and Prioritisation (IMAP) framework.

The IMAP framework addresses the human health and environmental impacts of previously unassessed industrial chemicals listed on the Australian Inventory of Chemical Substances (the Inventory).

The framework was developed with significant input from stakeholders and provides a more rapid, flexible and transparent approach for the assessment of chemicals listed on the Inventory.

Stage One of the implementation of this framework, which lasted four years from 1 July 2012, examined 3000 chemicals meeting characteristics identified by stakeholders as needing priority assessment. This included chemicals for which NICNAS already held exposure information, chemicals identified as a concern or for which regulatory action had been taken overseas, and chemicals detected in international studies analysing chemicals present in babies' umbilical cord blood.

Stage Two of IMAP began in July 2016. We are continuing to assess chemicals on the Inventory, including chemicals identified as a concern for which action has been taken overseas and chemicals that can be rapidly identified and assessed by using Stage One information. We are also continuing to publish information for chemicals on the Inventory that pose a low risk to human health or the environment or both. This work provides efficiencies and enables us to identify higher risk chemicals requiring assessment.

The IMAP framework is a science and risk-based model designed to align the assessment effort with the human health and environmental impacts of chemicals. It has three tiers of assessment, with the assessment effort increasing with each tier. The Tier I assessment is a high throughput approach using tabulated electronic data. The Tier II assessment is an evaluation of risk on a substance-by-substance or chemical category-by-category basis. Tier III assessments are conducted to address specific concerns that could not be resolved during the Tier II assessment.

These assessments are carried out by staff employed by the Australian Government Department of Health and the Australian Government Department of the Environment and Energy. The human health and environment risk assessments are conducted and published separately, using information available at the time, and may be undertaken at different tiers.

This chemical or group of chemicals are being assessed at Tier II because the Tier I assessment indicated that it needed further investigation.

For more detail on this program please visit: www.nicnas.gov.au

Disclaimer

NICNAS has made every effort to assure the quality of information available in this report. However, before relying on it for a specific purpose, users should obtain advice relevant to their particular circumstances. This report has been prepared by NICNAS using a range of sources, including information from databases maintained by third parties, which include data supplied by industry. NICNAS has not verified and cannot guarantee the correctness of all information obtained from those databases. Reproduction or further distribution of this information may be subject to copyright protection. Use of this information without obtaining the permission from the owner(s) of the respective information might violate the rights of the owner. NICNAS does not take any responsibility whatsoever for any copyright or other infringements that may be caused by using this information.

ACRONYMS & ABBREVIATIONS

Grouping Rationale

The chemical, ammonia (CAS No. 7664-41-7) dissolves readily in water to form the solution described as ammonium hydroxide (CAS No. 1336-21-6). In biological fluids, the chemicals mostly exist in the ionised form as ammonium ions (NH_4^+), with approximately 99 % ionised in blood within the normal pH range (ASTDR, 2004; OECD, 2007). Although ammonia is a gas and ammonium hydroxide is a solution, given the high vapour pressure of ammonium hydroxide, inhalation is considered a relevant exposure route for assessing both chemicals.

The chemicals have similar uses.

Import, Manufacture and Use

Australian

The following Australian industrial uses were reported under previous mandatory and/or voluntary calls for information.

Ammonia has reported site-limited use as a laboratory chemical and in manufacturing other chemicals for uses such as:

- cleaning/washing agents and additives; and
- explosives.

Ammonia has reported commercial uses including as a:

- pH regulating agent;
- cleaning/washing agent;
- photochemical; and

- flotation agent.

Ammonia is listed on the 2006 High Volume Industrial Chemicals List (HVICL) with a total reported volume of 100000–999999 tonnes.

International

The following international uses have been identified through European Union Registration, Evaluation, Authorisation and Restriction of Chemicals (EU REACH) dossiers; the Organisation for Economic Cooperation and Development Screening Information Dataset Initial Assessment Report (OECD SIAR); Galleria Chemica; Substances and Preparations in the Nordic countries (SPIN) database; the European Commission Cosmetic Ingredients and Substances (CosIng) database; United States (US) Personal Care Product Council International Nomenclature of Cosmetic Ingredients (INCI) dictionary, the US National Library of Medicine's Hazardous Substances Data Bank (HSDB) and various international assessments (IPCS, 1986; ASTDR, 2004).

The chemicals have reported cosmetic use with the identified functions of buffering, masking and as denaturants. The majority of identified products for ammonium hydroxide are hair dye kits which are reported to contain the chemical at concentrations up to 7 % (Household Products Database, US Department of Health and Human Services).

The chemicals have reported domestic use in a range of domestic products up to a concentration of 2 % for ammonia and 5 % for ammonia hydroxide. Identified products include home maintenance products such as adhesives and sealants, auto products such as cleaners and tyre repair products, and a range of cleaning products (Household Products Database, US Department of Health and Human Services).

The chemicals have reported commercial uses including:

- in textile and leather processing;
- in paper processing;
- as corrosion inhibitors;
- as pH-regulating agents; and
- as photochemicals.

Ammonia has reported site-limited uses including:

- in the manufacturing of other chemicals including explosives and fertilisers;
- in condensation polymerisation;
- in metal extraction, refining and processing of metals;
- as complexing agents;
- as heat transferring agents/refrigerant;
- as laboratory chemicals; and
- as reducing agents.

The chemicals have reported non-industrial uses including:

- in disinfectants;
- in agricultural and pharmaceuticals products; and
- food processing.

Restrictions

Australian

The chemicals are listed in the *Poisons Standard*—the Standard for the Uniform Scheduling of Medicines and Poisons (SUSMP) in Schedules 5 and 6 (SUSMP, 2013).

Schedule 6:

'AMMONIA (excluding its salts and derivatives other than ammonium hydroxide) **except**:

- (a) when included in Schedule 5;
- (b) in preparations for human internal therapeutic use;
- (c) in preparations for inhalation when absorbed in an inert solid material; or
- (d) in preparations containing 0.5 per cent or less of ammonia.' (SUSMP, 2013)

Schedule 5

'AMMONIA (excluding its salts and derivatives other than ammonium hydroxide) in preparations containing 5 percent or less of ammonia **except**:

- (a) in preparations for human internal therapeutic use;
- (b) in preparations for inhalation when absorbed in an inert solid material; or
- (c) in preparations containing 0.5 per cent or less of free ammonia.' (SUSMP, 2013)

Schedule 6 chemicals are described as 'Substances with a moderate potential for causing harm, the extent of which can be reduced through the use of distinctive packaging with strong warnings and safety directions on the label'. Schedule 6 chemicals are labelled with 'Poison' (SUSMP, 2013).

Schedule 5 chemicals are described as 'Substances with a low potential for causing harm, the extent of which can be reduced through the use of appropriate packaging with simple warnings and safety directions on the label.' Schedule 5 chemicals are labelled with 'Caution' (SUSMP, 2013).

International

The chemicals are listed on EU Cosmetics Regulation 1223/2009 Annex III—List of substances which cosmetic products must not contain except subject to the restrictions laid down. The maximum concentration in ready for use preparations is 6 % (as NH₃). Products containing > 2 % must be labelled 'contains ammonia'.

Ammonia is listed on the following (Galleria Chemica):

- The Association of Southeast Asian Nations (ASEAN) Cosmetic Directive Annex III Part 1—List of substances which cosmetic products must not contain except subject to restriction and conditions laid down;
- Canada Environmental Protection Act (CEPA) 1999—Schedule 1 Toxic Substances List;
- New Zealand Cosmetics Group Standard (2006) Schedule 5— Components cosmetic products must not contain except subject to the restrictions and conditions laid down; and
- *New Zealand Hazardous Substances and New Organisms (HSNO) Act*—Scheduled Toxic Substances.

Existing Worker Health and Safety Controls

Hazard Classification

Ammonia is classified as hazardous, with the following risk phrases for human health in the Hazardous Substances Information System (HSIS) (Safe Work Australia):

T; R23 (Toxic by inhalation)

C; R34 (Causes burns)

Ammonium hydroxide is classified as hazardous, with the following risk phrases for human health in the (HSIS) (Safe Work Australia):

C; R34 (Causes burns)

Exposure Standards

Australian

Ammonia has an exposure standard of 17 mg/m³ (25 ppm) time weighted average (TWA) and 24 mg/m³ (35 ppm) short-term exposure limit (STEL).

International

The following exposure standards for ammonia are identified (Galleria Chemica).

An exposure limit of 14–18 mg/m³ (20–25 ppm) TWA and 24–36 mg/m³ (35–50 ppm) STEL in different countries such as Egypt, Canada (Yukon), Finland, Singapore and South Africa.

The American Conference of Governmental Industrial Hygienists (ACGIH) recommends a threshold limit value (TLV) of 25 ppm (17 mg/m³) TWA and 35 ppm (24 mg/m³) STEL for ammonia. 'These values are intended to minimize the potential for acute ocular and respiratory tract irritation' (ACGIH, 2011).

Health Hazard Information

As the chemicals exist in biological solutions as ammonium ions, data for ammonium chloride (CAS No. 12125-02-9) have been included where data relating to systemic effects are not available.

Toxicokinetics

Ammonia is present in body fluids of animals as a by-product of protein and nucleic acid metabolism and is a component of the diet. Metabolically-produced ammonia is conjugated and excreted. Toxicity only occurs when these mechanisms are defective or if excessive exposure to the chemical occurs.

The chemicals can be absorbed following inhalation and oral exposure. Reviews state that 'absorption through the eye has been documented' (refer to **Corrosivity: Animal data: eye** for more information) (ASTDR, 2004; OECD, 2007). Whilst in vitro studies in human skin indicate that it can act as a barrier to dermal absorption (REACH), the corrosive nature of the chemicals might destroy this protective barrier.

A significant proportion of inhaled ammonia is eliminated in expired air (without absorption). In biological fluids the chemicals mostly exist in the ionised form as ammonium ions (NH_4^+), with approximately 99 % ionised in blood within the normal pH range (ASTDR, 2004; OECD, 2007). After oral ingestion in rats, ammonium ions are rapidly absorbed through the gastrointestinal tract. Thereafter, they are metabolised to urea or glutamine and excreted in urine (within six hours) as urea (ASTDR, 2004; OECD, 2007).

Acute Toxicity

Oral

The chemical, ammonium hydroxide, has moderate acute toxicity based on a result from an animal test following oral exposure. The median lethal dose (LD50) for aqueous ammonia in rats is reported to be 350 mg/kg. In an acute oral toxicity study, 10 male Wistar rats received a single oral dose (by gavage) of ammonium hydroxide (concentration not specified) at various dose levels and were observed for 14 days. Clinical signs included sedation, staggering, abnormal posture, convulsions, tremors, salivation, diarrhoea, ataxia (a lack of voluntary muscle coordination during movement) and laboured breathing. The LD50 in rats is 350 mg/kg bw (OECD, 2007; REACH).

Oral exposure is not considered a relevant route of occupational exposure for ammonia gas.

Dermal

No data are available.

Inhalation

Ammonia is classified as hazardous with the risk phrase 'Toxic by inhalation' (T; R23) in HSIS (Safe Work Australia). Whilst the reported median lethal concentration (LC50) values in rats do not support this classification, substantially lower values were observed in mice. Levels that cause lethality are dependent on exposure duration and frequency. In addition, death has been observed in humans exposed to ammonia (see **Acute toxicity: Observation in humans**). Overall, the existing classification is supported.

A one hour study in various species of animals exposed to high concentrations of gaseous ammonia reported the LC50 values as 2961 mg/m^3 in mice and 5137 mg/m^3 in rats. Reported signs of toxicity included excessive respiratory irritation, nasal and mouth secretions, depressed respiratory rates, lung oedema and haemorrhage (OECD, 2007).

In another acute inhalation toxicity study using 10 Wistar rats (both sexes), the animals were exposed to varying concentrations of an ammonia/air mixture over various exposure periods (10, 20, 40 or 60 minutes). After a one hour exposure period, the LC50 values reported were: 9850 mg/m^3 in male and 13770 mg/m^3 in female rats. Reported signs of toxicity included eye irritation, laboured breathing and nasal discharge. In mice, an LC50 of 2940 mg/m^3 was observed after a one hour exposure (OECD, 2007; REACH).

Observation in humans

There are a number of reported cases of death from inhaling ammonia. Concentrations of 3480–6953 mg/m^3 (5000–10000 ppm) are reported to cause almost immediate death, with concentrations of 1738–4519 mg/m^3 (2500–6500 ppm) causing death within 30 minutes. Post-mortem examination findings include burns and oedema to the skin, eyes and respiratory, and blocked larynx and lungs (IPCS, 1986; ASTDR, 2004; OECD, 2007). Irritant effects have occurred following exposure to lower concentrations (see **Irritation/Corrosion: Observation in humans**). Death has also been observed following ingestion of an unknown amount of ammonium hydroxide (ASTDR, 2004).

Corrosion / Irritation

Corrosivity

The chemicals in this group are classified as hazardous with the risk phrase 'Causes burns' (R34) in HSIS (Safe Work Australia). The available data in humans and animals support this classification. In addition, based on effects observed in the respiratory tract, the Globally Harmonized System of Classification (GHS) classification 'Corrosive to the respiratory tract' (AUH071) should apply (refer to **Recommendation** section). The available data for ammonium hydroxide also support the concentration cut-off values currently in HSIS.

Animal data: Skin

In an acute dermal irritation/corrosion study (comparable to OECD Test Guidelines (TG) 404) in rabbits, a 20 % ammonium hydroxide solution tested positive for skin corrosion (OECD, 2007). In another similar study, 0.5 mL of various concentrations of ammonium hydroxide solution (10 % and 12 %) were applied to the shaved skin of 12 female New Zealand White rabbits in a occlusive patch. After a four hour exposure period, a positive corrosive response was observed with the 12 % solution—positive results were based on observed visible tissue destruction in at least two rabbits. The 10 % concentration was not corrosive (Vernot et al., 1977; REACH).

Animal data: Eye

In rabbits, ammonia has been shown to penetrate the cornea rapidly due to its lipid solubility, and to cause burns in the range of 200 –1000 ppm. Highly irritant effects were observed in the eye of a rabbit following exposure to ammonium hydroxide for 30 seconds (OECD, 2007).

Animal data: Respiratory tract

In acute toxicity studies, severe lesions in the respiratory tract were observed in animals exposed to acutely lethal concentrations (ASTDR, 2004).

Observation in humans

There have been several reported cases in which humans have been accidentally exposed to ammonia (as a gas or pressurised liquid). Reported effects include:

- full thickness burns on the skin;
- temporary blindness, corneal abrasions, sustained corneal damage; and
- damage to the respiratory tract such as stripping the bronchial walls of the epithelial lining (IPCS, 1986; ASTDR, 2004; OECD, 2007).

Respiratory and ocular irritation has been observed in workers exposed to lower concentrations, down to 50 ppm (35 mg/m³). Tolerance appears to develop with repeated exposure (IPCS, 1986; ASTDR, 2004; OECD, 2007).

Oesophageal lesions and oedema were reported in people who ingested ammonium hydroxide, and ulcerative lesions on the tongue and/or on the buccal mucosa were reported in children following biting ammonia pellets/capsules (ASTDR, 2004).

Sensitisation

Skin Sensitisation

No data are available.

Repeated Dose Toxicity

Oral

No data are available for the chemicals. Based on the data for ammonium chloride (CAS No: 12125-02-9) (NICNAS), the chemicals are not expected to cause systemic effects following oral exposure, although local effects in the gastrointestinal tract could occur. Metabolic acidosis has been observed following long-term exposure to ammonium salts such as ammonium chloride, but this is not relevant for these basic chemicals (NICNAS).

Dermal

No data are available.

Inhalation

A number of repeated dose inhalation studies are available for the chemicals. These are all older studies that were not conducted in accordance with current guidelines. In general, no systemic effects were observed, although irritation effects for the eye and respiratory tract were reported (IPCS, 1986; ASTDR, 2004; OECD, 2007).

Mild haematological changes were observed in a 12 week study (non-guideline) in rats following exposure to 100 mg/m³ for a period of 25 or 60 mins every 48 hours.

In a 90-day continuous exposure study, Sprague Dawley (SD) and Long-Evans rats (both sexes) were exposed to various concentrations of gaseous ammonia (127, 262, 455 and 470 mg/m³) 24 hours per day. Additionally, 15 Princeton-derived guinea pigs (both sexes), three male New Zealand White rabbits, two purebred male Beagle dogs and three male squirrel monkeys were exposed to a concentration of 470 mg/m³ of gaseous ammonia. At a dose of 455 mg/m³, mortalities were observed in 50/51 rats. At the highest concentration, mortalities occurred in 13/15 rats and 4/15 guinea pigs. Eye irritation (including; discharge, erythema, opacity and excessive lacrimation) was observed in both the dogs and rabbits. Nasal discharge was also observed in dogs. Irritation to the lungs was also observed in two rabbits (moderate lung congestion) and one dog (haemorrhagic lesion). In a similar study, but over a shorter exposure period, (30 days, eight hours per day, five days per week) no mortalities were observed up to an exposure concentration of 770 mg/m³.

Observation in humans

Ocular and respiratory tract irritation has been reported for workers repeatedly exposed to ammonia. Tolerance appears to develop with repeated exposure (see **Corrosivity/Irritation: Observation in humans** for more information).

Genotoxicity

Based on the weight of evidence of the limited available data, the chemicals are not considered to have significant genotoxic potential.

In an Ames assay (OECD TG 471) with five strains of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537, TA1538) and *Escherichia coli* (WP2uvrA) with varying test doses to a maximum dose of 25,000 ppm with and without metabolic activation—ammonia did not induce gene mutations in any of the strains tested (OECD, 2007). Whilst positive results were observed in an Ames assay with *E. coli*, this was only at toxic doses (ASTDR, 2004). Ammonium hydroxide was negative for genotoxic effects in a non-standard bacterial reverse mutation assay using *E. coli* (REACH).

Equivocal results were reported in studies (in vivo) (mutagenic lethality, sex-linked recessive lethal mutations and dominant lethality) in *Drosophila melanogaster*, with positive results only observed at toxic levels (ASTDR, 2004).

Increased frequency of chromosomal aberrations (CAs) and sister chromatid exchanges (SCEs), increased mitotic index (MI), and increased frequency of CAs and SCEs with increasing length of exposure, were observed in workers exposed to ammonia in a fertiliser factory (ASTDR, 2004). However, there is a number of limitations in this study including gaps in the analysis, small study size and possible confounding factors such as smoking and exposure to other chemicals (UK HPA, 2007).

The weight of evidence indicates that the analogue chemical, ammonium chloride, is not genotoxic (NICNAS).

Carcinogenicity

Based on the limited data available, the chemicals are considered to have a low potential to cause carcinogenic effects.

In a 24-week study, 40 male SD rats were exposed to ammonium hydroxide (0.01 %) in their drinking water daily ad libitum. A significantly higher number of gastric tumours were observed in the exposed group when compared with the control group (OECD, 2007).

In a lifetime study, 50 Swiss and 40 C3H mice (both sexes) were exposed to ammonium hydroxide in their drinking water (0.3 % and 0.1 % solution respectively) ad libitum. No carcinogenic effects were observed (OECD, 2007).

No carcinogenic effects were reported in a study in male and female rats (OECD TG 451), on the analogue chemical, ammonium chloride. In another study in rats and mice, this chemical was found to not be a promoter of carcinogenic effects (NICNAS).

Reproductive and Developmental Toxicity

Based on the available data, the chemicals are not expected to cause specific reproductive or developmental toxicity.

In a six-week reproductive study (non-guideline), two groups of 40 Chester White, Yorkshire and Hampshire female pigs were exposed to average atmospheric ammonia concentrations of 7 and 36 ppm. After six weeks of exposure, half of the pigs were sacrificed and half of the pigs were mated with non-exposed mature boars then sacrificed at day 30 of gestation. No adverse effects on reproductive parameters or foetal developmental effects were observed (ASTDR, 2007).

In developmental studies with the analogue chemical, ammonium chloride, limited effects on foetal development were observed. The reduced foetal growth observed in one study was considered due to maternal toxicity (NICNAS).

Risk Characterisation

Critical Health Effects

The critical health effects for risk characterisation include corrosivity and acute toxicity from inhalation exposure.

Public Risk Characterisation

Although use in cosmetic/domestic products in Australia is not known, the chemical is reported to be used in cosmetic/domestic products overseas at concentrations up to 7 %.

The chemicals are currently listed on Schedules 5 and 6 of the SUSMP. At concentrations between 0.5 % and 5 % the label 'Caution' applies. At concentrations >5 % (and particularly >20 %), a number of warning statements, first aid instructions and safety directions relating to eye, skin and respiratory exposure apply.

The current controls are considered adequate to minimise the risk to public health posed by domestic and cosmetic products containing the chemical, therefore, the chemical is not considered to pose an unreasonable risk to public health.

Occupational Risk Characterisation

During product formulation, dermal, ocular and inhalation exposure of workers to the chemicals can occur, particularly where manual or open processes are used. These could include transfer and blending activities, quality control analysis, and cleaning and maintaining equipment. Worker exposure to the chemicals at lower concentrations might also occur while using formulated products containing the chemicals. The level and route of exposure will vary depending on the method of application and work practices employed.

Given the critical systemic acute and local health effects, the chemicals could pose an unreasonable risk to workers unless adequate control measures to minimise dermal, ocular and inhalation exposure to the chemicals are implemented. The chemicals should be appropriately classified and labelled to ensure that a person conducting a business or undertaking (PCBU) at a workplace (such as an employer) has adequate information to determine appropriate controls.

The data available support an amendment to the hazard classification in HSIS for ammonium hydroxide (refer to **Recommendation** section).

NICNAS Recommendation

Assessment of the chemicals are considered to be sufficient, provided that the recommended amendment to the classification is adopted, and labelling and all other requirements are met under workplace health and safety and poisons legislation as adopted by the relevant state or territory.

Regulatory Control

Public Health

Products containing the chemicals should be labelled in accordance with state and territory legislation (SUSMP).

Work Health and Safety

The chemicals are recommended for classification and labelling under the current approved criteria and adopted GHS as below. This assessment does not consider classification of physical hazards and environmental hazards.

NOTE: The acute toxicity oral classification applies to ammonium hydroxide (CAS No. 1336-21-6) only.

Hazard	Approved Criteria (HSIS) ^a	GHS Classification (HCIS) ^b
Acute Toxicity	Harmful if swallowed (Xn; R22) Toxic by inhalation (T; R23)*	Harmful if swallowed - Cat. 4 (H302) Toxic if inhaled - Cat. 3 (H331)
Irritation / Corrosivity	Causes burns (C; R34)*	Corrosive to the respiratory tract (AUH071) Causes severe skin burns and eye damage - Cat. 1 (H314)

^a Approved Criteria for Classifying Hazardous Substances [NOHSC:1008(2004)].

^b Globally Harmonized System of Classification and Labelling of Chemicals (GHS) United Nations, 2009. Third Edition.

* Existing Hazard Classification. No change recommended to this classification

Advice for consumers

Products containing the chemicals should be used according to the instruction on the label.

Advice for industry

Control measures

Control measures to minimise the risk from oral/dermal/ocular/inhalation exposure to the chemicals should be implemented in accordance with the hierarchy of controls. Approaches to minimise risk include substitution, isolation and engineering controls. Measures required to eliminate or minimise risk arising from storing, handling and using a hazardous chemical depend on the physical form and the manner in which the chemical is used. Examples of control measures which may minimise the risk include, but are not limited to:

- using closed systems or isolating operations;
- using local exhaust ventilation to prevent the chemical from entering the breathing zone of any worker;
- air monitoring to ensure control measures in place are working effectively and continue to do so;
- minimising manual processes and work tasks through automating processes;
- work procedures that minimise splashes and spills;
- regularly cleaning equipment and work areas; and
- using protective equipment that is designed, constructed, and operated to ensure that the worker does not come into contact with the chemical.

Guidance on managing risks from hazardous chemicals are provided in the *Managing risks of hazardous chemicals in the workplace—Code of practice* available on the Safe Work Australia website.

Personal protective equipment should not solely be relied upon to control risk and should only be used when all other reasonably practicable control measures do not eliminate or sufficiently minimise risk. Guidance in selecting personal protective equipment can be obtained from Australian, Australian/New Zealand or other approved standards.

Obligations under workplace health and safety legislation

Information in this report should be taken into account to assist with meeting obligations under workplace health and safety legislation as adopted by the relevant state or territory. This includes, but is not limited to:

- ensuring that hazardous chemicals are correctly classified and labelled;
- ensuring that (material) safety data sheets ((m)SDS) containing accurate information about the hazards (relating to both health hazards and physicochemical (physical) hazards) of the chemical are prepared; and
- managing risks arising from storing, handling and using a hazardous chemical.

Your work health and safety regulator should be contacted for information on the work health and safety laws in your jurisdiction.

Information on how to prepare an (m)SDS and how to label containers of hazardous chemicals are provided in relevant codes of practice such as the *Preparation of safety data sheets for hazardous chemicals—Code of practice* and *Labelling of workplace hazardous chemicals—Code of practice*, respectively. These codes of practice are available from the Safe Work Australia website.

A review of the physical hazards of the chemicals has not been undertaken as part of this assessment.

References

Agency for Toxic Substances & Disease Registry (ATSDR) Toxicological Profile for ammonia. Accessed June 2014 at <http://www.atsdr.cdc.gov/toxprofiles/tp126-c3.pdf>

Galleria Chemica. Accessed June 2014 at <https://jr.chemwatch.net/galleria/>

Globally Harmonised System of Classification and Labelling of Chemicals (GHS) United Nations, 2013. Fifth edition. Accessed at http://www.unece.org/trans/danger/publi/ghs/ghs_welcome_e.html

International Programme on Chemical Safety (IPCS) 1986. Ammonia. Accessed June 2014
<http://www.inchem.org/documents/ehc/ehc/ehc54.htm>

National Industrial Chemicals Notification and Assessment Scheme (NICNAS). Inventory Multi-Tiered and Prioritisation (IMAP) Human Health Tier II Assessment for ammonium chloride (CAS No. 12125-02-9). Accessed June 2014 at <http://www.nicnas.gov.au/chemical-information/imap-assessments/imap-assessments>

OECD (2007). SIDS Initial Assessment Report (SIAR) ammonia (CAS No 7664-41-7). Accessed at http://webnet.oecd.org/HPV/UI/SIDS_Details.aspx?key=38d2d3b9-a169-44b9-9a6a-0f7e095e77f4&idx=0

Registration, Evaluation and Authorisation of Chemicals (REACH) Dossier. Ammonia, anhydrous (CAS: 7664-41-7). Accessed June 2014 at <http://echa.europa.eu/web/guest/information-on-chemicals/registered-substances>

Safe Work Australia (SWA). Hazardous Substances Information System (HSIS). Accessed May 2014 at <http://hsis.safeworkaustralia.gov.au/HazardousSubstance>

The Poisons Standard (the Standard for the Uniform Scheduling of Medicines and Poisons (SUSMP)) 2013. Accessed June 2014 at <http://www.comlaw.gov.au/Details/F2013L01607/Download>

United Kingdom Health Protection Agency (UK HPA) 2007. Ammonia Toxicological overview. Version 2. Accessed June 2014 at http://www.hpa.org.uk/webc/hpawebfile/hpaweb_c/1194947398510

Vernot EH, MacEwen JD, Haun CC and Kinkead ER 1977. Acute toxicity and skin corrosion data for some organic and inorganic compounds and aqueous solutions. Toxicology and Applied Pharmacology 42 pp. 417-423.

Last Update 04 July 2014

Chemical Identities

Chemical Name in the Inventory and Synonyms	Ammonium hydroxide Ammonia aqua Ammonia water Aqueous ammonia Ammonium liquor
CAS Number	1336-21-6
Structural Formula	



Molecular Formula	H5NO
Molecular Weight	35.04

Chemical Name in the Inventory and Synonyms	Ammonia Ammonia anhydrous Ammonia gas Ammoniac
CAS Number	7664-41-7
Structural Formula	



Molecular Formula	H3N
Molecular Weight	17.03

Share this page



Ammonia

Toxicological Overview

Key Points

Kinetics and metabolism

- ammonia dissolves in moisture in the air and on tissue or mucous membranes to form ammonium hydroxide
- systemic absorption following dermal or ocular exposure is not considered significant
- ammonia is readily metabolised in the liver to urea or glutamine
- ammonia is excreted primarily in the urine as urea

Health effects of acute exposure

- irritant, corrosive and may be harmful by all routes of exposure
- acute inhalation may initially cause upper respiratory tract irritation
- oral exposure results in pain, excessive salivation and burns to the aerodigestive tract
- substantial exposures can cause oral cavity, nasopharynx, larynx and trachea burns, with airway obstruction, respiratory distress and bronchiolar and alveolar oedema
- corrosive in contact with tissue and splashes to the eye may result in serious injury

Health effects of chronic exposure

- effects following chronic oral exposure have not been defined in humans
- animal studies suggest osteoporosis secondary to chronic metabolic acidosis may occur
- chronic inhalation is associated with increased cough, phlegm, wheeze and asthma

Summary of Health Effects

Ammonia and ammonium hydroxide are corrosive and can rapidly penetrate the eye and may cause permanent injury. Therefore, splashes in the eye should be considered an ophthalmic emergency.

Dermal exposure to ammonia or its solutions may result in irritation and, depending on the concentration, alkali burns. Pressurised ammonia (as a liquefied gas) may also cause cryogenic burns following skin or eye contact.

Ingestion of ammonia solution (ammonium hydroxide) causes rapid onset of signs and symptoms including pain in the mouth, throat and chest, excessive salivation and extensive alkali burns to the aerodigestive tract. In severe cases perforation of the stomach or oesophagus may occur, which can result in complications such as cardiac injury, mediastinitis and pneumonitis. Aspiration of ammonia following ingestion may also lead to respiratory complications. Chronic oral exposure to ammonia has not been characterised in humans. Limited animal data has shown that osteoporosis secondary to chronic metabolic acidosis may occur.

Inhalation of ammonia causes rapid onset of signs and its toxic effects are mediated through its irritant and corrosive properties. Features include irritation to the nose, throat and respiratory tract. Increased lacrimation, coughing, an increased respiratory rate as well as respiratory distress may occur. Substantial exposures can cause burns of all depths in the oral cavity, nasopharynx, larynx and trachea, together with airway obstruction and bronchiolar and alveolar oedema. Exposure to a massive concentration of ammonia gas may be fatal within minutes.

Ammonia has no structural alerts for DNA reactivity, and is not mutagenic.

Ammonia has not been classified as a human carcinogen. Ammonia is not considered to be an animal carcinogen, ingestion by rats of ammonia as ammonium hydroxide for 2 years did not result in an increase in cancers.

Kinetics and Metabolism

Ammonia is extremely soluble in water and dissolves in the mucus fluid covering the mucous lining of the respiratory system to produce ammonium hydroxide, a strong base. Following short-term inhalation exposure, ammonia is almost entirely retained in the upper nasal mucosa [1]. Inhalation of high concentrations of ammonia may exceed the capacity of this mechanism, leading to systemic absorption through the lungs.

Although ammonia rapidly enters the eye, systemic absorption is considered not to be quantitatively significant [1]. The toxicity findings after acute skin exposure to ammonia suggest that systemic absorption is not significant by this route either [2].

Ammonia is absorbed readily through mucous membranes and the intestinal tract but not through the skin [3].

Absorbed ammonia is well distributed throughout body compartments and reacts with hydrogen ions, depending on the pH of the compartment, to produce ammonium ions [3]. The ammonium ion is less mobile due to its charged nature [1].

Ammonia is endogenously produced in the gut from the bacterial breakdown of nitrogenous constituents of food; estimates of production range from 10 mg a day in the duodenum to 3 g a day in the colon [4]. In healthy adults the physiological ammonium level in blood is typically below 35 $\mu\text{mol/L}$ (approximately 0.67 mg/L) [5].

In the liver, ammonium ions are extensively metabolised to urea and glutamine. Consequently, levels of ammonia that reach the circulation are low [2]. The metabolites may be either excreted as urea or used in the synthesis of amino acids and other biomolecules [5]. Ammonia produced in peripheral tissues is not transported to the liver but metabolised into glutamine and alanine [4]. In individuals with deficiencies in ammonia metabolism or urea excretion (eg enzyme deficiencies, or severe renal or hepatic impairment) toxic ammonia levels may be reached within the body [5].

Ammonia reaching the circulation is principally excreted by humans as urinary urea, excretion of absorbed ammonia in exhaled breath and faeces is not significant [2, 3]. Small amounts of ammonia are excreted in urine; the average daily excretion for humans is approximately 2–3 μg , about 0.01% of the total body burden. Small amounts of unabsorbed ammonia may also be excreted from the gastrointestinal tract in faeces [2].

Sources and Route of Human Exposure

Apart from endogenously produced ammonia, the most common route of exposure to ammonia is inhalation [6].

Ammonia in the environment originates from both anthropogenic and natural sources. In 2013 agriculture contributed 81.7% of UK ammonia emissions [7]. The total emissions for the UK in 2013 were estimated at 271,000 tonnes, having fallen 22.7% from 1980 [7]. Local concentrations may be elevated where organic waste matter is concentrated, such as in intensive farming environments for cattle, pig and chickens [8]. Non-agricultural sources include sewage sludge, pets, industrial and combustion processes, and petrol vehicles fitted with catalytic converters [8].

High levels of ammonia are naturally present in certain foods – eg in vegetables levels can range from 4,000–15,000 mg/kg; however, higher levels have been reported [5]. In dairy products ammonia may form as a result of processing – eg cheese ripening, sterilisation and acidification – in some food products it may be added artificially [5].

Ammonia is used either directly or indirectly in many industrial processes. It is transported in bulk as a pressurised gas. Exposure to ammonia may occur in industrial settings or following an accidental spill or leak during transport.

Domestically, exposure may occur from certain cleaning agents and dyes.

Ammonia gas is not persistent and rapidly reacts in the environment to form ammonium compounds.

In light of the levels of ammonia produced endogenously and the ability to detoxify ammonia, environmental exposure is not considered to represent a risk to human health [5]. For vulnerable groups, endogenous ammonia still represents a more relevant exposure source than that from environmental sources [5]. The World Health Organization (WHO) does not consider exposure from ammonia in drinking water to be of immediate health relevance [9].

Health Effects of Acute/Single Exposure

Human data

General toxicity

The clinical manifestations of acute ammonia exposure are usually immediate in presentation and its toxic effects are mediated through its irritant and corrosive properties.

Inhalation

After inhalation of ammonia the damage to the respiratory tract depends on the concentration, pH, length of exposure and depth of inhalation [6].

Inhalation of ammonia will rapidly cause irritation to the nose, throat and respiratory tract. Increased lacrimation, coughing and increased respiratory rate as well as respiratory distress may occur [1]. Ammonia is water soluble and is therefore absorbed by the mucosa of the upper respiratory tract, this protects the lungs from exposure to low concentrations of ammonia [1].

Substantial exposures to concentrated aerosols of ammonium hydroxide, elevated levels of ammonia gas or anhydrous ammonia fumes can cause burns of all depths in the oral cavity, nasopharynx, larynx and trachea, together with airway obstruction, respiratory distress and pulmonary oedema [2, 10, 11].

Exposure to a massive concentration of ammonia gas may be fatal within minutes and asphyxiation may occur after exposure in poorly ventilated or enclosed spaces. Findings in fatal cases include extensive oedema, full thickness burns to the entire respiratory tract, purulent bronchitis and greatly distended lungs [2, 10, 12]. Bronchial walls may also be stripped of their epithelial lining [12, 13].

Lower levels of ammonia exposure that do not result in upper airway obstruction may cause significant alkali burns throughout the tracheobronchial tree [10].

Systemic effects following acute exposures to high concentrations of ammonia include an elevated pulse and blood pressure, bradycardia, cardiac arrest, cyanosis and haemorrhagic necrosis of the liver [2].

The primary features after ammonia exposure are summarised in table 1.

Ammonia has a pungent and characteristic odour of drying urine which is discernible at around 35 mg/m^3 (50 ppm) [1, 2]. However, ammonia causes olfactory fatigue (adaptation) making its presence difficult to detect when exposure is prolonged. Odour, therefore, is not to be considered as a reliable indicator of exposure, or the extent of an exposure.

Table 1: Summary of toxic effects following acute exposure to ammonia by inhalation

Exposure		Signs and symptoms
mg/m ³	ppm	
35	50	Irritation to eyes, nose and throat (2 hours' exposure)
70	100	Rapid eye and respiratory tract irritation
174	250	Tolerable by most people (30–60 minutes' exposure)
488	700	Immediately irritating to eyes and throat
>1,045	>1,500	Pulmonary oedema, coughing, laryngospasm
1,740–3,134	2,500–4,500	Fatal (30 minutes' exposure)
3,480–6,965	5,000–10,000	Rapidly fatal due to airway obstruction, may also cause skin damage
Values in mg/m ³ are approximate calculations from ppm, where $\text{mg/m}^3 = \text{ppm} \times \text{gram molecular weight}/24.45$ (molar volume of air at standard temperature and pressure)		
References [2, 14]		

Ingestion

Ingestion of ammonia solutions (ammonium hydroxide) causes rapid onset of signs and symptoms including pain in the mouth, throat and chest, excessive salivation and extensive alkali burns to the aerodigestive tract. Although there is little quantitative data, these features have been noted in one case involving ingestion (with a suicidal intent) of as little as 20–25 mL of 6% household ammonia solution [15]. In more severe cases perforation of the stomach or oesophagus may occur soon after ingestion, perforation may also follow ulceration with complications including cardiac injury, mediastinitis and pneumonitis [6].

In severe cases haemorrhagic or hypovolemic shock and airway obstruction from laryngeal or epiglottic oedema may occur [6]. Aspiration following ingestion may result in stridor and respiratory complications such as pneumonitis, pulmonary oedema, acute respiratory distress syndrome (ARDS) and pulmonary necrosis [6].

Paediatric exposures to ammonia capsules (used as “smelling salts”) or small volumes of ammonia solutions may cause the child to be drooling and irritable, dysphagic and with ulcerative lesions to the buccal cavity and first degree burns to the tongue or aerodigestive tract [16–18].

Adult fatalities have occurred from deliberate ingestion of ammonia solutions. In one case, ingestion of an unspecified volume of 3% ammonium ion resulted in aspiration pneumonia and laryngeal and epiglottic oedema and a friable and erythematous oesophagus with severe corrosive injury [2]. The individual died several days later from ARDS and renal failure. In another case, after the ingestion of an unspecified amount of ammonium hydroxide

solution (2.4% ammonium ion), findings at post-mortem included haemorrhagic oesophagus, stomach and duodenum [2].

Dermal/ocular exposure

Splashes of ammonia into the eye may cause serious and potentially irreversible damage [6]. Effects may range from increased lacrimation, conjunctivitis, palpebral oedema, photophobia and blepharospasm, through to corneal ulceration, corneal opacification, iritis, anterior and posterior synechia formation, retinal atrophy, glaucoma, cataract formation and blindness [19]. Irritation arising from low atmospheric concentrations of $>20 \text{ mg/m}^3$ ($>29 \text{ ppm}$) is considered to be readily reversible when exposure ceases [1].

Anhydrous ammonia gas stored under pressure as a compressed liquid expands rapidly on liberation, resulting in vaporisation and a large endothermic reaction. The result may be evaporative freezing of any tissue in contact with the ammonia [20]. Ammonia readily forms ammonium hydroxide on contact with moisture in the air and skin and the resultant hydroxide saponifies lipids of the epidermal fats and cell membranes [14]. The resultant liquifactive necrosis may appear pale and without charring or blistering and may cause an increased depth of injury [3]. Initially alkali burns may be painless, which could lead to a delay in treatment. Effects may develop over several hours and can be difficult to assess as a result of rapid skin discolouration [6]. The combination of both cryogenic effects and alkali burn can produce serious injuries. Skin damage may occur after exposure to ammonia gas at concentrations of approximately $7,634 \text{ mg/m}^3$ ($10,000 \text{ ppm}$) [6].

Individuals with extensive burns to the eyes and skin are likely to have obstruction of the airway [10].

Delayed effects following acute exposure

Inhalation exposures to low concentrations for a short period, from which an individual recovers quickly on removal to fresh air, are unlikely to result in delayed or long-term adverse health effects.

In severe cases pulmonary oedema, breathlessness, wheezing, hypoxia and cyanosis may take 36 hours to develop after the initial inhalation exposure [6].

Substantial inhalation exposures to ammonia may cause long-term health effects, including persistent airway obstruction, cough, exertional dyspnoea, bronchiolitis obliterans and bronchiectasis, which for some cases may persist for many years [2, 10, 13, 14]. Dysphonia may persist for many months as a result of burns to the aerodigestive tract [10, 12].

Ingestion of ammonia may lead to the development of strictures between 2 weeks and 2 months after exposure, though may not be clinically apparent for years [6]. Ammonia ingestion may lead to a pyloric stenosis with a small, scarred, immobile stomach [6].

Scarring to body tissue can be pronounced following burns from ammonia exposure [14].

Health Effects of Chronic/Repeated Exposure

Human data

Inhalation

There is limited data on the effects of chronic inhalation of ammonia. Inhaled ammonia is retained by the mucosal membranes of the upper respiratory tract, therefore effects on the lower respiratory tract are less common [21]. However, there are a few case reports of interstitial lung disease associated with chronic low level exposure to ammonia [22]. Occupational studies have reported an increase in prevalence of acute and chronic respiratory symptoms and a decline in respiratory function in fertiliser factory workers exposed to ammonia [21-24].

Acclimatisation to the irritant effects of ammonia at concentrations up to 70 mg/m³ (100 ppm) has been demonstrated after repeated exposure for 6 hours a day for 5 days each week over a 6-week period [25]. No further interpretation was possible due to the limited design of this study.

Chronic inhalation of ammonia may result in optic neuropathy [6].

Ingestion

There is no human data on which to assess the effects of chronic excessive ammonia intake.

Ammonia may be ingested in both food and water; however, as ammonia is readily metabolised to products of low toxicity (such as urea and glutamate) within the body, it is unlikely that chronic exposures to low levels will have a significant adverse health effect.

Genotoxicity

There is limited data in humans on the genotoxicity of ammonia. One small study in humans examining the exposure to ammonia at a fertiliser factory noted an increase in chromosomal aberrations, sister chromatid exchanges and increased mitotic index [26]. There was a weak association reported between increased length of exposure and increased frequency of chromosomal aberrations and sister chromatid exchanges. No detail was given as to how well the exposed and control group were matched for age, smoking habits, etc. Furthermore, it appears that gaps were included in the cytogenetic analysis. Given these limitations and the small size of this study, the low levels of ambient ammonia and the likely exposure to other chemicals, no conclusions can be drawn regarding the mutagenicity of ammonia.

There is no other in-vivo human data on which to assess the genotoxicity of ammonia and there is conflicting evidence between this study and in-vitro studies. However, ammonia has no structural alerts for DNA damage and when the in-vitro data is taken into account, ammonia can be considered as not having significant mutagenic potential.

Carcinogenicity

There is insufficient evidence to classify ammonia as a carcinogen in humans and it has not been classified by the International Agency for Research on Cancer (IARC).

Reproductive and developmental toxicity

There is no published data available on the potential effects of exposure to ammonia on reproductive function or human pregnancy [27]. A healthy adult with normal liver function and sufficient dietary arginine would be expected to be able to metabolise ammonia. Therefore, exposure to low levels of ammonia in the environment would not be expected to result in damage to the fetus [27].

The UK Teratology Information Service (UKTIS) followed up 24 cases of women exposed to ammonia during pregnancy [27]. Exposures ranged from acute to 20 weeks [27]. The rate of major congenital malformations was found to be higher than the background; however, owing to the small sample size and resultant wide confidence intervals, this finding may be due to chance [27].

Animal and in-vitro data

Inhalation

Inhalation exposure of several animal species to ammonia has been conducted. Repeated exposures (8 hours a day for 5 days a week for 30 days) to 155 mg/m³ (233 ppm) ammonia produced no adverse clinical effects in rats, guinea pigs, rabbits, dogs and monkeys [28]. The group sizes of higher order animals were, however, small (2 or 3). After continuous exposure to 40 mg/m³ (58 ppm) ammonia for 114 days, there were no adverse clinical observations noted and findings at post-mortem were normal. Histological examination revealed lipid-filled macrophages in the lungs of 2/2 dogs, 1/3 monkeys and 1/15 rats; these findings were considered to be of uncertain toxicological significance. No lung alterations were seen in the remaining experimental or control animals [28].

A limited study in pigs compared low atmospheric exposures to ammonia of approximately 5 mg/m³ (7 ppm) with moderate exposures of approximately 24 mg/m³ (35 ppm). Mean daily body weight was reduced in the moderate exposure group in the first 2 weeks of exposure, which resulted in small animals at slaughter after 6 weeks of exposure [29].

Ingestion

There is limited data available on the effects of chronic oral exposure to ammonia (as ammonium hydroxide).

In one study, rabbits were given ammonium hydroxide (100 mg/kg bw) as a 0.5–1% solution by oral gavage for up to 17 months. The key findings from this study included an initial fall in blood pressure, followed by an increase above the baseline and enlarged adrenal glands [1].

Long-term exposure of rats, rabbits and dogs to ammonium salts such as ammonium chloride can cause metabolic acidosis. This acidosis occurs from H⁺ ions released during

conversion to urea and may produce a range of non-specific effects on cardiovascular, pulmonary (including increased ventilation), gastrointestinal and musculoskeletal functions [2]. Osteoporosis has been noted, arising from the mobilisation of bone mineral to spare bicarbonate. However, these are considered to be secondary to prolonged metabolic acidosis [1].

Genotoxicity

Ammonia gas was negative in the Ames tests for *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 in both activated and non-activated systems. Concentrations in the range 714–35,700 mg/m³ (500–25,000 ppm) were employed. Tests conducted in *Escherichia coli* WRP uvrA were also negative [30].

Positive effects were noted in a separate reverse mutation study test in *E. coli* but only at treatments of ammonia that caused severe toxicity [1].

There is very limited in-vivo mammalian data on the effects of ammonia. One study with mice (a single intraperitoneal dose of ammonia at 12, 25 or 50 mg/kg) reported dose-dependent increases in the frequencies of micronuclei when compared to controls [26]. Few details were given (it was not stated if ammonia was given as a gas or a solution) and no conclusions can be drawn.

Neither ammonia nor its metabolites have any structural alerts for DNA reactivity. In view of this and the negative in-vitro data, it is concluded that ammonia does not have any significant mutagenic properties.

Carcinogenicity

Ammonia is considered not to be a carcinogen in animals.

The carcinogenic potential of ammonia gas has not been considered by IARC. Administering a daily oral dose to mice of 193 mg/kg bw of ammonia as ammonium hydroxide in drinking water for 2 years did not result in carcinogenic effects or increase the spontaneous incidence of breast cancer in the C3H female mice used in the study [2].

Reproductive and developmental toxicity

There is little data on the reproductive or developmental toxicity of ammonia. However, ammonia is not considered to be a developmental toxin.

Several studies have investigated the effects of ammonium ion on development using embryo culture techniques [31, 32]. There was a relationship between the concentration of ammonium in the culture medium and the incidence of abnormalities or toxicity to the blastocyst [31, 32]. The relevance of this to an in-vivo model or indeed to humans is doubtful, as high concentrations of ammonia are unlikely to reach the fetus in-vivo due to its rapid and extensive metabolism.

References

1. International Programme on Chemical Safety (IPCS). Ammonia. Environmental Health Criteria 54, 1986. World Health Organization: Geneva.
2. Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological Profile for Ammonia, 2004.
3. International Programme on Chemical Safety (IPCS). Ammonia. Health and Safety Guide, 1990. World Health Organization: Geneva.
4. EFSA Panel on Food Contact Materials, E Flavourings and Processing Aids (CEF). Scientific opinion on flavouring group evaluation 46. Revision 1 (FGE.46Rev1): Ammonia and three ammonium salts from chemical group 30. EFSA J, 2011; 9(2): 1925 [35 pp]. doi:10.2903/j.efsa.2011.1925. Available at www.efsa.europa.eu.
5. European Food Safety Authority (EFSA). Health risk of ammonium released from water filters. EFSA J, 2012; 10(10): 2918 [16 pp.] doi:10.2903/j.efsa.2012.2918. Available at www.efsa.europa.eu/efsajournal.
6. National Poisons Information Service (NPIS). Ammonia. TOXBASE®, 2014 (accessed 04/2014.)
7. Department for Environment, Food and Rural Affairs (Defra). National statistics release: emissions of air pollutants in the UK, 1970 to 2013, 2014. Available at http://www.defra.gov.uk/environment/airquality/ammonia/pdf/ammonia_uk.pdf.
8. Department for Environment, Food and Rural Affairs (Defra). Ammonia in the UK, 2002.
9. World Health Organization (WHO). Guidelines for drinking-water quality (4th edition), 2011. World Health Organization: Geneva.
10. Close LG, FI Catlin, and AM Cohn. Acute and chronic effects of ammonia burns on the respiratory tract. Arch Otolaryngol, 1980; 106(3): 151–8.
11. Latenser BA and TA Lucktong. Anhydrous ammonia burns: case presentation and literature review. J Burn Care Rehabil, 2000; 21(1 Part 1): 40–42.
12. Kass I, et al. Bronchiectasis following ammonia burns of the respiratory tract. A review of two cases. Chest, 1972; 62(3): 282–5.
13. Leduc D, et al. Acute and long term respiratory damage following inhalation of ammonia. Thorax, 1992; 47(9): 755–7.
14. Amshel CE, et al. Anhydrous ammonia burns case report and review of the literature. Burns, 2000; 26(5): 493–7.
15. Singh S, et al. Gastro-esophageal burns following domestic liquid ammonia ingestion: report of two cases. J Assoc Physicians India, 1999; 47(6): 647.
16. Rosenbaum AM, et al. Ammonia capsule ingestion causing upper aerodigestive tract injury. Otolaryngol Head Neck Surg, 1998; 119(6): 678–80.
17. Massa N and JP Ludemann. Pediatric caustic ingestion and parental cocaine abuse. Int J Pediatr Otorhinolaryngol, 2004. 68(12): 1513–17.
18. Wason S, M Stephan and C Breide. Ingestion of aromatic ammonia "smelling salts" capsules. Am J Dis Child, 1990; 144(2): 139–40.
19. George A, et al. Liquid ammonia injury. Burns, 2000; 26(4): 409–13.
20. Sotiropoulos G, et al. Cold injury from pressurized liquid ammonia: a report of two cases. J Emerg Med, 1998; 16(3): 409–12.
21. Rahman MDH, M Bråtveit and BE Moen. Exposure to ammonia and acute respiratory effects in a urea fertilizer factory. Int J Occup Environ Health, 2007; 13(2): 153–9.
22. Brautbar N, MP Wu and ED Richter. Chronic ammonia inhalation and interstitial pulmonary fibrosis: a case report and review of the literature. Arch Environ Health, 2003; 58(9): 592–6.
23. Ali BA, et al. Pulmonary function of workers exposed to ammonia: a study in the Eastern Province of Saudi Arabia. Int J Occup Environ Health, 2001; 7(1): 19–22.
24. Bhat MR and C Ramaswamy. Effect of ammonia, urea and diammonium phosphate (DAP) on lung functions in fertilizer plant workers. Indian J Physiol Pharmacol, 1993; 37(3): 221–4.

25. Ferguson WS, et al. Human physiological response and adaption to ammonia. *J Occup Med*, 1977; 19(5): 319–26.
26. Yadav JS and VK Kaushik. Genotoxic effect of ammonia exposure on workers in a fertilizer factory. *Indian J Exp Biol*, 1997; 35(5): 487–92.
27. UK Teratology Information Service (UKTIS). Ammonia exposure in pregnancy, 2012 (accessed 04/2014.)
28. Coon RA, et al. Animal inhalation studies on ammonia, ethylene glycol, formaldehyde, dimethylamine, and ethanol. *Toxicol Appl Pharmacol*, 1970; 16(3): 646–55.
29. Diekman MA, et al. Growth and reproductive performance, during exposure to ammonia, of gilts afflicted with pneumonia and atrophic rhinitis. *Am J Vet Res*, 1993; 54(12): 2128–31.
30. Shimizu H, et al. The results of microbial mutation test for forty-three industrial chemicals. *Sangyo Igaku*, 1985; 27(6): 400–419.
31. Sinawat S, et al. Fetal abnormalities produced after preimplantation exposure of mouse embryos to ammonium chloride. *Hum Reprod*, 2003; 18(10): 2157–65.
32. Lane M and DK Gardner. Increase in postimplantation development of cultured mouse embryos by amino acids and induction of fetal retardation and exencephaly by ammonium ions. *J Reprod Fertil*, 1994; 102(2): 305–12.

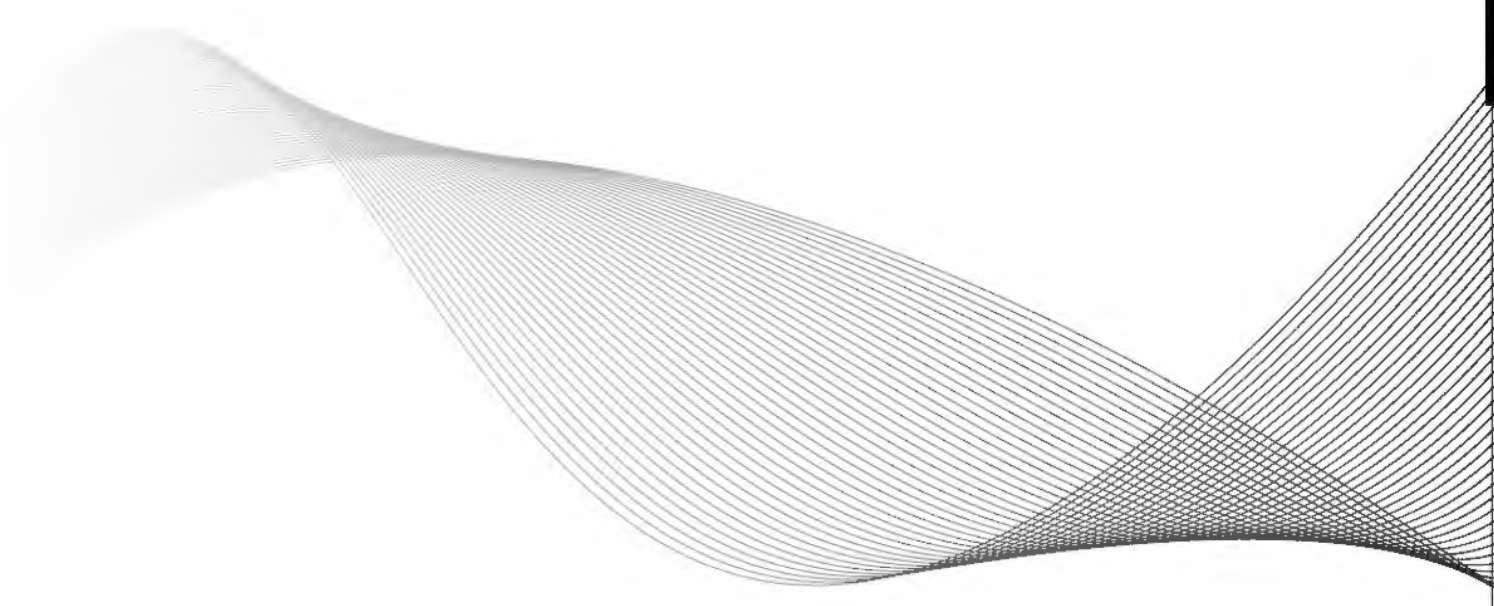
This document from the PHE Centre for Radiation, Chemical and Environmental Hazards reflects understanding and evaluation of the current scientific evidence as presented and referenced here.

First published: August 2015

For queries relating to this document, please contact generaltox@phe.gov.uk

© Crown copyright 2015, www.gov.uk/phe

Re-use of Crown copyright material (excluding logos) is allowed under the terms of the Open Government Licence, visit www.nationalarchives.gov.uk/doc/open-government-licence/version/3/ for terms and conditions.



Recommendation from the Scientific Expert Group on Occupational Exposure Limits for ammonia

SEG/SUM/20
1992





Table of Contents

1 Occurrence/use 4

2 Health Significance: 4

3 Recommendation 4

4 Bibliography 6



Recommendation from the Scientific Expert Group on Occupational Exposure Limits for ammonia

8 hour TWA:	20 ppm (14 mg/m ³)
STEL (15 mins):	50 ppm (36 mg/m ³)
Notation:	-

Substance:

Ammonia

Synonyms :

EINECS N° : 231-635-3

EECN° : 007-001-00-5

Classification : RIO T; R23

CAS N° : 7664-41-7

MWt : 17.03

Conversion factor (20°C, 101kPa) : 0.71 mg/m³ = 1 ppm



1 Occurrence/use

Ammonia is a colourless gas at ambient temperature and pressure with a strong, irritating odour, a MPt of -78°C, a BPt of -33°C and a vapour pressure of 8.7 atm. at 20°C. It is explosive over the range 16 - 27%. The odour threshold is about 5 ppm (3.6 mg/irr).

Ammonia is present in the environment as a result of biological, agricultural and human activity. Together with industrial sources, the total production of ammonia in the EEC is in excess of 10 million tonnes per annum. It is present in a number of industrial activities mainly related to fertilizer production. It is commercially available as the liquified gas or as aqueous solutions.

2 Health Significance

The critical effect of ammonia is irritation of the eyes, skin and upper respiratory tract. Studies in human volunteers have indicated that, in some individuals, subjective symptoms start to occur at exposures of around 50 ppm (36 mg/m³) for periods ranging from 10 min to 6 hours and that acclimatization occurs (MacEwen *et al*, 1970; Industrial Biotest Labs, 1973; Ferguson *et al*, 1977; Verbeck, 1977).

No signs of toxicity were reported in rats, rabbits, guinea-pigs, dogs and monkeys following continuous exposure to 56 ppm (40 mg/m³) ammonia for 114 days (Coon *et al*, 1970).

There is no evidence for mutagenicity or carcinogenicity of ammonia (Litton-Bionetics, 1975; Toth, 1972). Reproductive toxicity of ammonia has not been adequately investigated.

3 Recommendation

The studies cited above, indicating a LOAEL of 50 ppm (36 mg/m³) for mild irritation in humans, were considered to be the best available basis for setting exposure limits. An uncertainty factor of 2 was applied to allow for individual variability. According to the preferred value approach of the SEG, the recommended 8-hour TWA for ammonia is 20 ppm (14 mg/irr).



A STEL of 50 ppm (36 mg/m) is recommended to limit peaks in exposure which could result in irritation.

At the level recommended, no measurement difficulties are foreseen.



4 Bibliography

Principal reference

Scientific document on professional exposure limit for ammonia.
Prepared by S.Basilico and T. Garlanda, Milan.

Key Studies

Coon, R.A., Jones, R.A., Jenkins, L.J. jr., Siegel, J. (1970). Animal inhalation studies on ammonia, ethylene glycol, formaldehyde, dimethylamine and ethanol. *Toxicol. Appl. Pharmacol.* 16, 646-655.

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

Industrial Biotest Laboratories, Inc. (1973). Irritation threshold evaluation study with ammonia, IBL Inc. (Report to International Institute of Ammonia Refrigeration, Publ. No. 663-03161.

Litton Bionetics Inc. (1975). Mutagenic evaluation of compound FDA 73-42 ammonium sulfate, granular, food grade, Washington DC, LBI (prepared for U.S. Food and Drug Administration) (N'H8 PB-245 506).

MacEwen, J.D., Theodore, J., Vernot, E.H. (1970). Human exposure to EEL concentrations of monoethylhydrazine. In: Proceedings of the 1st Annual Conference on Environmental Toxicology, Ohio, Wright-Patterson Air Force Base, 9-11 September 1970, Aerospace Medical Research Laboratory, pp 355-363 (AMRL-TR-70-102, Paper No 23).

Toth, B. (1972). Hydrazine, methylhydrazine and methylhydrazine sulfate carcinogenesis in Swiss mice. Failure of ammonium hydroxide to interfere in the development of tumors. *Int. J. Cancer* 9, 109.

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

SIDS INITIAL ASSESSMENT PROFILE

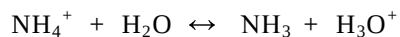
CAS Nos.	7664-41-7; 1336-21-6; 7783-18-8; 12593-60-1
Chemical Names	<u>Ammonia category</u> : ammonia, aqueous ammonia, ammonium thiosulfate, ammonium phosphate sulfate
Structural Formula	NH ₃ ; NH ₄ OH; (NH ₄) ₂ S ₂ O ₃ ; (NH ₄) ₂ H ₂ PO ₄ HSO ₄

SUMMARY CONCLUSIONS OF THE SIAR

Category Rationale

The ammonia category includes anhydrous ammonia, aqueous ammonia, ammonium thiosulfate and ammonium phosphate sulfate. In biological fluids and in aquatic environments, every substance of the category will dissociate to yield, respectively; ammonium ion (NH₄⁺), hydroxide ion (OH⁻), thiosulfate ion (S₂O₃²⁻), phosphate ion (H₂PO₄⁻) and sulfate ion (SO₄²⁻). Each of the compounds of the category contains the ammonia/ammonium ion functional group. The sulfate, thiosulfate and phosphate ions are normal constituents of the blood, and when in excess, are excreted via the urine. Among these compounds, *un-ionized* ammonia is considered the most toxic and is the basis for read-across for the category members.

The category is based on the dissociation of all the category members into the ammonium ion (NH₄⁺) and the corresponding anions in aqueous environments, where the ammonia/ammonium ion will exist in equilibrium between NH₃ and NH₄⁺, depending on the pH. At ambient environmental conditions, the category members are stable substances that show normal acid/base chemical activity with the following equilibria:



Thus for many environmental conditions (pH =5 to 8), the predominant form will be NH₄⁺. At pH =9 the ratio of ammonia to ammonium ion (NH₃/ NH₄⁺) should approach unity; at higher pHs the proportion of ammonia (NH₃) should increase. As inorganic salts that ionize in the aquatic environment, the category members can be considered chemically and structurally similar for the purposes of this category evaluation. As pH decreases, the concentration of ammonium ion increases with respect to decreases of unionized ammonia concentrations. However, the toxicity of the unionized ammonia is considered several orders of magnitude greater than the more abundant ammonium ion. The ammonium ion toxicity is considered to contribute to the overall toxicity.

Analog Rationale

Data for ammonium sulfate (SIAM 19; CAS No. 7783-20-2), sodium sulfate (SIAM 20; CAS No. 7757-82-6), ammonium chloride (SIAM 17; CAS No. 12125-02-9), sodium thiosulfate (CAS No.

7772-98-7) and di-ammonium phosphate (SIAM 24; CAS No. 7783-28-0) are used to fill the data gaps for SIDS endpoints. These substances all dissociate to their respective cations and anions at physiological pH, whose data are used to support the respective cations or anions of the category members.

Human Health

After uptake into a biological system the salts in the ammonia category will dissociate directly into ammonium ion and the corresponding anions, i.e. phosphate, thiosulfate and sulfate. The anions will enter the body electrolyte pool, and are not expected to play a significant toxicological role at low doses. After intestinal absorption, ammonium ions are converted to urea in the liver, and subsequently excreted in urine (within 6 hours). The ratio of ammonium ion to neutral ammonia is about 100 in blood of normal pH range.

The acute oral LD₅₀ values range from 1,950 to >2,000 mg/kg bw in rats for the ammonium salts. Aqueous ammonia has a higher oral toxicity (LD₅₀ of 350 mg/kg bw in rat) than the other compounds in the group. Clinical signs include sedation, staggering, abnormal posture, convulsions, tremors, ataxia, prostration, ptosis, exophthalmus, chromodacryorrhea, unilateral ocular opacity, salivation, labored and irregular breathing and diarrhea. Acute dermal LD₅₀ values are >2,000 mg/kg bw for ammonium sulfate and diammonium phosphate (DAP). The inhalation LC₅₀ values range from >900 mg/m³ for ammonium sulfate (guinea pig) to 13,770 mg/m³ for ammonia (rat). Clinical signs observed after inhalation include eye irritation, labored breathing and nasal discharge, pulmonary hemorrhages. Ammonia concentrations of 348-6953 mg/m³ may lead to death in humans. The LD₅₀ and LC₅₀ values are taken from studies whose reliability rating is a 4 (unassignable) and whose data are used on a weight of evidence basis.

Aqueous ammonia is corrosive to skin and eyes in animal studies and humans due to the high pH. The ammonium salts are slightly irritating to skin and moderately irritating to eyes in animal studies. In humans, slight respiratory irritation was observed at 50 mg/m³ ammonia and at 1 mg/m³ concentrations of ammonium sulfate, pulmonary function was affected. No data are available on the sensitization potential of the category members. However, human data suggest that DAP may be sensitizing to the respiratory tract. In repeated-dose inhalation studies, with ammonia (rats) and aqueous ammonia (rats, rabbits, guinea pigs and dogs), the main effects observed are irritation, and inflammation of the respiratory tract at 105 mg/m³ and above. For ammonia, the NOAEL of 6.4 mg/m³ was determined based on an occupational study and the lack of evidence of decreased pulmonary function or changes in subjective symptoms. No LOAEL was established. In rats, the LOAEL was determined to be 17.4 mg/m³ based on increased severity of rhinitis and pneumonia with respiratory lesions. A NOAEL was not established.

For ammonium sulfate, inhalation studies in rats showed respiratory effects (enlarged alveoli, alveolar ducts and sacs) at 1 mg/m³. In a semi-chronic study [input exposure duration], enhanced bronchiolar cell hyperplasia and fibrosis were reported at 0.5 mg/m³. In a sub-chronic inhalation study with rats, exposure to 26.8 mg/m³ of ammonium thiosulfate resulted in systemic effects such as growth reduction, increased levels of acid and alkaline phosphatase, effects on calcium, CNS effects and cardiovascular changes.

In a 13 week repeated-dose feeding study in rats given ammonium sulfate up to 1,975 mg/kg bw/day, no effects were observed on body weight, food consumption or hematological and clinical parameters. However, increased kidney weights (in males and females) and increased liver weights (females) were observed at the highest dose without histopathological changes. The NOAEL was 1,975 mg/kg bw/day for females and 886 mg/kg bw/day for males (diarrhea). In a combined repeated-dose/reproduction/developmental oral gavage screening study in rats exposed to 0, 250, 750 and 1,500 mg/kg bw DAP for 35 days, a NOAEL of 250 mg/kg bw was derived based on increased alkaline phosphatase and decreased total protein in blood.

Ammonia, ammonium thiosulfate and the analogues ammonium sulfate and DAP did not induce effects in tests on gene mutations and chromosomal aberrations. Ammonium sulfate was negative, *in vivo*, when tested for chromosomal effects. Ammonia is not carcinogenic when administered to rats in the drinking water. However, ammonia did have promoting effects in gastric cancer induced by N-methyl-N'-nitro-N-nitrosoguanidine (MMNG). Ammonium sulfate showed no promoting effect.

No histopathological changes to the reproductive organs were found in repeated-dose toxicity studies with ammonium sulfate. No effects on reproduction and development were found in studies with ammonia (at 24.3 mg/m³). In a reproduction/developmental toxicity screening test (similar to OECD 422) rats (5 males and 10 females/group) were administered DAP by oral gavage at doses of 0, 250, 750 and 1,500 mg/kg bw/day. Animals were exposed two weeks prior to mating, during mating (both sexes) and for an additional three and a half weeks through gestation until lactation day 4 (females). No effects on reproduction were reported up to 1,500 mg/kg bw/day. No developmental effects were observed up to 1,500 mg/kg bw/day. The NOAEL for reproductive and developmental toxicity for DAP was 1,500 mg/kg bw.

Environment

The melting points for ammonia, aqueous ammonia, ammonium thiosulfate, and ammonium phosphate sulfate are -78°C, -77°C, 150°C, and 235°C, respectively. The boiling points are -33°C, 36°C, for ammonia and aqueous ammonia, respectively, while ammonium thiosulfate and ammonium phosphate sulfate decompose at 150°C and >235°C, respectively. The vapor pressure for ammonia is 8611hPa at 20°C; aqueous ammonia, 2878hPa at 25°C; ammonium thiosulfate, 18 mm Hg at 70°F (21.1°C), and ammonium phosphate sulfate – (not applicable). The water solubility for the category substances is in the range of 430 to > 1000 g/L at 20°C. The pH for ammonia is 10.6-11.6 (0.01-1%) at 25°C and aqueous ammonia is expected to be similar. The pKa for ammonia is 9.25. Ammonium thiosulfate (200 g/L at 20°C) and ammonium phosphate sulfate (10% solution in water) pH values are in the range of 6.5-7.2 and 4.5-5.5°C, respectively.

All substances from the ammonia category are very soluble in water and dissociate upon release into water. Anhydrous and aqueous ammonia are volatile. Ammonia toxicity depends on the pH and temperature of the media, as well as the amount of ammonia already present. Increasing pH, and temperature to a lesser degree, results in the presence of more un-ionized ammonia (percentage of total ammonia present as NH₃ in aqueous solutions at 20 °C is 0.039% at pH 6 and 3.82% at pH 8).

Upon application of ammonium fertilizers, ammonium can be taken up by plants or adsorbed onto clay particles in the soil. Leaching or run-off may occur via cation exchange dependent on soil texture, clay content, pH and ionic strength of the irrigation water. Soil bacteria may convert ammonium to nitrate. Nitrate can be taken up by plants or denitrified again to yield nitrogen and nitrous oxide gas. Ammonia may also directly volatilize from the soil. Transport via the atmosphere will mainly be due to diffusion and air concentrations will decrease quickly with increasing distance from the source. Ammonium ion and the anions from the substances of the ammonia category are all subject to nitrification. The fate and behaviour of the substances, such as bioaccumulation, are also closely related to nitrogen cycles in air, soil and water. In anaerobic environments, sulfate is biologically reduced to (hydrogen) sulfide by sulfate reducing bacteria, or incorporated into living organisms as a source of sulfur, and thereby included in the sulfur cycle. In acidic aqueous environments, thiosulfate is similarly expected to dissociate to sulfur and sulfur dioxide and enter the sulfur cycle and/or the atmosphere. Phosphate salts released into the environment will be distributed between water and soil. Land-applied phosphate and ammonium are adsorbed to soil particles. In water the phosphate salts may result in eutrophication (caused by ammonium and phosphate ions), which may lead to increased algal growth. Decomposition of the algae may in turn result in lower dissolved oxygen concentrations. If dissolved oxygen concentrations are lowered significantly, suffocation of other aquatic organisms may occur.

LC₅₀ values for fish toxicity range between 6.9 and 175 mg total NH₃/L for the ammonium substances dependent on pH and temperature during the test. Long-term exposure of fish to ammonium compounds may induce reproductive changes starting at 100 mg/L. EC₅₀ values for invertebrates range from 21.8 mg total NH₃/L for ammonium chloride to > 25.7 mg total NH₃/L for ammonium sulfate. Long-term studies with invertebrates showed slightly lower NOEC values of 3.1-3.47 mg total NH₃/L. The algae *Chlorella vulgaris* EC₅₀ (biomass; 0-5d) was 1300mg/L using ammonium chloride). In a 21-day test with *Chlorella vulgaris* and ammonium sulfate, an EC₅₀ of 25,476 mg/L (2700 mg N/L) was established from exponential growth on day 11-18. Ammonia that is unionized is toxic to aquatic organisms at concentrations below 1 mg/L.

Exposure

For the year 2004 the global market for the ammonia category was estimated to be ca. 65,000 ktonnes. For the United States (Sponsor country) production was estimated at 9 ktonnes. The substances are mainly used as intermediates in the production of fertilizers, fibers and plastics, explosives, solvents and household cleaning products. Environmental exposure occurs through naturally occurring mechanisms whereby microorganisms break down organic nitrogen products such as urea and proteins found in manure as well as via the use of fertilisers and household cleaning

products. According to the information in the USEPA Toxics Release Inventory database for 2004, the total reported emissions of ammonia were 171,179,218 lbs (77,647 tonnes).

Occupational exposure may occur during the production, transportation and processing of ammonia containing compounds. Field exposure to workers is possible during use as fertilizers via both the dermal and inhalation routes of exposure. A generic Threshold Limit Value (TLV) of 10 mg/m³, as a nuisance dust, exists for the solids of the ammonia category. The Occupational Safety and Health Administration (OSHA) has set a Permissible Exposure Limit (PEL) of 50 ppm (35 mg/m³) averaged over 8 hours for ammonia and 15 mg/m³ for sodium thiosulfate. The National Institute for Occupational Safety and Health (NIOSH) has recommended an airborne exposure limit of 25 ppm (18 mg/m³) averaged over a 10 hour period and not to exceed 35 ppm (27 mg/m³) during any 15 minute work period for ammonia. Similarly, the American Conference of Governmental Industrial Hygienists (ACGIH) has recommended a 25 ppm (18 mg/m³) airborne exposure limit averaged over 8 hours with a short term exposure limit (STEL) of 35 (27 mg/m³) ppm.

Consumer exposure can occur during the use of cleaning products and fertilizers.

RECOMMENDATIONS AND RATIONALE FOR THE RECOMMENDATION AND NATURE OF FURTHER WORK RECOMMENDED

Human Health: The chemicals are currently of low priority for further work. The chemicals possess hazards for human health (irritant to corrosive, acute toxicity). Based on data presented by the Sponsor country, adequate risk management measures are being applied. Countries may desire to check their own risk management measures to find out whether there is a need for additional measures. In the environment, ammonia degrades to nitrite. It is recommended that the use of the chemicals as fertilizers be taken into account when assessing the exposure of nitrite and nitrate to humans through drinking water.

Environment: The chemicals are currently of low priority for further work. The chemicals possess properties indicating a hazard to the environment (acute aquatic EC/LC₅₀ values between 1 and 100 mg/L). Unionized ammonia toxicity to aquatic organisms is reported at < 1 mg/L. The pH and temperature of water bodies can affect the EC/LC₅₀ values. The chemicals are of low priority for further work for the environment because of their rapid nitrification. Ammonia has indirect and long-term effects on ecosystems, e.g. eutrophication, groundwater pollution and soil acidification due to the nitrification of ammonia.