TOXICOLOGICAL PROFILE FOR NITRATE AND NITRITE

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

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DISCLAIMER

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UPDATE STATEMENT

A Toxicological Profile for Nitrate and Nitrite, Draft for Public Comment was released in September 2015. 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 and Human Health Sciences
Environmental Toxicology Branch
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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 these toxic substances described therein. Each peer-reviewed profile identifies and reviews the key literature that describes a 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 the protection of public health are identified by ATSDR.

Each profile includes the following:

(A) The examination, summary, and interpretation of available toxicologic information and epidemiologic evaluations on a toxic 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. Staffs 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.

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*Legislative Background

The toxicological profiles are developed under the Comprehensive Environmental Response, Compensation, and Liability Act of 1980, as amended (CERCLA or Superfund). CERCLA section 104(i)(1) directs the Administrator of ATSDR to "…effectuate and implement the health related authorities" of the statute. This includes the preparation of 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. Section 104(i)(3) of CERCLA, as amended, directs the Administrator of ATSDR to prepare a toxicological profile for each substance on the list. In addition, ATSDR has the authority to prepare toxicological profiles for substances not found at sites on the National Priorities List, in an effort to "…establish and maintain inventory of literature, research, and studies on the health effects of toxic substances" under CERCLA Section 104(i)(1)(B), to respond to requests for consultation under section 104(i)(4), and as otherwise necessary to support the site-specific response actions conducted by ATSDR.

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 may 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 (e.g.,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:

Chapter 1	**How Can (Chemical X) Affect Children?**
Chapter 1	**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: 1-800-CDC-INFO (800-232-4636) or 1-888-232-6348 (TTY)
Internet: http://www.atsdr.cdc.gov

The following additional materials are available online:

Case Studies in Environmental Medicine are self-instructional publications designed to increase primary health care providers' knowledge of a hazardous substance in the environment and to aid in the evaluation of potentially exposed patients (see https://www.atsdr.cdc.gov/csem/csem.html).

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 (see https://www.atsdr.cdc.gov/MHMI/index.asp). 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 (see https://www.atsdr.cdc.gov/toxfaqs/Index.asp).

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 • Web Page: https://www.cdc.gov/nceh/.

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, 395 E Street, S.W., Suite 9200, Patriots Plaza Building, Washington, DC 20201 • Phone: 202-245-0625 or 1-800-CDC-INFO (800-232-4636) • Web Page: https://www.cdc.gov/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 • Web Page: https://www.niehs.nih.gov/.

Clinical Resources (Publicly Available Information)

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, 25 Northwest Point Boulevard, Suite 700, Elk Grove Village, IL 60007-1030 • Phone: 847-818-1800 • FAX: 847-818-9266 • Web Page: http://www.acoem.org/.

The American College of Medical Toxicology (ACMT) is a nonprofit association of physicians with recognized expertise in medical toxicology. Contact: ACMT, 10645 North Tatum Boulevard,

Suite 200-111, Phoenix AZ 85028 • Phone: 844-226-8333 • FAX: 844-226-8333 • Web Page: http://www.acmt.net.

The Pediatric Environmental Health Specialty Units (PEHSUs) is an interconnected system of specialists who respond to questions from public health professionals, clinicians, policy makers, and the public about the impact of environmental factors on the health of children and reproductive-aged adults. Contact information for regional centers can be found at http://pehsu.net/findhelp.html.

The American Association of Poison Control Centers (AAPCC) provide support on the prevention and treatment of poison exposures. Contact: AAPCC, 515 King Street, Suite 510, Alexandria VA 22314 • Phone: 701-894-1858 • Poison Help Line: 1-800-222-1222 • Web Page: http://www.aapcc.org/.

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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 Environmental Toxicology Branch reviews data needs sections to assure consistency across profiles and adherence to instructions in the Guidance.

4. Green Border Review. Green Border review assures the consistency with ATSDR policy.

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PEER REVIEW

A peer review panel was assembled for nitrate and nitrite. The panel consisted of the following members:

1. Dr. John Fawell, Visiting Professor, School of Applied Sciences, Cranfield University, Cranfield, Bedfordshire MK43 OAL, United Kingdom;

2. Dr. Richard B. Ferguson, Professor of Soil Science, Associate Head of the Department of Agronomy & Horticulture, University of Nebraska-Lincoln, Lincoln, Nebraska; and

3. Dr. Stephen M. Roberts, Director, Center for Environmental & Human Toxicology; Professor, College of Veterinary Medicine, College of Medicine, College of Public Health and Health Professions, University of Florida, Gainesville, Florida.

These experts collectively have knowledge of nitrate's and nitrite'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.

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.

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1. PUBLIC HEALTH STATEMENT FOR NITRATE AND NITRITE

This Public Health Statement summarizes the Agency for Toxic Substances and Disease Registry's (ATSDR) findings on inorganic nitrate and nitrite, including chemical characteristics, exposure risks, possible health effects from exposure, and ways to limit exposure. Nitrate and nitrite can be present in organic or inorganic compounds, depending on their chemical structures. This profile pertains to inorganic nitrate and nitrite, specifically the ionic forms of both nitrate and nitrite.

The U.S. Environmental Protection Agency (EPA) identifies the most serious hazardous waste sites in the nation. These sites make up the National Priorities List (NPL) and are sites targeted for long-term federal clean-up activities. Nitrate and nitrite are ubiquitous in the environment. Specific forms of nitrate and nitrite have occasionally been identified in hazardous waste sites. Ammonium nitrate, sodium nitrate, and sodium nitrite were identified in 7, 3, and 2 of the 1,832 hazardous waste sites, respectively, that have been proposed for inclusion on the NPL. The total number of NPL sites evaluated for nitrate and nitrite is not known. But the possibility remains that as more sites are evaluated, the number of sites at which nitrate and/or nitrite are found may increase. This information is important because these future sites may be sources of exposure, and overexposure to nitrate and/or nitrite may be harmful.

If you are exposed to nitrate and/or nitrite, many factors determine whether you'll be harmed. These include how much you are exposed to (dose), how long you are exposed (duration), how often you are exposed (frequency), and how you are exposed (route of exposure). You must also consider the other chemicals you are exposed to and your age, sex, diet, family traits, lifestyle, and state of health.

WHAT ARE NITRATE AND NITRITE?

Nitrate and nitrite are naturally occurring ionic species that are part of the earth's nitrogen cycle. They typically exist in the environment in highly water-soluble forms, in association with other ionic species such as sodium and potassium. Nitrate and nitrite salts completely dissociate in aqueous environments. Nitrite is readily oxidized (combines with oxygen) to form nitrate. Nitrate is generally stable in the environment; however, it may be reduced to nitrite through biological processes involving plants, microbes, etc.

In nature, plants utilize nitrate as an essential (key) nutrient. In commerce, the majority of nitrate is used in inorganic fertilizers. Additional uses of commercial nitrate and nitrite include food preservation and

the production of munitions and explosives. Sodium nitrite is also being used in medicines and therapeutics; for example, as an antidote for cyanide poisoning and as a treatment for pulmonary arterial hypertension.

WHAT HAPPENS TO NITRATE AND NITRITE WHEN THEY ENTER THE ENVIRONMENT?

Nitrate and nitrite ions naturally occur in the terrestrial (soil) and aquatic (water) environment as part of the earth's nitrogen cycle (see Figure 5-1) and can therefore be found in both soil and water. In nature, nitrate and nitrite can also be found in igneous and volcanic rocks. Nitrate is formed naturally as an end product of vegetable and animal decomposition, making this a principal source for nitrate ion in both terrestrial (soil) and aquatic (water) environments. Nitrate and nitrite can also be released into the atmospheric (air), terrestrial (soil), and aquatic (water) environments at places where human-made materials such as fertilizers are produced or used. Human and animal wastes are important sources of ammonia, a compound containing nitrogen, which undergoes chemical reaction to produce nitrite and subsequently nitrate. In aerobic (containing oxygen) environments, ammonia is readily oxidized to nitrite by ammonia-oxidizing bacteria; nitrite is oxidized to nitrate by nitrite-oxidizing bacteria. This two-stage process is known as nitrification. Both human-made and natural sources of nitrogen may contribute to nitrate aerosols in the atmosphere, as well as nitrate and nitrite ions in terrestrial (soil) and aquatic (water) environments.

Nitrate and nitrite have been detected in surface waters, drinking water (including public and private wells), and groundwater. Nitrate accounts for the majority of the total available nitrogen in surface waters. Contamination of waters is a result of agricultural runoff (use of chemical fertilizer or animal manure) and discharges from septic systems and municipal waste water treatment facilities. Nitrogen exists naturally in soils, typically bound to organic matter or mineral soil material such as rocks. Available forms of nitrogen, including nitrate and nitrite, are present in soils at a few kilograms (kg)/hectare.

Nitrate and nitrite are a normal part of the human diet and can be found in vegetables, fruits, cured meats, fish, dairy products, beers, cereals, and cereal products. Some salts, such as sodium nitrite, are intentionally added to foods and beverages to preserve or cure them; inhibiting the formation of microorganisms that may cause disease such as botulism. Additionally, nitrites and nitrates may be present in some medicines as they can be employed in medicinal and therapeutic uses.

HOW MIGHT I BE EXPOSED TO NITRATE AND NITRITE?

The major source of overexposure of the general population to nitrate and nitrite is via ingestion of water, foods, beverages, and/or medicines that contain nitrate and/or nitrite naturally or as an added preservative. Nitrate and nitrite can be taken up by plants, especially leafy vegetables such as lettuce and spinach and beet roots; vegetables account for about 80% of the nitrate in a typical human diet. Cured meats, meat products, cheeses, and beverages may contain sodium nitrate and/or sodium nitrite as preservatives. Relatively high nitrate concentrations are found in some privately owned wells with shallow depths and permeable soils. Drinking of water from such sources, combined with nitrate intake from the diet, may result in overexposure to nitrate in some individuals. Release of nitrate and/or nitrite to soil and water at waste disposal sites could result in contamination of drinking water sources and increased uptake by plants used for the human diet. Inhalation of nitrate or nitrite is not a likely exposure route of concern for the general population, although inhalation of dust from fertilizer products containing nitrate salts is possible. Dusts may also dissolve in sweat on skin, increasing the potential for dermal exposure.

HOW CAN NITRATE AND NITRITE ENTER AND LEAVE MY BODY?

Nitrate and nitrite could enter your body from the air you breathe; however, you are not likely to be exposed to amounts of nitrate or nitrite in the air that might cause adverse health effects. Nitrate and nitrite enter your body when you drink water or eat foods that contain these substances. Nitrate and nitrite are also present in smokeless tobacco products. Certain bacteria and fungi in these products can convert nitrate to nitrite, which can lead to the formation of carcinogenic nitrosamines. Neither nitrate nor nitrite is likely to enter your body from soil. However, nitrate or nitrite in soil could enter the body of young children if they put soil containing nitrate or nitrite in the mouth. Intake of some nitrate is a normal part of the nitrogen cycle in humans. Both nitrate and nitrite can be produced inside the body as well. Some of the nitrate in your body moves from blood to the salivary glands where some of it is changed to nitrite. Nitrate and nitrite are widely distributed in the body. Nitrate and nitrite that enter your body are no different chemically than nitrate and nitrite produced inside your body. Most nitrate in your body leaves in the urine the same day it enters your body. Some nitrite in the stomach forms other substances, some of which may be harmful. Nitrite in your blood can react with hemoglobin (which carries oxygen to body tissues) and reduce the ability of hemoglobin to carry oxygen. Nitrite can also form nitric oxide, which may be beneficial in some instances.

HOW CAN NITRATE AND NITRITE AFFECT MY HEALTH?

Most people are not exposed to levels of nitrate and/or nitrite that would cause adverse health effects. Young infants (<6 months of age) appeared to be particularly sensitive to the effects of nitrite on hemoglobin after consuming formula prepared with drinking water that contained nitrate at levels higher than recommended limits; some of these infants died. The cause of methemoglobinemia (a change to hemoglobin that decreases the ability to transport oxygen to tissues) in many of these infants may have been gastroenteritis from bacteria or viruses in the drinking water or from other sources not related to nitrate. Some children and adults who ate food or drank fluids that contained unusually high levels of nitrite experienced decreases in blood pressure, increased heart rate, reduced ability of the blood to carry oxygen to tissues, headaches, abdominal cramps, vomiting, and even death.

There is limited evidence that nitrite may cause some cancers of the gastrointestinal tract in humans and mice. Cancer could result from reactions between nitrite and certain other chemicals that may produce cancer-causing substances. The International Agency for Research on Cancer (IARC) determined that there is inadequate evidence for the carcinogenicity of nitrate in food or drinking water and limited evidence for the carcinogenicity of nitrite in food (based on association with increased incidence of stomach cancer). IARC determined that there is inadequate evidence for the carcinogenicity of nitrate, limited evidence for the carcinogenicity of nitrite *per se*, and sufficient evidence for the carcinogenicity of nitrite in combination with amines or amides. The overall conclusions of IARC were that "ingested nitrate and nitrite under conditions that result in endogenous nitrosation is probably carcinogenic to humans (Group 2A)." IARC noted that: (1) the endogenous nitrogen cycle in humans includes interconversion of nitrate and nitrite; (2) nitrite-derived nitrosating agents produced in the acid stomach environment can react with nitrosating compounds such as secondary amines and amides to generate N-nitroso compounds; (3) nitrosating conditions are enhanced upon ingestion of additional nitrate, nitrite, or nitrosatable compounds; and (4) some N-nitroso compounds are known carcinogens.

The U.S. EPA Integrated Risk Information System does not include a carcinogenicity evaluation for nitrate or nitrite.

See Chapters 2 and 3 for more information on health effects of nitrate and nitrite.

HOW CAN NITRATE AND NITRITE AFFECT CHILDREN?

This section discusses potential health effects of nitrate and nitrite exposure in humans from when they're first conceived to 18 years of age.

Children can experience the same effects as adults from overexposure to nitrate and/or nitrite. Young infants (<6 months of age) who were fed formula prepared using nitrate-contaminated drinking water sources appear to be particularly sensitive to the effects of nitrate on hemoglobin (i.e., methemoglobinemia), although bacterial infections may have been at least partially responsible for increased sensitivity in these infants. It is not known whether nitrate or nitrite can cause birth defects. Results of some studies suggest that ingestion of relatively high levels of nitrate or nitrite could cause developmental effects, but other studies found no evidence for nitrate- or nitrite-related developmental effects.

HOW CAN FAMILIES REDUCE THE RISK OF OVEREXPOSURE TO NITRATE AND NITRITE?

If your doctor finds that you have been exposed to significant amounts of nitrate and/or nitrite, ask whether your children might also be exposed. Your doctor might need to ask your state health department to investigate. You may also contact the state or local health department with health concerns.

Much of the diet contains food with nitrate and possibly small amounts of nitrite. Some processed food contains nitrate and/or nitrite as preservative. If you think that you are getting too much nitrate or nitrite in your diet, consider eating less of those foods that contain high levels of nitrate or nitrite. This consideration is particularly relevant to infants and small children. Don't drink water containing levels of nitrate or nitrite higher than guideline levels for drinking water.

ARE THERE MEDICAL TESTS TO DETERMINE WHETHER I HAVE BEEN OVEREXPOSED TO NITRATE AND/OR NITRITE?

Methods are available to detect nitrate and nitrite in plasma and urine; however, these are usually not available at a doctor's office and are not clinically useful.

Routine blood tests are available to detect a condition known as methemoglobinemia, which is caused by the presence of higher-than-normal levels of a form of hemoglobin. However, these tests cannot tell

whether the high methemoglobin levels were caused by nitrate and nitrite or by some other substance or disease.

For more information on the different substances formed by nitrate and nitrite breakdown and tests to detect these substances in the body, see Chapters 3 and 7.

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. Federal agencies that develop regulations for toxic substances include the Environmental Protection Agency (EPA), the Occupational Safety and Health Administration (OSHA), and the Food and Drug Administration (FDA). Recommendations provide valuable guidelines to protect public health but are not enforceable by law. Federal organizations that develop recommendations for toxic substances include the Agency for Toxic Substances and Disease Registry (ATSDR) and the National Institute for Occupational Safety and Health (NIOSH).

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 usually based on levels that affect animals; levels are then adjusted to help protect humans. Sometimes these not-to-exceed levels differ among federal organizations. Different organizations use different exposure times (e.g., an 8-hour workday or a 24-hour day), different animal studies, or emphasize some factors over others, depending on their mission.

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 issued the regulation or recommendation.

The EPA lists maximum contaminant levels (MCL) and maximum contaminant level goals (MCLG) of 10 mg/L (or ppm) for nitrate (as nitrate-nitrogen; ~44 mg nitrate/L) and 1 mg/L (or ppm) for nitrite (as nitrite-nitrogen; ~3.3 mg nitrite/L) in the 2012 Edition of the Drinking Water Standards and Health Advisories. The FDA lists 10 mg/L nitrate (as nitrogen; ~44 mg nitrate/L), 1 mg/L nitrite (as nitrogen; ~3.3 mg nitrite/L), and 10 mg/L total nitrate and nitrite (as nitrogen) as allowable levels in bottled water. OSHA has not set a legal limit for nitrate or nitrite in air. NIOSH has not set a recommended limit for nitrate or nitrite in air.

WHERE CAN I GET MORE INFORMATION?

If you have any questions or concerns, please contact your community or state health or environmental quality department, or contact ATSDR at the address and phone number below. You may also contact your doctor if experiencing adverse health effects or for medical concerns or questions. ATSDR can also provide publicly available information regarding medical specialists with expertise and experience recognizing, evaluating, treating, and managing patients exposed to hazardous substances.

- Call the toll-free information and technical assistance number at 1-800-CDCINFO (1-800-232-4636) or

- Write to:
  Agency for Toxic Substances and Disease Registry
  Division of Toxicology and Human Health Sciences
  1600 Clifton Road NE
  Mailstop F-57
  Atlanta, GA 30329-4027

Toxicological profiles and other information are available on ATSDR's web site: http://www.atsdr.cdc.gov.

1. PUBLIC HEALTH STATEMENT

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2. RELEVANCE TO PUBLIC HEALTH

2.1 BACKGROUND AND ENVIRONMENTAL EXPOSURES TO NITRATE AND NITRITE IN THE UNITED STATES

Nitrate and nitrite can be organic or inorganic chemicals depending on their chemical structures. This profile pertains to inorganic nitrate and nitrite, specifically the nitrate anion and the nitrite anion. Nitrate and nitrite occur naturally in the environment as part of the nitrogen cycle, and are produced both endogenously and exogenously. Ammonia-oxidizing bacteria convert ammonia into nitrite; nitrite-oxidizing bacteria convert nitrite into nitrate in aerobic environments. This two-stage process is known as nitrification. Main sources of ammonia in the environment are decaying organic matter and human and animal wastes. Nitrification, atmospheric fixation, and nitrogen fertilizers contribute to nitrite and nitrate concentrations in the environment. In nature, salts of nitrate and nitrite completely dissociate and these anions typically exist as ionic species. In the environment, nitrite is readily oxidized to nitrate. Nitrate is generally stable in the environment; however, it may be reduced through biotic (living systems; plants, microbes, etc.) processes to nitrite under anerobic conditions.

Nitrate and nitrite are ubiquitous in the environment and people are exposed to them primarily through the ingestion of food and drinking water. Significant uptake of nitrate and nitrite occurs in all varieties of plants; internal storage of nitrate (rather than metabolic conversion to ammonium and amino acids) can occur in some plants, especially leafy vegetables such as lettuce and spinach. Vegetables account for about 80% of the nitrate in a typical human diet. Nitrate and nitrite are also produced in the body as part of the natural nitrate-nitrite-nitric oxide cycle.

2.2 SUMMARY OF HEALTH EFFECTS

Hematological Effects. In humans, ingested nitrate is nearly completely absorbed into the blood from the small intestine and approximately 25% of the plasma nitrate enters the salivary glands where it is secreted in saliva. As much as 20% of salivary nitrate (5% of ingested nitrate) is reduced to nitrite by bacterial reductases in the mouth. This *in vivo* reduction of nitrate accounts for 80–85% of the body's nitrite and most of the rest comes from nitrite-containing food sources. Nitrite in the blood can react with ferrous (Fe^{2+}) hemoglobin (which transports oxygen) to form ferric (Fe^{3+}) hemoglobin (methemoglobin, a poor transporter of oxygen), and nitric oxide (which can also bind to deoxyhemoglobin) and nitrate.

2. RELEVANCE TO PUBLIC HEALTH

Methemoglobinemia is a condition in which increased methemoglobin as a percentage of total hemoglobin results in the expression of clinical signs that increase in severity with increasing percent methemoglobin. In normal healthy individuals, methemoglobin levels are <1% of total hemoglobin. Discoloration of the skin (cyanosis) is often observed at methemoglobin levels in the range of 3–15%; most patients tolerate methemoglobin levels <10%. Tachycardia, weakness, and other signs of tissue hypoxia may be observed at 10–20% methemoglobin levels. Symptoms involving the central nervous system (e.g., headache, dizziness, fatigue) and dyspnea and nausea appear at >20% methemoglobin; the severity of symptoms increases with increasing methemoglobin level. High risk of mortality occurs at levels >70% methemoglobin. It should be noted that a patient with comorbidities that decrease oxygen transport or delivery may develop moderate to severe symptoms at much lower methemoglobin levels than a previously healthy patient. Furthermore, due to differences in the oxygen carrying capacity between fetal hemoglobin and adult hemoglobin (which replaces fetal hemoglobin during the first year of postnatal life), cyanosis in young infants with mostly fetal hemoglobin may not be detected at methemoglobin levels eliciting clinical cyanosis in older infants with mostly adult hemoglobin.

As early as the mid-1900s, methemoglobinemia was reported in infants exposed to relatively large amounts of nitrate from drinking water sources. Available data identify young bottle-fed infants (1–3 months of age) as a subpopulation that is particularly susceptible to nitrate-induced methemoglobinemia, especially those consuming formula prepared from drinking water sources containing nitrate in excess of 10 mg nitrate-nitrogen/L (44 mg nitrate/L). Subsequent reports provide additional evidence of associations between ingestion of nitrate from drinking water sources and elevated methemoglobin levels in infants. Cyanosis and even death occurred in some of the reported cases.

Limited data are available regarding administration of controlled amounts of nitrate and methemoglobin levels. A study reported methemoglobin levels as high as 5.3% of total hemoglobin in a group of four infants (ages 11 days to 11 months) administered sodium nitrate in the formula for 2–18 days at a concentration resulting in a dose of 50 mg nitrate/kg/day and as high as 7.5% in another group of four infants (ages 2 days to 6 months) similarly treated at 100 mg nitrate/kg/day for 6–9 days. A study reported methemoglobin levels as high as 6.9–15.9% among three infants (ages not specified) fed formula prepared using water containing 108 mg nitrate/L.

Young children are somewhat less sensitive than infants to nitrate-induced methemoglobinemia. A study evaluated methemoglobin levels in 102 children 1–8 years of age. Sixty-four of the children lived in households where drinking water contained 22–111 mg nitrate-nitrogen/L (97–488 mg nitrate/L);

drinking water sources for the other 38 children (controls) contained <10 mg nitrate-nitrogen/L (<44 mg nitrate/L). Methemoglobin measured 1.0–1.36% in those children 1–4 years of age and appeared to increase with increasing nitrate intake, although there was no statistically significant change. Methemoglobin levels in those children 5–8 years of age averaged 0.9–0.95%, independent of level of exposure to nitrate.

Endocrine Effects. There is some evidence for nitrate-induced effects on thyroid function and development. Nitrate is one of the substances that act as dose-dependent competitive inhibitors of the sodium iodide symporter (NIS), which mediates the uptake of iodine by the thyroid. Sufficiently decreased iodine uptake by the thyroid may result in decreased production of thyroid hormones triiodothyronine (T3) and thyroxine (T4). Decreased thyroid hormone production causes increased release of thyroid stimulating hormone (TSH) from the anterior pituitary gland leading to increased uptake of iodine by the thyroid gland. Sufficiently inhibited uptake of iodine by the thyroid could result in effects associated with thyroid dysfunction (e.g., hypothyroidism). Concern for nitrate-induced effects on thyroid function has prompted scientists to perform studies designed to assess thyroid function relative to drinking water and/or dietary nitrate levels. Some human studies provide suggestive evidence that elevated levels of nitrate in drinking water and/or nitrate-rich diets may be associated with signs of thyroid dysfunction. However, limitations of these studies include lack of individual dose-response data, quantification of iodine intake, and control for other substances that may affect the thyroid; one study relied on self-reported thyroid status and self-reported dietary nitrate intake. A study found no evidence for nitrate-induced effects on thyroid function in adults ingesting sodium nitrate for 38 days at 15 mg/kg/day (which is 3 times the maximum acceptable daily intake of 5 mg sodium nitrate/kg/day set by the Joint Expert Committee on Food Additives [JECFA] of the Food and Agriculture Organization of the United Nations/World Health Organization and the European Commission's Scientific Committee on Food).

Thyroid status has been assessed to some extent in animals consuming drinking water or food to which nitrate salts had been added. There were no clinical signs of hypothyroidism or effects on serum T3 or T4 levels in adult Beagles or their puppies during exposure of the breeding dogs to sodium nitrate in the drinking water for 1 year at concentrations in the range of 300–1,000 ppm (equivalent to 219–730 mg nitrate/L). Decreased thyroidal ^{131}iodine uptake was noted in rats given food containing 0.5–2.5% potassium nitrate (approximately 3,000–15,000 mg nitrate/kg food). Significantly increased uptake of thyroidal ^{131}iodine; decreased serum T3, T4, and TSH levels; increased thyroid weight; and follicular hyperplasia were noted in female Wistar rats administered sodium nitrate in the drinking water for

30 weeks at concentrations ≥250 mg/L (≥182 mg nitrate/L). In another study, significantly increased serum T3 (34–44% lower than controls), increased thyroid weight (45–77% greater than controls), and histopathologic thyroid lesions (glandular hypertrophy accompanied by vacuolization, increased colloidal volume of the follicles, and flattened follicular epithelium) were observed in male Wistar rats receiving drinking water for 5 months to which potassium nitrate had been added at concentrations ≥100 mg/L. Significantly decreased serum T3 and T4 levels were observed in all groups of weanling male Wistar rats with intakes in the range of 8.7–47.4 mg sodium nitrate/kg/day (equivalent to 6.4–34.6 mg nitrate/kg/day). At doses ≥15.8 mg nitrate/kg/day, significantly increased serum TSH was also noted. Groups of similarly-treated young adult male Wistar rats exhibited significantly decreased T3 and T4 levels and increased serum TSH at doses ≥15.8 mg nitrate/kg/day. Significantly increased thyroid gland weight, increased TSH, decreased serum T3 and T4 levels, and decreased thyroid peroxidase activity were reported in rats administered 3% potassium nitrate in the diet.

In a 13-week study of rats receiving drinking water to which potassium nitrite had been added, doses in the range of 8.9–241.7 mg/kg/day (4.8–130.5 mg nitrite/kg/day), oral doses ≥13.3 mg nitrite/kg/day (males) and ≥61.8 mg nitrite/kg/day (females) resulted in hypertrophy in the zona glomerulosa of the adrenal gland. The effect on the adrenal gland was not observed in untreated controls or potassium chloride controls. Similar results were obtained at estimated doses of 105.1 mg nitrite/kg/day (males) and 130.1 mg nitrite/kg/day (females) in a subsequent similarly-designed study. Results of a subsequent study indicate that the effect on the adrenal gland of the rat is a physiological adaptation to repeated episodes of hypotension caused by nitrite.

Metabolic Effects. Possible associations between nitrate and/or nitrite in drinking water and/or food sources and risk of type 1 diabetes have been investigated in a number of case-control studies. Some studies found no significant risk for childhood type 1 diabetes. In one case-control study that included estimates of nitrate intake based on food frequency questionnaire results for children 0–14 years of age, a significantly increased risk of type 1 diabetes was noted for children at the high end (≥75th percentile) of estimated nitrate intake compared to those at the low end (<25th percentile). In an ecological study of type 1 diabetes incidence rates by county in Colorado, children (<18 years of age) in counties with water nitrate levels in the range of 0.77–8.2 mg/L had a significantly increased risk of type1 diabetes compared to those in counties with water nitrate levels in the range of 0.0–0.084 mg/L. In another ecological study, a significantly increased association between nitrate in drinking water (highest tertile versus lowest tertile) and incidence of childhood type 1 diabetes was reported for children diagnosed between 1978 and 1994 in the Yorkshire Regional Health Authority in England. In a subsequent ecological study that

included portions of England and Scotland, the Drinking Water Inspectorate found no evidence for an association between nitrate in the drinking water and incidence of childhood type 1 diabetes.

Cardiovascular Effects. Cardiovascular health is an end point of concern for nitrate and nitrite because some nitrate is converted to nitrite in the body. Nitrite is a smooth muscle relaxant that can cause hypotension and plasma nitrite is involved in the oxidation of hemoglobin to methemoglobin, which is associated with hypotension, rapid pulse, and rapid breathing at high enough concentrations. Ingestion of nitrite (from potassium nitrite or sodium nitrite sources) has been associated with severe methemoglobinemia in adults and children; in some of these cases, symptoms included hypotension and/or tachycardia. These cases were the result of consumption of food or drink that contained unusually high levels of nitrite via contamination, inadvertent use of sodium nitrite instead of table salt, or ingestion of a single sodium nitrite tablet (1 g; equivalent to 667 mg nitrite).

In a hospital-based study in Colorado that included 226 cases of hypertension among patients living in areas where drinking water contained nitrate at concentrations ranging from 19 to 125 ppm (mean 52 ppm) and 261 cases from patients living in areas without nitrate in the drinking water, the mean annual incidence rate for hypertension in the nitrate-exposed patients was only 5.9/1,000 compared to 7.9/1,000 for the control patients. However, the nitrate-exposed patients exhibited an earlier mean age at hospitalization for hypertension (58.5 versus 65.2 years for controls); the toxicological significance of this finding is uncertain because the incidence rate for hypertension was higher among control patients than among patients exposed to nitrate in the drinking water.

In a study designed to evaluate the oral bioavailability of sodium nitrite in healthy volunteers (seven females and two males; mean age 22.9 years), ingestion of 0.06 sodium nitrite per mmol hemoglobin (~1.5–1.8 mg nitrite/kg) resulted in an average heart rate increase from 55 to 63 beats per minute (bpm) and average mean arterial blood pressure decrease from 78 to 70 mmHg. At a higher intake (~2.9–3.6 mg nitrite/kg), the average heart rate increased from 57 to 67 bpm and the average mean arterial blood pressure decreased from 80 to 69 mmHg. The maximum effects on heart rate and blood pressure occurred between 15 and 20 minutes following ingestion; heart rate and blood pressure returned to near-baseline levels approximately 2 hours following ingestion at the low dose, but the effects had not returned to baseline at 4 hours following ingestion at the high dose. The blood pressure-lowering effect of short-term dietary supplementation of inorganic nitrate appears to be beneficial; however, there is some uncertainty regarding potential health benefits of long-term nitrate supplementation to treat cardiovascular diseases.

Gastrointestinal Effects. Ingestion of nitrite (from potassium nitrite or sodium nitrite sources) has been associated with severe methemoglobinemia in adults and children; in many of these cases, symptoms included abdominal cramps and vomiting. These cases were the result of consumption of food or drink that contained unusually high levels of nitrite via contamination, inadvertent use of sodium nitrite instead of table salt, or ingestion of a single sodium nitrite tablet (667 mg nitrite). In a study designed to evaluate the oral bioavailability of sodium nitrite in healthy volunteers (seven females and two males; mean age 22.9 years), one subject became nauseous and vomited within 20 minutes following ingestion of 0.12 mmol sodium nitrite per mmol hemoglobin (~3.2 mg nitrite/kg); another subject reported nausea within 30 minutes following ingestion of 0.12 mmol sodium nitrite per mmol hemoglobin (~2.9 mg nitrite/kg).

Epithelial hyperplasia was noted in the forestomach of male and female B6C3F1 mice provided sodium nitrite in the drinking water for 14 weeks at a concentrations resulting in estimated doses of 663.3 and 824.1 mg nitrite/kg/day, respectively); the no-observed-adverse-effect levels (NOAELs) for these lesions in the males and females were 435.5 and 562.8 mg nitrite/kg/day, respectively. Similar results were noted for male and female F344/N rats and male B6C3F1 mice treated for 104–105 weeks at estimated doses of 87.1, 100.5, and 147.4 mg nitrite/kg/day, respectively; the NOAELs for these lesions in the male and female rats and male mice were 46.9, 53.6, and 80.4 mg nitrite/kg/day, respectively. Sodium nitrite treatment did not result in increased incidences of forestomach lesions in other groups of male F344 rats provided sodium nitrite in the drinking water at 2,000 mg/L (estimated dose of 208.4 mg nitrite/kg/day) for 35 weeks or 51 weeks.

Neurological Effects. Neurological effects have been reported in humans and animals following ingestion of nitrite; however, these effects may be secondary to nitrite-induced reductions in oxygen-carrying capacity. Ingestion of nitrite (from potassium nitrite or sodium nitrite sources) has been associated with severe methemoglobinemia in adults and children; in many of these cases, clinical signs included dizziness, loss of consciousness, and/or convulsions. These cases were the result of consumption of food or drink that contained unusually high levels of nitrite via contamination, inadvertent use of sodium nitrite instead of table salt, or ingestion of a single sodium nitrite tablet (667 mg nitrite).

Headache was induced in a male subject following consumption of a 10 mg sodium nitrite solution. Headaches were induced in 8 out of 13 such tests. In a study designed to evaluate the oral bioavailability

of sodium nitrite in healthy volunteers (seven females and two males; mean age 22.9 years), headache was reported by four out of the nine people after ingestion of 0.12 mmol sodium nitrite per mmol hemoglobin (~2.9–3.6 mg nitrite/kg) and by four of nine subjects after ingestion of 0.06 mmol sodium nitrite per mmol hemoglobin (~1.5–1.8 mg nitrite/kg).

Abnormalities in electroencephalograms (EEGs) were reported in male albino rats provided sodium nitrite in the drinking water for 2 months at concentrations resulting in ≥9.38 mg nitrite/kg/day. The abnormal readings persisted during up to 4.5 months following cessation of exposure to sodium nitrite. At the highest dose (187.6 mg nitrite/kg/day), rats exhibited clinical signs of sedation and became motionless during periods of electrical outbursts. Increased aggressive behavior was observed in male C57B1 mice provided sodium nitrite in the drinking water at 1,000 mg/L for up to 13 weeks postweaning. The mice had also been exposed via their parents during mating and their mothers during gestation and lactation. Significantly reduced motor activity was reported in male mice provided sodium nitrite in the drinking water. Sodium nitrite levels tested ranged from 100 to 2,000 mg/L; however, the study report did not include specific information regarding the exposure levels that resulted in reduced motor activity.

Developmental Effects. A number of studies evaluated possible associations between developmental end points and exposure to nitrate. The results provide some evidence of nitrate-related developmental effects. The results are not adequate for quantitative risk assessment because estimations of nitrate intakes were typically based on measurements of nitrate levels in drinking water sources at selected time points and self-reported estimates of water consumption, possible confounding by other potential toxicants was not evaluated, and most studies did not account for dietary nitrate or nitrite intake, which is typically the major source of ingested nitrate and nitrite. Some studies reported significant associations between selected developmental end points and nitrate in drinking water sources. One study reported increased risk of intercalary limb defect associated with estimated total nitrite intake. Other studies found no evidence of associations between nitrate and risk of developmental effects.

Cancer. Numerous case-control and cohort studies of carcinogenicity of ingested nitrate and nitrite in humans have been reported. Many ecological studies have also been reported; however, interpretation of outcomes of these studies is more uncertain because of various factors that contribute to ecologic bias (group-based associations between exposure and cancer outcomes may not apply to individuals). In general, outcomes of case-control and cohort studies have found no or weak associations between exposure to nitrate and cancer in humans, with stronger associations with exposures to nitrite or intake of high nitrite foods such as cured meat. Mechanistically, this outcome is consistent with nitrite being an

intermediate in the cancer mode of action of nitrate (see Section 3.5.2). This is further supported by studies that have found interactions between cancer risk attributed to nitrite and exposure to antioxidants. Uncertainties in estimates of cancer risk from exposure to nitrate or nitrite include those typical of epidemiological studies in general: uncertainties in estimation of exposure (e.g., estimating long-term dietary intakes from food frequency questionnaires or levels in public water supplies [PWS]), exposure misclassification of individual outcomes (e.g., assigning group-level exposure estimates to individuals), and adequacy of controlling for confounders (e.g., other factors contributing to the cancer). One potentially important class of confounders is antioxidants that can influence the degree of nitrosation of dietary amines and, thereby, the cancer risk from exposure to nitrate or nitrite.

The strongest and most consistent evidence of a carcinogenic role for nitrite is from studies of gastrointestinal cancers and, in particular, gastric cancer. In general, these studies found significant positive trends for cancer risk (risk increases with increasing intake), and three studies found elevated cancer risk. Relative risks (RRs) were 1.71 (95% confidence interval [CI]: 1.24, 2.37) at a nitrite intake of 1 mg/day and 2.5 (95% CI: 1.4, 4.3) at nitrite intakes ≥6 mg/day. Risk was modified by dietary vitamin E and folate intake, with increased risk in association with higher nitrate/vitamin E or folate ratios. Associations between exposure to nitrate or nitrite and colorectal cancer have been studied in cohort and case-control studies and results are less consistent than for gastric cancer. Two studies found elevated risk: 1.16 (95% CI: 1.04, 1.30) for colon cancer at nitrate-nitrogen levels >0.6 mg/L (>2.65 mg nitrate/L drinking water; 1.5 (95% CI: 1.0, 2.1) for colon cancer at a dietary nitrite intake >1.26 mg/day, and 1.7 (95% CI: 1.1, 2.5) at a dietary nitrite intake >1.26 mg/day. Risks were higher in populations exposed to drinking water that had a calcium level >34.6 mg/L (odds ratio [OR] 1.37, 95% CI: 1.11; 1.69) for nitrate <2.65 mg/L; or in populations exposed to nitrate in drinking water at levels >5 mg/L in combination with a low vitamin C intake (OR 2.0, 95% CI: 1.2, 3.3).

Results have been mixed for other types of cancer. Some case-control or cohort studies found associations between exposure to nitrite (or foods high in nitrite such as cured meat) and brain cancer in children and adults, breast cancer, kidney cancer, testicular cancer, and non-Hodgkin's lymphoma. Of these studies, the highest risks were reported for brain cancers. Two case-control studies found elevated relative risk of brain cancer in children in association with maternal exposure: 3.0 (95% CI: 1.2, 7.9) for nitrite intakes >3.0 mg/day and 5.7 (95% CI: 1.2, 27.2) for astroglial tumors at drinking water exposures ≥5 mg/L. In general, case-control and cohort studies of cancers of larynx, liver, lung, mouth, pancreas, and pharynx have found no consistent associations with exposures to nitrate or nitrite.

2. RELEVANCE TO PUBLIC HEALTH

The potential carcinogenicity of nitrate has been investigated in several animal studies that employed the oral exposure route. Studies in which negative results were reported include MCR-derived rats (15/sex/group) provided 5,000 mg sodium nitrate/L (3,650 mg nitrate/L) in the drinking water for 84 weeks and sacrificed 20 weeks later, male white rats provided 4,000 mg sodium nitrate in the drinking water for 273 days and sacrificed at 10 months, strain A male mice (n=40) provided 12,300 mg sodium nitrate/L in the drinking water for 25 weeks and sacrificed 13 weeks later, female NMRI mice provided 1,000 mg calcium nitrate/L in the drinking water for 18 months, Fischer 344 rats (50/sex/group) fed diet containing up to 5% sodium nitrate (1,517–1,730 mg nitrate/kg/day) for 2 years, and ICR mice (10/sex/group) fed diets containing up to 5% sodium nitrate for 2 years. In one study, some groups of male white rats were treated with drinking water containing 0.05% N-butyl-N-(4-hydroxybutyl)-nitrosamine (BBNA, an inducer of urinary bladder cancer in laboratory animals) for 30 days, either alone or followed by 4,000 mg sodium nitrate/L drinking water for 273 days. The group treated with BBNA followed by sodium nitrate exhibited significantly increased incidence of urinary bladder carcinoma (6/20 rats versus 1/18 rats treated with 0.05% BBNA only. These results indicate that sodium nitrate may have promoted BBNA-induced bladder tumors.

The potential carcinogenicity of ingested nitrite has been investigated in numerous animal studies. Nitrite treatment alone did not result in increased incidences of tumors in most studies. There was no evidence of sodium nitrite-induced forestomach neoplasms among male and female F344/N rats provided sodium nitrite in the drinking water for 2 years at concentrations of 750, 1,500, or 3,000 ppm (average doses in the range of 35–150 mg sodium nitrite/kg/day or 23.3–100 mg nitrite/kg/day). Although the mid-dose group of female rats exhibited a significantly increased incidence of mammary gland fibroadenoma, the incidence in the high-dose group was not significantly different from that of controls; based on this finding and the high historical background incidence of mammary gland fibroadenomas, the incidence in the mid-dose group was not considered treatment related. Significantly decreased incidences of mononuclear cell leukemia were observed in mid- and high-dose male and female rats. It was speculated that increased methemoglobin concentrations may have played a role in the decreased incidences of mononuclear cell leukemia. Significantly increased incidence of fibroma of the subcutis was noted in mid-dose male rats; however, several factors (the incidence only slightly exceeded the historical range of NTP controls, there was a lack of a dose-response characteristic, combined incidences of fibroma or fibrosarcoma were within the historical range for NTP controls, and fibromas and fibrosarcomas are common neoplasms in the skin of F344/N rats) suggested that the fibroma was not related to sodium nitrite exposure. It was concluded that there was "no evidence of carcinogenic activity" of sodium nitrite in the male or female F344/N rats under the conditions of the study.

In a similarly-designed study of B6C3F1 mice provided sodium nitrite in the drinking water (average doses ranging from 45 to 220 mg sodium nitrite/kg/day or 30–146.7 mg nitrite/kg/day), female mice exhibited a significantly positive trend for increased incidence of forestomach squamous cell papilloma or carcinoma (combined) and the incidence in the high-dose female mice exceeded the historical range for NTP controls; however, based on concurrent controls, incidences of squamous cell adenoma (1/50, 0/50, 1/50, and 3/50 for controls, 750, 1,500, and 3,000 ppm groups, respectively), squamous cell carcinoma (0/50, 0/50, 0/50, and 2/50 for controls, 750, 1,500, and 3,000 ppm groups, respectively), and squamous cell adenoma or carcinoma (1/50, 0/50, 1/50, and 5/50 for controls, 750, 1,500, and 3,000 ppm groups, respectively) were not statistically significantly increased for any sodium nitrite exposure group. The positive trend for incidences of forestomach squamous cell papilloma or carcinoma (combined) in the female B6C3F1 mice was considered to provide "equivocal evidence of carcinogenic activity" of sodium nitrite; there was "no evidence of carcinogenic activity" in the male B6C3F1 mice under the conditions of the study. Incidences of alveolar/bronchiolar adenoma or carcinoma (combined) in sodium nitrite-exposed groups of female mice were slightly greater than that of controls (incidences of 1/50, 6/50, 5/50, and 6/50 for controls, 750, 1,500, and 3,000 ppm groups, respectively); however, incidences were within that of historical NTP controls. Because the incidences did not exhibit exposure concentration-response characteristics and were not accompanied by increased incidences of preneoplastic lesions, the study authors did not consider them to be sodium nitrite exposure-related effects. Significantly increased incidence of fibrosarcoma of the subcutis was noted in mid-dose female mice (incidences of 0/50, 5/50, 1/50, and 2/50 for 0, 750, 1,500, and 3,000 ppm groups, respectively) and exceeded the historical range for controls; however, lack of exposure concentration-response characteristics and the fact that combined incidence of fibroma or fibrosarcoma (0/50, 5/50, 1/50, and 3/50 for 0, 750, 1,500, and 3,000 ppm groups, respectively) were within the historical range for controls suggest that these neoplasms were not related to sodium nitrite exposure.

In two other studies of male and female F344 rats, addition of sodium nitrite to the drinking water at concentrations as high as 2,000–3,000 ppm for up to 2 years did not result in significant increases in tumor incidences at any site. Conversely, incidences of mononuclear cell leukemia were significantly lower in the nitrite-treated groups relative to controls. In a 26-month study of male and female Sprague-Dawley rats provided drinking water to which up to 2,000 ppm sodium nitrite was added, the study author reported increased incidence of lymphomas, but not other types of tumors; however, two studies noted that a working group sponsored by the U.S. FDA reevaluated the histology and did not confirm the results of another study. A study reported that the working group considered the incidences of lymphomas to be

similar to those arising spontaneously in Sprague-Dawley rats. Increased incidences of total tumors and lymphoreticular tumors were reported in rats fed diet to which sodium nitrite was added at 1,000 ppm (total tumors: 58/96 versus 28/156 controls; lymphoreticular tumors 26/96 versus 9/156 controls); the results were reported for F1 and F2 offspring that had been exposed via their mothers during gestation and lactation and directly from the diet thereafter. In a 96-week study, a significantly increased incidence of benign liver tumors among male CBA mice administered drinking water to which sodium nitrite was added at a concentration resulting in author-estimated total dose of 1,600 mg sodium nitrite/mouse compared to a group of untreated controls; however, there was no apparent sodium nitrite treatment-related effect at a higher estimated dose (2,000 mg sodium nitrite/mouse).

Significantly increased incidences of forestomach squamous papillomas were reported for male and female MRC Wistar rats provided drinking water to which sodium nitrite was added at 3,000 ppm on 5 days/week for life (5/22 males and 3/23 females versus 2/47 control males and 0/44 control females). Dose-related decreases in time of onset and incidence of lymphomas, mononuclear cell leukemia, and testicular interstitial-cell tumors were reported for male and female F344 rats administered reduced-protein diet to which sodium nitrite was added for up to 115 weeks, compared to a group of controls receiving reduced-protein only diet. There was no evidence of increased tumor incidences in male or female ICR mice provided sodium nitrite in the drinking water for up to 109 weeks at concentrations as high as 0.5% (5,000 ppm sodium nitrite), or in male or female Swiss mice or their offspring following a single gavage administration of 10 mg/kg nitrite and subsequent exposure to 0.1% sodium nitrite (1,000 ppm) in the drinking water during gestation days 15–21; terminal sacrifices occurred 10 months following the initiation of treatment. There was no evidence of treatment-related effects on incidences of nervous system tumors among male and female VM mice (susceptible to spontaneous development of cerebral gliomas) provided drinking water to which sodium nitrite was added at 0.2% (2,000 ppm) from weaning for a lifetime and others exposed via their mothers during gestation and lactation as well.

The potential carcinogenicity of combined exposure to sodium nitrite and selected nitrosatable substances (oral exposures via combinations of drinking water, diet, and/or gavage dosing) has been well-studied in laboratory animals. Many of the studies included sodium nitrite-only treatment groups for which there was no evidence of sodium nitrite-induced carcinogenicity. However, one study reported significantly increased incidence of hepatocellular neoplasms in female (but not male) F344 rats administered diet to which sodium nitrite was added at 2,000 ppm for 2 years; significantly decreased incidence of mononuclear-cell leukemia was observed as well.

2. RELEVANCE TO PUBLIC HEALTH

Significantly increased incidences of selected tumor types were observed in some studies of laboratory animals that employed coexposure to various amino compounds and sodium nitrite. These results were typically attributed to *in vivo* nitrosation of amines by nitrite to produce carcinogenic N-nitrosoamines; some of the studies did not include sodium nitrite-only treatment groups. Addition of sodium nitrite or potassium nitrite to the food of rats in three other studies resulted in increased incidences of selected tumors; analysis of the food revealed the presence of N-nitroso compounds (likely formed by nitrosation in the presence of nitrite and selected amine compounds in the food), which were considered the probable principal cause of the tumors. One study reported 30–70% incidences of malignant lymphomas, lung adenomas, and hepatomas among maternal mice and their offspring following gavage treatment of the dams with the fungicide, dodecylguanidine acetate, together with 0.05% sodium nitrite; the frequency of spontaneous tumors in untreated controls was 6%. Dodecylguanidine acetate alone had no effect on cancer incidence. One study found no significant increase in tumor incidences among male and female MCR rats provided drinking water comprised of 0.5% nitrilotriacetic acid or iminodiacetic acid and 0.2 or 0.5% sodium nitrite on 5 days/week for a lifetime. There were no signs of treatment-related effects on incidences of tumors at any site among groups of pregnant Syrian golden hamsters and their offspring fed diets to which up to 1,000 ppm sodium nitrite and/or up to 1,000 ppm morpholine were added throughout production of an F2 generation.

Based on available human data, one study determined that there is *inadequate evidence* for the carcinogenicity of nitrate in food or drinking water and *limited evidence* for the carcinogenicity of nitrite in food (based on association with increased incidence of stomach cancer). Evaluation of available animal data resulted in the determination that there is *inadequate evidence* for the carcinogenicity of nitrate, *limited evidence* for the carcinogenicity of nitrite *per se*, and *sufficient evidence* for the carcinogenicity of nitrite in combination with amines or amides. The overall conclusions of a study were that "ingested nitrate and nitrite under conditions that result in endogenous nitrosation is *probably carcinogenic to humans (Group 2A)*." One study noted that: (1) the endogenous nitrogen cycle in humans includes interconversion of nitrate and nitrite; (2) nitrite-derived nitrosating agents produced in the acid stomach environment can react with nitrosating compounds such as secondary amines and amides to generate N-nitroso compounds; (3) nitrosating conditions are enhanced upon ingestion of additional nitrate, nitrite, or nitrosatable compounds; and (4) some N-nitroso compounds are known carcinogens.

The U.S. EPA does not include a carcinogenicity evaluation for nitrate or nitrite.

2.3 MINIMAL RISK LEVELS (MRLs)

Estimates of exposure levels posing minimal risk to humans (MRLs) have been established for nitrate and nitrite. 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.

Inhalation MRLs

Inhalation MRLs were not derived for nitrate or nitrite due to lack of adequate human or animal data. Limited human data are available. Al-Dabbagh et al. (1986) evaluated mortality rates among a cohort of 1,327 male workers involved in the manufacture of nitrate fertilizer for at least 1 year between 1946 and 1981 for a chemical company in northeast England and found no evidence of an association between exposure to nitrate dusts and death from all respiratory diseases, ischemic heart disease, or other circulatory diseases compared to mortality rates for the northern region of England. There was no evidence of an association between exposure to nitrate dust and death from ischemic heart disease, cerebrovascular disease, or all circulatory diseases in a census-based (England and Wales) mortality study of workers involved in the production of nitrate fertilizers (Fraser et al. 1982, 1989). The study included a cohort of 866 men from the 1961 census and 651 men from the 1971 census. These cohorts were followed through 1985. Studies of workers in which outcomes are compared to the general population (e.g., observed versus expected deaths) may be biased by a healthy worker effect, which may lower estimated risks.

Available animal data are limited to a study in which dogs and sheep were exposed to aerosols of sodium nitrate for short periods (Sackner et al. 1979). No signs of exposure-related pulmonary effects (e.g., respiratory resistance, static lung performance, functional residual capacity) were seen in anesthetized dogs exposed at 10 mg sodium nitrate/m^3 (2.88 ppm) for 7.5 minutes or anesthetized dogs and conscious sheep exposed for 4 hours at 5 mg sodium nitrate/m^3 (1.44 ppm). There was no evidence of exposure-related cardiac effects (pulmonary and systemic arterial pressure, cardiac output, heart rate, arterial blood gases) in anesthetized dogs or conscious sheep exposed at 5 mg sodium nitrate/m^3 (1.44 ppm) for 4 hours.

Oral MRLs

Nitrate

- An MRL of 4 mg/kg/day has been derived for acute-duration oral exposure (14 days or less) to nitrate.

- An MRL of 4 mg/kg/day has been derived for intermediate-duration oral exposure (15–364 days) to nitrate.

- An MRL of 4 mg/kg/day has been derived for chronic-duration oral exposure (365 days or more) to nitrate.

Results from studies in laboratory animals are not an appropriate basis for oral MRL derivation due to significant interspecies differences in kinetics of the nitrate-nitrite-nitric oxide pathway.

Most human exposure to nitrate and nitrite is through the diet. Vegetables are the major source of exposure to nitrate; both nitrate and nitrite may be found in some meat, fish, and dairy products as well. Estimates of daily dietary intake in the United States range from 103 mg nitrate/day from the normal diet to as high as 367 mg nitrate/day for a vegetarian diet and from 1.2 mg nitrite/day for the normal and vegetarian diets to 2.6 mg nitrite/day for a diet high in cured meat (Gangolli et al. 1994). Nitrate-contaminated drinking water is another source of exposure to nitrate and nitrite; estimated oral intake from drinking water sources may be as high as 319 mg nitrate/day and 1.2 mg nitrite/day (Gangolli et al. 1994).

Methemoglobinemia is the hallmark effect of overexposure to nitrate or nitrite. Although available human data are limited by lack of information regarding bacterial contamination in drinking water and its possible influence on methemoglobin levels, the weight-of-evidence indicates that bottle-fed infants (0–

<3 months of age) ingesting formula prepared using drinking water sources containing >44 mg nitrate/L are at risk of methemoglobinemia (e.g., Bosch et al. 1950; Walton 1951). Proposed explanations for increased susceptibility of infants to methemoglobinemia following ingestion of nitrate include: (1) increased reduction of nitrate to nitrite in the newborn, (2) increased tendency for nitrite-induced methemoglobin formation by fetal hemoglobin compared to adult hemoglobin, (3) lower levels of NADH-dependent methemoglobin reductase (the major enzyme responsible for reduction of methemoglobin to normal hemoglobin; also termed NADH-diaphorase, a soluble form of cytochrome-b5 reductase) in the newborn compared to older infants and adults, and (4) incompletely developed hepatic microsomal enzyme system in the infant and consequent lower rate of hepatic reduction of circulating nitrite compared to that of older children and adults. A portion of ingested nitrate is reduced to nitrite by commensal bacteria in the mouth; however, the acid environment of the normal stomach does not support the growth of such bacteria and most of the nitrate that reaches the stomach passes to the small intestine from which it is nearly completely absorbed into the blood. Although Kanady et al. (2012) reported little or no bacterial conversion of nitrate to nitrite in the saliva of a group of 10 infants during the first 2 postnatal months (considered mainly due to lower numbers of major nitrate-reducing oral bacteria than adults), a higher pH in the stomach of the newborn may favor growth of nitrate-reducing bacteria, resulting in increased reduction of nitrate to nitrite and increased plasma methemoglobin. Most hemoglobin in the newborn is in the form of fetal hemoglobin, which appears to be more readily oxidized to methemoglobin than adult hemoglobin; fetal hemoglobin is replaced by adult hemoglobin during early postnatal life. Levels of NADH-dependent methemoglobin reductase (the major enzyme responsible for reduction of methemoglobin to normal hemoglobin) in the newborn increase approximately 2-fold during the first 4 months of postnatal life to reach adult levels. During the period of relatively lower methemoglobin reductase levels, methemoglobin would not be expected to be as readily reduced, resulting in increased susceptibility to methemoglobinemia. In apparent contrast, Ibrahim et al. (2012) reported that blood nitrite levels in newborns approximately 1–2 days of age were 35–55% lower than that of adults. However, one study that evaluated reduction rates of methemoglobin in human adult blood and cord blood from term newborns estimated methemoglobin half-lives of 162 and 210 minutes, respectively, indicating that methemoglobin reduction occurs more slowly in newborns than adults (Power et al. 2007). Although specific mechanisms have not been elucidated, the increased susceptibility to nitrite-induced methemoglobinemia in infants is well-documented.

Available human data provide some evidence of nitrate-induced developmental effects, limited human data provide only suggestive evidence that elevated levels of nitrate in drinking water and/or nitrate-rich diets may be associated with signs of thyroid dysfunction (Aschebrook-Kilfoy et al. 2012; Gatseva and

Argirova 2008; Rádiková et al. 2008; Tajtáková et al. 2006; Ward et al. 2010). Significant associations between nitrate levels in drinking water and risk of childhood type 1 diabetes were reported by some investigators (Kostraba et al. 1992; Parslow et al. 1997; Virtanen et al. 1994); others found no evidence for such an association (Casu et al. 2000; Dahlquist et al. 1990; Moltchanova et al. 2004; van Maanen et al. 2000; Zhao et al. 2001).

Although available data suggest that reports of methemoglobinemia among infants exposed to nitrate from the drinking water may involve factors other than (or in addition to) nitrate exposure, the study of Walton (1951) is selected as the principal study and methemoglobinemia is selected as the critical effect for deriving acute-, intermediate-, and chronic-duration oral MRLs for nitrate to be protective of particularly sensitive subpopulations (e.g., infants from birth to <3 months of age), including those with gastrointestinal infections. Following ingestion of relatively large amounts of nitrate by healthy normal individuals, blood methemoglobin levels increase rapidly, followed by a return to normal within several hours following intake. Repeated ingestion for intermediate- or chronic-duration time periods would be expected to result in changes in methemoglobin levels similar to those elicited from a single exposure. Therefore, the acute-, intermediate- and chronic-duration oral MRL values are equivalent.

There is some evidence that methemoglobinemia in infants drinking formula prepared using drinking water with relatively high levels of nitrate may be related to bacterial contamination of such water sources and consequent gastrointestinal disorders, as well as overproduction of nitric oxide due to gastrointestinal infection and inflammation (Avery 1999; Gupta et al. 1998; L'hirondel and L'hirondel 2002; Yano et al. 1982). On behalf of the World Health Organization (WHO), Fewtrell (2004) performed a literature-based investigation of methemoglobinemia and drinking water concentrations >50 mg nitrate/L and concluded that nitrate may be one of a number of cofactors in causing methemoglobinemia. Fewtrell (2004) noted a paucity of information since the early 1990s linking methemoglobinemia to nitrate in drinking water, although numerous reports describe water supplies worldwide that contain nitrate at levels >50 mg/L.

The acute-, intermediate-, and chronic-duration oral MRLs were calculated using estimated mean values for drinking water ingestion rates (Kahn and Stralka 2009) and body weight (EPA 2008) and the assumption that a drinking water level of 44 mg nitrate/L is a concentration not expected to cause methemoglobinemia. A NOAEL of 4.33 mg nitrate/kg/day for infants <3 months of age was calculated based on a drinking water NOAEL of 44 mg nitrate/L and estimations of water intake (0.525 L/day) and body weight (5.33 kg) (i.e., [44 mg nitrate/L x 0.525 L/day] / 5.33 kg = 4.33 mg nitrate/kg/day). The dose of 4.33 mg nitrate/kg/day for infants from birth to <3 months of age is selected as the point of

departure for deriving acute-, intermediate-, and chronic-duration oral MRLs for nitrate because infants <3 months of age are particularly sensitive to nitrate-induced adverse effects. Application of a total uncertainty factor of 1 is justified because the point of departure is a NOAEL for nitrate-induced effects on methemoglobin in a sensitive human subpopulation (i.e., <3 month-old infants, which in many cases may have been at increased risk of methemoglobinemia due to microbial contamination and associated gastrointestinal infection, or which may have had gastroenteritis-associated methemoglobinemia unrelated to nitrate intake). The resulting acute-, intermediate-, and chronic-duration oral MRLs for nitrate are 4 mg/kg/day and are considered to be highly conservative because they were derived using results from a particularly sensitive population exhibiting nitrate-induced methemoglobinemia (infants <3 months of age), and because increased risk of methemoglobinemia in the most sensitive population may have been due in part to exposure to contaminants other than nitrate in the drinking water (refer to Appendix A for additional details regarding derivation of oral MRLs for nitrate).

A physiologically based pharmacokinetic (PBPK) model approach to derivation of oral MRLs for nitrate was initially considered, in which case a methemoglobin level of 10% of total hemoglobin would have been considered a threshold for nitrate-induced methemoglobinemia in infants. However, although the model of Zeilmaker et al. (1996, 2010b) simulates the kinetics of methemoglobin formation resulting from gastrointestinal absorption of nitrate in adult humans, the model is not considered adequate for the purpose of simulating the kinetics in infants.

Nitrite

- An MRL of 0.1 mg/kg/day has been derived for acute-duration oral exposure (14 days or less) to nitrite.

- An MRL of 0.1 mg/kg/day has been derived for intermediate-duration oral exposure (15–364 days) to nitrite.

- An MRL of 0.1 mg/kg/day has been derived for chronic-duration oral exposure (365 days or more) to nitrite.

Ingestion of nitrite (from potassium nitrite or sodium nitrite sources) has been associated with severe methemoglobinemia in adults and children (Aquanno et al. 1981; CDC 1997, 2002; Gautami et al. 1995; Gowans 1990; Greenberg et al. 1945; Kaplan et al. 1990; Ringling et al. 2003; Sevier and Berbatis 1976; Ten Brink et al. 1982; Walley and Flanagan 1987). In many of these cases, clinical signs included dizziness, loss of consciousness, and/or convulsions (CDC 1997, 2002; Gautami et al. 1995; Greenberg et

al. 1945; Sevier and Berbatis 1976; Ten Brink et al. 1982). All cases were the result of consumption of food or drink that contained unusually high levels of nitrite via contamination, inadvertent use of sodium nitrite instead of table salt, or ingestion of a single sodium nitrite tablet (667 mg nitrite). Headache was induced in a male subject following consumption of a 10 mg sodium nitrite solution (Henderson and Raskin 1972). Headaches were induced in 8 out of 13 such tests. No information was located regarding methemoglobin concentrations in infants following oral exposure to nitrite. The ingestion of nitrate results in the formation of nitrite, which is the moiety responsible for methemoglobinemia. The study of Walton (1951) is selected as the principal study and methemoglobinemia as the critical effect for deriving acute-, intermediate-, and chronic-duration oral MRLs for nitrite to be protective of particularly sensitive subpopulations (e.g., infants from birth to <3 months of age), including those with gastrointestinal infections. On average, approximately 25% of an ingested dose of nitrate enters the saliva of an adult where a portion (ca. 20% g/g) is reduced by commensal bacteria to nitrite (i.e., approximately 5% g/g of ingested nitrate is reduced to nitrite in the saliva of an adult) (Spiegelhalder et al. 1976); most salivary nitrite is absorbed into the blood in the small intestine. Therefore, the ingestion of nitrate at the oral MRL of 4 mg/kg/day would be expected to result in the production of 0.2 mg nitrite/kg/day by an adult (i.e., 0.2 mg nitrite/kg/day is 5% (g/g) of an oral dose of nitrate at the oral MRL of 4 mg/kg/day). Although quantitative data are lacking regarding the effective blood nitrite level in a young infant from an ingested dose of nitrate, young infants exhibit increased susceptibility to methemoglobinemia following nitrate ingestion. Mechanisms responsible for increased susceptibility in infants may include greater reduction of nitrate to nitrite (which may be higher in the stomach of an infant due to a higher pH), lower levels of NADH-dependent methemoglobin reductase, slower rate of hepatic reduction of circulating nitrite, and/or increased tendency for nitrite-induced methemoglobin formation by fetal hemoglobin compared to adult hemoglobin. To account for increased susceptibility to methemoglobinemia following ingestion of nitrate by infants, a modifying factor of 2 is applied to the point of departure (0.2 mg nitrite/kg/day ÷ 2 = 0.1 mg/kg/day). The modifying factor assumes that the effective methemoglobin level from a given intake of nitrate by an infant is twice that of an adult (e.g., approximately 5% of an oral dose of nitrate is converted to nitrite in the adult; the modifying factor of 2 accounts for up to 10% conversion in the infant). The resulting acute-, intermediate-, and chronic-duration oral MRLs of 0.1 mg nitrite/kg/day are considered protective of nitrite-induced methemoglobinemia for particularly sensitive subpopulations (e.g., infants <3 months of age). The oral MRLs for nitrite are considered to be highly conservative because they were derived using results from a particularly sensitive population exhibiting nitrate-induced methemoglobinemia (infants <3 months of age), and because increased risk of methemoglobinemia in the infants may have been due in part to exposure to contaminants other than nitrate in the drinking water (refer to Appendix A for additional details regarding derivation of oral MRLs for nitrite).

2. RELEVANCE TO PUBLIC HEALTH

Drinking water and dietary sources may contain both nitrate and nitrite; furthermore, as discussed in Section 3.4, some nitrate is converted to nitrite in the body and nitrite can be converted to nitrate as well. Overexposure to either nitrate or nitrite can result in elevated methemoglobin levels. At a worldwide level, WHO (2011a, 2011b) provides guidance for combined exposure to nitrate and nitrite in drinking water, which states that the sum of the ratios of the concentration of each to its guideline value should not exceed 1.

2. RELEVANCE TO PUBLIC HEALTH

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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 nitrate and nitrite. 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 (e.g., death, systemic, immunological, neurological, reproductive, developmental, 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 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.

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.

Nitrate (NO_3^-) and nitrite (NO_2^-) are naturally-occurring oxidation products of nitrogen. Nitrate may be expressed in terms of ionic concentration (i.e., mg nitrate/L), or elemental concentration (i.e., mg nitrate-nitrogen/L or mg nitrogen as nitrate/L). A concentration of nitrate expressed in elemental concentration can be converted to its ionic concentration according to the following relationship: 1 mg nitrate-nitrogen is equivalent to 4.4 mg nitrate. In aqueous environments, nitrate and nitrite salts such as sodium nitrate, potassium nitrate, sodium nitrite, and potassium nitrite rapidly ionize. Sodium nitrate is approximately 27% sodium and 73% nitrate. To determine a nitrate dose from a sodium nitrate source, the quantity of sodium nitrate is multiplied by the nitrate proportion (0.73). Thus a nitrate dose from a 5 mg sodium nitrate source is 5x0.73=3.65 mg nitrate. The conversion factor for nitrate from a potassium nitrate source is 0.61. Conversion factors for nitrite from sodium nitrite and potassium nitrite are 0.67 and 0.54, respectively.

3.2.1 Inhalation Exposure

3.2.1.1 Death

No information was located regarding death in humans following inhalation exposure to nitrate or nitrite.

An inhalation LC_{50} is an exposure level expected to result in 50% mortality. RTECS (2014) lists a rat 4-hour LC_{50} of 5.5 mg/m^3 (1.95 ppm) for sodium nitrite and a rat 2-hour LC_{50} of 85 mg/m^3 (24.42 ppm) for potassium nitrite. No additional information was located regarding death in animals exposed to nitrate or nitrite.

3.2.1.2 Systemic Effects

No studies were located regarding gastrointestinal, hematological, musculoskeletal, hepatic, renal, endocrine, dermal, ocular, or body weight effects in humans or animals after inhalation exposure to nitrate or nitrite.

Respiratory Effects. Limited human data are available. Al-Dabbagh et al. (1986) evaluated the mortality of a cohort of 1,327 male workers involved in the manufacture of nitrate fertilizer for at least 1 year between 1946 and 1981 for a chemical company in northeast England. There was no evidence of an association between exposure to nitrate dusts and death from all respiratory diseases compared to mortality rates for the northern region of England.

Available information in animals is limited to a study in which dogs and sheep were exposed to aerosols of sodium nitrate for short periods (Sackner et al. 1979). There was no evidence of exposure-related pulmonary effects (e.g., respiratory resistance, static lung performance, functional residual capacity) in anesthetized dogs exposed at up to 10 mg sodium nitrate/m^3 (2.88 ppm) for 7.5 minutes or anesthetized dogs or conscious sheep exposed at 5 mg sodium nitrate/m^3 (1.44 ppm) for 4 hours.

Cardiovascular Effects. Available information in humans is limited to results of mortality studies of workers involved in the production of nitrate fertilizers. In general, studies of workers in which outcomes are compared to the general population (e.g., observed versus expected deaths) may be biased by a healthy worker effect, which may lower estimated risks. There was no evidence of an association between exposure to nitrate dust and death from ischemic heart disease, cerebrovascular disease, or all circulatory diseases in a census-based (England and Wales) mortality study of workers involved in the production of nitrate fertilizers (Fraser et al. 1982, 1989). The study included a cohort of 866 men from the 1961 census and 651 men from the 1971 census. These cohorts were followed through 1985. Al-Dabbagh et al. (1986) evaluated the mortality of a cohort of 1,327 male workers involved in the manufacture of nitrate fertilizer for at least 1 year between 1946 and 1981 for a chemical company in northeast England. There was no evidence of an association between exposure to nitrate dusts and death from ischemic heart disease or other circulatory diseases compared to mortality rates for the northern region of England.

Available information in animals is limited to a study in which dogs and sheep were exposed to aerosols of sodium nitrate for short periods (Sackner et al. 1979). There was no evidence of exposure-related cardiac effects (pulmonary and systemic arterial pressure, cardiac output, heart rate, arterial blood gases) in anesthetized dogs or conscious sheep exposed at 5 mg sodium nitrate/m^3 (1.44 ppm) for 4 hours.

No information was located regarding the following effects in humans or animals exposed to nitrate or nitrite via the inhalation route:

3.2.1.3 Immunological and Lymphoreticular Effects
3.2.1.4 Neurological Effects
3.2.1.5 Reproductive Effects
3.2.1.6 Developmental Effects

3.2.1.7 Cancer

Available information in humans is limited to results of mortality studies of workers involved in the production of nitrate fertilizers. In general, studies of workers in which outcomes are compared to the general population (e.g., observed versus expected deaths) may be biased by a healthy worker effect, which may lower estimated risks. A census-based (England and Wales) mortality study of workers involved in the production of nitrate fertilizers included 866 men from the 1961 census and 651 men from the 1971 census; mortality rates among these workers were compared to mortality rates of men from England and Wales (Fraser et al. 1982). At follow-up until 1978, slight excess of death from intestinal cancer was noted among men from the 1961 census (6 observed versus 4.5 expected); excess of death from all cancers, (19 versus 14.4 expected), esophageal cancer (1 versus 0.4 expected), gastric cancer (2 versus 1.5 expected), intestinal cancer (1 versus 0.9 expected), rectal cancer (2 versus 0.6 expected), and lung cancer (9 versus 6.4 expected) were observed in the 1971 census cohort. However, follow-up through 1985 revealed no significant increased risk for cancer at any site (Fraser et al. 1989).

Al-Dabbagh et al. (1986) evaluated mortality rates within a cohort of 1,327 male workers involved in the manufacture of nitrate fertilizer for at least 1 year between 1946 and 1981 for a chemical company in northeast England; mortality rates were compared with those of the male population of the region. Among 537 workers described as having been heavily exposed to nitrate dust (i.e., working in an environment likely to have contained >10 mg nitrate/m^3 [>2.88 ppm]), slight excesses were noted for deaths from lung cancer (25 observed versus 21.04 expected) and death from all malignant neoplasms (59 observed versus 51.36 expected), but not for cancers of the esophagus, stomach, or bladder. After categorizing the heavily-exposed workers by duration of exposure and time since first exposure, excess

death from lung cancer was noted for those exposed for ≥10 years, with a lag time of ≥20 years since first exposure (13 observed versus 8.11 expected). The study authors indicated that they were unable to adjust for smoking.

Hagmar et al. (1991) evaluated mortality rates within a cohort of 2,131 male workers at a nitrate fertilizer production facility in Sweden and compared them to mortality rates for men in the same county. Death from prostate cancer (26 observed versus 16.1 expected) was in excess (standardized mortality ratio [SMR] 161, 95% CI: 107, 239); however, risk of prostate cancer within this cohort was not enhanced following application of a ≥10-year latency period. There was no significant increase in death from tumors of the lips, oral cavity, pharynx, salivary glands, gastrointestinal tract, stomach, respiratory tract, lung, urinary bladder, blood, or all sites combined.

Fandrem et al. (1993) evaluated incidences of selected cancers among 2,023 male workers who had been employed for >1 year at a Norwegian nitrate fertilizer plant between 1945 and 1979. The average historical concentration of nitrate in the workplace air was estimated to have been 10 mg/m^3. The cohort was followed from 1953 through 1988 and incidences of cancer among the workers were compared to national rates. The study authors reported 30 incidences of lung cancer (27.5 expected: standardized incidence ratio [SIR] 1.09; 95% CI 0.73, 1.53), 9 incidences of kidney cancer (7.6 expected: SIR 1.18; 95% CI 0.54, 2.25), and 9 incidences of pancreatic cancer (7.3 expected: SIR 1.23; 95% CI 0.56, 2.34). There were fewer than expected cancers of the oesophagus, stomach, colon/rectum, pleura, bladder, malignant melanoma, and all cancers combined. No association was found between gastric cancer and cumulative exposure to nitrate, duration of employment, or time since first exposure.

Rafnsson and Gunnarsdóttir (1990) evaluated mortality rates among 603 male workers at a nitrate fertilizer plant in Iceland who had been employed for >1 year between 1954 and 1985. Mortality data were compared to national rates for men. The study authors reported nonstatistically significant excesses of cancers of the large intestine (2 observed versus 1.25 expected: SMR 160; 95% CI 19, 578), rectum (1 observed versus 0.61 expected: SMR 164; 95% CI 4, 913), pancreas (3 observed versus 1.31 expected: SMR 229; 95% CI 47, 669), and respiratory tract (4 observed versus 2.88 expected: SMR 139; 95 CI 38, 356). There was no excess of death from stomach cancer (4 observed versus 4.32 expected: SMR 93; 95% CI 25, 237). This study is limited by low incidences of selected cancers and possible confounding by the healthy worker effect.

3.2.2 Oral Exposure

3.2.2.1 Death

As early as the mid-1900s, methemoglobinemia was reported in infants exposed to relatively large amounts of nitrate from drinking water sources (e.g., Bosch et al. 1950; Bucklin and Myint 1960; Chapin 1947; Comly 1987; Donahoe 1949; Faucett and Miller 1946; Ferrant 1946; McLetchie and Robertson 1949; Medovy 1948; Robertson and Riddell 1949; Stafford 1947). Deaths occurred in some of these cases. Ingestion of nitrite (from potassium nitrite or sodium nitrite sources) has been associated with severe methemoglobinemia in adults and children (Aquanno et al. 1981; CDC 1997, 2002; Gautami et al. 1995; Gowans 1990; Greenberg et al. 1945; Kaplan et al. 1990; Ringling et al. 2003; Sevier and Berbatis 1976; Ten Brink et al. 1982; Walley and Flanagan 1987). Deaths occurred in some of these cases following consumption of food or drink that contained unusually high levels of nitrite via contamination, inadvertent use of sodium nitrite instead of table salt, or ingestion of a single sodium nitrite tablet (667 mg nitrite).

An oral LD_{50} is the dose expected to result in 50% mortality. Single oral doses of sodium nitrite at multiple dose levels resulted in LD_{50} values of 150 mg/kg (100 mg nitrite/kg) in rats (Imaizumi et al. 1980) and 265 mg/kg (178.2 mg nitrite/kg) in mice (Sheehy and Way 1974). RTECS (2014) lists oral LD_{50} values for sodium nitrate of 1,267, 3,500, and 2,680 mg/kg for the rat, mouse, and rabbit, respectively; LD_{50} values for sodium nitrite of 157.9, 175, and 186 mg/kg for the rat, mouse, and rabbit, respectively; LD_{50} values for potassium nitrate of 3,540 and 3,750 for the rat and 1,901 mg/kg for the rabbit; and an LD_{50} for potassium nitrite of 200 mg/kg. Among rats provided sodium nitrate in the drinking water for 6 weeks, concentrations of sodium nitrate resulting in an estimated dose of 14,600 mg nitrate/kg/day was lethal to 7/10 male rats; an estimated dose of 16,483.9 mg nitrate/kg/day was lethal to 10/10 female rats. Among male rats similarly treated with sodium nitrite, an estimated dose of 1,080.6 mg nitrite/kg/day was lethal to 4/10 rats. Inai et al. (1979) reported 100% mortality in male and female mice (10/sex) provided sodium nitrite in the drinking water at concentrations resulting in estimated doses of 330.8 and 354.1 mg nitrite/kg/day, respectively; the deaths occurred within the first 3 weeks of a 6-week study.

3.2.2.2 Systemic Effects

No studies were located regarding musculoskeletal or ocular effects in humans or animals after oral exposure to nitrate or nitrite.

The highest NOAEL values and all LOAEL values from each reliable study for systemic effects in each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1.

Respiratory Effects. No studies were located regarding respiratory effects in humans or animals following oral exposure to nitrate or nitrite.

Cardiovascular Effects. Malberg et al. (1978) investigated possible associations between hypertension and levels of nitrate in the drinking water in a hospital-based study in Colorado that included 226 cases of hypertension among patients living in areas where drinking water contained nitrate at concentrations ranging from 19 to 125 ppm (mean 52 ppm) and 261 cases from patients living in areas without nitrate in the drinking water. The mean annual incidence rate for the nitrate-exposed patients was 5.9/1,000 population versus 7.9/1,000 for the control patients. However, the nitrate-exposed patients exhibited an earlier mean age at hospitalization for hypertension (58.5 years versus 65.2 years for controls); the toxicological significance of this finding is uncertain because the incidence rate for hypertension was higher among control patients than among patients exposed to nitrate in the drinking water.

Cardiovascular health is an end point of concern for nitrate because some nitrate is converted to nitrite in the body. Nitrite is a smooth muscle relaxant that can cause hypotension and plasma nitrite is involved in the oxidation of hemoglobin to methemoglobin, which is associated with hypotension, rapid pulse, and rapid breathing at high enough concentrations. Ingestion of nitrite (from potassium nitrite or sodium nitrite sources) has been associated with severe methemoglobinemia in adults and children; in some of these cases, symptoms included hypotension and/or tachycardia (Gowans 1990; Sevier and Berbatis 1976; Ten Brink et al. 1982). These cases were the result of consumption of food or drink that contained unusually high levels of nitrite via contamination, inadvertent use of sodium nitrite instead of table salt, or ingestion of a single sodium nitrite tablet (667 mg nitrite).

In a study designed to evaluate the oral bioavailability of sodium nitrite in healthy volunteers (seven females and two males; mean age 22.9 years), ingestion of 0.06 sodium nitrite per mmol hemoglobin (~2.2–2.7 mg sodium nitrite/kg, or 1.5–1.8 mg nitrite/kg) resulted in an average heart rate increase from 55 to 63 bpm and average mean arterial blood pressure decrease from 78 to 70 mmHg (Kortboyer et al. 1997b). At a higher intake (0.12 mmol sodium nitrite per mmol hemoglobin; ~4.4–5.4 mg sodium nitrite/kg, or 2.9–3.6 mg nitrite/kg), the average heart rate increased from 57 to 67 bpm and the average

Table 3-1 Levels of Significant Exposure to Nitrate And Nitrite - Oral

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
ACUTE EXPOSURE								
Death								
1	Rat (Sprague-Dawley)	Once (GW)				100.5 (LD50)	Imaizumi et al. 1980 Sodium Nitrite	
2	Mouse (Swiss-Webster)	Once (GW)				178.2 M (LD50)	Sheehy and Way 1974 Sodium Nitrite	
Systemic								
3	Human	NS (F)	Hemato	4.33[b]			Walton 1951 Nitrate	Dose based on a drinking water level (44 mg nitrate/L) above which nitrate could cause methemoglobinemia in infants <3 months old.
4	Human	NS (F)	Hemato	0.2[c]			Walton 1951 Nitrite	The NOAEL represents the estimated nitrite dose to an infant <3 months of age consuming nitrate from drinking water at up to 44 mg/L.
5	Rat (Sprague-Dawley)	Once (GW)	Hemato	6.7	16.75 (8.6% methemoglobin)		Imaizumi et al. 1980 Sodium Nitrite	
6	Rat (Wistar)	1 or 3 d 1 x/d (GW)	Hepatic	104.2 M			Lijinsky and Greenblatt 1972 Sodium Nitrite	

Table 3-1 Levels of Significant Exposure to Nitrate And Nitrite - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
7	Mouse HaM/ICR	Once (GW)	Hepatic	150 M			Asahina et al. 1971 Sodium Nitrite	
			Bd Wt	150 M				
Developmental								
8	Rat (NS)	Gd 15 Once (GW)		53.6			Khera 1982 Sodium Nitrite	
9	Mouse (CD-1)	Gd 1-14, 16, or 18 1 x/d (GW)		13			Globus and Samuel 1978 Sodium Nitrite	
10	Mouse (ICR)	Gd 7-18 (W)		113.2			Shimada 1989 Sodium Nitrite	
INTERMEDIATE EXPOSURE								
Death								
11	Rat (Fischer- 344)	6 wk (W)				1080.6 F (4/10 died)	Maekawa et al. 1982 Sodium Nitrite	
12	Rat (Fischer- 344)	6 wk (F)				14600 M (7/10 died) 16483.9 F (10/10 died)	Maekawa et al. 1982 Sodium Nitrate	
13	Mouse (ICR)	6 wk (W)				330.8 M (death during first 3 treatment weeks) 354.1 F (death during first 3 treatment weeks)	Inai et al. 1979 Sodium Nitrite	

Table 3-1 Levels of Significant Exposure to Nitrate And Nitrite - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
14	Gn Pig (NS)	143-204 d (W)				4972 F (1/3 died)	Sleight and Atallah 1968 potassium nitrate	
Systemic								
15	Human	NS (F)	Hemato	4.33[b]			Walton 1951 Nitrate	Dose based on a drinking water level (44 mg nitrate/L) above which nitrate could cause methemoglobinemia in infants <3 months old.
16	Human	NS (F)	Hemato	0.2[c]			Walton 1951 Nitrite	The NOAEL represents the estimated nitrite dose to an infant <3 months of age consuming nitrate from drinking water at up to 44 mg/L.
17	Rat (Sprague-Dawley)	12wk 1x/d (G)	Metab			80 M (hyperglycemia, insulin resistance)	Al-Gayyar et al. 2015 Sodium Nitrite	
18	Rat (albino)	2 mo (W)	Hemato	28.14 M	187.6 M (12.16% methemoglobin)		Behroozi et al. 1972 Sodium Nitrite	
19	Rat (Sprague-Dawley)	16 wk (W)	Hemato	40.5 M			Chow et al. 1980 Sodium Nitrate	

Table 3-1 Levels of Significant Exposure to Nitrate And Nitrite - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
20	Rat (Sprague-Dawley)	16 wk (W)	Hemato	18.6 M			Chow et al. 1980 Sodium Nitrite	
21	Rat (Wistar)	4 mo (W)	Renal	6.4 M	15.8 M (increased urinary urea and creatinine levels)		El-Wakf et al. 2008 Sodium Nitrate	
			Endocr		6.4 M (decreased serum T3 and T4; increased serum TSH)			
			Bd Wt		6.4 M (11-12% depressed mean body weight and body weight gain)			
22	Rat (Wistar)	4mo Continuous (W)	Bd Wt		34.8 M (9 and 30% depressed mean body weight and body weight gain, respectively, among adult rats)	34.8 M (24 and 39% depressed mean body weight and body weight gain, respectively, among young rats)	El-Wakf et al. 2015 Sodium Nitrate	
			Metab			34.8 M (hyperglycemia)		
23	Rat (Wistar)	30 wk (W)	Endocr	60.16 F	158.77 F (decreased serum T3, T4, and TSH levels; increased thyroid weight; follicular hyperplasia)		Eskiocak et al. 2005 Sodium Nitrate	
24	Rat (Sprague-Dawley)	6 mo (W)	Hemato			167.5 (peak methemoglobin of 33-88%)	Imaizumi et al. 1980 Sodium Nitrite	

Table 3-1 Levels of Significant Exposure to Nitrate And Nitrite - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
25	Rat (Fischer- 344)	51 wk (W)	Gastro	208.4 M			Kawabe et al. 1994 Sodium Nitrite	
26	Rat (Sprague-Dawley)	10 mo (F)	Hepatic	183.1			Lin and Ho 1992 Sodium Nitrite	
			Bd Wt	183.1				
27	Rat (Fischer- 344)	6 wk (F)	Hemato	3650 M 4121 F	7300 M (discolored blood and spleen indicative of methemoglobinemia) 8241.9 F (discolored blood and spleen indicative of methemoglobinemia)		Maekawa et al. 1982 Sodium Nitrate	
			Bd Wt	7300 M 4121 F	14600 M (at least 10% depressed body weight gain) 8241.9 F (at least 10% depressed body weight gain)			

Table 3-1 Levels of Significant Exposure to Nitrate And Nitrite - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
28	Rat (Fischer- 344)	6 wk (W)	Hemato	186.1 M 270.2 F	372.2 M (discolored blood and spleen indicative of methemoglobinemia)		Maekawa et al. 1982 Sodium Nitrite	
					540.3 F (discoloration of blood and spleen indicative of methemoglobinemia)			
			Bd Wt	372.2 M 540.3 F	744.4 M (at least 10% depressed body weight gain)			
					1080.6 F (at least 10% depressed body weight gain)			
29	Rat (Fischer- 344)	35 wk (W)	Gastro	208.4 M			Miyauchi et al. 2002 Sodium Nitrite	
30	Rat (Wistar)	4 wk (F)	Endocr		2416.6 (increased thyroid weight, decreased thyroid peroxidase activity, decreased serum T3 and T4, increased serum TSH)		Mukhopadhyay et al. 2005 potassium nitrate	
			Bd Wt	2416.6				

Table 3-1 Levels of Significant Exposure to Nitrate And Nitrite - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
31	Rat (Fischer- 344)	14 wk (W)	Hemato	77.1 M 53.6 F	134 M (up to 10% methemoglobin) 87.1 F (up to 13% methemoglobin)		NTP 2001 Sodium Nitrite	
32	Rat (Wistar)	13 wk (W)	Hemato	41.9 M 61.8 F	107.6 M (5.7% methemoglobin) 130.5 F (7.6% methemoglobin)		Til et al. 1988 potassium nitrite	
			Endocr	4.8 M 16.8 F	13.3 M (hypertrophy in zona glomerulosa of adrenal gland) 61.8 F (hypertrophy in zona glomerulosa of adrenal gland)			

Table 3-1 Levels of Significant Exposure to Nitrate And Nitrite - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
33	Rat (Wistar)	13 wk (W)	Endocr	4.59 M 5.94 F	105.1 M (hypertrophy in zona glomerulosa of adrenal gland) 130.1 F (hypertrophy in zona glomerulosa of adrenal gland)		Til et al. 1997 potassium nitrite	
34	Rat (Wistar)	13 wk (W)	Hemato	5.2 M 7.1 F	106.3 M (increased methemoglobin, magnitude not specified) 124.8 F (increased methemoglobin, magnitude not specified)		Til et al. 1997 Sodium Nitrite	
			Endocr	5.2 M 7.1 F	106.3 M (hypertrophy in zona glomerulosa of adrenal gland) 124.8 F (hypertrophy in zona glomerulosa of adrenal gland)			
35	Rat (Sprague-Dawley)	F0 males: 15-28 d F0 females: 58-71 d F1 pups: 69 d (F)	Bd Wt	28.1			Vorhees et al. 1984 Sodium Nitrite	

Table 3-1 Levels of Significant Exposure to Nitrate And Nitrite - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
36	Rat (Wistar)	5 mo (W)	Endocr	9 M	13.5 M (increases in serum T3 and thyroid weight; nonneoplastic lesions in thyroid gland)		Zaki et al. 2004 potassium nitrate	
			Bd Wt	9 M	13.5 M (16% lower mean body weight than controls)			
37	Mouse Swiss	26 wk (W)	Bd Wt	82.5			Greenblatt and Lijinsky 1974 Sodium Nitrite	
38	Mouse Strain A	25 wk 5 d/wk (W)	Bd Wt	118.1 M			Greenblatt and Mirvish 1973 Sodium Nitrite	
39	Mouse Strain A	25 wk 5 d/wk (W)	Bd Wt	1583 M			Greenblatt and Mirvish 1973 Sodium Nitrate	
40	Mouse Strain A	20 wk 5 d/wk (W)	Bd Wt	236.3 M			Greenblatt and Mirvish 1973 Sodium Nitrite	

Table 3-1 Levels of Significant Exposure to Nitrate And Nitrite - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
41	Mouse (B6C3F1)	14 wk (W)	Gastro	435.5 M 562.8 F	663.3 M (focal hyperplasia in forestomach) 824.1 F (focal hyperplasia in forestomach)		NTP 2001 Sodium Nitrite	
			Hemato	231.2 M 160.8 F	435.5 M (extramedullary hematopoiesis in spleen) 298.1 F (extramedullary hematopoiesis in spleen)			
			Bd Wt	435.5 M 824.1 F	663.3 M (10% depressed final mean body weight and body weight gain)			
Neurological								
42	Rat (albino)	2 mo (W)			9.38 M (altered EEG)		Behroozi et al. 1972 Sodium Nitrite	
43	Rat C57B1	F0: Mating, gestation, lactation F1: 14 wk postweaning (W)			165.4 M (increased aggressive behavior)		Gruener 1974 Sodium Nitrite	

Table 3-1 Levels of Significant Exposure to Nitrate And Nitrite - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
Reproductive								
44	Rat (Sprague-Dawley)	12wk 1x/d (G)				80 M (Increases in testicular weight and serum FSH, LH, and prolactin; decreases in sperm count and serum testosterone)	Alyoussef and Al-Gayyar 2016a Sodium Nitrite	
45	Rat (Sprague-Dawley)	12wk 1x/d (G)				80 M (decreased serum testosterone; increases in testicular weight; increased testicular levels of pro-inflammatory cytokines, oxidative stress markers, and enzymes involved in programmed cell death)	Alyoussef and Al-Gayyar 2016b Sodium Nitrite	
46	Rat (Wistar)	2 generations (F)		160 F			Hugot et al. 1980 Sodium Nitrite	
47	Rat (Fischer- 344)	14 wk (W)		77.1 M	134 M (7% decreased sperm motility)		NTP 2001 Sodium Nitrite	
48	Mouse (B6C3F1)	14 wk (W)		231.2 M		435.5 M (degeneration in testis, characterized by increased size of residual bodies within the lumen of the seminiferous tubules)	NTP 2001 Sodium Nitrite	

Table 3-1 Levels of Significant Exposure to Nitrate And Nitrite - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)		Reference Chemical Form	Comments
49	Gn Pig (NS)	143-204 d (W)		2230.8 F		4972 F		Sleight and Atallah 1968 potassium nitrate	
50	Gn Pig (NS)	100-240 d (W)		59.4 F		148.5 F	(decreased number of litters and live fetuses)	Sleight and Atallah 1968 potassium nitrite	
Developmental									
51	Rat (Wistar)	2 generations (F)		160				Hugot et al. 1980 Sodium Nitrite	
52	Rat (Sprague-Dawley)	F0 males: 15-28 d F0 females: 58-71 d F1 pups: 69 d (F)		7.2		14.4	(increased pup mortality, depressed preweaning pup body weight, delayed swimming development)	Vorhees et al. 1984 Sodium Nitrite	
CHRONIC EXPOSURE									
Systemic									
53	Human	NS (F)	Hemato	4.33[b]				Walton 1951 Nitrate	Dose based on a drinking water level (44 mg nitrate/L) above which nitrate could cause methemoglobinemia in infants <3 months old.

Table 3-1 Levels of Significant Exposure to Nitrate And Nitrite - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
54	Human	NS (F)	Hemato	0.2[c]			Walton 1951 Nitrite	The NOAEL represents the estimated nitrite dose to an infant <3 months of age consuming nitrate from drinking water at up to 44 mg/L.
55	Rat (Fischer- 344)	115 wk (F)	Bd Wt	60.5 M	178.2 M (approximately 15% depressed mean body weight)		Grant and Butler 1989 Sodium Nitrite	
56	Rat (Wistar)	67 wk 5 d/wk (W)	Bd Wt	14.5 M 22.6 F			Greenblatt et al. 1973 Sodium Nitrite	
57	Rat (Fischer- 344)	104 wk (W)	Bd Wt	82.4 M 60.3 F	101 F (more than 10% lower mean body weight than controls)		Maekawa et al. 1982 Sodium Nitrite	Study authors did not specify whether reported nitrite consumption was nitrite or sodium nitrite
58	Rat (Fischer- 344)	104 wk (F)	Bd Wt	1517 M 832 F	1730 F (up to 13% lower mean body weight than controls)		Maekawa et al. 1982 Sodium Nitrate	

Table 3-1 Levels of Significant Exposure to Nitrate And Nitrite - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
59	Rat (Fischer- 344)	105 wk (W)	Gastro	46.9 M 53.6 F	87.1 M (epithelial hyperplasia in the forestomach) 100.5 F (epithelial hyperplasia in the forestomach)		NTP 2001 Sodium Nitrite	
60	Rat NS	24 mo (W)	Hemato Hepatic	 86.4 M	172.8 M (12% methemoglobin)		Shuval and Gruener 1972 Sodium Nitrite	
61	Rat (Wistar)	29 mo (F)	Gastro Hemato Hepatic Bd Wt	176.8 M 204.5 F 176.8 M 204.5 F 176.8 M 204.5 F 204.5 F	 176.8 M (10% lower mean body weight)		van Logten et al. 1972 Sodium Nitrite	

Table 3-1 Levels of Significant Exposure to Nitrate And Nitrite - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
62	Mouse (B6C3F1)	104-105 wk (W)	Gastro	80.4 M	147.4 M (epithelial hyperplasia in glandular stomach)		NTP 2001 Sodium Nitrite	
			Bd Wt	147.4 M 110.6 F				
63	Dog (Beagle)	1 yr (W)	Endocr	38.5 M 39 F			Kelley et al. 1974 Sodium Nitrate	
Reproductive								
64	Dog (Beagle)	1 yr (W)		38.5 M 39 F			Kelley et al. 1974 Sodium Nitrate	
Cancer								
65	Rat (Fischer- 344)	106 wk (F)				108.4 F (CEL; hepatocellular neoplasms)	Lijinsky 1984a; Lijinsky et al. 1983 Sodium Nitrite	
66	Rat (Fischer- 344)	104 wk (F)				110.4 F (CEL: hepatocellular neoplasms)	Lijinsky 1984b; Lijinsky et al. 1983 Sodium Nitrite	
67	Rat (Wistar)	Lifetime (W)				298 (CEL; forestomach tumors)	Mirvish et al. 1980 Sodium Nitrite	

Table 3-1 Levels of Significant Exposure to Nitrate And Nitrite - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
68	Mouse (Hybrid)	Lifetime (W)				207.7 M (CEL: lung carcinoma)	Anderson et al. 1985 Sodium Nitrite	

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

b NOAEL of of 4 mg/kg/day for nitrate was used to derive acute-, intermediate, and chronic-duration oral minimal risk levels (MRLs) of 4 mg/kg/day for nitrate, as described in detail in Chapter 2 and Appendix A. The NOAEL was divided by an uncertainty factor of 1 for human variability because the NOAEL accounted for exposure of a particularly sensitive subpopulation (infants <3 months of age).

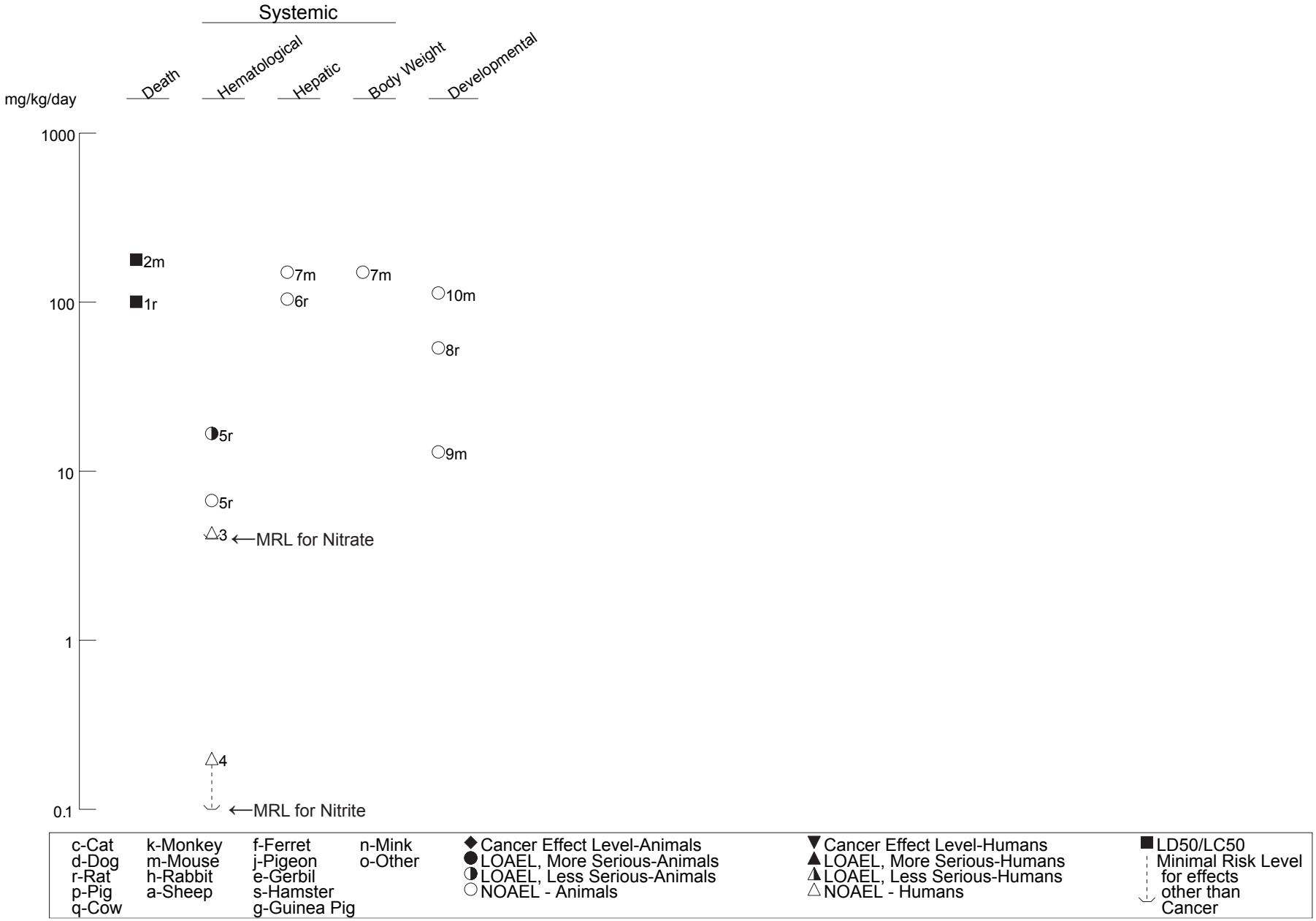
c NOAEL of 0.2 mg/kg/day for nitrite was used to derive acute-, intermediate, and chronic-duration oral minimal risk levels (MRLs) of 0.1 mg/kg/day for nitrite, as described in detail in Chapter 2 and Appendix A. The NOAEL represents the dose of nitrite that would be expected to enter the blood following ingestion of nitrate by an adult at the oral MRL value of 4 mg nitrate/kg/day assuming 5% reduction of an oral dose of nitrate to nitrite in the adult saliva complete absorption of nitrite from the digestive tract. The NOAEL of 0.2 mg/kg/day for nitrite was divided by an uncertainty factor of 1 for human variability because the NOAEL was for exposure of a particularly sensitive subpopulation (infants <3 months of age). A modifying factor of 2 was applied based on the assumption that the effective methemoglobin level from a given intake by an infant may be up to twice that of an adult.

Bd Wt = body weight; CEL = cancer effect level; d = day(s); EEG = electroencephalogram; Endocr = endocrine; (F) = feed; F = Female; Gastro = gastrointestinal; Gd = gestational day; Gn pig = guinea pig; (GW) = gavage in water; Hemato = hematological; LD50 = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; mo = month(s); NOAEL = no-observed-adverse-effect level; NS = not specified; T3 = triiodothyronine ; T4 = thyroxine; TSH = thyroid stimulating hormone ; (W) = drinking water; wk = week(s); x = time(s); yr = year(s)

3. HEALTH EFFECTS

Figure 3-1 Levels of Significant Exposure to Nitrate And Nitrite - Oral

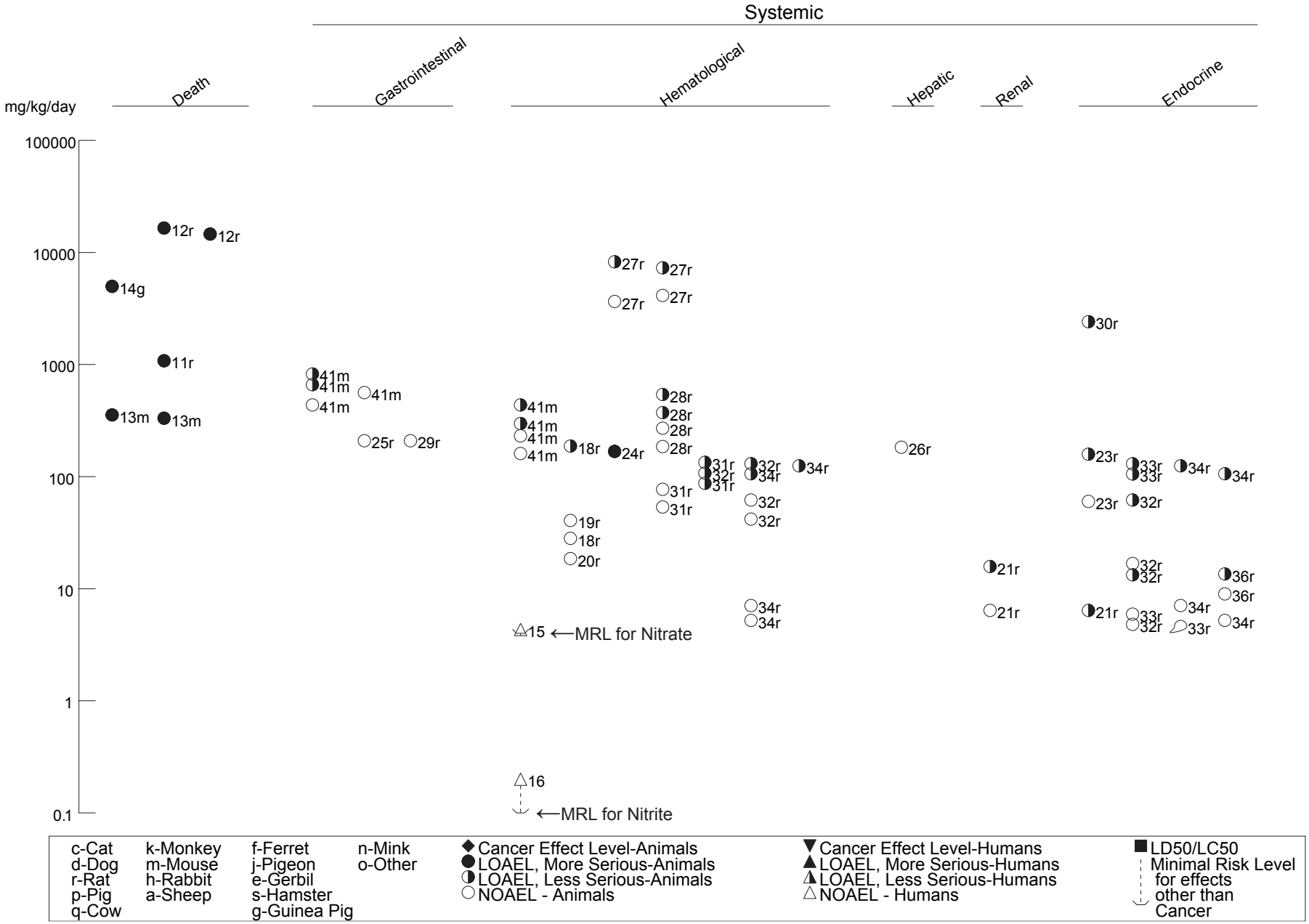
Acute (≤14 days)



3. HEALTH EFFECTS

Figure 3-1 Levels of Significant Exposure to Nitrate And Nitrite - Oral *(Continued)*

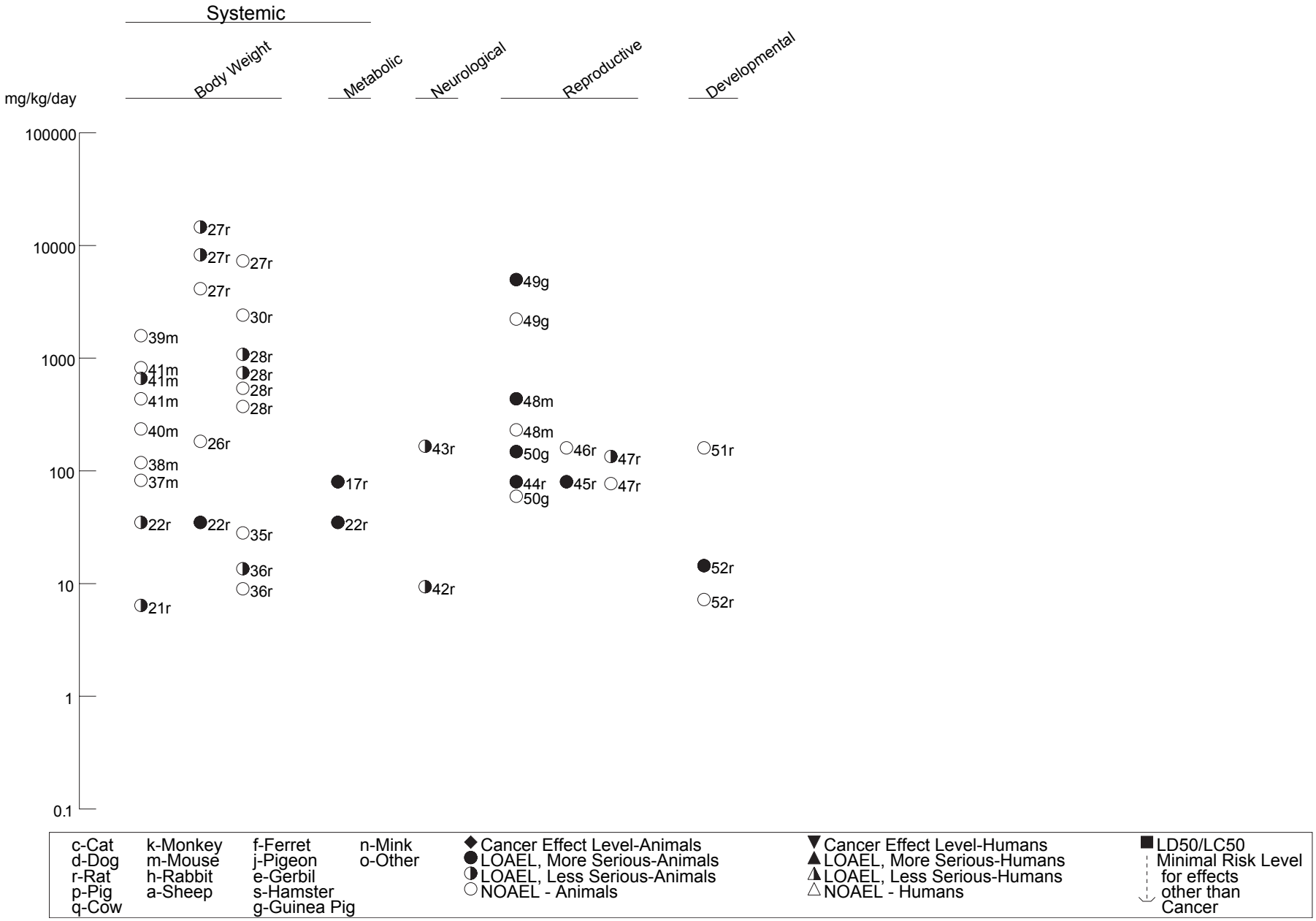
Intermediate (15-364 days)



3. HEALTH EFFECTS

Figure 3-1 Levels of Significant Exposure to Nitrate And Nitrite - Oral *(Continued)*

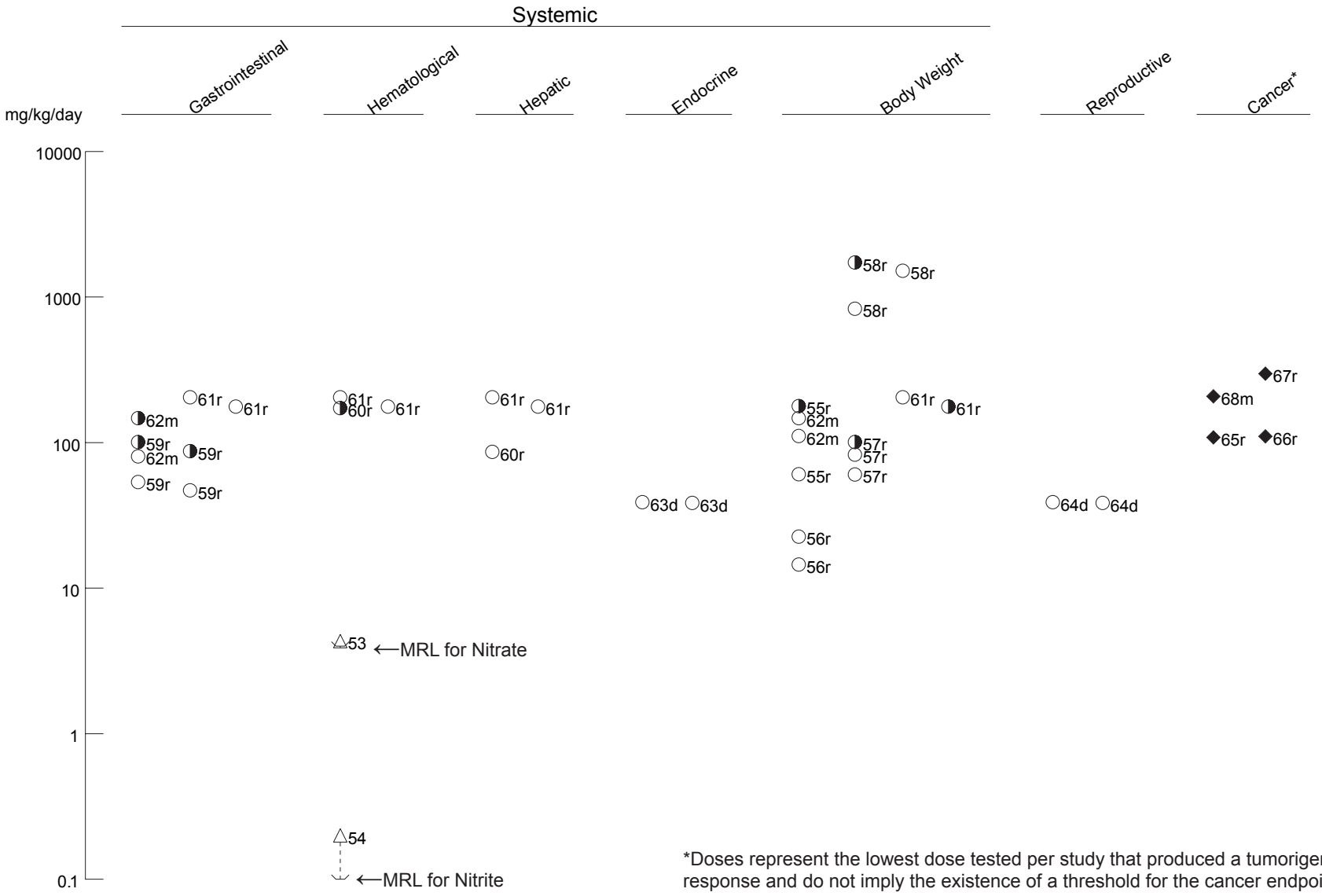
Intermediate (15-364 days)



3. HEALTH EFFECTS

Figure 3-1 Levels of Significant Exposure to Nitrate And Nitrite - Oral *(Continued)*

Chronic (≥365 days)



mean arterial blood pressure decreased from 80 to 69 mmHg. The maximum effects on heart rate and blood pressure occurred between 15 and 20 minutes following ingestion; heart rate and blood pressure returned to near-baseline levels approximately 2 hours following ingestion at the low dose, but the effects had not returned to baseline at 4 hours following ingestion at the high dose. The blood pressure-lowering effect of short-term dietary supplementation of inorganic nitrate appears to be beneficial; however, there is some uncertainty regarding potential health benefits of long-term nitrate supplementation to treat cardiovascular diseases (Maccha and Schecter 2012; Siervo et al. 2013).

Gastrointestinal Effects. Ingestion of nitrite (from potassium nitrite or sodium nitrite sources) has been associated with severe methemoglobinemia in adults and children; in many of these cases, symptoms included abdominal cramps and vomiting (CDC 1997, 2002; Gautami et al. 1995; Gowans 1990; Greenberg et al. 1945; Sevier and Berbatis 1976; Ten Brink et al. 1982). These cases were the result of consumption of food or drink that contained unusually high levels of nitrite via contamination, inadvertent use of sodium nitrite instead of table salt, or ingestion of a single sodium nitrite tablet (667 mg nitrite). In a study designed to evaluate the oral bioavailability of sodium nitrite in healthy volunteers (seven females and two males; mean age 22.9 years), one subject became nauseous and vomited within 20 minutes following ingestion of 0.12 mmol sodium nitrite per mmol hemoglobin (~4.8 mg sodium nitrite/kg, or 3.2 mg nitrite/kg); another subject reported nausea within 30 minutes following ingestion of 0.12 mmol sodium nitrite per mmol hemoglobin (~4.4 mg sodium nitrite/kg, or 2.9 mg nitrite/kg) (Kortboyer et al. 1997b).

In a population-based study, Nasseri-Moghaddam et al. (2011) evaluated the prevalence of acid regurgitation and/or heartburn in regions of Tehran categorized by nitrate levels in drinking water sources. The study authors reported a significantly increased prevalence of frequent (at least weekly) acid regurgitation among residents living in areas with drinking water nitrate concentrations >100 mg/L compared to those living in areas with drinking water nitrate concentrations <100 mg/L (OR 3.65; 95% CI 1.32, 10.09).

NTP (2001) observed epithelial hyperplasia in the forestomach of male and female B6C3F1 mice provided sodium nitrite in the drinking water for 14 weeks at a concentration (5,000 ppm) that resulted in estimated sodium nitrite doses of 990 and 1,230 mg/kg/day, respectively (663.3 and 824.1 mg nitrite/kg/day, respectively); NOAELs for these lesions in the males and females were 435.5 and 562.8 mg nitrite/kg/day, respectively. Similar results were noted for male and female F344/N rats and male B6C3F1 mice treated for 104–105 weeks at estimated doses of 87.1, 100.5, and 147.4 mg

nitrite/kg/day, respectively; NOAELs for these lesions in the male and female rats and male mice were 46.9, 53.6, and 80.4 mg nitrite/kg/day, respectively. Sodium nitrite treatment did not result in increased incidences of forestomach lesions in other groups of male F344 rats provided sodium nitrite in the drinking water at 2,000 mg/L (estimated dose of 208.4 mg nitrite/kg/day) for 35 weeks (Miyauchi et al. 2002) or 51 weeks (Kawabe et al. 1994).

Hematological Effects. As discussed in detail in Section 3.4 (Toxicokinetics) and Section 3.5 (Mechanisms of Action), some plasma nitrite, arising from reduction of ingested nitrate and via endogenous production, is involved in the oxidation of hemoglobin-Fe^{2+} (which transports oxygen) to hemoglobin-Fe^{3+} (methemoglobin, incapable of binding oxygen).

Methemoglobinemia is a condition in which increased methemoglobin as a percentage of total hemoglobin results in the expression of clinical signs that increase in severity with increasing percent methemoglobin (ATSDR 2013a; Bloom et al. 2013; Denshaw-Burke et al. 2013; Haymond et al. 2005). In normal healthy individuals, methemoglobin levels are <1% of total hemoglobin. Discoloration (e.g., pale, gray blue) of the skin is often observed at methemoglobin levels in the range of 3–15%; most patients tolerate methemoglobin levels <10%. Tachycardia, weakness, and other signs of tissue hypoxia may be observed at 10–20% methemoglobin levels. Effects on the central nervous system (e.g., headache, dizziness, fatigue) and dyspnea and nausea appear at >20% methemoglobin; the severity of symptoms increases with increasing methemoglobin level. High risk of mortality occurs at levels >70% methemoglobin).

As early as the mid-1900s, methemoglobinemia was reported in infants exposed to relatively large amounts of nitrate from drinking water sources (e.g., Bailey 1966; Bosch et al. 1950; Bucklin and Myint 1960; Chapin 1947; Comly 1987; Donahoe 1949; Faucett and Miller 1946; Ferrant 1946; McLetchie and Robertson 1949; Medovy 1948; Robertson and Riddell 1949; Stafford 1947; Walton 1951). Available data identify young bottle-fed infants (1–3 months of age) as a subpopulation that is particularly susceptible to nitrate-induced methemoglobinemia, especially those consuming formula prepared from drinking water sources containing nitrate in excess of 10 mg nitrate-nitrogen/L (44 mg nitrate/L) (e.g., Bosch et al. 1950; Walton 1951); EPA established a maximum contaminant level (MCL) of 10 mg/L for nitrate-nitrogen in drinking water (EPA 2009c). Subsequent reports provide additional evidence of associations between ingestion of nitrate from drinking water sources and elevated methemoglobin levels in infants (e.g., Craun et al. 1981; Fan and Steinberg 1996; Fan et al. 1987; Gruener and Toeplitz 1975; Gupta et al. 1999; Johnson et al. 1987; Jones et al. 1973; Miller 1971; Shuval

and Gruener 1972; Simon et al. 1964; Super et al. 1981; Winton et al. 1971; Zeman et al. 2002). Cyanosis and even death occurred in some of the reported cases. However, there is some evidence that methemoglobinemia in infants who drank formula prepared using drinking water with relatively high levels of nitrate may be related to bacterial contamination of such water sources and consequent gastrointestinal disorders, as well as endogenous overproduction of nitric oxide due to gastrointestinal infection and inflammation (Avery 1999; Gupta et al. 1998; Hegesh and Shiloah 1982; L'hirondel and L'hirondel 2002; Yano et al. 1982).

Walton (1951) reviewed available literature and found 278 reported cases of infant methemoglobinemia. Among those infants for whom data on nitrate levels in water sources used to prepare infant formula were available (n=214), levels >50 mg nitrate-nitrogen/L (220 mg nitrate/L) were associated with 173 cases (81%), levels of 21–50 mg/L (92–220 mg nitrate/L) were associated with 36 cases (17%), and levels of 11–20 mg nitrate-nitrogen (48–88 mg nitrate/L) were associated with 5 cases (2%). There were no cases among those infants consuming water containing <10 mg nitrate-nitrogen/L (<44 mg nitrate/L). Limitations include lack of information regarding the actual ages of the infants, total nitrate doses, and other water source contaminants (e.g., bacterial levels).

Bosch et al. (1950) evaluated 139 reported cases of cyanosis among infants in Minnesota (90% of which were <2 months of age; range 8 days to 5 months). Samples from 129 wells that served as water sources to the cases revealed nitrate-nitrogen concentrations >100 mg/L (>440 mg nitrate/L) in 49 wells, 50–100 mg/L (220–440 mg nitrate/L) in 53 wells, 21–50 mg/L (92–220 mg nitrate/L) in 25 wells, and 10–20 mg/L (44–88 mg nitrate/L) in the other 2 wells. A major limitation of this study was the detection of coliform organisms in 45 of 51 well water samples tested for bacterial contamination; bacteria in the water source might have been a causal factor for gastrointestinal tract disturbances in some of the infants and may have been at least partially responsible for increased susceptibility to nitrate-induced cyanosis (e.g., gastrointestinal tract disturbances could have influenced conversion of ingested nitrate to nitrite or absorption of nitrite).

Subsequent reports provide additional evidence of associations between ingestion of nitrate from drinking water sources and elevated methemoglobin levels in infants (e.g., Craun et al. 1981; Fan and Steinberg 1996; Fan et al. 1987; Gruener and Toeplitz 1975; Gupta et al. 1999; Johnson et al. 1987; Jones et al. 1973; Miller 1971; Shuval and Gruener 1972; Simon et al. 1964; Super et al. 1981; Winton et al. 1971; Zeman et al. 2002). Cyanosis and even death occurred in some of the reported cases.

Simon et al. (1964) evaluated methemoglobin levels from 89 healthy infants with a nitrate-free water source (group 1), 38 infants whose water source contained 50–100 mg nitrate/L (group 2), and 25 infants whose water source contained >100 mg nitrate/L (group 3). Nitrite levels in the water sources measured less than 0.3 mg/L (with the exception of a single measurement of 1 mg nitrite/L). For groups 1, 2, and 3, methemoglobin levels averaged 1.0, 1.3, and 2.9%, respectively, in the first postnatal trimester (0–3 months of age) and 0.8, 0.8, and 0.7 %, respectively, in the second trimester. Significantly increased methemoglobin was observed only in the highest exposure group (>100 mg nitrate/L) and only during the first trimester.

Super et al. (1981) evaluated associations between methemoglobin levels among infants 1–12 months of age (relatively evenly distributed by month) and estimates of nitrate intake (based on measured drinking water nitrate levels and considerations of liquid intake from other sources). When divided into two groups according to estimated nitrate intake (310 infants ingesting ≤2.93 mg nitrate/kg/day and 102 infants ingesting >2.93 mg nitrate/kg/day), mean methemoglobin levels were 1.54 and 3.03%, respectively. There were no striking age-related differences in frequency of infants with methemoglobin levels >3%.

A nested case-control study included 26 cases of infants diagnosed with methemoglobinemia at ≤2 months of age and 45 age-matched controls (Zeman et al. 2002). Nitrate exposure levels were categorized as low (<0.5 ppm), medium (1–10 ppm), or high (>10 ppm) according to estimated nitrate levels reconstructed from parental responses to dietary questionnaires and environmental sampling (1 ppm in the diet is equivalent to 1 mg/kg diet; 1 ppm in drinking water is equivalent to 1 mg/L). Numbers of methemoglobinemia cases in the low-, medium-, and high-exposure categories were 0/26, 4/26, and 22/26, respectively, and estimated dietary nitrate intake ranged from 2.83 to 451.20 mg/kg/day (mean 103.6 mg nitrate/kg/day); diarrheal disease was reported for 14/26 methemoglobinemia cases. Numbers of controls in the low-, medium-, and high-exposure categories were 21/45, 11/45, and 13/45, respectively, and estimated dietary nitrate intake ranged from 0 to 182 mg/kg/day (mean 11.2 mg nitrate/kg/day) for the controls; diarrheal disease was reported for 13/45 controls. Univariate and multifactorial analysis of risk factors for methemoglobinemia indicated that methemoglobinemia was most strongly associated with dietary exposure to nitrate/nitrite ($p=0.0318$), but also significantly associated with diarrheal disease ($p=0.0376$). Controls in the high-exposure category were less likely than high-exposure methemoglobinemia cases to have experienced severe diarrhea and were more likely to have been breastfed for >2 weeks. Major limitations to the study include the collection of information

contributing to the exposure estimates several years following the occurrences of methemoglobinemia and reliance on parental recollection of infant nutritional intake.

Sadeq et al. (2008) measured methemoglobin levels in children ranging in age from birth to 8 years of age who either lived in a region where nitrate levels in 78 tested wells ranged from 15.39 to 246.9 mg/L or a region supplied by municipal water with a mean nitrate level of 2.99 mg/L. The mean methemoglobin level (0.205 g/dL) among 100 children in the region supplied by well water was slightly higher than that of 37 children in the region supplied by municipal water (0.166 g/dL). The study authors stated that 0.24 g methemoglobin/dL is the equivalent of 2% methemoglobin, in which case mean methemoglobin among the children in the region supplied by well water was approximately 1.7% of total hemoglobin compared to a mean of 1.4% for the children in the region supplied by municipal water. The slight increases in mean methemoglobin among the children in the region supplied by well water were consistent within various age ranges (0–6, 7–11, 13–35, 36–71, and 72–95 months). The study authors stated that methemoglobin ≤0.24 g/dL (2%) was considered to be within normal limits.

Craun et al. (1981) evaluated methemoglobin levels in 102 children 1–8 years of age. Sixty-four of the children lived in households where drinking water contained 22–111 mg nitrate-nitrogen/L (97–488 mg nitrate/L); drinking water sources for the other 38 children (controls) contained <10 mg nitrate-nitrogen/L (<44 mg nitrate/L). Methemoglobin measured 1.0–1.36% in those children 1–4 years of age and appeared to increase with increasing nitrate intake, although there was no statistically significant change. Methemoglobin levels in those children 5–8 years of age averaged 0.9–0.95% independent of level of exposure to nitrate.

In one longitudinal study of 357 pregnant women in south-central Minnesota, there was no apparent association between estimated intake of nitrate from tap water and methemoglobin levels (Manassaram et al. 2010). However, only four of the women used tap water with nitrate-nitrogen content above the EPA (2009c) MCL of 10 mg/L.

Elevated methemoglobin levels and methemoglobinemia have been associated with consumption of foods high in nitrate (e.g., borage, carrots, kohlrabi, spinach) by infants and small children (Greer and Shannon 2005; Keating et al. 1973; Martinez et al. 2013; Sanchez-Echaniz et al. 2001). In the study of Sanchez-Echaniz et al. (2001), a homemade purée of mixed vegetables with high nitrate content was considered the source of elevated methemoglobin levels (10–58% of total hemoglobin) among seven infants 7–13 months of age.

Limited data are available regarding administration of controlled amounts of nitrate and methemoglobin levels. Cornblath and Hartmann (1948) administered sodium nitrate in the formula fed to four infants (ages 11 days to 11 months) for 2–18 days at a concentration resulting in a dose of 50 mg nitrate/kg/day. The highest observed level of methemoglobin was 5.3% of total hemoglobin; there was no evidence of cyanosis. Among four other infants (ages 2 days to 6 months) similarly treated at 100 mg nitrate/kg/day for 6–9 days, the only reported effect was that of 7.5% methemoglobin in a 10-day-old infant following 8 days of treatment in the absence of clinical cyanosis. Gruener and Toeplitz (1975) fed 104 infants (1 week to 10 months of age) for 1 day with formula prepared using water containing 15 mg nitrate/L (~0.8–1.5 mg nitrate/kg, based on age-specific values for water consumption [Kahn and Stralka 2009] and body weight [EPA 2008]), increased to 108 mg nitrate/L for the next 3 days (~5.5–10.6 mg nitrate/kg/day, based on age-specific values for water consumption [Kahn and Stralka 2009] and body weight [EPA 2008], and returned to 15 mg nitrate/L for one additional day. Mean methemoglobin levels were 0.89% after the first day of feeding, 1.3, 0.91, and 0.93% after days 2, 3, and 4, and dropped to 0.8% on the fifth day. Among three of these infants (ages not specified), methemoglobin levels reached 6.9, 13.9, and 15.9% during the high-dose days. Limitations of this study include the use of a wide range of ages and the fact that only 57 of the 104 infants supplied blood samples on all 5 treatment days.

Ingestion of nitrite (from potassium nitrite or sodium nitrite sources) has been associated with severe methemoglobinemia in adults and children (Aquanno et al. 1981; CDC 1997, 2002; Finan et al. 1998; Gautami et al. 1995; Gowans 1990; Greenberg et al. 1945; Kaplan et al. 1990; Ringling et al. 2003; Sevier and Berbatis 1976; Ten Brink et al. 1982; Walley and Flanagan 1987). These cases were the result of consumption of food or drink that contained unusually high levels of nitrite via contamination, inadvertent use of sodium nitrite instead of table salt, inadvertent use of sodium nitrite-contaminated sugar, or ingestion of a single sodium nitrite tablet (667 mg nitrite).

In a study designed to evaluate the oral bioavailability of sodium nitrite in healthy volunteers (seven females and two males; mean age 22.9 years), ingestion of 0.06 sodium nitrite per mmol hemoglobin (~2.2–2.7 mg sodium nitrite/kg, or 1.5–1.8 mg nitrite/kg) resulted in a mean maximum methemoglobin concentration of 0.309 mmol/L (range of 3.4–4.5% of total hemoglobin) at approximately 0.70 hours following ingestion, and a mean half-life of approximately 1.07 hours for methemoglobin reduction (Kortboyer et al. 1997b). At a higher intake (0.12 mmol sodium nitrite per mmol hemoglobin; ~4.4–5.4 mg sodium nitrite/kg, or 2.9–3.6 mg nitrite/kg), the mean maximum methemoglobin concentration

was 0.727 mmol/L (range of 7.7–10.9% of total hemoglobin) at approximately 1.14 hours following ingestion, and a mean half-life of approximately 1.13 hours for methemoglobin reduction.

Increased methemoglobin levels have been reported in rats administered sodium nitrite orally. Imaizumi et al. (1980) administered aqueous sodium nitrite to fasted Sprague-Dawley rats by gavage at 20, 25, 50, 100, or 150 mg/kg (6.7, 16.75, 33.5, 67, and 100.5 mg nitrite/kg, respectively) and observed methemoglobin levels of 4.3, 8.6, 40.3, 64.7, and 45–80%, respectively, at 1 hour posttreatment. The highest dose resulted in 50% mortality. Among surviving rats, methemoglobin levels returned to normal after 24 hours. Imaizumi et al. (1980) administered sodium nitrite in the drinking water of other rats for 6 months at 0.5% (5,000 mg sodium nitrite/L or 3,333 mg nitrite/L). Methemoglobin levels as high as 88% were observed during evening hours of treatment day 18 when the rats were likely drinking water and as low as 4% during morning and afternoon hours of the following day. The study authors did not provide information regarding clinical signs or mortality, but stated that there was no effect on growth.

In a 14-week study of male and female Fischer-344 rats administered sodium nitrite in the drinking water, clinical signs of cyanosis and brownish discoloration of mucous membranes and skin were noted at concentrations ≥1,500 ppm (≥130 mg/kg or 87.1 mg nitrite/kg) in the females and ≥3,000 ppm (≥225 mg/kg or 134 mg nitrite/kg) in the males (NTP 2001). The clinical signs were consistent with increased methemoglobin, which measured as high as 13, 24, and 50% in the 1,500, 3,000, and 5,000 ppm groups, respectively. Til et al. (1988) reported methemoglobin levels of 5.7 and 7.6% in male and female Wistar rats, respectively, administered potassium nitrite in the drinking water for 13 weeks at concentrations resulting in approximate doses of 107.6 and 130.5 mg nitrite/kg/day, respectively. Til et al. (1997) reported similar effects on methemoglobin in rats similarly exposed to either potassium nitrite or sodium nitrite; however, quantitative data were not included in the study report.

Behroozi et al. (1972) provided sodium nitrite in the drinking water of male albino rats for 2 months at concentrations resulting in sodium nitrite doses of 0, 14, 42, and 280 mg/kg/day (0, 9.38, 28.14, and 187.6 mg nitrite/kg/day, respectively). Methemoglobin in all groups was approximately 0.5% prior to the initiation of sodium nitrite treatment and remained at that level in the control group throughout the study. Methemoglobin in the low-, mid- and high-dose groups averaged 1.1, 3.0, and 12.16%, respectively, during the treatment period; following cessation of sodium nitrite exposure, methemoglobin levels in all sodium nitrite-treated groups decreased to 0.3–0.7%.

Chow et al. (1980) provided drinking water to male Sprague-Dawley rats for 16 weeks that contained 0 or 200 mg sodium nitrite/L (calculated dose of 18.6 mg nitrite/kg/day, based on EPA [1988] subchronic reference values for body weight and food consumption). Methemoglobin averaged 0.5–3.0% in the sodium nitrite-treated group and 0–1.2% in the controls.

Shuval and Gruener (1972) provided sodium nitrite in the drinking water to male rats for 24 months at 0, 100, 1,000, 2,000, or 3,000 mg/L (calculated doses of 0, 8.64, 86.4, 172.8, and 259.2 mg nitrite/kg/day, based on EPA [1988] chronic default reference values for body weight and food consumption). Methemoglobin levels in the three highest exposure groups averaged 5, 12, and 22% of total hemoglobin; there were no treatment-related effects on hemoglobin levels.

Maekawa et al. (1982) added sodium nitrite to the food of male and female F-344 rats for 6 weeks at concentrations ranging from 0.06 to 1% and sodium nitrate to the food of other rats at concentrations ranging from 1.25 to 20%. Discoloration in blood and spleen were noted in rats from the two highest exposure levels for sodium nitrite and sodium nitrate. These exposure levels were equivalent to doses ≥370 mg nitrite/kg/day and ≥7,300 mg nitrate/kg/day (based on EPA [1988] subchronic reference values for body weight and food consumption in male and female F-344 rats). The study report did not include information regarding methemoglobin levels.

Chow et al. (1980) provided drinking water to male Sprague-Dawley rats for 16 weeks that contained 0 or 400 mg sodium nitrate/L (calculated dose of 40.5 mg nitrate/kg/day, based on EPA [1988] subchronic reference values for body weight and food consumption). There were no treatment-related effects on mean methemoglobin levels.

Other hematological effects were noted in some animal studies that employed exposure to sodium nitrite or potassium nitrite in the drinking water for periods of 13–115 weeks. Imaizumi et al. (1980) reported decreased hemoglobin and irregularities in erythrocytes (irregular sizes and marked Heinz body formation) in rats receiving 167.5 mg nitrite/kg/day. Til et al. (1988, 1997) noted slightly decreased hemoglobin in male rats at ≥42 mg nitrite/kg/day, decreased packed cell volume and erythrocyte count at approximately 108 mg nitrite/kg/day, and decreases in erythrocyte count, mean corpuscular volume and mean corpuscular hemoglobin in female rats at 130 mg nitrite/kg/day. Initially decreased erythrocyte counts were noted in male rats at ≥60 mg nitrite/kg/day (as much as 44% lower than controls at 8 weeks of treatment, but returning to control levels by 52 weeks); significant decreases in mean corpuscular volume, and hemoglobin in these rats were noted throughout the 115-week treatment period (Grant and

Butler 1989). Significantly increased spleen weights were noted in male mice receiving sodium nitrite for 14 weeks at ≥ 435 mg nitrite/kg/day (39% greater than that of controls) and in male and female mice at 663 or 824 mg nitrite/kg/day (approximately 66% greater than their controls). The study authors suggested that the increased spleen weights may have represented increased erythropoietic activity in response to increased methemoglobin; however, methemoglobin data were not included in the study report.

Hepatic Effects. No information was located regarding hepatic effects in humans following oral exposure to nitrate or nitrite.

No indications of sodium nitrite-induced liver effects were observed in animal studies that included assessment of liver function and/or histopathology (Asahina et al. 1971; Lijinsky and Greenblatt 1972; Lin and Ho 1992; Shuval and Gruener 1972; van Logten et al. 1972).

Renal Effects. No information was located regarding renal effects in humans following oral exposure to nitrate or nitrite.

El-Wakf et al. (2008) reported significantly increased urinary levels of urea and creatinine in male rats provided sodium nitrate in the drinking water for 4 months at author-estimated doses of 21.7 and 47.4 mg sodium nitrate/kg/day (15.8 and 34.6 mg nitrate/kg/day, respectively).

Endocrine Effects. Nitrate acts as a dose-dependent competitive inhibitor of the sodium iodide symporter (NIS) that mediates the uptake of iodine by the thyroid. Sufficiently decreased iodine uptake by the thyroid may result in decreased production of thyroid hormones T3 and T4. Decreased thyroid hormone production causes increased release of TSH from the anterior pituitary gland and consequent increased uptake of iodine by the thyroid gland. Sufficiently inhibited uptake of iodine by the thyroid could result in effects associated with thyroid dysfunction (e.g., hypothyroidism). Concern for nitrate-induced effects on thyroid function has prompted scientists to perform studies designed to assess thyroid function relative to drinking water and/or dietary nitrate levels. Available human data provide suggestive evidence that elevated levels of nitrate in drinking water and/or nitrate-rich diets may be associated with signs of thyroid dysfunction. However, limitations of these studies include lack of individual dose-response data, quantification of iodine intake, and control for other potential substances that may affect the thyroid; one study relied on self-reported thyroid status and self-reported dietary nitrate intake.

Tajtáková and coworkers evaluated thyroid function among schoolchildren (boys and girls 10 or 13 years of age) from three areas in Slovakia; an agricultural area with drinking water sources containing nitrate at 51–274 mg/L (n=324), an area from a neighboring area where drinking water sources contained <2 mg nitrate/L (n=168), and the city of Košice supplied with drinking water reported to be low in nitrate (n=596) (Rádiková et al. 2008; Tajtáková et al. 2006). At the time of the study, measurements of urinary iodine indicated that the children in the high- and low-nitrate areas were ingesting sufficient iodine. Thyroid volume and density were estimated with the assistance of ultrasound equipment. Mean thyroid volume was significantly higher in the 10- and 13-year-old children from the high-nitrate area (5.10±0.14 mL for the 10-year-olds and 5.97±0.11 mL for the 13-year-olds) compared to that of the children from the low-nitrate area (4.58±0.17 and 5.23±0.15 mL, respectively) and from the city of Košice (4.77±0.10 and 4.87±0.10 mL, respectively). The frequency of hypoechogenicity (ultrasound indicator of decreased thyroid density typically indicating destruction of normal thyroid tissue) was significantly greater in children from the high-nitrate area compared to those from the low-nitrate areas (13.7 versus 4.7% for the 10-year-olds and 10.6 versus 5.7% for the 13-year-olds). Blood samples revealed TSH in the range of subclinical hypothyroidism in 13/324 children and positive anti-thyroperoxidase antibodies (an indicator of subclinical thyroid disorder) in 8/324 of the children from the high-nitrate area versus no cases in 109 children from the low-nitrate area. There were no significant differences between children from the low- and high-nitrate areas regarding serum T3 or T4 levels.

Iodine status and goiter prevalence were evaluated in 156 schoolchildren (7–14 years of age) in an area of rural Bulgaria where nitrate in the drinking water averaged 75 mg/L and 163 schoolchildren in a nearby area drinking water nitrate averaged 8 mg/L at the time of the study (Gatseva and Argirova 2008). At the time of the study, perchlorate was below the detection limit (1 µg/mL). Urinary iodine measurements indicated that iodine intake was satisfactory for most children from each group. The goiter rate within the high-nitrate areas was significantly higher than the goiter rate within the low-nitrate area (13.5 versus 5.9%). Familial thyroid disorders and chronic diseases were reported by families of 7.7% of the children from the high-nitrate area and only 3.06% of the children from the low-nitrate area. In a similar study that included two areas of Bulgaria, one with high nitrate in the drinking water (average of 93 mg/L) and one with low nitrate in the drinking water (average 8 mg/L), pregnant women from the high- (n=26) and low- (n=22) nitrate areas and children (3–6 years of age) from the high- (n=50) and low- (n=49) areas were evaluated for iodine status and goiter frequency. Mean urinary iodine in the women from the high-nitrate area was significantly lower than that of the women from the low-nitrate area (147.85±56.38 versus 230.55±61.56 µg/L). Iodine deficiency was indicated for 5/26 women and 11/50 of the children from the high-nitrate area and 1/22 women and 5/49 children from the low-nitrate area. Goiter was

reported for 9/26 women and 14/50 children from the high-nitrate area and 2/22 women and 7/49 children from the low-nitrate area. Familial thyroid disorders and chronic diseases were reported for 9/26 women and 3/50 children from the high-nitrate area and 2/22 women and 1/49 children from the low-nitrate area. The differences in goiter rates may be the result of differences in iodine intake and reported familial thyroid disorder and chronic disease prevalence.

Aschebrook-Kilfoy et al. (2012) reported an association between nitrate in private wells at estimated levels >6.5 mg nitrate-nitrogen /L (>28.6 mg nitrate/L) and elevated serum TSH in women (but not men) as an indicator of subclinical hypothyroidism (OR 1.60, 95% CI: 1.11, 2.32). The study included 2,543 Old Order Amish residing in several counties in Pennsylvania for whom TSH levels were available. Nitrate levels in the wells were estimated by modeling data provided by the U.S. Geological Survey (USGS) that monitored nitrate levels in 3,613 wells in the study area.

In one cohort of 21,977 older women in Iowa who had used the same water supply for >10 years, there were no significant differences in prevalence of self-reported hypothyroidism or hyperthyroidism between those using private wells as drinking water source (n=5,436) and those using public water sources (n=16,541) (Ward et al. 2010). Sufficient data for public water sources were available from which to evaluate prevalence of thyroid disorders by quartile of nitrate concentration in public water sources defined as mean concentrations <0.36, 0.36–1.00, 1.01–2.46, and >2.46 mg nitrate-nitrogen/L (<1.58, 1.58–4.4, 4.41–10.82, and >10.82 mg nitrate/L, respectively). There was no apparent association between nitrate in the drinking water and prevalence of self-reported hypothyroidism or hyperthyroidism when comparing results by quartile. No nitrate measurement data were available for women using private wells. Data for these women were compared to data for women in the lowest quartile of public water sources, although it was estimated at the time of the study that 18% of the rural private wells in Iowa had nitrate levels >10 mg nitrate-nitrogen/L (>44 mg nitrate/L). In the same study (Ward et al. 2010), dietary nitrate intake was estimated using a food frequency questionnaire and published nitrate levels for various food sources and the study subjects (3,018 cases of hypothyroidism and 937 cases of hyperthyroidism) were divided into quartiles according to dietary nitrate intake (≤17.4, 17.5–27.7, 27.8–41.1, and >41.1 mg nitrate-nitrogen/day; approximately equivalent to <77, 77–121.9, 122–181, and >181 mg nitrate mg/day, respectively). Using the lowest quartile as a referent, associations were found for prevalence of hypothyroidism (but not hyperthyroidism) for the second quartile (OR 1.13, 95% CI: 1.01, 1.27), third quartile (OR 1.19, 95% CI: 1.06, 1.33), and fourth quartile (OR 1.24, 95% CI: 1.10, 1.40). A significant trend was noted as well for increasing prevalence of hypothyroidism with increasing quartile of dietary nitrate (p=0.001).

In a randomized controlled study, 10 volunteers consumed sodium nitrate in aqueous solution at a dose of 15 mg/kg/day for 28 days; 10 other volunteers receiving distilled water served as controls. There were no sodium nitrate treatment-related effects on thyroidal ^{131}iodine uptake or plasma thyroid hormone concentrations (Hunault et al. 2007).

Thyroid status has been assessed to some extent in animals consuming drinking water or food to which nitrate salts had been added. There were no clinical signs of hypothyroidism or effects on serum T3 or T4 levels in adult Beagles or their puppies during exposure of the breeding dogs to sodium nitrate in the drinking water for 1 year at concentrations in the range of 300–1,000 ppm (equivalent to 219–730 mg nitrate/L) (Kelley et al. 1974). Decreased thyroidal ^{131}iodine uptake was noted in rats given food containing 0.5–2.5% potassium nitrate (equivalent to 3,000–15,000 mg nitrate/kg food) (Bloomfield et al. 1961). Significantly increased uptake of thyroidal ^{131}iodine; decreased serum T3, T4, and TSH levels; increased thyroid weight; and follicular hyperplasia were noted in female Wistar rats administered sodium nitrate in the drinking water for 30 weeks at concentrations ≥250 mg/L (≥159 mg nitrate/kg/day, based on reported average water intake and EPA [1988] subchronic reference body weight of 0.156 kg for the female Wistar rat) (Eskiocak et al. 2005). In another study (Zaki et al. 2004), significantly decreased serum T3 (34–44% lower than controls), increased thyroid weight (45–77% greater than controls), and histopathologic thyroid lesions (glandular hypertrophy accompanied by vacuolization, increased colloidal volume of the follicles, and flattened follicular epithelium) were observed in male Wistar rats receiving drinking water for 5 months to which potassium nitrate had been added at concentrations resulting in estimated doses ≥13.5 mg nitrate/kg/day (based on EPA [1988] subchronic reference values for body weight and water consumption for the male Wistar rat).

El-Wakf et al. (2008) reported significantly decreased serum T3 and T4 levels (17–41% lower than controls) in all groups of weanling male Wistar rats provided sodium nitrate in the drinking water for 4 months at concentrations resulting in author-estimated intakes in the range of 8.7–47.4 mg sodium nitrate/kg/day (equivalent to 6.4–34.6 mg nitrate/kg/day). At estimated doses ≥15.8 mg nitrate/kg/day, significantly increased serum TSH was also noted (26–30% higher than that of controls). Groups of similarly-treated young adult male Wistar rats exhibited significantly decreased T3 and T4 levels (24–47% lower than controls) and increased serum TSH (30–35% higher than controls) at estimated doses ≥15.8 mg nitrate/kg/day.

In a 28-day study of rats receiving food to which potassium nitrate had been added to constitute 3% of the diet, thyroid effects included significantly increased thyroid gland weight (45% greater than controls), increased TSH (nearly 7-fold higher than that of controls), decreased serum T3 and T4 levels (61–63% lower than controls), and decreased thyroid peroxidase activity (84% lower than controls) (Mukhopadhyay et al. 2005). Based on reported body weight data and the EPA (1988) allometric equation for calculating a food consumption rate for laboratory mammals (0.056 x body weight$^{0.6611}$), an estimated dose was 2,416 mg nitrate/kg/day.

Til et al. (1988) added potassium nitrite to the drinking water of male and female rats for 13 weeks at concentrations resulting in estimated doses in the range of 8.9–199.2 mg/kg/day (4.8–108 mg nitrite/kg/day) to the males and 10.9–241.7 mg/kg/day (5.9–130.5 mg nitrite/kg/day) to the females. Doses ≥13.3 mg nitrite/kg/day (males) and ≥61.8 mg nitrite/kg/day (females) resulted in hypertrophy in the zona glomerulosa of the adrenal gland. In this study, potassium was added to the drinking water of each treatment group up to the level of potassium in the drinking water of the highest dose group. Controls included groups with untreated drinking water and groups with potassium chloride-treated water. The effect on the adrenal gland was not observed in the untreated controls or the potassium chloride controls, indicating that the effect was the result of nitrite ion. Similar results were obtained at estimated doses of 105.1 mg nitrite/kg/day (males) and 130.1 mg nitrite/kg/day (females) in a subsequent similarly-designed study (Til et al. 1997) to evaluate effects at lower doses than those employed in the earlier study (Til et al. 1988). Results of a subsequent study indicate that the effect on the adrenal gland of the rat is a physiological adaptation to repeated episodes of hypotension caused by nitrite (RIVM 1996).

Dermal Effects. Available information regarding dermal effects following oral exposure to nitrate or nitrite is limited to a case report in which ingestion of ammonium nitrate was considered a possible cause of erythema dyschromicum perstans (ashy dermatosis) (Jablonska 1975).

Body Weight Effects. No information was located regarding body weight effects in humans following ingestion of nitrate or nitrite.

No body weight effects were observed in some studies of laboratory animals provided sodium nitrate, sodium nitrite, or potassium nitrite in the drinking water for intermediate exposure durations (4 weeks to 10 months) at concentrations resulting in estimated doses in the range of 1,583–7,300 mg nitrate/kg/day (Maekawa et al. 1982; Mukhopadhyay et al. 2005) or 28–435.5 mg nitrite/kg/day (Greenblatt and Lijinsky 1974; Greenblatt and Mirvish 1973; Greenblatt et al. 1971; Lin and Ho 1992; Maekawa et al.

1982; NTP 2001; Vorhees et al. 1984). Depressed body weight and/or body weight gain (approximately 10% less that of controls) were observed in other studies at estimated doses of 8,241.9–14,600 mg nitrate/kg/day (Maekawa et al. 1982) and 663.3–1,080.6 mg nitrite/kg/day (Maekawa et al. 1982; NTP 2001). In chronic-duration studies (≥365 days), doses in the range of 101–178.2 mg nitrite/kg/day and 1,730 mg nitrate/kg/day resulted in 10–15% depressed body weight in rats and mice (Grant and Butler 1989; Maekawa et al. 1982; van Logten et al. 1972).

Body weight data in the study report of Zaki et al. (2004) indicate as much as 16–25% depressed mean body weight among male Wistar rats provided drinking water for 5 months that contained 150 or 500 mg potassium nitrate/L (estimated doses of 13.5 and 45 mg nitrate/kg/day); however, data regarding food and water consumption were not included in the study report. El-Wakf et al. (2008) provided drinking water to weanling male Wistar rats for 4 months that contained 100, 250, or 500 mg sodium nitrate/L (estimated doses of 6.4, 15.8, and 34.6 mg nitrate/kg/day) and reported mean final body weights that were 11, 29, and 46%, respectively, less than that of control; however, data regarding food and water consumption were not included in the study report. El-Wakf et al. (2015) provided young (3-week-old) and adult (12-week-old) male Wistar rats with drinking water to which sodium nitrate was added at 550 mg/L (estimated daily intake of 47.7 mg sodium nitrate/kg/day or 34.8 mg nitrate/kg/day) for 4 months; controls received drinking water without added sodium nitrate. The sodium nitrate treatment resulted in depressed body weight (24 and 9% less among the young and adult rats, respectively, compared to controls) and depressed body weight gain (39 and 30% less among the young and adult rats, respectively, compared to controls).

Metabolic Effects. Possible associations between nitrate and/or nitrite in drinking water and/or food sources and risk of type 1 diabetes have been investigated in a number of epidemiological studies (Casu et al. 2000; Dahlquist et al. 1990; Kostraba et al. 1992; Moltchanova et al. 2004; Parslow et al. 1997; van Maanen et al. 2000; Zhao et al. 2001). Statistically significant associations between estimated nitrate and/or nitrite intake were reported by some investigators, but were not observed by others. Limitations of studies include the lack of quantitative dose-response data and the likelihood of confounding by other potential toxicants. Therefore, there is considerable uncertainty regarding nitrate or nitrite intake and risk of type 1 childhood diabetes.

A study in the Netherlands involved 1,064 cases of type 1 diabetes in a total of 2,829,020 children (0–14 years of age) included in the analysis (van Maanen et al. 2000). Nitrate levels in drinking water were determined by postal code. Two exposure categories were used. One category was based on equal

numbers of children exposed to various levels of nitrate in the drinking water (0.25–2.08, 2.10–6.42, and 6.44–41.19 mg nitrate/L); the other category was based on cutoff values of 10 and 25 mg nitrate/L. The study authors concluded that there was little evidence that nitrate in the drinking water was a risk factor for childhood type 1 diabetes under the conditions of the study.

Zhao et al. (2001) found no significant association between nitrate in the drinking water and risk for childhood type 1 diabetes in a study of 517 cases (0–15 years of age). The mean concentration of nitrate in the drinking water was 6.62 mg/L (range 0.49–31.9 mg/L). Casu et al. (2000) found no significant association between nitrate in tap water or bottled water and risk of type 1 diabetes among 1,975 cases (0–29 years of age), 1,142 of which were <15 years of age. In this study, nitrate concentrations in tap and bottled water were below the acceptable maximal concentration of 50 mg/L established by the European Community and the recommended level of 25 mg/L. Moltchanova et al. (2004) found no significant association between childhood type 1 diabetes and nitrate in the groundwater in Finland. The study included 3,598 cases of childhood type 1 diabetes (ages 0–14 years) and 9,601,164 children at risk; drinking water nitrate levels averaged 6.228 mg/L.

Dahlquist et al. (1990) evaluated a variety of nutrients and food additives (including nitrate) as possible risk factors for type 1 diabetes among 339 children under 15 years of age and matched with 528 control children in Sweden. Estimates of intake of the various nutrients and food additives were made based on parental responses to food frequency questionnaires. Upon dividing the subjects into three groups according to estimated nitrate intake (low=<25th percentile; medium=25–75th percentile; high=>75th percentile), a significant nonlinear trend for increased risk of type 1 diabetes with increasing nitrate intake was noted. The high-nitrate intake group exhibited a significantly increased risk (crude OR 2.14, 95% CI: 1.64, 3.54) compared to the low-nitrate intake group; adjustment for age, sex, maternal age, maternal education, and family history of type 1 diabetes did not significantly alter the results.

Kostraba et al. (1992) calculated incidence rates by county in Colorado (63 counties) for type 1 diabetes in children (<18 years of age at diagnosis during the years 1978 and 1988; n=1,280) and compared the rates to nitrate levels in potable water supplies. Children in counties with water nitrate levels in the range of 0.77–8.2 mg/L had a significantly increased risk of type1 diabetes compared to those in counties with water nitrate levels in the range of 0.0–0.084 mg/L.

Parslow et al. (1997) reported a significant increase association (SIR 115, 95% CI: 107,124) between nitrate in drinking water (highest tertile versus lowest tertile) and incidence of childhood type 1 diabetes

diagnosed between 1978 and 1994 in the Yorkshire Regional Health Authority in England. The study subjects were 0–16 years of age, and nitrate levels in the drinking water were divided into tertiles (1.48–<3.22, 3.22–<14.85, 14.85–40.01 mg/L). The study included 498 cases in a population of 225,708 children in the lowest tertile, 591 cases in a population of 232,373 children in the middle tertile, and 708 cases in a population of 237,951 children in the highest tertile.

Virtanen et al. (1994) reported a significant association between estimated dietary nitrite intake by children and mothers and risk for type 1 diabetes in all age groups of boys and girls (ages 0–4, 5–9, and 10–14 years). The study included 684 children with Type 1 diabetes, 595 control children, 548 case-control pairs of fathers, and 620 case-control pairs of mothers in a nationwide Finnish study. Nitrate and nitrite levels were estimated based on results from food frequency questionnaires and household water data provided by the Finnish waterworks. Nitrate intake of the mother was associated with decreased risk for childhood type 1 diabetes.

El-Wakf et al. (2015) provided young (3-week-old) and adult (12-week-old) male Wistar rats with drinking water to which sodium nitrate was added at 550 mg/L (estimated daily intake of 47.7 mg sodium nitrate/kg/day or 34.8 mg nitrate/kg/day) for 4 months; controls received drinking water without added sodium nitrate. The sodium nitrate treatment induced hyperglycemia in both age groups. In a study of Sprague-Dawley rats administered sodium nitrite by gavage at 80 mg/kg/day for 12 weeks, nitrite-induced effects included inhibition of liver glycogenesis (generation of glycogen from glucose molecules) and enhanced liver glycogenolysis (breakdown of glycogen) and gluconeogenesis (generation of glucose from non-carbohydrate carbon substrates), accompanied by hyperglycemia and insulin resistance (Al-Gayyar et al. 2015).

3.2.2.3 Immunological and Lymphoreticular Effects

No information was located regarding immunological or lymphoreticular effects in humans or animals following oral exposure to nitrate or nitrite.

3.2.2.4 Neurological Effects

Ingestion of nitrite (from potassium nitrite or sodium nitrite sources) has been associated with severe methemoglobinemia in adults and children; in many of these cases, clinical signs included dizziness, loss of consciousness, and/or convulsions (CDC 1997, 2002; Gautami et al. 1995; Greenberg et al. 1945; Sevier and Berbatis 1976; Ten Brink et al. 1982). These cases were the result of consumption of food or

drink that contained unusually high levels of nitrite via contamination, inadvertent use of sodium nitrite instead of table salt, or ingestion of a single sodium nitrite tablet (667 mg nitrite).

Headache was induced in a male subject following consumption of a 10 mg sodium nitrite solution (Henderson and Raskin 1972). Headaches were induced in 8 out of 13 such tests. The tests were performed to evaluate whether nitrite in frankfurters that the subject had previously ingested might be cause for the headache he had developed shortly thereafter. In a study designed to evaluate the oral bioavailability of sodium nitrite in healthy volunteers (seven females and two males; mean age 22.9 years), headache was reported by four of the nine people ingesting 0.12 mmol sodium nitrite per mmol hemoglobin (~4.4–5.4 mg sodium nitrite/kg, or 2.9–3.6 mg nitrite/kg) and by four of the nine subjects ingesting 0.06 mmol sodium nitrite per mmol hemoglobin (~2.2–2.7 mg sodium nitrite/kg, or 1.5–1.8 mg nitrite/kg) (Kortboyer et al. 1997b).

Abnormalities in electroencephalograms (EEGs) were reported in male albino rats provided sodium nitrite in the drinking water for 2 months at concentrations resulting in author-reported doses ≥14 mg sodium nitrite (≥9.38 mg nitrite/kg/day) (Behroozi et al. 1972). The abnormal readings persisted during up to 4.5 months following cessation of exposure to sodium nitrite. At the highest dose (187.6 mg nitrite/kg/day), rats exhibited clinical signs of sedation and became motionless during periods of electrical outbursts.

Gruener (1974) reported increased aggressive behavior in male C57B1 mice provided sodium nitrite in the drinking water at 1,000 mg/L (estimated dose of 165.4 mg nitrite/kg/day) for up to 13 weeks postweaning. The mice had also been exposed via their parents during mating and their mothers during gestation and lactation. Shuval and Gruener (1972) reported significantly reduced motor activity in male mice provided sodium nitrite in the drinking water. Sodium nitrite levels tested ranged from 100 to 2,000 mg/L; however, the study report did not include specific information regarding the exposure levels that resulted in reduced motor activity.

3.2.2.5 Reproductive Effects

See Section 3.2.2.6 for information regarding results of case-control studies that evaluated reproductive/ developmental end points.

Several animal studies included evaluation of selected reproductive end points. Among three female guinea pigs provided potassium nitrate in the drinking water for up to 204 days of cohabitation at a concentration resulting in estimated intake of 4,972 mg nitrate/kg/day, one female died and the other two females produced a total of two litters (one live birth per litter) (Sleight and Atallah 1968). During 191 days of cohabitation, four control females produced eight litters and a total of 31 live births. There was no gross or histopathologic evidence of treatment-related effects on reproductive organs. Sleight and Atallah (1968) provided other guinea pigs with drinking water that contained potassium nitrite at concentrations ranging from 300 to 10,000 ppm. Exposure levels ≥1,000 ppm potassium nitrite (estimated doses ≥148.5 mg nitrite/kg/day) resulted in decreases in number of litters and live births; histopathologic evaluations of reproductive organs revealed placental, uterine, and cervical lesions.

No treatment-related effects on implantations or resorptions were seen in female Wistar rats provided sodium nitrite in the food throughout the production of two litters at concentrations resulting in estimated doses as high as 160 mg nitrite/kg/day (Hugot et al. 1980). No treatment-related effects on fertility were seen in breeding dogs provided sodium nitrate in the drinking water for 1 year at concentrations resulting in doses as high as 39 mg nitrate/kg/day (Kelley et al. 1974).

Alavantić et al. (1988a) treated male mice with sodium nitrate or sodium nitrite by gavage for 3 days at doses of 0, 600, or 1,200 mg/kg/day (sodium nitrate) or 0, 60, or 120 mg/kg/day (sodium nitrite); sperm-head abnormalities were evaluated at 11 and 17 days following treatment. Frequencies of sperm-head abnormalities in the low- and high-dose sodium nitrate-treated and the low-dose sodium nitrite-treated groups were not significantly different from controls. However, the high-dose group of sodium nitrite-treated mice exhibited significantly increased frequency of sperm-head abnormalities at 11 and 17 days following treatment (approximately 1.5-fold greater than controls). Alavantić et al. (1988b) treated male mice with sodium nitrate or sodium nitrite by gavage for 2 weeks at doses of 0, 600, or 1,200 mg/kg/day (sodium nitrate) or 0, 60, or 120 mg/kg/day (sodium nitrite) and subsequently mated them to virgin females. Evaluation of primary spermatocytes from parental males revealed significantly increased frequency of sperm-head abnormalities in the high-dose sodium nitrate-treated group (1.4-fold greater than controls) and the low- and high-dose sodium nitrite-treated groups (1.2- and 1.4-fold greater, respectively, than controls). There was no treatment-related effect on frequency of sperm-head abnormalities in F1 males. Fertility in the high-dose sodium nitrite-treated group was significantly affected; only 31 of 49 females mated to the high-dose males became pregnant compared to 45 of 50 females mated to control males.

Alyoussef and Al-Gayyar (2016a, 2016b) administered sodium nitrite to male Sprague-Dawley rats by gavage at 0 or 80 mg/kg/day for 12 weeks. Sodium nitrite treatment resulted in increased testicular weight (1.6–1.7-fold greater than controls); decreased serum testosterone levels (36–44% less than controls); decreased epididymal sperm count (48% less than controls); decreased testicular anti-inflammatory cytokine levels; increased serum luteinizing hormone (LH), follicle stimulating hormone (FSH), and prolactin levels; and increased testicular levels of pro-inflammatory cytokines, oxidative stress markers, and enzymes involved in programmed cell death.

NTP (2001) reported degeneration of the testis (characterized by increased size of residual bodies within the lumen of the seminiferous tubules) in male mice provided sodium nitrite in the drinking water for 14 weeks at concentrations resulting in estimated doses ≥435.5 mg nitrite/kg/day; the biological significance of this lesion was uncertain. In similarly-treated female mice, estrous cycles were significantly increased (11 and 15%, respectively, longer than controls) at estimated doses of 298.1 and 824.1 mg nitrite/kg/day, but not at 562.8 mg nitrite/kg/day. Among similarly-treated male and female rats, the males exhibited 7–18% decreased sperm motility at doses ≥134 mg nitrite/kg/day; there were no treatment-related effects on vaginal cytology end points in the females at doses as high as 231 mg nitrite/kg/day.

3.2.2.6 Developmental Effects

Several population-based, case-control studies evaluated possible associations between developmental end points and exposure to nitrate from drinking water sources. The results are not adequate for quantitative risk assessment because estimations of nitrate intakes were typically based on measurements of nitrate levels in drinking water sources at selected time points and self-reported estimates of water consumption, possible confounding by other potential toxicants was not evaluated, and most studies did not account for dietary nitrate or nitrite intake which is typically the major source of ingested nitrate and nitrite. Statistically significant associations between nitrate in the drinking water and selected developmental end points (e.g., birth defects, spontaneous abortions) were reported by some investigators, but were not observed by others.

Brender et al. (2013) evaluated possible relationships between prenatal exposure to nitrate in drinking water and selected birth defects in a large population-based, case-control study that included 3,300 case mothers and 1,121 control mothers who were participants in the National Birth Defects Prevention Study. Nitrate levels were measured in public water supplies and in representative samples of bottled water sold

in local stores; daily nitrate consumption was estimated from self-reported water consumption at home and work. The lowest tertile of nitrate intake from water (<0.91 mg/day at conception or <1.0 mg/day during preconception and the first trimester of pregnancy) represented the referent group. Within the highest tertile (≥5.0 mg/day at conception; ≥5.42 mg/day during preconception and the first trimester of pregnancy), significant associations were noted for risk of spina bifida (OR 2.02, 95% CI: 1.01, 2.04), any limb deficiency (OR 1.79, 95% CI: 1.05, 3.08), any oral cleft defect (OR 1.45, 95% CI: 1.10, 1.92), cleft lip without cleft palate (OR 1.82, 95% CI: 1.08, 3.07), cleft palate (OR 1.90, 95% CI: 1.17, 3.09), and any neural tube defect (OR 1.43, 95% CI: 1.01, 2.04). Cases in the various tertiles ranged in number from 23 to 173. The study authors noted that higher estimated nitrate intakes from drinking water did not increase associations between reported maternal intake of nitrosatable drugs and birth defects.

Dorsch et al. (1984) evaluated 218 cases of congenital malformations and matched controls between 1951 and 1979 in an area of South Australia. In an analysis of data by estimated level of nitrate in the drinking water, the risk of malformations was significantly greater at nitrate levels of 5–15 mg/L (OR 2.6, 95% CI: 1.6, 4.1; 138 cases, 106 controls) and >15 mg/L (OR 4.1, 95% CI: 1.3, 13.1; 10 cases, 5 controls) compared to those with nitrate levels <5 mg/L (70 cases, 107 controls).

Scragg et al. (1982) evaluated possible associations between maternal water source and frequency of congenital malformations (mainly neural tube defects) in a locality in South Australia (258 cases and matched controls). A referent group consisted of those women who used rainwater as the drinking water source. Significantly increased risk of occurrence of a malformation was noted for those women who drank water from a lake source (RR 2.8, 95% CL: 1.6, 5.1) and for women who used water from private wells with nitrate levels typically >15 ppm (RR 4.1, 95% CL: 1.7, 10.0).

Cedergren et al. (2002) reported nonstatistically significant increased risk of cardiac defects among infants of mothers exposed to nitrate in the drinking water at levels ≥2 mg/L (OR 1.18, 95% CI: 0.97, 1.44; 392 cases, 27,962 controls) compared to those with nitrate levels <2 mg/L; all measured nitrate concentrations were below the Swedish maximum contaminant level. The study population included 75,832 infants born in a Swedish county between January 1982 and December 1996.

Croen et al. (2001) evaluated 538 cases of neural tube defects and 539 normal controls in an area of California between June 1989 and May 1991. Exposure to nitrate in drinking water at concentrations >45 mg/L was associated with statistically significantly increased risk of anencephaly (OR 4.0, 95% CI:

1.0, 15.4), but no increased risk for spina bifida. Increased risk was also noted at nitrate levels <45 mg/L among groundwater drinkers.

Arbuckle et al. (1988) evaluated mothers in the area of New Brunswick where private wells averaged 26 mg/L nitrate and public municipal sources averaged 0.1 mg/L nitrate. There was no statistically significant increased risk for delivering a central nervous system-malformed infant by mothers using private wells (OR 2.30, 95% CI: 0.73, 7.29). The study included 130 cases of central nervous system birth defects for the years 1973–1983, each matched to 2 controls.

Aschengrau et al. (1993) found no statistically significant association between drinking water nitrate levels (up to 4.5 mg/L) or nitrite levels (up to 0.15 mg/L) and frequency of congenital anomalies, stillbirth, or neonatal death among 1,171 cases and 1,177 controls who delivered at a Massachusetts hospital between August 1977 and March 1980.

Holtby et al. (2014) evaluated possible associations between nitrate in drinking water sources and incidence of congenital anomalies in the agricultural region of Kings County, Nova Scotia, Canada between 1988 and 2006. A mean level of 6.44 mg nitrate-nitrogen/L was calculated for rural wells (equivalent to 28.34 mg nitrate/L), based on 1,113 water samples from 140 wells. A mean level of 2.03 mg nitrate-nitrogen/L was calculated for municipal water supplies (equivalent to 8.93 mg nitrate/L), based on 53 water samples from 20 water sources (19 groundwater sources and 1 surface water source). Nitrate-nitrogen concentration estimates were divided into tertiles (<1, 1–5.56, and >5.56 mg nitrate-nitrogen/L; equivalent to <4.4, 4.4–24.46, and >24.46 mg nitrate/L). Overall, no significant association was found between nitrate levels in drinking water sources and incidences of congenital malformations. However, stratification of the data by conception before or after the onset of food fortification with folate in Canada (instituted in 1998) resulted in an OR of 2.44 (95% CI 1.05, 5.66) for risk of congenital anomalies with exposure of 1–5.56 mg nitrate-nitrogen/L (4.4–24.46 mg nitrate/L) for the time period (1998–2006).

Ericson et al. (1988) found no association between frequency of neural tube defects and levels of nitrate in the drinking water in a case-control study that included 1,458 cases of neural tube defects and 280 matched controls. The reported average nitrate levels in the water were 4.9 mg/L among the cases and 5.1 mg/L among the controls.

Super et al. (1981) evaluated the status of 486 infants in a geographical area of southwest Africa served by 153 wells divided into regions of high nitrate (>20 mg/L) and low nitrate (≤20 mg/L). There was no significant association between nitrate levels in drinking water sources and incidence of stillbirths, prematurity, or birth size; however, an increased incidence of deaths during the first year of life was noted for the high-nitrate region.

Winchester et al. (2009) investigated whether U.S. live births are at increased risk for birth defects when conception occurs during months when surface water agrichemicals (including nitrate, atrazine, and other pesticides) are at greatest concentrations (April–July). For the years 1996–2002, monthly agrichemical concentrations were calculated using USGS's National Water Quality Assessment data and live birth data collected from the Centers for Disease Control and Prevention (CDC) natality data sets. Birth defects were more likely to occur in live births conceived between April and July. However, this finding does not necessarily implicate nitrate in the drinking water.

Brender et al. (2004) found no significant association between dietary nitrate or nitrite intake and risk of offspring with neural tube defects at estimated total nitrite doses (dietary nitrite plus 5% dietary nitrate) ranging from <7.5 to >10.53 mg/day. However, the risk of neural tube defect was significant among those women with total nitrite doses >10.53 mg/day who also reported taking nitrosatable drugs (OR 7.5, 95% CI: 1.8, 45).

Huber et al. (2013) estimated daily nitrate and nitrite intakes among 6,544 mothers of infants with neural tube defects, oral clefts, or limb deficiencies and 6,807 mothers of unaffected control infants, based on results of food frequency questionnaires. The study included areas of 10 U.S. states, and the population was divided into quartiles of estimated nitrate intake and nitrite intake. There was no statistically significant increased risk of neural tube defect with any estimate of nitrate or nitrite intake. Similar results were obtained for oral cleft and limb deficiency, with the exceptions of increased risk at the highest quartile of cleft lip only (OR 1.32, 95% CI: 1.01, 1.72) and cleft lip with or without cleft palate (OR 1.24, 95% CI: 1.05, 1.48) at the highest quartile of animal-based nitrite intake, and increased risk of intercalary limb defect (OR 4.70, 95% CI: 1.23, 17.93) at the highest quartile of total nitrite intake.

Aschengrau et al. (1989) found no nitrate-related increased risk of spontaneous abortion in a study of 286 women who presented at a Massachusetts hospital between July 1976 and February 1978 with a spontaneous loss through gestation week 27 and 1,391 controls.

The CDC (1996) investigated a small cluster of spontaneous abortions (three women, six spontaneous abortions) in close proximity to one another and to a hog farm in LaGrange County, Indiana during 1991–1993. Well water on the hog farm contained >50 mg nitrate/L. Water samples from wells supplying the women who aborted contained 19–26 mg nitrate/L. A mean concentration of 3.1 mg nitrate/L (1.6–8.4 mg/L) was determined for well water supplies to residences of a comparison group of five women, each having full-term birth within the same time period. During the investigation, another case was identified in which a 35-year-old woman, living approximately 10 miles from the other three women, had two spontaneous abortions after having five previous live births. Well water during the first four pregnancies was found to contain 1.2 mg nitrate-nitrogen/L (5.3 mg nitrate/L); the spontaneous abortions occurred after installation of a new well that was found to contain 28.7 mg nitrate-nitrogen/L (126 mg nitrate/L). Although all four women delivered full-term, live-born infants after changing to nitrate-free drinking water sources, the occurrences of spontaneous abortion may have been unrelated to nitrate-containing drinking water.

George et al. (2001) evaluated the geographical and seasonal distribution of sudden infant death syndrome (SIDS) in Sweden during the period 1990–1996 in relation to nitrate levels in drinking water and changes in groundwater nitrate content. The local incidence of SIDS was correlated to maximally recorded concentrations of nitrate in the drinking water. However, in addition to lack of dose-response data for individuals, the SIDS incidence was declining during the study period, numbers of SIDS cases were small in scarcely populated areas, and nitrate concentrations in groundwater sources may have changed rapidly with weather changes and other factors.

Tabacova et al. (1997) evaluated maternal health among 61 pregnant women who lived near an ammonium nitrate fertilizer plant and presented at a local prenatal care clinic. Tabacova et al. (1998) evaluated the status of 51 mother-infant pairs in the same region. Nitrogen oxides in the air averaged 23.1 $\mu g/m^3$ with short-term peak levels as high as 238.5; nitrate concentrations in the public drinking water supply measured 8–54 mg/L; nitrate levels in private wells measured as much as 13–400 mg/L. Of the 61 pregnant mothers in the sample of Tabacova et al. (1997), only 10 had "normal" pregnancies. Mothers diagnosed with anemia (41 cases), toxemia (20 cases), and/or threatened abortion/premature labor (20 cases) exhibited ≥2-fold higher serum methemoglobin than those with "normal" pregnancies. Of the 51 mothers in the sample of Tabacova et al. (1998), there were 38 full-term and normal-weight infants, 7 full-term and low-weight infants, 6 premature deliveries, 1 Caesarean delivery, and 1 breech delivery. Elevated methemoglobin was observed in serum from 28/51 of the mothers, and 24/51 cord blood samples. Both maternal and cord blood methemoglobin levels were higher in cases of abnormal

birth outcome. These results could not be directly linked to elevated nitrate intake from drinking water or food sources.

Developmental end points have been assessed in some animal studies. No indications of treatment-related developmental toxicity were seen in fetuses from pregnant mice administered sodium nitrite in the drinking water during gestation days 7–18 at concentrations as high as 1,000 mg/L (approximate doses as high as 113.2 mg nitrite/kg/day) (Shimada 1989). There were no signs of toxicity in offspring of pregnant rats administered 80 mg sodium nitrite/kg (53.6 mg nitrite/kg) on gestation day 15; offspring were observed for up to 140 days postpartum (Khera 1982). There were no signs of treatment-related developmental effects during the production of two litters by female Wistar rats provided sodium nitrite in the food at concentrations resulting in estimated doses as high as 160 mg nitrite/kg/day (Hugot et al. 1980). Among three female guinea pigs provided potassium nitrate in the drinking water for up to 204 days of cohabitation at a concentration resulting in estimated intake of 4,972 mg nitrate/kg/day, one female died and the other two females produced a total of two litters (one live birth per litter) (Sleight and Atallah 1968). During 191 days of cohabitation, four control females produced eight litters and a total of 31 live births. The only indication of a treatment-related effect on the offspring of pregnant mice administered sodium nitrite by gavage at 0.5 mg/mouse/day (approximate dose of 13 mg nitrite/kg/day) on gestation days 1–14, 16, or 18 was increased fetal hepatic erythropoiesis at gestation days 14 and 16, which was thought to have been a response to nitrite-induced fetal methemoglobinemia (Globus and Samuel 1978).

Significantly impaired auditory and visual discrimination learning behavior and retention of passive avoidance responses (Nyakas et al. 1990), and delay in cholinergic and serotonergic fiber outgrowth in cortical target areas of the brain (Nyakas et al. 1994), presumably due to nitrite-induced hypoxia, were reported in offspring of Wistar rats provided sodium nitrite in the drinking water at 2,000 mg/L (1,334 mg nitrite/L) during gestation day 13 until parturition. However, lack of information regarding body weight and water consumption of the pregnant rats precludes estimation of nitrite doses to the pregnant dams.

Shuval and Gruener (1972) provided sodium nitrite in the drinking water of pregnant rats for 6 weeks (that presumably included gestation and lactation) at concentrations of 2,000 or 3,000 mg/L (1,334 or 2,001 mg nitrite/L, respectively). There were no treatment-related effects on group litter sizes or pup birth weights. However, during 3 weeks postpartum, 30 and 53% of the low- and high-dose pups died (compared to 6% of control pups); surviving pups from the low- and high-dose groups exhibited 43 and 66% lower mean body weight than controls at 3 weeks postpartum. Lack of information regarding body

weight and water consumption of the pregnant rats precludes estimation of nitrite doses to the pregnant dams.

Increased pup mortality, depressed preweaning pup body weight, and delayed swimming development were observed in offspring of male and female rats provided sodium nitrite in the diet at 0.025 or 0.05% (estimated dose levels of 14.4 and 28.1 mg nitrite/kg/day, based on author-reported dose of 43 mg sodium nitrite/kg/day for the high-dose group) (Vorhees et al. 1984). There were no treatment-related effects on preweaning behavior that included surface righting, pivoting, negative geotaxis, or auditory startle and no effects on postweaning survival, body weight, or most behavioral indices, with the exception of decreased open-field behavior on days 40–45 in groups from dams exposed to 0.0125 or 0.05% (but not 0.025%) sodium nitrite in the diet.

3.2.2.7 Cancer

Human Data. Numerous studies are available in which the carcinogenicity of ingested nitrate and nitrite in humans was assessed. A comprehensive review of the cancer epidemiology studies of nitrate and nitrite, published up to approximately 2007, is provided in IARC (2010). Up to that point, most studies employed ecological designs and fewer case-control or cohort studies were available on cancers other than gastrointestinal cancers. Since then, several cohort and case-control studies have been reported that examine a variety of different cancer types (Aschebrook-Kilfoy et al. 2011, 2013a, 2013b; DellaValle et al. 2013; Espejo-Herrera et al. 2015, 2016a, 2016b; Inoue-Choi et al. 2012, 2015; Kilfoy et al. 2010, 2011; Kim et al. 2007; Michaud et al. 2009; Ward et al. 2007, 2008; Wu et al. 2013; Yang et al. 2010; Zeegers et al. 2006; Table 3-2). Ecological studies measure exposure and outcomes at the group level rather than the individual level. Interpretation of outcomes of these studies is more uncertain because of various factors that contribute to ecologic bias (group-based associations between exposure and cancer outcomes may not apply to individuals). Ecological studies can be valuable for exploring causal relationships when the exposures within exposure groups have low variability (homogenous), differences in exposure are large between exposure groups, and when groups are assigned based on geography and migration in and out of exposure areas is minimal (IARC 2010). A typical example of an ecological design assigns group exposures based on residence within a public water supply (PWS) district, where the average (or median) concentration of nitrate or nitrite in the PWS is the exposure metric and outcomes are measured at the level of the PWS area (e.g., cancer incidences in two areas served by public water supplies that have different nitrate or nitrite levels). The major limitation of this approach is that the group-based exposure estimate may (and probably does not) apply to individuals and their cancer

Table 3-2. Selected Cohort and Case-Control Studies Published Since 2006 Examining Possible Associations Between Nitrate and Nitrite Intake and Cancer

Reference	Cancer type	Study design	Nitrate and nitrite intakes	Outcomes[a]
Aschebrook-Kilfoy et al. 2011	Pancreatic	Cohort from NIH-AARP Diet and Health Study, 1995–2006 303,156 cohort, 1,728 cases	Quintile median intake: Nitrate from food: 34.8, 56.9, 75.0, 95.3, 150.3 mg/day; 19.3, 29.9, 40.9, 57.4, 94.8 mg/1,000 kcal Nitrite from food: 0.8, 1.0, 1.2, 1.2, 1.6 mg/day; 0.45, 0.57, 0.65, 0.74, 0.9 mg/1,000 kcal Based on food frequency questionnaire, 24-hour recall, and published food nitrate and nitrite levels	Highest quintile vs lowest quintile: Nitrate: OR 1.01 (95% CI 0.85, 1.20) Nitrite: OR 0.92 (95% CI 0.78, 1.08) No increased risk when accounting for nitrite intake from plant sources, animal sources, or processed meats Adjustments: age, race, caloric intake, smoking, family history of cancer and diabetes, BMI; intakes of saturated fat, folate, vitamin C
Aschebrook-Kilfoy et al. 2013a	Thyroid	Cohort from Shanghai Women's Health Study, 1996–2000 73,317 cohort, 164 cases	Quartile median intake: Nitrate from food: 165.8, 257.8, 350.6, 506.8 mg/day; 108.6, 164.2, 217.6, 250.9 mg/1,000 kcal Nitrite from food: 0.89, 1.27, 1.61, 2.14 mg/day; 0.62, 0.81, 0.95, 1.12 mg/1,000 kcal Based on food frequency questionnaire, 24-hour dietary recall, and published food nitrate and nitrite levels	Highest quartile vs lowest quartile: Nitrate: RR 0.93 (95% CI 0.42, 2.07) Nitrite: RR 2.05 (95% CI 1.20, 3.51) with total nitrite intake RR 1.96 (95% CI 1.28, 2.99) for nitrite intake from processed meat Adjustments: age, caloric intake, education, history of thyroid disease; intakes of vitamin C, carotene, folate

Table 3-2. Selected Cohort and Case-Control Studies Published Since 2006 Examining Possible Associations Between Nitrate and Nitrite Intake and Cancer

Reference	Cancer type	Study design	Nitrate and nitrite intakes	Outcomes[a]
Aschebrook-Kilfoy et al. 2013b	Non-Hodgkin's lymphoma	Case-control with subjects from Nebraska between 1999 and 2002 348 cases, 470 controls	Quartile median intake: Nitrate from food: 22.0, 39.1, 57.5, 106.1 mg/day; 22.2, 38.2, 55.5, 88.3 mg/1,000 kcal Nitrite from food: 0.5, 0.6, 0.7, 0.9 mg/day; 0.49, 0.61, 0.71, 0.86 mg/1,000 kcal Based on food frequency questionnaire and published food nitrate and nitrite levels	Highest quartile vs lowest quartile: Nitrate: OR 0.8 (95% CI 0.5, 1.3; p-trend 0.6) Nitrite: OR 1.3 (95% CI 0.8, 1.9; p-trend 0.4) No significant associations for nitrate or nitrite by lymphoma subtype t(14;18)-positive or -negative) Adjustments: sex, age, BMI, caloric intake, education, family history of cancer, vitamin C intake
Chiu et al. 2011	Colon	Case-control from Taiwan Provincial Department of Health, 2003–2007 3,707 cases, 3,707 controls	Tertile median Nitrate-nitrogen ranges in drinking water: <0.38, 0.39–0.57, ≥0.60 mg/L (<1.67, 1.72–2.51, ≥2.64 mg nitrate/L) Based on PWS data	Highest tertile vs lowest tertile: OR 1.16 (95% CI 1.04, 1.30; p-trend 0.001) OR 1.37 (95% CI 1.11, 1.69) with drinking water calcium levels <34.6 mg/L Adjustments: age, gender, marital status, urbanization level of residence

Table 3-2. Selected Cohort and Case-Control Studies Published Since 2006 Examining Possible Associations Between Nitrate and Nitrite Intake and Cancer

Reference	Cancer type	Study design	Nitrate and nitrite intakes	Outcomes[a]
DellaValle et al. 2013	Kidney (RCC)	Cohort from NIH-AARP Diet and Health Study, 1995–2006 491,841cohort, 488 cases	Quintile median intake: Nitrate intake from food: 19.3, 40.9, and 94.8 mg/1,000 kcal, for quintiles 1, 3, and 5, respectively Nitrite intake from food: 0.5, 0.7, and 0.9 mg/1,000 kcal, for quintiles 1, 3, and 5, respectively Based on food frequency questionnaire and published food nitrate and nitrite levels	Highest quintile vs lowest quintile: Nitrate: No increased risk (HR 0.98; 95% CI 0.84, 1.14 for total RCC) Nitrite: No increased risk for total nitrite (HR 1.02; 95% CI 0.87, 1.19 for total RCC) or nitrite from plant sources (HR 0.89; 95% CI 0.76, 1.04 for total RCC) Increased risk for nitrite from animal sources (HR 1.28; 95% CI 1.10, 1.49; p-trend <0.01 for total RCC), nitrite from processed meat sources (HR 1.16; 95% CI 1.00, 1.35; p-trend 0.04 for total RCC), nitrite from animal sources other than processed meat (HR 1.23 (95% CI 1.06, 1.43; p-trend 0.02 for total RCC), nitrate and nitrite from processed meat sources (HR 1.17; 95% CI 1.00, 1.37; p-trend 0.03 for total RCC) Risk of RCC mainly associated with clear cell histological subtype (e.g., HR 1.68; 95% CI 1.25, 2.27; p-trend <0.01 for nitrite from animal sources and clear cell subtype) Adjustments: age, sex, caloric intake, race, smoking, family history of cancer, BMI, alcohol intake, education; history of hypertension, diabetes

Table 3-2. Selected Cohort and Case-Control Studies Published Since 2006 Examining Possible Associations Between Nitrate and Nitrite Intake and Cancer

Reference	Cancer type	Study design	Nitrate and nitrite intakes	Outcomes[a]
Espejo-Herrera et al. 2015	Bladder	Case-control. Spain, 1998–2001 556 controls, 531 cases	Average residential ranges in drinking water by tertiles: ≤5 mg/L, >5–10 mg/L, >10 mg/L Based on historical records of nitrate levels in municipal water sources	No associations between risk of bladder cancer and average nitrate level (OR 1.09; 95% CI 0.63, 1.87) for highest versus lowest level For subjects with longest exposure duration (>20 years) to highest levels (>9.5 mg/L), OR=1.42; 95% CI 0.989 2.26 Stratification by intake of vitamin C, vitamin E, meat, and gastric ulcer did not modify the results Adjustments: age, sex and area of residence smoking status, NSAIDs use, night-time urinary frequency, time working in farm/agriculture activities, tap water and vitamin C daily intake, urinary infections (ever)
Espejo-Herrera et al. 2016b	Breast	Case-control. Spain, 2008–2013 1,520 controls, 1,245 cases	Average waterborne ingested nitrate ranged from 2.9 ±1.9 mg/day (mean ± SD) to 13.5 ±7.5 mg/day Based on historical records of nitrate levels in municipal water sources and monitoring of other sources Average dietary nitrate intake ranged from 88.5±48.7 mg/day to 154±87.8 mg/day Based on food frequency questionnaire and published food nitrate and nitrite levels	No associations between dietary nitrate intake or waterborne ingested nitrate and risk of breast cancer overall, but increased risk (OR 1.64; 95% CI 1.08, 2.49) among postmenopausal women with both high waterborne nitrate intake (>6 mg/day) and high red meat intake (≥20 g/day) Adjustments: study area, age, education, BMI, family history of breast cancer, age at first birth, age at menopause, oral contraceptives use, energy intake

Table 3-2. Selected Cohort and Case-Control Studies Published Since 2006 Examining Possible Associations Between Nitrate and Nitrite Intake and Cancer

Reference	Cancer type	Study design	Nitrate and nitrite intakes	Outcomes[a]
Espejo-Herrera et al. 2016a	Colorectal	Case-control. Spain and Italy, 2008–2013 3,530 controls, 1,869 cases (1,285 colon; 557 rectal)	Average waterborne ingested nitrate ranged from 3.4±3.3 mg/day to 19.7±22.6 mg/day Tertiles: ≤5, <5–10, >10 mg/day Based on historical records of nitrate levels in municipal water sources and monitoring of other sources Mean dietary nitrate intake was 118±72 mg/day overall (102±70.5 mg/day from vegetables and 6.2±3.3 mg/day from animal sources) Tertiles: <4.5, 4.5–6.8, >6.8 mg/day Based on food frequency questionnaire and published food nitrate and nitrite levels	For highest versus lowest waterborne nitrate intake: OR 1.49 (95% CI 1.24, 1.78) for colorectal cancer OR 1.52 (95% CI 1.24, 1.86) for colon cancer OR 1.62 (95% CI 1.23, 2.14) for rectal cancer For risk of rectal cancer among subjects with dietary intake of nitrate from animal sources: OR 1.59 (95% CI 1.22, 2.06) for mid tertile OR 1.55 (95% CI 1.17, 2.05) for highest tertile Greater risk among men than women Adjustments: sex, age, education, physical activity, BMI, family history of colorectal cancer, NSAIDs use, energy intake, oral contraceptives use

Table 3-2. Selected Cohort and Case-Control Studies Published Since 2006 Examining Possible Associations Between Nitrate and Nitrite Intake and Cancer

Reference	Cancer type	Study design	Nitrate and nitrite intakes	Outcomes[a]
Inoue-Choi et al. 2012	Breast	Cohort of post-menopausal women from Iowa Women's Health Study, 1989–2008 34,388 cohort, 2,875 cases	Quintile median nitrate intake from drinking water: 1.6, 4.1, 9.4, 21.2, and 57.8 mg/2 L Based on historical database of Iowa municipal water supplies Quintile median nitrate intake from food: 49.3, 78.7, 106.1, 140.2, 209.9 mg/day Quintile median nitrite intake from food: 0.6, 0.9, 1.1, 1.4, 1.8 mg/day Based on food frequency questionnaire and published food nitrate and nitrite levels	Highest quintile vs lowest quintile: Overall, no increased risk of breast cancer with intake of nitrate and/or nitrite from diet and/or drinking water Significant trend for increasing HR with increasing nitrite intake HR 1.40 (95% CI 1.05, 1.87) for nitrate and folate ≥400 µg/day HR 1.0 95% CI (0.79, 125) for nitrate and folate <400 µg/day Adjustments: age; caloric intake; BMI; waist-hip ratio; education; smoking; physical activity level; alcohol intake; family history of breast cancer; age at menopause; age at first live birth; estrogen use; intakes of alcohol, vitamin C, vitamin E, flavonoids, cruciferae, red meat

Table 3-2. Selected Cohort and Case-Control Studies Published Since 2006 Examining Possible Associations Between Nitrate and Nitrite Intake and Cancer

Reference	Cancer type	Study design	Nitrate and nitrite intakes	Outcomes[a]
Inoue-Choi et al. 2015	Ovarian	Cohort of post-menopausal women from Iowa Women's Health Study, 1986–2010 28,555 cohort, 315 cases	Quartile median nitrate levels in drinking water: 0.31, 0.75, 1.68, 3.81 mg nitrate-nitrogen/L (1.36, 3.3, 7.39, 16.76 mg nitrate/L) Historical database of Iowa municipal water supplies Quintile median nitrate intake from food: 49.5, 78.9, 106.2, 140.2, 209.2 mg/day Quintile medium nitrite dietary intake: 0.7, 0.9, 1.1, 1.4, 1.8 mg/day Based on food frequency questionnaire and published food nitrate and nitrite levels	Highest quartile/quintile vs lowest quartile/quintile: HR 2.03 (95% CI 1.22, 3.38) for highest quartile of nitrate in public drinking water; association was stronger when vitamin C intake was ≤190 mg/day and when red meat servings exceeded 5 per week Overall, no increased risk of ovarian cancer with total intake of nitrate or nitrite. Adjustments: age, BMI, family history of ovarian cancer, number of live births, age at menarche, age at menopause, age at first live birth, oral contraceptive use, estrogen use, history of unilateral oophorectomy, and/or total energy intake
Kilfoy et al. 2010	Non-Hodgkin's lymphoma	Case-control of women in Connecticut between 1995 and 2001 594 cases, 710 controls	Quartile ranges: Nitrate from food: <63.9, 63.9 to <93.0, 93.0 to <140.5, ≥140.5 mg/day Nitrite from food: <0.77, 0.77 to <0.99, 0.99 to <1.32, ≥1.32 mg/day Based on food frequency questionnaire and published food nitrate and nitrite levels	Highest quartile vs lowest quartile: Overall non-Hodgkin's lymphoma: OR 0.9 (95% CI 0.6, 1.2) for nitrate OR 1.4 (95% CI 0.9, 2.2) for nitrite Significant trend (p=0.04) for follicular lymphoma with increasing nitrate intake OR 2.3 (95% CI 1.1, 4.9; p-trend 0.008) for follicular lymphoma with nitrite intake Adjustments: age; family history of cancer; calories; intakes of vitamins C, vitamin E, protein

Table 3-2. Selected Cohort and Case-Control Studies Published Since 2006 Examining Possible Associations Between Nitrate and Nitrite Intake and Cancer

Reference	Cancer type	Study design	Nitrate and nitrite intakes	Outcomes[a]
Kilfoy et al. 2011	Thyroid	Cohort from NIH-AARP Diet and Health Study, 1995–1996 with average of 7 years of follow-up 490,194 cohort (292,125 men; 198,069 women), 370 cases (170 men; 200 women)	Quintile median ranges: Nitrate from food: 29.6, 49.8, 70.2, 100.9, 166.8 mg/day (19.4, 29.9, 40.9, 57.4, 94.8 mg/1,000 kcal) Nitrite from food: 0.6, 0.9, 1.1, 1.4, 1.9 mg/day (0.5, 0.6, 0.7, 0.7, 0.9 mg/1,000 kcal) Based on food frequency questionnaire and published food nitrate and nitrite levels	Highest quintile versus lowest quintile: Nitrate: Men: RR 2.28 (95% CI 1.29, 4.04; p-trend <0.01) for thyroid cancer RR 2.10 (95% CI 1.09, 4.05; p-trend 0.05) for papillary thyroid cancer RR 3.42 (95% CI 1.03, 11.4; p-trend <0.01) for follicular thyroid cancer Women: RR 0.76 (95% CI 0.48, 1.10) for thyroid cancer Nitrite: Men: RR 1.36 (95% CI 0.78, 2.37) for thyroid cancer RR 2.74 (95% CI 0.86, 8.77; p-trend 0.04) for follicular thyroid cancer Women: RR 1.19 (95% CI 0.71, 1.98) for thyroid cancer Adjustments: sex; age; smoking status; race; physical activity; alcohol use; BMI; caloric intake; education; family history of cancer; intakes of vitamin C, beta-carotene, folate
Kim et al. 2007	Stomach	Case-control with subjects from two Korean hospitals, 1997–1998 136 controls, 136 cases	Tertile median values: Nitrate from food: 240, 458, and 811 mg/day Based on food frequency questionnaire and published food nitrate and nitrite levels	Highest tertile vs lowest tertile: OR 1.13 (95% CI 0.42, 3.06) OR 2.78 (95% CI 1.01, 7.67) for nitrate/86.7 mg/mg vitamin E OR 3.37 (95% CI 1.28, 8.87) for nitrate/2.47 mg/µg folate Adjustments: age; sex; SES; family history of gastric cancer; refrigerator use; *Helicobacter pylori* infection; intakes of charcoal grilled beef, Korean cabbage *kimichi*, *Dongchimi*, spinach, garlic, mushroom, salty foods

Table 3-2. Selected Cohort and Case-Control Studies Published Since 2006 Examining Possible Associations Between Nitrate and Nitrite Intake and Cancer

Reference	Cancer type	Study design	Nitrate and nitrite intakes	Outcomes[a]
McElroy et al. 2008	Colorectal	Wisconsin, United States, 1990–2001 4,297 controls, 1,476 cases, all females	Quintile cutoff range: Nitrate in water: <0.5, 0.5–1.9, 2.0–5.9, 6.0–9.9, ≥10 mg/L Based on groundwater nitrate data and spatial interpolation to individual residences	Highest quintile versus lowest quintile: OR 1.52 (95% CI 0.95, 2.44) for all colon cancer sites OR 2.91 (95% CI 1.52, 5.56) for all proximal colon sites Adjustments: age
Michaud et al. 2009	Brain (glioma)	Combined analysis of cohorts from NHS I (1980–2004), NHS II (1991–2005), and HPFS (1986–2004) 230,655 cohort, 335 cases	Quintile cutoff ranges, based on baseline values: Nitrate from food: 43–205 mg/day Nitrite from food: 1.1–2.4 mg/day NDMA from food: 0.02–0.09 mg/day Based on food frequency questionnaire and published food nitrate and nitrite levels	Highest tertile vs lowest tertile: Nitrate: RR 1.02 (95% CI 0.66, 1.58) Nitrite: RR 1.26 (95% CI 0.89, 1.79) NDMA: RR 0.88 (95% CI: 0.57, 1.36) Processed meat: RR 0.92 (95% CI 0.48, 1.77) No effect of vitamin C, vitamin E, or ferric-reducing ability of plasma Adjustments: age, caloric intake

Table 3-2. Selected Cohort and Case-Control Studies Published Since 2006 Examining Possible Associations Between Nitrate and Nitrite Intake and Cancer

Reference	Cancer type	Study design	Nitrate and nitrite intakes	Outcomes[a]
Ward et al. 2007	Kidney (RCC)	Case-control, Iowa, United States, 1986–1989 2,434 controls, 406 cases	Quartile cutoff ranges: Nitrate from food: <59.32, 59.32–86.62, 86.63–122.00, ≥122.01 mg/day Nitrite from food: <0.70, 0.70–0.93, 0.94–1.25, ≥1.26 mg/day Nitrate in water: <0.62, 0.62–<1.27, 1.27–≤2.78, ≥2.78 mg/L Based on food frequency questionnaire and published food nitrate and nitrite levels, and PWS data	Highest quartile versus lowest quartile: Nitrate: OR 0.41 (95% CI 0.28, 0.60) for dietary nitrate OR 0.89 (95% CI 0.57, 1.39) for nitrate in water Nitrite: OR 0.82 (95% CI 0.50, 1.33) OR 1.00 (95% CI 0.63, 1.59) for nitrite from animal sources OR 1.91 (95% CI 1.04, 3.51) for red meat intake ≥1.2 servings/day and PWS nitrate >5 mg/L for >10 years Adjustments: age, sex, BMI, caloric intake, intakes of sodium and fat

Table 3-2. Selected Cohort and Case-Control Studies Published Since 2006 Examining Possible Associations Between Nitrate and Nitrite Intake and Cancer

Reference	Cancer type	Study design	Nitrate and nitrite intakes	Outcomes[a]
Ward et al. 2008	Esophagus, Stomach	Case-control, Nebraska, United States, 1988–1993 321 controls, 79 stomach cases, 84 esophagus cases	Quartile cutoff ranges: Nitrate-nitrogen in water: <2.45, 2.45–<2.58, 2.58–4.32, >4.32 mg/L (<10.78, 10.78–<11.35, 11.35–19.01, >19.01 mg nitrate/L) Dietary nitrate from plant sources: <16.9, 16.9–<26.2, 26.2–<38.8, >38.8 mg/day nitrate-nitrogen (<74.4, 74.4–<115.3, 115.3–<170.7, >170.7 mg nitrate/day) Nitrate and nitrite from animal sources: <3.8, 3.8–<5.7, 5.7–<8.3, ≥8.3 mg/day Based on food frequency questionnaire and published food nitrate and nitrite levels, and PWS data	Highest quartile versus lowest quartile: Nitrate in water: OR 1.2 (95% CI 0.5, 2.7) for stomach cancer OR 1.2 (95% CI 0.6, 2.7) for esophageal cancer Nitrate from plant sources: OR 1.6 (95% CI 0.7, 3.6) for stomach cancer OR 0.8 (95% CI 0.3, 1.8) for esophageal cancer Nitrate and nitrite from animal sources: OR 1.6 (95% CI 0.7, 3.7) for stomach cancer OR 2.2 (95% CI 0.9, 5.7; p-trend 0.015) for esophageal cancer Adjustments: year of birth; sex; BMI; smoking; alcohol; caloric intake; intakes of vitamin A, folate, riboflavin, zinc, protein, carbohydrate
Wu et al. 2013	Prostate	Case-control with subjects from HPFS (1997–2005) 630 controls, 630 cases	Quartile median range: Plasma nitrate (cases): 29.39, and 51.47 µmol/L (1.82 and 3.19 mg/L)	Adjusted RR not significant; no significant trend RR 0.99 (95% CI 0.68, 1.48) for highest plasma nitrate quintile Adjustments: age, BMI, caloric intake, time of blood draw, hours since last meal before blood draw year of blood draw, family history of prostate cancer, smoking, history of hypertension, history of diabetes, physical activity

Table 3-2. Selected Cohort and Case-Control Studies Published Since 2006 Examining Possible Associations Between Nitrate and Nitrite Intake and Cancer

Reference	Cancer type	Study design	Nitrate and nitrite intakes	Outcomes[a]
Yang et al. 2010	Breast	Case-control. Seoul, South Korea, 2004–2006 362 controls, 362 cases	Quintile median nitrate intake from food: 179.4, 299.7, 372.1, 492.5, 716.1 mg/day Based on food frequency questionnaire and published food nitrate levels	Adjusted OR not significant for dietary nitrate, no significant trend. Significant trend for increasing OR with increasing nitrate/folate ratio; OR significantly elevated in highest nitrate/folate quintile. OR 1.54 (95% CI 0.88, 2.70) for nitrate OR 2.03 (95% CI 1.16, 3.54) for nitrate/folate intake ratio (2.10) Adjustments: age, education, physical activity, family history of breast cancer, parity, breast feeding, menopause, oral contraceptive use; intakes of soy protein, mushroom, fat
Zeegers et al. 2006	Bladder	Cohort from Netherlands Cohort Study, 1986–1996 cohort 120,852, 889 cases, 4,441 subcohort	Quintile median nitrate intakes: Nitrate from food: 57.4, 78.6, 97.8, 119.5, 158.9 mg/day Nitrate from water: 0.5, 1.4, 3.4, 5.6, 10.6 mg/day Based on food frequency questionnaire and published food nitrate and nitrite levels, and PWS data	Adjusted RR not significant for nitrate in food or water; no significant trend Highest quintile vs lowest quintile: RR 1.06 (95% CI 0.81, 1.37) for nitrate from food RR 1.06 (95% CI 0.82, 1.37) for nitrate from water RR 1.09 (95% CI 0.84, 1.42) for total nitrate intake Adjustments: age, sex, smoking

[a]Risk estimates (95% confidence limits)

AARP = American Association of Retired Persons; BMI = body mass index; HPFS = Health Professionals Follow-Up Study; HR = multivariate hazard ratio; NDMA = nitrosodimethylamine; NIH = National Institutes of Health; NHS = Nurses' Health Study; NSAIDs = non-steroidal anti-inflammatories; OR = odds ratio; PWS = public water supply; RCC = renal cell carcimoma; RR = relative risk; SD = standard deviation; SES = socioeconomic status

outcomes. Exposure misclassification can occur for various reasons including dietary factors that contribute to variability in dose of nitrosation precursors (e.g., nitrate or nitrite in fish, meat, and vegetables) and nitrosatable compounds; consumption of antioxidants that can inhibit nitrosation (e.g., vitamin C, flavenoids, polyphenols); migration in and out of the PWS district; and ingestion of other water sources (e.g., bottled water). Estimates of exposure from drinking water can be made at the individual subject level. This can be accomplished with surveys of the individual's residence history and consumption patterns (e.g., percentage of drinking water consumed from the PWS and other sources, such as bottled water), along with data on nitrate concentrations in the water supply (Inoue-Choi et al. 2012). Dietary surveys (e.g., food frequency questionnaires, 24-hour recalls), coupled with data from residue monitoring studies of market basket foods, can be used to estimate individual exposures to nitrosation precursors in foods. However, in this approach, exposure misclassification can occur as a result of ingestion of nitrosation precursors from non-market basket foods. Also, the diet survey is typically cross-sectional, even in longitudinal studies, and results may not accurately reflect the average diets during the entire follow-up period. Exposure misclassification can also occur in studies that examine associations at the individual level. However, in these studies, exposure misclassification is likely to be non-differential or independent of cancer status. As a result, exposure comparisons (exposed versus unexposed) would tend to be biased towards the null if there truly is an effect of the exposure on cancer outcome, and if more than two levels of exposure are being evaluated (e.g., high, low, versus no exposure), then the bias can be in either direction for the middle levels of exposure and tend to be biased towards the null at the highest level so that exposure-response relationships are distorted (e.g., the risk would be attenuated or fall at the highest levels of exposure because of this bias). Most of the nitrate and nitrite ingested comes from the diet (Zeegers et al. 2006); therefore, studies that quantify exposure only from drinking water are weak designs for assessing cancer risk unless the water supply is extraordinarily contaminated (>20 mg nitrate/L). Some studies have employed biomarkers (blood, plasma, saliva, or urine) as exposure metrics (Armijo et al. 1981; Cuello et al. 1976; Forman et al. 1985; Joossens et al. 1996; Kamiyama et al. 1987; Knight et al. 1990; Lin et al. 2003; Lu et al. 1986; Sierra et al. 1993; Tsugane et al. 1992; Wu et al. 1993, 2013). Biomarkers can provide more accurate estimates of the steady-state levels of nitrate (or nitrite) in an individual; however, they may not reflect the cumulative absorbed dose or the dose of nitrosation products that may contribute to cancers (e.g., N-nitrosodimethylamine) (Zeilmaker et al. 2010a). An additional uncertainty that applies to all studies described in this summary is that cancer risk may be mis-attributed to nitrite (or nitrosation precursors) as a result of other factors that contribute cancer risk that co-vary with exposure to nitrite or nitrite precursors. These may include other carcinogens in drinking water or diet. However, unless these risk factors have extremely strong associations with exposures to nitrate or nitrite (or nitrosation precursors), confounding from these factors is unlikely to be a major

source of uncertainty in interpretation of cancer risk estimates. One potentially important class of confounders is anti-oxidants, which can interfere with nitrosation of dietary amines and, thereby, the mode of carcinogenicity of nitrite, and may also interfere with other carcinogenic process that involve reactive intermediates. In the discussions of individual studies, the terms "statistically significant" refer to relative risks that are estimated to be ≥1 or trends that were reported by the investigators to be statistically significant, typically $p<0.05$).

In general, outcomes of cohort and case-control studies have found no or weak associations between nitrate intakes and cancer in humans, with stronger associations for exposures to nitrite or intake of high-nitrite foods such as cured meat (Aschebrook et al. 2013; DellaValle et al. 2013; IARC 2010; Inoue-Choi et al. 2012). Mechanistically, this outcome is consistent with nitrite being a reactive intermediate in the cancer mode of action of nitrate (see Section 3.5.2).

Studies that form the basis for evidence of carcinogenicity of nitrate or nitrite are briefly described below. Most of these studies are described in greater detail in IARC (2010). Studies published since IARC (2010) are summarized in Table 3-2. Studies included in Table 3-2 estimated nitrate or nitrite intakes from dietary survey instruments of individuals, in some cases, supplemented with estimates from drinking water based on well water or PWS data and geographic location of the residence, or with biomarkers of exposure. The table summarizes major features of the design of each study and the major outcomes. Complete details of the outcomes for various design strata can be obtained from the cited references.

This summary of carcinogenicity of nitrate and nitrite in humans is intentionally biased for the sake of brevity, in that it is restricted to case-control and cohort studies and emphasizes studies that have found associations between nitrate or nitrite and cancer, while most studies that found no associations are not described. Descriptions of important ecological studies and negative outcome studies can be found in IARC (2010). In the summary below, reported risks are adjusted for co-variables, which differed across studies. Most studies adjusted for age, sex, body mass index (BMI), caloric intake, family history of cancer, smoking, and alcohol consumption. Some studies also adjusted for socioeconomic status, education, and various dietary intakes (e.g., vitamin C, vitamin E, flavenoids, folate), as well as cancer specific-adjustments (e.g., reproductive history in breast cancer studies). Estimates of risk for studies not included in Table 3-2 were those reported in IARC (2010) where they were expressed as relative risk (RR) without specification of the actual risk metric estimated in the study. Risk metrics reported in Table 3-2 are ORs for case-control studies and RR or hazard ratio (HR) for cohort studies.

Gastrointestinal Cancer. Associations between intake of nitrite and a variety of cancer types has been studied; however, the strongest and most consistent evidence for carcinogenicity of nitrite derives from studies of gastrointestinal cancers and, in particular, gastric cancer (Buiatti et al. 1990; Engel et al. 2003; La Vecchia et al. 1994, 1997; Mayne et al. 2001; Palli et al. 2001; Risch et al. 1985; Rogers et al. 1995; Ward et al. 2007, 2008). In general, these studies have found significant positive trends for cancer risk (risk increases with increasing intake), and three studies found elevated cancer risk (Engel et al. 2003; Kim et al. 2007; Risch et al. 1985). In the Risch et al. (1985) case-control study (246 cases, 246 controls), relative risk was 1.71 (95% CI: 1.24, 2.37) for a nitrite intake of 1 mg/day. In another case-control study (369 cases, 695 controls) (Engel et al. 2003; Mayne et al. 2001), risk for stomach cancer (non-cardia) was elevated at nitrite intakes ≥6 mg/day (OR 2.5, 95% CI: 1.4, 4.3). Risk increased with decreasing vitamin C intake (RR 2.95, 95% CI: 1.90, 4.59). Additional support for antioxidants as effect modifiers comes from a case-control study (136 cases, 136 controls) in which stomach cancer risk increased in association with increasing ratio of nitrate to antioxidants in the diet (e.g., vitamin C, vitamin E, folate) (Kim et al. 2007). Risk (OR) at the highest nitrate/vitamin E ratio (86.7 mg nitrate/mg vitamin E) was 2.78 (95% CI: 1.01, 7.67). At the highest nitrate/folate ratio (2.47 mg nitrate/µg folate), an OR of 3.37 (95% CI: 1.28, 8.87) was determined.

Associations between exposure to nitrate or nitrite and colorectal cancer have been studied in cohort and case-control studies (Chiu et al. 2011; De Roos et al. 2003; Knekt et al. 1999; Weyer et al. 2001). The largest of the case-control studies (3,707 cases, 3,707 controls) (Chiu et al. 2011) found a significant positive trend (chi-square for trend =13.26, p=0.001) for mortality from colon cancer with increasing nitrate levels in drinking water (OR 1.16, 95% CI: 1.04, 1.30 at nitrate-nitrogen levels >0.6 mg/L; >2.65 mg nitrate/L). Risks were higher in a stratum exposed to drinking water that had a calcium level >34.6 mg/L (OR 1.37, 95% CI: 1.11, 1.69 for nitrate <2.64 mg/L). The De Roos et al. (2003) case-control study (685 cases of colon cancer, 655 cases of rectal cancer, 2,434 controls) found elevated risk of colon (RR 1.5, 95% CI: 1.0, 2.1) and rectal cancer (RR 1.7, 95% CI: 1.1, 2.5) at a dietary nitrite intake >1.26 mg/day. Risk of colon cancer was higher in a stratum exposed to nitrate in drinking water at levels >5 mg/L in combination with a low vitamin C intake (RR 2.0, 95% CI: 1.2, 3.3). Two meta-analyses reported in IARC (2010) concluded that ingestion of cured meats was associated with increased risk of colorectal cancer (Norat et al. 2002; Sandhu et al. 2001).

Central Nervous System Cancer. Cancer of the central nervous system has been studied extensively in case-control studies (IARC 2010). Some studies found significant positive trends with nitrite and/or cured meat intake; elevated risk was reported in a few studies (Blowers et al. 1997; Giles et al. 1994, Lee

et al. 1997; Mueller et al. 2004; Pogoda and Preston-Martin 2001a, 2001b; Preston-Martin et al. 1996). Risks increased with higher nitrite intake or cured meat/antioxidant ratios (Blowers et al. 1997; Preston-Martin et al. 1996). The study of Preston-Martin and coworkers (Pogoda and Preston-Martin 2001a, 2001b; Preston-Martin et al. 1996) included 540 cases and 801 controls. Significantly increased risk (OR 3.0, 95% CI: 1.2, 7.9) was observed for central nervous system cancers (brain, cranial nerves, or cranial meninges) in children of mothers reporting a nitrite intake >3.0 mg/day from cured meat during pregnancy. The Mueller et al. (2004) case-control study (1,218 cases, 2,223 controls) found elevated risk (RR 5.7, 95% CI: 1.2, 27.2) for astroglial tumors in children in association with maternal exposure to drinking water to nitrite concentrations ≥5 mg/L during pregnancy. Risks for other types of brain tumors were not elevated. A smaller case-control study (94 cases, 94 controls) found elevated risk of glioma in women (with trend p=0.07) in association with intake of nitrite from cured meat (RR 2.1, 95% CI: 1.0, 4.6). Results of meta-analyses of brain cancer studies also support associations between intake of cured meat during pregnancy and brain tumors in children and cured meat ingestion and brain tumors in adults (Huncharek and Kupelnick 2004; Huncharek et al. 2003). A large cohort study (230,655 subjects, 335 cases) of associations between intakes of nitrate, nitrite, and nitrosodimethylamine (NDMA) and glioma in adults did not find significant trends or elevated risk for glioma (Michaud et al. 2009, Table 3-2).

Urinary Tract Cancer. Cancer of the urinary tract has been studied in several case-control and large cohort studies (DellaValle et al. 2013; Espejo-Herrera et al. 2015; IARC 2010; Ward et al. 2007, Zeegers et al. 2006). Positive trends for risk or elevated risk were found in some studies (DellaValle et al. 2013; Ward et al. 2007; Wilkens et al. 1996). In the Wilkens et al. (1996) case-control study (272 cases, 522 controls), risk was elevated (trend p=0.05) in association with dietary nitrite intake (RR 2.0, 95% CI: 1.0, 4.0). In the Ward et al. (2007) case-control study (406 cases, 2,434 controls), risk of kidney cancer was elevated in the strata who consumed >1.2 servings of red meat/day and who resided for >10 years in a PWS district that had nitrate concentrations >5 mg/L (OR 1.91, 95% CI: 1.04, 3.51; see Table 3-2). A large cohort study (491,841 subjects, 488 cases) found a significant positive trend and elevated risk for renal cell carcinoma in association with nitrite intake from animal sources (HR 1.28, 95% CI: 1.10, 1.49 for renal cell carcinoma; HR 1.68, 95% CI: 1.25, 2.27 for clear cell carcinoma, both at 0.9 mg nitrite/1,000 kcal) (DellaValle et al. 2013). The Zeegers et al. (2006) cohort study (120,852 subjects, 889 cases) found no association between bladder cancer and intake of nitrate from food or drinking water. Wang et al. (2012) evaluated possible association between nitrate in drinking water and risk of bladder cancer in a meta-analysis that included results from one ecological study (Morales et al. 1993), two cohort studies (Weyer et al. 2001; Zeegers et al. 2006), and two case-control studies (Chiu et al.2007; Ward et al.

2003) and found no evidence that nitrate in the drinking water was associated with risk of bladder cancer (combined RR 1.27; 95% CI 0.75, 2.15) based on data for highest nitrate levels reported relative to reference values from each study.

Reproductive Organ Cancer. A small number of case-control and cohort studies have examined associations between exposure to nitrate or nitrite and cancers of breast, ovary, uterus, prostate, and testis (Barbone et al. 1993; IARC 2010; Espejo-Herrera et al. 2016b; Inoue-Choi et al. 2012, 2015; Moller 1997;Wu et al. 2013; Yang et al. 2010). A cohort study of post-menopausal women (34,388 subjects, 2,875 cases) found a significant positive trend (p=0.04) and elevated risk (HR 1.40, 95% CI: 1.05, 1.87) for breast cancer in association with consumption of public drinking water at ≥33.5 mg nitrate/2 L (median 57.8 mg nitrate/2 L) among women who consumed folate at rates ≥400 µg/day; risk was not elevated among those women who ingested folate at <400 µg/day (Inoue-Choi et al. 2012). Similarly increased risk (HR 1.38, 95% CI: 1.05, 1.82) was noted for private well users who ingested folate at >400 µg/day when compared to the lowest quintile of users of the public drinking water sources who ingested folate at >400 µg/day. In contrast, Yang et al. (2010) reported elevated risk for breast cancer in association with increasing dietary nitrate/folate ratio, with significantly elevated risk (OR 2.03, 95% CI: 1.16, 3.54) at nitrate/folate ratios in the range of 1.79–8.19. The contrasting effects of folate in these two studies may reflect dose-dependent effect modification: an antioxidant effect at lower folate intakes and a tumor promoting effect of folate at higher folate intakes (Inoue-Choi et al. 2012). Inoue-Choi et al. (2015) reported increased risk of ovarian cancer (HR 2.03; 95% CI 1.22, 3.38) among subjects with public water containing ≥2.98 mg nitrate-nitrogen/L (≥13.1 mg nitrate/L) in a cohort study of 28,555 post- menopausal women (315 ovarian cancer cases) in the Iowa Women's Health Study. Associations were stronger when vitamin C intake was ≤190 mg/day and when red meat servings exceeded five per week. Espejo-Herrera et al. (2016b) reported increased risk (OR 1.64; 95% CI 1.08, 2.49) of breast cancer among postmenopausal women with both high waterborne nitrate intake (>6 mg/day) and high red meat intake (≥20 g/day) in a case control study in Spain (1,245 cases, 1,520 controls). A case-control study of prostate cancer (630 cases, 630 controls) did not find significant associations between prostate cancer risk and plasma nitrate concentrations (1.8–3.8 mg/L) (Wu et al. 2013). In the Moller (1997) case-control study (514 cases, 720 controls), elevated risk of testicular cancer (OR 1.51, 95% CI: 1.03, 2.20) was found among men who had lived in areas during childhood with drinking water containing >25 mg nitrate/L. Barbone et al. (1993) conducted a case-control study of endometrial cancer (168 cases, 334 controls) and found a negative trend for risk (risk decreased with increasing dietary nitrate intake).

Reticuloendothelial Cancer. Associations between exposure to nitrate or nitrite and leukemia or non-Hodgkin's lymphoma have been studied in population-based case-control studies (Aschebrook-Kilfoy et al. 2013; Chiu et al. 2008; Freedman et al. 2000; Kilfoy et al. 2010; Ward et al. 1996, 2006) and a prospective cohort study (Weyer et al. 2001). One case-control study (181 cases, 142 controls) reported elevated risk (OR 3.1, 95% CI: 1.7, 5.5) of non-Hodgkin's lymphoma in association with dietary nitrite (but not nitrate) at dietary nitrite intake >1.21 mg/day (Ward et al. 2006). Another case-control study (156 cases, 527 controls) reported elevated risk (OR 2.0; 95% CI 1.1, 3.6) of non-Hodgkin's lymphoma in association with average nitrate levels ≥4 mg/L nitrate-nitrogen (17.6 mg nitrate/L) in the community drinking water supply (Ward et al. 1996). Chiu et al. (2008) evaluated possible associations between diet and non-Hodgkin's lymphoma according to t(14;18) status (one of the most common chromosomal abnormalities in non-Hodgkin's lymphoma. Dietary factors in 60 t(14;18)-positive and 87 t(14; 18)-negative cases were compared with 1,075 controls. The study authors reported increased risk (OR 2.8; 95% CI 1.3, 6.1) of t(14;18)-positive non-Hodgkin's lymphoma for the highest tertile of dietary nitrite (>1 mg/day) versus the lowest tertile (<1 mg/day). The Freedman et al. (2000) case-control study (73 cases, 147 controls) found no association between non-Hodgkin's lymphoma and nitrate levels in public drinking water. Kilfoy et al. (2010) evaluated risk of non-Hodgkin's lymphoma overall and by histological type in relation to self-reported dietary nitrate and nitrite intake in a case-control study of 1,304 women. No significant association was found between risk of non-Hodgkin's lymphoma overall and dietary nitrate or nitrite. Significant positive trends were reported for follicular lymphoma and increasing intakes of nitrate (p-trend =0.04) and nitrite (p-trend <0.01); a significant association (OR 2.3; 95% CI 1.1, 4.9) was noted for the highest nitrite intake quartile (≥1.32 mg/day). Aschebrook-Kilfoy et al. (2013) estimated dietary intake of nitrate and nitrite intake via food frequency questionnaire among 348 non-Hodgkin's lymphoma cases and 470 controls in Nebraska in 1999–2002 and reported nonsignificant excess risk of non-Hodgkin's lymphoma (OR 1.6; 95% CI 0.8, 2.9) among women in the highest quartile of nitrite intake (median nitrite intake 0.86 mg/1,000 kcal) compared to the lowest quartile (median nitrite intake 0.49 mg/kcal). An OR of 1.9 (95% CI 1.0, 3.4) was estimated for the highest quartile based on nitrite intake from animal sources (median nitrite intake 0.41 mg/kcal versus 0.16 mg/kcal for the lowest quartile). There were no significant associations between estimated nitrate or nitrite intake and risk of non-Hodgkin's lymphoma subtypes. The Weyer et al. (2001) cohort study (21,977 subjects, 105 cases of non-Hodgkin's lymphoma, 94 cases of leukemia) did not find positive associations or elevated risk of non-Hodgkin's lymphoma or leukemia in association with dietary or drinking water nitrate.

Thyroid Cancer. Kilfoy et al. (2011) evaluated possible associations between dietary intake of nitrate and nitrite and risk of thyroid cancer in a cohort of 292,125 men (170 thyroid cancer cases) and 198,069 women (200 thyroid cancer cases) from the NIH-AARP Diet and Health Study 1995–1996. The study authors reported increased risk of thyroid cancer overall with nitrate intake among men (RR 2.28; 95% CI 1.29, 4.04; p-trend <0.01), but not women (RR 0.69; 95% CI 0.42, 1.15; p-trend 0.61). For nitrate intake among the men, thyroid cancer risk was increased by subtype as well; RR 2.10; 95% CI 1.09, 4.05; p-trend 0.05 for papillary cancer and RR 2.74; 95% CI 0.86, 8.77; p-trend 0.04 for follicular cancer. There were no significant associations between nitrite intake and risk of thyroid cancer among men or women. Aschebrook-Kilfoy et al. (2013a) evaluated possible associations between dietary intake of nitrate and nitrite and risk of thyroid cancer in a cohort of 73,317 women enrolled in the Shanghai Women's Health Study in 1996–2000 and followed-up for 11 years (164 thyroid cancer cases). The study authors reported increased risk of thyroid cancer among the group with highest nitrite intake (RR 2.05; 95% CI 1.20, 3.51). The risk was strongest for nitrite intake from processed meats (RR 1.96; 95% CI 1.28, 2.99). Nitrate intake was not associated with increased risk (RR 0.93; 95% CI 0.42, 2.07). Meta-analysis of the results from selected studies that evaluated risk of thyroid cancer with nitrate intake (Aschebrook-Kilfoy et al. 2013a; Kilfoy et al. 2011; Ward et al. 2010) or nitrite intake (Aschebrook-Kilfoy et al. 2013a; Kilfoy et al. 2011) indicated increased risk of thyroid cancer with nitrite intake (RR 1.48; 95% CI 1.09, 2.02), but not with nitrate intake (RR 1.36; 95% CI 0.67, 2.75) (Bahadoran et al. 2015).

Other Cancers. In general, case-control and cohort studies of cancers of larynx, liver, lung, mouth, pancreas, or pharynx have found no consistent associations with exposure to nitrate or nitrite (Aschebrook-Kilfoy et al. 2011; IARC 2010).

Studies of Laboratory Animals. The potential carcinogenicity of nitrate has been investigated in several animal studies that employed the oral exposure route. Studies in which negative results were reported include MCR-derived rats (15/sex/group) provided 5,000 mg sodium nitrate/L (3,650 mg nitrate/L) in the drinking water for 84 weeks and sacrificed 20 weeks later (Lijinsky et al. 1973a), male white rats provided 4,000 mg sodium nitrate in the drinking water for 273 days and sacrificed at 10 months (Pliss and Frolov 1991), strain A male mice (n=40) provided 12,300 mg sodium nitrate/L in the drinking water for 25 weeks and sacrificed 13 weeks later (Greenblatt and Mirvish 1973), female NMRI mice provided 1,000 mg calcium nitrate/L in the drinking water for 18 months (Mascher and Marth 1993), Fischer 344 rats (50/sex/group) fed diets containing up to 5% sodium nitrate (1,517–1,730 mg nitrate/kg/day) for 2 years (Maekawa et al. 1982), and ICR mice (10/sex/group) fed diets containing up to 5% sodium nitrate

for 2 years (IARC 2010). In the study of Pliss and Frolov (1991) some groups of male white rats were treated with drinking water containing 0.05% N-butyl-N-(4-hydroxybutyl)nitrosamine (BBNA, an inducer of urinary bladder cancer in laboratory animals) for 30 days, either alone or followed by 4,000 mg sodium nitrate/L drinking water for 273 days. The group treated with BBNA followed by sodium nitrate exhibited a significantly increased incidence of urinary bladder carcinoma (6/20 rats versus 1/18 rats treated with 0.05% BBNA only). These results indicate that sodium nitrate promoted BBNA-induced bladder tumors.

The potential carcinogenicity of ingested nitrite has been investigated in numerous animal studies. Nitrite treatment alone did not result in increased incidences of tumors in most studies. Nitrite doses (expressed as nitrite/kg/day) reported in this Toxicological Profile for Nitrate and Nitrite were either provided by the study authors or estimated using available body weight and oral intake data; otherwise, EPA (1988) default reference values for body weight, food consumption, and water intake were used to calculate doses.

NTP (2001) performed a cancer bioassay of male and female F344/N rats (50/sex/group) provided sodium nitrite in the drinking water for 2 years at concentrations of 0, 750, 1,500, or 3,000 ppm. Author-reported average doses were 35–130 mg sodium nitrite/kg/day (23.5–87.1 mg nitrite/kg/day) to the males and 40–150 mg sodium nitrite/kg/day (26.8–100.5 mg nitrite/kg/day) to the females. There was no evidence of sodium nitrite-induced forestomach neoplasms. Although the mid-dose group of female rats exhibited a significantly increased incidence of mammary gland fibroadenoma, the incidence in the high-dose group was not significantly different from that of controls; based on this finding and the high historical background incidence of mammary gland fibroadenomas, the incidence in the mid-dose group was not considered treatment related. Significantly decreased incidences of mononuclear cell leukemia were observed in mid- and high-dose male and female rats. It was speculated that increased methemoglobin concentrations may have played a role in the decreased incidences of mononuclear cell leukemia. Significantly increased incidence of fibroma of the subcutis was noted in mid-dose male rats; however, several factors (the incidence only slightly exceeded the historical range of NTP controls, lacked a dose-response characteristic, combined incidences of fibroma or fibrosarcoma were within the historical range for NTP controls, and fibromas and fibrosarcomas are common neoplasms in the skin of F344/N rats) suggested that the fibroma was not related to sodium nitrite exposure. NTP (2001) concluded that there was "no evidence of carcinogenic activity" of sodium nitrite in the male or female F344/N rats under the conditions of the study.

NTP (2001) also provided sodium nitrite in the drinking water of B6C3F1 mice (50/sex/group) for 2 years at concentrations of 0, 750, 1,500, or 3,000 ppm. Author-reported average doses were 60–220 mg sodium nitrite/kg/day (40.2–107.2 mg nitrite/kg/day) to the males and 45–160 mg sodium nitrite/kg/day (30.2–107.2 mg nitrite/kg/day) to the females. Female mice exhibited a significant positive trend for increased incidence of forestomach squamous cell papilloma or carcinoma (combined) and the incidence in the high-dose female mice exceeded the historical range for NTP controls; however, based on concurrent controls, incidences of squamous cell adenoma (1/50, 0/50, 1/50, and 3/50 for controls, 750, 1,500, and 3,000 ppm groups, respectively), squamous cell carcinoma (0/50, 0/50, 0/50, and 2/50 for controls, 750, 1,500, and 3,000 ppm groups, respectively), and squamous cell adenoma or carcinoma (1/50, 0/50, 1/50, and 5/50 for controls, 750, 1,500, and 3,000 ppm groups, respectively) were not statistically significantly increased for any sodium nitrite exposure group. NTP (2001) considered the positive trend for incidences of forestomach squamous cell papilloma or carcinoma (combined) in the female B6C3F1 mice to provide "equivocal evidence of carcinogenic activity" of sodium nitrite and noted that there was "no evidence of carcinogenic activity" in the male B6C3F1 mice under the conditions of the study. Incidences of alveolar/bronchiolar adenoma or carcinoma (combined) in sodium nitrite-exposed groups of female mice were slightly greater than that of controls (incidences of 1/50, 6/50, 5/50, and 6/50 for controls, 750, 1,500, and 3,000 ppm groups, respectively); however, incidences were within that of historical NTP controls. Because the incidences did not exhibit exposure concentration-response characteristics and were not accompanied by increased incidences of preneoplastic lesions, the study authors did not consider them to be sodium nitrite exposure-related effects. Significantly increased incidence of fibrosarcoma of the subcutis was noted in mid-dose female mice (incidences of 0/50, 5/50, 1/50, and 2/50 for 0, 750, 1,500, and 3,000 ppm groups, respectively) and exceeded the historical range for NTP controls; however, lack of exposure concentration-response characteristics and the fact that combined incidence of fibroma or fibrosarcoma (0/50, 5/50, 1/50, and 3/50 for 0, 750, 1,500, and 3,000 ppm groups, respectively) were within the historical range for NTP controls suggest that these neoplasms were not related to sodium nitrite exposure.

In two other studies of male and female F344 rats, addition of sodium nitrite to the drinking water at concentrations as high as 2,000–3,000 ppm for up to 2 years did not result in significant increases in tumor incidences at any site (Lijinsky 1984a, 1984b; Lijinsky et al. 1983; Maekawa et al. 1982). Conversely, incidences of mononuclear cell leukemia were significantly lower in the nitrite-treated groups relative to controls. In a 26-month study of male and female Sprague-Dawley rats provided drinking water to which up to 2,000 ppm sodium nitrite was added, the study author reported increased incidence of lymphomas, but not other types of tumors (Newberne 1979); however, IARC (2010) and

NTP (2001) noted that a working group sponsored by the U.S. FDA reevaluated the histology and did not confirm the results of Newberne (1979). IARC (2010) reported that the working group considered the incidences of lymphomas to be similar to those arising spontaneously in Sprague-Dawley rats. Shank and Newberne (1976) reported increased incidences of total tumors and lymphoreticular tumors in rats fed diet to which sodium nitrite was added at 1,000 ppm (total tumors: 58/96 versus 28/156 controls; lymphoreticular tumors: 26/96 versus 9/156 controls); the results were reported for F1 and F2 offspring that had been exposed via their mothers during gestation and lactation and directly from the diet thereafter. In a 96-week study, Iurchenko et al. (1986) reported significantly increased incidences of benign liver tumors among male CBA mice administered drinking water to which sodium nitrite was added at a concentration resulting in author-estimated total dose of 1,600 mg sodium nitrite/mouse compared to a group of untreated controls; however, there was no apparent sodium nitrite treatment-related effect at a higher estimated dose (2,000 mg sodium nitrite/mouse).

Significantly increased incidences of forestomach squamous papillomas (by the life-table method) were reported for male and female MRC Wistar rats provided drinking water to which sodium nitrite was added at 3,000 ppm on 5 days/week for life (5/22 males and 3/23 females versus 2/47 control males and 0/44 control females) (Mirvish et al. 1980). The study authors stated that the sodium nitrite-treated rats received a total dose of 63 g sodium nitrite/kg. Total numbers of rats and incidences of rats with papillomas were small.

Grant and Butler (1989) added sodium nitrite to a reduced-protein diet and administered the diet to male and female F344 rats for up to 115 weeks; a control group received reduced-protein diet alone. The study authors reported dose-related decreases in time of onset and incidence of lymphomas, mononuclear cell leukemia, and testicular interstitial-cell tumors in the nitrite-treated groups.

There was no evidence of increased tumor incidences in male or female ICR mice provided sodium nitrite in the drinking water for up to 109 weeks at concentrations as high as 0.5% (5,000 ppm sodium nitrite) (Inai et al. 1979), or in male or female Swiss mice or their offspring following a single gavage administration of 10 mg/kg nitrite and subsequent exposure to 0.1% sodium nitrite (1,000 ppm) in the drinking water during gestation days 15–21; terminal sacrifices occurred 10 months following the initiation of treatment (Börzsönyi et al. 1978). Hawkes et al. (1992) found no evidence of treatment-related effects on incidences of nervous system tumors among male and female VM mice (susceptible to spontaneous development of cerebral gliomas) provided drinking water to which sodium nitrite was

added at 0.2% (2,000 ppm) from weaning for a lifetime and others exposed via their mothers during gestation and lactation as well.

The potential carcinogenicity of combined exposure to sodium nitrite and selected nitrosatable substances (oral exposures via combinations of drinking water, diet, and/or gavage dosing) has been well-studied in laboratory animals. Many of the studies included sodium nitrite-only treatment groups for which there was no evidence of sodium-nitrite induced carcinogenicity (Anderson et al. 1985; Börzsönyi and Pintér 1977; Börzsönyi et al. 1976; Greenblatt and Lijinsky 1972, 1974; Greenblatt and Mirvish 1973; Greenblatt et al. 1971, 1973; Hirose et al. 2002; Ivankovic 1979; Ivankovic and Preussman 1970; Kitano et al. 1997; Murthy et al. 1979; Lijinsky 1984a, 1984b; Lijinsky and Reuber 1980; Mirvish et al. 1972; Miyauchi et al. 2002; Rijhsinghani et al. 1982; Scheunig et al. 1979; Taylor and Lijinsky 1975a, 1975b; van Logten et al. 1972; Yada et al. 2002; Yoshida et al. 1993, 1994). However, Lijinsky et al. (1983) reported significantly increased incidences of hepatocellular neoplasms in female (but not male) F344 rats administered diet to which sodium nitrite was added at 2,000 ppm for 2 years; significantly decreased incidences of mononuclear-cell leukemia was observed as well.

Significantly increased incidences of selected tumor types were observed in some studies of laboratory animals that employed coexposure to various amino compounds and sodium nitrite (Anderson et al. 1985; Börzsönyi and Pintér 1977; Börzsönyi et al. 1976, 1978; Chan and Fong 1977; Greenblatt and Mirvish 1973; Greenblatt et al. 1971; Hirose et al. 1990; Iurchenko et al. 1986; Ivankovic 1979; Ivankovic and Preussmann 1970; Kawabe et al. 1994; Murthy 1979; Lijinsky 1984a, 1984b; Lijinsky and Reuber 1980; Lijinsky and Taylor 1977; Lijinsky et al. 1973b; Lin and Ho 1992; Maekawa et al. 1977; Mirvish et al. 1972, 1976, 1980; Miyauchi et al. 2002; Mokhtar et al. 1988; Newberne and Shank 1973; Nishiyama et al. 1998; Nixon et al. 1979; Oka et al. 1974; Rijhsinghani et al. 1982; Rustia and Shubik 1974; Scheunig et al. 1979; Shank and Newberne 1976; Tahira et al. 1988; Taylor and Lijinsky 1975a, 1975b; Weisburger et al. 1980; Xiang et al. 1995; Yada et al. 2002; Yamamoto et al. 1989; Yoshida et al. 1993, 1994). These results were typically attributed to *in vivo* nitrosation of amines by nitrite to produce carcinogenic N-nitrosoamines; some of the studies did not include sodium nitrite-only treatment groups. Addition of sodium nitrite or potassium nitrite to the food of rats in three other studies resulted in increased incidences of selected tumors; analysis of the food revealed the presence of N-nitroso compounds (likely formed by nitrosation in the presence of nitrite and selected amine compounds in the food), which were considered the probable principal cause of the tumors (Aoyagi et al. 1980; Matsukura et al. 1977; Olsen et al. 1984). Börzsönyi et al. (1978) reported 30–70% incidences of malignant lymphomas, lung adenomas, and hepatomas among maternal mice and their offspring following gavage treatment of the dams with the

fungicide, dodecylguanidine acetate, together with 0.05% sodium nitrite; the frequency of spontaneous tumors in untreated controls was 6%. Dodecylguanidine acetate alone had no effect on cancer incidence. Lijinsky et al. (1973a) found no significant increase in tumor incidences among male and female MCR rats provided drinking water comprised of 0.5% nitrilotriacetic acid or iminodiacetic acid and 0.2 or 0.5% sodium nitrite on 5 days/week for a lifetime.

There were no signs of treatment-related effects on incidences of tumors at any site among groups of pregnant Syrian golden hamsters and their offspring fed diets to which sodium nitrite and/or morpholine were added throughout production of an F2 generation (Shank and Newberne 1976). Fresh diet was prepared every 2–7 days and 25% of the initial concentration of sodium nitrite was lost during 7 days after preparation of the diet.

Based on available human data, IARC (2010) determined that there is *inadequate evidence* for the carcinogenicity of nitrate in food or drinking water and *limited evidence* for the carcinogenicity of nitrite in food (based on association with increased incidence of stomach cancer). Evaluation of available animal data by IARC (2010) resulted in the determination that there is *inadequate evidence* for the carcinogenicity of nitrate, *limited evidence* for the carcinogenicity of nitrite *per se*, and *sufficient evidence* for the carcinogenicity of nitrite in combination with amines or amides. The overall conclusions of IARC (2010) were that "ingested nitrate and nitrite under conditions that result in endogenous nitrosation is *probably carcinogenic to humans (Group 2A)*." IARC (2010) noted that: (1) the endogenous nitrogen cycle in humans includes interconversion of nitrate and nitrite; (2) nitrite-derived nitrosating agents produced in the acid stomach environment can react with nitrosating compounds such as secondary amines and amides to generate N-nitroso compounds; (3) nitrosating conditions are enhanced upon ingestion of additional nitrate, nitrite, or nitrosatable compounds; and (4) some N-nitroso compounds are known carcinogens.

The U.S. EPA IRIS (2002) does not include a carcinogenicity evaluation for nitrate or nitrite.

3.2.3 Dermal Exposure

No relevant information was located regarding the following effects in humans or animals exposed to nitrate or nitrite via the dermal route:

3.2.3.1 Death
3.2.3.2 Systemic Effects
3.2.3.3 Immunological and Lymphoreticular Effects
3.2.3.4 Neurological Effects
3.2.3.5 Reproductive Effects
3.2.3.6 Developmental Effects
3.2.3.7 Cancer

3.3 GENOTOXICITY

No studies were located regarding genotoxicity in human populations exposed to exogenous nitrite. Limited information is available for nitrate. Kleinjans et al. (1991) examined the association between nitrate levels in drinking water and frequency of sister chromatid exchanges (SCEs) in peripheral lymphocytes from women from the Netherlands. Three groups were formed, low- (n=30), medium- (n=30), and high- (n=18) exposure groups, based on the levels of nitrate in their drinking water. The corresponding nitrate levels were 0.13, 32.0, and 133.5 mg/L. Regression analysis showed a good correlation between levels on nitrate in water and nitrate body burden monitored by 24-hour urine levels of nitrate. Examination of peripheral lymphocytes showed no significant association between 24-hour urine excretion of nitrate and frequency of SCEs. Another study examined the frequency of hypoxanthine-guanine phosphoribosyltransferase (HPRT) variants (an index of genetic risk) in peripheral lymphocytes in groups of women from the Netherlands in relation to levels of nitrate in drinking water (van Maanen et al. 1996a). A total of 50 subjects were exposed to concentrations of nitrate of 0.02 mg/L (n=14), 17.5 mg/L (n=21), 25 mg/L (n=6), or 135 mg/L (n=9). The two lower concentrations were from PWS, whereas the two highest originated from private wells. Analysis of 24-hour urine samples showed a positive correlation between nitrate in drinking water and urinary nitrate. Also, salivary nitrate and nitrite were similarly increased. Results of multiple regression analysis showed that the mean log frequency of HPRT variants was significantly higher in the group exposed to 25 mg/L nitrate than in the groups exposed to 0.02 and 17.5 mg/L nitrate. The analyses also showed a significant correlation between frequency of HPRT variants and 24-hour urinary nitrate and salivary nitrite levels and between 24-hour urinary excretion of N-nitrosopyrrolidine and 24-hour urinary excretion of nitrate. The results suggested that drinking water with nitrate poses a genetic risk due to the potential formation of nitrosamines after endogenous reduction of nitrate to nitrite and reaction with amino compounds. A third

study examined the frequency of SCEs and chromosomal aberrations in peripheral blood lymphocytes from70 male and female Greek children (12–15 years of age) who were exposed to high nitrate in drinking water (55.7–88.0 mg/L) (Tsezou et al. 1996). Controls consisted of 20 children from areas with low nitrate content in the drinking water (0.70 mg/L). No measurements of nitrate or nitrite in biological fluids were conducted in this study. Analyses of the results showed a significant increase in chromatid and chromosome breaks in children exposed to nitrate levels ≥70.5 mg/L of drinking water. However, levels of SCEs showed no significant increase with increasing nitrate levels. IARC (2010) noted that the possibility that chemicals other than nitrate could have been responsible for the elevated chromosomal aberrations could not be ruled out.

A limited number of studies have examined the *in vivo* genotoxicity of nitrate in laboratory animals. Gavage administration of up to 500 mg/kg/day sodium nitrate to pregnant Syrian Golden hamsters on gestation days 11 and 12 did not significantly affect the frequency of micronuclei, chromosomal aberrations, morphological or malignant cell transformation, or drug-resistant mutations in embryonic cells (Inui et al. 1979). In another *in vivo* study, oral administration of 150 mg/kg sodium nitrate (only dose tested) to male Swiss mice did not inhibit testicular DNA synthesis measured 3.5 hours after dosing (Friedman and Staub 1976). Gavage administration of up to 2,120 mg/kg/day sodium nitrate for 2 days to male Wistar rats did not induce chromosomal aberrations in bone marrow cells examined 24 hours after the last dose (Luca et al. 1985). A similar experiment with male Swiss mice showed induction of chromosomal aberrations at 706.6 mg/kg/day sodium nitrate but not at 2,120 mg/kg/day (Luca et al. 1985). Daily administration of ≥78.5 mg/kg sodium nitrate for 2 weeks to rats resulted in a significant dose-dependent increase in chromosomal aberrations in bone marrow cells 24 hours after the last dose (Luca et al. 1985). Evaluation of micronuclei in mice treated daily for 2 weeks showed significant increases (approximately 2-fold greater than controls) at the low concentrations tested, 78.5 and 235.5 mg/kg/day sodium nitrate, but not at 706.6 or 2,120 mg/kg/day, which the investigators attributed to possible induction of cytotoxic effects (Luca et al. 1985). Alavantić et al. (1988a) treated male mice with sodium nitrate by gavage for 3 days at doses of 0, 600, or 1,200 mg/kg/day; there was no sign of treatment-related unscheduled DNA synthesis in spermatids analyzed 17 days following treatment. Alavantić et al. (1988b) treated male mice with sodium nitrate by gavage for 2 weeks doses of 0, 600, or 1,200 mg/kg/day and subsequently mated them to virgin females; evaluation of primary spermatocytes from F1 males revealed no sign of treatment-related heritable translocations.

In studies *in vitro*, neither potassium nitrate nor sodium nitrate in concentrations of up to 20 and 5 mg/plate, respectively, was mutagenic in various strains of *Salmonella typhimurium* (TA92, TA94,

TA98, TA100, TA1535) (Ishidate et al. 1984), tested with and without metabolic activation. Lanthanum nitrate hexahydrate also yielded negative results in *S. typhimurium* strains TA100 and TA1535 (Zeiger et al. 1992). Tests for chromosomal aberrations in Chinese hamster fibroblast cells were positive for sodium nitrate, but negative for potassium nitrate (Ishidate et al. 1984). IARC (2010) noted that since sodium chlorite also yielded positive results in the same assay, the chromosomal aberrations induced by sodium nitrate could have been due to the high osmotic pressure and sodium ion concentration. In another study, incubation of Chinese hamster ovary cells with up to 10 mM ammonium nitrate for up to 24 hours in the presence of metabolic activation or up to 48 hours without metabolic activation did not induce chromosomal aberrations (Kim et al. 2011).

Several studies have examined the *in vivo* genotoxicity of nitrite using a variety of tests, a summary is shown in Table 3-3. The results have been mixed, and at times inconsistent, between laboratories that used the same tests. Administration of up to 7.3 mg sodium nitrite to pregnant mice (~290 mg/kg assuming 0.025 kg body weight) on gestation days 7–18 via the drinking water did not induce chromosomal aberrations in maternal bone marrow cells or in fetal liver cells (Shimada 1989). Negative results for chromosomal aberrations were also reported in embryonic hamster cells after administration of a single dose of up to 500 mg/kg sodium nitrite on gestation day 11 or 12 (Inui et al. 1979). However, significantly increased incidences of chromosomal aberrations were reported in bone marrow cells from male rats (ca. 2.1–2.4 times greater than controls), mice (ca. 4–5 times greater than controls), and rabbits (ca. 2–3.6 times greater than controls) dosed with ≥1.7 mg/kg sodium nitrite (Luca et al. 1987). Rats and mice were dosed twice by gavage, whereas rabbits received sodium nitrite via the drinking water for 3 months. No dose-response was apparent in the studies by Luca et al. (1987) over an approximately 27-fold dose range, suggesting that maximum response was already achieved with the lowest dose, 1.7 mg/kg. Sodium nitrite also induced micronuclei in polychromatic erythrocytes of mice dosed twice at ≥1.7 mg/kg (Luca et al. 1987) and in embryonic hamster cells after a single administration of 250 mg/kg sodium nitrite to the pregnant dams (Inui et al. 1979). However, in another study (NTP 2001), sodium nitrite did not induce micronuclei in male rat or mouse bone marrow cells after three intraperitoneal injections at nonlethal doses up to 50 mg/kg/day (rats) and 125 mg/kg/day (mice). Evaluation of SCEs also provided seemingly conflicting results. In a study by Giri et al. (1986), single doses of ≥5 mg/kg sodium nitrite by gavage induced dose-related significant increases in SCEs in mouse bone marrow cells, but Bambrilla et al. (1983) reported that a single gavage dose of 80 mg/kg sodium nitrite did not induce SCEs in mouse bone marrow cells. Results from assays for DNA repair, DNA damage, or DNA synthesis in mammalian cells from rats or mice generally yielded negative results (Bambrilla et al. 1983; Friedman and Staub 1976; Hellmér and Bolcsfoldi 1992; Robbiano et al. 1990). Sodium nitrite induced

Table 3-3. Genotoxicity of Sodium Nitrite *In Vivo*

Species (test system)	End point	Results	Reference
Mammalian cells:			
Pregnant mouse bone marrow cells	Chromosomal aberrations	−	Shimada 1989
Mouse fetal liver cells	Chromosomal aberrations	–	Shimada 1989
Embryonic hamster cells	Chromosomal aberrations	−	Inui et al. 1979
Rat bone marrow cells	Chromosomal aberrations	+	Luca et al. 1987
Mouse bone marrow cells	Chromosomal aberrations	+	Luca et al. 1987
Rabbit bone marrow cells	Chromosomal aberrations	+	Luca et al. 1987
Mouse polychromatic erythrocytes	Micronuclei	+	Luca et al. 1987
Rat bone marrow cells	Micronuclei	−	NTP 2001
Mouse bone marrow cells	Micronuclei	−	NTP 2001
Embryonic hamster cells	Micronuclei	+	Inui et al. 1979
Embryonic hamster cells	Malignant cell transformation	+	Inui et al. 1979
Embryonic hamster cells	Drug-resistant mutations	+	Inui et al. 1979
Mouse bone marrow cells	Sister chromatid exchange	+	Giri et al. 1986
Mouse bone marrow cells	Sister chromatid exchange	−	Brambrilla et al. 1983
Mouse host-mediated assay	Mutations in Salmonella	−	Couch and Friedman 1975
Mouse host-mediated assay	DNA repair in *E. coli* K-12 *uvrb*/*rec*A	−	Hellmér and Bolcsfoldi 1992
Rat liver cells	DNA damage	−	Robbiano et al. 1990
Rat liver and gastric mucosa cells	DNA damage	−	Brambrilla et al. 1983
Mouse testicular cells	DNA synthesis	−	Friedman and Staub 1976
Male mouse germ cells	Unscheduled DNA synthesis	−	Alavantić et al. 1988a
Male mouse germ cells	Heritable translocations	−	Alavantić et al. 1988b
Insect systems:			
Drosophila melanogaster (wing spot test)	Somatic mutation	+	Graf et al. 1989

+ = positive results; – = negative results

malignant cell transformation and produced drug-resistant mutations in embryonic hamster cells following treatment of the pregnant dams on gestation day 11 or 12 with a single dose of ≥125 mg/kg (Inui et al. 1979). Alavantić et al. (1988a) treated male mice with sodium nitrite by gavage for 3 days at doses of 0, 60, or 120 mg/kg/day; there was no sign of treatment-related unscheduled DNA synthesis in spermatids analyzed 17 days following treatment. Alavantić et al. (1988b) treated male mice with sodium nitrite by gavage for 2 weeks doses of 0, 60, or 120 mg/kg/day and subsequently mated them to virgin females; evaluation of primary spermatocytes from F1 males revealed no sign of treatment-related heritable translocations. In a host-mediated assay, mice were intraperitoneally inoculated with *S. typhimurium* strain G46 and gavaged with sodium nitrite (Couch and Friedman 1975); the sodium nitrite treatment did not induced increased frequency in *S. typhimurium* mutation rate in this host-mediated assay. Finally, feeding sodium nitrite to larvae of *Drosophila melanogaster* induced somatic mutations as assessed by the wing spot test (Graf et al. 1989).

Numerous studies have examined the genotoxicity of nitrite in *in vitro* assays. As shown in Table 3-4, there seem to be more positive results than negative results in tests of gene mutations in prokaryotic organisms, but it is difficult to draw a firm conclusion (Andrews et al. 1980, 1984; Balimandawa et al. 1994; Brams et al. 1987; De Flora 1981, De Flora et al. 1984; Ehrenberg et al. 1980; Ishidate et al. 1981, 1984; McCann et al. 1975; Törnqvist et al. 1983; Zeiger et al. 1992). However, it appears that the addition of metabolic activation systems to the incubation mixtures did not make a difference in the results. That is, tests that were positive without activation were also positive with activation; tests that were negative without activation were also negative with activation. This would indicate that nitrite can be a direct mutagenic chemical. *In vitro* tests that assessed chromosomal aberrations, SCEs, DNA repair, and cell transformations in sodium nitrite-treated mammalian cells yielded positive results (Inoue et al. 1985; Ishidate et al. 1984; Luca et al. 1987; Lynch et al. 1983; Tsuda and Kato 1977; Tsuda et al. 1973, 1981). Nitrite enhanced neutrophil-induced DNA strand breakage in rat lung type II epithelial cells; the enhancement was associated with an inhibition of neutrophil-derived myeloperoxidase (Knaapen et al. 2005).

3.4 TOXICOKINETICS

No information was located regarding the pharmacokinetics of nitrate or nitrite following inhalation or dermal exposure. However, numerous reports are available regarding the pharmacokinetics of ingested nitrate and nitrite. Comprehensive reviews of the available data (Bailey et al. 2012; Bryan and van Grinsven 2013; IARC 2010; JECFA 2003a, 2003b; Lundberg and Weitzberg 2013; Lundberg and Govoni

Table 3-4. Genotoxicity of Sodium Nitrite *In Vitro*

Species (test system)	End point	Results		Reference
		With activation	Without activation	
Prokaryotic organisms:				
Salmonella typhimurium TA98	Gene mutation	–	–	NTP 2001; Zeiger et al. 1992
S. typhimurium TA100	Gene mutation	+	+	NTP 2001; Zeiger et al. 1992
S. typhimurium TA98, TA100, TA1537	Gene mutation	+	+	Ishidate et al. 1981
S. typhimurium TA100, TA1535	Gene mutation	+	+	Ishidate et al. 1984
S. typhimurium TA98, TA100, TA1535, TA1537, TA1538	Gene mutation	−	−	Andrews et al. 1980, 1984
S. typhimurium TA100, TA1530, TA1535	Gene mutation	+	+	Balimandawa et al. 1994
S. typhimurium TA102, YG1024, DJ400, DJ460	Gene mutation	−	−	Balimandawa et al. 1994
S. typhimurium TA100	Gene mutation	+	NT	Brams et al. 1987
S typhimurium TA97, TA98	Gene mutation	−	NT	Brams et al. 1987
S. typhimurium TA1530	Gene mutation	NT	+	Ehrenberg et al. 1980
S typhimurium TA100, TA1535	Gene mutation	–[a]	+	De Flora 1981, 1984
S typhimurium TA98, TA1537, TA1538	Gene mutation	−	−	De Flora 1981, 1984
S. typhimurium TA1535	Gene mutation	NT	(+)	McCann et al. 1975
Escherichia coli WP2, WP67, CM871	DNA repair	+	+	De Flora et al. 1984
Eukaryotic organisms:				
Cultured human lymphocytes	Sister chromatid exchange	NT	+	Inoue et al. 1985
Chinese hamster ovary cells	Sister chromatid exchange	NT	+	Tsuda et al. 1981
Chinese hamster ovary cells	Chromosomal aberrations	NT	+	Tsuda et al. 1981
Monkey BS-C-1 fetal liver cells	Chromosomal aberrations	NT	+	Luca et al. 1987
HeLa cells	Chromosomal aberrations	NT	+	Luca et al. 1987
Chinese hamster fibroblasts	Chromosomal aberrations	NT	+	Ishidate et al.1984
Syrian hamster embryo cells	Chromosomal aberrations	NT	+	Tsuda and Kato 1977

Table 3-4. Genotoxicity of Sodium Nitrite *In Vitro*

Species (test system)	End point	Results		Reference
		With activation	Without activation	
HeLa S3 carcinoma cells	DNA repair	NT	+	Lynch et al. 1983
Syrian hamster embryo cells	Cell transformation	NT	+	Tsuda et al. 1973

[a]Reported as a decrease in mutagenicity in the presence of S9 mix; however, it was not specified whether the decrease was relative to controls or sodium nitrite treatment in the absence of S9 mix.

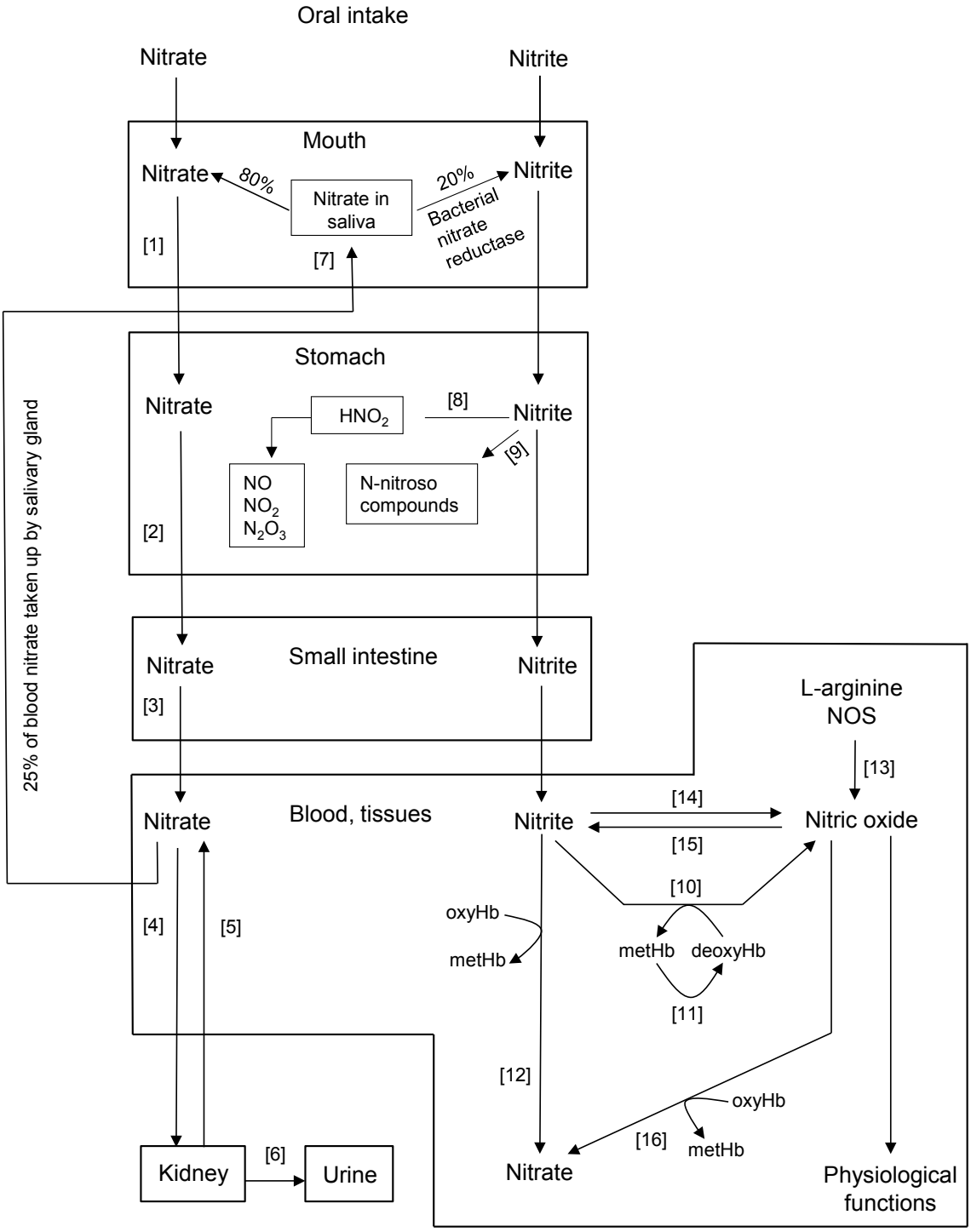
+ = positive results; (+) = weakly positive; – = negative results; DNA = deoxyribonucleic acid; NT = not tested

2004; Lundberg et al. 2008, 2009; Weitzberg and Lundberg 2013; Weitzberg et al. 2010; WHO 2011b) serve as references for the major portion of toxicokinetic data presented in this section of the ATSDR Toxicological Profile for Nitrate and Nitrite.

Ingestion is the major source of exposure to nitrate and nitrite. Vegetables are the main source of nitrate in the diet (approximately 60–80% of total nitrate intake); nitrate in some drinking water sources may contribute 15–20% of total nitrate intake. Small amounts of nitrate and nitrite are added to some animal-based products to serve as preservatives and to enhance taste. Approximately 80–85% of nitrite in humans is produced from *in vivo* reduction of nitrate.

The nitrate-nitrite-nitric oxide pathway in mammals includes a dietary component and an endogenous component. Figure 3-2 depicts the metabolic pathways for ingested nitrate and nitrite, as well as the endogenous production of nitric oxide via nitric oxide synthase (NOS). Numbers in brackets in the figure coincide with those in the following description of the pathways. Ingested nitrate passes through the stomach [1] to the small intestine [2] where it is nearly completely absorbed into the blood [3]. Following a nitrate-containing meal, circulating nitrate concentrations are normally in the range of 20–40 μM, depending on the type of diet and activity of nitric oxide synthases. Peak plasma nitrate levels are reached 15–30 minutes following ingestion; the half-time of plasma nitrate is on the order of 5–6 hours. Most nitrate that passes through the kidney [4] is reabsorbed into the blood [5]. However, some is excreted in the urine [6]. In humans, approximately 25% of plasma nitrate is taken up by the salivary glands and secreted in the saliva [7]; concentrations salivary nitrate can be as much as 10–20 times that of plasma nitrate. Approximately 20% of the nitrate in saliva undergoes anaerobic, nitrate reductase-catalyzed reduction to nitrite by commensal bacteria; thus, salivary secretion and reduction in saliva results in conversion of approximately 5% of ingested nitrate to nitrite (Gangolli et al. 1994; Walker 1996). *In vitro* results using selected rat and mouse tissues and human liver tissues suggest a possible metabolic pathway whereby some plasma nitrate could be reduced to nitrite by enzymes such as xanthine oxidase (Jansson et al. 2008). Most salivary nitrate, however, passes to the small intestine and is absorbed into the blood. A portion of nitrite (either produced from reduction of nitrate or ingested from food sources) that enters the stomach is rapidly protonated to nitrous acid (HNO_2), which decomposes spontaneously to nitric oxide and other biologically active nitrogen oxides (e.g., nitrogen dioxide [NO_2]; dinitrogen trioxide [N_2O_3]) in the acid environment of the stomach [8]; this process is enhanced in the presence of reducing compounds such as ascorbic acid and polyphenols. Nitrite can also react with proteins, amines, and amides in the stomach. Reaction of nitrite with some low-molecular-weight amines

Figure 3-2. The Nitrate-Nitrite-Nitric Oxide Cycle in Humans*



*Numbers in brackets coincide with those in the descriptive text.

deoxyHb = deoxyhemoglobin; HNO_2 = nitrous acid; metHb = methemoglobin; NO = nitric oxide; NO_2 = nitrogen dioxide; N_2O_3 = dinitrogen trioxide; NOS = nitric oxide synthase; oxyHb = oxyhemoglobin

(nitrosation) produces N-nitroso derivatives [9], including carcinogenic compounds, portions of which can be absorbed and distributed via systemic circulation. However, most nitrite passes to the small intestine where it is absorbed into the blood. Plasma levels of nitrite increase within 30 minutes following ingestion of nitrate. Although the biological half-time of plasma nitrite is only 20–30 minutes, plasma levels remain elevated for several hours due to the enterosalivary circulation of nitrate. Plasma nitrite concentrations, which are normally 50–100 nM, may increase as much as 5 times after a nitrate-rich meal. The production of N-nitroso derivatives from plasma nitrite occurs to some extent in selected tissues.

Nitrite in the blood and tissues can be reduced to nitric oxide, which is involved in a variety of physiological processes. In the presence of deoxyhemoglobin, reduction of nitrite to nitric oxide occurs via oxidation of ferrous (Fe^{2+}) hemoglobin (which transports oxygen) to ferric (Fe^{3+}) hemoglobin (methemoglobin, a poor transporter of oxygen) [10]. Methemoglobin is converted to deoxyhemoglobin [11] in a reaction catalyzed by methemoglobin reductase. Nitrite can also react with oxyhemoglobin to form nitrate and methemoglobin [12].

In addition to exogenous sources of nitrate and nitrite (e.g., diet), nitrate, nitrite, and nitric oxide are produced endogenously. A major endogenous production mechanism is oxygen-dependent reduction of L-arginine (a biologically-relevant amino acid) to nitric oxide [13], which occurs in most cells of the body in the presence of nicotinamide adenine dinucleotide phosphate (NADPH) and cofactors flavin adenine dinucleotide (FAD), tetrahydrobiopterin (BH_4), heme, and calmodulin. Nitric oxide is involved in a variety of physiological functions that include regulation of blood flow, platelet function, pulmonary function, nerve function, host defense, and metabolic control. Nitric oxide may also be formed via an oxygen-independent one-electron reduction of nitrite in acidic and hypoxic tissues [14]. Nitrite may serve as an important source of nitric oxide under such acidic and hypoxic conditions because the half-time for plasma nitrite (15–20 minutes) is much longer than that of nitric oxide (<6 seconds). Nitric oxide is rapidly oxidized to nitrite in the presence of oxygen and ceruloplasmin [15]. Nitric oxide can also react with oxyhemoglobin to form nitrate and methemoglobin [16]. Various physiological processes are involved in maintaining a balance between systemic levels of nitrate, nitrite, and nitric oxide. The endogenous nitrate-nitrite-nitric oxide pathway provides baseline levels of nitrate and nitrite in the body which are supplemented by dietary intake. The total plasma nitrate and nitrite content consists of portions entering the blood from oral intake and portions generated endogenously from nitric oxide in the body.

As much as 60–75% of plasma nitrate is excreted unchanged in the urine within 24 hours following ingestion. Under normal physiological conditions, nitrite is not detected in the urine and its presence in urine is an indication of infection by nitrate-reducing organisms. Zhou et al. (2014) reported increased urinary excretion of N-nitroso compounds following ingestion of nitrite from the drinking water of rats. Minor urinary products of nitrate and nitrite metabolism include ammonia and urea. Nitrate and nitrite are secreted to some extent in breast milk and perspiration. Fecal excretion of nitrate and nitrite is negligible.

3.4.1 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 (Clewell 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 and Krishnan 1994; Andersen et al. 1987). These models are biologically and mechanistically based and can 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 parameterization, (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.

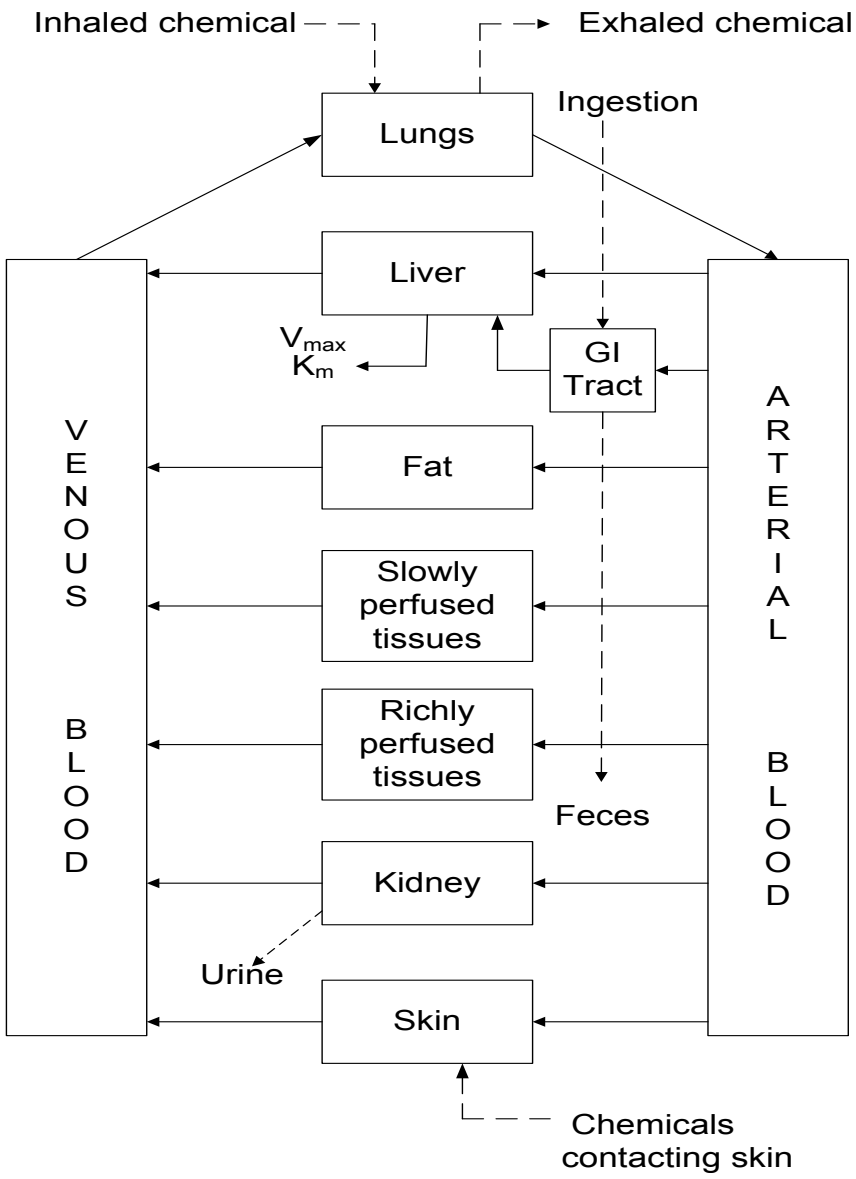
The structure and mathematical expressions used in PBPK models significantly simplify the true complexities of biological systems. However, if the uptake and disposition of the chemical substance(s) are adequately described, this simplification is desirable because data are often unavailable for many 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 3-3 shows a conceptualized representation of a PBPK model.

If PBPK models for nitrate and nitrite exist, the overall results and individual models are discussed in this section in terms of their use in risk assessment, tissue dosimetry, and dose, route, and species extrapolations.

Kinetics of absorption of nitrate from the gastrointestinal tract and elimination in urine can be described mathematically with simple one-compartment first-order models (Schultz et al. 1985; Wagner et al. 1983). The complex kinetics of salivary secretion of nitrate, reduction and absorption in the gastrointestinal tract, and binding to hemoglobin and formation of methemoglobin have been described with a multicompartment model (Zeilmaker et al. 1996, 2010b).

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



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.

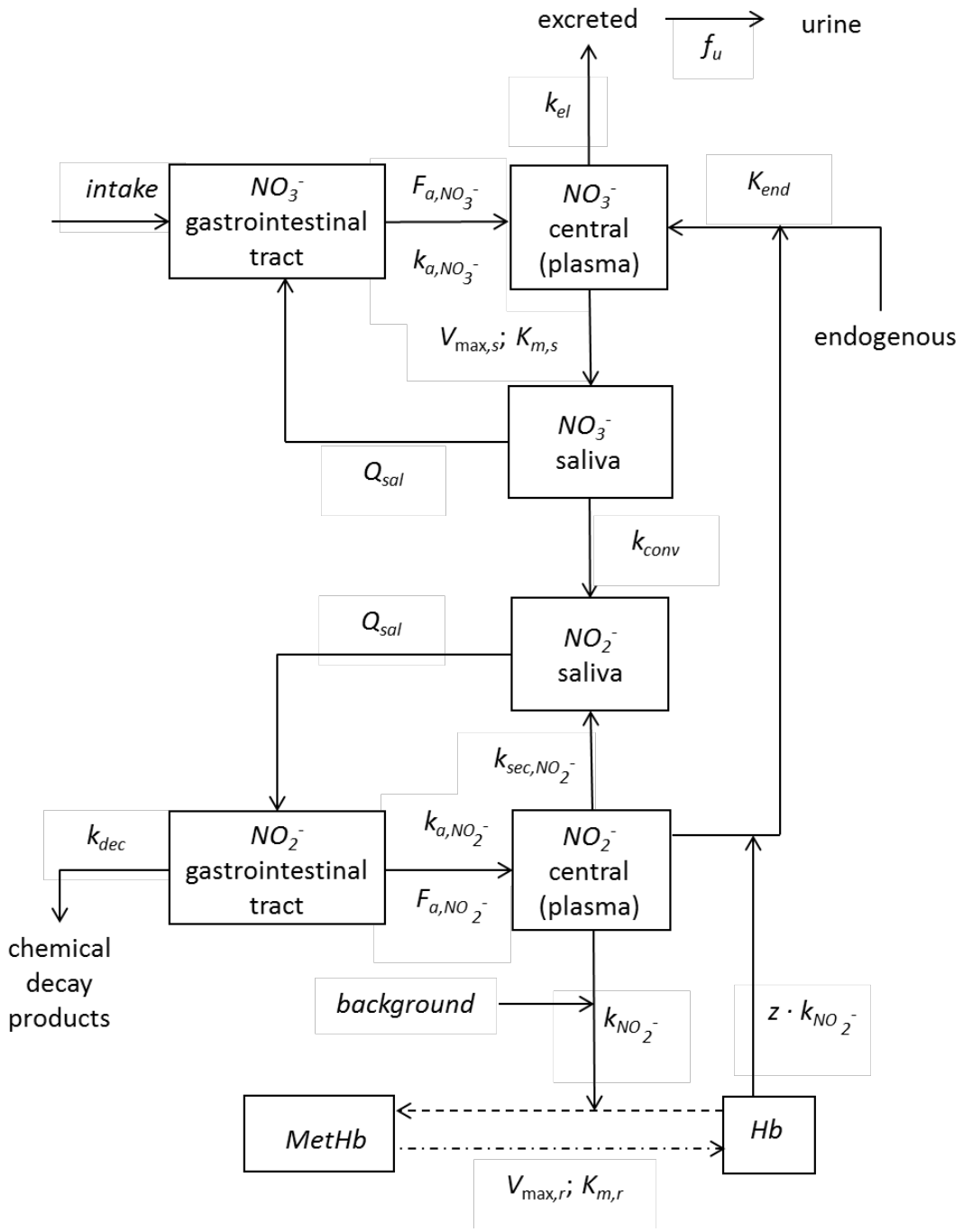
Source: adapted from Krishnan and Andersen 1994

The Zeilmaker et al. (1996, 2010b) Model

Description of the Model. Zeilmaker et al. (1996, 2010b) developed a PBPK model for simulating kinetics of methemoglobin formation resulting from absorption of nitrate in adult humans. The structure of the model is depicted in Figure 3-4. Parameters and parameter values for the model are presented in Table 3-5.

The model simulates absorption of nitrate from the gastrointestinal tract as a first-order transfer to a central nitrate distribution compartment, which is assumed to be in equilibrium with blood plasma ($k_{a,NO3}$, $hour^{-1}$). The fraction of ingested nitrate that is absorbed is assumed to be 100% (F_a=1). The model also simulates delivery of endogenously produced nitrate to blood (zero-order K_{end} = 162 mg NO_3/24 hours). Absorbed nitrate is eliminated from the central compartment by excretion into urine, metabolism (tissues and gastrointestinal bacteria), and secretion into saliva. The metabolism and urinary pathways are combined in the model into a single first-order pathway (k_{el}, $hour^{-1}$), a fraction of which goes to urine (f_u=0.56). Secretion of nitrate into saliva is simulated as a separate pathway. Secretion occurs by capacity-limited transport mediated by a sodium/iodide (Na^+/I^- symporter, NIS) in the salivary gland epithelium. Although the NIS has limited capacity for nitrate, relatively large nitrate doses and blood nitrate concentrations are required to exceed linear blood-to-saliva kinetics *in vivo*, indicative of saturation of the carrier. The model can simulate salivary secretion of nitrate as either a first-order process ($k_{sec,NO3}$, $hour^{-1}$), or a capacity-limited process (K_m, mM; $C_{s,max,NO3}$, mg/L), depending on the dose (<1,000 mg/70 kg; 14 mg/kg) or plasma nitrate concentration (<34 mg NO_3/L). Nitrate is eliminated from saliva by transfer to the gastrointestinal tract (flow-limited *B*, L/hour) or reduction to nitrite (first-order k_{conv}, $hour^{-1}$). Nitrite in saliva undergoes transfer to the gastrointestinal tract (flow-limited *B*, L/hour), from where it can be absorbed into blood (first-order $k_{a,NO2}$, $hour^{-1}$) or be converted to other metabolites and reaction products (first-order k_{dec}, $hour^{-1}$). Nitrite in blood is secreted into saliva (first-order $k_{sec,NO2}$, $hour^{-1}$) or reacts with hemoglobin to produce methemoglobin (first-order k_{NO2}, $hour^{-1}$) and nitrate. Methemoglobin is regenerated as a product of methemoglobin reductase (capacity-limited $K_{m,r}$, mM). Nitrate formed in the reaction of nitrite with hemoglobin is returned to blood (first-order $z \cdot k_{NO2}$, $hour^{-1}$). Background production of methemoglobin from reactants other than nitrite is accounted for as a background concentration of reactants (C_{bg}, mM), which combines additively with the concentration of nitrite (C_{NO2}, mM) to react with hemoglobin.

Figure 3-4. Structure of the Zeilmaker et al. (1996, 2010b) Model*



*See Table 3-5 for explanation of symbols; solid lines = mass flows; dotted lines = functional relationships

Hb = hemoglobin; MetHb = methemoglobin

Source: Adapted from Zeilmaker et al. (2010b)

Table 3-5. Parameter Values for the Zeilmaker et al. (1996, 2010) PBPK Model of Nitrate and Nitrite in Humans

Parameter	Value (standard deviation or range)
Physiological parameters	
Volume of saliva compartment (V_s)	0.001 L
Salivary flow (B)	0.069 L/hour (0.042–0.120)
Nitrate parameters	
Volume fraction (of body weight) of central nitrate distribution compartment (V_{NO3})	0.30 (0.29–0.33)
Nitrate dose averaging time (Δt)	0.1 hours (drinking water); 0.8 hours (vegetables)
Nitrate gastrointestinal absorption rate ($k_{a,NO3}$)	>5 $hour^{-1}$
Nitrate gastrointestinal absorption fraction ($F_{a,NO3}$)	1
Nitrate endogenous production (K_{end})	162 mg/24 hours
Nitrate elimination rate (k_{el})	0.14±0.01 $hour^{-1}$
Nitrate urinary elimination fraction (f_u)	0.56±0.029
Nitrate blood-to-saliva secretion rate ($k_{sec,NO3}$)	0.045±0.003 $hour^{-1}$
Nitrate blood-to-saliva half-maximum ($K_{M,s}$)	104 mg/L
Nitrate blood-to-saliva maximum ($C_{max,s}$)	2,258 mg/L
Nitrate-to-nitrite conversion rate in saliva (k_{conv})	19.95±1.75 $hour^{-1}$
Nitrite parameters	
Volume fraction (of body weight) of central nitrite distribution compartment (V_{NO2})	0.65±0.03
Nitrite gastrointestinal absorption rate ($k_{a,NO2}$)	>5 $hour^{-1}$
Nitrite gastrointestinal absorption fraction ($F_{a,NO2}$)	1
Nitrite blood-to-saliva secretion rate ($k_{sec,NO2}$)	0.045±0.003 $hour^{-1}$
Nitrite gastrointestinal conversion rate to other products (k_{dec})	0.67 $hour^{-1}$ (at pH 1.5)
Hemoglobin/methemoglobin parameters	
Nitrite reaction rate with hemoglobin (k_{NO2})	4.23±0.15 $mM^{-1}hour^{-1}$
Methemoglobin reductase half maximum ($K_{M,r}$)	0.012±0.0018 mM
Methemoglobin reductase maximum ($V_{max,r}$)	4.23±0.15 mM/hour
Stoichiometric constant for regeneration of nitrate from methemoglobin (z)	0.5±0.01
Hemoglobin concentration in blood (C_{Hg})	8 mM
Background methemoglobin concentration in blood ($C_{MetHb,bg}$)	0.03 mM
Background concentration of hemoglobin oxidizing reactants in blood(C_{bg})	0.0058 mM

[a]Based on Zeilmaker et al. (2010b)

Following ingestion of nitrate in a given medium (e.g., drinking water or vegetables), the ingested nitrate dose is assumed to enter the absorption compartment at a rate (mg/hour) given by the oral dose (mg) divided by a dose averaging time, Δt (hour), where the parameter, Δt, is assigned a value specific for the ingested medium.

Sources for model parameter estimates are presented in Table 3-5. Nine parameters were derived by statistical optimization to experimental *in vivo* data (Kortboyer et al. 1997b, 1998b; Wagner et al. 1983). Data from Wagner et al. (1983) were used to optimize parameters Δt_{water}, $k_{a,NO3}$, $k_{sec,NO3}$, and k_{conv}. Wagner et al. (1983) measured plasma, saliva, and urine, and nitrite in plasma and saliva, in 12 healthy adults following a single oral dose of ^{15}N-nitrate in drinking water. The parameter, Δt_{veg} (for vegetables), was optimized with data from a study in which plasma nitrate was measured in six adults before and following a vegetable meal (Kortboyer et al. 1998b). The parameter, V_{NO2}, was optimized with data from a study in which plasma nitrite concentrations were measured in nine adults before and following an intravenous dose of sodium nitrite (Kortboyer et al. 1997b). Parameters describing reactions with hemoglobin and methemoglobin (k_{NO2}, [$K_{m,r}$, $V_{max,r}$, z]) were derived by statistical optimization to experimental *in vitro* studies in which reaction kinetics of nitrite with hemoglobin were measured in whole human blood (Kosaka et al. 1979; Rodkey 1976). The remaining parameters were estimated from reported literature or calculated from other parameters (Cortas and Wakid 1991; Kortboyer et al. 1995, 1997a, 1997b, 1998a, 1998b; Lambers et al. 2000; McKnight et al. 1997; Mirvish et al. 1975; Schultz et al. 1985; Wagner et al. 1983).

Validation of the Model. The optimized model was evaluated by comparing predictions of plasma nitrate and nitrite concentrations and blood methemoglobin concentrations in nine adults who consumed a single oral dose of sodium nitrite (2.42 or 4.84 mg sodium nitrite/kg) (Kortboyer et al. 1997b). The results of the evaluation are reported in Zeilmaker et al. (2010b) as overlay plots of observations and predictions. Statistical evaluations of the agreement between predictions and observations were not reported.

Risk Assessment. The model has been used to predict concentrations of methemoglobin that would result from a vegetable meal and to evaluate whether the average daily intake of nitrate would result in clinically significant methemoglobinemia (JECFA 2003a). JECFA (2003a) applied the model to make predictions in adults and infants. In order to apply the model to infants, blood volume and volumes of the central nitrate and nitrite compartments were scaled to infants (the exact scaling procedure or scaled parameter values were not reported). JECFA (2003a) also applied the model to predict methemoglobin

concentrations that might occur in patients who have inflammatory reactions to absorbed nitrite. Absorbed doses of nitrate in patients were simulated in the model as an intravenous infusion of nitrite.

Target Tissues. The model was calibrated to predict concentrations of nitrate and nitrite in plasma and blood methemoglobin concentrations in humans.

Species Extrapolation. The model simulates nitrate and nitrite kinetics in humans. Applications to other species would require development of appropriate scaling methods, optimization, and validation.

Interroute Extrapolation. The model is currently configured to simulate kinetics associated with intravenous and oral dosing. Simulation of other potential routes of exposure (e.g., inhalation, dermal) would require development of models for the absorption of inhaled nitrate or nitrate deposited on the skin.

3.5 MECHANISMS OF ACTION

3.5.1 Pharmacokinetic Mechanisms

Ingestion is the major route of exposure to exogenous nitrate and nitrite. Nitrate is assumed to enter the blood from the upper small intestinal tract via active transport (EPA 1990b), which may involve active transport systems such as the sodium iodide symporter (NIS) because nitrate has been shown to be a relatively weak competitive inhibitor of NIS (e.g., Eskandari et al. 1997) and the NIS-mediated uptake of iodine from the intestine has been demonstrated (Nicola et al. 2009). Nitrite is readily absorbed via diffusion across the gastric mucosa and wall of the small intestine (EPA 1990b). As described in detail in Section 3.4, nitrate and nitrite are readily distributed throughout the body and a portion of plasma nitrate is concentrated in the salivary gland at concentrations as much as 10 times that of plasma nitrate. Qin et al. (2012) demonstrated that the scialic acid (SA)/H^+ cotransporter, sialin, is endogenously localized in the plasma membrane of salivary gland cells and functions as an electrogenic $2NO_3^-/H^+$ cotransporter; this active transport mechanism may be responsible for high concentrations of nitrate in the salivary gland. Refer to Section 3.4 for information regarding metabolic pathways involved in the nitrate-nitrite-nitric oxide cycle. No information was located regarding specific mechanisms involved in transfer of nitrate to the urine.

3.5.2 Mechanisms of Toxicity

The most sensitive and widely-recognized toxic effect of nitrate and nitrite is that of nitrite-induced methemoglobinemia in which nitrite (ingested as nitrite, formed via bacterial reduction of ingested nitrate, and/or produced as an endogenous product of the nitric oxide oxidation) reacts with ferrous (Fe^{2+}) hemoglobin (which transports oxygen) to form ferric (Fe^{3+}) hemoglobin (methemoglobin, a poor transporter of oxygen) (refer to Section 3.4 for additional information regarding the nitrate-nitrite-nitric oxide pathway).

As stated in Section 3.2.2.2 (Endocrine Effects), nitrate is a dose-dependent competitive inhibitor of the NIS, which mediates the uptake of iodine by the thyroid. Sufficiently decreased iodine uptake by the thyroid might result in decreased production of thyroid hormones T3 and T4 and consequent adverse effects associated with thyroid dysfunction (e.g., hypothyroidism), including effects on developing fetuses.

Proposed mechanisms of carcinogenicity involve the production of N-nitrosamines via nitrosating reactions that involve nitrite and amines or amides. Such reactions may occur within some food items during storage or preparation or in the body (usually in the stomach) (Mirvish 1975). The National Toxicology Program's 12th Report on Carcinogens (NTP 2011) lists 17 N-nitroso compounds (mostly nitrosamines) as *reasonably anticipated to be a human carcinogen* based on sufficient evidence of carcinogenicity from studies in experimental animals and one nitrosourea compound as *known to be a human carcinogen* and one nitrosourea compound (1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea) as *known to be a human carcinogen* based on sufficient evidence of carcinogenicity from studies in humans. The International Agency for Research on Cancer (IARC 2014) lists eight of these compounds in Group 2A (probably carcinogenic to humans), another eight in Group 2B (possibly carcinogenic to humans), and two compounds (N-nitrosopiperadine and 4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butanone) in Group 1 (carcinogenic to humans). Interactions between nitrite and a variety of drugs have been shown to result in the formation of carcinogenic N-nitroso compounds (Brambilla and Martelli (2007).

3.5.3 Animal-to-Human Extrapolations

Interspecies differences in nitrate-nitrite-nitric acid pathways indicate that laboratory animals do not represent reliable models of nitrate-nitrite-nitric oxide pathways for humans (EPA 1990b; Health Canada 2012; Kortboyer et al. 1997a, 1997b; Walker 1995; WHO 2011b). For example, Til et al. (1988) reported

that the rate of conversion of nitrate to nitrite is much lower in rats than humans. Cohen and Myant (1959) reported that the rat lacks the active transport mechanism (sodium iodide symporter) responsible for secretion of plasma nitrate to the salivary gland in humans. Therefore, the rate of reduction of salivary nitrate to nitrite in the rat is likely much less than the estimate of 25% reduction in human saliva.

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 Thomas and Colborn (1992), was also used in 1996 when Congress mandated the 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), and in 1998, the EDSTAC 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 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).

As discussed in detail in Section 3.2.2.2 (Endocrine Effects), available human data provide some evidence that elevated levels of nitrate in drinking water and/or nitrate-rich diets may be associated with signs of thyroid dysfunction (Aschebrook-Kilfoy et al. 2012; Gatseva and Argirova 2008; Rádiková et al. 2008; Tajtáková et al. 2006; Ward et al. 2010). In animals, orally-administered nitrate has been demonstrated to cause decreased iodine uptake by the thyroid and changes in serum thyroid hormone levels (e.g., Bloomfield et al. 1961; El-Wakf et al. 2008; Eskiocak et al. 2005; Mukhopadhyay et al. 2005; Zaki et al. 2004).

No *in vitro* studies were located regarding endocrine disruption of nitrate or nitrite.

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 most 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.

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 adverse health effects from exposure to hazardous chemicals, but whether there is a difference depends on the chemical(s) (Guzelian et al. 1992; NRC 1993). Children may be more or less susceptible than adults to exposure-related 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 that are most sensitive to disruption from exposure to hazardous substances. Damage from exposure in one stage 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). Past literature has often described the fetus/infant as having an immature (developing) blood-brain barrier that is leaky and poorly intact (Costa et al. 2004). However, current evidence suggests that the blood-brain barrier is anatomically and physically intact at this stage of development, and the restrictive intracellular junctions that exist at the blood-CNS interface are fully formed, intact, and functionally effective (Saunders et al. 2008, 2012).

However, during development of the brain, there are differences between fetuses/infants and adults that are toxicologically important. These differences mainly involve variations in physiological transport systems that form during development (Ek et al. 2012). These transport mechanisms (influx and efflux) play an important role in the movement of amino acids and other vital substances across the blood-brain barrier in the developing brain; these transport mechanisms are far more active in the developing brain than in the adult. Because many drugs or potential toxins may be transported into the brain using these same transport mechanisms—the developing brain may be rendered more vulnerable than the adult. Thus, concern regarding possible involvement of the blood-brain barrier with enhanced susceptibility of the developing brain to toxins is valid. It is important to note however, that this potential selective vulnerability of the developing brain is associated with essential normal physiological mechanisms; and not because of an absence or deficiency of anatomical/physical barrier mechanisms.

The presence of these unique transport systems in the developing brain of the fetus/infant is intriguing; whether these mechanisms provide protection for the developing brain or render it more vulnerable to toxic injury is an important toxicological question. Chemical exposure should be assessed on a case-by-case basis. Research continues into the function and structure of the blood-brain barrier in early life (Kearns et al. 2003; Saunders et al. 2012; Scheuplein et al. 2002).

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 given their low glomerular filtration rate and not having 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 alveoli being less developed, which results in a disproportionately smaller surface area for alveolar absorption (NRC 1993).

As discussed in detail in Section 3.4 (Toxicokinetics), a portion of ingested nitrate is reduced to nitrite by commensal bacteria in the mouth; however, the acid environment of the normal stomach does not support the growth of such bacteria. Most nitrite (ingested or reduced from nitrate) is absorbed from the upper gastrointestinal tract and enters the blood; plasma nitrite readily reacts with hemoglobin to form methemoglobin. Sufficiently high levels of methemoglobin levels result in poor oxygen supply to tissues. Clinical methemoglobinemia is generally indicated at methemoglobin levels >10% of total hemoglobin and cyanosis is an early clinical sign. The first 6 months of postnatal life is a period of increased susceptibility to methemoglobinemia (termed infantile methemoglobinemia or blue baby syndrome); possible contributing factors to this increased susceptibility (pH of the infant stomach, proportion of fetal hemoglobin to adult hemoglobin, and concentration of NADH-dependent methemoglobin reductase) (Greer and Shannon 2005) are discussed below.

A portion of ingested nitrate is reduced to nitrite by commensal bacteria in the mouth; however, the acid environment of the normal stomach does not support the growth of such bacteria and most of the nitrate that reaches the stomach passes to the small intestine from which it is nearly completely absorbed into the blood. However, a higher pH in the stomach of the newborn may favor growth of nitrate-reducing bacteria and increased reduction of nitrate to nitrite and consequent increased plasma methemoglobin. Most hemoglobin in the newborn is in a form termed fetal hemoglobin, which appears to be more readily oxidized to methemoglobin than adult hemoglobin; fetal hemoglobin is replaced by adult hemoglobin during early postnatal life. Levels of NADH-dependent methemoglobin reductase (the major enzyme responsible for reduction of methemoglobin to normal hemoglobin) in the newborn increase approximately 2-fold during the first 4 month of postnatal life to reach adult levels.

There is some evidence that methemoglobinemia in infants drinking formula prepared using drinking water with relatively high levels of nitrate may be related to bacterial contamination of such water sources and consequent gastrointestinal disorders, as well as gastrointestinal infection and inflammation and the ensuing overproduction of nitric oxide (Avery 1999). Kanady et al. (2012) reported little or no bacterial conversion of nitrate to nitrite in the saliva of a group of 10 infants during the first 2 postnatal months that was considered mainly due to lower numbers of major nitrate-reducing oral bacteria than adults. Ibrahim et al. (2012) found that blood nitrite levels of newborns approximately 1–2 days of age were 35–55% lower than that of adults.

Some investigators have reported significant associations between nitrate levels in drinking water (or living in areas presumed to have elevated nitrate levels in drinking water sources) and risk of childhood type 1 diabetes (Dahlquist et al. 1990; Kostraba et al. 1992; Parslow et al. 1997; Virtanen et al. 1994). However, no such relationship was observed in two other studies (van Maanen et al. 2000; Zhao et al. 2001). Refer to Section 3.2.2.2 (Metabolic Effects) for summaries of these study reports.

Results of studies designed to assess possible associations between nitrate levels in drinking water sources and developmental end points in humans provide equivocal evidence of nitrate-related effects on the developing fetus and infant (see Section 3.2.2.6, Developmental Effects). There is limited evidence of nitrate-induced thyroid dysfunction (see Section 3.2.2.2, Endocrine Effects), which could result in adverse effects on the developing fetus of a pregnant mother.

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).

The National Report on Human Exposure to Environmental Chemicals provides an ongoing assessment of a generalizable sample of the exposure of the U.S. population to environmental chemicals using biomonitoring. This report is available at http://www.cdc.gov/exposurereport/. The biomonitoring data for nitrate from this report is discussed in Section 6.5. 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, 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 nitrate and nitrite 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 nitrate and nitrite are discussed in Section 3.8.2.

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 Nitrate and Nitrite

There are no biomarkers of exposure that are specific to nitrate or nitrite. Although nitrate and nitrite can be detected in blood, saliva, and urine (mostly nitrate), nitrate and nitrite are also produced endogenously via the nitrate-nitrite-nitric oxide pathway. Sources for nitrate and nitrite levels in the body may therefore include not only ingested food and drinking water, but also oxidation of nitric oxide produced endogenously. Similarly, N-nitroso compounds that may be detected in the blood or urine may indicate exposure to nitrate or nitrite; however, these compounds may also be products of the endogenous nitrate-nitrite-nitric oxide pathway.

3.8.2 Biomarkers Used to Characterize Effects Caused by Nitrate and Nitrite

Biomarkers of effects from exposure to nitrate or nitrite are not specific to nitrate or nitrite. Blood methemoglobin level has been used as a biomarker of nitrate and nitrite toxicity; however, methemoglobinemia may be elicited by other substances such as selected drugs, pesticides, industrial and commercial products, and medical conditions such as pediatric gastrointestinal infection, sepsis, and sickle cell crisis (ATSDR 2013a). Methemoglobinemia may also be inherited (genetic conditions that result in decreased activity of enzymes that reduce methemoglobin or the presence of hemoglobin M). Jansen et al. (1995) reported a rapid 6-fold increase in urinary N-methylnicotinamide (a metabolite of tryptophan) in four of eight volunteers following the ingestion of sodium nitrate at 10 mg/kg; however, little to no increase in urinary N-methylnicotinamide was observed in the other four volunteers. Urinary levels of various other N-nitroso compounds (e.g., nitrosoproline) have been measured as an index of nitrosation (Ohshima and Bartsch 1988); however, N-nitroso compounds can form via endogenous nitrosation and do not require the intake of nitrate or nitrite.

3.9 INTERACTIONS WITH OTHER CHEMICALS

Information regarding interactions between nitrate or nitrite and other substances is comprised mainly of studies that assessed the tumorigenicity of oral exposure to sodium nitrite in the presence of selected amino compounds or other substances suspected or known to cause cancer and studies that assessed modulation of tumorigenicity by selected antioxidants. As discussed in Section 3.5 (Mechanisms of Action), nitrosating reactions that involve nitrite and amines or amides may result in the production of N-nitrosamines, some of which may be carcinogenic. Interactions between nitrite and a variety of drugs may also result in the formation of carcinogenic N-nitroso compounds (Brambilla and Martelli (2007).

Adverse effects elicited in laboratory animals exposed to selected substances were enhanced or diminished upon co-exposure to nitrite, although mechanisms for such nitrite-induced enhanced or diminished responses have not been identified. For example, Kawabe et al. (1994) observed increased severity of forestomach hyperplasia in groups of catechol- or 3-methoxycatechol-treated rats coadministered sodium nitrite and increased thickness of forestomach mucosa (indication of cellular proliferation) in rats treated with sodium nitrite in combination with phenolic compounds such as *t*-butylhydroquinone, catechol, gallic acid, 1,2,4-benzenetriol, *dl*-3-(3,4-dihydroxyphenyl)-alanine, and hydroquinone. Coadministration of sodium nitrite with catechol resulted in enhanced cellular proliferation. Pregnant Syrian golden hamsters fed a diet containing nitrite and morpholine exhibited a

higher incidence of liver-cell carcinoma (5/16 hamsters) compared to those fed diets containing morpholine in the absence of nitrite (0/22) (Shank and Newberne 1976). Sodium nitrite treatment resulted in increased incidences of forestomach papillomas and decreased incidences of glandular stomach epithelial adenomas in rats provided drinking water to which sodium nitrite and either catechol or 3-methoxycatechol were added either with or without coexposure to known carcinogens (Hirose et al. 1990, 1993). IARC (2010) summarized results from a Russian study (Ilnitsky and Kolpakova 1997) in which sodium nitrite appeared to enhance the carcinogenic effect of leukemia viruses in mice. Hirose et al. (2002) observed a reduction of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine-induced mammary gland tumors in rats coexposed to sodium nitrite in the drinking water. Commoner et al. (1970) reported an inhibition of the tumorigenic action of 2-acetylaminofluorene in rats co-treated with nitrite.

Nitrate, thiocyanate, and perchlorate are dose-dependent competitive inhibitors of the sodium-iodide symporter (NIS), which mediates the uptake of iodine by the thyroid (De Groef et al. 2006). Overexposure to any one of these competitive inhibitors could decrease iodine uptake and result in thyroid dysfunction; this effect could be more severe during exposures to combinations of these substances (and possibly other NIS competitive inhibitors).

3.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to nitrate and nitrite than will most persons exposed to the same level of nitrate and nitrite in the environment. Factors involved with increased susceptibility 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 nitrate and nitrite, or compromised function of organs affected by nitrate and nitrite. Populations who are at greater risk due to their unusually high exposure to nitrate and nitrite are discussed in Section 6.7, Populations with Potentially High Exposures.

Infants 1–6 months of age appear to be particularly sensitive to nitrite-induced methemoglobinemia following ingestion of formula prepared from drinking water containing elevated levels of nitrate (see Section 3.7 for detailed discussion of biological factors that may be responsible for increased sensitivity of infants). Infants with gastroenteritis may be at increased risk for nitrite-induced methemoglobinemia, although nitrite and nitrate generation from oxidation of endogenous nitric oxide produced under inflammatory conditions may be a major contributory factor (Avery 1999). Individuals with higher-than-normal gastric pH (e.g., achlorhydria, a condition whereby gastric acid production is low or absent;

individuals taking antacids) may be at increased risk of methemoglobinemia if the gastric environment supports survival of nitrate-reducing bacteria.

Some epidemiological studies provide suggestive evidence of associations between exposure to nitrates in drinking water and spontaneous abortions, intrauterine growth restriction, and selected birth defects (e.g., Brender et al. 2013; Bukowski et al. 2001; CDC 1996; Dorsch et al. 1984; Schmitz 1961; Tabacova et al. 1997, 1998). Results from these studies suggest that the pregnant mother and her developing fetus might be particularly susceptible to nitrate/nitrite toxicity. However, estimates of nitrate intakes were typically based on measurements of nitrate levels in drinking water sources at selected time points and self-reported estimates of water consumption. Furthermore, possible confounding by other potential toxicants was not evaluated and studies did not typically account for dietary nitrate or nitrite.

Other factors that may contribute to increased risk of methemoglobinemia include glucose-6-phosphate dehydrogenase deficiency (which can result in decreased numbers of red blood cells); deficiency in NADH-dependent methemoglobin reductase (the major enzyme responsible for the reduction of methemoglobin to normal hemoglobin); diseases such as anemia, cardiovascular disease, lung disease, and sepsis; and abnormal hemoglobin species including carboxyhemoglobin, sulfhemoglobin, and sickle hemoglobin. Individuals consuming diets deficient in selected antioxidants (e.g., vitamin C, vitamin E) might be at increased risk of cancer associated with the production of potentially carcinogenic N-nitroso compounds (WHO 2011b).

3.11 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to nitrate and nitrite. 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 nitrate and nitrite. When specific exposures have occurred, poison control centers, board certified medical toxicologists, board-certified occupational medicine physicians and/or other medical specialists with expertise and experience treating patients overexposed to nitrate and nitrite can be consulted for medical advice. The following texts provide specific information about treatment following exposures to nitrate and nitrite:

Barclay PJ. 1998. Nitrates and nitrites. In: Viccellio P, ed. Emergency toxicology. 2nd ed. Philadelphia, PA: Lippincott-Raven Publishers, 315-323.

Leikin JB, Paloucek FP, eds. 2008. Poisoning and toxicology handbook. 4th ed. Boca Raton, FL: CRC Press, 830.

Seifert SA. 2004. Nitrates and nitrites. In: Dart RC, ed. Medical toxicology. 3rd ed. Philadelphia, PA: Lippincott Williams & Williams, 1174-1180.

Additional relevant information can be found in the front section of this profile under QUICK REFERENCE FOR HEALTH CARE PROVIDERS.

3.11.1 Reducing Peak Absorption Following Exposure

Ingestion is the most likely route of overexposure to nitrate or nitrite. Nitrate and nitrite bind to activated charcoal, which may be administered (1 g/kg without cathartic) within 1–2 hours following significant ingestion (Seifert 2004). Use of mouthwash containing chlorhexidine (an active antibacterial) resulted in a large decrease in the mean percent reduction of salivary nitrate to nitrite (van Maanen et al. 1996b).

3.11.2 Reducing Body Burden

No information was located regarding methods to reduce the body burden of nitrate or nitrite.

3.11.3 Interfering with the Mechanism of Action for Toxic Effects

Severe methemoglobinemia (methemoglobin levels generally >30% of total hemoglobin) can be reduced by intravenous administration of methylene blue (1–2 mg/kg) (Barclay 1998; Leikin and Paloucek 2008; Seifert 2004). Exchange transfusions may be considered for patients who do not respond to methylene blue (particularly patients with glucose-6-phosphate dehydrogenase deficiency or hemoglobin M), and patients where methylene blue is contraindicated (e.g., patients on serotonin uptake inhibitors) (ATSDR 2013a; Barclay 1998). In symptomatic patients, 100% oxygen and assisted ventilation should be considered; seizures can be treated with oxygen and benzodiazepines, followed by phenobarbital (Seifert 2004). Hyperbaric oxygen therapy may be of some benefit, but has not been demonstrated in controlled studies (Leikin and Paloucek 2008; Seifert 2004). Management of nitrite-induced hypotension involves placement of the patient in Trendelenburg position, administration of intravenous isotonic fluids at 10–20 mL/kg bolus and as required thereafter, and pressors such as dopamine or norepinephrine, as needed (Seifert 2004).

In several rat studies, tumorigenicity associated with concurrent exposure to nitrite and various amino compounds was modulated by coexposure to selected antioxidants such as ascorbic acid, catechol, 3-methoxycatechol, tert-butylhydroquinone, α-tocopherol, and propyl gallate (Chan and Fong 1977;

Mirvish et al. 1976, 1983; Miyauchi et al. 2002; Mohktar et al. 1988; Yada et al. 2002; Yoshida et al. 1994); thioproline (which may serve as a nitrite scavenger when nitrosated to nitrosothioproline) (Tahira et al. 1988); or soy bean (Mokhtar et al. 1988).

3.12 ADEQUACY OF THE DATABASE

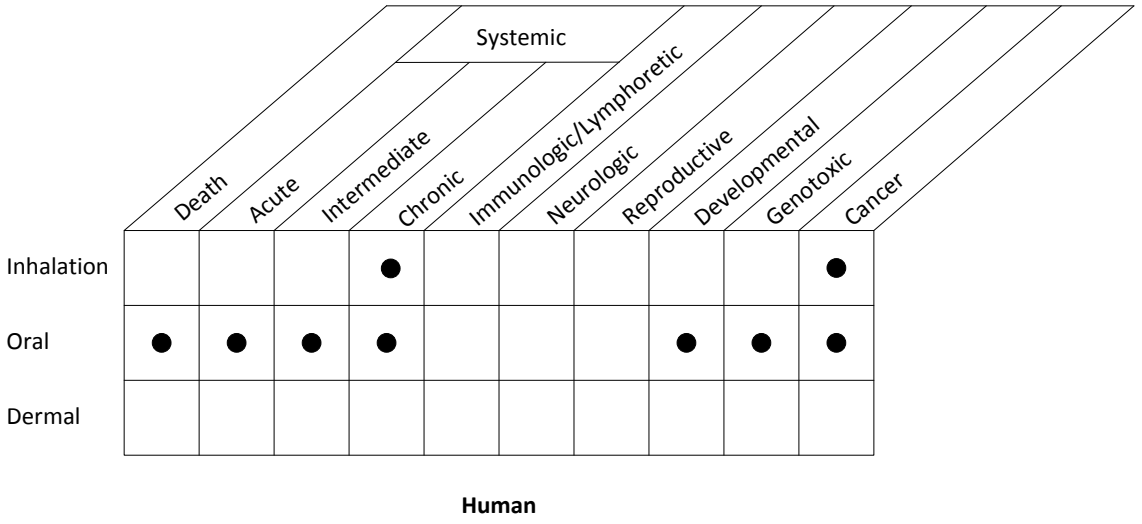
Section 104(I)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of nitrate and nitrite is available. Where adequate information 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 adverse health effects (and techniques for developing methods to determine such health effects) of nitrate and nitrite.

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 risk 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.

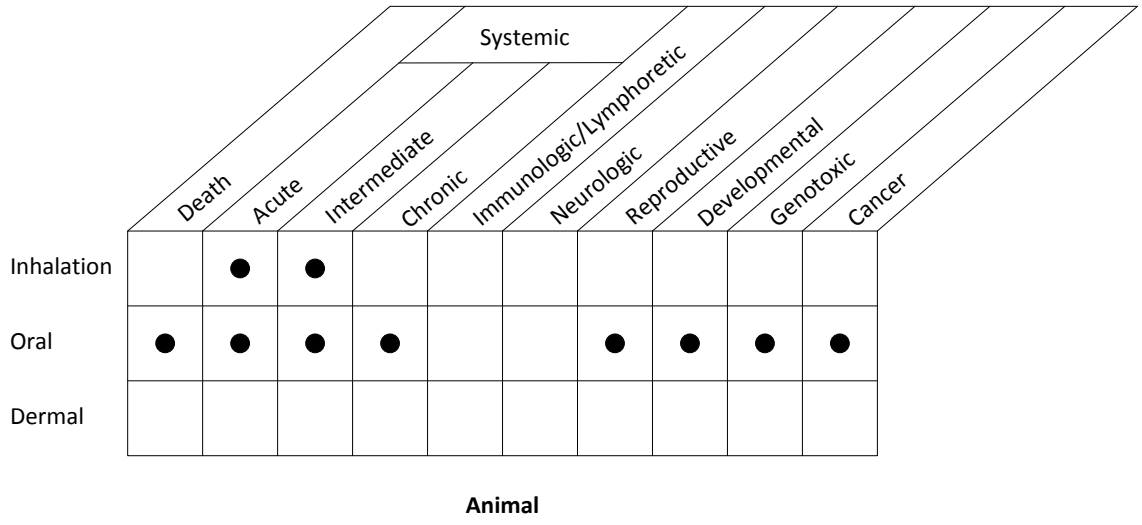
3.12.1 Existing Information on Health Effects of Nitrate and Nitrite

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to nitrate and nitrite are summarized in Figures 3-5 and 3-6. The purpose of this figure is to illustrate the existing information concerning the health effects of nitrate and nitrite. 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.

Figure 3-5. Existing Information on Health Effects of Nitrate



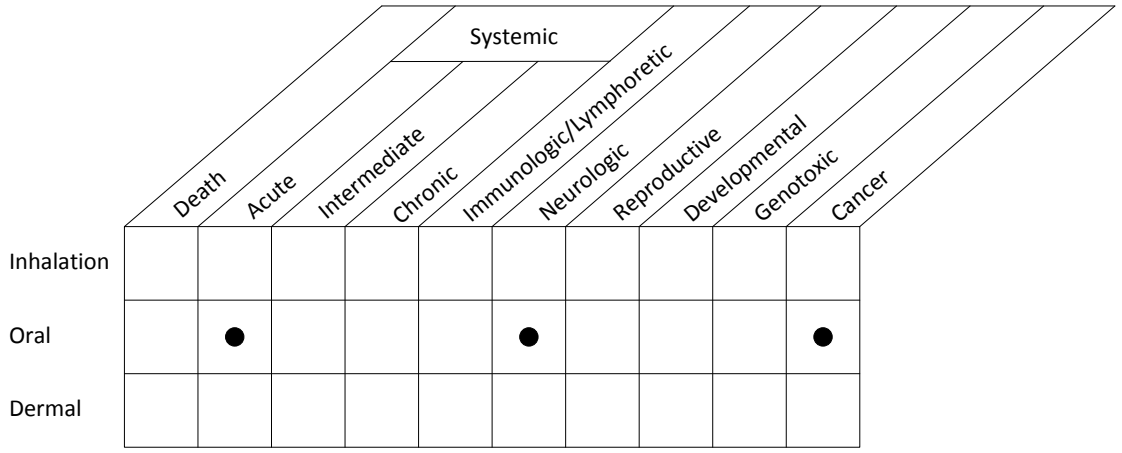
Human



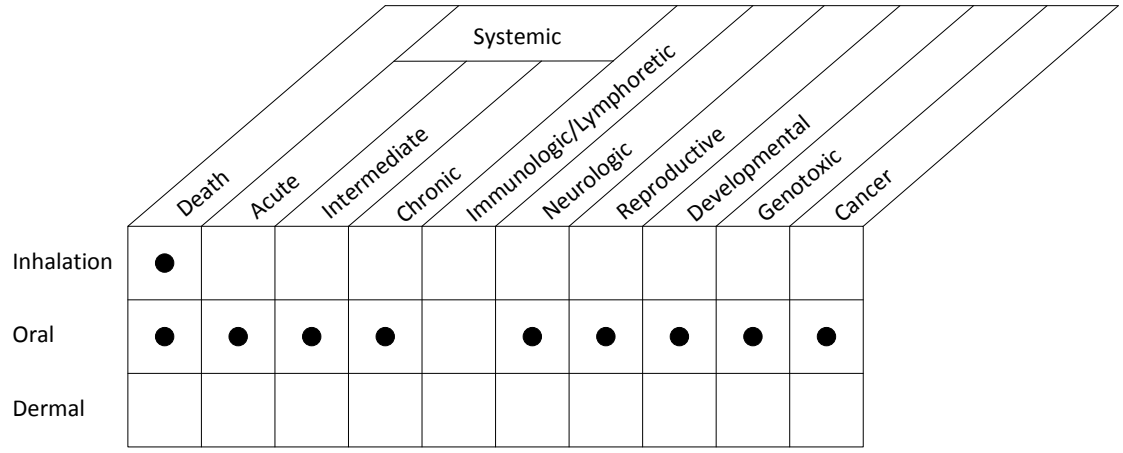
Animal

● Existing Studies

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



Human



Animal

● Existing Studies

3.12.2 Identification of Data Needs

Acute-Duration Exposure. No information was located regarding the effects of acute-duration inhalation exposure to nitrate or nitrite in humans. Available information in laboratory animals is limited. RTECS (2014) lists a rat 4-hour LC_{50} of 5.5 mg/m^3 (1.95 ppm) for sodium nitrite and a rat 2-hour LC_{50} of 85 mg/m^3 (24.42 ppm) for potassium nitrite. There was no evidence of exposure-related pulmonary or cardiac effects in anesthetized dogs exposed at up to 10 mg sodium nitrate/m^3 (2.88 ppm) for 7.5 minutes or anesthetized dogs or conscious sheep exposed at 5 mg sodium nitrate/m^3 (1.44 ppm) for 4 hours. Additional information regarding the effects of acute-duration inhalation exposure to nitrate or nitrite is not considered necessary because the general population is not likely to be exposed to airborne nitrate or nitrite concentrations at levels that might cause adverse health effects.

Refer to the section titled "Epidemiological and Human Dosimetry Studies" for a summary of available information regarding noncancer effects in humans following oral exposure to nitrate or nitrite.

Among laboratory animals, acute oral LD_{50} values range from 1,267 to 3,750 mg/kg for selected nitrate salts (RTECS 2014) and from 150 to 200 mg/kg for selected nitrite salts (Imaizumi et al. 1980; RTECS 2014; Sheehy and Way 1974). Imaizumi et al. (1980) administered aqueous sodium nitrite to fasted Sprague-Dawley rats by gavage and observed dose-related increased methemoglobin levels. Additional studies regarding the effects of acute-duration oral exposure of laboratory animals to nitrate or nitrite are not considered necessary, in part due to interspecies differences in kinetics of the nitrate-nitrite-nitric oxide pathway.

No information was located regarding health effects in humans or animals following acute-duration dermal exposure to nitrate or nitrite. Information regarding the effects of acute-duration dermal exposure to nitrate or nitrite is not considered necessary because the general population is not likely to be dermally-exposed to nitrate or nitrite concentrations at levels that might cause adverse health effects.

Intermediate-Duration Exposure. No information was located regarding the effects of intermediate-duration inhalation exposure to nitrate or nitrite in humans or animals. The general population is not likely to be exposed to airborne nitrate or nitrite concentrations at levels that might cause adverse health effects.

Refer to the section titled "Epidemiological and Human Dosimetry Studies" for a summary of available information regarding noncancer effects in humans following oral exposure to nitrate or nitrite.

Epithelial hyperplasia was noted in the forestomach of mice provided sodium nitrite in the drinking water for 14 weeks (NTP 2001). Another study found no evidence of treatment-related forestomach lesions in male rats provided sodium nitrite in the drinking water for 35 weeks (Kawabe et al. 1994). Increased methemoglobin levels and other evidence of hematological effects have been reported in laboratory animals administered sodium nitrite or potassium nitrite orally for intermediate-duration time periods (Behroozi et al. 1972; Chow et al. 1980; Grant and Butler 1989; Imaizumi et al. 1980; NTP 2001; Shuval and Gruener 1972; Til et al. 1988, 1997). Several animal studies found no indications of sodium nitrite-induced effects on liver function or histopathology (Asahina et al. 1971; Lijinsky and Greenblatt 1972; Lin and Ho 1992; Shuval and Gruener 1972; van Logten et al. 1972). El-Wakf et al. (2008) reported significantly increased urinary levels of urea and creatinine in male rats provided sodium nitrate in the drinking water for 4 months. Sodium or potassium nitrate-induced effects on the endocrine system of laboratory animals have been reported by several groups of investigators; effects include decreased serum thyroidal iodine uptake, decreased serum thyroid hormone levels, increased thyroid weight, and follicular hyperplasia (El-Wakf et al. 2008; Eskiocak et al. 2005; Mukhopadhyay et al. 2005; Zaki et al. 2004). Til et al. (1988, 1997) observed adrenal gland hypertrophy in rats administered potassium nitrite in the drinking water for 13 weeks; results of a subsequent study indicated that this effect was a physiological adaptation to repeated episodes of hypotension caused by nitrite (RIVM 1996). Depressed body weight and/or body weight gain were observed in some laboratory animals receiving nitrate or nitrite from the drinking water for intermediate exposure durations (El-Wakf et al. 2008; Maekawa et al. 1982; Zaki et al. 2004). Intermediate-duration oral exposure to sodium nitrite in the drinking water of laboratory animals has been associated with neurological effects such as abnormalities in EEGs (Behroozi et al. 1972), increased aggressive behavior (Gruener 1974), and reduced motor activity (Shuval and Gruener 1972). Available intermediate-duration oral studies in laboratory animals adequately characterize nitrate- and nitrite-induced effects; additional animal studies do not appear necessary.

No information was located regarding health effects in humans or animals following intermediate-duration dermal exposure to nitrate or nitrite. Information regarding the effects of intermediate-duration dermal exposure to nitrate or nitrite is not considered necessary because the general population is not likely to be dermally-exposed to nitrate or nitrite concentrations at levels that might cause adverse health effects.

Chronic-Duration Exposure and Cancer. Information regarding the effects of chronic-duration inhalation exposure is limited. A cohort mortality study of male workers involved in the manufacture of nitrate fertilizer for at least 1 year between 1946 and 1981 found no evidence of associations between exposure to nitrate dusts and death from respiratory or circulatory diseases (Al-Dabbagh et al. 1986). Among workers described as having been heavily exposed to nitrate dust, slight excesses were noted for death from lung cancer and death from all malignant neoplasms, but not for cancers of the esophagus, stomach, or bladder. After categorizing the heavily-exposed workers by duration of exposure and time since first exposure, excess death from lung cancer was noted for those exposed for ≥10 years, with a lag time of ≥20 years since first exposure. The study authors indicated that they were unable to adjust for smoking. In a census-based mortality study of workers involved in production of nitrate fertilizer, there was no evidence of associations between exposure to nitrate dust and death from circulatory diseases; slight excesses were noted for deaths from lung cancer and death from all malignant neoplasms, but not for cancers of the esophagus, stomach, or bladder (Fraser et al. 1982, 1989). No significant increased risk for cancer at any site was observed at 7-year follow-up evaluation. In yet another cohort of workers at a nitrate fertilizer production facility (Hagmar et al. 1991), death from prostate cancer was in excess; however, risk of prostate cancer within this cohort was not enhanced following application of a ≥10-year latency period, and there was no significant increase in death from tumors of the lips, oral cavity, pharynx, salivary glands, gastrointestinal tract, stomach, respiratory tract, lung, urinary bladder, blood, or all sites combined. The general population is not likely to be exposed to airborne nitrate or nitrite concentrations at levels that might cause adverse health effects.

Refer to the section titled “Epidemiological and Human Dosimetry Studies” for a summary of available information regarding noncancer effects in humans following oral exposure to nitrate or nitrite.

Numerous case-control and cohort studies regarding the carcinogenicity of ingested nitrate and nitrite in humans have been reported (IARC 2010). Many ecological studies have also been reported; however, interpretation of outcomes of these studies is more uncertain because of various factors that contribute to ecologic bias (group-based associations between exposure and cancer outcomes may not apply to individuals). In general, outcomes of case-control and cohort studies have found no or weak associations between exposure to nitrate and cancer in humans, with stronger associations with exposures to nitrite or intake of high nitrite foods such as cured meat (Aschebrook et al. 2013; DellaValle et al. 2013; IARC 2010; Inoue-Choi et al. 2012). Mechanistically, this outcome is consistent with nitrite and being a reactive intermediate in the cancer mode of action of nitrate. This is further supported by studies that found interactions between cancer risk attributed to nitrite and exposure to antioxidants (IARC 2010;

Inoue-Choi et al. 2012; Kim et al. 2007; Yang et al. 2010). Uncertainties in estimates of cancer risk from exposure to nitrate or nitrite include those typical of epidemiological studies in general: uncertainties in estimation of exposure (e.g., estimating long-term dietary intakes from food frequency questionnaires or levels in PWS), exposure misclassification of individual outcomes (e.g., assigning group-level exposure estimates to individuals), and adequacy of controlling for confounders (e.g., other factors contributing to the cancer). One potentially important class of confounders is antioxidants that can interfere with nitrosation of dietary amines, and thereby the mode of carcinogenicity of nitrite, and may also interfere with other carcinogenic processes that involve reactive intermediates.

The strongest and most consistent evidence for carcinogenicity of nitrite is from studies of gastrointestinal cancers and, in particular, gastric cancer (Buiatti et al. 1990; Engel et al. 2003; La Vecchia et al. 1994, 1997; Mayne et al. 2001; Palli et al. 2001; Risch et al. 1985; Rogers et al. 1995; Ward et al. 2007, 2008). Results have been mixed for other types of cancer. Some case-control or cohort studies found associations between exposure to nitrite (or foods high in nitrite such as cured meat) and brain cancer in children and adults (Blowers et al. 1997; Giles et al. 1994, Huncharek and Kupelnick 2004; Huncharek et al. 2003; Lee et al. 1997; Pogoda and Preston-Martin 2001a, 2001b; Preston-Martin et al. 1996; Mueller et al. 2004), breast cancer (Inoue-Choi et al. 2012; Yang et al. 2010), kidney cancer (DellaValle et al. 2013; Ward et al. 2007; Wilkens et al. 1996), testicular cancer (Moller 1997), and non-Hodgkin's lymphoma (Ward et al. 2006). Of these studies, the highest risks were reported for brain cancers. Two case-control studies found elevated relative risk of brain cancer in children in association with maternal nitrite intake (Mueller et al. 2004; Pogoda and Preston-Martin 2001a, 2001b; Preston-Martin et al. 1996). In general, case-control and cohort studies of cancers of larynx, liver, lung, mouth, pancreas, and pharynx have found no consistent associations with exposures to nitrate or nitrite (IARC 2010).

The potential carcinogenicity of nitrate has been investigated in several animal studies that employed the oral exposure route. Studies in which negative results were reported include MCR-derived rats provided sodium nitrate in the drinking water for 84 weeks (Lijinsky et al. 1973a), male white rats provided sodium nitrate in the drinking water for 273 days (Pliss and Frolov 1991), strain A male mice provided sodium nitrate in the drinking water for 25 weeks (Greenblatt and Mirvish 1973), female NMRI mice provided calcium nitrate in the drinking water for 18 months (Mascher and Marth 1993), Fischer 344 rats fed diet containing sodium nitrate for 2 years (Maekawa et al. 1982), and ICR mice fed diets containing sodium nitrate for 2 years (IARC 2010). In the study of Pliss and Frolov (1991), some groups of male rats were treated with drinking water containing BBNA (an inducer of urinary bladder cancer in laboratory animals) for 30 days, either alone or followed by sodium nitrate in the drinking water for 273 days. The group

treated with BBNA followed by sodium nitrate exhibited significantly increased incidence of urinary bladder carcinoma. These results indicate that sodium nitrate promoted BBNA-induced bladder tumors.

The potential carcinogenicity of ingested nitrite has been investigated in numerous animal studies. Nitrite treatment alone was not associated with tumor incidences in most studies (Börzsönyi et al. 1978; Hawkes et al. 1992; Inai et al. 1979; Lijinsky 1984a, 1984b; Lijinsky et al. 1983; Maekawa et al. 1982; NTP 2001). Significantly increased incidences of forestomach squamous papillomas were reported for male and female MRC Wistar rats provided drinking water to which sodium nitrite was added for life (Mirvish et al. 1980). Dose-related decreases in time of onset and incidence of lymphomas, mononuclear cell leukemia, and testicular interstitial-cell tumors were reported for male and female F344 rats administered reduced-protein diet to which sodium nitrite was added for up to 115 weeks (Grant and Butler 1989). In a 96-week study, Iurchenko et al. (1986) reported a significantly increased incidence of benign liver tumors among male CBA mice receiving sodium nitrite from the drinking water at an author-estimated total dose of 1,600 mg sodium nitrite/mouse; however, there was no apparent sodium nitrite treatment-related effect at a higher estimated dose (2,000 mg sodium nitrite/mouse). Increased incidences of total tumors and lymphoreticular tumors were reported in rats fed diet to which sodium nitrite was added; the results were reported for F1 and F2 offspring that had been exposed via their mothers during gestation and lactation and directly from the diet thereafter (Shank and Newberne 1976). A positive trend for incidences of forestomach squamous cell papilloma or carcinoma (combined) in female B6C3F1 mice administered sodium nitrite in the drinking water for 2 years was considered to provide "equivocal evidence of carcinogenic activity" of sodium nitrite (NTP 2001). In a 26-month study of male and female Sprague-Dawley rats provided drinking water to which sodium nitrite was added, the study author reported increased incidence of lymphomas, but not other types of tumors (Newberne 1979); however, IARC (2010) and NTP (2001) noted that a working group sponsored by the U.S. FDA reevaluated the histology and did not confirm the results of Newberne (1979). IARC (2010) reported that the working group considered the incidences of lymphomas to be similar to those arising spontaneously in Sprague-Dawley rats.

The potential carcinogenicity of combined exposure to sodium nitrite and selected nitrosatable substances (oral exposures via combinations of drinking water, diet, and/or gavage dosing) has been well-studied in laboratory animals. Many of the studies included sodium nitrite-only treatment groups for which there was no evidence of sodium-nitrite induced carcinogenicity (Anderson et al. 1985; Börzsönyi and Pintér 1977; Börzsönyi et al. 1976; Greenblatt and Lijinsky 1972, 1974; Greenblatt and Mirvish 1973; Greenblatt et al. 1971, 1973; Hirose et al. 2002; Ivankovic 1979; Ivankovic and Preussman 1970; Kitano

et al. 1997; Murthy et al. 1979; Lijinsky 1984a, 1984b; Lijinsky and Reuber 1980; Mirvish et al. 1972; Miyauchi et al. 2002; Rijhsinghani et al. 1982; Scheunig et al. 1979; Taylor and Lijinsky 1975a, 1975b; van Logten et al. 1972; Yada et al. 2002; Yoshida et al. 1993, 1994). However, Lijinsky et al. (1983) reported significantly increased incidences of hepatocellular neoplasms in female (but not male) F344 rats administered diet to which sodium nitrite was added for 2 years.

Significantly increased incidences of selected tumor types were observed in some studies of laboratory animals that employed coexposure to various amino compounds and sodium nitrite (Anderson et al. 1985; Aoyagi et al. 1980; Börzsönyi and Pintér 1977; Börzsönyi et al. 1976, 1978; Chan and Fong 1977; Greenblatt and Mirvish 1973; Greenblatt et al. 1971; Hirose et al. 1990; Iurchenko et al. 1986; Ivankovic 1979; Ivankovic and Preussmann 1970; Kawabe et al. 1994; Matsukura et al. 1977; Murthy 1979; Lijinsky 1984a, 1984b; Lijinsky and Reuber 1980; Lijinsky and Taylor 1977; Lijinsky et al. 1973b; Lin and Ho 1992; Maekawa et al. 1977; Mirvish et al. 1972, 1976, 1980; Miyauchi et al. 2002; Mokhtar et al. 1988; Newberne and Shank 1973; Nishiyama et al. 1998; Nixon et al. 1979; Oka et al. 1974; Olsen et al. 1984; Rijhsinghani et al. 1982; Rustia and Shubik 1974; Scheunig et al. 1979; Shank and Newberne 1976; Tahira et al. 1988; Taylor and Lijinsky 1975a, 1975b; Weisburger et al. 1980; Xiang et al. 1995; Yada et al. 2002; Yamamoto et al. 1989; Yoshida et al. 1993, 1994). These results were typically attributed to *in vivo* nitrosation of amines by nitrite to produce carcinogenic N-nitrosoamines; some of the studies did not include sodium nitrite-only treatment groups.

Based on available human data, IARC (2010) determined that there is inadequate evidence for the carcinogenicity of nitrate in food or drinking water and limited evidence for the carcinogenicity of nitrite in food (based on association with increased incidence of stomach cancer). Evaluation of available animal data by IARC (2010) resulted in the determination that there is inadequate evidence for the carcinogenicity of nitrate, limited evidence for the carcinogenicity of nitrite *per se*, and sufficient evidence for the carcinogenicity of nitrite in combination with amines or amides. The overall conclusions of IARC (2010) were that "ingested nitrate and nitrite under conditions that result in endogenous nitrosation is probably carcinogenic to humans (Group 2A)." IARC (2010) noted that: (1) the endogenous nitrogen cycle in humans includes interconversion of nitrate and nitrite; (2) nitrite-derived nitrosating agents produced in the acid stomach environment can react with nitrosating compounds such as secondary amines and amides to generate N-nitroso compounds; (3) nitrosating conditions are enhanced upon ingestion of additional nitrate, nitrite, or nitrosatable compounds; and (4) some N-nitroso compounds are known carcinogens. The U.S. EPA IRIS (2002) does not include a carcinogenicity evaluation for nitrate or nitrite.

No information was located regarding health effects in humans or animals following chronic-duration dermal exposure to nitrate or nitrite. Information regarding the effects of chronic-duration dermal exposure to nitrate or nitrite is not considered necessary because the general population is not likely to be dermally-exposed to nitrate or nitrite concentrations at levels that might cause adverse health effects.

Genotoxicity. Limited information is available regarding the potential genotoxicity of nitrate in human studies. One study found no significant association between urinary excretion of nitrate and frequency of SCEs in peripheral lymphocytes (Kleinjans et al. 1991). In another study, frequency of HPRT variants in peripheral lymphocytes was associated with nitrate levels in drinking water, urinary and salivary nitrite levels, and urinary excretion of nitrate and N-nitrosopyrrolidine (van Maanen et al. 1996a). The results suggest that drinking water with nitrate poses a genetic risk due to the potential formation of nitrosamines after endogenous reduction of nitrate to nitrite and reaction with amino compounds. Tsezou et al. (1996) reported a significant increase in chromatid and chromosome breaks in children exposed to nitrate in drinking water.

A limited number of studies have examined the *in vivo* genotoxicity of nitrate in laboratory animals; results were negative for frequency of micronuclei, chromosomal aberrations, morphological or malignant cell transformation, or drug-resistant mutations in embryonic cells in one study (Inui et al. 1979), inhibition of testicular DNA synthesis in another study (Friedman and Staub 1976), and chromosomal aberrations in bone marrow cells in a 2-day study (Luca et al. 1985). However, daily administration of sodium nitrate for 2 weeks resulted in significant dose-dependent increase in chromosomal aberrations in bone marrow cells (Luca et al. 1985). Gavage administration of 706.6 mg/kg/day sodium nitrate for 2 days to male Swiss mice showed induction of chromosomal aberrations; however, this effect was not observed at a much higher dose (Luca et al. 1985). Evaluation of micronuclei in mice treated daily for 2 weeks showed significant increases at the low concentrations tested (78.5 and 235.5 mg/kg/day sodium nitrate), but not at 706.6 or 2,120 mg/kg/day; the investigators attributed the result to possible induction of cytotoxic effects (Luca et al. 1985).

Neither potassium nitrate, sodium nitrate, nor lanthanum nitrate hexahydrate were mutagenic to multiple strains of *S. typhimurium* either with or without metabolic activation (Ishidate et al. 1984; Zeiger et al. 1992). Tests for chromosomal aberrations in Chinese hamster fibroblast cells were positive for sodium nitrate, but negative for potassium nitrate (Ishidate et al. 1984). IARC (2010) noted that since sodium chlorite also yielded positive results in the same assay, the chromosomal aberrations induced by sodium

nitrate could have been due to the high osmotic pressure and sodium ion concentration. Ammonium nitrate did not induce chromosomal aberrations in Chinese hamster ovary cells with or without metabolic activation (Kim et al. 2011).

In vivo tests for nitrite conducted in mammalian cells yielded negative results for chromosomal aberrations, SCEs, DNA repair, and cell transformations (Inoue et al. 1985; Ishidate et al. 1984; Lynch et al. 1983; Tsuda and Kato 1977; Tsuda et al. 1973, 1981). Numerous studies have examined the *in vitro* genotoxicity of nitrite; more positive results than negative results were found in tests of gene mutations in prokaryotic organisms, but it is difficult to draw a firm conclusion (Andrews et al. 1980, 1984; Balimandawa et al. 1994; Brams et al. 1987; De Flora 1981, De Flora et al. 1984; Ehrenberg et al. 1980; Ishidate et al. 1984; Törnqvist et al. 1983; Zeiger et al. 1992). However, it appears that the addition of metabolic activation systems to the incubation mixtures did not make a difference in the results. This would indicate that nitrite could be a direct mutagenic chemical.

Additional *in vivo* and *in vitro* studies could be designed to further assess the genotoxicity of nitrate and nitrite.

Reproductive Toxicity. Refer to the section titled "Developmental Toxicity" for information regarding results of case-control studies that evaluated reproductive/developmental end points.

Several animal studies included evaluation of selected reproductive end points. Sleight and Atallah (1968) reported death and reduced litter production among female guinea pigs provided potassium nitrate in the drinking water for up to 204 days of cohabitation at a concentration resulting in estimated intake of 4,972 mg nitrate/kg/day. Reduced litter production was the likely result of maternal toxicity rather than reproductive toxicity *per se*. Sleight and Atallah (1968) also reported decreases in number of litters and live births and histopathologic lesions in reproductive organs (placenta, uterus, and cervix) of guinea pigs administered sodium nitrite in the drinking water. No treatment-related reproductive effects were seen in female Wistar rats provided sodium nitrite in the food throughout the production of two litters (Hugot et al. 1980) or in breeding dogs provided sodium nitrate in the drinking water for 1 year (Kelley et al. 1974). NTP (2001) reported degeneration of the testis in male mice provided sodium nitrite in the drinking water for 14 weeks, and significantly increased estrous cycles in similarly-treated female mice. Among similarly-treated male and female rats, the males exhibited decreased sperm motility.

A multi-generation reproductive toxicity study in laboratory animals could be designed to more comprehensively assess the reproductive toxicity potential of ingested nitrate and nitrite.

Developmental Toxicity. A number of studies evaluated possible associations between developmental end points and exposure to nitrate in humans. The results provide some evidence of nitrate-related developmental effects. The results are not adequate for quantitative risk assessment because (1) estimations of nitrate intakes were typically based on measurements of nitrate levels in drinking water sources at selected time points and self-reported estimates of water consumption; (2) possible confounding by other potential toxicants was not evaluated; and (3) most studies did not account for dietary nitrate or nitrite intake, which is typically the major source of ingested nitrate and nitrite. Some studies reported significant associations between selected developmental end points and nitrate in drinking water sources (Brender et al. 2013; Croen et al. 2001; Dorsch et al. 1984; Scragg et al. 1982). One study reported increased risk of intercalary limb defect associated with estimated total nitrite intake (Huber et al. 2013). Other studies found no evidence of associations between nitrate and risk of developmental effects (Arbuckle et al. 1988; Aschengrau et al. 1989, 1993; Brender et al. 2004; Cedergren et al. 2002; Ericson et al. 1988; Huber et al. 2013; Super et al. 1981). Tabacova et al. (1997, 1998) evaluated maternal health among pregnant women and their infants who lived near an ammonium nitrate fertilizer plant. Nitrogen oxides in the air averaged 23.1 $\mu g/m^3$ with short-term peak levels as high as 238.5; nitrate concentrations in the public drinking water supply measured 8–54 mg/L and nitrate levels in private wells measured as much as 13–400 mg/L. Results indicated that both maternal and cord blood methemoglobin levels were higher in cases of abnormal birth outcome.

Developmental end points have been assessed in some animal studies. Some studies found no indication of nitrite treatment-related developmental toxicity (Hugot et al. 1980; Khera 1982; Shimada 1989). One study reported increased fetal hepatic erythropoiesis, which was thought to have been a response to nitrite-induced fetal methemoglobinemia (Globus and Samuel 1978). Significantly impaired auditory and visual discrimination learning behavior and retention of passive avoidance responses (Nyakas et al. 1990), and delay in cholinergic and serotonergic fiber outgrowth in cortical target areas of the brain (Nyakas et al. 1994), presumably due to nitrite-induced hypoxia, were reported in offspring of Wistar rats provided sodium nitrite in the drinking water. Shuval and Gruener (1972) reported decreases in postpartum survival and pup body weight during 3 weeks postpartum following addition of sodium nitrite to the drinking water of pregnant rats for 6 weeks; no treatment-related effects were observed regarding group litter sizes or pup birth weights. Increased pup mortality, depressed preweaning pup body weight, and delayed swimming development were observed in offspring of male and female rats provided sodium

nitrite in the diet (Vorhees et al. 1984). There were no treatment-related effects on preweaning behavior (surface righting, pivoting, negative geotaxis, or auditory startle) and no effects on postweaning survival, body weight, or most behavioral indices among pups from dams exposed to sodium nitrite in the diet.

Additional human data are needed to comprehensively assess the developmental toxicity potential of ingested nitrate and nitrite.

Immunotoxicity. No information was located regarding immunological or lymphoreticular effects in humans or animals following exposure to nitrate or nitrite by any route. An animal study could be designed to assess the potential immunotoxicity of ingested nitrate and nitrite.

Neurotoxicity. No information was located regarding the neurotoxicity of nitrate in humans or animals. Ingestion of nitrite has been associated with severe methemoglobinemia in adults and children; in many of these cases, clinical signs included dizziness, loss of consciousness, and/or convulsions (CDC 1997, 2002; Gautami et al. 1995; Greenberg et al. 1945; Sevier and Berbatis 1976; Ten Brink et al. 1982). These cases were the result of consumption of food or drink that contained unusually high levels of nitrite via contamination, inadvertent use of sodium nitrite instead of table salt, or ingestion of a single sodium nitrite tablet (667 mg nitrite). Headache was induced in a male subject following consumption of a 10 mg sodium nitrite solution (Henderson and Raskin 1972). In a study designed to evaluate the oral bioavailability of sodium nitrite in healthy volunteers, headache was reported after ingestion of nitrite at doses as low as approximately 1.5–1.8 mg nitrite/kg (Kortboyer et al. 1997b).

Abnormalities in EEGs were reported in male albino rats provided sodium nitrite in the drinking water for 2 months at concentrations resulting in ingestion of ≥9.38 mg nitrite/kg/day (Behroozi et al. 1972). At the highest dose (187.6 mg nitrite/kg/day), rats exhibited clinical signs of sedation and became motionless during periods of electrical outbursts. Increased aggressive behavior was observed in male C57B1 mice provided sodium nitrite in the drinking water at 1,000 mg/L for up to 13 weeks postweaning (Gruener 1974). The mice had also been exposed via their parents during mating and via their mothers during gestation and lactation. Significantly reduced motor activity was reported in male mice provided sodium nitrite in the drinking water (Shuval and Gruener 1972).

The nervous system is not expected to be a particularly sensitive target of nitrate toxicity; available data for nitrite appear adequate for the purpose of hazard identification. Additional neurotoxicity studies do not appear necessary.

Epidemiological and Human Dosimetry Studies. Oral exposure to nitrate and nitrite is ubiquitous because nitrate and nitrite are part of the normal diet. Elevated methemoglobin levels are commonly associated with levels of nitrate in drinking water sources or ingestion of nitrate; clinical signs of methemoglobinemia may be observed at sufficiently high nitrate levels, particularly among newborn infants (e.g., Bosch et al. 1950; Chapin 1947; Comly 1987; Craun et al. 1981; Donahoe 1949; Fan and Steinberg 1996; Fan et al. 1987; Faucett and Miller 1946; Ferrant 1946; Gruener and Toeplitz 1975; Gupta et al. 1999; Johnson et al. 1987; Jones et al. 1973; Medovy 1948; Miller 1971; Robertson and Riddell 1949; Sadeq et al. 2008; Shuval and Gruener 1972; Simon et al. 1964; Stafford 1947; Super et al. 1981; Walton 1951; Winton et al. 1971; Zeman et al. 2002). Although oral exposure to nitrate has been associated with methemoglobinemia in bottle-fed infants receiving drinking water containing measurable levels of nitrate, available studies are limited by lack of accounting for substances in the drinking water (e.g., bacteria) that may have contributed to the methemoglobinemia and the fact that many of the infants exhibited gastroenteritis, which in itself can trigger increased methemoglobin levels. Therefore, additional information regarding the effects of oral exposure of infants to nitrate would serve to reduce uncertainty as to the role of nitrate in the observed methemoglobinemia cases reported in the literature.

Available human data provide suggestive evidence that elevated levels of nitrate in drinking water and/or nitrate-rich diets may be associated with signs of thyroid dysfunction (Aschebrook-Kilfoy et al. 2012; Gatseva and Argirova 2008; Rádiková et al. 2008; Tajtáková et al. 2006; Ward et al. 2010). However, limitations of these studies include lack of individual dose-response data, quantification of iodine intake, and control for other potential substances that may affect the thyroid; one study relied on self-reported thyroid status and self-reported dietary nitrate intake. Additional studies should focus on possible associations between nitrate and/or nitrite and thyroid status.

Possible associations between nitrate and/or nitrite in drinking water and/or food sources and risk of type 1 diabetes have been investigated in a number of epidemiological studies. Significant associations were reported in some studies (Dahlquist et al. 1990; Kostraba et al. 1992; Parslow et al. 1997; Virtanen et al. 1994), but not in other studies (Casu et al. 2000; Moltchanova et al. 2004; van Maanen et al. 2000; Zhao et al. 2001). Limitations of studies include the lack of quantitative dose-response data and the likelihood of confounding by other potential toxicants. Additional studies should focus on possible associations between nitrate and/or nitrite and risk of type 1 diabetes.

Ingestion of nitrite has been associated with severe methemoglobinemia in adults and children (Aquanno et al. 1981; CDC 1997, 2002; Gautami et al. 1995; Gowans 1990; Greenberg et al. 1945; Kaplan et al. 1990; Ringling et al. 2003; Sevier and Berbatis 1976; Ten Brink et al. 1982; Walley and Flanagan 1987), typically following consumption of food or drink that contained unusually high levels of nitrite via contamination, inadvertent use of sodium nitrite instead of table salt, or ingestion of a single sodium nitrite tablet (667 mg nitrite). Other effects noted in some of these cases include hypotension and/or tachycardia, abdominal cramps, vomiting, dizziness, loss of consciousness, convulsions, and even death. In a study designed to evaluate the oral bioavailability of sodium nitrite in healthy volunteers, ingestion of approximately 1.5–1.8 mg nitrite/kg resulted in increased percent methemoglobin and average heart rate, and decreased mean arterial blood pressure (Kortboyer et al. 1997b). Higher ingested doses resulted in more pronounced effects and included nausea and vomiting. Additional information regarding effects of oral exposure to nitrite at lower dose levels would be useful in determining minimal risk levels for nitrite toxicity if populations with such exposure characteristics are identified.

Data needs relating to both prenatal and childhood exposures, and developmental effects expressed either prenatally or during childhood, are discussed in detail in the Developmental Toxicity subsection above.

Biomarkers of Exposure and Effect

Exposure. There are no biomarkers of exposure that are specific to nitrate or nitrite. Although nitrate and nitrite can be detected in blood, saliva, and urine (mostly nitrate), nitrate and nitrite are also produced endogenously via the nitrate-nitrite-nitric oxide pathway. Sources for nitrate and nitrite levels in the body may therefore include not only ingested food and drinking water, but also oxidation of nitric oxide produced endogenously. Similarly, N-nitroso compounds that may be detected in the blood or urine may indicate exposure to nitrate or nitrite; however, these compounds may also be products of the endogenous nitrate-nitrite-nitric oxide pathway.

Effect. Biomarkers of effects from exposure to nitrate or nitrite are not specific to nitrate or nitrite. Blood methemoglobin level has been used as a biomarker of nitrate and nitrite toxicity; however, methemoglobinemia may be elicited by other substances such as selected drugs, pesticides, industrial and commercial products, and medical conditions such as pediatric gastrointestinal infection, sepsis, and sickle cell crisis (ATSDR 2013a). Methemoglobinemia may also be inherited (genetic conditions that result in decreased activity of enzymes that reduce methemoglobin or the presence of hemoglobin M). Urinary levels of various N-nitroso compounds have been measured as an index of nitrosation; however,

N-nitroso compounds can form via endogenous nitrosation and do not require the intake of nitrate or nitrite.

Absorption, Distribution, Metabolism, and Excretion. No information was located regarding the pharmacokinetics of nitrate or nitrite following inhalation or dermal exposure. However, numerous reviews are available regarding the pharmacokinetics of ingested nitrate and nitrite (Bailey et al. 2012; Bryan and van Grinsven 2013; IARC 2010; JECFA 2003a, 2003b; Lundberg and Govoni 2004; Lundberg and Weitzberg 2013; Lundberg et al. 2008, 2009; Weitzberg and Lundberg 2013; Weitzberg et al. 2010; WHO 2011b). Ingestion is the major source of exposure to nitrate and nitrite. The data adequately describe the pharmacokinetics of nitrate and nitrite; additional studies do not appear necessary.

A PBPK model (Zeilmaker et al. 1996, 2010b) simulates the kinetics of methemoglobin formation resulting from gastrointestinal absorption of nitrate in adult humans. The model is adequate for this purpose; however, the model is not considered adequate for the purpose of simulating the kinetics in infants. Additional information is needed to adapt the model to infants for the purpose of quantitative risk assessment.

Comparative Toxicokinetics. Significant differences exist regarding the kinetics of the nitrate-nitrite-nitric oxide pathway in humans and laboratory animals, thus precluding the usefulness of results from laboratory animals to evaluate the toxicokinetics of nitrate or nitrite in humans.

Methods for Reducing Toxic Effects. Ingestion is the most likely route of overexposure to nitrate or nitrite. Methods for reducing peak absorption include oral administration of activated charcoal within a short period following significant ingestion (Seifert 2004) and use of mouthwash containing chlorhexidine (an active antibacterial), which may decrease the reduction of salivary nitrate to nitrite (van Maanen et al. 1996b). No information was located regarding methods to reduce the body burden of nitrate or nitrite. Adequate data are available regarding methods for reducing nitrate- or nitrite-induced methemoglobinemia (e.g., Barclay 1998; Leikin and Paloucek 2008; Seifert 2004). In several rat studies, tumorigenicity associated with concurrent exposure to nitrite and various amino compounds was modulated by coexposure to selected antioxidants such as ascorbic acid, catechol, 3-methoxycatechol, tert-butylhydroquinone, α-tocopherol, and propyl gallate (Chan and Fong 1977; Mirvish et al. 1976, 1983; Miyauchi et al. 2002; Mohktar et al. 1988; Yada et al. 2002; Yoshida et al. 1994); thioproline (which may serve as a nitrite scavenger when nitrosated to nitrosothioproline) (Tahira et al. 1988); or soy bean (Mokhtar et al. 1988).

Children's Susceptibility. Data needs relating to both prenatal and childhood exposures, and developmental effects expressed either prenatally or during childhood, are discussed in detail in the Developmental Toxicity subsection above.

Ingestion of relatively large amounts of nitrate or nitrite can result in methemoglobinemia. The first 6 months of postnatal life is a period of increased susceptibility to methemoglobinemia; possible contributing factors to this increased susceptibility include a higher pH in the infant stomach, greater proportion of fetal hemoglobin (which appears to be more readily oxidized to methemoglobin than adult hemoglobin), and higher concentration of NADH-dependent methemoglobin reductase (an enzyme involved in the reduction of methemoglobin to hemoglobin). Some investigators have reported significant associations between nitrate levels in drinking water (or living in areas presumed to have elevated nitrate levels in drinking water sources) and risk of childhood type 1 diabetes (Dahlquist et al. 1990; Kostraba et al. 1992; Parslow et al. 1997; Virtanen et al. 1994). However, no such relationship was observed in two other studies (van Maanen et al. 2000; Zhao et al. 2001). Refer to Section 3.2.2.2 (Metabolic Effects) for summaries of these study reports.

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

The following ongoing study pertaining to nitrate was identified in National Institutes of Health (NIH) Research Portfolio Online Reporting Tools (RePORTER 2014): Dr. Paul A Romitti, College of Public Health, University of Iowa, is evaluating risk of birth defects associated with nitrate in drinking water.

4. CHEMICAL AND PHYSICAL INFORMATION

4.1 CHEMICAL IDENTITY

Information regarding the chemical identity of nitrate and nitrite is provided in Table 4-1 and information regarding the chemical identity of selected inorganic nitrate and nitrite compounds is provided in Table 4-2. Information regarding ammonia and urea is provided in Table 4-3.

Inorganic nitrate and nitrite are naturally occurring ionic species that are part of the earth's nitrogen cycle (see Figure 5-1). These anions are the products formed via the fixation of nitrogen and oxygen. Chemical processes, biological processes, and microbial processes in the environment convert nitrogen compounds to nitrite and nitrate via nitrogen fixation and nitrification. Compounds such as urea are converted via hydrolysis to ammonia, protonation of ammonia to ammonium (cation), followed by oxidation of ammonium to form nitrite, and then oxidation to form nitrate. Nitrate and nitrite are not neutral compounds, but rather the ionic (anionic; negatively charged) portions of compounds, commonly found in commerce as organic and inorganic salts. As used in this profile, the word "ion" is implied and not used, unless added for clarity.

Nitrate and nitrite typically exist in the environment as highly water-soluble inorganic salts, often bound when not solubilized to metal cations such as sodium or potassium. The nitrate ion is the more stable form as it is chemically unreactive in aqueous solution; however, it may be reduced through biotic processes with nitrate reductase to the nitrite ion. The nitrite ion is readily oxidized back to the nitrate ion via *Nitrobacter* (a genus of proteobacteria), or conversely, the nitrite ion may be reduced to various compounds (IARC 2010; WHO 2011b).

Under certain conditions, nitrite may be converted to a class of compounds called N-nitrosamines. In foods, endogenous production of N-nitrosamines occurs when nitrite reacts with secondary amines or amides. Several factors, including the presence of antioxidants, such as vitamin C, affect the rate of formation. N-nitrosamines are a class of chemical compounds that have a nitroso (N=O) group bonded to an amine (-N(R)R') with a general chemical structure of RN(R')-N=O (IARC 94).

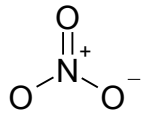
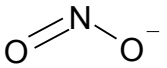
There is a wide range of both organic and inorganic nitrate and nitrite compounds. Common nitrate and nitrite salts include potassium nitrate, potassium nitrite, sodium nitrate, sodium nitrite, and ammonium nitrate; these salts are highly soluble in water, dissociate under environmental conditions, and exist as

ions (WHO 1978, 2011b). Common inorganic fertilizers that contribute to environmental concentrations of nitrate and nitrite include ammonia and urea.

4.2 PHYSICAL AND CHEMICAL PROPERTIES

Information regarding the physical and chemical properties of selected inorganic nitrate and nitrite compounds is provided in Table 4-4 and information regarding the physical and chemical properties of ammonia and urea is provided in Table 4-5.

Table 4-1. Chemical Identity of Nitrate and Nitrite Ions[a]

Characteristic	Nitrate ion	Nitrite ion
Synonym(s)	Nitrate ion; nitrate(1-); nitrate ion (NO_3^-); nitrate ion(1-); nitrato; nitric acid, ion (1-)	Nitrite ion; nitrite (1-); nitrite anion; nitrite ion (NO_2^-); nitrite ion (1-); nitrogen dioxide(1-); nitrogen peroxide ion (1-); nitrous acid, ion (1-)
Registered trade name(s)	No data	No data
Chemical formula	NO_3^-	NO_2^-
Chemical structure[b]		
Ionic weight	62.005	45.995
Identification numbers:		
CAS registry	14797-55-8	14797-65-0
NIOSH RTECS	No data	No data
EPA hazardous waste	No data	No data
DOT/UN/NA/IMDG shipping[c]	UN3218; UN1447	No data
HSDB	Not applicable	Not applicable
NCI	No data	No data
EPA Pesticide Chemical Code	No data	No data

[a]All information obtained from IARC (2010), except where noted.
[b]HSDB 2007.
[c]ChemIDplus 2014.

CAS = Chemical Abstracts Service; DOT/UN/NA/IMDG = 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; RTECS = Registry of Toxic Effects of Chemical Substances

Table 4-2. Chemical Identity of Selected Inorganic Nitrate and Nitrite Compounds[a]

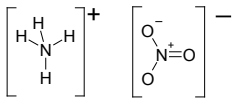
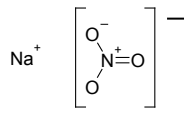
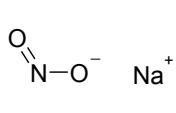
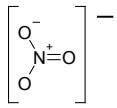
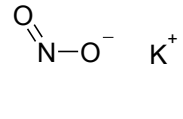
Characteristic	Ammonium nitrate	Sodium nitrate	Sodium nitrite	Potassium nitrate	Potassium nitrite
Synonym(s)	Nitric acid, ammonium salt; ammonium nitrate (NH_4NO_3); Emulite; EXP 200; German saltpeter; Norge saltpeter; Norway saltpeter; Norwegian saltpeter; Plenco 12203; Varioform I; ZhVK	Nitric acid, sodium salt; Chile saltpeter; niter; nitric acid sodium salt(1:1); saltpeter; soda niter; nitrate of soda; cubic niter; nitratine	Nitrous acid, sodium salt; nitrous acid soda; nitrous acid sodium salt (1:1)	Nitric acid, potassium salt; niter; nitre; nitric acid potassium salt (1:1); saltpeter; saltpetre; nitrate of potash	Nitrous acid, potassium salt; Chile saltpeter; niter; nitric acid sodium salt (1:1); salpeter; soda niter
Registered trade name(s)	No data	No data	No data	No data	No data
Chemical formula	NH_4NO_3	$NaNO_3$	$NaNO_2$	KNO_3	KNO_2
Chemical structure	Trigonal $NH_4^+NO_3^-$ 	$Na^+NO_3^-$ 	Trigonal $Na^+NO_2^-$ 	Orthorhombic $K^+NO_3^-$ 	$K^+NO_2^-$ 
Identification numbers:					
CAS registry	6484-52-2	7631-99-4	7632-00-0	7757-79-1	7758-09-0
NIOSH RTECS	BR9050000	WC5600000	RA1225000	TT3700000	TT3750000
EPA hazardous waste	No data	No data	No data	No data	No data
DOT/UN/NA/IMDG shipping	UN 2426; UN 0223; UN 1942; UN 2067; UN 2068; UN 2069; UN 2070; UN 2071; UN 2072; UN 0222 IMO 5.1; NA 1942; IMO 1.1; IMO 9.0	UN 1498; IMO 5.1	UN 1500; IMO 5.1	UN 1486; IMO 5.1	UN 1488; IMO 5.1
HSDB	475	726	757	1227	1216
NCI	No data	No data	No data	No data	No data
NFPA instability hazard[b]	3	No data	No data	No data	No data

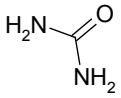
Table 4-2. Chemical Identity of Selected Inorganic Nitrate and Nitrite Compounds[a]

Characteristic	Ammonium nitrate	Sodium nitrate	Sodium nitrite	Potassium nitrate	Potassium nitrite
EPA Pesticide Chemical Code	076101[c]	076104[c]	076204[c]	076103[c]	076203[c]

[a]All information obtained from IARC 2010 and HSDB 2007, except where noted.
[b]NFPA 2002; instability hazard 3 = materials that in themselves are capable of detonation or explosive decomposition or explosive reaction, but that require a strong initiating source or that must be heated under confinement before initiation.
[c]EPA 2014f.

CAS = Chemical Abstracts Service; DOT/UN/NA/IMDG = 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; NFPA = National Fire Protection Association; NIOSH = National Institute for Occupational Safety and Health; RTECS = Registry of Toxic Effects of Chemical Substances

Table 4-3. Chemical Identity of Ammonia[a] and Urea[b]

Characteristic	Ammonia	Urea
Synonym(s)	Anhydrous ammonia, ammonia gas; aqua ammonia; liquid ammonia	Alphahydrate; carbamide; carbonyl diamide; carbonyldiamine; isourea
Registered trade name(s)	BCMW; BUSAN 1215	UAL-37; N-Dure; UF-Concentrate-85; Ureacin-20
Chemical formula	NH_3	CH_4N_2O
Chemical structure		
Ionic weight	17.03	60.06
Identification numbers:		
CAS registry	7664-41-7	57-13-6
NIOSH RTECS	No data	No data
EPA hazardous waste	No data	No data
DOT/UN/NA/IMDG shipping	UN 1005; UN 3318; UN 2672; UN 2073	No data
HSDB	162	163
NCI	No data	No data
EPA Pesticide Chemical Code	005302	085702

[a]HSDB 2012.
[b]HSDB 2003.

CAS = Chemical Abstracts Service; DOT/UN/NA/IMDG = 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; RTECS = Registry of Toxic Effects of Chemical Substances

Table 4-4. Physical and Chemical Properties of Selected Inorganic Nitrate and Nitrite Compounds[a]

Characteristic	Ammonium nitrate	Sodium nitrate	Sodium nitrite	Potassium nitrate	Potassium nitrite
CAS	6484-52-2	7631-99-4	7632-00-0	7757-79-1	7758-09-0
Molecular weight	80.043	84.995	68.985	101.103	85.093
Color	White; colorless (pure); gray or brown (fertilizer grade)	White; colorless	White to pale yellow	Colorless	Pale yellow
Physical state	Solid	Solid	Solid	Solid	Solid
Melting point	169.7°C	306°C; 308°C	271°C	334°C; 337°C	440°C
Boiling point	Decomposes at ~210°C (200–260°C)	380°C; decomposes	320°C; decomposes	400°C; decomposes	537°C; explodes
Density: at 20°C/4°C	1.725 g/cm^3	2.26 g/cm^3	2.17 g/cm^3	2.11 g/cm^3	1.915 g/cm^3
Odor	Odorless	Odorless[b]	No data	Odorless	No data
Odor threshold:	No data	No data	No data	No data	No data
Taste threshold	No data	No data	No data	No data	No data
Solubility:					
Water at 25°C	213 g/100 g	91.2[c] g/100 g	84.8 g/100 g	38.3 g/100 g	312 g/100 g
Organic solvent(s)	Acetone, ammonia, ethanol, isopropanol, methanol	Ammonia, hydrazine, ethanol, methanol, acetone, glycerol	Ammonia, ethanol, methanol	Ammonia; glycerol; sl sol ethanol	Ammonia; alcohol
Partition coefficients	Not available	Not available	Not available	Not available	Not available
Vapor pressure	Not available	Not available	Not available	Not available	Not available
Henry's law constant	Not available	Not available	Not available	Not available	Not available
Flashpoint	Not available	Flames up when heated to 540°C	498ºC; May explode above 530°C	Not flammable	Not flammable
Flammability limits	Not available	Not available	Not available	Not available	Not available
Explosive limits	Not available	Not available	>1,000°C	Not available	Not available

[a]All information obtained from HSDB 2007, unless otherwise noted.
[b]Lewis 2002.
[c]Lide 2013.

CAS = Chemical Abstracts Service; HSDB = Hazardous Substances Data Bank

Table 4-5. Physical and Chemical Properties of Ammonia[a] and Urea[b]

Characteristic	Ammonia	Urea
CAS	7664-41-7	57-13-6
Molecular weight	17.03	60.06
Color	Colorless	White
Physical state	Gas	Crystal/powder
Melting point	-77.7°C	132.70°C
Boiling point	-33.35 °C at 760 mm Hg	Decomposes
Density:		
at 20°C/4°C	0.696 g/L (liquid)	1.3230
Odor	Sharp, pungent, irritating	Slight odor of ammonia; odorless
Odor threshold:	Water: 1.5 mg/L; Air: 5.2 µL/L	No data
Taste threshold	No data	No data
Solubility:		
Water	4.82×10^5 mg/L at 24°C	5.45×10^5 mg/L at 25°C
Organic solvent(s)	Alcohol, chloroform, ether	Alcohol; acetic acid; pyrimidine
Partition coefficients	No data	-2.11
Vapor pressure at 25°C	7.51×10^3 mm Hg	1.2×10^{-5} mm Hg
Henry's law constant	1.61×10^{-5} atm m^3/mole at 25°C	No data
Flashpoint	No data	No data
Flammability limits	No data	No data
Explosive limits	No data	No data

[a]HSDB 2012
[b]HSDB 2003

CAS = Chemical Abstracts Service; HSDB = Hazardous Substances Data Bank

Nitrate is the most oxidized form of nitrogen present in the environment (oxidation state of nitrogen +5). It accounts for the majority of the total available nitrogen in surface waters (Environment Canada 2012), perhaps due to its formation by converting the ammonium ion (e.g., from fertilizer and manure) through a 2-step oxidation process, first to nitrite and then to nitrate. In compounds, nitrate and nitrite typically exist in an oxidation state of 1^-. Nitrate is the conjugate base of nitric acid (HNO_3), a strong acid with pKa of -1.38 at 25°C (Dean 1985). Nitric acid and salts of nitric acid completely dissociate in aqueous solutions, except for nitrates of mercury and bismuth (Environment Canada 2012; WHO 1978). Nitrite is the conjugate base of nitrous acid (HNO_2), a weak acid with a pKa of 3.14 at 25°C (Dean 1985); nitrite readily decomposes to yield water and dinitrogen trioxide (N_2O_3), or nitric acid, nitric oxide (NO), and water (H_2O) (WHO 1978, 2011b).

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

5.1 PRODUCTION

Table 5-1 lists the production year, number of facilities, the state where each facility is located, and the range (in pounds) for each domestic manufacturer that reported the production or formulation of nitrate compounds in 2012 (TRI12 2014). Table 5-2 lists Toxics Release Inventory (TRI) data for sodium nitrite, a common nitrite salt. Table 5-3 lists the TRI data for ammonia. Manufacturers are required to report Toxics Release Inventory (TRI) data to satisfy EPA requirements. The TRI data should be used with caution since only certain types of facilities are required to report (EPA 2005). Facilities that must report to the TRI include industries in a specific business sector such as manufacturing, mining, or electric generation, employ ≥10 full-time employees, and manufacture or process 25,000 pounds of a TRI-listed chemical or use >10,000 pounds of a TRI listed chemical per calendar year. Therefore, there are some facilities that may be processing or using nitrate and/or nitrite, but are not required to report to TRI because they do not meet the regulatory criteria. The amounts reported in Tables 5-1, 5-2, and 5-3 represent those reported by all facilities in each state that are required to report to the TRI and represent the range of minimum to maximum amounts of each chemical present on-site at these facilities during the year. This is not an exhaustive list.

Nitrate and nitrite are not stable compounds, but rather the ionic portions of compounds such as inorganic salts. As used in this profile, the word "ion" is implied and not used, unless added for clarity. Nitrate and nitrite occur naturally in the environment as a part of the nitrogen cycle. Nitrogen fixation is part of the natural process by which free nitrogen gas (N_2) is converted to nitrite, then to nitrate, used by plants, and returned as free N_2 to the atmosphere. This is called the nitrogen cycle, and is shown in Figure 5-1. This cycle occurs through the global environment (Newton 2005). Nitrogen exists naturally in soils. Topsoils contain nitrogen, at content levels as high as 2 to 4 tons/hectare (roughly 1.2–2.4 kg/m^3 in the upper 15 cm of soil; topsoil depths can range between 0 and 30 cm [Hill Laboratories 2014]), typically bound to organic matter and mineral soil material; available forms of nitrogen, including nitrate, are present in soils at a few kg/hectare (Taylor 2004). Nitrate is also formed naturally as an end product of oxidation of vegetable debris and animal and human waste, mainly urine disposed of in waste water. This process is known as nitrification, which is a microbial process that converts ammonia to nitrate and is the principal source for nitrate in the terrestrial and aquatic environment (Environment Canada 2012). Under aerobic conditions, the ammonium ion (e.g., from fertilizer or manure, or discharge from municipal and onsite waste water treatment systems) is converted to nitrite ion via ammonia-oxidizing bacteria (Nolan 1999).

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

State[a]	Number of facilities	Minimum amount on site in pounds[b]	Maximum amount on site in pounds[b]	Activities and uses[c]
AK	2	1,000,000	9,999,999	1, 5, 12, 14
AL	56	0	49,999,999	1, 2, 3, 4, 5, 6, 7, 8, 10, 11, 12, 13, 14
AR	26	0	49,999,999	1, 3, 4, 5, 7, 9, 11, 12, 13
AZ	32	0	99,999,999	1, 4, 5, 6, 7, 11, 12
CA	139	0	499,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
CO	39	0	10,000,000,000	1, 5, 7, 11, 12, 13, 14
CT	26	0	99,999	1, 3, 5, 7, 8, 10, 12
DC	4	1,000	9,999	7, 8
DE	6	0	9,999,999	1, 5, 7, 13, 14
FL	36	0	9,999,999	1, 3, 4, 5, 6, 7, 9, 11, 12, 13, 14
GA	57	0	99,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
GU	1	100,000	999,999	1, 5
HI	7	0	99,999	1, 5, 9
IA	46	0	999,999,999	1, 3, 4, 5, 7, 8, 10, 11, 12, 13
ID	25	0	9,999,999	1, 2, 3, 5, 6, 7, 8, 10, 12, 13, 14
IL	108	0	499,999,999	1, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
IN	62	0	9,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
KS	27	0	999,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13
KY	44	0	999,999	1, 2, 3, 5, 6, 7, 8, 10, 11, 12, 13
LA	48	0	999,999,999	1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14
MA	42	0	9,999,999	1, 3, 5, 6, 7, 11, 12, 13
MD	21	0	999,999	1, 3, 4, 5, 6, 7, 8, 10, 13, 14
ME	12	0	99,999	1, 5, 11, 12
MI	105	0	9,999,999	1, 5, 6, 7, 8, 9, 10, 11, 12, 14
MN	53	0	999,999,999	1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 14
MO	37	0	49,999,999	1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14
MS	28	100	49,999,999	1, 2, 3, 4, 5, 6, 7, 8, 10, 11, 12
MT	10	100	999,999	1, 3, 5, 7, 11, 12, 13
NC	41	0	9,999,999	1, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13
ND	10	100	999,999	1, 5, 7, 8, 13
NE	25	100	99,999,999	1, 3, 4, 5, 6, 7, 10, 12, 13
NH	7	0	99,999	1, 5, 7, 10, 11, 12
NJ	40	100	999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14
NM	14	0	499,999,999	1, 5, 6, 10, 11, 12
NV	27	0	499,999,999	1, 2, 3, 5, 6, 7, 10, 11, 12, 13, 14
NY	73	0	999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
OH	111	0	499,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
OK	37	100	99,999,999	1, 3, 4, 5, 6, 7, 10, 11, 12, 14
OR	40	0	49,999,999	1, 2, 3, 4, 5, 6, 7, 8, 10, 11, 12

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

State[a]	Number of facilities	Minimum amount on site in pounds[b]	Maximum amount on site in pounds[b]	Activities and uses[c]
PA	67	0	499,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13
PR	7	100	999,999	1, 3, 5, 7, 8, 14
RI	6	100	99,999	1, 2, 3, 5, 6, 12
SC	43	0	999,999	1, 2, 3, 5, 6, 7, 10, 11, 12, 13, 14
SD	8	1,000	9,999,999	1, 5
TN	45	0	49,999,999	1, 3, 4, 5, 6, 7, 8, 10, 11, 12, 13, 14
TX	132	0	49,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
UT	38	0	49,999,999	1, 2, 3, 4, 5, 6, 7, 9, 10, 12, 13
VA	42	0	9,999,999	1, 3, 5, 7, 8, 9, 10, 11, 12, 13
VT	6	100	9,999,999	1, 5, 6, 10, 12
WA	46	0	99,999,999	1, 3, 4, 5, 6, 7, 9, 12, 13, 14
WI	127	0	499,999,999	1, 4, 5, 7, 9, 10, 11, 12, 13, 14
WV	18	0	9,999,999	1, 3, 4, 5, 6, 7, 10, 12, 13, 14
WY	5	10,000	99,999,999	1, 3, 4, 6, 7, 11

[a]Post office state abbreviations used.
[b]Amounts on site reported by facilities in each state.
[c]Activities/Uses:

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

Source: TRI13 2014 (Data are from 2013)

Table 5-2. Facilities that Produce, Process, or Use Sodium Nitrite

State[a]	Number of facilities	Minimum amount on site in pounds[b]	Maximum amount on site in pounds[b]	Activities and uses[c]
AL	7	1,000	999,999	1, 5, 6, 7, 10, 11, 12
AR	8	0	99,999	2, 3, 5, 6, 8, 9, 12
AZ	3	1,000	9,999	12
CA	11	1,000	499,999,999	1, 3, 6, 7, 9, 11
CO	2	1,000	99,999	7, 9, 11
FL	1	10,000	99,999	12
GA	11	1,000	99,999	1, 5, 6, 7, 9, 11
IA	3	100	99,999	6, 10
ID	1	10,000	99,999	11
IL	32	0	999,999	1, 3, 4, 5, 6, 7, 9, 10, 11, 12
IN	20	100	9,999,999	1, 2, 3, 5, 6, 7, 9, 10, 11, 12, 13
KS	2	1,000	9,999	10, 12
KY	10	100	999,999	6, 7, 10, 11, 12
LA	9	1,000	9,999,999	1, 5, 6, 7, 10, 11, 12
MA	5	1,000	99,999	6, 12
MD	1	10,000	99,999	2, 3, 11
MI	43	0	9,999,999	2, 3, 6, 7, 8, 9, 10, 11, 12
MN	6	10,000	999,999	10, 12
MO	13	0	99,999,999	2, 3, 6, 7, 10, 11, 12
MS	6	1,000	99,999	7, 10, 11, 12
NC	4	1,000	99,999	1, 5, 7, 12
NE	4	1,000	99,999	7, 8, 9
NJ	9	1,000	999,999	7, 9, 11, 12
NM	1	1,000	9,999	12
NV	1	10,000	99,999	2, 3, 12
NY	8	0	9,999,999	1, 4, 5, 7, 10, 11, 12
OH	35	100	999,999	1, 2, 3, 5, 6, 7, 10, 11, 12
OK	3	100	999,999	1, 5, 7, 11
OR	2	10,000	99,999	11
PA	15	0	9,999,999	1, 5, 6, 7, 10, 11, 12
RI	1	100	999	1, 5, 12
SC	19	100	9,999,999	1, 2, 3, 5, 6, 7, 8, 10, 11, 12
SD	3	1,000	99,999	1, 5, 7
TN	5	1,000	999,999	2, 3, 4, 7, 8, 9, 10, 11, 12
TX	38	0	9,999,999	1, 5, 6, 7, 8, 9, 10, 11, 12, 13
UT	1	0	0	0
VA	4	1,000	999,999	7, 8, 11, 12
WA	1	0	0	0

Table 5-2. Facilities that Produce, Process, or Use Sodium Nitrite

State[a]	Number of facilities	Minimum amount on site in pounds[b]	Maximum amount on site in pounds[b]	Activities and uses[c]
WI	13	100	999,999	7, 9, 11, 12
WV	4	1,000	9,999,999	1, 5, 7, 11, 13

[a]Post office state abbreviations used.
[b]Amounts on site reported by facilities in each state.
[c]Activities/Uses:

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

Source: TRI13 2014 (Data are from 2013)

Table 5-3. 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	0	999,999	1, 2, 3, 5, 11, 12
AL	70	0	49,999,999	1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14
AR	49	0	49,999,999	1, 2, 3, 5, 6, 7, 9, 10, 11, 12, 13
AS	1	1,000	9,999	12
AZ	20	0	49,999,999	1, 5, 6, 7, 9, 10, 11, 12
CA	120	0	99,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
CO	19	0	9,999,999	1, 2, 3, 4, 5, 7, 9, 10, 11, 12, 13, 14
CT	15	0	999,999	1, 2, 3, 5, 6, 7, 8, 10, 11, 12
DC	2	10,000	99,999	12
DE	7	1,000	9,999,999	1, 3, 5, 6, 7, 11, 12
FL	64	0	499,999,999	1, 2, 3, 5, 6, 7, 9, 10, 11, 12, 13
GA	81	0	99,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13
HI	9	0	999,999	1, 3, 5, 6, 7, 9, 10, 11, 12, 13, 14
IA	79	100	999,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
ID	19	100	49,999,999	1, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13
IL	112	100	99,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14
IN	65	0	9,999,999	1, 2, 3, 5, 6, 7, 9, 10, 11, 12, 13, 14
KS	37	0	99,999,999	1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14
KY	47	0	49,999,999	1, 2, 3, 5, 6, 7, 9, 10, 11, 12, 13, 14
LA	72	0	499,999,999	1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14
ME	10	0	999,999	1, 2, 3, 5, 6, 7, 8, 10, 11, 12, 13
MI	70	0	9,999,999	1, 2, 3, 5, 6, 7, 8, 10, 11, 12, 13, 14
MN	61	0	49,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13
MO	46	0	9,999,999	1, 2, 3, 5, 6, 7, 8, 9, 10, 11, 12, 13
MS	34	0	99,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13
MT	10	0	9,999,999	1, 2, 3, 5, 6, 9, 10, 12, 13
NC	86	0	49,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
ND	13	0	99,999,999	1, 3, 4, 5, 6, 9, 10, 11, 12
NE	45	100	499,999,999	1, 2, 3, 4, 5, 6, 7, 8, 10, 11, 12, 13, 14
NH	9	0	9,999,999	1, 3, 5, 6, 10, 11, 12
NJ	43	0	999,999	1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13
NM	6	0	99,999	1, 3, 5, 6, 11, 12, 13, 14
NV	12	0	9,999,999	1, 2, 3, 5, 6, 7, 9, 12, 13, 14
NY	50	0	999,999	1, 2, 3, 5, 6, 7, 8, 9, 10, 11, 12, 14
OH	115	0	10,000,000,000	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
OK	24	0	99,999,999	1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14
OR	31	0	9,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13
PA	95	0	49,999,999	1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14

Table 5-3. Facilities that Produce, Process, or Use Ammonia

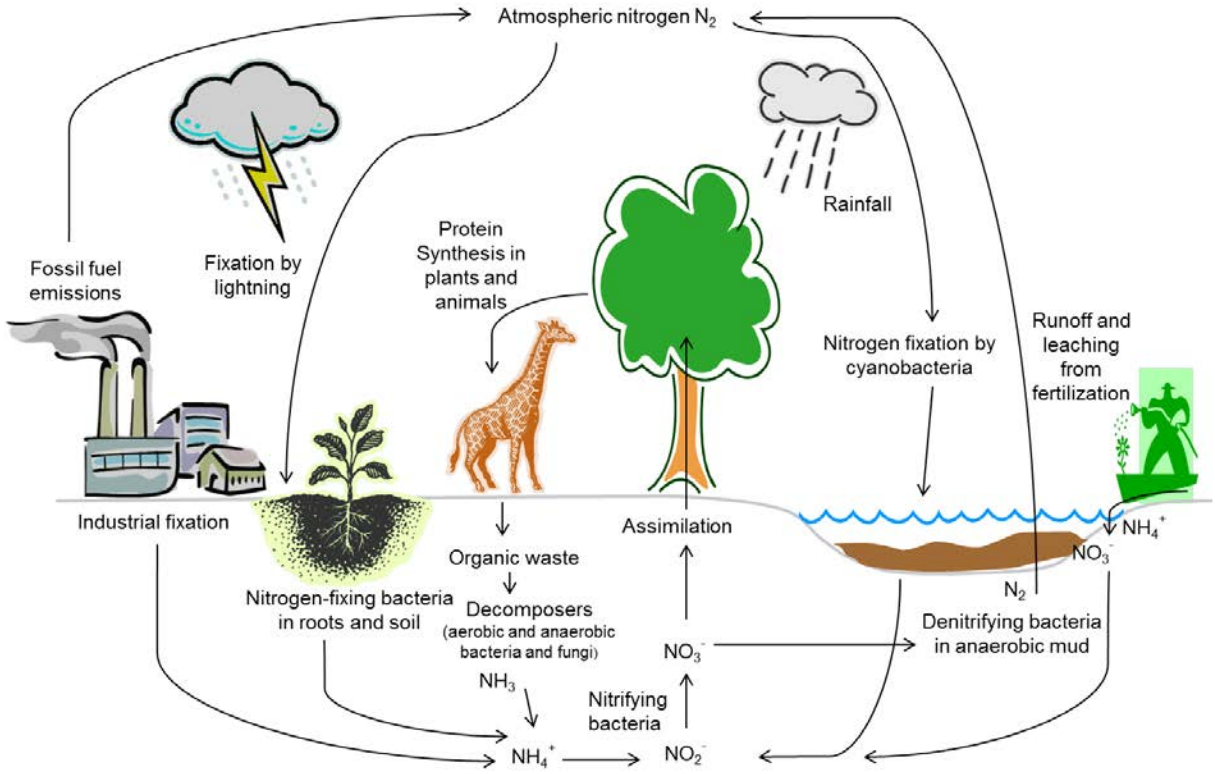
State[a]	Number of facilities	Minimum amount on site in pounds[b]	Maximum amount on site in pounds[b]	Activities and uses[c]
PR	10	0	999,999	1, 2, 3, 4, 5, 6, 7, 10, 12
RI	10	1,000	9,999,999	1, 2, 3, 4, 5, 6, 9, 10, 11
SC	54	0	499,999,999	1, 3, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
SD	10	1,000	999,999	1, 2, 5, 7, 10, 11, 13
TN	70	0	999,999	1, 2, 3, 5, 6, 7, 8, 9, 10, 11, 12, 13
TX	211	0	99,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
UT	27	0	9,999,999	1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12
WA	29	0	9,999,999	1, 2, 3, 5, 6, 7, 9, 10, 11, 12, 13
WI	81	0	49,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
WV	34	0	49,999,999	1, 2, 3, 5, 6, 7, 8, 10, 11, 12, 13, 14
WY	14	0	99,999,999	1, 2, 3, 4, 5, 6, 7, 10, 12, 13

[a]Post office state abbreviations used.
[b]Amounts on site reported by facilities in each state.
[c]Activities/Uses:

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

Source: TRI13 2014 (Data are from 2013)

Figure 5-1. Simplified Schematic of the Nitrogen Cycle



Adapted from EEA 2010; EPA 2012a; Vitousek et al. 1997

This oxidation process is an intermediate step in the nitrogen cycle, followed by further oxidation of nitrite to nitrate ion via nitrite-oxidizing bacteria. These two reactions are mediated by aerobic chemolithotrophs, *Nitrosomonas* and *Nitrobactor*, respectively (WHO 1978). Microbial conversion of nitrate to nitrite (reduction) may also occur, especially after prolonged storage of vegetables that make the environment anaerobic.

In nature, nitrate can also be found in igneous and volcanic rocks; however, the high solubility of nitrogen salts makes minerals containing nitrate rare. Major minerals known are saltpeter (KNO_3) found in India, and Chile saltpeter ($NaNO_3$) found in deserts of northern Chile (Environment Canada 2012; Hammerl and Klapotke 2006).

Plants and mammals naturally contain nitrate and nitrite (WHO 2011b). Assimilation of nitrite from soils occurs via reduction of nitrate to nitrite, which is facilitated by various bacteria and catalyzed by nitrate reductase (WHO 1978). Mammals endogenously produce nitrate and excrete it in their waste products (WHO 1978, 2011b).

Various industrial process produce nitrate in their waste streams. Specifically, potassium nitrate, calcium nitrate, silver nitrate, and sodium nitrate used in several industrial applications have waste waters with high-nitrate concentrations (Environment Canada 2012).

A major source of anthropogenic nitrate and nitrite is artificial fertilizers (WHO 1978). The majority of nitrate in the environment derived from fertilizers does not solely originate from nitrate-containing fertilizers; it also comes from ammonium and urea fertilizers. Nitrate from ammonium and urea fertilizers is produced through biological processes involving hydrolysis of urea to ammonium and ammonium nitrification (Kissel et al. 2008). Approximately 11.5 million tons of nitrogen are applied yearly (as of 1994) in the United States as fertilizer in agricultural areas (Nolan et al. 1997). The Association of American Plant Food Control Officials and The Fertilizer Institute reported that the United States used 13.5 thousand tons of nitrogen fertilizer in 2012 (TFI 2014). Ammonium, calcium, potassium, and sodium salts are all used in commercial fertilizers compounds (IARC 2010; WHO 2011b). The most common nitrite salt, sodium nitrite, is produced commercially via the reaction of nitrogen oxides with sodium carbonate or sodium hydroxide solution, typically at a pH higher than 8 (Hammerl and Klapotke 2006). In 2004, global production of sodium nitrate was about 63 kilotons (IARC 2010). Ammonium nitrate is manufactured through the reaction of nitric acid and ammonium (HSDB 2007). Global production of ammonium nitrate in 2002 was reported at 13,608 kilotons (IARC 2010). Between

1998 and 1999, 90 kilotons of Canadian fertilizers were nitrate compounds: 82% as ammonium nitrate and the remaining 18% from calcium nitrate, calcium ammonium nitrate, and potassium nitrate (Environment Canada 2012).

According to the 2011 SRI Directory of Chemical Producers, there are 15 domestic producers of ammonium nitrate in the United States, with an annual capacity of 2,290 metric tons (SRI 2011). There were six producers of sodium nitrate, two producers of sodium nitrite, and one producer of potassium nitrite; however, no production volumes or capacities were reported for any of these substances (SRI 2011). Production of ammonium nitrate in 2004 by the United States chemical industry was reported as 6,558 thousands of metric tons and preliminary production data reported 6,353 thousands of metric tons for the year 2005 (HSDB 2007). Production of ammonium nitrate by the U.S. chemical industry in 1994 through 2003 is listed in Table 5-4. U.S. production of sodium nitrate in 1982 was estimated as $4.75x10^7$ kg and at least $5.0x10^7$ kg in 1977; U.S. production of sodium nitrite in 1977 was reported as at least $5.0x10^6$ kg; U.S. production of potassium nitrate in 1972 and 1975 were reported as $4.23x10^7$ and $9.89x10^7$ kg, respectively (HSDB 2007).

Production of ammonia by the U.S. chemical industry in 1995 through 2002 is listed in Table 5-5. According to 2012 Chemical Data Reporting (CDR) data, the total reported production volumes for ammonia and urea were 1.75 $x10^{10}$ kg/year and 1.17 $x10^{10}$, respectively (EPA 2014g) Consumption patterns indicate that the major use for these chemicals is in the fertilizer industry (HSDB 2003, 2012).

5.2 IMPORT/EXPORT

In 1984, United States imports of ammonium nitrate were $1.14x10^{11}$ g (109,247 metric tons) and exports in 1975 were reported as $3.18x10^{10}$ (31,298 metric tons) (HSDB 2007). In 1986, U.S. imports of potassium nitrate were $3.62x10^6$ g (3.56 metric tons) and exports in 1975 were reported as negligible (HSDB 2007). In 1985, U.S. imports of sodium nitrate were $6.44x10^7$ g (63.4 metric tons) and exports in 1985 were reported as $4.81x10^6$ (4.73 metric tons) (HSDB 2007). In 1984, U.S. imports of sodium nitrite were $8.14x10^9$ g (8,011 metric tons) and exports in 1984 were reported as $4.03x10^{11}$ (396,635 metric tons) (exports related to general sodium compounds) (HSDB 2007).

Table 5-4. Production of Ammonium Nitrate by the U.S. Chemical Industry

Year	Thousands of metric tons
1994	7,771
1995	7,700
1996	7,708
1997	7,804
1998	8,235
1999	6,920
2000	7,237
2001	5,833
2002	6,436
2003	5,733

Source: HSDB 2007

Table 5-5. Production of Ammonia by the U.S. Chemical Industry

Year	Millions of metric tons
1994	64,510
1995	35,600
1999	16.6
2000	15.7
2001	9.5
2002	10.8

Source: HSDB 2012

The U.S. Department of Agriculture (USDA 2013) has compiled annual import/export data on nitrate fertilizers (ammonium nitrate, potassium nitrate, and sodium nitrate) for years 2000–2012. The volumes for ammonium nitrate are provided in Table 5-6, followed by a 2012 comparison for all three fertilizers (Table 5-7).

5.3 USE

The majority of nitrate in commerce is used in common inorganic fertilizers. Ammonia, urea, ammonium nitrate, sodium nitrate, potassium nitrate, and calcium nitrate are used as commercial fertilizers; ammonium nitrate and sodium nitrate are also used in munitions and explosives. These chemicals have uses defined in several other industrial and consumer categories. Nitrate and nitrite salts are used as preservatives in beverages. Additional uses include oxidizing agents, in instant cold packs and for the production of nitrous oxide (ammonium nitrate), and for glass making (potassium nitrate) (EPA 2009a; IARC 2010; Taylor 2004; WHO 2011b). Potassium and ammonium nitrate may also be used in pyrotechnics, herbicides, and insecticides (HSDB 2007). Sodium nitrite is mainly used in the food industry as a preservative, in cured meats for preventing botulism (e.g., it inhibits microbial activity of certain *Clostridium* species in cheeses), and in the chemical, pharmaceutical, and agricultural industries (Hammerl and Klapotke 2006; HSDB 2007; WHO 2011b). Sodium nitrite also has therapeutic uses such as an antidote for cyanide poisoning and as an antifungal topical agent, for example against MRSA strains (HSDB 2007; Ormerod et al. 2011; Pokorny and Maturana 2006). Due to the bioactivity of NO, an endogenous metabolite of nitrite produced under hypoxic conditions, sodium nitrite is being used in medicinal applications, such as for the treatment of pulmonary arterial hypertension (Blood and Power 2015; Lundberg et al. 2008; Rix et al. 2015). Potassium nitrate has been added to some toothpastes for cavity prevention and to reduce sensitivity, as well as being used as a curing agent and color fixative in meats (HSDB 2007). In nature, plants utilize nitrate as an essential nutrient (WHO 2011b).

5.4 DISPOSAL

Disposal methods for anthropogenic sources of nitrate and nitrite are general; unused portions of the material should be recycled for the approved use or returned to the manufacturer or supplier, while leaks or spills should be resolved wearing appropriate protective equipment and taking care not to create a flammable or explosive environment. Response to a small liquid spill involves stopping the leak, soaking up the liquid with vermiculite or sand, and placing it in a non-combustible container. Response to a large liquid spill on land involves diking, product recovery, treating residue with soda ash and neutralizing it with HCl, and flushing residue from the area with water. Response to a solid spill involves picking up the

Table 5-6. U.S. Imports and Exports (Metric Tons) of Selected Fertilizers 2000–2012

Year	Ammonium nitrate exports	Ammonium nitrate imports	Calcium nitrate exports	Calcium nitrate imports	Potassium nitrate exports	Potassium nitrate imports	Sodium nitrate exports	Sodium nitrate imports
2012	335,080	851,196	Not reported	38,550	15,746	159,135	3,348	148,898
2011	314,764	633,974	Not reported	33,998	16,449	114,861	3,286	90,470
2010	317,737	548,976	Not reported	34,490	9,991	76,849	2,429	70,156
2009	195,455	450,664	Not reported	123,168	8,449	73,871	2,536	79,741
2008	188,818	706,955	Not reported	204,552	4,322	132,571	5,783	149,467
2007	194,038	1,107,220	Not reported	187,640	Not reported	135,912	3,139	72,892
2006	127,244	1,150,523	Not reported	156,997	Not reported	149,633	2,827	68,416
2005	82,237	907,618	Not reported	119,448	Not reported	86,961	2,289	66,655
2004	109,972	1,055,949	Not reported	126,498	Not reported	66,381	2,838	62,812
2003	51,856	1,203,985	Not reported	90,989	Not reported	78,754	2,465	85,565
2002	98,218	989,507	Not reported	99,200	Not reported	100,712	2,810	72,568
2001	19,277	925,534	Not reported	127,586	Not reported	50,791	2,199	89,422
2000	21,611	838,035	Not reported	108,269	Not reported	40,941	2,264	96,067

Source: USDA 2013

Table 5-7. U.S. Exports and Imports for Nitrate Fertilizers in 2012 (Short Tons)

Fertilizer	Exports	Imports
Ammonium nitrate	369,362	938,283
Potassium nitrate	17,357	175,416
Sodium nitrate	3,691	164,132
Urea	370,694	7,654,464
Anhydrous ammonia	41,504	6,938,744
Aqua ammonia	6,549	96,517
All fertilizers[a]	10,783,383	35,552,395

[a]Includes nitrogen, potassium and phosphate fertilizers

Source: USDA 2013

material with implements (e.g., shovels, broom, and pan), placing in a non-combustible container, and loosely capping the container. Spills to water can be treated with activated charcoal. Ultimate disposal of the chemicals should take into account several factors (the material's impact on air quality; migration characteristics; effects on animal, aquatic, and plant life) and must take into account compliance with environmental and public health regulations. Generally, this involves treatment with sodium carbonate, neutralization with HCl, and disposal of the resulting sludge in a secure landfill. If incineration is used, processes to remove nitrogen dioxide and nitrogen oxide should be included (HSDB 2007).

6. POTENTIAL FOR HUMAN EXPOSURE

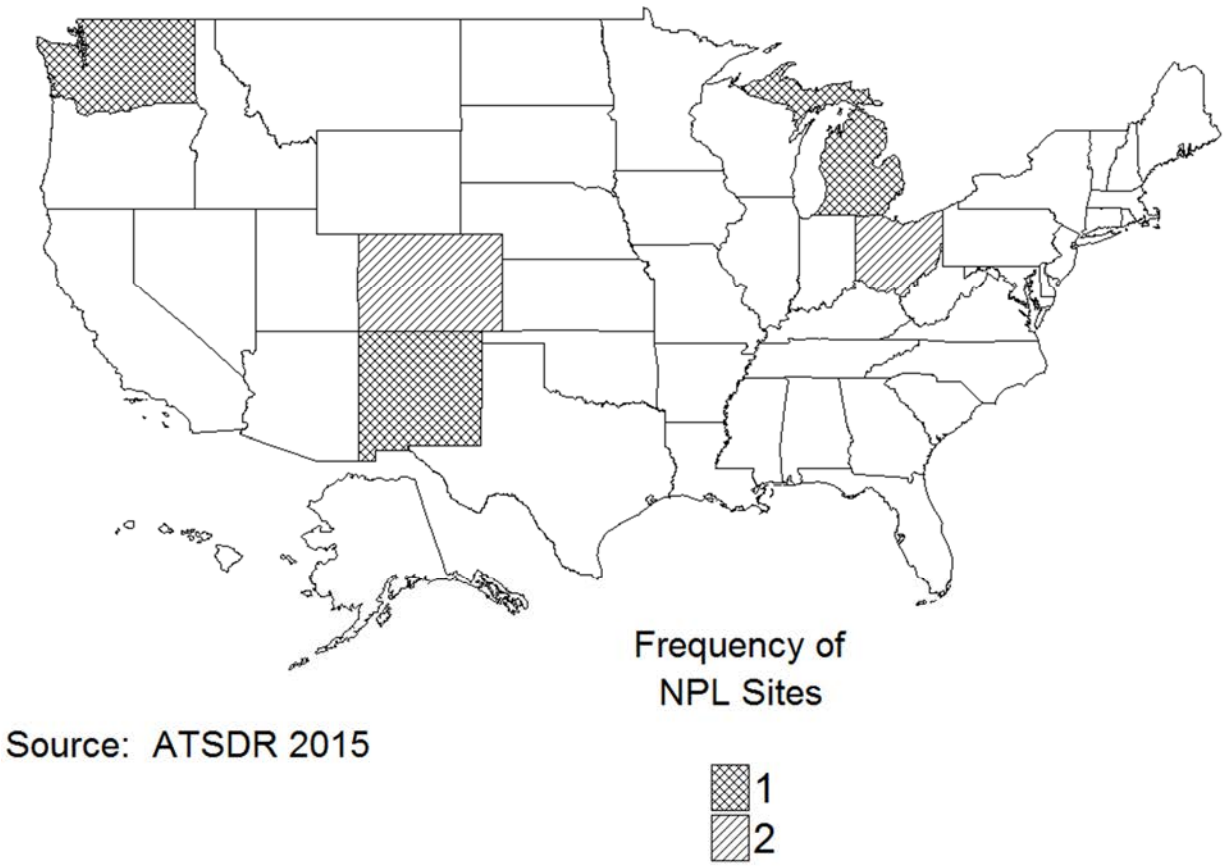
6.1 OVERVIEW

Nitrate and nitrite are ubiquitous in the environment. Specific salts have occasionally been identified in hazardous waste sites. Ammonium nitrate, sodium nitrate, and sodium nitrite were identified in 7, 3, and 2, of the 1,832 hazardous waste sites, respectively that have been proposed for inclusion on the EPA National Priorities List (NPL) (ATSDR 2015). However, the number of sites evaluated for these substances is not known. The frequency of these sites can be seen in Figures 6-1, 6-2, and 6-3.

Nitrate may enter the environment via natural and anthropogenic sources. Nitrate and nitrite occur naturally in the environments as a part of the earth's nitrogen cycle. A major source of anthropogenic nitrate and nitrite is artificial fertilizers, and various industrial processes also produce nitrate in their waste streams (Environment Canada 2012; WHO 1978). Inorganic fertilizer and nitrification of animal waste are the principal sources of nitrate in the environment (Environment Canada 2012; Nolan et al. 1997). However, contributions from human waste must be taken into account as well. Point and non-point anthropogenic sources that contribute include industrial waste water, mining (explosives) waste water, agricultural and urban runoff, feedlot discharges, septic system and landfill leachate, lawn fertilizers, storm sewer overflow, and nitric oxide and nitrogen dioxide from vehicle exhaust (Environment Canada 2012). Additionally, organic forms of nitrogen in the environment from various sources may undergo ammonification to form inorganic ammonia and ammonium, and nitrification to form nitrate, and have the potential to be released into surface waters (Environment Canada 2012). Inorganic nitrate and nitrite in soil and water can be taken up by plants used for human consumption (ATSDR 2013a).

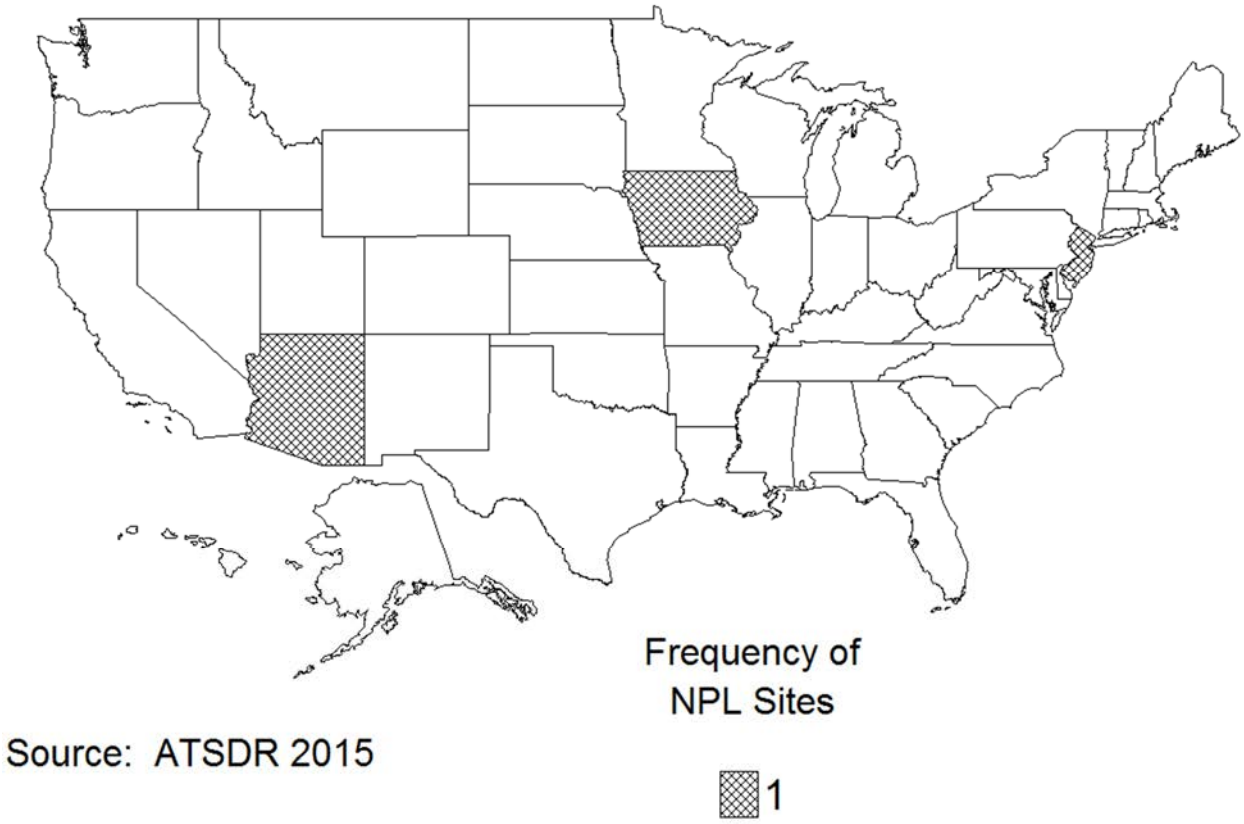
Exposure from drinking water of private wells is a source of concern as elevated concentrations have been reported in some wells, yet these water sources are not routinely tested, monitored, or regulated since they are not covered by the Safe Drinking Water Act (SDWA). About 15% of Americans use private wells as a source of drinking water and an important percentage of them may have a septic system serving their homes. Additionally, nitrate and nitrite exposure can occur from the ingestion of foods containing high levels of these chemicals (ATSDR 2013a).

Figure 6-1. Frequency of NPL Sites with Ammonium Nitrate Contamination



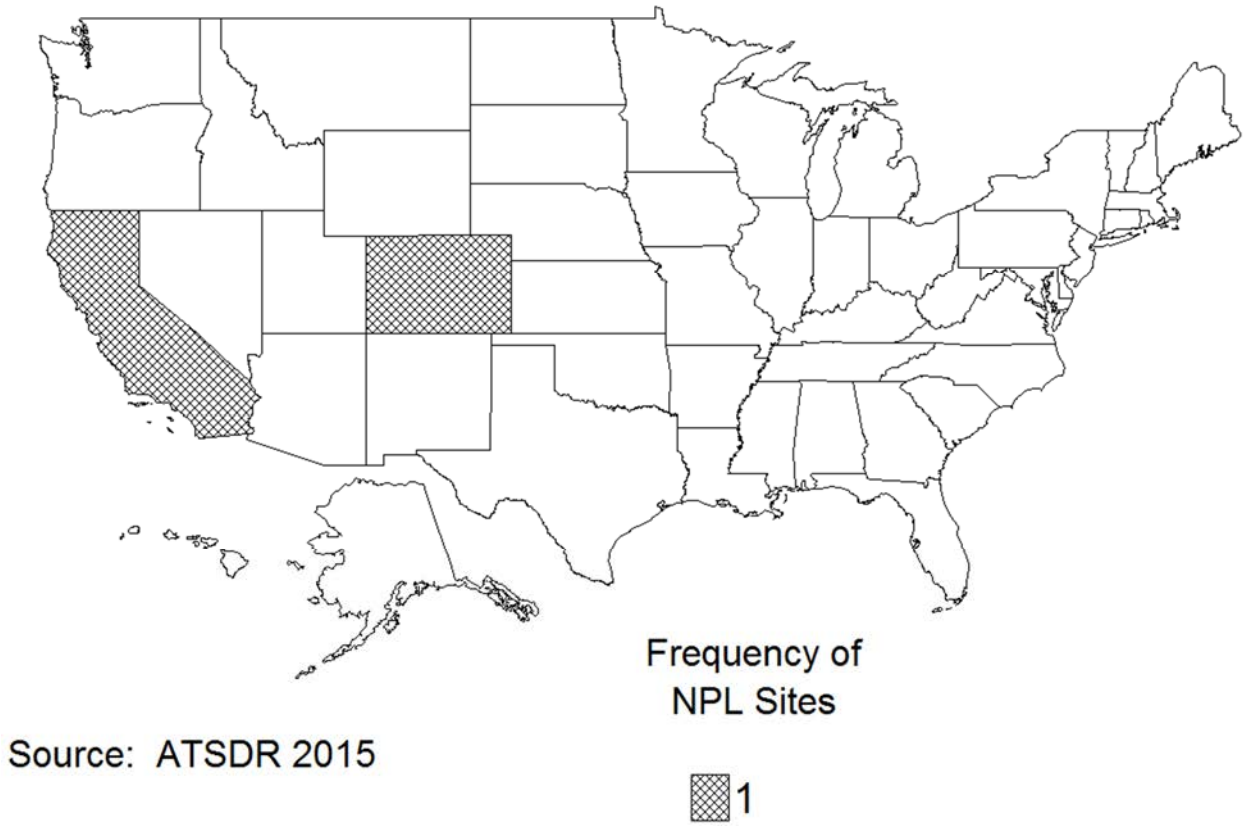
Source: ATSDR 2015

Figure 6-2. Frequency of NPL Sites with Sodium Nitrate Contamination



Source: ATSDR 2015

Figure 6-3. Frequency of NPL Sites with Sodium Nitrite Contamination



Source: ATSDR 2015

6.2 RELEASES TO THE ENVIRONMENT

The Toxics Release Inventory (TRI) data should be used with caution because only certain types of facilities are required to report (EPA 2005). This is not an exhaustive list. Manufacturing and processing facilities are required to report information to the TRI only if they employ 10 or more full-time employees; if their facility is included in Standard Industrial Classification (SIC) Codes 10 (except 1011, 1081, and 1094), 12 (except 1241), 20–39, 4911 (limited to facilities that combust coal and/or oil for the purpose of generating electricity for distribution in commerce), 4931 (limited to facilities that combust coal and/or oil for the purpose of generating electricity for distribution in commerce), 4939 (limited to facilities that combust coal and/or oil for the purpose of generating electricity for distribution in commerce), 4953 (limited to facilities regulated under RCRA Subtitle C, 42 U.S.C. section 6921 et seq.), 5169, 5171, and 7389 (limited S.C. section 6921 et seq.), 5169, 5171, and 7389 (limited to facilities primarily engaged in solvents recovery services on a contract or fee basis); and if their facility produces, imports, or processes ≥25,000 pounds of any TRI chemical or otherwise uses >10,000 pounds of a TRI chemical in a calendar year (EPA 2005).

Nitrate is released into the environment through both natural and anthropogenic sources. Naturally occurring nitrate and nitrite are part of the earth's nitrogen cycle. Anthropogenic sources, including animal and human organic wastes as well as nitrogen-containing fertilizers, increase the concentrations of nitrate in the environment. Nitrate and nitrite are present in the environment, in soils and water, and to a lesser extent, in air, plant materials, and meat products. Concentrations of nitrite in plants and water are low relative to nitrate concentration due to the fact that nitrite is easily oxidized to nitrate (WHO 1978).

Nitrate is the ion detected in the majority of groundwater and surface water samples because the nitrite ion is easily oxidized to nitrate in the environment; the nitrate ion is stable and is chemically unreactive under most environmental conditions (IARC 2010; WHO 2011b).

6.2.1 Air

Estimated releases of 301,654 pounds (~137 metric tons) of nitrate compounds to the atmosphere from 2,110 domestic manufacturing and processing facilities in 2013, accounted for about 0.1% of the estimated total environmental releases from facilities required to report to the TRI (TRI13 2014). These releases are summarized in Table 6-1. Estimated releases of 65,201 (~30 metric tons) pounds of sodium nitrite were released to the atmosphere from 363 domestic manufacturing and

Table 6-1. Releases to the Environment from Facilities that Produce, Process, or Use Nitrate Compounds[a]

		Reported amounts released in pounds per year[b]							
							Total release		
State[c]	RF[d]	Air[e]	Water[f]	UI[g]	Land[h]	Other[i]	On-site[j]	Off-site[k]	On- and off-site
AK	2	0	310,000	0	1,730,003	0	2,040,003	No data	2,040,003
AL	56	5	10,970,520	5,000	589,240	1,003	11,012,324	553,444	11,565,768
AR	26	0	3,776,021	0	14,399	4,217	3,790,167	4,470	3,794,637
AZ	32	3,295	0	0	39,894	78	43,189	78	43,267
CA	139	7,172	1,967,150	20,684	956,477	65,682	2,831,868	185,297	3,017,165
CO	39	1	1,850,266	0	54,944	50	1,904,854	407	1,905,261
CT	26	58,098	202,953	0	698	54,000	261,051	54,698	315,749
DC	4	0	0	0	0	0	0	No data	0
DE	6	0	2,850,359	0	0	0	2,850,359	No data	2,850,359
FL	36	479	850,888	23,373,722	264,705	0	24,261,115	228,680	24,489,794
GA	57	511	12,284,962	0	352,756	196,086	12,573,225	261,090	12,834,315
GU	1	0	181,244	0	196	0	181,440	No data	181,440
HI	7	0	439,915	0	0	0	439,915	No data	439,915
IA	46	23,005	6,737,465	0	133,387	28	6,886,605	7,280	6,893,885
ID	25	53	2,462,856	0	1,590,643	0	4,021,989	31,564	4,053,553
IL	108	28,202	6,428,670	14,007	454,232	2,346	6,875,892	51,565	6,927,456
IN	62	439	19,965,218	0	3,287,114	12,527	19,965,662	3,299,636	23,265,298
KS	27	38,262	108,068	340,905	98,569	21	585,186	639	585,825
KY	44	990	5,031,265	0	313,935	532	5,305,617	41,104	5,346,721
LA	48	4,502	10,169,890	1,576,528	55,015	0	11,753,676	52,259	11,805,934
MA	42	10	115	25,928	27,091	217,377	145	270,376	270,520
MD	21	0	739,290	0	84,199	35	739,687	83,837	823,524
ME	12	1,209	2,854,965	0	63	0	2,856,209	28	2,856,237
MI	103	10,021	1,714,827	0	228,050	33,121	1,732,091	253,928	1,986,019
MN	53	1,076	1,471,856	0	78,276	250	1,536,094	15,364	1,551,458
MO	37	2,852	1,752,877	0	241,834	5,100	1,977,139	25,524	2,002,663
MS	28	372	6,495,644	0	329	0	6,496,345	No data	6,496,345
MT	10	0	234,169	0	43,891	0	272,860	5,200	278,060
NC	41	1	6,563,023	0	236,361	236,692	6,793,505	242,572	7,036,077
ND	10	0	113,400	0	15,640	0	129,040	No data	129,040
NE	25	187	11,785,649	0	243,650	40	12,022,108	7,418	12,029,526
NH	7	125	0	0	0	39	125	39	164
NJ	40	0	5,313,118	0	41,966	376	5,354,858	602	5,355,460
NM	14	55,000	42,240	0	662,620	0	406,331	353,529	759,860
NV	27	6	2,800	0	3,947,279	2	3,729,262	220,825	3,950,087
NY	73	5,337	5,797,905	0	437,314	28,473	5,804,333	464,696	6,269,029
OH	111	1,987	6,081,057	134,614	182,994	64,665	6,219,720	245,598	6,465,318
OK	37	13,010	4,246,811	534,620	180,969	0	4,970,061	5,349	4,975,410
OR	40	1,000	542,093	0	6,833	0	545,071	4,855	549,926

Table 6-1. Releases to the Environment from Facilities that Produce, Process, or Use Nitrate Compounds[a]

		Reported amounts released in pounds per year[b]							
							Total release		
State[c]	RF[d]	Air[e]	Water[f]	UI[g]	Land[h]	Other[i]	On-site[j]	Off-site[k]	On- and off-site
PA	67	9,139	7,212,765	0	66,541	2,428	7,224,885	65,988	7,290,874
PR	7	0	0	1,465	120	34	1,465	154	1,619
RI	6	0	121	0	0	20,098	121	20,098	20,219
SC	43	3,001	2,346,088	0	178,745	458	2,376,696	151,596	2,528,293
SD	8	0	2,995,074	2,000	338,782	6	3,280,324	55,538	3,335,862
TN	45	270	2,748,175	470,956	125,258	1,678	2,772,445	573,892	3,346,337
TX	131	788	14,234,564	7,762,004	876,395	7,165	21,822,716	1,058,200	22,880,916
UT	37	329	97,000	0	1,282,016	29	714,559	664,815	1,379,374
VA	42	3,558	10,978,189	0	4,587	1	10,981,823	4,511	10,986,334
VT	6	0	124,890	0	57,395	0	124,890	57,395	182,285
WA	46	8,770	1,190,505	0	981,730	0	1,631,774	549,231	2,181,004
WI	127	591	2,162,376	0	2,339,390	31,174	3,734,315	799,216	4,533,531
WV	18	18,000	2,140,714	0	2,138	0	2,160,673	179	2,160,852
WY	5	0	633	6,569,900	249	0	6,570,782	No data	6,570,782
Total	2,110	301,654	188,570,641	40,832,332	22,848,913	985,811	242,566,590	10,972,761	253,539,351

[a]The 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.
[b]Data in TRI are maximum amounts released by each facility.
[c]Post office state abbreviations are used.
[d]Number of reporting facilities.
[e]The sum of fugitive and point source releases are included in releases to air by a given facility.
[f]Surface water discharges, waste water treatment-(metals only), and publicly owned treatment works (POTWs) (metal and metal compounds).
[g]Class I wells, Class II-V wells, and underground injection.
[h]Resource Conservation and Recovery Act (RCRA) subtitle C landfills; other onsite landfills, land treatment, surface impoundments, other land disposal, other landfills.
[i]Storage only, solidification/stabilization (metals only), other off-site management, transfers to waste broker for disposal, unknown
[j]The sum of all releases of the chemical to air, land, water, and underground injection wells.
[k]Total amount of chemical transferred off-site, including to POTWs.

RF = reporting facilities; UI = underground injection

Source: TRI13 2014 (Data are from 2013)

processing facilities in 2013, accounted for about 0.8% of the estimated total environmental releases from facilities required to report to the TRI (TRI13 2014). These releases are summarized in Table 6-2. Estimated releases of 125,680,001 pounds (~57,007 metric tons) of ammonia were released to the atmosphere from 2,292 domestic manufacturing and processing facilities in 2013, accounted for about 77% of the estimated total environmental releases from facilities required to report to the TRI (TRI13 2014). These releases are summarized in Table 6-3.

6.2.2 Water

Estimated releases of 188,570,641 pounds (~85,534 metric tons) of nitrate compounds to surface water from 2,110 domestic manufacturing and processing facilities in 2013, accounted for about 74% of the estimated total environmental releases from facilities required to report to the TRI (TRI13 2014). These releases are summarized in Table 6-1. Estimated releases of 2,472,668 pounds (~1,122 metric tons) of sodium nitrite compounds to surface water from 363 domestic manufacturing and processing facilities in 2013, accounted for about 30% of the estimated total environmental releases from facilities required to report to the TRI (TRI13 2014). These releases are summarized in Table 6-2. Estimated releases of 4,221,440 pounds (~1,914 metric tons) of ammonia to surface water from 2,292 domestic manufacturing and processing facilities in 2013, accounted for about 2.6% of the estimated total environmental releases from facilities required to report to the TRI (TRI13 2014). These releases are summarized in Table 6-3.

EPA (2009d) reported that the Mississippi River drains >40% of the area of the contiguous 48 states and carries roughly 15 times more nitrate than any other river in the country. EPA (2009d) noted that the nitrate load in the Mississippi rose from 200,000 to 500,000 tons per year in the 1950s and 1960s to an average of approximately 1,000,000 tons per year during the 1980s and 1990s; the data indicate that the nitrate load decreased slightly in the early 2000s.

Nitrate is commonly detected in various surface waters and groundwaters such as shallow, rural domestic wells. Contamination of water systems is a consequence of inorganic fertilizer use, animal manures, septic systems, and waste water treatment (ATSDR 2013a; Nolan 1999; WHO 2011b). Ammonium ions in sludge from waste water treatment plants, as well as effluents from those plants and septic systems, are rapidly converted to nitrate (WHO 1978). Various industrial process produce nitrate in their waste streams. For example, potassium nitrate, calcium nitrate,

Table 6-2. Releases to the Environment from Facilities that Produce, Process, or Use Sodium Nitrite[a]

		Reported amounts released in pounds per year[b]							
							Total release		
State[c]	RF[d]	Air[e]	Water[f]	UI[g]	Land[h]	Other[i]	On-site[j]	Off-site[k]	On- and off-site
AL	7	0	0	0	360	0	0	360	360
AR	7	0	254	0	0	0	254	No data	254
AZ	3	0	0	0	9,117	0	9,117	No data	9,117
CA	11	7	0	0	5	0	7	5	12
CO	2	255	0	0	0	0	255	No data	255
FL	1	0	0	0	0	0	0	No data	0
GA	11	98	648,256	0	101,148	68	749,502	68	749,570
IA	3	0	2,517	0	0	0	2,517	No data	2,517
ID	1	0	0	0	0	0	0	No data	0
IL	32	1,637	18,600	0	90,065	15	20,237	90,080	110,317
IN	20	1	1,130,853	0	3,312,017	4,896	1,130,854	3,316,913	4,447,767
KS	2	0	0	0	0	0	0	No data	0
KY	10	1,016	0	0	41,435	36,320	27,291	51,480	78,771
LA	9	0	48,000	1,500,000	107	0	1,548,000	107	1,548,107
MA	5	0	0	0	0	0	0	No data	0
MD	1	16	0	0	0	103	16	103	119
MI	42	12,992	4	0	181,207	2,490	18,745	177,948	196,693
MN	6	0	194,173	0	0	0	194,173	No data	194,173
MO	13	2,871	0	0	412	3,685	2,871	4,097	6,968
MS	6	8,489	7,895	0	26,296	3	16,384	26,299	42,683
NC	4	0	4,455	0	0	0	4,455	No data	4,455
NE	4	0	21,200	0	1,182	1,637	21,467	2,552	24,019
NJ	9	200	68,032	0	2,898	0	68,290	2,840	71,130
NM	1	0	0	0	0	0	0	No data	0
NV	1	2	0	0	33,641	0	33,642	No data	33,642
NY	8	11,807	4,925	0	2,800	220	16,732	3,020	19,752
OH	35	13,067	731	0	77,303	1,134	13,798	78,437	92,235
OK	3	289	9,010	0	17,405	0	9,299	17,405	26,704
OR	2	0	0	0	430	0	0	430	430
PA	15	250	0	0	7	0	250	7	257
RI	1	0	0	0	0	0	0	No data	0
SC	19	1,530	69,403	0	787	190	70,933	977	71,910
SD	3	6	121	25	5,500	0	5,652	0	5,652
TN	5	239	0	0	153	0	239	153	392
TX	38	34	199,659	198,969	54,487	0	449,818	3,331	453,149
UT	1	No data	No data	No data	No data	No data	No data	No data	No data
VA	4	0	0	0	40	0	0	40	40
WA	1	No data	No data	No data	No data	No data	No data	No data	No data

Table 6-2. Releases to the Environment from Facilities that Produce, Process, or Use Sodium Nitrite[a]

		Reported amounts released in pounds per year[b]							
							Total release		
State[c]	RF[d]	Air[e]	Water[f]	UI[g]	Land[h]	Other[i]	On-site[j]	Off-site[k]	On- and off-site
WI	13	10,231	28	0	69,018	0	10,259	69,018	79,277
WV	4	165	44,552	0	3	0	44,717	3	44,720
Total	363	65,201	2,472,668	1,698,994	4,027,823	50,760	4,469,774	3,845,673	8,315,446

[a]The 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.
[b]Data in TRI are maximum amounts released by each facility.
[c]Post office state abbreviations are used.
[d]Number of reporting facilities.
[e]The sum of fugitive and point source releases are included in releases to air by a given facility.
[f]Surface water discharges, waste water treatment-(metals only), and publicly owned treatment works (POTWs) (metal and metal compounds).
[g]Class I wells, Class II-V wells, and underground injection.
[h]Resource Conservation and Recovery Act (RCRA) subtitle C landfills; other onsite landfills, land treatment, surface impoundments, other land disposal, other landfills.
[i]Storage only, solidification/stabilization (metals only), other off-site management, transfers to waste broker for disposal, unknown
[j]The sum of all releases of the chemical to air, land, water, and underground injection wells.
[k]Total amount of chemical transferred off-site, including to POTWs.

RF = reporting facilities; UI = underground injection

Source: TRI13 2014 (Data are from 2013)

Table 6-3. Releases to the Environment from Facilities that Produce, Process, or Use Ammonia[a]

		Reported amounts released in pounds per year[b]							
							Total release		
State[c]	RF[d]	Air[e]	Water[f]	UI[g]	Land[h]	Other[i]	On-site[j]	Off-site[k]	On- and off-site
AK	6	23,099	7,012	136	24,075	0	54,323	No data	54,323
AL	70	4,060,001	209,278	9,343	43,646	294	4,287,915	34,647	4,322,563
AR	47	1,837,750	114,277	0	5,499	511	1,956,076	1,961	1,958,037
AS	1	20	0	0	0	0	20	No data	20
AZ	20	336,273	0	0	722	0	336,989	6	336,995
CA	118	2,801,456	26,830	2,870	165,409	369	2,978,123	18,811	2,996,934
CO	19	269,063	17,833	0	101,218	2,430	385,942	4,602	390,544
CT	15	74,775	155	0	0	0	74,930	No data	74,930
DC	2	165	0	0	0	0	165	No data	165
DE	7	58,659	6,071	0	23	0	64,730	23	64,753
FL	63	5,607,959	244,014	464,183	960,078	0	6,343,579	932,655	7,276,234
GA	81	12,615,696	268,976	0	166,494	153	12,967,436	83,883	13,051,319
HI	9	100,496	1,000	1,200	0	0	102,696	No data	102,696
IA	79	7,333,058	108,999	0	210,562	6,621	7,545,358	113,882	7,659,240
ID	19	2,844,444	27,824	0	167,548	0	3,023,068	16,749	3,039,817
IL	112	3,081,028	110,035	0	69,293	4,620	3,246,667	18,309	3,264,976
IN	64	1,600,854	45,833	707,485	77,195	0	2,423,117	8,250	2,431,367
KS	37	3,056,601	6,490	38,214	49,185	15,483	3,130,592	35,381	3,165,972
KY	47	971,022	55,064	0	48,663	1,845	1,036,520	40,073	1,076,593
LA	72	13,461,749	585,745	4,582,747	326,520	0	18,630,317	326,444	18,956,761
MA	30	178,727	41	0	1,622	0	178,768	1,622	180,390
MD	16	523,890	48,675	0	2	0	572,565	2	572,567
ME	10	753,203	89,718	0	0	0	842,921	No data	842,921
MI	70	1,681,959	36,582	9,790	7,181	7,875	1,731,377	12,010	1,743,387
MN	61	1,870,843	49,112	0	35,092	2,270	1,938,690	18,627	1,957,317
MO	46	422,032	228,193	0	44,490	251	654,800	40,166	694,966
MS	34	4,700,259	188,194	0	2,181	0	4,889,803	830	4,890,633
MT	10	449,711	5,420	0	264,118	0	719,205	44	719,249
NC	86	2,661,108	85,427	0	73,410	110	2,781,752	38,302	2,820,054
ND	13	16,199,585	4,476	11,500	474,130	0	16,689,625	66	16,689,691
NE	44	974,508	26,655	0	162,935	4,643	1,021,771	146,970	1,168,741
NH	9	127,211	447	0	0	2	127,658	2	127,660
NJ	43	527,774	9,790	0	24,984	74	537,605	25,017	562,622
NM	6	98,819	0	2,300	11,561	0	112,680	No data	112,680
NV	12	132,670	560	0	228,759	1	361,989	1	361,990
NY	50	687,039	50,197	0	974	274	737,570	915	738,485
OH	115	6,430,630	100,258	1,715,361	76,575	2,560	8,244,156	81,229	8,325,384
OK	24	5,433,575	18,832	696,880	137,282	0	6,282,692	3,877	6,286,569
OR	31	1,156,034	35,599	0	9,980	0	1,192,997	8,616	1,201,613

Table 6-3. Releases to the Environment from Facilities that Produce, Process, or Use Ammonia[a]

		Reported amounts released in pounds per year[b]							
							Total release		
State[c]	RF[d]	Air[e]	Water[f]	UI[g]	Land[h]	Other[i]	On-site[j]	Off-site[k]	On- and off-site
PA	95	1,739,875	77,649	59	710,403	4,905	1,829,424	703,467	2,532,891
PR	10	366,211	0	0	1,499	0	366,211	1,499	367,710
RI	10	7,130	0	0	0	2,500	7,130	2,500	9,630
SC	54	3,581,123	163,436	0	15,085	60,935	3,747,126	73,453	3,820,579
SD	10	102,302	1,809	1	21,448	0	104,819	20,741	125,560
TN	70	2,512,358	418,374	0	90,697	0	2,932,376	89,053	3,021,430
TX	209	4,853,142	297,133	17,187,730	397,858	468	21,789,218	947,114	22,736,332
UT	27	520,482	1,484	8	1,162,922	120	1,684,865	151	1,685,016
VA	49	4,358,663	105,431	0	30,198	32,318	4,471,823	54,787	4,526,610
VT	2	4,543	5,357	0	76	0	9,900	76	9,976
WA	29	878,866	55,744	0	100,110	3,880	940,195	98,405	1,038,600
WI	81	593,142	45,129	15,241	14,091	0	640,527	27,076	667,603
WV	34	332,238	227,614	137,238	47,192	13	602,602	141,693	744,294
WY	14	686,181	8,666	297,557	2,791	0	995,195	No data	995,195
Total	2,292	125,680,001	4,221,440	25,879,844	6,565,774	155,524	158,328,597	4,173,986	162,502,583

[a]The 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.
[b]Data in TRI are maximum amounts released by each facility.
[c]Post office state abbreviations are used.
[d]Number of reporting facilities.
[e]The sum of fugitive and point source releases are included in releases to air by a given facility.
[f]Surface water discharges, waste water treatment-(metals only), and publicly owned treatment works (POTWs) (metal and metal compounds).
[g]Class I wells, Class II-V wells, and underground injection.
[h]Resource Conservation and Recovery Act (RCRA) subtitle C landfills; other onsite landfills, land treatment, surface impoundments, other land disposal, other landfills.
[i]Storage only, solidification/stabilization (metals only), other off-site management, transfers to waste broker for disposal, unknown
[j]The sum of all releases of the chemical to air, land, water, and underground injection wells.
[k]Total amount of chemical transferred off-site, including to POTWs.

RF = reporting facilities; UI = underground injection

Source: TRI13 2014 (Data are from 2013)

silver nitrate, and sodium nitrate used in several industrial applications have waste waters with high-nitrate concentrations (Environment Canada 2012). Discharges of these waste streams increase the concentrations of nitrate and nitrite in surface waters. Treatment of these waste streams may only remove a portion of nitrogen. Factors such as nitrogen loading, population density, soil drainage characteristics, and woodland to cropland ratios, affect the transport of nitrogen from land to water (Nolan et al. 1997; Zhang et al. 1998). Increased levels of nitrite in drinking water may also be a consequence of contamination by boiler fluid additives (ATSDR 2013a). High risk waters for nitrate contamination include areas having soils with high permeability, high-nitrogen input, and low woodland to cropland ratios (Nolan et al. 1997; Zhang et al. 1998).

Natural sources of nitrate and nitrite include wet and dry deposition of atmospheric nitric acid and nitrate ion. These are formed in the atmosphere as a result of nitrogen cycling. Atmospheric deposition is a factor for nitrate concentrations in water systems (Momen et al. 1999). Atmospheric nitrogen (NO_3^-, NO_2^-, and NH_4^+), mainly from natural sources but a result of anthropogenic sources as well, have been estimated to contribute 182 kilotons of inorganic nitrogen per year to Canadian surface waters via wet and dry deposition (Environment Canada 2012). In the United States, deposition contributes an estimated 3.2 million tons (3,200 kilotons) of nitrogen per year to watersheds (Momen et al. 1999; Nolan 1999; Nolan et al. 1997). Owens et al. (1994) reported that nitrogen input to a grass pasture from precipitation was equivalent to 10% of the nitrogen fertilizer applied during a 5-year period. The concentration of nitrate-nitrogen in the precipitation during 1975–1980 was reported as 1.1 mg/L (ppm), which correlated to an input of 12.0 kg nitrate-nitrogen/hectare. A 10-year average was also evaluated for the years 1980–1990, which resulted in an input of 8.9 kg nitrate-nitrogen/hectare (Owens et al. 1994).

Stagnation of nitrate-containing and oxygen-poor drinking water in galvanized steel pipes and chlorination disinfectant residues can lead to conditions where nitrite is formed via chemical reactions in the distribution pipes by *Nitrosomonas* bacteria (WHO 2011b).

A U.S. Geological Survey (USGS) study across the United States showed that 7% of 2,388 domestic wells and about 3% of 384 public-supply wells were contaminated with nitrate levels above the EPA drinking water standard of 10 mg/L (10 ppm) (ATSDR 2013a). Between 1994 and 1996, 24 lakes in the Adirondack Park, United States, were studied to assess the contribution of in-lake processes, atmospheric deposition, and watershed cover on the lakes' nitrate concentrations (Momen et al. 1999). Weighted means for nitrate concentrations as a result of precipitation near the lakes were reported for all seasons during the study period and ranged from 13.86 to 35.52 μeq/L. Nitrogen concentrations throughout the

study period ranged from 2.1 to 22 µmol/L. Both atmospheric deposition and average lake depth were considered strong factors in concentrations of nitrate in lakes. It was concluded that the average lake depth was the most important factor; greater average depths correlated to higher nitrate concentrations. This was attributed to decreased contact time with lake sediment, decreasing the potential for removal processes (Momen et al. 1999).

Concentrations of nitrate in freshwater downstream from an open-pit coal mining operation have been reported to exceed 44 mg nitrate/L (Nordin and Pommen 1986). This is attributed to high nitrate levels in waste streams due to explosive residues. Monitoring studies conducted by the USGS indicate that nitrate and nitrite levels are several times greater in streams and groundwater in areas classified as agricultural use rather than as urban use, mixed use, or undeveloped land (USGS 2010a, 2010b).

Policies implemented by the European Union (EU) to reduce nitrogen emissions from agricultural point sources were reviewed by Velthof et al. (2014). The Nitrates Directive (ND) was implemented to protect water quality across Europe by inhibiting nitrates released by agricultural sources from leaching into groundwater and surface waters through the use of good farming practices. Although regional differences in emissions were large throughout the entire EU, nitrate leaching into groundwater and surface waters was estimated to decrease by 16% in nitrate leaching vulnerable zones over the period of 2000–2008, primarily as a result of lower nitrogen emissions from fertilizers and manures (Velthof et al. 2014).

Seawater nitrate concentrations that occur naturally due to nitrification processes can be as high as 2.4 mg nitrate/L (Environment Canada 2012). Assimilation into biological systems can deplete nitrate concentrations in marine environments, causing seasonal variations in nitrate concentrations. Winter concentrations off the Canadian Atlantic coast were reported to be 0.54 mg nitrate/L, a magnitude higher than summer concentrations of <0.03 mg nitrate/L (Environment Canada 2012).

6.2.3 Soil

Estimated releases of 22,848,913 pounds (~10,364 metric tons) of nitrate compounds to soils from 2,110 domestic manufacturing and processing facilities in 2013, accounted for about 9% of the estimated total environmental releases from facilities required to report to the TRI (TRI13 2014). An additional 40,832,332 pounds (~18,521 metric tons), constituting about 16% of the total environmental emissions, were released via underground injection (TRI13 2014). These releases are summarized in Table 6-1. An estimated release of 4,027,823 pounds (~1,826 metric tons) of sodium nitrite were emitted to soils from

363 domestic manufacturing and processing facilities in 2013, accounted for about 48% of the estimated total environmental releases from facilities required to report to the TRI (TRI13 2014). An additional 1,698,994 pounds (~771 metric tons), constituting about 20% of the total environmental emissions, were released via underground injection (TRI13 2014). These releases are summarized in Table 6-2. An estimated release of 6,565,774 pounds (~2,978 metric tons) of ammonia were emitted to soils from 2,292 domestic manufacturing and processing facilities in 2013, accounted for about 4% of the estimated total environmental releases from facilities required to report to the TRI (TRI13 2014). An additional 25,879,844 pounds (~11,739 metric tons), constituting about 16% of the total environmental emissions, were released via underground injection (TRI13 2014). These releases are summarized in Table 6-3.

In 2012, 13.5 million tons of nitrogen was added to soils as fertilizer (TFI 2014). Therefore, it should be noted that the totals provided here, for nitrate compounds, ammonia, and ammonium nitrite alone, may be insignificant, yet contribute to and are representative of, nitrogen releases to the environment.

6.3 ENVIRONMENTAL FATE

Nitrate and nitrite occur naturally in water and soils as part of the nitrogen cycle. Plants and mammals naturally contain nitrate and nitrite (WHO 2011b). Nitrate is the primary source of nitrogen for plants (EPA 2009a). Assimilation of nitrite from soils occurs via reduction of nitrate to nitrite, which is facilitated by various bacteria and catalyzed via nitrate reductase (WHO 1978). The most common forms of nitrogen that plants assimilate include ammonium (NH_4^+), nitrate (NO_3^-), and urea ($(NH_2)2CO$) (Cornell University 2009). Transport, partitioning, and transformation are controlled by various physicochemical properties, degradation, and other loss processes. Mammals endogenously produce nitrate and excrete them in their waste products (WHO 1978). Anthropogenic and natural sources of ammonia in the environment, such as fertilizers or animal waste products, are converted to nitrite via *Nitrosomonas* bacteria and then to nitrate via *Nitrobactor* bacteria. These products may be assimilated into plants and subsequently the atmosphere, or may leach into groundwater when they are present in excessive amounts (WHO 2011b).

Nitrate is the most oxidized form of nitrogen present in the environment (oxidation state of +5) and accounts for the majority of the total available nitrogen in surface waters (Environment Canada 2012). Nitrate is the conjugate base of nitric acid, HNO_3, a strong acid with pKa of -1.37 (WHO 1978). Nitric acid and salts of nitric acid completely dissociate in aqueous solutions (Environment Canada 2012; WHO 1978). Nitrite is the conjugate base of nitrous acid, HNO_2, a weak acid with a pKa of 3.37; nitrite readily

decomposes to yield water and dinitrogen trioxide or nitric acid, nitric oxide, and water (WHO 1978; WHO 2011b).

6.3.1 Transport and Partitioning

Nitrate and nitrite are inorganic water-soluble salts with the potential for rapid migration through soils to surface water and groundwater (Nolan 1999; Taylor 2004; EPA 2009a). Sorption of anions such as nitrate is insignificant in most soils; therefore, leaching of excess soil nitrate into oceans, lakes, streams, and groundwater is an important consideration (Taylor 2004). Drainage characteristics of soils are strongly related to nitrate levels in shallow wells near agricultural areas (Nolan et al. 1997; Zhang et al. 1998). Other factors affecting leaching potential include the texture of the soil, pH, precipitation rates, tillage, and the types of crops or vegetation that may be planted in the soils.

The mobility of nitrate in a mid-European semi-natural grassland ecosystem as a function of plant diversity was investigated (Scherer-Lorenzen et al. 2003). The greatest leaching was observed in bare ground plots as well as plots planted only with legumes. Experiments with plots containing a wider variety of plant species indicated that total nitrate plant uptake increased and leaching losses decreased with increasing plant diversity due to greater root biomass within the soils. The leaching of nitrate decreased in the following order: bare plots > pure legumes > legumes + grasses > legumes + grasses + herbs (Scherer-Lorenzen et al. 2003). Annual nitrate leaching in an apple orchard was 4.4–5.6 times greater in plots treated with conventional farming practices (calcium nitrate fertilizer) as compared to plots treated by organic farming practices, in which nitrogen application was accomplished by loadings of chicken manure and alfalfa meal (Kramer et al. 2006). Reduced leaching was accompanied by increased denitrification in the organic treatment areas. Kitchen et al. (2015) investigated groundwater nitrate as a result of leaching due to agriculture cropping systems over time (1994–2004) and found the greatest decreases in groundwater nitrate concentration occurred as groundwater moved through an in-field tree line or through a riparian zone.

Nitrate leaching from croplands with high fertilizer use is a major source of groundwater nitrate concentrations. In Nebraska, groundwater concentrations of nitrate have been correlated with nitrogen-containing fertilizer application rates and residual nitrogen in surface soils (Schepers et al. 1991). The reduction of nitrate concentrations in groundwater through agricultural management practices was assessed in Nebraska's central Platte River valley (Exner et al. 2010). Groundwater nitrate concentration reports were studied from 1986 to 2003. Peak levels, during 1988, in the primary aquifers were

26.8 mg nitrate-nitrogen/L. A gradual decline was observed with the implementation of fertilizer management regulations. In 2003, nitrate-nitrogen levels in the aquifer averaged 22.0 mg/L.

Nitrate in soils and surface water are susceptible to denitrification resulting in gaseous losses to the atmosphere (Taylor 2004). Nitrate in the atmosphere, emitted by denitrification, industrial processes, and vehicle exhaust, is deposited on land and water in precipitation, gases, and dry particles (Nolan 1999; Taylor 2004). Atmospheric deposition is a factor for nitrate concentrations in water systems (Momen et al. 1999; Nolan 1999; Nolan et al. 1997).

6.3.2 Transformation and Degradation

Nitrate and nitrite has the potential to move into various environmental compartments and are subject to abiotic and biotic degradation processes. Transformation and degradation processes include denitrification to atmospheric nitrogen and plant uptake (Newton 2005; Nolan 1999). Conversion is achieved via biotic process carried out by auto- and heterotrophic bacteria (Hammerl and Klapotke 2006). Under aerobic conditions in aquatic systems, ammonia and nitrite are converted to nitrate via nitrification. Conversion is achieved through a biotic process carried out by autotrophic nitrifying bacteria. Under anaerobic conditions in aquatic systems, bacteria convert nitrate to nitrite, which is further reduced to the gaseous compounds nitric oxide (NO), nitrous oxide (N_2O), and N_2 (nitrogen). These compounds are subsequently released to the atmosphere. Results from a study of denitrification in riverbed sediments found that potential rates for denitrification are limited by environmental conditions such as available organic carbon and temperature, rather than concentration of nitrate itself (Pfenning and McMahon1997). Higher rates were demonstrated in experiments with added carbon sources. Additionally, higher rates of denitrification were measured at 22°C compared to those at 4°C (Pfenning and McMahon1997).

6.3.2.1 Air

Nitrogen compounds are formed in the air by natural phenomena such as lightning (Hord et al. 2011), or may be discharged into air from industrial processes, motor vehicles, agricultural practices, or emitted by denitrification processes. Nitrate is present in air primarily as nitric acid and inorganic aerosols, as well as nitrate radicals and organic gases or aerosols (WHO 2011b). Nitrate in the atmosphere is subject to wet and dry deposition and are deposited on land via precipitation, gases, and dry particles (Nolan 1999; Taylor 2004).

6.3.2.2 Water

In surface waters, assimilation by plants and algae accounts for the majority of nitrate loss. Reducing conditions of water system including dissolved oxygen (DO) and dissolved organic carbon (DOC), as well as temperature and pH, influence the extent of bacterially mediated nitrate loss processes in water systems (Nolan 1999; WHO 2011b). Biologically mediated reduction processes of nitrate and nitrite were found to be positively related to DO and inversely related to iron, manganese, ammonium, and DOC concentrations (Nolan 1999).

Rates of denitrification were examined by measuring N_2O production using riverbed sediment and groundwater or surface water collected from the South Platte alluvial aquifer in Colorado, an area with high nitrate levels due to anthropogenic activity such as fertilizer use, farming practices, and septic drainage and runoff (Pfenning and McMahon 1996). The greatest N_2O production was observed in microcosms containing high levels of organic carbon. The type of organic carbon source was also shown to be correlated with the denitrification rate. Higher N_2O production rates were observed with acetate-amended sediments as compared to sediments amended with fulvic acids. Surface water-derived fulvic acids resulted in higher denitrification rates as compared to groundwater-derived fulvic acids. Reduction in microbial activity due to temperature gradients was also investigated. Denitrification rates decreased by nearly 80% in laboratory experiments when the temperature was lowered from 22 to 4°C.

6.3.2.3 Sediment and Soil

In soils, nitrite is oxidized to nitrate and the majority of nitrate is assimilated by plants and algae (WHO 2011b). Under aerobic conditions, residual or excess nitrate is expected to leach into groundwater and is not expected to undergo considerable degradation and/or denitrification. Under anaerobic conditions degradation of nitrate into atmospheric nitrogen is an important removal process (WHO 2011b). Poorly drained soils, which lack oxygen, promote nitrate conversion to gaseous nitrogen (Nolan et al. 1997).

Denitrification rates were examined using soil samples obtained from different areas of an apple orchard and measuring the rates of gaseous nitrogen and N_2O production as well as nitrate leaching (Kramer et al. 2006). Specifically denitrification rates were measured at different locations of the orchard treated with organic farming practices, conventional farming practices for orchards in the state of Washington, and integrated treatments from horticultural and pest management practices of conventional and organic farming practices. Nitrogen application in the conventional plots used calcium nitrate based fertilizer, while the organic plots used chicken manure and alfalfa meal. The integrated plots used equal parts of

calcium nitrate fertilizer and chicken manure. Nitrogen emissions were higher in the organically treated plots, as compared to the conventional and integrated plots. Nitrate leaching was much greater (4.4–5.6 times higher) in the conventional plots as compared to the organically treated plots.

6.4 LEVELS MONITORED OR ESTIMATED IN THE ENVIRONMENT

Reliable evaluation of the potential for human exposure to nitrate and nitrite depends in part on the reliability of supporting analytical data from environmental samples and biological specimens. Concentrations of nitrate and nitrite in unpolluted atmospheres and in pristine surface waters are often so low as to be near the limits of current analytical methods. In reviewing data on nitrate and nitrite levels monitored or estimated in the environment, it should also be noted that the amount of chemical identified analytically is not necessarily equivalent to the amount that is bioavailable. The analytical methods available for monitoring nitrate and nitrite in a variety of environmental media are detailed in Chapter 7.

Nitrate occurs naturally in the environments as a part of the earth's nitrogen cycle. Elevated levels may be present due to anthropogenic sources such as fertilizers, and human or animal wastes. High levels of nitrate in drinking water pose a health risk to infants, children, and pregnant or nursing women (EPA 2009a).

6.4.1 Air

Anthropogenic emissions of nitrogen oxides (NO_x) are now of the same order of magnitude as natural emissions (Hammerl and Klapotke 2006). Air pollution is considered a minor source of exposure to nitrate (WHO 2011b). Nitrate in the atmosphere is generally a result of nitrogen oxides released into the atmosphere that are oxidized to nitric acid, in turn forming nitrate particles (Matsumoto and Tanaka 1996). Atmospheric levels of particulate nitrate are highly dependent on temperature and the chemical composition of aerosol and gases in the atmosphere, especially particulate ammonium nitrate and gaseous nitric acid (Matsumoto and Tanaka 1996). Reported atmospheric nitrate concentrations range from low concentrations of 0.1–0.4 μg/m^3 up to higher-level concentrations ranging from 1 to 40 μg/m^3 (WHO 1978, 2011b). Concentrations in Netherland air samples have been reported to range from 1 to 14 μg/m^3. Indoor nitrate aerosol concentrations of 1.1–5.6 μg/m^3 appear to be related to outdoor concentrations (WHO 2011b). Zhuang et al. (1999) evaluated the concentrations of fine and coarse particle nitrate in the atmosphere over Hong Kong. The average daily concentrations for fine and coarse particle nitrate were found to be 0.583 and 1.663 μg/m^3, respectively.

6.4.2 Water

Nitrate and nitrite concentrations in water are typically expressed as either mg nitrate/L (ppm nitrate) and mg nitrite/L (ppm nitrite), or mg nitrate as nitrogen (nitrate-nitrogen/L) and mg nitrite as nitrogen (nitrite-nitrogen/L) (IARC 2010). The federal drinking-water standard maximum contaminant level (MCL) for nitrate is 10 mg nitrate-nitrogen/L and the MCL for nitrite is 1 mg nitrite-nitrogen/L (EPA 2009c; USGS 2010a; WHO 2011b). Inorganic nitrate and nitrite are very soluble in water and occur naturally in groundwater and surface water as a result of the earth's nitrogen cycle. Naturally occurring background levels of nitrate (concentrations expected if there were no effects of human development and anthropogenic sources) have been estimated as 1.0 and 0.24 mg nitrate-nitrogen/L for groundwater and streams, respectively, in the United States (USGS 2010a).

A comprehensive report analyzed nutrient levels in 5,101 wells from 51 different study areas (Burow et al. 2010; USGS 2010a). Monitoring data from 1993 to 2003 indicated that nitrate levels in groundwater varied widely across the nation, with some of the highest levels observed in the Northeast (particularly southern Pennsylvania), the Midwest, the state of California, and select regions of the Northwest (Washington state and Idaho). The report concluded that nitrate levels in deep aquifers were likely to continue to increase as shallow groundwater with high levels of nitrate gravitate downward (USGS 2010a). The highest levels of nitrate were observed in oxic groundwater (water containing >0.5 ppm DO) as opposed to anoxic groundwaters and shallow wells in agricultural areas, which tended to have greater levels than in urban areas (USGS 2010a). Burow et al. (2010) analyzed these data and reported that nitrate concentrations exceeded the MCL (10 mg nitrate-nitrogen/L) in 437 wells (8%). Levels exceeded the MCL in 20% of wells classified as agricultural land-use setting, and 3% were above the MCL in wells classified as urban use. In monitoring data from bank and in-stream wells in the San Joaquin River in California, collected between 2006 and 2008, the concentration of nitrate exceeded the detection limit (0.01 mg/L) in 5% of the groundwater samples and the concentrations in surface waters ranged from 1 to 3 mg/L. It was reported that 17 of the 26 nested monitoring wells, along the river bed and river bank, had no detectable concentrations of nitrate during the monitoring period (USGS 2013a).

Monitoring data obtained from 1991 to 1995 in shallow groundwater of coastal plains in the Albemarle-Pamlico Drainage Unit, in North Carolina and Virginia, have indicated the presence of increased nitrate concentrations as a result of agriculture and anthropogenic sources. Shallow groundwater concentrations are higher at inner coastal sites with well-drained soils compared with outer coastal sites. Areas with anthropogenic nitrogen sources, such as fertilizer and manure, had aquifer concentrations >3 mg nitrate-

nitrogen/L. Levels <2 mg nitrate-nitrogen/L were reported in aquifer waters with greater DOC concentrations. Groundwaters from areas having well-drained soils had a median concentration of approximately 0.4 mg nitrate-nitrogen/L. Two of the 20 inner coastal wells had levels >10 mg nitrate-nitrogen/L. Groundwater concentration of nitrate was nearly undetectable in waters underneath poorly drained soils in the outer coast (median 0.05 mg nitrate-nitrogen/L). The North Carolina Division of Water Quality (NCDWQ) selected groundwater samples susceptible to contamination; 40% of the 15 wells in the inner coastal plain of the Albemarle-Pamlico Drainage had levels >10 mg nitrate-nitrogen/L (USGS 2012).

Nitrate and nitrite were the two most detected inorganic chemicals reported in public water systems (PWSs) in an analysis supporting the U.S. EPA's second Six-Year Review of National Primary Drinking Water Regulations. Occurrence data for nitrate from a Six-Year Review-ICR Dataset include 1,052,487 analytical results from 119,537 public water systems (groundwater 114,764; surface water 4,773) across 44 states during the time period from 1998 to 2005 (EPA 2009a). These water systems are reported to serve a combined population of 229,508,036. Nitrate was detected in approximately 70% of the water systems (groundwater 69.4%; surface water 81.3%) at a median concentration of 1.8 mg nitrate-nitrogen/L (groundwater systems 1.6 mg nitrate-nitrogen/L; surface water systems 2.71 mg nitrate-nitrogen/L). Maximum concentrations detected in groundwater and surface water systems were 99 and 48.5 mg nitrate-nitrogen/L, respectively. Seven states in the review reported at least one detection of nitrate greater than the MCL of 10 mg nitrate-nitrogen/L in >5% of their systems. Overall, the 2,973 systems with detections exceeding the MCL serve a combined population of 16,777,093 (EPA 2009a). Occurrence data for nitrite from the Six-Year Review-ICR Dataset includes 397,175 analytical results from 86,313 public water systems (groundwater 82,738; surface water 3,575) across 44 states during the time period from 1998 to 2005 (EPA 2009a). These water systems are reported to serve a combined population of 207,984,813. Nitrite was detected in approximately 22% of the water systems (groundwater 22%; surface water 23%) at a median concentration of 0.02 mg nitrite-nitrogen/L. Maximum concentrations detected in groundwater and surface water systems were 13 and 8.68 mg nitrite-nitrogen/L, respectively. Four states in the review reported at least one nitrite detection greater than the MCL (1 mg nitrite-nitrogen/L) in more than 1% of their systems. Overall, the 635 systems with detections exceeding the MCL serve a combined population of 10,067,031 (EPA 2009a).

In 1991, 12% of 631 private wells located on farmlands across 18 states in the United States reported concentrations >10.2 mg nitrate-nitrogen/L (Bruning-Fann et al. 1994; IARC 2010). Additionally, in 1994, levels of nitrate found in drinking waters across nine mid-western U.S. states ranged from 0.01 to

266 mg nitrate-nitrogen/L, with a mean value of 8.4 mg nitrate-nitrogen/L and 10% of the water supplies had concentrations >10 mg nitrate-nitrogen/L (CDC 1998). California groundwater is relied on for drinking water in 70% of its cities. A study in 1987 indicated that ~10% of the sampled California wells and >7% of the public water systems in Tulare County, California had levels >45 mg nitrate/L (>10 mg nitrate-nitrogen/L) (Zhang et al. 1998).

Studies regarding nitrate levels in drinking water outside the United States were summarized (IARC 2010). Zhang et al. (1996) reported that several cities in China (with populations between 10,000 and 100,000) had water supplies with concentrations >11.3 mg nitrate-nitrogen/L. Twenty-eight percent of public wells monitored in India and 13% in Saudi Arabia had reported levels >11.2 mg nitrate-nitrogen/L as well. In 1990, average concentrations in Canadian municipal drinking waters were reported to range from 0.1 to 3.3 mg nitrate/L (0.02–0.75 mg nitrate-nitrogen/L) (Environment Canada 2012). It has been estimated that 2% of the European population receive their drinking water from private wells and an estimated 2.4 million people are exposed to water supplies containing nitrate concentrations above guideline levels (Gangolli et al. 1994). Nitrate concentrations in many European countries have been reported to be gradually increasing over the last few decades. An average annual increase of 0.7 mg nitrate/L (0.2 mg nitrate-nitrogen/L) has been observed in some rivers of the United Kingdom (WHO 2011b). Nitrate levels in drinking water in Denmark have increased about 400% over the period from 1940 to 1983 (Moller et al. 1989).

Nitrate was reported to be the most frequently detected nutrient in U.S. streams that exceeded its MCL. It exceeded the MCL in 2% of all samples obtained (566 out of 27,555) and in at least 1 sample of 50 of the 499 streams surveyed from 1992 to 2001 (USGS 2010a). Many of the streams with levels above the MCL were located in the upper Midwestern Corn Belt where application rates of fertilizer and manure are high. Nitrite samples from five streams exceeded the nitrite MCL (USGS 2010a). Flow-adjusted nitrate concentrations decreased in 25% of 166 streams and rivers sampled by the USGS over the period 1993–2003; however, concentrations increased over this time period in 20 of the 166 sites (12%) surveyed (USGS 2009). Decreases in levels were attributed to changes in nitrogen use patterns and implementation of pollution control strategies. Multiple factors such as land use, nitrogen loading from fertilizer, manure use, and atmospheric deposition affected the trends observed.

Annual trends of nitrate levels at eight sites along the Mississippi River Basin were studied by the USGS from 1980 to 2010 (USGS 2013b). Flow-normalized nitrate concentrations were generally reported to be level or increasing at all of the monitoring sites from 1980 to 2000; however, select locations showed

greater increases or actual decreases in levels since 2000. The greatest increases over the 30-year period were observed in the upper Mississippi River (Clinton, Iowa) and Missouri River (Herman, Missouri). Decreasing flow-normalized nitrate levels over the 30-year period were observed at the Iowa River near Wapello, Iowa and Illinois River at Valley City, Illinois, suggesting that recent land management and farming practices may be reducing the nitrate fluxes in these areas (USGS 2013b).

Many surface water bodies in the United States have a large percentage of total nitrate load contributed by base flow (groundwater discharge, release from other watershed storages, and long-term interflow) in addition to surface runoff. Mean annual base-flow nitrate levels for 148 surface water and shallow groundwater sites in the United States from 1990 to 2006 were typically reported to be <1 mg nitrate-nitrogen/L; however, values as high as 8.48, 11.44, 8.29, and 8.25 mg nitrate-nitrogen/L were reported for Tulpehocken Creek, Pennsylvania; Indian Creek, Illinois; Salt Creek, Illinois; and Clifty Creek, Indiana, respectively (USGS 2010b). The highest levels typically were reported for agricultural land use sites that had frequent fertilizer and manure applications and highly permeable underlying bedrock.

Due to assimilation of nitrate by algae and other plant-life, concentrations of nitrate in surface water are typically lower than that detected in groundwaters (IARC 2010). Tables 1.4 and 1.5 in the IARC report summarize concentrations of nitrate from various regions around the globe. The global concentrations in groundwater were reported to range from 0.02 to 110 mg nitrate-nitrogen/L; mean values ranged from 2.2 to 42.9 mg nitrate-nitrogen/L. Global concentrations of nitrate in surface water were reported to range from 0 to 22 mg nitrate-nitrogen/L; mean values range from 0.1 to 8.3 mg nitrate-nitrogen/L). Nitrate levels in rainwater as high as 5 mg nitrate/L (1 mg nitrate-nitrogen/L) have been observed in various industrial areas (WHO 2011b).

6.4.3 Sediment and Soil

Levels of nitrate and nitrite in soil vary considerably as a function of soil properties, temperature, precipitation rates, nitrogen loadings, farming practices (tillage, crops planted), and seasonal changes. In well-drained aerobic soils, the conversion of ammonia into nitrate (nitrification) increases the soil-nitrate content and in anaerobic soils with high organic matter (such as waterlogged soils or wetlands), denitrification decreases the levels of nitrate and nitrite in soils. Acidic soils tend to have lower levels of nitrate since the nitrification process ceases at pH levels below 4.5 (USDA 2014). Typical nitrate levels in humid temperate soils fluctuate from about 20–65 kg-nitrogen/hectare in cropped soils and 25–150 kg-

nitrogen/hectare in bare soils (USDA 2014). Certain locations near the South Platte River in northeastern Colorado had nitrate levels exceeding 500 kg-nitrogen/hectare (Shaffer et al. 1995).

6.4.4 Other Environmental Media

Nitrate and nitrite are common food preservatives. Levels of nitrite and nitrate were evaluated through several processing steps and storage conditions. Nitrite is oxidized to nitrate during storage. Nitrate remained relatively constant over a 2-week storage period, while nitrite levels declined over the same time period. The study also observed minimal influence of the nitrate levels formed from the amount of nitrite added. Additionally, using nitrate alone resulted in the formation of nitrite after thermal processing. Cooking resulted in losses of both nitrite and nitrate; 50% nitrite and 10–15% nitrate remained in the prepared sausage analyzed (Perez-Rodriguez et al. 1996).

Nitrate and nitrite are present in vegetables, fruits, cured meats, fish, dairy products, beers, cereals, and cereal products (Gangolli et al. 1994). Nitrate content of foodstuffs is typically higher than nitrite content (ATSDR 2013a; WHO 2011b). Cured meats have concentrations of <2.7–945 mg of nitrate/kg and <0.2–1.7 mg nitrite/kg (IARC 2010; WHO 2011b). Concentrations of nitrate in vegetables and fruit is strongly affected by processing of the food, fertilizer use, and growing conditions and range from 30 to 6,000 mg/kg (ppm) (IARC 2010; WHO 2011b). Celery, lettuce, red beetroot, and spinach have high levels of nitrate (200–>2,500 mg/kg [ppm]). Parsley, leek, endive, Chinese cabbage, and fennel have high levels of nitrate ranging from 100 to 250 mg/kg (ppm). Cabbage, dill, and turnips have medium levels of nitrate ranging from 50 to 100 mg/g. Vegetables with low levels of nitrate (20–50 mg/g) include broccoli, carrots, cauliflower, cucumber, and pumpkin. Very low levels of nitrate (<20 mg/g) are found in artichokes, asparagus, eggplant, garlic, onions, green beans, mushrooms, peas, peppers, potatoes, summer squash, sweet potatoes, tomatoes, and watermelons (ATSDR 2013a). Nitrite concentrations are typically <10 mg/kg (ppm) and rarely reach 100 mg/kg (ppm); however, exceptions include damaged, outdated, pickled, and fermented foods in which levels may be as high as 400 mg/kg (ppm) (WHO 2011b). Data for nitrate and nitrite in foodstuffs are not lacking and several papers have been published that summarize numerous studies, including IARC (94 2010) and Gangolli et al. (1994). Gangolli et al. (1994) cited a paper reporting the average daily intakes of nitrate and nitrite in the United States to be 106 and 4.1 mg/day, respectively.

Marshall and Trenerry (1996) analyzed several food types purchased from local supermarkets in Australia for both nitrate and nitrite. Nitrite was not detected in fruit juices in this study. Nitrate and nitrite was

detected in various cheeses at levels <10 mg/kg (ppm). Canned meat products had small amounts of nitrite (<10 mg/kg [ppm]), while nitrate levels were higher (10–25 mg/kg [ppm]). Several samples of ice were also analyzed for the presence of nitrite and nitrate, and all samples were below Australian food standards (10 mg/L nitrate; 1 mg/L nitrite).

Nitrite and nitrate levels were determined in several whey-containing food products (Oliveira et al. 1995). In total, 231 samples from powdered modified milk, powdered non-fat milk, a dairy beverage, and strawberry- and chocolate-flavored instant mixes were evaluated. Mean nitrate levels ranged between 7.3 and 532 mg/kg (ppm). Mean nitrite levels ranged between 1.1 and 2.5 mg/kg (ppm). One serving of the product with the highest reported nitrate levels (a chocolate-flavored instant mix) was calculated to be 51.9 mg/serving. One serving of the product with the highest reported nitrite levels (powdered non-fat milk) was calculated to be 0.1 mg/serving.

From 1993 to 1997, nitrate and nitrite levels were monitored in Danish lettuce, leek, potato, beetroot, Chinese cabbage, and white cabbage, and spinach (Peterson and Stoltze 1999). Seasonal variation was observed in nitrate levels. Lettuce exhibited higher concentrations in winter as opposed to summer. Overall nitrite concentrations were low. Average nitrate concentrations were 2,760, 1,783, 198, and 158 mg/kg fresh weight for lettuce, fresh spinach, leeks, and potatoes, respectively. Average nitrite concentrations were 11, 0.91, 0.80, 0.15, and 0.14 mg/kg fresh weight for fresh spinach, beetroot, potatoes, leeks, and lettuce, respectively. The average daily intakes, estimated from consumption surveys of the vegetables in the study, were 40 mg/day nitrate and 0.09 mg/day nitrite.

Table 6-4 contains data on infant foods examined for nitrate and nitrite (Cortesi et al. 2015). Food samples of animal origin were composed of a variety of sources such as poultry, beef, rabbit, lamb, and turkey. Foods samples of plant origin were composed of a variety of sources such peas, legumes, vegetable broths, cream of pumpkin and carrots, and mixed vegetables. Mixed-origin samples were composed of both plant and animal sources. The highest average concentration of nitrate was found in foods of plant origin (45.5 mg/kg), while the highest average concentration of nitrite was found in foods of animal origin (14.82 mg/kg).

Jones et al. (2014) reported nitrate and nitrite concentrations in fresh breast milk, freeze-thawed breast milk, freeze thawed colostrum, and several commercially available infant formulas. Data are tabulated in Table 6-5. Fresh breast milk was collected from 11 mothers of term infants and 13 mothers of preterm infants. Samples of colostrum (milk expressed days 1–3), transition milk (expressed days 4–7), and

Table 6-4. Concentrations of Nitrate and Nitrite in Infant Food Products

Food type	Nitrate (mg/kg)	Nitrite (mg/kg)
Homogenized samples of animal origin	0.35–83.2	6.6–48.87
Freeze dried samples of animal origin	2.01–80.26	1.3–74.74
Homogenized samples of plant origin	4.82–131.68	2.26–20.71
Freeze dried samples of plant origin	19.41–85.03	1.34–6.62
Homogenized samples of mixed origin	3.77–67.31	1.98–80.22

Source: Cortesi et al. 2015

Table 6-5. Average Concentrations of Nitrate and Nitrite in Human Milk and Infant Formula

Milk type	Nitrate (μmol/L)	Nitrite (μmol/L)
Fresh breast milk	16	0.1
Freeze-thawed breast milk	20	0.04
Preterm fresh	12	0.07
Preterm freeze-thawed	22	0.03
Term fresh	11.5	0.13
Term freeze-thawed	12.5	0.04
Freeze-thawed colostrum	41	0.16
Infant formula	43	0.29
Freeze-thawed colostrum	44	0.15
Transition milk	Not reported	0.05
Mature milk	Not reported	0.025

Source: Jones et al. 2014

mature milk (expressed days >7) were analyzed. Concentrations of nitrate and nitrite in the 10 formulas evaluated were 9–61 and not detected–1.4 μmol/L, respectively.

6.5 GENERAL POPULATION AND OCCUPATIONAL EXPOSURE

The general public is typically exposed to nitrate and nitrite via ingestion of water and foods that contain these chemicals. Inhalation and dermal exposure may be possible; however, these routes are not as prominent. Oral exposure to nitrate and nitrite from contaminated drinking water and food is the prominent route. Nitrate and nitrite overexposure may occur through ingestion of foods containing high levels of nitrate and nitrite (ATSDR 2013a). Inorganic nitrate and nitrite can be taken up by plants, especially leafy vegetables such as lettuce and spinach as well as beet root; vegetables account for about 80% of the nitrate in a typical human diet (ATSDR 2013a; Hord 2011; Lundberg et al. 2009; Peterson and Stoltze 1999). Contaminated foodstuffs from improper storage of commercial and prepared baby foods have caused overexposure in children (Dusdieker et al. 1994; Greer and Shannon 2005; Sanchez-Echaniz et al. 2001).

Iammarino et al. (2014) analysed 75 samples of spinach and 75 samples of lettuce, collected from June 2010 to December 2011, for nitrate and nitrite. Spinach had a greater number of detections compared with the lettuce samples. Mean nitrate concentrations ranged from 155.5 to 2,149.6 mg/kg; mean nitrite concentrations ranged from 16.3 to 101.6 mg/kg. Four spinach samples and five lettuce samples had concentrations of nitrate >2,000 mg/kg. Quantifiable concentrations of nitrite were detected in 15 samples of spinach (28.5–197.5 mg/kg) and one sample of lettuce (66.5 mg/kg).

The remainder of the nitrate in a typical diet comes from drinking water (about 21%) and from meat and meat products (about 6%) in which sodium nitrate is used as a preservative and color-enhancing agent (ATSDR 2013a; Lundberg et al. 2008; Saito et al. 2000). For bottle-fed infants, the major source of nitrate exposure is from contaminated drinking water used to dilute formula, especially when the water is boiled prior to use (ATSDR 2013a; Fewtrell 2004). A review by Jones et al. (2015) reported daily nitrate ingestion concentrations for adults. An intake of approximately 3 mg/kg/day for adults was based on a typical adult diet.

The Fourth National Report on Human Exposures to Environmental Chemicals, published and updated by the Centers for Disease Control and Prevention (CDC 2013), reported the following data from the National Health and Nutrition Examination Survey (NHANES) 1999–2008. Nitrate levels in the urine

(see Table 6-6), and urine (creatinine corrected) (see Table 6-7) were evaluated for various ages and ethnicities. Mean values of nitrate in the urine were 42.7 and 46.3 mg/L for 7,697 members of the general U.S. population sampled during 2005–2006 and 7,629 members of the general U.S. population sampled during 2007–2008, respectively. The highest geometric mean (creatinine corrected) during 2001–2002 of 72.0 mg/L was determined from 374 samples from 6–11 year olds; the highest geometric mean (creatinine corrected) during 2005–2006 of 60.8 mg/L was determined from 1,054 samples from 6–11 year olds; and the highest geometric mean during 2007–2008 of 70.2 mg/L was determined from 1,143 samples from 6–11 year olds. Throughout all survey years, females had a higher geometric mean than males. In the survey years 2007–2008, 3,789 female samples yielded a mean of 51.0 mg/g, while 5,351 male samples yielded a mean of 44.6 mg/g (CDC 2013).

No information was located regarding absorption of inhaled inorganic nitrate or nitrite in humans or laboratory animals. Inhalation of inorganic nitrate or nitrite is not a likely exposure route of concern for the general population, although inhalation of dust from fertilizer products containing nitrate salts is possible.

Occupational exposure is primarily via inhalation and dermal routes. Industrial workers and farmers may be exposed via inhalation of dusts. Dusts may also dissolve in sweat on skin, increasing the potential for dermal exposure.

Vegetable consumption is a considerable source of nitrate, and drinking water with high levels of nitrate is also a major contributing factor. Several studies have been conducted assessing exposure to drinking waters with high levels of nitrate. In 1986 until 1987, Moller et al. (1989) studied 294 Danish adults between the ages of 20 and 64 years who were exposed to various levels of nitrate in their drinking water and diet. Twenty-one drinking water supplies contained low (0–5 mg nitrate/L [ppm nitrate]) intermediate (35–59 mg nitrate/L [ppm nitrate]) and high (≥60 mg nitrate/L [ppm nitrate]) nitrate levels, with mean concentrations of 0.3, 46.5, and 84.4 mg nitrate/L (ppm nitrate), respectively. The median exposures of total dietary nitrate for the low, intermediate, and high water categories were 37, 89, and 123 mg nitrate/day, respectively. Mean nitrate levels detected in the participant's 24-hour urine samples for the low, intermediate, and high water concentration categories were reported as 36, 55, and 73 mg nitrate, respectively. Overall, the dietary contribution was calculated to be 17% from water and 83% from food for the low group, and increased to approximately 60% from water and 40% from food for the intermediate and high groups. Fifty-nine Canadian adults between the ages of 20–74 years used tap water with low (<3 mg nitrate-nitrogen/L) and high (>3 mg nitrate-nitrogen/L) concentrations of nitrate. The

Table 6-6. Geometric Mean and Selected Percentiles of Urine Concentrations of Urinary Nitrate (in mg/L) for the U.S. Population from the National Health and Nutrition Examination Survey (NHANES)

	Survey years	Geometric mean (95% CI)	Selected percentiles (95% CI)				Sample size
			50th	75th	90th	95th	
Total	2001–2002	48.2 (46.2–50.3)	49.0 (46.0–52.0)	78.0 (73.0–83.0)	100 (100–130)	140 (130–150)	1,617
	2005–2006	42.7 (39.6–46.1)	47.8 (44.4–51.2)	74.6 (69.8–79.4)	108 (101–114)	133 (125–144)	7,697
	2007–2008	46.3 (44.6–48.1)	50.3 (48.4–52.0)	76.0 (72.7–79.1)	110 (104–116)	138 (132–146)	7,629
Age group							
6–11 years	2001–2002	62.2 (53.8–71.8)	68.0 (58.0–79.0)	94.0 (84.0–100)	130 (100–160)	150 (120–380)	374
	2005–2006	51.2 (47.4–55.4)	54.7 (51.9–58.2)	79.2 (72.8–87.2)	113 (101–128)	141 (120–158)	1,054
	2007–2008	55.2 (51.7–58.9)	60.2 (56.1–64.3)	84.5 (80.1–92.5)	117 (107–135)	149 (132–189)	1,143
12–19 years	2001–2002	57.4 (53.5–61.6)	66.0 (60.0–69.0)	91.0 (86.0–95.0)	120 (100–130)	150 (130–160)	827
	2005–2006	52.5 (48.5–56.8)	57.5 (52.3–62.6)	84.2 (79.9–88.4)	119 (111–124)	144 (129–153)	2,106
	2007–2008	55.5 (51.5–59.7)	56.8 (51.9–61.5)	84.1 (76.2–94.5)	119 (107–133)	144 (133–162)	1,135
≥20 years	2001–2002	45.4 (43.3–47.5)	49.0 (46.0–52.0)	78.0 (73.0–83.0)	100 (100–130)	140 (130–150)	1,617
	2005–2006	40.5 (37.4–43.9)	45.0 (41.3–48.3)	71.7 (67.1–77.4)	105 (98.0–113)	129 (122–142)	4,537
	2007–2008	44.2 (42.5–45.9)	48.1 (46.0–49.7)	73.2 (70.1–76.5)	107 (101–113)	135 (128–146)	5,351
Gender							
Males	2001–2002	57.5 (54.6–60.6)	63.0 (59.0–67.0)	89.0 (83.0–94.0)	130 (100–140)	150 (140–170)	1,335
	2005–2006	48.4 (44.6–52.6)	52.7 (48.3–57.8)	79.4 (72.8–86.5)	110 (103–121)	136 (123–152)	3,765
	2007–2008	51.9 (49.9–54.1)	56.1 (53.8–58.0)	79.5 (75.7–83.5)	112 (105–119)	137 (131–149)	3,839
Females	2001–2002	40.7 (38.4–43.2)	43.0 (41.0–48.0)	72.0 (68.0–76.0)	100 (98.0–120)	130 (120–150)	1,483
	2005–2006	37.9 (35.1–40.8)	42.0 (38.2–46.0)	69.2 (65.5–73.4)	104 (96.6–110)	130 (124–140)	3,932
	2007–2008	41.4 (39.3–43.7)	43.9 (41.5–46.3)	71.3 (67.5–74.8)	108 (99.8–115)	138 (132–149)	3,790

Table 6-6. Geometric Mean and Selected Percentiles of Urine Concentrations of Urinary Nitrate (in mg/L) for the U.S. Population from the National Health and Nutrition Examination Survey (NHANES)

	Survey years	Geometric mean (95% CI)	Selected percentiles (95% CI)				Sample size
			50th	75th	90th	95th	
Race/ethnicity							
Mexican Americans	2001–2002	53.2 (48.7–58.2)	59.0 (52.0–66.0)	84.0 (79.0–91.0)	120 (100–150)	160 (130–180)	707
	2005–2006	47.8 (44.7–51.2)	52.4 (49.9–56.2)	77.9 (75.1–83.1)	113 (104–120)	148 (133–156)	1,972
	2007–2008	48.7 (45.0–52.6)	51.9 (47.4–56.7)	75.6 (69.7–81.3)	111 (100–122)	148 (127–164)	1,505
Non-Hispanic blacks	2001–2002	53.8 (47.8–60.5)	58.0 (51.0–64.0)	84.0 (77.0–93.0)	120 (100–130)	140 (130–170)	680
	2005–2006	45.9 (42.1–50.0)	50.4 (46.6–54.9)	75.0 (68.8–80.8)	101 (95.3–110)	127 (114–148)	2,078
	2007–2008	47.5 (45.0–50.3)	50.3 (48.6–52.5)	74.7 (71.9–77.3)	105 (97.1–116)	134 (125–150)	1,707
Non-Hispanic whites	2001–2002	46.3 (44.1–48.6)	51.0 (47.0–53.0)	81.0 (78.0–85.0)	120 (100–130)	140 (130–150)	1,228
	2005–2006	41.2 (37.6–45.2)	46.2 (41.3–50.6)	73.3 (67.3–80.1)	107 (98.2–115)	129 (122–142)	3,056
	2007–2008	45.0 (42.7–47.5)	49.2 (46.1–52.6)	75.5 (70.5–80.0)	108 (101–116)	134 (128–140)	3,190

CI = confidence interval

Source: CDC 2013

Table 6-7. Geometric Mean and Selected Percentiles of Urine Concentrations of Urinary Nitrate (Creatinine Corrected) (in mg/g of creatinine) for the U.S. Population from the National Health and Nutrition Examination Survey (NHANES)

	Survey years	Geometric mean (95% CI)	Selected percentiles (95% CI)				Sample size
			50th	75th	90th	95th	
Total	2001–2002	49.8 (47.7–51.9)	46.9 (44.2–49.6)	63.8 (61.2–67.7)	90.9 (84.3–98.8)	120 (111–128)	1,616
	2005–2006	42.6 (40.2–45.1)	42.4 (40.1–44.7)	59.7 (55.8–64.1)	85.5 (81.3–91.3)	113 (106–118)	7,697
	2007–2008	47.7 (45.9–49.7)	46.0 (44.0–48.3)	66.5 (62.4–70.5)	98.0 (92.3–102)	127 (119–135)	7,628
Age group							
6–11 years	2001–2002	72.0 (66.1–78.4)	66.0 (62.6–70.4)	87.0 (80.2–97.7)	129 (96.5–144)	144 (130–235)	374
	2005–2006	60.8 (57.4–64.5)	57.3 (53.6–60.6)	76.5 (70.9–82.1)	109 (95.0–123)	134 (121–164)	1,054
	2007–2008	70.2 (65.7–74.9)	65.9 (62.4–69.5)	89.0 (83.2–96.5)	128 (112–152)	173 (140–216)	1,143
12–19 years	2001–2002	44.8 (43.4–46.2)	43.8 (42.6–45.0)	56.2 (52.2–59.7)	73.2 (65.2–85.1)	93.4 (79.0–104)	826
	2005–2006	39.8 (37.8–41.9)	38.1 (36.3–40.3)	51.8 (47.7–56.0)	70.6 (63.1–78.8)	88.9 (79.4–103)	2,106
	2007–2008	43.4 (41.3–45.5)	40.5 (38.6–43.5)	56.0 (52.7–59.2)	76.6 (69.0–86.2)	98.0 (85.4–121)	1,134
≥20 years	2001–2002	48.3 (45.9–50.9)	46.9 (44.2–49.6)	63.8 (61.2–67.7)	90.9 (84.3–98.8)	120 (111–128)	1,616
	2005–2006	41.4 (38.9–43.9)	41.0 (38.8–43.7)	58.6 (54.8–63.1)	85.3 (80.2–91.0)	111 (105–116)	4,537
	2007–2008	46.5 (44.6–48.4)	44.7 (42.5–47.0)	65.0 (60.7–69.2)	96.1 (90.0–102)	125 (117–132)	5,351
Gender							
Males	2001–2002	47.6 (44.7–50.7)	46.1 (43.4–48.7)	61.3 (58.0–64.5)	86.7 (77.1–97.3)	114 (97.3–125)	1,335
	2005–2006	40.1 (37.5–42.9)	39.5 (36.7–42.8)	55.3 (51.6–59.5)	77.2 (70.7–83.0)	95.9 (89.6–102)	3,765
	2007–2008	44.6 (42.8–46.6)	42.8 (40.7–45.1)	60.6 (58.1–64.4)	85.6 (81.1–91.3)	111 (101–121)	3,839
Females	2001–2002	51.9 (49.9–54.1)	51.2 (48.4–53.0)	69.1 (66.7–71.2)	100 (91.7–111)	129 (118–140)	1,481
	2005–2006	45.1 (42.8–47.6)	45.0 (42.4–47.4)	64.4 (60.0–69.6)	96.8 (87.8–105)	128 (117–134)	3,932
	2007–2008	51.0 (48.9–53.2)	50.0 (47.5–52.7)	72.3 (67.8–76.8)	107 (101–116)	146 (129–163)	3,789

Table 6-7. Geometric Mean and Selected Percentiles of Urine Concentrations of Urinary Nitrate (Creatinine Corrected) (in mg/g of creatinine) for the U.S. Population from the National Health and Nutrition Examination Survey (NHANES)

	Survey years	Geometric mean (95% CI)	Selected percentiles (95% CI)				Sample size
			50th	75th	90th	95th	
Race/ethnicity							
Mexican Americans	2001–2002	50.9 (45.7–56.8)	48.1 (44.9–51.4)	67.4 (60.3–77.6)	97.3 (85.7–117)	135 (100–161)	707
	2005–2006	44.6 (42.6–46.7)	44.0 (42.2–45.3)	60.4 (58.1–62.9)	89.9 (82.0–95.4)	120 (111–128)	1,972
	2007–2008	48.7 (45.1–52.7)	47.0 (43.5–50.5)	64.8 (59.7–69.9)	93.5 (88.9–101)	128 (108–156)	1,505
Non-Hispanic blacks	2001–2002	38.7 (36.3–41.3)	38.0 (34.6–41.3)	53.5 (50.3–57.4)	70.3 (64.9–79.3)	91.7 (78.9–100)	679
	2005–2006	32.9 (30.9–35.0)	31.9 (29.8–34.1)	45.4 (41.5–49.6)	64.0 (60.1–68.0)	81.2 (75.3–89.6)	2,078
	2007–2008	35.9 (34.2–37.7)	34.8 (33.3–36.6)	49.0 (44.8–53.6)	69.1 (63.5–77.4)	87.8 (78.1–97.4)	1,706
Non-Hispanic whites	2001–2002	51.4 (49.4–53.4)	49.1 (46.9–51.5)	66.9 (64.2–69.4)	95.2 (87.7–100)	124 (115–132)	1,227
	2005–2006	43.7 (40.8–46.8)	43.9 (40.8–46.8)	61.4 (56.5–66.2)	85.5 (80.6–91.9)	110 (102–116)	3,056
	2007–2008	49.0 (46.7–51.4)	47.4 (44.9–50.5)	68.2 (63.3–73.1)	98.3 (91.2–105)	126 (118–135)	3,190

CI = confidence interval

Source: CDC 2013

mean urinary nitrate excretion of the participants who consumed drinking water with low-nitrate levels was 15.0 mg nitrate-nitrogen/day, while the mean value for the participants who consumed drinking water with higher nitrate levels was 22 mg nitrate-nitrogen/day (Levellois et al. 2000). Higher correlations for total nitrate intake and urinary excretion were found with dietary nitrate intake as opposed to water nitrate intake.

Various scenarios for nitrate and nitrite intake have been considered and it has been found that dietary intake contributes the majority of exposure occurrences. Approximately 89–99% of an adult's daily intake of both nitrate and nitrite is from their food when an average to high vegetable diet is consumed with average water intake. The daily intake contribution from food decreases to 33–56% for nitrate and 7.7–14% for nitrite when average to high consumption of water with nitrate levels (50 mg nitrate/L [ppm nitrate]) is considered (IARC94_2010). Gangolli et al. (1994) also reported that 88–96% of the average dietary intake of nitrate comes from food sources (85% of which is attributed to vegetables), while 4–12% comes from drinking waters. Exposure to dietary nitrate may increase exposure to nitrite due to endogenous production. Ingested nitrate is readily absorbed from the upper gastrointestinal tract into the blood and is mainly excreted in the urine (Gangolli et al. 1994). Portions of blood nitrate are transported to human saliva where it is mostly metabolized to nitrite; approximately 5% of dietary nitrate is metabolized to nitrite (Gangolli et al. 1994). Gangolli et al. (1994) estimated that the human adult intakes of nitrate are 2.4 mg nitrate/kg (ppm nitrate) body weight/day from food and 0.33–4.1 mg nitrate/kg body weight/day from water; the human adult intakes of nitrite are 0.04–0.07 mg nitrite/kg body weight/day from food and <0.002–0.07 mg nitrite/kg body weight/day from water. These estimates do not account for the endogenous production of nitrite. Worldwide dietary exposures were estimated from data collected in the 1997 Total Diet Study in the United Kingdom and additional dietary studies. Representative exposure estimates for nitrate and nitrite were reported as 58–218 and 0.7–1.6 mg nitrite/day, respectively (IARC 2010).

Estimated daily intake values of nitrate and nitrite have been calculated based on data from the United Kingdom and the United States. An individual with an average water intake (1.4 L/day) and average food consumption or a high vegetable diet is estimated to consume levels 52–80 or 140–220 mg nitrate/day, respectively. An individual with an average water intake (1.4 L/day) and average food consumption or a high vegetable diet is estimated to consume levels 0.74 or 2.2 mg nitrite/day, respectively (IARC 2010).

Dermal exposure to inorganic nitrate or nitrite is not a likely route of concern for the general population, although absorption following dermal exposure to dust from fertilizer products containing nitrate salts is possible.

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 than adults. 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 may spend more time outdoors. Children also are generally closer to the ground and have not yet developed the adult capacity to judge and take actions to avoid hazards (NRC 1993).

Children will be exposed to nitrate and nitrite through ingestion of food and drinking water. Nitrate is commonly detected in various surface waters and groundwaters. High nitrate concentrations in drinking water are commonly found in privately owned wells, with shallow depths and permeable soils. It has been estimated that about 15 million families in the United States use private well drinking water. Based on monitoring data and birthrates from 2000, it was estimated that 40,000 infants <6 months old would be living in households using drinking water with nitrate levels that exceed the federal standard (10 mg nitrate-nitrogen/L) (Fewtrell 2004). Additionally, boiling water from private wells may concentrate nitrate in the water, which may lead to higher exposure of children whose infant foods are prepared using water that is boiled first (Fewtrell 2004). Gangolli et al. (1994) estimated that the infant intake of nitrate and nitrite from food is negligible, while the infant intakes of nitrate and nitrite from water are 1.7–8.3 mg nitrate/kg body weight/day and <0.02 mg nitrite/kg body weight/day, respectively. A review by Jones et al. (2015) reported dietary nitrate and nitrite concentrations for newborn infants. An intake of approximately 0.15 mg/kg/day for infants was based on a mean of reported concentrations in breast milk and formula. Ingestion based on breast milk intake of approximately 150 mL/kg/day, was reported as 0.12 mL/kg/day for nitrate and 0.0007 mL/kg/day for nitrite.

Infants may have higher exposures as compared to adults if the water source used for formula has high levels of nitrates and nitrites. Human breast milk has been shown to contain, although not concentrate, nitrate and is not considered a significant source of infant exposure (IARC 2010).

6.7 POPULATIONS WITH POTENTIALLY HIGH EXPOSURES

Populations using well water in agricultural areas may be exposed to greater levels of nitrate and nitrite as compared to populations living in urban areas since groundwater in agricultural communities typically has greater levels of nitrate and nitrite than urban water (Burow et al. 2010). Furthermore, workers who are employed in occupations where fertilizer use is common (e.g., farming, greenhouse operations) may be exposed to nitrate and nitrite through dermal routes and inhalation of dust particles.

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 nitrate and nitrite is available. Where adequate information is not available, ATSDR, in conjunction with 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 nitrate and nitrite.

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 nitrate salts are discussed in Chapter 4. These salts are highly soluble in water and dissociate under environmental conditions and exist as ions (WHO 1978, 2011b). No data needs are identified.

Production, Import/Export, Use, Release, and Disposal. According to the Emergency Planning and Community Right-to-Know Act of 1986, 42 U.S.C. Section 11023, industries are required to submit substance release and off-site transfer information to the EPA. The TRI, which contains this information for 2012, became available in November of 2013. This database is updated yearly and should provide a list of industrial production facilities and emissions. Import/export data are available for ammonium nitrate (USDA 2013). Data for the other compounds assessed in this profile would be useful.

Environmental Fate. The transport and fate of nitrate and nitrite compounds have been studied (Kramer et al. 2006; Pfenning and McMahon 1996; WHO 2011b). These substances are highly mobile in soils. Transformation and degradation processes include denitrification to atmospheric nitrogen and plant uptake (Newton 2005; Nolan 1999). Conversion is achieved via biotic process carried out by auto- and heterotrophic bacteria (Hammerl and Klapotke 2006). Under aerobic conditions in aquatic systems, ammonia and nitrite are converted to nitrate via nitrification. Conversion is achieved through a biotic process carried out by autotrophic nitrifying bacteria. Under anaerobic conditions in aquatic systems, bacteria convert nitrate to nitrite, which is further reduced to the gaseous compounds nitric oxide (NO), nitrous oxide (N_2O), and N_2 (nitrogen). No data needs are identified.

Bioavailability from Environmental Media. Nitrate and nitrite are readily absorbed following ingestion from water or food sources.

Data assessing absorption from intake of food sources and water containing nitrate and nitrite has been studied (Gangolli et al. 1994; Kortboyer et al. 1997b). Several reports have indicated the correlation of methemoglobinemia in adults and children and elevated nitrite levels in the blood (CDC 1997, 2002; Gautami et al. 1995; Gowans 1990; Greenberg et al. 1945; Sevier and Berbatis 1976; Ten Brink et al. 1982). Adequate data for intake of nitrate and nitrite from drinking water and food are available (ATSDR 2013a; Gangolli et al. 1994; Hord 2011; JECFA 2003c; Lundberg et al. 2009; Peterson and Stoltze 1999). Data are lacking for absorption from the lungs and skin. Further data may be useful to establish whether uptake via inhalation or dermal contact of dust is a notable source of exposure, since this may occur during application of fertilizers containing these chemicals.

Food Chain Bioaccumulation. Nitrate ion and nitrite ion are both a natural part of the earth's nitrogen cycle. Plants and mammals naturally contain nitrate and nitrite (WHO 2011b). Assimilation of nitrite from soils occurs via reduction of nitrate to nitrite, which is facilitated by various bacteria and catalyzed by nitrate reductase (WHO 1978). Data are available to indicate that nitrate and nitrite may be

concentrated in several plants and waters intended for human consumption (JECFA 2003c; Peterson and Stoltze 1999; Zhang et al. 1996, 2003). No data needs are identified.

Exposure Levels in Environmental Media. Reliable monitoring data for the levels of nitrate and nitrite in contaminated media at hazardous waste sites are needed so that the information obtained on levels of nitrate and nitrite in the environment can be used in combination with the known body burden of nitrate and nitrite to assess the potential risk of adverse health effects in populations living in the vicinity of hazardous waste sites.

Exposure Levels in Humans. Humans are exposed to nitrate and nitrite primarily through the ingestion of drinking water and consumption of food. Estimated intakes are available (Gangolli et al. 1994; IARC 2010). Biomonitoring data for nitrate levels in urinary samples have been reported (CDC 2013). Continued monitoring of nitrate and nitrite levels in humans is needed.

This information is necessary for assessing the need to conduct health studies on these populations.

Exposures of Children. Children are exposed to nitrate and nitrite by the same exposure routes as adults (e.g., ingestion of food and water). Data from the NHANES survey discussed in Section 6.5 indicated that higher urinary nitrate levels were typically observed in children as compared to adults. Continued monitoring of nitrate and nitrite levels in children is needed.

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 nitrate or nitrite were located. This substance is not currently one of the compounds for which a sub-registry has been established in the National Exposure Registry. The substance will be considered in the future when chemical selection is made for sub-registries 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 substance.

6.8.2 Ongoing Studies

No ongoing environmental fate studies for nitrate or nitrite were identified using NIH RePORTER or the Defense Technical Information Center (DTIC) online database. Nitrate and nitrite levels and trends are monitored in major watersheds and drinking water by organizations such as the USGS and USDA. These reports are typically available from their websites.

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7. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting, measuring, and/or monitoring nitrate and nitrite, and other biomarkers of exposure and effect to nitrate and nitrite. 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

Several methods are available for the analysis of nitrate and nitrite in biological media; details of selected methods are provided in Table 7-1.

Following the ingestion of nitrate and nitrite, they are readily absorbed from the upper gastrointestinal tract into the blood and readily excreted in human urine as nitrate. This process is essentially complete at 18 hours following ingestion; minor urinary products of nitrate and nitrite metabolism include ammonia and urea (Gangolli et al. 1994). Portions of blood nitrate are transported to human saliva where it is mostly metabolized to nitrite. In human blood and tissues, nitrite is typically oxidized to nitrate. Concentrations of nitrate in urine and saliva fluctuate; therefore, in order to evaluate exposure more precisely, a 24-hour collection of urine is recommended. Analysis is achieved via hydrazine reduction (IARC 2010; Levallois et al. 2000).

Levels of nitrate and nitrite in plasma, urine, and saliva can be measured by gas chromatography/mass spectrometry (GC/MS) (Bondonno et al. 2012; Tsikas 2005). Frozen samples are treated with tetraoctylammonium bromide and derivatizing reagent pentafluorobenzyl bromide in acetone solutions at elevated temperature. Acetone is removed by evaporation under a nitrogen atmosphere and the remaining aqueous phase is extracted with an isooctane/toluene solution and analyzed by GC/MS (m/z = 62 for nitrate and 46 for nitrite). Sample procedures must involve precautionary steps to minimize the

Table 7-1. Analytical Methods for Determining Nitrate and Nitrite in Biological Materials

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Urine	Hydrazine reduction	GC-MS	5 ng/L	Not reported	Levallois et al. 2000
Plasma, urine, or saliva	Sample derivitization with tetraoctyl-ammonium bromide and pentafluorobenzyl bromide	GC-MS	Not reported	Not reported	Bondonna et al. 2012
Plasma (serum)	Deproteinization; Griess reaction	HPLC-UV spectrometry; GC-MS	0.3–20 µM (nitrite) 4–81 µM (nitrate)	Not reported	Hibbs et al. 1992; Sun et al. 2003; Tsikas 2005
Urine	Griess	GC-MS	690 µmol/ 24 hours	Not reported	Hibbs et al. 1992
Urine	International standard dilution	IC-MS/MS	500 µg/L (nitrate)	95	Valentín-Blasini et al. 2007
Urine (metabolite-ammonia)	24-Hour specimen collected, preserve with HCl and refrigerate	Colorimetric (Berthelot reaction)	Not reported	Not reported	Tietz 1970
Urine (metabolite-ammonia)	24-Hour specimen analyzed immediately, or stored up to 8 weeks at -20 °C	Indophenol reaction	Not reported	Not reported	Huizenga et al. 1994)
Whole blood	Deprotonized using acetonitrile followed by purification	HPLC/direct conductivity detection	0.4 µmol/L (nitrite)		Yan et al. 2016

GC-MS = gas chromatography-mass spectrometry; HPLC = high-performance liquid chromatography; IC-MS/MS = ion chromatography-mass spectrometry/mass spectrometry; UV = ultraviolet

endogenous contribution of analytes in the laboratory chemicals and materials (glassware, filtration equipment, etc.) used for sample collection and work up (Tietz 1970; Tsikas 2005). Chemical interferences in samples resulting in reduction of nitrate to nitrite, or conversely, the oxidation of nitrite to nitrate during analysis should be evaluated and accounted for. Microbial conversion via hydrolysis may cause an increase in values (Tietz 1970). Preparation of blood samples must involve procedures that limit the oxidation of nitrite by oxyhaemoglobin and loss due to methods requiring acidification or derivatization. Possible interferences in ammonia quanitification and to the Griess assay, such as anticoagulants, must also be factored (Huizenga 1994; Tsikas 2005).

The Griess assay is one of the first methods used to measure levels of nitrate and nitrite in biological and environmental samples. The method involves reduction of nitrate to nitrite followed by a diazotization reaction and then measuring the absorbance of the diazo chromophore in the visible spectrum. To determine the levels of nitrate and nitrite separately, the procedure is first carried out without the preliminary reduction step in order to quantify the level of nitrite solely. This assay was originally performed using sulfanilic acid, which forms a diazonium cation with nitrite under acidic conditions followed by coupling with α-naphthylamine to form a diazo compound, which contains a strong absorption band at about 540 nm (Tsikas 2005). Other methods include diazotizing with sulfanilamide and coupling with N-(1-naphthyl)-ethylenediamine dihydrochloride to form the diazo compound (EPA 1993). GC/MS methods were shown to provide superior quantification of nitrate and nitrite in human plasma and urine samples when compared to the Griess assays (Tsikas 2005)

Reverse-phase high performance liquid chromatography (HPLC) by means of ion pairing in the mobile phase without derivitization followed by ultraviolet (UV) detection around 210 nm has also been used to detect nitrate in urine samples (Tsikas 2005). Urinary nitrate levels can be measure using ion chromatography-tandem mass spectrometry (IC-MS/MS) by means of internal standard dilution (Valentín-Blasini et al. 2007).

The level of methemoglobin in the blood is often the biomarker for assessing nitrate exposure (Manassaram et al. 2010). Methemoglobin can be measured in blood collected via finger stick samples. Samples are analyzed with portable AVOXimeter 4000 whole-blood oximeter devices. The device measures total hemoglobin, and further characterizes percentages of oxyhemoglobin, carboxyhemoglobin, and methemoglobin. The accuracy and precision of the method were reported as ±0.5 and ±0.7%, respectively. Refer to ATSDR (Agency for Toxic Substance and Disease Registry 2013b) for discussion of other nitrate and nitrite laboratory tests.

Yan et al. (2016) developed a simple method for the quantitative determination of nitrite in whole blood samples employing ion chromatography and electrochemical detection. The blood sample is prepared by adding acetonitrile followed by purification using mini-cartridges to remove interfering compounds. The detection limit for the method is reported as 0.4 μmol/L.

7.2 ENVIRONMENTAL SAMPLES

Methods are available for determining the level of nitrate and nitrite in a variety of environmental matrices. A summary of representative methods is shown in Table 7-2.

Ion chromatography and spectrometry methods are the most common analytical techniques employed for the detection and quantification of nitrate and nitrite in environmental samples; detection limits range from 0.01 to 1 mg/L (ppm) (IARC 2010; WHO 2011b). Samples must be analyzed as soon as is reasonably possible in order to minimize any changes in the sample due to microbial transformations. Sample preservation using chemicals and or deep freezing methods have been reported; however, interference with the analysis can occur in certain methods (Mulvaney 1996).

Methods based on the Griess assay are available for the determination of nitrate and nitrite in potable water, raw water and wastewater (EPA 1993; WHO 2011b). The limit of detection for the International Organization for Standardization ISO method 6777/1 lies within the range of 0.005–0.01 mg/L (ppm) (WHO 2011b). A continuous-flow spectrometric method (ISO method 7890-1) for the determination of nitrite, nitrate or the sum of both in various types of water is suitable at concentrations ranging from 0.05 to 5 mg/L (ppm) for nitrite and from 1 to 100 mg/L (ppm) for nitrite and nitrate, both in the undiluted sample (WHO 2011b).

NIOSH method 7903 employs ion chromatography for the determination of nitric acid in air (NIOSH 1994a). Method 7903 is an analytical technique for determining inorganic acids by measuring the total concentration of airborne anions. Particulate nitrate has been successfully detected and quantified in atmospheric samples via ion chromatographic techniques and NO_x chemiluminescent analyzers (Small et al. 1975; Yoshizumi et al. 1985)

Table 7-2. Analytical Methods for Determining Nitrate and Nitrite in Environmental Samples

Sample matrix[a]	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air/water (nitrate, nitrite)	Nitrite prepared using Griess-Ilosvay reaction; nitrate prepared using hydrazine reduction; aqueous extracts from aerosol filters are analyzed without pretreatment	UV spectrometry	0.07 ppm (nitrite); 0.2 ppm (nitrate)	Not reported	Oms et al. 1995
Air (nitrate)	Personal air sampled at 0.2–0.5 L/minute for total sample size of 3–100 L using silica gel sample tube; boil sorbent from sample tube in bicarbonate/ carbonate buffer for 10 minutes	Ion chromatography/ conductivity detector; NIOSH 7903	0.7 µg/sample	Not reported	NIOSH 1994a
Air (nitrite)	Ambient air is sampled at 0.025 L/minute for 3-L air sample using glass sorbent tubes with glass wool retainers; add adsorbing solution; add solution of hydrogen peroxide, sulfanilamide and NEDA to extracted sample, set for 10 minutes	Visible absorption spectro-photometry; NIOSH 6014	1 µg/sample	Not reported	NIOSH 1994b
Air (nitrite)	Ambient air is sampled at 0.025 L/minute for 3-L air sample using diffusive sampler tubes with three triethanolamine screens; add adsorbing solution; add solution of hydrogen peroxide, sulfanilamide and NEDA to extracted sample, set for 10 minutes	Visible absorption spectro-photometry; NIOSH 6700	0.01 µg/sample	Not reported	NIOSH 1998
Water (nitrate)	Drinking water or river water samples are prepared with lanthanum (III) chloride and placed into the cell	Voltammetry/ static mercury drop electrode	20 µg/L	Not reported	Markusova et al. 1996

Table 7-2. Analytical Methods for Determining Nitrate and Nitrite in Environmental Samples

Sample matrix[a]	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Drinking water	Clean samples can be used directly	IC/CD	Not reported	93–114 (nitrite-N) 83–113 (nitrate-N)	IARC 2010
Soil (nitrate)	Soil samples are added to Gohler solutions followed by neutralization before analysis	Voltammetry/ static mercury drop electrode	20 µg/L	Not reported	Markusova et al. 1996
Soil (nitrite, nitrate)	Extraction of field samples with 2 M KCL; nitrate reduction; diazoitization and coupling with resulting dye formation	UV-Vis (λ=540 nm)	Not reported	Not reported	Mulvaney 1996
Foods and juices (nitrate, nitrite)	Blended/pureed food samples are mixed with water and filtered	Capillary ion electrophoresis	Not reported	>73 (nitrate); >88 (nitrite)	Marshall and Trenerry 1996
Food (nitrate, nitrite)	Direct injection	HePI-MS	Not reported	Not reported	Pavlov and Attygalle 2013
Fish and water (nitrate, nitrite)	Fish muscle homogenized, digested with perchloric acid and centrifuged; nitrate reduction to nitrite using copperised cadmium redactor; reaction with phosphomolybdenum blue complex and ammonium chloride	FIA spectrometry	0.01 µg/mL (nitrite); 0.025 µg/mL (nitrate)	97.8–102.1 (nitrite); 98.5–101.6 (nitrate)	Monser et al. 2002
Water and vegetables (nitrate)	Food: homogenization with deionized water and heated at 80°C; cooled and diluted with deionized water Water: direct analysis	Potentiometry with solid contact ISE	0.0037 µ/mL (nitrate)	98.9–105.9 (nitrate)	Wardak and Grabarczky 2016
Cured meat (nitrite)	Reaction with sulfanilamide followed by reaction with NEDA	Colorimetry; absorbance 540 nm	Not reported	Not reported	IARC 2010
Milk and milk products (nitrate, nitrite)	Suspension in buffer solution; centrifuge and reduce with cadmium; react with sulfanilamide followed by reaction with NEDA	FIA spectrometry; absorbance 540 nm	0.5 mg/kg (nitrate) 1.0 mg/kg (nitrite)	Not reported	IARC 2010

Table 7-2. Analytical Methods for Determining Nitrate and Nitrite in Environmental Samples

Sample matrix[a]	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Dairy products and cheese (nitrate, nitrite)	Extract cheese slurry using $ZnSO_4$ and NaOH; reduce in Jones reductor using zinc and $CdSO_4$	Spectro-photometry; absorbance at 522 nm	≥1 µg/g nitrate	Not reported	IARC 2010
Fried bacon (N-nitros-amines)	Grind frozen sample; vacuum distill with NaOH and mineral oil; extract and dry with DCM and anhydrous $NaSO_4$; concentrate	GC	Not reported	Not reported	IARC 2010

DCM = dichloromethane; FIA = flow injection analysis; GC = gas chromatography; HePI-MS = helium-plasma ionization-mass spectrometry; IC/CD = ion chromatography/conductivity detector; ISE = ion-selective electrodes; NEDA = N-1-naphthylethylenediamine dihydrochloride; NIOSH = National Institute for Occupational Safety and Health; UV = ultraviolet absorbance detection

A sequential injection method coupled with spectrophotometry has been developed for the detection of nitrate and nitrite in environmental samples such as atmospheric aerosol filter extracts and waste water samples (Oms et al. 1995). The method is advantageous due to the small volumes of sample and reagents required for analysis. The detection limits were reported as 0.07 ppm for nitrite and 0.2 ppm for nitrate. Nitrite is analyzed using the Griess-Ilosvay reaction; nitrate is reduced to nitrite using hydrazine sulphate.

Markusova et al. (1996) developed a sensitive voltammetric method that can determine nitrate levels in drinking water, river water, or soil extracts three orders of magnitude lower than the allowed levels of nitrate in drinking water. The method employs a multi-purpose electrochemical analyzer and a voltammetric cell. Water samples are prepared with lanthanum (III) chloride and placed into the cell; soil samples are added to Gohler solutions followed by neutralization before analysis. The reported limit of detection is 20 µg nitrate/L (20 ppb) (5 µg NO_3^-N/L).

The most commonly used method for soil and soil extract analysis of nitrite is a modified Griess-Ilosvay colorimetric method using a continuous flow analyzer. Nitrites react with primary aromatic amines to form a diazonium salt which is then coupled with an aromatic compound; the resulting complex has a characteristic absorbance band in the UV-Vis spectrum. The concentration of nitrite is proportional to the color intensity of the resulting azo compound measured using a spectrophotometer or colorimeter. This technique is also used for sensitive analysis of nitrate following reduction to nitrite. Cadmium reduction to nitrite is achieved in a column of copperized cadmium with an ammonium chloride (NH4Cl) matrix at pH between 5 and 10. Other various reducing agents have been reported. Analysis for nitrate must account for initial concentrations of nitrite in the sample prior to reduction. Maximum accuracy is seen when absorbance is measured at wavelengths of 540 nm; however, wavelengths between 510 and 550 nm are acceptable (Mulvaney 1996).

Pavlov and Attygalle (2013) developed an analytical method with minimal sample preparation employing helium-plasma ionization-mass spectrometry. Nitrate was successfully identified and quantified using this solvent-less ambient pressure mass spectrometry technique in various foodstuffs. Samples of fruit juice and meat pieces (i.e., tomato and celery juice, hot dog and beef) can be placed onto glass slides and analyzed directly without any modification. Quantification of nitrate in such complex matrices is suggested to be determined with accuracy by spiking with known quantities of radiolabeled nitrate. The method detection limit for determining the nitrate concentration is in the range of 20 ng/sample and depends on the specific sample matrix.

Capillary ion electrophoresis has been successfully employed for the determination of nitrite and nitrate in foods and juices (Marshall and Trenerry 1996). The authors tested the procedure using cheese, cabbage, fruit juices, and meats. Percent recovery for three processed meat samples ranged from 88 to 118% for nitrite and from 73 to 106% for nitrate.

7.3 ADEQUACY OF THE DATABASE

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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.

Exposure. Nitrate and nitrite may be converted to many other compounds in the body, such as N-nitroso compounds, including nitrosamines. Approximately 25% of absorbed nitrate is secreted to saliva and about 20% of this is reduced to nitrite. Nitrite is converted to nitric oxide by the acidic environment on the stomach. Methods exist for the measurement of nitroso compounds and nitrite in plasma and salivary nitrite (Bondonno et al. 2012). Nitrate in the diet may contribute to nitric oxide levels in the body, and increases in these levels can be a biomarker of exposure. Ammonia is a minor urinary product of nitrite and nitrate in which analytical methods are available (Huizenga et al. 1994; Tietz 1970). N-Methyl-nicotinamide has also been shown to be a potential biomarker of exposure to nitrate and nitrite and there are methods to measure this (Jansen et al. 1995). No data needs were identified.

Effect. Methemoglobinemia caused by the presence of higher-than-normal levels of methemoglobin is a biomarker of effect for exposure to high levels of nitrate; however, this effect is not unique for nitrate and nitrite since other substances may also cause this condition (Bruning-Fann and Kaneene 1993). Methods are available to measure methemoglobin in the blood (Manassaram et al. 2010).

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Methods are available for determining nitrate and nitrite levels in environmental samples such as air (NIOSH 1994a; Small et al. 1975; Yoshizumi et al. 1985) and water (EPA 1993; Markusova et al. 1996; WHO 2011b).

7.3.2 Ongoing Studies

No ongoing analytical methodologies for nitrate or nitrite were identified using the NIH RePORTER version 6.1.0 or the DTIC online database.

8. REGULATIONS, ADVISORIES, AND GUIDELINES

MRLs are substance-specific estimates that are intended to serve as screening levels. They are used by ATSDR health assessors and other responders to identify contaminants and potential health effects that may be of concern at hazardous waste sites.

MRLs of 4 mg nitrate/kg/day have been derived for acute-, intermediate, and chronic-duration oral exposure (≤14 days) to nitrate. The MRLs are based on a no-adverse-effect concentration (NOAEC) of 10 mg nitrate-nitrogen/L (44 mg nitrate/L) in drinking water used to prepare formula for infants <6 months of age (Walton 1951). A NOAEL of 4.33 mg nitrate/kg/day at the NOAEC of 44 mg nitrate/L was calculated based on estimates of 0.525 L/day for water intake (Kahn and Stralka 2009) and 5.33 kg for body weight (EPA 2008) of an infant from birth to <3 months of age. A total uncertainty factor of 1 was applied because the point of departure is a NOAEL for nitrate-induced effects on methemoglobin in a sensitive human subpopulation (i.e., <3-month-old infants, which in many cases may have been at increased risk of methemoglobinemia due to microbial contamination and associated gastrointestinal infection). Following ingestion of relatively large amounts of nitrate by healthy normal individuals, blood methemoglobin levels increase rapidly, followed by a return to normal within several hours following intake. Repeated ingestion for intermediate- or chronic-duration time periods would be expected to result in changes in methemoglobin levels similar to those elicited from a single exposure. Therefore, the acute-, intermediate- and chronic-duration oral MRL values are equivalent. Refer to Appendix A for additional information regarding derivation of oral MRLs for nitrate.

MRLs of 0.1 mg nitrite/kg/day have been derived for acute-, intermediate, and chronic-duration oral exposure (≤14 days) to nitrite. The ingestion of nitrate results in the formation of nitrite, which is the moiety responsible for methemoglobinemia. In adults, approximately 5% of an oral dose of nitrate is reduced to nitrite in the saliva, most of which is absorbed into the blood in the small intestine. Based on the assumption of 100% absorption of ingested nitrite, an oral dose of 0.2 mg nitrite/kg/day by an adult would be expected to result in a nitrite blood level similar to that achieved following ingestion of nitrate at the oral MRL dose of 4 mg nitrate/kg/day (i.e., 0.2 mg nitrite/kg/day is 5% of an oral dose of nitrate at the MRL of 4 mg nitrate/kg/day). A modifying factor of 2 was applied to the point of departure (0.2 mg nitrite/kg/day ÷ 2 = 0.1 mg nitrite/kg/day) because young infants exhibit increased susceptibility to methemoglobinemia following nitrate ingestion; the modifying factor assumes that the effective methemoglobin level from a given intake of nitrate by an infant is up to twice that of an adult. Following ingestion of relatively large amounts of nitrate by healthy normal individuals, blood methemoglobin

levels increase rapidly, followed by a return to normal within several hours following intake. Repeated ingestion for intermediate- or chronic-duration time periods would be expected to result in changes in methemoglobin levels similar to those elicited from a single exposure. Therefore, the acute-, intermediate-, and chronic-duration oral MRL values are equivalent. Refer to Appendix A for additional information regarding derivation of oral MRLs for nitrite.

EPA (IRIS 2002) derived an oral reference dose (RfD) of 1.6 mg nitrate-nitrogen/kg/day (i.e., 1.6 mg nitrogen from nitrate; ~7 mg nitrate/kg/day) based on a NOAEL of 1.6 mg nitrate-nitrogen/kg/day and a LOAEL of 1.8–3.2 mg nitrate-nitrogen/kg/day (7.92–14.08 mg nitrate/kg/day) for early clinical signs of methemoglobinemia in excess of 10% among formula-fed infants 0–3 months of age (Bosch et al. 1950; Walton 1951). An uncertainty factor of 1 was employed because available data defined the NOAEL for the critical effect in the most sensitive human subpopulation.

EPA (IRIS 2002) derived an RfD of 0.1 mg nitrite-nitrogen/kg/day (~0.33 mg nitrite/kg/day) based on a NOAEL of 10 mg nitrate-nitrogen/L and a LOAEL of 11–20 mg nitrate-nitrogen/L for early clinical signs of methemoglobinemia in excess of 10% (Walton 1951). The NOAEL of 10 mg nitrate-nitrogen/L was converted to an estimated dose of 1 mg nitrate-nitrogen/kg/day based assumptions that a 10-kg child would ingest 1 L of water/day. EPA applied a modifying factor of 10 to the NOAEL of 1 mg nitrate-nitrogen/kg/day from the Walton (1951) study to account for the direct toxicity of nitrite, resulting in an RfD of 0.1 mg nitrite-nitrogen/kg/day. As described in a Drinking Water Criteria Document for Nitrate/Nitrite (EPA 1990a), the modifying factor of 10 was used to account for an estimated rate of 10% conversion of ingested nitrate to nitrite in infants compared to an estimated rate of 5% conversion in adults.

Based on available human data, IARC (2010) determined that there is *inadequate evidence* for the carcinogenicity of nitrate in food or drinking water and *limited evidence* for the carcinogenicity of nitrite in food (based on association with increased incidence of stomach cancer). Evaluation of available animal data by IARC (2010) resulted in the determination that there is *inadequate evidence* for the carcinogenicity of nitrate, *limited evidence* for the carcinogenicity of nitrite *per se*, and *sufficient evidence* for the carcinogenicity of nitrite in combination with amines or amides. The overall conclusions of IARC (2010) were that "ingested nitrate and nitrite under conditions that result in endogenous nitrosation is *probably carcinogenic to humans (Group 2A)*." IARC (2010) noted that: (1) the endogenous nitrogen cycle in humans includes interconversion of nitrate and nitrite; (2) nitrite-derived nitrosating agents produced in the acid stomach environment can react with nitrosating compounds such as secondary

amines and amides to generate N-nitroso compounds; (3) nitrosating conditions are enhanced upon ingestion of additional nitrate, nitrite, or nitrosatable compounds; and (4) some N-nitroso compounds are known carcinogens.

Neither nitrate nor nitrite have been classified as to their carcinogenicity by the U.S. EPA Integrated Risk Information System (IRIS 2002), the National Toxicology Program (NTP, 2011), or the American Conference of Governmental Industrial Hygienists (ACGIH 2013).

The EPA lists maximum contaminant levels (MCL) and maximum contaminant level goals (MCLG) of 10 mg/L for nitrate (as nitrate-nitrogen; ~44 mg nitrate/L) and 1 mg/L for nitrite (as nitrite nitrogen; ~3.3 mg nitrite/L) in the 2012 Edition of the Drinking Water Standards and Health Advisories (EPA 2012b).

The international and national regulations, advisories, and guidelines regarding nitrate and nitrite in air, water, and other media are summarized in Table 8-1.

Table 8-1. Regulations, Advisories, and Guidelines Applicable to Nitrate and Nitrite

Agency	Description	Information	Reference
INTERNATIONAL			
Guidelines:			
IARC	Carcinogenicity classification		IARC 2014
	Nitrate or nitrite (ingested) under conditions that result in endogenous nitrosation	Group 2A[a]	
WHO	Air quality guidelines	No data	WHO 2010
	Drinking water quality guidelines		WHO 2011a
	Nitrate (as NO_3^-)	50 mg/L[b]	
	Nitrite (as NO_2^-)	3 mg/L[c]	
	Combined nitrate plus nitrite	The sum of the ratios of the concentrations as reported or detected in the sample of each to its guideline value should not exceed 1	
NATIONAL			
Regulations and guidelines:			
a. Air			
ACGIH	TLV-TWA	No data	ACGIH 2013
AIHA	ERPGs	No data	AIHA 2013
DOE	Nitrate(s)		DOE 2012
	PAC-1[d]	30 mg/m^3	
	PAC-2	330 mg/m^3	
	PAC-3	2,000 mg/m^3	
	Ammonium nitrate		
	PAC-1[d]	6.7 mg/m^3	
	PAC-2	73 mg/m^3	
	PAC-3	440 mg/m^3	
	Potassium nitrate		
	PAC-1[d]	0.074 mg/m^3	
	PAC-2	0.82 mg/m^3	
	PAC-3	600 mg/m^3	
	Sodium nitrate		
	PAC-1[d]	12 mg/m^3	
	PAC-2	130 mg/m^3	
	PAC-3	250 mg/m^3	

Table 8-1. Regulations, Advisories, and Guidelines Applicable to Nitrate and Nitrite

Agency	Description	Information	Reference
NATIONAL *(cont.)*			
EPA	Sodium nitrite		
	PAC-1[d]	2.3 mg/m^3	
	PAC-2	26 mg/m^3	
	PAC-3	280 mg/m^3	
EPA	AEGLs	No data	EPA 2013a
	Hazardous air pollutant	No data	EPA 2014a 42 USC 7412
	NAAQS	No data	EPA 2014d
NIOSH	REL	No data	NIOSH 2014
	STEL	No data	
	IDLH	No data	
OSHA	PEL (8-hour TWA) for general industry	No data	OSHA 2013b 29 CFR 1910.1000, Table Z-2
	Highly hazardous chemicals	No data	OSHA 2013a 29 CFR 1910.119, Appendix A
b. Water			
EPA	Designated as hazardous substances in accordance with Section 311(b)(2)(A) of the Clean Water Act	No data	EPA 2013b 40 CFR 116.4
	Drinking water contaminant candidate list	No data	EPA 2009b 74 FR 51850
	Drinking water standards and health advisories		EPA 2012b
	Nitrate		
	MCL	10 mg nitrogen/L (~44 mg nitrate/L)[e]	
	MCLG	10 mg nitrogen/L (~44 mg nitrate/L)[f]	
	Health advisory for 1 day for 10-kg child	100 mg nitrogen/L (~440 mg nitrate/L)[e]	
	Health advisory for 10 days for 10-kg child	100 mg nitrogen/L (~440 mg nitrate/L)[e]	
	DWEL	No data	

Table 8-1. Regulations, Advisories, and Guidelines Applicable to Nitrate and Nitrite

Agency	Description	Information	Reference
NATIONAL *(cont.)*			
EPA	Nitrite		
	MCL	1 mg nitrogen/L (~3.3 mg nitrite/L)[f]	
	MCLG	1 mg nitrogen/L (~3.3 mg nitrite/L)[f]	
	Health advisory for 1 day for 10-kg child	10 mg nitrogen/L (~33 mg nitrite/L)[f]	
	Health advisory for 10 days for 10-kg child	10 mg nitrogen/L (~33 mg nitrite/L)[f]	
	DWEL	No data	
	Nitrate + nitrite (both as nitrogen)		
	MCL	10 mg/L	
	MCLG	10 mg/L	
	National primary drinking water standards		EPA 2009c
	Nitrate		
	MCL	10 mg nitrogen/L (~44 mg nitrate/L)[e]	
	Potential health effects from long-term exposure above the MCL	Serious illness; symptoms include shortness of breath and blue-baby syndrome[g]	
	Common sources of contaminant in drinking water	Runoff from fertilizer use; leaching from septic tanks, sewage; erosion of natural deposits	
	Public Health Goal	10 mg nitrogen/L (~44 mg nitrate/L)[e]	
	Nitrite		
	MCL	1 mg nitrogen/L (~3.3 mg nitrite/L)[f]	
	Potential health effects from long-term exposure above the MCL	Serious illness; symptoms include shortness of breath and blue-baby syndrome[g]	
	Common sources of contaminant in drinking water	Runoff from fertilizer use; leaching from septic tanks, sewage; erosion of natural deposits	
	Public Health Goal	1 mg nitrogen/L (~3.3 mg nitrite/L)[f]	

Table 8-1. Regulations, Advisories, and Guidelines Applicable to Nitrate and Nitrite

Agency	Description	Information	Reference
NATIONAL *(cont.)*			
EPA	National recommended water quality criteria: human health for the consumption of (at 10^{-4} risk)		EPA 2014e
	Nitrates		
	Water + organism	10,000 µg nitrogen/L	
	Organism only	No data	
	Reportable quantities of hazardous substances designated pursuant to Section 311 of the Clean Water Act		EPA 2013d 40 CFR 117.3
	Sodium nitrite	100 pounds	
c. Food			
FDA	Bottled water (allowable limits)		FDA 2013 21 CFR 165.110
	Nitrate	10 mg nitrogen/L (~44 mg nitrate/L)[e]	
	Nitrite	1 mg nitrogen/L (~3.3 mg nitrite/L)[f]	
	Total nitrate and nitrite (as nitrogen)	10 mg/L	
	EAFUS[h]		FDA 2014
	Potassium nitrate, sodium nitrate, potassium nitrite, and sodium nitrite	Yes	
d. Other			
ACGIH	Carcinogenicity classification	No data	ACGIH 2013
EPA	Nitrate		EPA 1990a; IRIS 2002
	Carcinogenicity classification	No data	
	RfC	No data	
	RfD	1.6 mg nitrogen/kg/day (~7 mg nitrate/kg/day)[e]	
	Nitrite		
	Carcinogenicity classification	No data	
	RfC	No data	
	RfD	0.1 mg nitrogen/kg/day (~0.33 mg nitrite/kg/day)[f]	
	Identification and listing of hazardous waste	No data	EPA 2013c 40 CFR 261, Appendix VIII

Table 8-1. Regulations, Advisories, and Guidelines Applicable to Nitrate and Nitrite

Agency	Description	Information	Reference
NATIONAL *(cont.)*			
EPA	Inert pesticide ingredients in pesticide products approved for nonfood use only		EPA 2014b
	Ammonium nitrate, potassium nitrate, sodium nitrate, potassium nitrite, and sodium nitrite	Yes	
	Master Testing List	No data	EPA 2014c
	RCRA waste minimization PBT priority chemical list	No data	EPA 1998 63 FR 60332
	Standards for owners and operators of hazardous waste TSD facilities; groundwater monitoring list	No data	EPA 2013e 40 CFR 264, Appendix IX
	Superfund, emergency planning, and community right-to-know		
	Designated CERCLA hazardous substance and reportable quantity pursuant to Section 311(b)(2) of the Clean Water Act		EPA 2013f 40 CFR 302.4
	Sodium nitrite	100 pounds	
	Nitrate compounds (water dissociable; reportable only when in aqueous solution); sodium nitrite	Effective date of toxic chemical release reporting; 01/01/1995	EPA 2013h 40 CFR 372.65
	Superfund, emergency planning, and community right-to-know		
	Extremely hazardous substances and its threshold planning quantity	No data	EPA 2013g 40 CFR 355, Appendix A
	TSCA chemical lists and reporting periods	No data	EPA 2013i 40 CFR 712.30
	TSCA health and safety data reporting	No data	EPA 2013j 40 CFR 716.120

Table 8-1. Regulations, Advisories, and Guidelines Applicable to Nitrate and Nitrite

Agency	Description	Information	Reference
NATIONAL *(cont.)*			
NTP	Carcinogenicity classification	No data	NTP 2011

[a]Group 2A: probably carcinogenic to humans.
[b]As nitrate ion (or 11 mg/L as nitrate-nitrogen) to protect against methemoglobinemia in bottle-fed infants (short-term exposure).
[c]As nitrite ion (or 0.9 mg/L as nitrite-nitrogen) to protect against methemoglobinemia in bottle-fed infants (short-term exposure).
[d]PAC-1: mild, transient health effects; PAC-2: irreversible or other serious health effects that could impair the ability to take protective action; PAC-3: life-threatening health effects (DOE 2012).
[e]1 mg nitrate-nitrogen/L (i.e., nitrogen from nitrate) ~4.4 mg nitrate/L
[f]1 mg nitrite-nitrogen/L (i.e., nitrogen from nitrite) ~3.3 mg nitrite/L
[g]Infants below the age of 6 months who drink water containing nitrate and/or nitrite in excess of the MCL could become seriously ill and, if untreated, may die (EPA 2009b).
[h]The EAFUS list of substances that contains ingredients added directly to food that FDA has either approved as food additives or listed or affirmed as GRAS.

ACGIH = American Conference of Governmental Industrial Hygienists; AEGL = acute exposure guideline level; AIHA = American Industrial Hygiene Association; CERCLA = Comprehensive Environmental Response, Compensation, and Liability Act; CFR = Code of Federal Regulations; DOE = Department of Energy; DWEL = drinking water equivalent level; EAFUS = Everything Added to Food in the United States; EPA = Environmental Protection Agency; ERPG = emergency response planning guidelines; FDA = Food and Drug Administration; FR = Federal Register; GRAS = generally recognized as safe; IARC = International Agency for Research on Cancer; IDLH = immediately dangerous to life or health; IRIS = Integrated Risk Information System; MCL = maximum contaminant level; MCLG = maximum contaminant level goal; NAAQS = National Ambient Air Quality Standards; NIOSH = National Institute for Occupational Safety and Health; NTP = National Toxicology Program; OSHA = Occupational Safety and Health Administration; PAC = protective action criteria; PBT = persistent, bioaccumulative, and toxic; PEL = permissible exposure limit; RCRA = Resource Conservation and Recovery Act; REL = recommended exposure limit; RfC = inhalation reference concentration; RfD = oral reference dose; STEL = short-term exposure limit; TLV = threshold limit value; TSCA = Toxic Substances Control Act; TSD = treatment, storage, and disposal; TWA = time-weighted average; USC = United States Code; WHO = World Health Organization

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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 (Kd)—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 BMD_{10} 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-control study, a group of people with a specified and well-defined outcome is identified and compared to a similar group of people without the 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.

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 that must not be exceeded.

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 risk 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)—A condition that poses a threat of life or health, or conditions that pose an immediate threat of severe exposure to contaminants that are likely to have adverse cumulative or delayed effects on health.

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 new cases 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$_{(50)}$ (LC_{50})—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$_{(50)}$ (LD_{50})—The dose of a chemical that has been calculated to cause death in 50% of a defined experimental animal population.

Lethal Time$_{(50)}$ (LT_{50})—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.

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 hazardous substance.

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) regulatory limit on the amount or concentration of a substance not to be exceeded in workplace air averaged over any 8-hour work 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 (insects or other organisms harmful to cultivated plants or animals).

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.

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 blood:air 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.

q_1*—The upper-bound estimate of the low-dose slope of the dose-response curve as determined by the multistage procedure. The q_1* 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/m^3 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/m^3 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 hazardous substance. 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.

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 hazardous substance.

Risk Factor—An aspect of personal behavior or lifestyle, an environmental exposure, existing health condition, 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)—A STEL is a 15-minute TWA exposure that should not be exceeded at any time during a workday.

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 it is believed that nearly all workers may be repeatedly exposed, day after day, for a working lifetime without adverse effect. The TLV may be expressed as a Time Weighted Average (TLV-TWA), as a Short-Term Exposure Limit (TLV-STEL), or as a ceiling limit (TLV-C).

Time-Weighted Average (TWA)—An average exposure within a given time period.

Toxic Dose$_{(50)}$ (TD$_{50}$)—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.

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 substance 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 route and 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 substance-induced endpoint 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.

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 and Human Health Sciences, 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 and Human Health Sciences, Agency for Toxic Substances and Disease Registry, 1600 Clifton Road NE, Mailstop F-57, Atlanta, Georgia 30329-4027.

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: Nitrate
CAS Numbers: 14797-55-8
Date: July 2017
Profile Status: Final
Route: [ ] Inhalation [x] Oral
Duration: [x] Acute [x] Intermediate [x] Chronic
Graph Key: 3 (Acute), 15 (Intermediate), 53 (Chronic)
Species: Human

Minimal Risk Level: 4 [x] mg/kg/day [ ] ppm

Reference: Walton G. 1951. Survey of literature relating to infant methemoglobinemia due to nitrate-contaminated water. Am J Public Health 41:986-996.

Experimental design: Walton (1951) reviewed available literature and found 278 reported cases of infant methemoglobinemia in a total of 14 U.S. states from which information was available. Cases were grouped by state according to ranges of nitrate levels in drinking water sources.

Effect noted in study and corresponding doses: Among methemoglobinemia cases for which nitrate levels in water sources used to prepare infant formula were available, 173 cases were associated with >50 mg nitrate-nitrogen/L (220 mg nitrate/L), 36 cases with 21–50 mg nitrate-nitrogen/L (92–220 mg nitrate/L), and 5 cases with 11–20 mg nitrate-nitrogen (48–88 mg nitrate/L). None of the methemoglobinemia cases were associated with drinking water sources measuring <10 mg nitrate-nitrogen/L (<44 mg nitrate/L). Limitations of the contributing studies include lack of information regarding the actual ages of the infants, total nitrate doses, and other water source contaminants (e.g., bacterial levels).

Following ingestion of relatively large amounts of nitrate by healthy normal individuals, blood methemoglobin levels increase rapidly, followed by a return to normal within several hours following intake. Repeated ingestion for intermediate- or chronic-duration time periods would be expected to result in changes in methemoglobin levels similar to those elicited from a single exposure. Therefore, the acute-, intermediate-, and chronic-duration oral MRL values are equivalent.

Dose and end point used for MRL derivation: 4.33 mg nitrate/kg/day

[x] NOAEL [ ] LOAEL

Uncertainty Factors used in MRL derivation:

[ ] 10 for use of a LOAEL
[ ] 10 for extrapolation from animals to humans
[x] 1 for human variability

A total uncertainty factor of 1 is justified because the point of departure is a NOAEL for nitrate-induced effects on methemoglobin in a particularly sensitive human subpopulation (i.e., <3-month-old infants, which in many cases may have been at increased risk of methemoglobinemia due to microbial contamination and associated gastrointestinal infection).

Was a conversion factor used from ppm in food or water to a mg/body weight dose? Nitrate may be expressed in terms of ionic concentration (i.e., mg nitrate/L), or elemental concentration (i.e., mg nitrate-nitrogen/L or mg nitrogen as nitrate/L). A concentration of nitrate expressed in elemental concentration (mg nitrogen per liter from nitrate source) can be converted to its ionic concentration (mg NO_3^-) according to the following relationship: 1 mg nitrate-nitrogen = 4.4 mg nitrate (i.e., the proportion of N in NO_3^- is 14 [atomic mass of N] ÷ 62 [molecular mass of NO_3^-] = 0.226).
Table A-1 presents estimated nitrate doses to infants (birth–<3 months of age) calculated using estimated mean values for drinking water ingestion rates (Kahn and Stralka 2009) and body weight (EPA 2008) and assuming a drinking water level of 44 mg nitrate/L as a concentration not expected to cause methemoglobinemia; the calculated doses of 4.31–4.34 mg nitrate/kg/day represent NOAELs for the age ranges. The TWA-based calculated dose of 4.33 mg nitrate/kg/day for the age range of birth–<3 months is selected as the point of departure for deriving acute-, intermediate-, and chronic-duration oral MRLs for nitrate.

Table A-1. Estimated Nitrate Dose to Infants of Selected Age Ranges Assuming a Drinking Water Level of 44 mg Nitrate/L[a]

Age range	Water intake (L/day)[b]	Body weight (kg)[c]	Nitrate dose (mg/kg/day)[d]
Birth–<1 month	0.470	4.8	4.31
1–<3 months	0.552	5.6	4.34
Birth–<3 months	0.525[e]	5.33[e]	4.33

[a]Considered a no-adverse-effect concentration for nitrate intake by infants up to 6 months of age, based on weight-of-evidence analysis of available human data.
[b]Estimated mean water intake (combined direct intake [ingested largely as a beverage] and indirect intake [added in preparation of food or beverages]) from community water; data from Table 3-14 of EPA (2008) and Table 2 of Kahn and Stralka (2009).
[c]Estimated mean body weight; data from Table 8-1 of EPA (2008).
[d]Nitrate dose = 44 mg nitrate/L (NOAEL) x water intake (L/day) / body weight (kg).
[e]Calculated TWA for birth–<1 month and 1–<3 months (e.g., TWA water intake for birth–<3 months = (0.470 L/day x 1 month) + (0.552 L/day x 2 months)/3 months = 0.525 L/day.

EPA = Environmental Protection Agency; NOAEL = no-observed-adverse-effect level; TWA = time-weighted average

If an inhalation study in animals, list conversion factors used in determining human equivalent dose: Not applicable.

Was a conversion used from intermittent to continuous exposure? Not applicable.

Other additional studies or pertinent information that lend support to this MRL: Methemoglobinemia is a condition in which increased methemoglobin as a percentage of total hemoglobin results in the expression of clinical signs that increase in severity with increasing percent methemoglobin (ATSDR 2013a; Bloom et al. 2013; Denshaw-Burke et al. 2013; Haymond et al. 2005). In normal healthy individuals, methemoglobin levels are <1% of total hemoglobin. Discoloration (e.g., pale, gray blue) of the skin is often observed at methemoglobin levels in the range of 3–15%; most patients tolerate methemoglobin levels <10%. Tachycardia, weakness, and other signs of tissue hypoxia may be observed at 10–20% methemoglobin levels. Effects on the central nervous system (e.g., headache, dizziness, fatigue) and dyspnea and nausea appear at >20% methemoglobin; the severity of symptoms increases with increasing methemoglobin level. High risk of mortality occurs at levels >70% methemoglobin.

Proposed explanations for increased susceptibility of infants to methemoglobinemia following ingestion of nitrate include: (1) increased reduction of nitrate to nitrite in the newborn, (2) increased tendency for

nitrite-induced methemoglobin formation by fetal hemoglobin compared to adult hemoglobin, (3) lower levels of NADH-dependent methemoglobin reductase (the major enzyme responsible for reduction of methemoglobin to normal hemoglobin; also termed NADH-diaphorase, a soluble form of cytochrome-b5 reductase) in the newborn compared to older infants and adults, and (4) incompletely developed hepatic microsomal enzyme system in the infant and consequent lower rate of hepatic reduction of circulating nitrite compared to that of older children and adults. A portion of ingested nitrate is reduced to nitrite by commensal bacteria in the mouth; however, the acid environment of the normal stomach does not support the growth of such bacteria and most of the nitrate that reaches the stomach passes to the small intestine from which it is nearly completely absorbed into the blood. Although Kanady et al. (2012) reported little or no bacterial conversion of nitrate to nitrite in the saliva of a group of 10 infants during the first 2 postnatal months (considered mainly due to lower numbers of major nitrate-reducing oral bacteria than adults), a higher pH in the stomach of the newborn may favor growth of nitrate-reducing bacteria, resulting in increased reduction of nitrate to nitrite and increased plasma methemoglobin. Most hemoglobin in the newborn is in the form of fetal hemoglobin, which appears to be more readily oxidized to methemoglobin than adult hemoglobin; fetal hemoglobin is replaced by adult hemoglobin during early postnatal life. Levels of NADH-dependent methemoglobin reductase in the newborn increase approximately 2-fold during the first 4 months of postnatal life to reach adult levels. During the period of relatively lower methemoglobin reductase levels, methemoglobin would not be expected to be as readily reduced, resulting in increased susceptibility to methemoglobinemia. In apparent contrast, Ibrahim et al. (2012) reported that blood nitrite levels in newborns approximately 1–2 days of age were 35–55% lower than that of adults. However, one study that evaluated reduction rates of methemoglobin in human adult blood and cord blood from term newborns estimated methemoglobin half-lives of 162 and 210 minutes, respectively, indicating that methemoglobin reduction occurs more slowly in newborns than adults (Power et al. 2007). Although specific mechanisms have not been elucidated, the increased susceptibility to nitrite-induced methemoglobinemia in infants is well-documented.

Bosch et al. (1950) evaluated 139 reported cases of cyanosis among infants in Minnesota (90% were <2 months of age; range 8 days to 5 months). Samples from 129 wells that served as water sources to the cases revealed nitrate-nitrogen concentrations >100 mg/L (>440 mg nitrate/L) in 49 wells, 50–100 mg/L (220–440 mg nitrate/L) in 53 wells, 21–50 mg/L (92–220 mg nitrate/L) in 25 wells, and 10–20 mg/L (44–88 mg nitrate/L) in the other 2 wells. A major limitation of this study was the detection of coliform organisms in 45 of 51 well water samples tested for bacterial contamination.

A nested case-control study included 26 cases of infants diagnosed with methemoglobinemia at ≤2 months of age and 45 age-matched controls (Zeman et al. 2002). Nitrate exposure levels were categorized as low (<0.5 ppm), medium (1–10 ppm), or high (>10 ppm) according to estimated nitrate levels reconstructed from parental responses to dietary questionnaires and environmental sampling. Numbers of methemoglobinemia cases in the low, medium, and high exposure categories were 0/26, 4/26, and 22/26, respectively, and estimated dietary nitrate intake ranged from 2.83 to 451.20 mg/kg/day (mean 103.6 mg nitrate/kg/day). Diarrheal disease was reported for 14/26 methemoglobinemia cases. Numbers of controls in the low, medium, and high exposure categories were 21/45, 11/45, and 13/45, respectively, and estimated dietary nitrate intake ranged from 0 to 182 mg/kg/day (mean 11.2 mg nitrate/kg/day) for the controls; diarrheal disease was reported for 13/45 controls. Univariate and multifactorial analysis of risk factors for methemoglobinemia indicated that methemoglobinemia was most strongly associated with dietary exposure to nitrate/nitrite ($p=0.0318$), but also significantly associated with diarrheal disease ($p=0.0376$). Controls in the high exposure category were less likely than high exposure methemoglobinemia cases to have experienced severe diarrhea and were more likely to have been breastfed for >2 weeks. Major limitations to the study include the collection of information contributing to the exposure estimates several years following the occurrences of methemoglobinemia and reliance on parental recollection of infant nutritional intake.

Results from other studies suggest an association between nitrate in drinking water sources and elevated methemoglobin among infants. Average methemoglobin levels of 1.0, 1.3, and 2.9% during the first postnatal trimester (0–3 months of age) were reported among groups healthy infants with water sources that were nitrate-free or contained 50–100 mg nitrate/L or >100 mg nitrate/L, respectively (Simon et al. 1964). At the end of the second trimester (6 months), methemoglobin averaged 0.7–0.8% for each group. Super et al. (1981) reported mean methemoglobin levels of 1.54% among infants ingesting ≤2.93 mg nitrate/kg/day and 3.03% among infants ingesting >2.93 mg nitrate/kg/day.

Limited data are available regarding administration of controlled amounts of nitrate and methemoglobin levels. Cornblath and Hartmann (1948) administered sodium nitrate in the formula fed to four infants (ages 11 days to 11 months) for 2–18 days at a concentration resulting in a dose of 50 mg nitrate/kg/day. The highest observed level of methemoglobin was 5.3% of total hemoglobin; there was no evidence of cyanosis. Among four other infants (ages 2 days to 6 months) similarly treated at 100 mg nitrate/kg/day for 6–9 days, the only reported effect was that of 7.5% methemoglobin in a 10-day-old infant following 8 days of treatment in the absence of clinical cyanosis. Gruener and Toeplitz (1975) fed 104 infants (1 week to 10 months of age) for 1 day with formula prepared using water containing 15 mg nitrate/L (~0.8–1.5 mg nitrate/kg, based on age-specific values for water consumption [Kahn and Stralka 2009] and body weight [EPA 2008]), increased to 108 mg nitrate/L for the next 3 days (~5.5–10.6 mg nitrate/kg/day, based on age-specific values for water consumption [Kahn and Stralka 2009] and body weight [EPA 2008], and returned to 15 mg nitrate/L for 1 additional day. Mean methemoglobin levels were 0.89% after the first day of feeding, 1.3, 0.91, and 0.93% after days 2, 3, and 4, and dropped to 0.8% on the fifth day. Among three of these infants (ages not specified), methemoglobin levels reached 6.9, 13.9, and 15.9% during the high-dose days. Limitations of this study include the use of a wide range of ages and the fact that only 57 of the 104 infants supplied blood samples on all 5 treatment days.

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APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: Nitrite
CAS Numbers: 14797-65-0
Date: July 2017
Profile Status: Final
Route: [ ] Inhalation [x] Oral
Duration: [x] Acute [x] Intermediate [x] Chronic
Graph Key: 4 (Acute), 16 (Intermediate), 54 (Chronic)
Species: Human

Minimal Risk Level: 0.1 [x] mg/kg/day [ ] ppm

Reference: Walton G. 1951. Survey of literature relating to infant methemoglobinemia due to nitrate-contaminated water. Am J Public Health 41:986-996.

Experimental design: Walton (1951) reviewed available literature and found 278 reported cases of infant methemoglobinemia in a total of 14 U.S. states from which information was available. Cases were grouped by state according to ranges of nitrate levels in drinking water sources.

Effect noted in study and corresponding doses: Among methemoglobinemia cases for which nitrate levels in water sources used to prepare infant formula were available, 173 cases were associated with >50 mg nitrate-nitrogen/L (220 mg nitrate/L), 36 cases with 21–50 mg nitrate-nitrogen/L (92–220 mg nitrate/L), and 5 cases with 11–20 mg nitrate-nitrogen (48–88 mg nitrate/L). None of the methemoglobinemia cases were associated with drinking water sources measuring <10 mg nitrate-nitrogen/L (<44 mg nitrate/L). Limitations of the contributing studies include lack of information regarding the actual ages of the infants, total nitrate doses, and other water source contaminants (e.g., bacterial levels).

Following ingestion of relatively large amounts of nitrate by healthy normal individuals, blood methemoglobin levels increase rapidly, followed by a return to normal within several hours following intake. Repeated ingestion of nitrate or nitrite for intermediate- or chronic-duration time periods would be expected to result in changes in methemoglobin levels similar to those elicited from a single exposure. Therefore, the acute-, intermediate-, and chronic-duration oral MRL values are equivalent.

Dose and end point used for MRL derivation: 0.2 mg nitrite/kg/day. The ingestion of nitrate results in the formation of nitrite, which is the moiety responsible for methemoglobinemia. On average, approximately 25% of an ingested dose of nitrate enters the saliva of an adult where a portion (ca. 20% g/g) is reduced by commensal bacteria to nitrite (i.e., approximately 5% g/g of ingested nitrate is reduced to nitrite in the saliva of an adult (Spiegelhalder et al. 1976); most salivary nitrite is absorbed into the blood in the small intestine. Therefore, the ingestion of 0.2 mg nitrite/kg/day by an adult would be expected to result in a nitrite blood level similar to that achieved following ingestion of 4 mg nitrate/kg/day, based on essentially 100% absorption of the ingested dose of nitrite (i.e., 0.2 mg nitrite/kg/day is 5% of an oral dose of nitrate at the oral MRL of 4 mg nitrate/kg/day).

[x] NOAEL [ ] LOAEL

Uncertainty Factors used in MRL derivation:

[ ] 10 for use of a LOAEL
[ ] 10 for extrapolation from animals to humans

[x] 1 for human variability

A total uncertainty factor of 1 is justified because the point of departure is a NOAEL for nitrate-induced effects on methemoglobin in a particularly sensitive human subpopulation (i.e., <3-month-old infants, which in many cases may have been at increased risk of methemoglobinemia due to microbial contamination and associated gastrointestinal infection).

Modifying factor used in MRL derivation:

[x] 2 because young infants exhibit increased susceptibility to methemoglobinemia following nitrate ingestion; the modifying factor assumes that the effective methemoglobin level from a given intake of nitrate by an infant is up to twice that of an adult; however, quantitative data regarding conversion of nitrate to nitrite in the infant are lacking.

Was a conversion factor used from ppm in food or water to a mg/body weight dose? Nitrate may be expressed in terms of ionic concentration (i.e., mg nitrate/L), or elemental concentration (i.e., mg nitrate-nitrogen/L or mg nitrogen as nitrate/L). A concentration of nitrate expressed in elemental concentration (mg nitrogen per liter from nitrate source) can be converted to its ionic concentration (mg NO_3^-) according to the following relationship: 1 mg nitrate-nitrogen = 4.4 mg nitrate (i.e., the proportion of N in NO_3^- is 14 [atomic mass of N] ÷ 62 [molecular mass of NO_3^-] = 0.226).

A concentration of 44 mg nitrate/L (10 mg nitrate-nitrogen/L) in drinking water used to prepare infant formula represents a NOAEC for infants <3 months of age. Table A-1 presents estimated nitrate doses to infants (birth–<3 months of age) calculated using estimated mean values for drinking water ingestion rates (Kahn and Stralka 2009) and body weight (EPA 2008) and assuming a drinking water level of 44 mg nitrate/L as a concentration not expected to cause methemoglobinemia; the calculated doses of 4.31–4.34 mg nitrate/kg/day represent NOAELs for the age ranges. The TWA-based calculated dose of 4.33 mg nitrate/kg/day for the age range of birth–<3 months is selected as the point of departure for deriving acute-, intermediate-, and chronic-duration oral MRLs for nitrite.

If an inhalation study in animals, list conversion factors used in determining human equivalent dose: Not applicable.

Was a conversion used from intermittent to continuous exposure? Not applicable.

Other additional studies or pertinent information that lend support to this MRL: Methemoglobinemia is a condition in which increased methemoglobin as a percentage of total hemoglobin results in the expression of clinical signs that increase in severity with increasing percent methemoglobin (ATSDR 2013a; Bloom et al. 2013; Denshaw-Burke et al. 2013; Haymond et al. 2005). In normal healthy individuals, methemoglobin levels are <1% of total hemoglobin. Discoloration (e.g., pale, gray blue) of the skin is often observed at methemoglobin levels in the range of 3–15%; most patients tolerate methemoglobin levels <10%. Tachycardia, weakness, and other signs of tissue hypoxia may be observed at 10–20% methemoglobin levels. Effects on the central nervous system (e.g., headache, dizziness, fatigue) and dyspnea and nausea appear at >20% methemoglobin; the severity of symptoms increases with increasing methemoglobin level. High risk of mortality occurs at levels >70% methemoglobin.

Proposed explanations for increased susceptibility of infants to methemoglobinemia following ingestion of nitrate include: (1) increased reduction of nitrate to nitrite in the newborn, (2) increased tendency for nitrite-induced methemoglobin formation by fetal hemoglobin compared to adult hemoglobin, (3) lower levels of NADH-dependent methemoglobin reductase (the major enzyme responsible for reduction of methemoglobin to normal hemoglobin; also termed NADH-diaphorase, a soluble form of cytochrome-b5

reductase) in the newborn compared to older infants and adults, and (4) incompletely developed hepatic microsomal enzyme system in the infant and consequent lower rate of hepatic reduction of circulating nitrite compared to that of older children and adults. A portion of ingested nitrate is reduced to nitrite by commensal bacteria in the mouth; however, the acid environment of the normal stomach does not support the growth of such bacteria and most of the nitrate that reaches the stomach passes to the small intestine from which it is nearly completely absorbed into the blood. Although Kanady et al. (2012) reported little or no bacterial conversion of nitrate to nitrite in the saliva of a group of 10 infants during the first 2 postnatal months (considered mainly due to lower numbers of major nitrate-reducing oral bacteria than adults), a higher pH in the stomach of the newborn may favor growth of nitrate-reducing bacteria, resulting in increased reduction of nitrate to nitrite and increased plasma methemoglobin. Most hemoglobin in the newborn is in the form of fetal hemoglobin, which appears to be more readily oxidized to methemoglobin than adult hemoglobin; fetal hemoglobin is replaced by adult hemoglobin during early postnatal life. Levels of NADH-dependent methemoglobin reductase in the newborn increase approximately 2-fold during the first 4 months of postnatal life to reach adult levels. During the period of relatively lower methemoglobin reductase levels, methemoglobin would not be expected to be as readily reduced, resulting in increased susceptibility to methemoglobinemia. In apparent contrast, Ibrahim et al. (2012) reported that blood nitrite levels in newborns approximately 1–2 days of age were 35–55% lower than that of adults. However, one study that evaluated reduction rates of methemoglobin in human adult blood and cord blood from term newborns estimated methemoglobin half-lives of 162 and 210 minutes, respectively, indicating that methemoglobin reduction occurs more slowly in newborns than adults (Power et al. 2007). Although specific mechanisms have not been elucidated, the increased susceptibility to nitrite-induced methemoglobinemia in infants is well-documented.

Bosch et al. (1950) evaluated 139 reported cases of cyanosis among infants in Minnesota (90% were <2 months of age; range 8 days to 5 months). Samples from 129 wells that served as water sources to the cases revealed nitrate-nitrogen concentrations >100 mg/L (>440 mg nitrate/L) in 49 wells, 50–100 mg/L (220–440 mg nitrate/L) in 53 wells, 21–50 mg/L (92–220 mg nitrate/L) in 25 wells, and 10–20 mg/L (44–88 mg nitrate/L) in the other 2 wells. A major limitation of this study was the detection of coliform organisms in 45 of 51 well water samples tested for bacterial contamination.

A nested case-control study included 26 cases of infants diagnosed with methemoglobinemia at ≤2 months of age and 45 age-matched controls (Zeman et al. 2002). Nitrate exposure levels were categorized as low (<0.5 ppm), medium (1–10 ppm), or high (>10 ppm) according to estimated nitrate levels reconstructed from parental responses to dietary questionnaires and environmental sampling. Numbers of methemoglobinemia cases in the low, medium, and high exposure categories were 0/26, 4/26, and 22/26, respectively, and estimated dietary nitrate intake ranged from 2.83 to 451.20 mg/kg/day (mean 103.6 mg nitrate/kg/day). Diarrheal disease was reported for 14/26 methemoglobinemia cases. Numbers of controls in the low, medium, and high exposure categories were 21/45, 11/45, and 13/45, respectively, and estimated dietary nitrate intake ranged from 0 to 182 mg/kg/day (mean 11.2 mg nitrate/kg/day) for the controls; diarrheal disease was reported for 13/45 controls. Univariate and multifactorial analysis of risk factors for methemoglobinemia indicated that methemoglobinemia was most strongly associated with dietary exposure to nitrate/nitrite ($p=0.0318$), but also significantly associated with diarrheal disease ($p=0.0376$). Controls in the high exposure category were less likely than high exposure methemoglobinemia cases to have experienced severe diarrhea and were more likely to have been breastfed for >2 weeks. Major limitations to the study include the collection of information contributing to the exposure estimates several years following the occurrences of methemoglobinemia and reliance on parental recollection of infant nutritional intake.

Results from other studies suggest an association between nitrate in drinking water sources and elevated methemoglobin among infants. Average methemoglobin levels of 1.0, 1.3, and 2.9% during the first postnatal trimester (0–3 months of age) were reported among groups healthy infants with water sources

that were nitrate-free or contained 50–100 mg nitrate/L or >100 mg nitrate/L, respectively (Simon et al. 1964). At the end of the second trimester (6 months), methemoglobin averaged 0.7–0.8% for each group. Super et al. (1981) reported mean methemoglobin levels of 1.54% among infants ingesting ≤2.93mg nitrate/kg/day and 3.03% among infants ingesting >2.93 mg nitrate/kg/day.

Limited data are available regarding administration of controlled amounts of nitrate and methemoglobin levels. Cornblath and Hartmann (1948) administered sodium nitrate in the formula fed to four infants (ages 11 days to 11 months) for 2–18 days at a concentration resulting in a dose of 50 mg nitrate/kg/day. The highest observed level of methemoglobin was 5.3% of total hemoglobin; there was no evidence of cyanosis. Among four other infants (ages 2 days to 6 months) similarly treated at 100 mg nitrate/kg/day for 6–9 days, the only reported effect was that of 7.5% methemoglobin in a 10-day-old infant following 8 days of treatment in the absence of clinical cyanosis. Gruener and Toeplitz (1975) fed 104 infants (1 week to 10 months of age) for 1 day with formula prepared using water containing 15 mg nitrate/L (~0.8–1.5 mg nitrate/kg, based on age-specific values for water consumption [Kahn and Stralka 2009] and body weight [EPA 2008]), increased to 108 mg nitrate/L for the next 3 days (~5.5–10.6 mg nitrate/kg/day, based on age-specific values for water consumption [Kahn and Stralka 2009] and body weight [EPA 2008], and returned to 15 mg nitrate/L for 1 additional day. Mean methemoglobin levels were 0.89% after the first day of feeding, 1.3, 0.91, and 0.93% after days 2, 3, and 4, and dropped to 0.8% on the fifth day. Among three of these infants (ages not specified), methemoglobin levels reached 6.9, 13.9, and 15.9% during the high-dose days. Limitations of this study include the use of a wide range of ages and the fact that only 57 of the 104 infants supplied blood samples on all 5 treatment days.

In a study designed to evaluate the oral bioavailability of sodium nitrite in healthy volunteers (seven females and two males; mean age 22.9 years), ingestion of ~2.2–2.7 mg sodium nitrite/kg (1.5–1.8 mg nitrite/kg) resulted in maximum methemoglobin concentrations ranging from 3.4 to 4.5% of total hemoglobin at approximately 0.70 hours following ingestion (Kortboyer et al. 1997b). At a higher intake (~4.4–5.4 mg sodium nitrite/kg, or 2.9–3.6 mg nitrite/kg), the maximum methemoglobin concentrations ranged from 7.7 to 10.9% of total hemoglobin at approximately 1.14 hours following ingestion.

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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 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 hazardous substance 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 include 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 adverse effects were seen in the organ system studied. Key number 18 reports a NOAEL of 3 ppm for the respiratory system, 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 an adverse 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/m^3 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.

(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

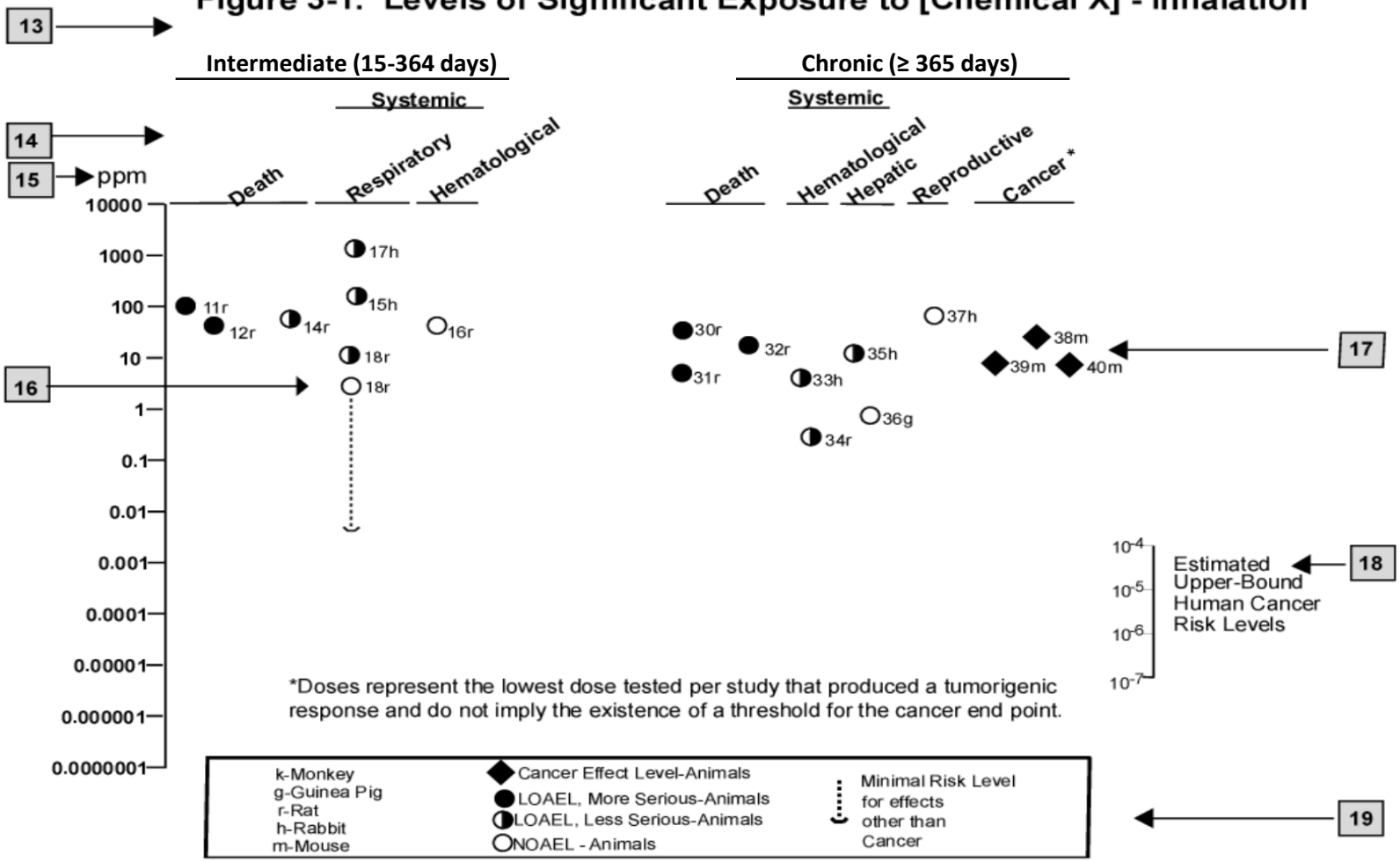
1 → **Table 3-1. Levels of Significant Exposure to [Chemical x] – Inhalation**

Key to figure[a]	Species	Exposure frequency/ duration	System	NOAEL (ppm)	LOAEL (effect) 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 $5x10^{-3}$ 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



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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/C	benchmark dose or benchmark concentration
BMD_X	dose that produces a X% change in response rate of an adverse effect
$BMDL_X$	95% lower confidence limit on the BMD_X
BMDS	Benchmark Dose Software
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
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

DOT/UN/ NA/IMDG	Department of Transportation/United Nations/ North America/Intergovernmental 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
Kd	adsorption ratio
kg	kilogram
kkg	kilokilogram; 1 kilokilogram is equivalent to 1,000 kilograms and 1 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	luteinizing hormone
LOAEL	lowest-observed-adverse-effect level
LSE	Levels of Significant Exposure
LT_{50}	lethal time, 50% kill
m	meter
MA	*trans,trans*-muconic acid
MAL	maximum allowable level
mCi	millicurie
MCL	maximum contaminant level

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
mt	metric ton
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
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
PEL-C	permissible exposure limit-ceiling value
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
REL-C	recommended exposure level-ceiling value
RfC	reference concentration (inhalation)
RfD	reference dose (oral)
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 (same as aspartate aminotransferase or AST)
SGPT	serum glutamic pyruvic transaminase (same as alanine aminotransferase or ALT)
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
TLV-C	threshold limit value-ceiling 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
>	greater than
≥	greater than or equal to
=	equal to
<	less than
≤	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