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).

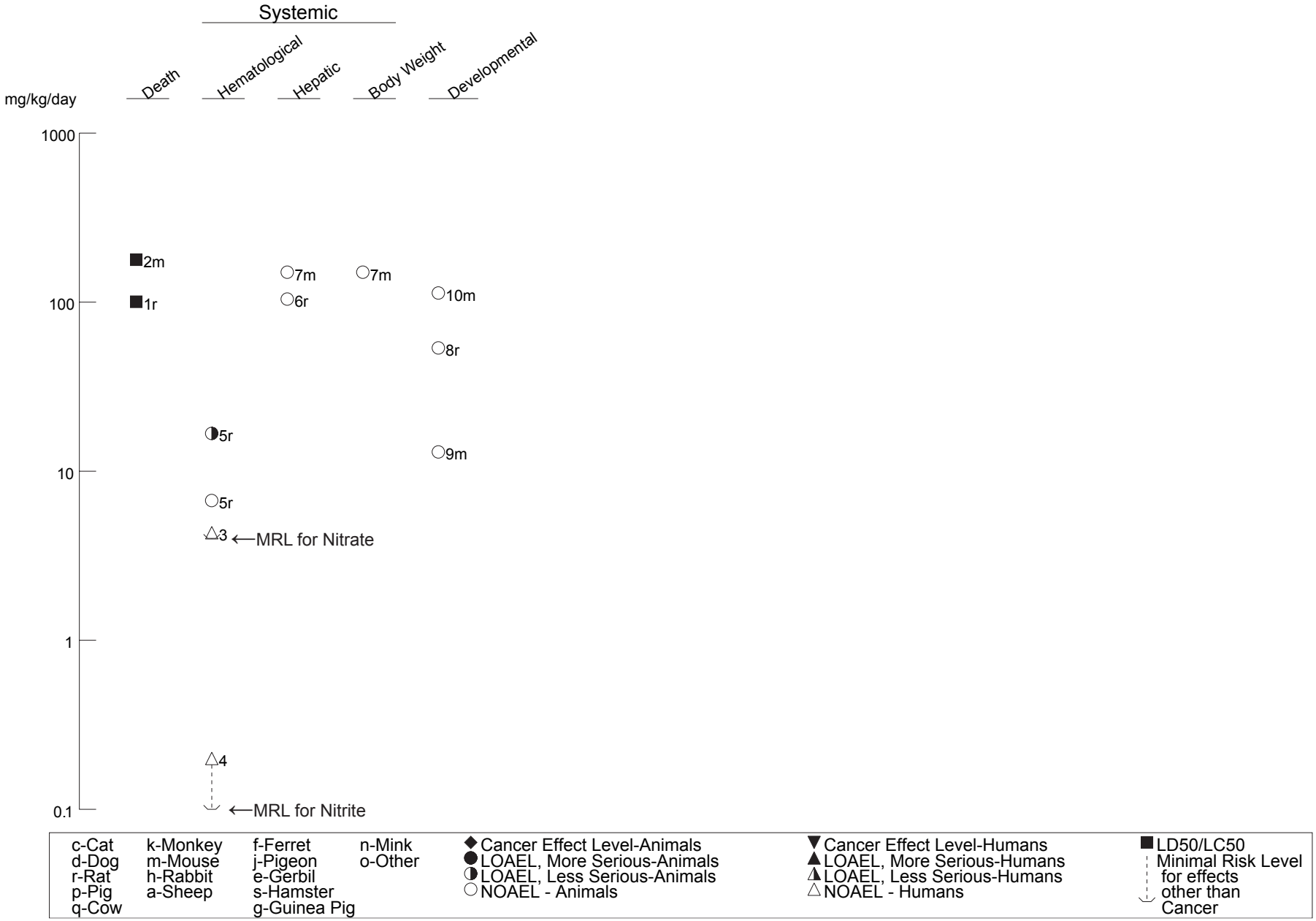
c NOAEL of 0.2 mg/kg/day for nitrite was used to derive acute-, intermediate, and chronic-duration oral minimal risk levels (MRLs) of 0.1 mg/kg/day for nitrite, as described in detail in Chapter 2 and Appendix A. The NOAEL represents the dose of nitrite that would be expected to enter the blood following 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

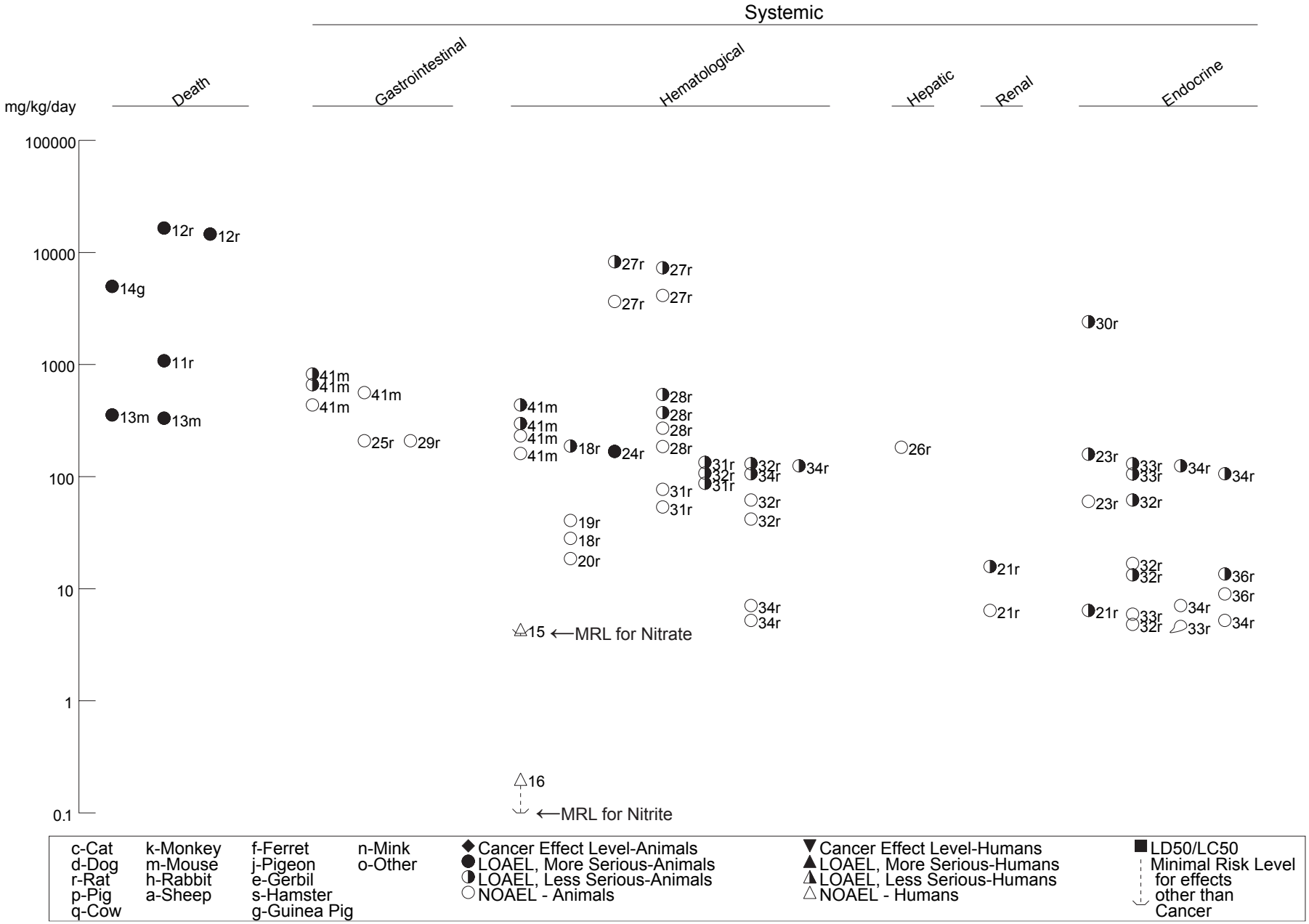
Acute (≤14 days)



3. HEALTH EFFECTS

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

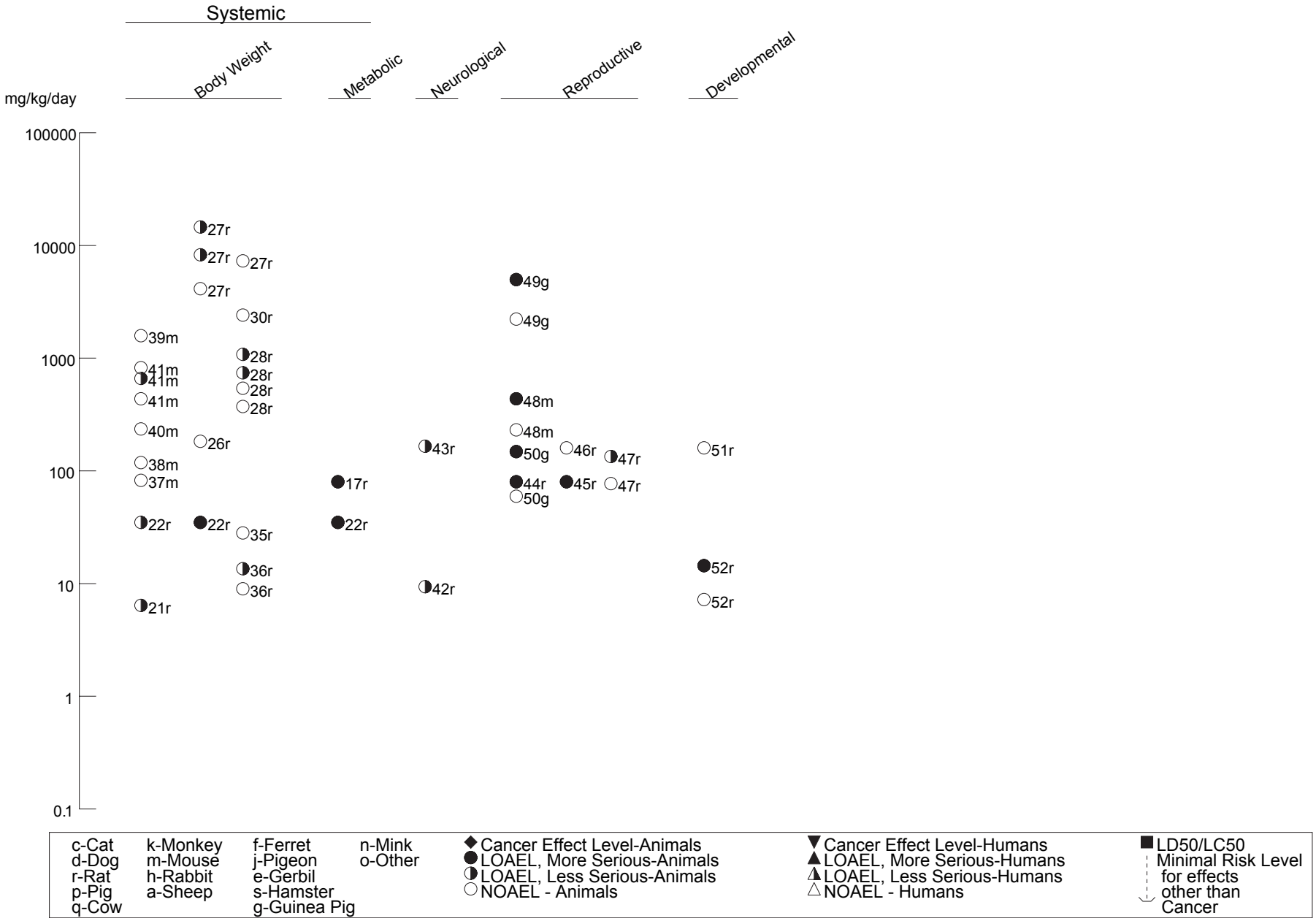
Intermediate (15-364 days)



3. HEALTH EFFECTS

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

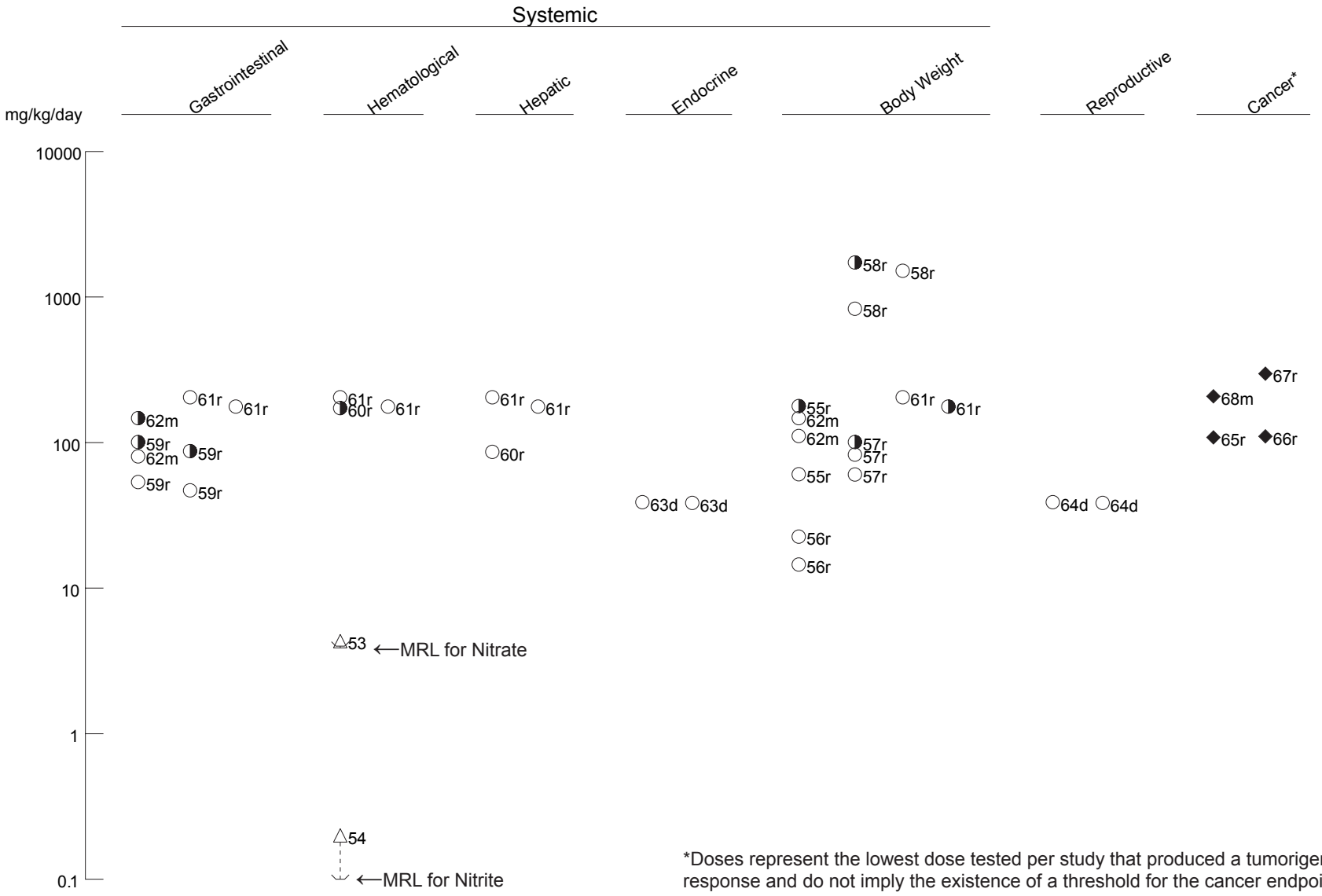
Intermediate (15-364 days)



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.

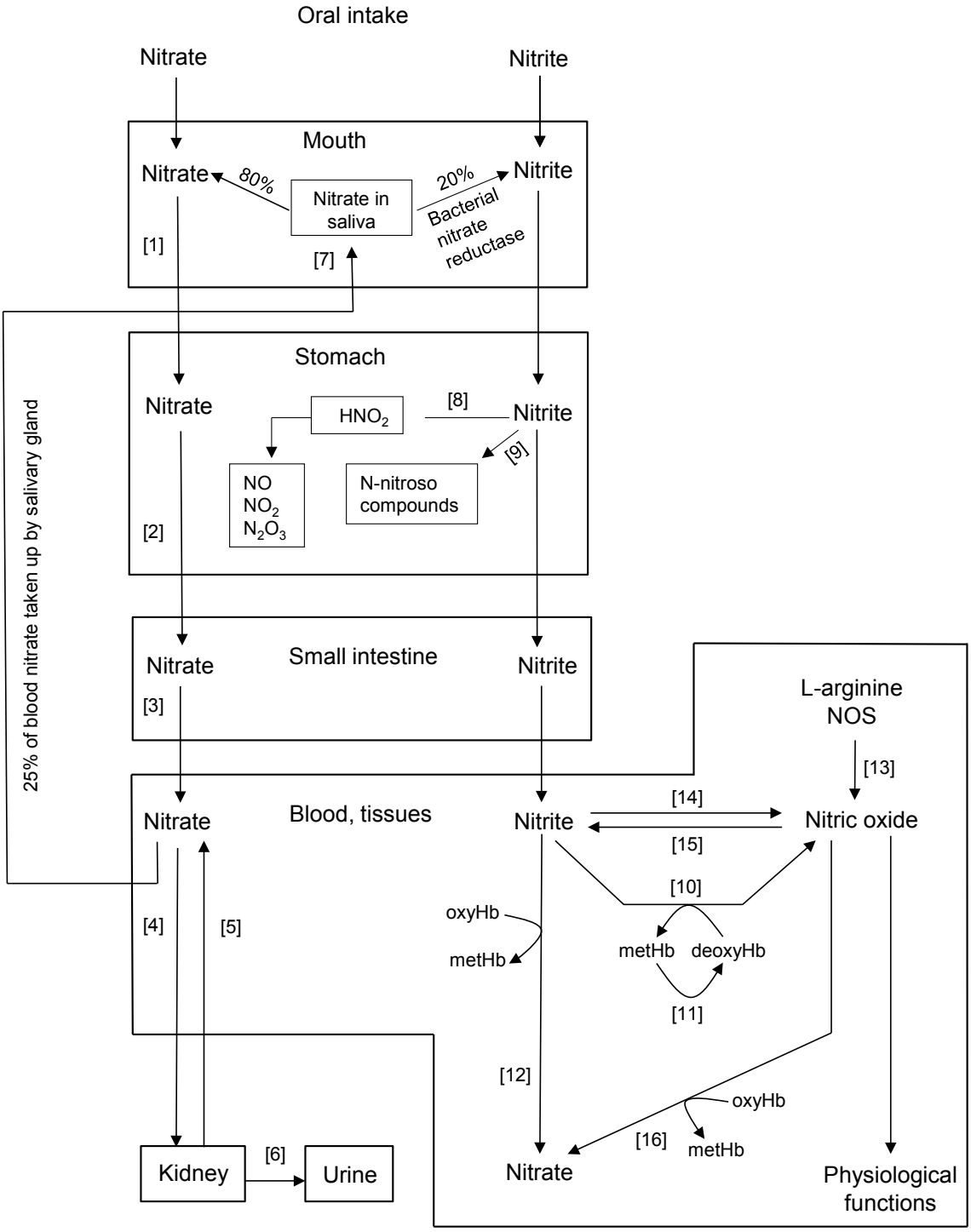
+ = positive results; (+) = weakly positive; – = negative results; DNA = deoxyribonucleic acid; NT = not tested

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.

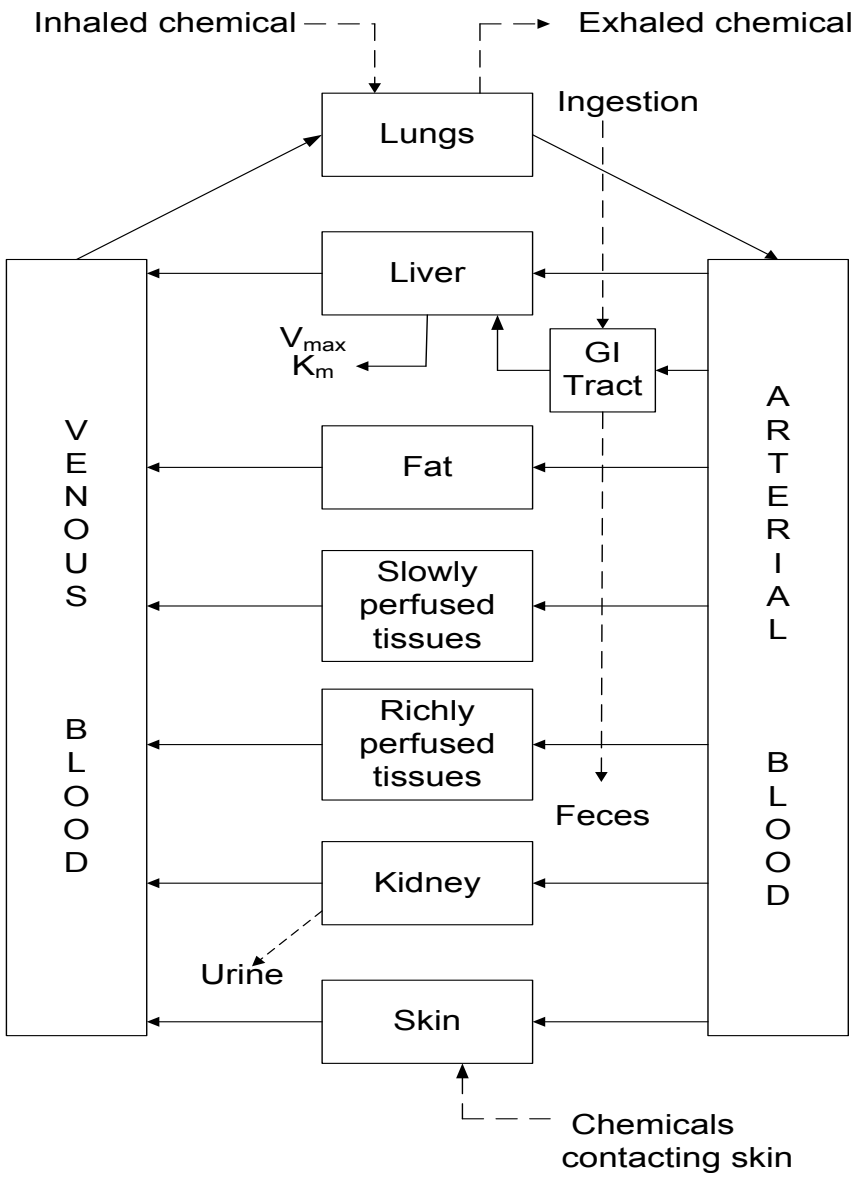
The structure and mathematical expressions used in PBPK models significantly simplify the true complexities of biological systems. However, if the uptake and disposition of the chemical substance(s) are adequately described, this simplification is desirable because data are often unavailable for many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

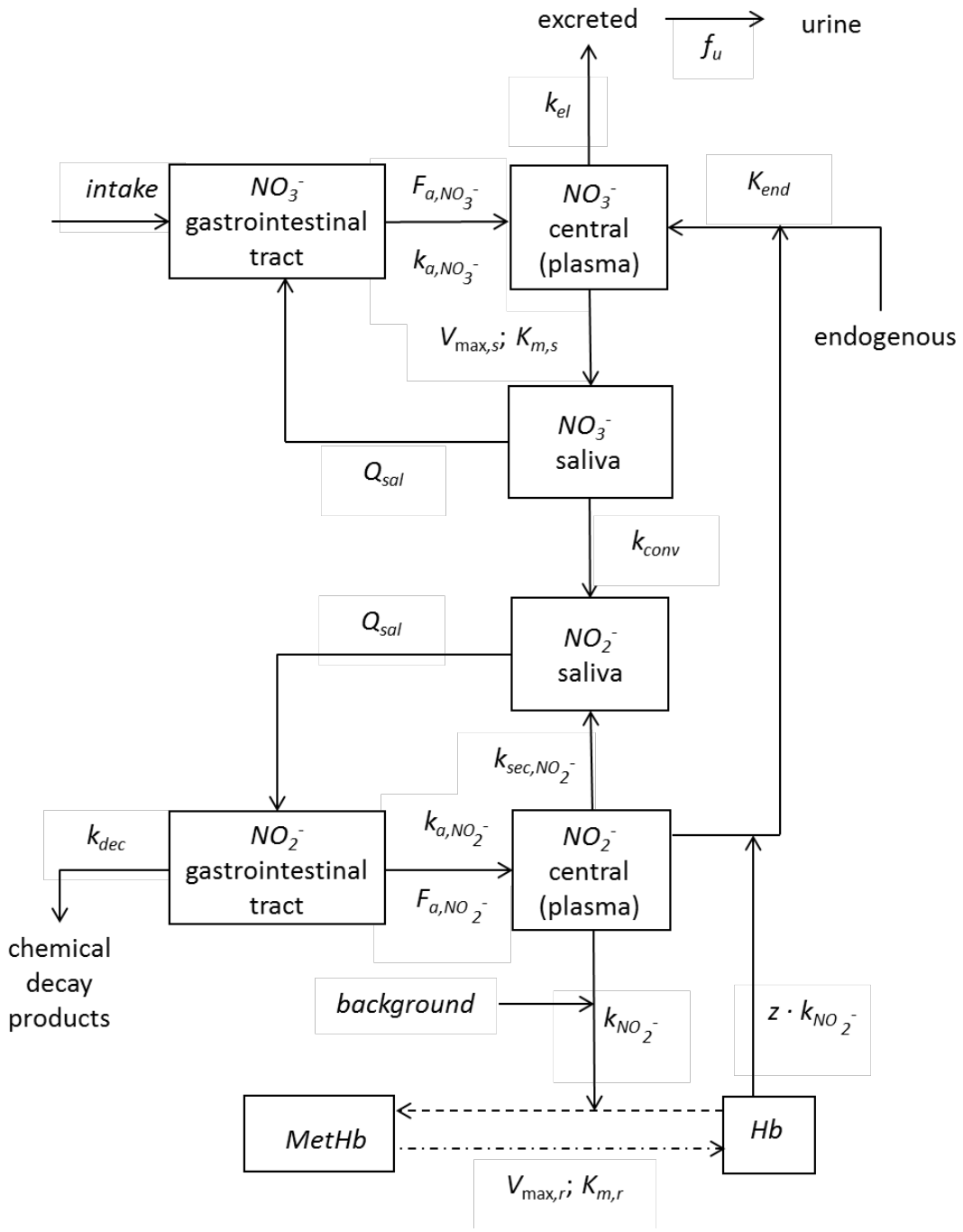
Source: adapted from Krishnan and Andersen 1994

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

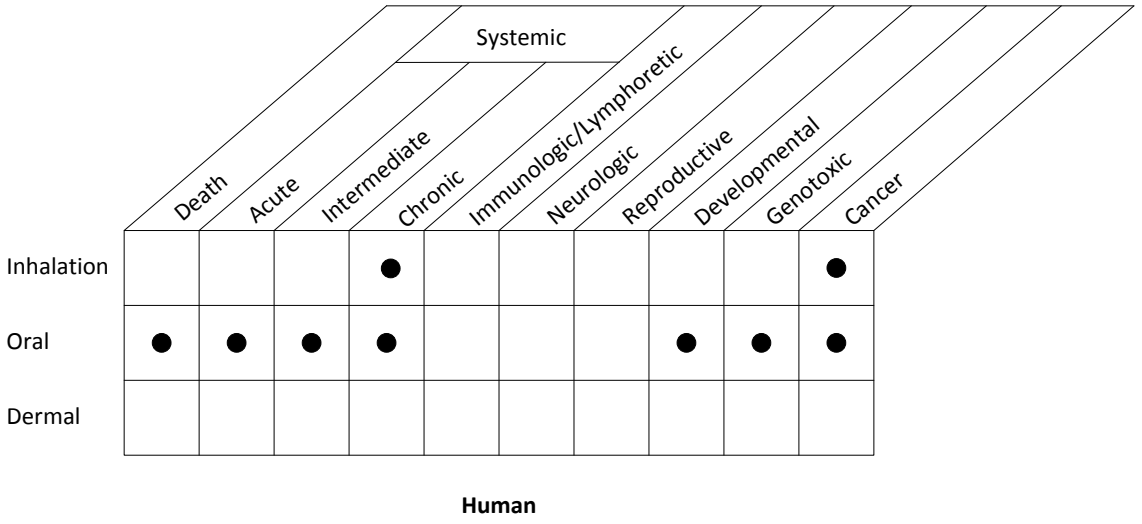
Section 104(I)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of nitrate and nitrite is available. Where adequate information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

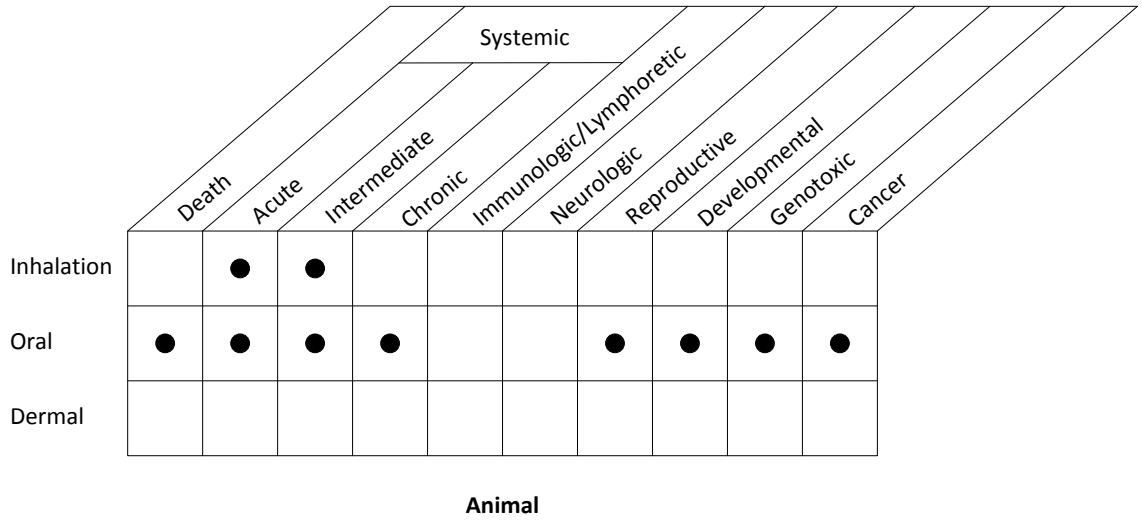
3.12.1 Existing Information on Health Effects of Nitrate and Nitrite

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



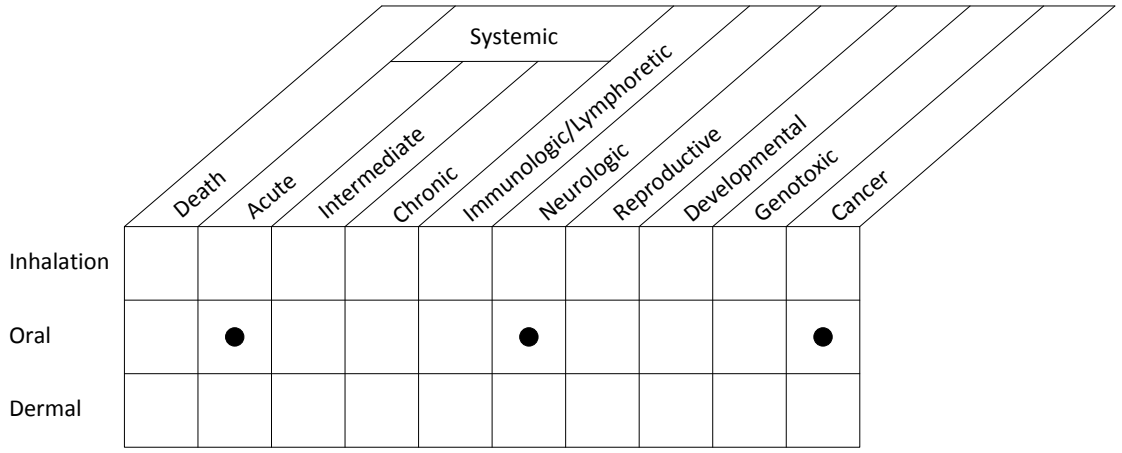
Human



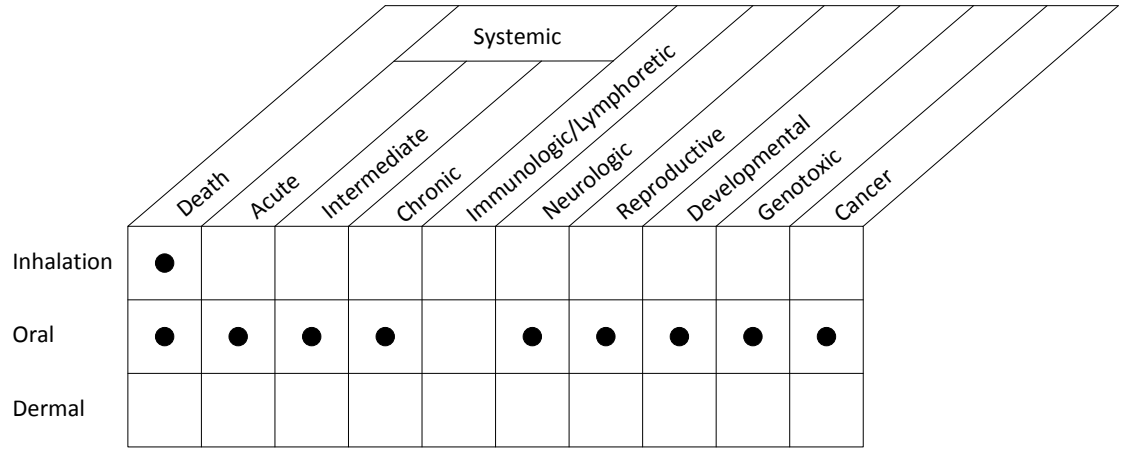
Animal

● 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.