2. RELEVANCE TO PUBLIC HEALTH

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

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

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

2.2 SUMMARY OF HEALTH EFFECTS

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

2. RELEVANCE TO PUBLIC HEALTH

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

2. RELEVANCE TO PUBLIC HEALTH

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

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

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

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

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

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

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

2. RELEVANCE TO PUBLIC HEALTH

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

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

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

2.3 MINIMAL RISK LEVELS (MRLs)

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

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

Inhalation MRLs

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

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

Oral MRLs

Nitrate

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Nitrite

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

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

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

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

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

2. RELEVANCE TO PUBLIC HEALTH

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