Public Health Goals

DRAFT

For Review Only

Nitrate/Nitrite in Drinking Water

December 2016

Pesticide and Environmental Toxicology Branch
Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

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Public Health Goals for Nitrate and Nitrite in Drinking Water

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PREFACE

This Public Health Goal (PHG) technical support document provides information on health effects from contaminants in California drinking water. PHGs are developed for chemical contaminants based on the best available data in the scientific literature and using the most current principles, practices, and methods used by public health professionals. These documents and the analyses contained therein provide estimates of the levels of contaminants in drinking water that would pose no significant health risk to individuals consuming the water on a daily basis over a lifetime.

Under the California Safe Drinking Water Act of 1996 (Health and Safety Code, Section 116365), the Office of Environmental Health Hazard Assessment (OEHHA) develops PHGs for drinking water contaminants in California based exclusively on public health considerations. OEHHA periodically reviews PHGs and revises them as necessary based on the availability of new scientific data. This document presents an update for nitrate and nitrite, for which PHGs have been previously developed.

PHGs published by OEHHA are for use by the State Water Resources Control Board (SWRCB) in establishing primary drinking water standards (California Maximum Contaminant Levels, or CA MCLs). Whereas PHGs are to be based solely on scientific and public health considerations without regard to economic cost considerations, drinking water standards adopted by SWRCB are to consider economic factors and technological feasibility. Each standard adopted shall be set at a level that is as close as feasible to the corresponding PHG, placing emphasis on the protection of public health. PHGs established by OEHHA are not regulatory in nature and represent only non-mandatory goals. By federal law, CA MCLs established by SWRCB must be at least as stringent as the federal MCL if one exists.

In July 2014, responsibility for the state's drinking water regulatory program was transferred to SWRCB from the California Department of Public Health. References in this document to drinking water monitoring and regulation may cite either or both entities as appropriate.

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SUMMARY

This document presents a proposed update of the public health goals (PHGs) for nitrate and nitrite published in 1997. The Office of Environmental Health Hazard Assessment (OEHHA) is proposing to retain the original PHG value of 45 milligrams/liter (mg/L or 45 parts-per-million (ppm) for nitrate. Nitrate can also be expressed in terms of its concentration as nitrogen. When expressed as nitrogen, 45 ppm nitrate is equivalent to 10 mg/L or 10 ppm nitrogen. The PHG of 3 mg/L for nitrite, which when expressed as nitrogen is 1 mg/L (1 ppm), remains the same. The joint nitrate/nitrite PHG of 10 mg/L (10 ppm, expressed as nitrogen), which accounts for the additive toxicity of nitrate and nitrite, is also retained. It does not replace the individual values, and the maximum contribution from nitrite should not exceed 1 mg/L nitrite-nitrogen. These PHGs protect against the occurrence of infant methemoglobinemia, a blood disorder that results in decreased oxygen distribution to tissues.

The purpose of this document is to update the PHG using current methodologies and the best available data. Many new studies on nitrate/nitrite toxicity have been published since the previous PHG, and the pertinent studies are critically evaluated in this PHG update. However, there are no new studies that support a change in the critical endpoint or critical studies used in the 1997 PHG. Therefore, the 1997 PHG values are retained in this proposed update. Although there are no proposed changes to the PHG values, this updated document examines toxicological studies published after 1997, and provides a thorough analysis of the critical endpoints of nitrate/nitrite toxicity.

INTRODUCTION

OEHHA performs health risk assessments and develops PHGs for drinking water contaminants in California. A PHG is the concentration of a contaminant in drinking water that is estimated to pose no significant health risk to individuals consuming the water on a daily basis over a lifetime. This document presents a proposed PHG update for nitrate/nitrite. This update incorporates a review of the scientific literature and the use of current risk assessment practices and methods.

Chemical Identity

Nitrate (NO_3^-) and nitrite (NO_2^-) are anions and can form salts with many cations, such as ammonium, sodium, potassium, calcium, and magnesium. The Chemical Abstracts Service (CAS) numbers for nitrate and nitrite are 14797-55-8 and 14797-68-0, respectively. Their molecular weights are 62.00 and 46.01, respectively.

Production and Uses

Nitrate and nitrite are ubiquitous in nature and are formed by the oxidation of nitrogen by microbes in soil and water and by lightning. Since 1910, the Haber-Bosch process has been used to react hydrogen and nitrogen gases at high temperature and pressure to form ammonia. Ammonia can be subsequently oxidized to nitrite and nitrate.

Nitrate is predominantly used as fertilizer, but also has uses as a food preservative, a coloring agent, a corrosion inhibitor, and for odor control in aqueous systems. It is also a component in the manufacture of a multitude of products, including explosives, pyrotechnics, matches, freezing mixtures and special cements, as well as a precursor for nitrite and nitrous oxide synthesis (IPCS, 1996; HSDB, 2016a). Nitrite has many commercial applications, including use in photography, as a corrosion inhibitor, and in the production of diazo dyes and rubber. It is also used as a food preservative and coloring agent, and can be found in cured meats (IPCS, 1996; HSDB, 2016a).

Environmental Occurrence and Human Exposure

Nitrate and nitrite are ubiquitous in the environment, since both chemicals are components of the global nitrogen cycle. The net losses in soil nitrogen, due to denitrifying bacteria reducing nitrate to nitrogen gas, and the leaching of nitrate into groundwater, are offset by net gains of nitrogen fixation by symbiotic bacteria such as *Rhizobia* and asymbiotic bacteria such as *Azobacter* and *Clostridium*. However, the equilibrium level is sub-optimal for many plants, and for that reason, nitrate and other nitrogen compounds have a long history of use in agricultural fertilizers. In this form, they also contribute to the global nitrogen balance. Furthermore, US EPA's Toxics Release Inventory (TRI) reported that approximately 4.5 million pounds of nitrate compounds were released to air, 59.6 million pounds were released to landfills, and 192 million pounds were released to surface water in the United States in 2014.[1]

Food

Human exposure to nitrate is principally from ingestion of water and food. In food, the main sources of exposure are leafy vegetables, with additional exposures coming from baked and processed cereal products and cured meat. Nitrate levels vary among different vegetable types (Table 1; Santamaria, 2006). There are many factors that contribute to nitrate intake from vegetables, including the types and quantities of vegetables consumed, and the levels of nitrate present in the vegetables (which are often related to nitrate levels in fertilizer and soil). Nitrate content in vegetables may vary considerably based on several different factors, including genetics, soil conditions, how the vegetables are stored and transported, and how the vegetables are harvested. This can complicate the analyses of epidemiological studies where populations eat similar vegetable types from different sources (Hord et al., 2009). In the United States, intake from food has been estimated to be 40-100 mg/day for nitrate, and 0.3-2.6 mg/day for nitrite (OEHHA, 1997). Based on several international studies, Pennington (1998) reports that daily intake is between 53-350 mg for nitrate, and 0-20 mg for nitrite. In areas with low nitrate in drinking water, estimates of nitrate intake can range from 60-90 mg/day, with high vegetable consumers potentially ingesting up to 200 mg/day

[1] Available online at: http://iaspub.epa.gov/triexplorer/tri_release.chemical

(IARC, 2010). The bioavailability of nitrate from spinach, lettuce, and beet root has been estimated to be approximately 100% (van Velzen et al., 2008).

Table 1. Classification of vegetables according to nitrate content (mg nitrate/kg fresh weight) (Santamaria, 2006)

	Vegetable Varieties
Very low, <200	artichoke, asparagus, broad bean, eggplant, garlic, onion, green bean, mushroom, pea, pepper, potato, summer squash, sweet potato, tomato, watermelon
Low, 200 to <500	broccoli, carrot, cauliflower, cucumber, pumpkin, chicory
Medium 500 to <1000	cabbage, dill, turnip, savoy cabbage
High, 1000 to <2500	celeriac, Chinese cabbage, endive, fennel, kohlrabi, leek, parsley
Very high, >2500	celery, cress, chervil, lettuce, red beet, spinach, rocket (rucola)

The European Food Safety Authority (EFSA, 2008) constructed different exposure scenarios to estimate daily exposure to nitrates from vegetable consumption. The "base" scenario assumed vegetable consumption (with the exception of potatoes) at the recommended level of 400 g/day, with a nitrate concentration of 392 milligrams per kilogram (mg/kg) (the median value calculated for most vegetables) applied. Other scenarios included elevated potato and leafy vegetable (spinach and lettuce) consumption. The "base" scenario estimated a nitrate intake of 157 mg/person/day, whereas exposures from other scenarios ranged from 82-457 mg/person/day. These values were calculated from vegetable consumption only, and did not consider additional sources of nitrate (a value estimated to be 44 mg/person/day).

Cured or processed meat products such as bacon, sausage, ham and luncheon meat also contain nitrate and nitrite. The World Health Organization (WHO) reports that meat products contain <2.7–945 mg of nitrate per kilogram and <0.2–6.4 mg of nitrite per kilogram, whereas dairy products contain <3–27 mg of nitrate per kilogram and <0.2–1.7 mg of nitrite per kilogram (WHO, 2011).

Hord et al. (2009) calculated a daily intake of 174 to 1,222 mg of nitrate from vegetables and fruits based on DASH (Dietary Approaches to Stop Hypertension), an eating plan that is low in fat but rich in low-fat dairy foods, fruits, and vegetables. The authors determined the nitrate and nitrite concentrations in a sample of foods and estimated the hypothetical dietary intake for a 60 kg individual on the DASH diet based on high-nitrate or low-nitrate vegetable and fruit consumption. The findings for the hypothetical high-nitrate DASH diet pattern intake would exceed the WHO's Acceptable Daily Intake for nitrate (3.7 mg/kg) by 550% for a 60-kg adult (Hord et al., 2009).

It is recommended that infants <3 months of age avoid particular vegetables that are naturally high in nitrates, such as spinach, carrots, and squash. Nitrates are present in commercial infant food, and private industry will often voluntarily screen food for nitrate content. A target concentration of <100 ppm nitrate-nitrogen in baby food is considered acceptable for infants (Greer et al., 2005).

Dietary exposure to nitrite (NO_2^-) is typically very low compared to nitrate, commonly <2 mg NO_2^-/day (Walker, 1990). Exceptionally, higher levels may result from foods rich in nitrates stored under inappropriate conditions.

Water

WHO (2011) reports that background nitrate levels in the United States rarely exceed 4-9 mg/L. However, because nitrate is associated with agricultural activities (e.g. fertilizers and animal excreta), both ground and surface water contamination can occur. Nitrate is a widespread groundwater contaminant in California, and elevated levels have been reported statewide. Monitoring data from the past three years show that a number of public water supply wells within every regional water quality control board boundary across the state reported groundwater nitrate levels that exceeded the California MCL of 45 mg/L, with maximum levels reaching 103,050 mg/L nitrate.[2]

A report prepared for the State Water Resources Control Board (SWRCB), *Addressing Nitrate in California's Drinking Water with a Focus on Tulare Lake Basin and Salinas Valley Groundwater*, noted that nitrate contamination of ground water is widespread and is expected to increase, particularly in agricultural areas, as nitrate from fertilizer use and animal excreta can take decades or more to percolate into wells and aquifers (Harter et al., 2012). The report further noted that public water systems serving 57% of the population in the study area had nitrate concentrations in their untreated water that exceeded the MCL at least once between 2006 and 2010, and projected that the affected population may rise to 80% by 2050 (Harter et al., 2012). Private wells are not monitored by the SWRCB, and may contain nitrate levels above the MCL that are unreported (SWRCB, 2011).

Nitrate levels are usually significantly higher in groundwater in regions with intensive agricultural activities, and in these regions nitrate concentrations may exceed the current WHO guideline of 50 mg/L. For the Fresno and Modesto area aquifers, Burow et al. (2008) found that increased nitrogen fertilizer use generally corresponds with increased nitrate groundwater concentrations. In addition, nitrate levels tend to be

[2]Based on monitoring data over the last three years for public water supply wells, accessed October 2016 with GeoTracker GAMA (http://geotracker.waterboards.ca.gov/gama/). The data do not indicate whether the source is raw (untreated) water or treated water; therefore, the results in the dataset may not be representative of the water delivered to customers.

much higher in the shallow part of the aquifer than in the deep part (Burow et al., 2008; Burow et al., 2010).

Although the majority of nitrate exposure comes from ingestion of vegetables, high levels in drinking water can significantly contribute to total nitrate exposure. For infants fed exclusively on formula reconstituted with tap water, exposure to nitrate may be substantial, whereas nitrate exposure during breast-feeding is considered negligible (OEHHA, 1997). L'hirondel and L'hirondel (2002) estimate that the percentage of total daily nitrate exposure from contaminated drinking water is directly related to the concentration of nitrate in drinking water, as shown in Table 2.

Table 2. Relative importance of nitrate in drinking water to total nitrate intake (L'hirondel and L'hirondel, 2002)

Drinking water nitrate concentration (mg NO_3^-/L)	Estimation of the contribution of drinking water nitrate to total nitrate intake (%)
0	0
10	20
50	55
75	65
100	71
150	79

Nitrite is not frequently detected in drinking water; it has not been detected in public water supply wells in California at levels above its MCL of 1 mg/L in the last three years. However, nitrite may be formed due to microbial reduction of nitrates in hygienically poor quality well water.

Air

Nitrates can be formed from emissions of nitric oxide (NO) from motor vehicles and power plants. Particulate nitrate has been monitored in various regions of California, including Fresno, the San Joaquin Valley, the Sacramento Valley, and areas of southern California, with average levels varying diurnally and seasonally (Blanchard and Tanenbaum, 2003; Turkiewicz et al., 2006). Examinations of $PM_{2.5}$ (particulate matter less than 2.5 micrometers in diameter) in the San Joaquin Valley in December 1999 revealed that ammonium nitrate was the predominant constituent, and its concentration depended on location from primary emission source and geographic region. During an episode of high $PM_{2.5}$ levels in December 1999, maximum particulate ammonium nitrate levels ranged from 48-72 $\mu g/m^3$ at sampling sites across the state (Turkiewicz et al., 2006). In Fresno from 2001-2005, mean particulate nitrate levels in the air were reported to be between 2.6-6.7 $\mu g/m^3$ depending on the year and the type of measurement done (Chow et al., 2008).

Soil

Nitrate is an important metabolite in the biological nitrogen cycle, and is a natural constituent of soil and vegetation. Many soils do not contain enough natural nitrogen to support plant and crop growth, so commercial fertilizers containing nitrates are often added to soil in agricultural areas. Because nitrates/nitrites do not bind tightly to soil, there is a high potential for them to migrate to groundwater or surface water. Nitrates can also be removed from soil via denitrification to nitrogen oxide or gaseous nitrogen by nitrate reducing bacteria.

BASIS FOR THE 1997 PHG

In 1997, OEHHA developed PHG values of 45 mg/L (or 45 ppm) for nitrate (equivalent to 10 mg/L or 10 ppm nitrate-nitrogen), 1 mg/L or 1 ppm for nitrite-nitrogen, and 10 mg/L or 10 ppm for combined nitrate/nitrite (expressed as nitrogen) in drinking water based on the protection of infants from the occurrence of methemoglobinemia. Methemoglobinemia is the principal acute toxic effect that has been observed in humans exposed to nitrate or nitrite (NAS, 1981; US EPA, 1990; NRC, 1995). In the body, nitrate can be converted to nitrite, which causes the oxidation of normal hemoglobin to methemoglobin (metHb) and results in a deficiency of oxygen delivery from the lungs to tissues (i.e., methemoglobinemia). The PHG was derived from a series of epidemiological studies that examined incidences of infant methemoglobinemia and nitrate-contaminated water (Bosch et al., 1950; Walton, 1951). No cases of infant methemoglobinemia were reported when water nitrate concentrations were <10 mg/L nitrate-nitrogen, thus 10 mg/L nitrate-nitrogen (45 mg/L nitrate) was the no-observed-adverse-effect level (NOAEL). Because the NOAEL was based on human data in the most sensitive population (infants), an uncertainty factor of 1 was applied. Body weight, relative source contribution, and daily water intake were not factored in the PHG calculation because the NOAEL was based on a drinking water concentration instead of a daily dose.

UPDATED TOXICOLOGICAL REVIEW

Since the publication of the nitrate/nitrite PHGs in 1997, many new studies and reports from government agencies have been published covering a broad range of topics related to nitrate/nitrite toxicity. The primary toxicological endpoints include methemoglobinemia, toxicity of the thyroid, and cancer. Additional effects seen in animals exposed to nitrate/nitrite include changes in organ weights and stomach hyperplasia. Nitrate does not appear to be genotoxic, but nitrite produced positive results in several genotoxicity assays. There is very weak evidence that nitrate or nitrite induces developmental and reproductive toxicity in humans and animals. The relevant studies and reports are reviewed and summarized under the following categories: pharmacokinetics, methemoglobinemia, thyroid toxicity, carcinogenicity, and additional toxicities.

Pharmacokinetics

Absorption

Following oral ingestion, nitrate is quickly absorbed from the duodenum and jejunum in humans (Bartholomew and Hill, 1984; Walker, 1990; Mensinga et al., 2003), with peak nitrate levels observed in blood, saliva, and urine 0.5-3 hours after administration (Ellen et al., 1982a; Wagner et al., 1983; Bartholomew and Hill, 1984; Mensinga et al., 2003). Absorption from the stomach and lower intestine is minimal (Witter and Balish, 1979). A similar rapid uptake of nitrate from the upper small intestine occurs in rats (Walker, 1990). Nitrates can also be absorbed by inhalation (e.g., from cigarette smoke and car exhaust) but this route of exposure is not expected to contribute much to the total exposure. There is no information on the dermal absorption of nitrate.

Nitrite is absorbed in the stomach, but not in the caecum or colon in rats. However, the overall absorption rate of nitrite following oral exposure is slower than that of nitrate (Witter and Balish, 1979; Walker, 1990). In humans, 90-95% absorption of orally administered sodium nitrite was observed under fasting conditions (WHO, 2003). In some cases, nitrite may be removed before absorption via reaction with gastric contents or reduction by gastrointestinal (GI) tract flora (WHO, 1995), which can reduce bioavailability.

Distribution

Once absorbed, nitrate rapidly enters the bloodstream. In most laboratory animals (with the notable exception of rats), nitrate in blood is actively transported to the salivary glands, possibly by sialin (Qin et al., 2012). Approximately 20 percent of nitrate in saliva is reduced to nitrite by bacteria in the oral cavity (Spiegelhalder et al., 1976; Mensinga et al., 2003; EFSA, 2008). When saliva is swallowed, nitrate and nitrite re-enter the stomach. Nitrite may subsequently enter systemic circulation or react with the acidic content in the stomach to form reactive chemicals, including nitric oxide and S-nitrosothiols (Lundberg, 2012). This cycle is known as the entero-salivary recirculation of nitrate/nitrite. There is no active transport of nitrate into salivary glands in rats (Cohen and Myant, 1959; Vittozzi, 1992). However, rats can secrete nitrate into the lower intestines, and nitrate can be found in gastric and lower intestinal secretions, such as bile (Witter and Balish, 1979; Witter et al., 1979; WHO, 1995).

In human breast milk, levels of nitrate rise rapidly after parturition, peaking on days 2–5 postpartum at concentrations higher than those in plasma. This may be due to nitric oxide production in the breast in preparation for breast feeding (Iizuka et al., 1997; L'hirondel and L'hirondel, 2002; Ohta et al., 2004). However, dietary exposure to nitrate (<100 mg/L) does not appear to increase levels in breast milk (Dusdieker et al., 1996).

There is evidence from animal studies that nitrite can travel through the placenta to the fetus. Nitrite was found in fetal blood following maternal sodium nitrite administration to rats (Shuval and Gruener, 1972) and mice (Globus and Samuel, 1978).

Metabolism

The plasma half-life of nitrate is around 5-8 hours in humans (Lauer et al., 2001; Mensinga et al., 2003), and approximately four hours in dogs (Zeballos et al., 1995). The half-life for nitrite in humans was estimated to be 30-45 minutes in plasma, and around 50 minutes in whole blood (Mensinga et al., 2003; Pluta et al., 2011).

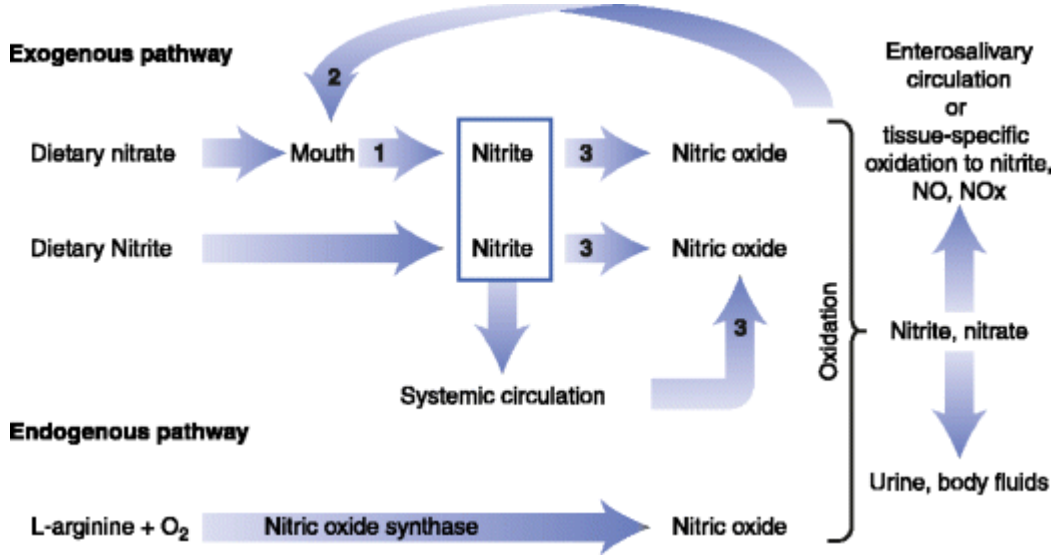
The characterization of nitrate and nitrite metabolism in humans is challenging for several reasons:

- Nitrate is endogenously synthesized.
- Entero-salivary recirculation of nitrate/nitrite.
- Nitrate, nitrite, and NO can be interconverted in the body through bacterial, enzymatic, and non-enzymatic reactions.

In mammals, the primary pathway of endogenous nitrate formation is the L-arginine-nitric oxide synthase (NOS) pathway (L'hirondel and L'hirondel, 2002; Lundberg et al., 2008), which is constitutively active in numerous cell types (macrophages, endothelial cells of blood vessels, and some nerve cells) throughout the body. This enzyme is capable of converting L-arginine into L-citrulline and nitric oxide. NO is unstable in the body and is quickly converted to nitrite or reacts with thiols and secondary amines to form nitrosothiols and nitrosamines respectively. Nitrite can be further oxidized to nitrate through reactions with hemoglobin. It has been estimated that for a healthy adult, approximately 62 mg nitrate/day from endogenous synthesis is excreted in the urine (Mensinga et al., 2003), suggesting that a greater amount is produced daily. Inflammation and exercise can increase the production of NO, nitrite, and nitrate (Allen et al., 2005; Hord et al., 2009). Nitrate and nitrite can act as reserves of NO for the body, as nitrate can be reduced to nitrite and NO when needed physiologically or as part of a pathological process (Hord, 2011).

Entero-salivary recirculation of nitrate/nitrite is an integral part of nitrate metabolism. Approximately 25% of the absorbed nitrate is actively transported into saliva, and about 20% of the nitrate in saliva (approximately 5% of dietary nitrate intake) is reduced to nitrite by bacteria in the oral cavity (Mensinga et al., 2003; Lundberg et al., 2008). When saliva is swallowed, nitrate and nitrite re-enter the gastrointestinal (GI) tract. When nitrite gets to the stomach, it reacts with the gastric juice and forms nitrous acid, which breaks down to NO and other reactive products. The disposition and interconversion of nitrate, nitrite, and nitric oxide are shown in Figure 1. It has been hypothesized that these reactive products can have antibacterial and antifungal effects (L'hirondel and L'hirondel, 2002), or may serve to regulate mucosal blood flow and mucus regeneration (Lundberg et al., 2008). It has also been proposed that the gastric environment of infants is more hospitable to nitrate-reducing bacteria because the gastric pH is higher than in adults, and thus nitrite can be generated in the infant gut (Cornblath and Hartmann, 1948).

Figure 1. The disposition of nitrate, nitrite, and nitric oxide (NO) from exogenous (dietary) and endogenous sources, taken from Hord et al. (2009)
The number 1 refers to bacterial nitrate reductase activity on the tongue or mammalian enzymes with nitrate reductase activity. The number 2 refers to bacterial nitrate reductase activity. The number 3 refers to mammalian enzymes with nitrite reductase activity.



Excretion

In humans, the main route of nitrate excretion occurs through the urine. Following single oral doses of various nitrate compounds (e.g., potassium nitrate, ammonium nitrate), several investigators reported that 60% to 75% of the nitrate was excreted in the urine (Ellen et al., 1982a; Wagner et al., 1983; Bartholomew and Hill, 1984; L'hirondel and L'hirondel, 2002). Urinary excretion was reported to be maximal within 5 hours of ingestion, with levels returning to baseline within 24 to 48 hours (Ellen et al., 1982a; Wagner et al., 1983). The remaining nitrate may be excreted in sweat and feces, or undergo bacterial or endogenous metabolism (Saul et al., 1981; Wagner et al., 1983). Turek et al. (1980) reported slightly higher urinary excretion levels (80-100% of the average intake) in infants, and lower urinary levels in adults (30-35%).

Pharmacokinetic Modeling

A pharmacokinetic model for nitrate and nitrite in humans was developed by Zeilmaker et al. (1996). The model was calibrated using data from several human studies, and accounts for endogenous synthesis of nitrate, in addition to uptake from food and water. The movement of nitrate from blood to saliva, and the interconversions of nitrate/nitrite are also included. Bioavailability of 100% is assumed for nitrate absorption from the "absorption compartment" (stomach and lower GI tract). The model predicted the following: an average human endogenously synthesizes 121 mg nitrate/day; 32-60% of

orally administered nitrate is transported to saliva from blood; 12-22% of salivary nitrate is converted to nitrite (7-9% of an oral nitrate dose). Based on this model, daily nitrate exposure at the Acceptable Daily Intake (ADI, 3.65 mg nitrate/kg-day) level determined by the Joint Food and Agriculture Organization of the United Nations (FAO)/WHO Expert Committee on Food Additives (JECFA) and WHO results in nitrite levels that exceed the ADI for nitrite (0.06 mg nitrite/kg-day) by 4-6 fold.

Kohn et al. (2002) developed a mathematical pharmacokinetic model to simulate methemoglobin formation and the distribution and clearance of sodium nitrite following intravenous or oral exposure in rats. The model predicted that intravenous doses of 8.9 mg/kg for male rats and 7.1 mg/kg for female rats, and oral doses of 15.9 mg/kg for males and 11.0 mg/kg for females would convert 10% of hemoglobin to metHb. The model predicted the recovery half-life to range from 60-100 minutes, depending on the dose and route of administration. The rat model was adapted for humans by inputting human parameters. Using a representative human Vmax value, the model accurately simulated the intravenous dose (7 mg/kg sodium nitrite) needed for 10% conversion of hemoglobin to methemoglobin in humans, reported to be 5.71 mg/kg sodium nitrite (Kohn et al., 2002). However, there are fundamental differences in the absorption, metabolism and distribution of nitrate/nitrite between humans and rats and it is not clear if this model fully accounted for that. Furthermore, the human model was validated with data from only one individual, so it is uncertain whether the model is truly appropriate for predicting the human pharmacokinetics of nitrite.

Methemoglobinemia

Methemoglobinemia is a blood disorder in which an abnormal amount of hemoglobin is transformed to metHb. MetHb is a form of hemoglobin that has a reduced capacity to release oxygen to tissues, due to the oxidation of the iron in the heme group from Fe^{2+} to Fe^{3+}. Normal metHb levels in human blood range from 1% to 3% of total hemoglobin, and symptoms of methemoglobinemia are sometimes seen when metHb levels exceed 10% (Manassaram et al., 2010). Excess metHb can cause ischemia and cyanosis, and may be fatal if levels exceed 70% (Nishiguchi et al., 2015).

Methemoglobinemia is a rare condition that can be caused by various factors and agents. Several chemicals have been linked to metHb formation, including nitrates/nitrites, sulfonamides, and various local anesthetics (Rehman, 2001). Inherited metabolic disorders, such as a deficiency of NADH diaphorase (Kumar et al., 1989) and genetically controlled deficiencies of the enzymes glucose-6-phosphate dehydrogenase or metHb reductase have also been described to cause methemoglobinemia. Methemoglobinemia has also been observed in ruminant animals following nitrate ingestion, and in a wide range of species (e.g., cattle, sheep, swine, dogs, guinea pigs, rats, chickens, and turkeys) following nitrite ingestion (Bruning-Fann and Kaneene, 1993).

Human Studies

The individuals considered most at risk of methemoglobinemia are young infants. Other populations at risk of increased metHb formation are pregnant women, the elderly, and persons with genetically controlled deficiencies of the enzymes glucose-6-phosphate dehydrogenase or metHb reductase. However, healthy adults can still develop methemoglobinemia with exposure to higher amounts of nitrate or nitrite.

There are several factors that contribute to the enhanced susceptibility of infants to metHb formation:

- Fetal hemoglobin, found in infants younger than 3 months, is more readily oxidized to metHb compared to adult hemoglobin.
- Infants have a higher gastric pH, which promotes the presence of nitrate-reducing bacteria that are typically absent under acidic conditions.
- Neonatal erythrocytes have less NADPH-metHb reductase, and are less capable of converting metHb to hemoglobin.
- Compared to adults, infants have larger food and water intake rates relative to body weight, and can have greater exposure to metHb-inducing chemicals, on a body weight basis (Greer et al., 2005; OEHHA, 2012).
- Certain infant illnesses, such as gastroenteritis and diarrhea have also been associated with the development of methemoglobinemia (L'hirondel and L'hirondel, 2002).

Infant metHb has been shown to correlate with blood nitrate levels. A toxic nitrate dose ranged from 1.5-2.7 mg/kg in young infants when a 10% formation of metHb was the toxic endpoint (Corré and Breimer, 1979 as cited in OEHHA, 1997). However, in reviewing case reports, WHO (2011) determined an average of 56.7 mg nitrate/kg in infants (amount of nitrate ingested ranged from 37.1-108.6 mg/kg) was associated with methemoglobinemia. In most clinical cases with a relationship between infant methemoglobinemia and nitrate concentrations in drinking water, the nitrate levels were reported to be 44.3 to 88.6 mg/L or higher (Walton, 1951; WHO, 2011). Literature reviews by Fan and Steinberg (1996) and Fan et al. (1986) found no cases of methemoglobinemia in the United States attributed to nitrate in drinking water when levels were below the MCL of 45 mg/L nitrate (10 mg/L nitrate-nitrogen).

Several studies have reported on methemoglobinemia in the U.S. as well as other countries. Bosch et al. (1950) evaluated 139 cases of cyanosis due to methemoglobinemia reported in Minnesota from 1947 to 1949. These cases, which included 14 deaths, occurred in infants under six months of age. Nitrate concentrations were measured in 129 wells used to supply water to the infants with methemoglobinemia. None of the wells contained nitrate concentrations less than 45

mg/L (10 mg nitrate-nitrogen/L). Only two of the wells contained levels as low as 10 to 20 mg nitrate-nitrogen/L. However, in both of these cases the diagnosis of methemoglobinemia was considered questionable. Coliform organisms were detected in 45 of 51 samples tested for bacterial contamination. The significance of bacterial contamination of the water is discussed in detail below.

Walton (1951) reported 278 cases of infantile methemoglobinemia linked to nitrate-contaminated water in the United States, but nitrate concentrations were only available in 214 cases. Of these 214 cases, no methemoglobinemia was observed at nitrate-nitrogen concentrations of 0-10 mg/L nitrate-nitrogen, and five cases (2.3%) were observed at concentrations of 11 to 20 mg nitrate-nitrogen/L (about 50-90 mg/L nitrate). Neither levels of bacterial contamination of the water supplies, nor the incidences of enteritis among infants were reported.

In Germany, Toussaint and Selenka (1970 as cited in OEHHA, 1997) gave 34 healthy infants (ages 1-3 months) formula prepared with water containing 150 mg nitrate/L, corresponding to 5.5 mg/kg-day of nitrate. Average metHb levels rose from about 1% to 2-3% within the first two days, and remained at this level for up to 10 days. They observed no clinical signs of methemoglobinemia.

Shuval and Gruener (1972) examined 2,473 infants in multiple communities in Israel. The nitrate levels in the water supply of the study communities varied from 50-90 mg/L, whereas the nitrate level in the control area was 5 mg/L. There were no significant differences in metHb levels between the two groups, and mean metHb levels were below 2%. However, only 6% of infants were given powdered formula reconstituted with tap water (Shuval and Gruener, 1972).

In a report describing multiple studies, Shuval and Gruener (1977) examined infants in Gaza, where reconstituted powdered milk consumption was prevalent. MetHb levels in infants consuming powdered milk reconstituted with water containing high levels of nitrate (>56 mg/L) were significantly higher than infants in areas with low nitrate levels (<44 mg/L), regardless of what type of milk was consumed. Exposure to medium nitrate levels (45-55 mg/L) in reconstituted milk elevated metHb levels, but was not statistically significant. However, all metHb levels measured were below 2%, and no signs of methemoglobinemia were observed.

Shuval and Gruener (1977) also described an experiment where 104 infants in an Israeli hospital were administered formula made with nitrate-contaminated water (at varying levels) for five days. The nitrate concentration on the first and fifth days was 15 mg/L, whereas the nitrate concentration on the second, third and fourth days was 108 mg/L. MetHb levels were measured every day, but only 57 infants were measured on all five days, due to patient turnover and technical reasons. Mean metHb levels modestly increased between the first and second day (where the nitrate concentration in formula was increased), but the authors reported three individual cases where metHb levels reached 6.9%, 13.9%, and 15.9%. Mean metHb levels dropped on the third through

fifth days despite continued nitrate exposure, and no clinical methemoglobinemia was observed at any point.

Craun et al. (1981) reported no significant differences in metHb in US child populations exposed to either <10 mg/L nitrate-nitrogen or >22 mg/L nitrate-nitrogen in drinking water. MetHb levels were <2%. Interestingly, most water samples tested in the study were substantially contaminated with bacteria, regardless of nitrate concentration.

Johnson and Kross (1990) noted an ongoing problem with nitrate contamination of well water in rural areas of the U.S., including infant deaths "still occurring as recently as 1986 in South Dakota." Kross et al. (1992) warned of infant methemoglobinemia in the Midwest associated with nitrate exposure, which could be from well water as well as consumption of vegetables.

The Centers for Disease Control and Prevention's (CDC's) Morbidity and Mortality Weekly Report for March 7, 1997, listed two cases of methemoglobinemia due to water contaminated with boiler fluids high in nitrite. In the first case, 29 students in first through fourth grades were diagnosed with methemoglobinemia after consuming commercially canned soup diluted with tap water. Nitrite levels in the soup were 459 mg/L, and the source was determined to be boiler fluids that mixed with potable water due to a faulty backflow check valve. In the second case, four office workers were diagnosed with methemoglobinemia after drinking coffee prepared with nitrite-contaminated water. The nitrite level in the coffee was 300 mg/L, and nitrate levels in hot water samples were >50 mg/L. A faulty backflow valve was also responsible for the water contamination (CDC, 1997). An additional publication described a fatal case of methemoglobinemia in an adult male after ingestion of an unknown amount of sodium nitrite. The subject's metHb levels reached 83.4%. Nitrate levels were 71.69 and 83.48 µg/ml in the heart and femoral blood respectively, and nitrite levels were <0.05 and 0.09 µg/ml in the heart and femoral blood respectively (Nishiguchi et al., 2015).

Ayebo et al. (1997) described three case studies of infant methemoglobinemia in Romania. All three cases reported ingestion of well water with nitrate levels >45 mg/L (two of the cases reported well water concentrations >250 mg/L), but only one infant had diarrhea. The sanitary condition of each well was questionable, and bacterial testing was not done to confirm microbiological contamination.

Gupta et al. (1999a) evaluated metHb levels in 178 individuals in five age groups from five villages in the state of Rajasthan, India, with five different nitrate water concentrations. Table 3 shows the average metHb levels observed for the various age groups (standard deviations were not reported), with the highest value in infants <1 year of age (26.99% metHb). Clinical cyanosis was observed in all age groups, and metHb levels were strongly correlated with cytochrome-b_5 reductase activity (enzyme involved in the conversion of methemoglobin to hemoglobin) in infants ≤1 year and adults >18 years of age (Gupta et al., 1999a). However, a clear dose-response is not noted among the different exposure groups and there is also a poor correlation between the nitrate concentrations and the metHb levels. Additionally, the reported metHb levels seem

high at the lower nitrate levels, considering that normal values were reported to be ≤2% metHb. It is unknown what background metHb levels in comparable populations without appreciable drinking water nitrate contamination were because those control values were not assessed/reported. Furthermore, the water was not tested for bacterial contamination.

Table 3. Average methemoglobin levels (% metHb) in 178 people in five age groups exposed to different concentrations of nitrate in drinking water[a] (adapted from Gupta et al., 1999a)

Nitrate concentration (mg/L)	Average % metHb in each age group				
	≤1 yr	>1 to ≤8 yrs	>8 to ≤18 yrs	>18 to ≤45 yrs	>45 yrs
26	12.69	8.94	4.81	9.33	7.90
45	19.52	15.17	16.52	19.01	10.72
95	26.99	15.13	9.69	12.73	7.00
222	7.06	8.07	5.76	6.93	7.06
459	15.38	15.46	11.06	9.87	10.0

[a]Correlation coefficients for nitrate concentration and metHb were: ≤1 yr = not reported; >1 to ≤8 yrs = 0.23; >8 to ≤18 yrs = 0.01; >18 to ≤45 yrs = -0.57; >45 yrs = -0.58.

Knobeloch et al. (2000) described two cases of blue baby syndrome in Wisconsin in which the infants became ill after ingesting formula reconstituted with water from private wells containing 22.9 and 27.4 mg/L nitrate-nitrogen. The well containing 22.9 mg/L nitrate-nitrogen was described as bacteriologically safe but the one with 27.4 mg/L nitrate-nitrogen tested positive for *E. coli* bacteria. However, the water from this well was boiled for several minutes prior to being used to prepare the infant formula.

Knobeloch and Proctor (2001) reviewed eight infant methemoglobinemia cases that occurred in Wisconsin between January 1990 and September 1999. Three of these cases involved infants whose formula was prepared with nitrate-contaminated well water. Two of the cases were previously described in Knobeloch et al. (2000) while the third case involved an infant whose well water was treated with a reverse osmosis (R/O) filtration system due to a history of nitrate contamination in the well. While the infant was hospitalized, nitrate-nitrogen concentrations of 58 and 9.9 mg/L were found in the well and from the R/O system, respectively. Several weeks after the infant's illness, nitrate-nitrogen levels from the R/O system were 12.5 and 23.5 mg/L, indicating varying levels depending on the frequency of use. Investigators in the case concluded that the infant's methemoglobinemia was due to nitrate exposure.

A more recent study examined the relationship between nitrate concentrations in drinking water and infant metHb levels in Gaza (Abu Naser et al., 2007). MetHb levels were measured in 338 infants from three different areas in the Gaza Strip, Palestine. Mean nitrate concentrations were 124 ppm (range 71-248 ppm) in Jabalia, 119 ppm (range 18-244 ppm) in Gaza City, and 195 ppm (18-440 ppm) in Khan-Younis. The

authors reported that families using tap water had the highest proportion (68.9%) of infants with metHb levels >5% whereas for families using treated or filtered water (nitrate levels not provided), the proportion with metHb was about 20%. Thus, the authors suggest there is a relationship between nitrate levels in drinking water and metHb levels in infants. Interestingly, the study also showed that for infants in the >5% metHb group, 94.4% were given boiled water and 87.8% did not have diarrhea.

Sadeq et al. (2008) surveyed incidence of methemoglobinemia in 411 children, ages 1-7 years in Morocco from low- and high-nitrate drinking water areas, where water sources were municipal water and well water, respectively. In the high-nitrate area the 78 wells tested contained 15 to 247 mg/L of nitrate; the nitrate was above 50 mg/L in 69% of the surveyed wells. In the low-nitrate area, the municipal water had a mean nitrate level of 2.99 mg/L. Methemoglobinemia was more likely to occur in children drinking well water with a nitrate concentration >50 mg/L than in those drinking municipal water (prevalence ratio = 1.60, $p < 0.01$; 95% CI, 1.16-2.21). The percent of children with elevated metHb levels (>2%) were as follows: 27.4% in the control group (exposed to an average nitrate concentration in drinking water of 2.99 mg/L); 24.5% in the <50 mg/L nitrate group; and 43.9% in the >50 mg/L group. Mean metHb levels in the <50 mg/L nitrate group (0.165 g/dL) were comparable to controls (0.166 g/dL), whereas metHb levels were significantly higher when the nitrate level in water was >50 mg/L (0.258 g/dL for 50-90 mg/L nitrate and 0.229 g/dl for >90 mg/L nitrate) (Sadeq et al., 2008). The authors also noted that the mean metHb levels increased with age ($R^2 = 0.79$, $p = 0.04$) in the high exposure area children as compared to the children in the low exposure area (i.e., $R^2 = 0.21$, $p = 0.44$). The high metHb incidence in controls and in older individuals seems to indicate the presence of additional sources of nitrate or perhaps an issue with the metHb diagnostic method, since these results are inconsistent with expected findings. Furthermore, the water was not tested for bacterial contamination.

Manassaram et al. (2010) did not find any association between metHb levels in pregnant women and exposure to nitrate in drinking water at levels below the federal MCL of 10 mg/L nitrate-nitrogen in Minnesota. In this longitudinal study, 357 pregnant women followed throughout pregnancy showed a decrease in metHb levels with increasing gestational duration.

Several researchers have pointed out that infantile methemoglobinemia is closely associated with gastrointestinal infection and diarrhea and that nitrate exposure may not be the driving factor (Hegesh and Shiloah, 1982; Avery, 1999; L'hirondel and L'hirondel, 2002; Powlson et al., 2008; Richard et al., 2014). L'hirondel and L'hirondel (2002) argue that nitrates are not responsible for infant methemoglobinemia for the following reasons: 1) the gastric pH of infants is alkaline immediately after birth, but the pH drops a few hours later to lower levels that are inhospitable to nitrate-reducing bacteria, thus preventing the formation of nitrite which is necessary for metHb formation; 2) methemoglobinemia cases in the US and Europe have dramatically decreased since the 1960s despite the current use of nitrate-contaminated well water; 3) municipal water supplies, which are monitored and controlled for bacterial contamination, have not been associated with infant methemoglobinemia; 4) nitrate levels in water and

methemoglobinemia incidence are poorly correlated; 5) enteritis and endogenous nitrite production may have contributed to elevated metHb levels in diagnosed cases; and 6) nitrate-contaminated well water associated with infant methemoglobinemia may have been contaminated with bacteria that facilitate the conversion of nitrate to nitrite.

Conversely, other researchers believe that infection, while associated with methemoglobinemia, is unlikely to be its primary cause and that vigilance on nitrate contamination should continue (Knobeloch et al., 2000; Knobeloch and Proctor, 2001). Knobeloch et al. (2000) point out that the low incidence of recent methemoglobinemia cases may be due to underreporting, and that investigations examining the causes of the illness are not conducted.

OEHHA acknowledges that microbiological contamination appears to be a potential contributing factor in the development of methemoglobinemia, but does not repudiate the role of nitrate in drinking water in relation to methemoglobinemia induction. There are case reports of elevated metHb levels attributed to nitrates where bacterial contamination was absent (Cornblath and Hartmann, 1948; Vigil et al., 1965; Ayebo et al., 1997; Knobeloch et al., 2000; Knobeloch and Proctor, 2001; Abu Naser et al., 2007). This suggests that ingested nitrates alone can generate high levels of metHb. In another study, metHb levels were unaffected despite high levels of bacteria in nitrate-contaminated water (Craun et al., 1981), indicating that the presence of bacteria in drinking water is not always a contributing factor for methemoglobinemia. Data suggest that the interplay between metHb, nitrates, and bacterial contamination is complex. When exposed to nitrate-contaminated water (both sterile water and water contaminated with bacteria), previously cyanotic infants (who had made a full recovery) again had high levels of metHb and displayed signs of cyanosis (Cornblath and Hartmann, 1948). In these particular infants, it appears that nitrate exposure is the primary factor in methemoglobinemia induction, regardless of the presence of bacteria. Furthermore, concern regarding the conversion of nitrate to nitrite by bacteria in unsanitary water strengthens the argument to keep background nitrate levels low.

L'hirondel and L'hirondel (2002) state that infant gastric pH is comparable to adult pH hours after birth, and therefore the infant stomach would not be a suitable environment for nitrate reducing bacteria to colonize and produce nitrite. Although gastric pH drops within hours after birth, infants subsequently go through a period of relative achlorhydria, where the pH slowly increases over 10 days. The gastric pH will eventually drop to adult levels by 2 years of age (Kenner and Lott, 2007), but remains more alkaline than the adult gastric pH during infancy. Cornblath and Hartmann (1948) showed that bacteria cultured from stool, saliva, and gastric juices of cyanotic infants only grow at a pH above 4, and that these bacteria are capable of reducing nitrate to nitrite. This suggests that the gastric environment of infants is more basic than in adults, and that infants are more susceptible to gastric colonization by these nitrate-reducing bacteria.

Infant methemoglobinemia has been attributed to nitrates in municipal water free of bacterial coliform (Vigil et al., 1965). In this case report, a cyanotic infant was

diagnosed with methemoglobinemia, which was attributed to nitrate contamination in the tap water used to reconstitute evaporated milk (63 mg/L in tap water and 73 mg/L in formula). L'hirondel and L'hirondel (2002) argue that the cause of methemoglobinemia cannot be determined because the infant had diarrhea. However, it seems unlikely that the infant's diarrhea would be related to infection since the case report noted that all bacteriological samples from water supply wells were negative for coliform organisms and the water used for preparing the formula was boiled for approximately 20 minutes.

A recent study by Kanady et al. (2012) showed that neonates and young infants have lower levels of nitrite in saliva than adults due to reduced bacterial nitrate reductase activity. Although nitrate-reducing bacteria were identified in the mouths of newborns, and salivary nitrate levels in infants were comparable to adults, salivary cultures taken from infants showed less nitrite production than adults. This difference in nitrate reductase activity may explain the reported differences between adult and neonatal infant blood nitrite concentrations (Ibrahim et al., 2012). One could argue that infants are not as susceptible to nitrate because they do not convert nitrate to nitrite in the mouth as efficiently as adults. However, the authors do not specifically comment on the source of nitrate in this study, and it is presumed that the nitrate/nitrite measured was produced endogenously. It is unknown whether the nitrate-reducing capability of the bacteria in the neonatal/infant oral cavity would be affected by larger concentrations of nitrate (from exogenous sources). Furthermore, nitrate-reducing bacteria in the infant gut may still convert nitrate to nitrite.

Animal Studies

Rats are less likely to develop methemoglobinemia because they lack the active transport of nitrate to the saliva, which limits their ability to convert nitrate to nitrite. However, increased metHb levels have been reported in mice acutely exposed via inhalation to butyl nitrate (McFadden et al., 1981). Methemoglobinemia has been observed in chronic and subchronic studies with nitrite in animals (Shuval and Gruener, 1972; Til et al., 1988). Additionally, sodium nitrite (120 µl, 140 g/L) applied to abraded skin increased metHb levels in rats, but did not alter metHb levels when applied to normal skin (Saito et al., 1997).

Summary

Overall, several studies have shown a higher incidence of methemoglobinemia in infants and children in areas with drinking water nitrate concentrations >50 mg/L. There were no documented cases of methemoglobinemia associated with a nitrate concentration of ≤45 mg/L. Some researchers have claimed that gastrointestinal infection from bacteria in contaminated drinking water is the cause of infantile methemoglobinemia rather than nitrate ingestion. However, there are several studies that show methemoglobinemia induction following nitrate exposure without bacterial contamination, indicating that nitrate is the causative agent behind methemoglobinemia. The studies and case reports of methemoglobinemia in infants and children present

consistent evidence of an association with exposure to nitrate, thus, methemoglobinemia is an appropriate critical endpoint for PHG development.

Thyroid Toxicity

Nitrate has the potential to affect thyroid function through inhibition of iodide uptake by the sodium-iodide symporter (NIS). The NIS is a plasma membrane glycoprotein responsible for active iodide uptake into the thyroid, lactating breast, small intestine, and other tissues (Dohan et al., 2003; Dohan et al., 2007; Nicola et al., 2009). The first step in iodide utilization is the absorption of iodide from dietary iodine in the salivary cells and small intestine. Once in the bloodstream, iodide uptake into the thyroid through the NIS leads to biosynthesis of thyroid hormones (triiodothyronine and thyroxine, also known as T3 and T4, respectively), which are essential for proper central nervous system (CNS) development as well as metabolism in other tissues. Thyroid hormone regulation is controlled via a negative feedback loop between the thyroid gland, the hypothalamus, and the pituitary gland. When thyroid hormone levels are low, thyrotropin-releasing hormone is released by the hypothalamus, which in turn induces the pituitary gland to release thyroid-stimulating hormone (TSH). Imbalances in thyroid hormone production due to iodine deficiency or other chemical exposures can lead to illness. Hypothyroidism can be caused by reduced output of thyroid hormones from the thyroid gland, and can lead to cretinism in children. Prolonged TSH activity can lead to hypertrophy and hyperplasia of thyroid follicular cells, which can lead to enlargement of the thyroid gland or goiter.

The NIS is also responsible for supplying the iodide anion to nursing newborns through breast milk, since NIS is present in the milk glands of lactating breasts (Dohan et al., 2007), and is thought to be responsible for iodide transport across the placenta (Gersten, 1954; Mitchell et al., 2001; Richard et al., 2012). A thorough review of thyroid physiology, thyroid changes during pregnancy, and the effects of iodine deficiency during pregnancy can be found in the Public Health Goal for perchlorate (OEHHA, 2015).

Other chemicals, including perchlorate and thiocyanate, can also block iodide uptake into the thyroid and reduce thyroid hormone production. In vitro data involving human NIS transfected into Chinese hamster ovary (CHO) cells suggest that perchlorate, thiocyanate, and nitrate can combine to inhibit NIS iodide uptake in a manner that is approximately additive (Tonacchera et al., 2004). However, because it is uncertain whether human NIS transfected into non-human, non-thyroid cells grown in a cell culture dish can actually recapitulate NIS function in humans, it is unknown whether these findings can be directly applied to humans. Interestingly, very high iodine intakes have also been shown to paradoxically decrease thyroid iodide uptake, at least temporarily (Wolff and Chaikoff, 1948). The results of several human studies also suggest these NIS blockers, in conjunction with low or very high iodine intakes, may combine to inhibit thyroid iodide uptake and decrease thyroid hormone production (Blount et al., 2006; Steinmaus et al., 2007; Steinmaus et al., 2013). Importantly, these human studies were not enough to distinguish whether the combined impacts were

additive, less than additive, or greater than additive. Overall, based on the currently available in vitro, animal, and human evidence, it seems possible that subjects with high intakes of thiocyanate or perchlorate, or subjects with low or very high iodine intakes, may be more sensitive to the thyroid effects of a given level of nitrate exposure than people without these factors. To date however, adequate studies detailing the dose-response relationship of nitrate on thyroid function in these potentially susceptible populations have not been done. As such, the possible thyroid effects of nitrate in these groups cannot be quantitatively evaluated at this time.

Thyroid hormone levels during pregnancy affect key neonatal developmental stages or lead to possible thyroid-related effects. Haddow and colleagues reported that children born to women with TSH levels above the 98th percentile during gestation had lower cognitive, attention, and motor performance scores (Haddow et al., 1999). Furthermore, low maternal serum free T4 levels early in pregnancy (12 weeks gestation) have been linked to impaired neurodevelopment in the infant (Pop et al., 1999; Pop et al., 2003).

Human Studies

The consumption of drinking water containing high nitrate levels (>50 mg/L nitrate) has been reported to adversely affect the thyroid in humans (van Maanen et al., 1994; Vladeva et al., 2000; Gatseva and Argirova, 2005; Tajtakova et al., 2006; Gatseva and Argirova, 2008b; Gatseva and Argirova, 2008a; Radikova et al., 2008; Cao et al., 2010; WHO, 2011). However, there are also several studies that reported no adverse association between nitrate ingestion and thyroid dysfunction (Lambers et al., 2000; Hampel et al., 2003; Hunault et al., 2007; Steinmaus et al., 2007; Below et al., 2008; Blount et al., 2009; Bruce et al., 2013).

van Maanen et al. (1994) reported an increase in the volume of the thyroid gland in women (on average 40-43 years of age) from the Netherlands who drank well water with nitrate concentrations in the range of 50-300 mg/L, compared to women who consumed water with lower nitrate concentrations. Thyroid volume, serum concentrations of TSH, total T4 and free T4, and urine concentrations of nitrate and iodine were determined in four groups of women (described in Table 4).

Thyroid volume was assessed by a physician using portable ultrasonograph and palpation. It was not reported whether the physician was blinded to the drinking water nitrate status of the subjects when assessing thyroid volume. The mean thyroid volume in Group D was statistically significantly larger than those in the other three groups based on the Mann-Whitney U-test ($p<0.006$, $p<0.02$, and $p<0.03$, respectively). When outlier (very large) values of thyroid volume were included (all outliers were in Groups B and C), these results remained statistically significant only for the difference between Groups A and D. Urinary iodine levels were lower in Group A than in Group D. Mean serum total T4 concentrations were higher and mean serum thyroid TSH levels were lower in Group D than in the other groups, although some comparisons were not statistically significant (van Maanen et al., 1994). These results are in the opposite direction of that which would be expected if nitrate were blocking the NIS and

decreasing thyroid hormone production.

Table 4. Subject characteristics and significant findings of van Maanen et al. (1994)

Group	N	Drinking water source	Drinking water nitrate concentration (mean, mg/L)	Thyroid volume (cm^3)	TSH (mU/L)	Total T4 (nmol/L)	Urinary iodine (µmol/24 hr)
A	21	Municipal	0.02	8.3 ± 1.7	2.6 ± 1.3	98 ± 25	0.75 ± 0.36
B	23	Municipal	17.5	8.9 ± 1.8	2.4 ± 1.1	100 ± 17	0.98 ± 0.26[a]
C	6	Private well	22 ± 10	8.2 ± 1.5	3.2 ± 1.1	90 ± 23	0.94 ± 0.31
D	10	Private well	131 ± 76	11.1 ± 3.0[b]	2.1 ± 1.1[b]	105 ± 17[b]	1.02 ± 0.45

Measurements are shown as mean values ± SD; standard deviations not given for all means
[a]p<0.05 when compared with group A (van Maanen et al., 1994)
[b]p<0.05 when compared with group C (van Maanen et al., 1994)

The authors collected information on several potential risk factors for goiter and thyroid disease including iodine intake and excretion, familial or historical incidence of goiter, and smoking, but it is not clear how or if these factors were incorporated into the analyses. A substantial proportion of the study participants were smokers, which may lead to increased serum thiocyanate levels (Rubab and Rahman, 2006), although smokers were distributed across all study groups. Other parameters were not accounted for in the analyses, such as normalizing thyroid volume to body weight or surface area, and presence of other potential goitrogens in the drinking water or food. "Normal" thyroid volume has been reported to vary depending on region, age, body surface area or body weight, and especially iodine sufficiency, and it is not clear that these factors were accounted for in this study (Berghout et al., 1987; Langer, 1999; Seker and Tas, 2010). Overall, although this study reports some evidence of an association between nitrate concentrations in drinking water and markers of thyroid health, the nitrate concentrations in the high exposure group were substantially higher than the current nitrate PHG. Major weaknesses of this study include the small sample size, the potential lack of a blinded assessment of thyroid volume, thyroid hormone results inconsistent with the proposed mechanism of NIS inhibition, and the lack of information on possibly important confounders and effect modifiers such as iodine intake level, diet, and body weight.

Vladeva et al. (2000) reported a statistically significant higher prevalence of goiter in children from Bulgarian villages with higher nitrate levels in drinking water compared to children from Bulgarian villages with lower nitrate levels. The prevalence of goiter in children 3-14 years of age in 1995 and in 1998 from the village of Karadjalovo (high drinking water nitrate concentrations) was compared to the prevalence of goiter in

children in the same age range from Tatrovo, with low drinking water nitrate concentrations. The same comparisons were also made between 6- to 14-year-old children from the high nitrate village of Ivailo versus Mokrishte (low nitrate). Goiter was assessed by clinical examination, including inspection and palpation of the thyroid. It was not stated whether the researchers assessing goiter status were blinded to the nitrate concentrations of the villages. Nitrate exposure was based only on the village in which the subjects lived and data on individual nitrate exposures were not provided. Nitrate in drinking water had been recorded from 1983 to 1999 for the villages in the study. The reported levels of nitrate in the drinking water were 70-90 mg/L in the high nitrate villages and 20-41 mg/L in the low nitrate villages.

Comparing the first set of villages (Karadjalovo vs. Tatrovo), the odds ratio (OR) for goiter was 3.97 (95% CI, 2.41-7.03) when data from 1995 and 1998 were combined. The OR decreased from 6.11 in 1995 to 2.06 in 1998. For Ivailo versus Mokrishte, the OR for goiter was 1.97 (95% CI, 1.42–2.74). A decrease in the OR in these villages was also noted between the two time periods: 2.11 in 1995 to 1.84 in 1998. The decrease in goiter frequency between these periods was attributed by the authors to increasing prophylactic use of iodine during this time. The large drops in the ORs between 1995 and 1998 after iodine prophylaxis suggest that a portion of the excess odds that remained in 1995 may have been related to differences in iodine intake between the villages rather than just nitrate. Overall, this study suggests that the higher prevalence of goiter in the towns with higher nitrate drinking water concentrations may be due to the nitrate, but the lack of data on individual exposure and other potential confounding factors such as iodine status, smoking status or diet, and the possible lack of blinded assessment of goiter status, all limit the interpretation of these findings.

In studies of iodine nutrition and goiter in Bulgaria, Gatseva et al*.* (1998) and Gatseva and Argirova (2005) reported an association of goiter with high consumption of nitrate in drinking water, especially in children with low iodine intake. These studies were expanded and are discussed more extensively in the reports of Gatseva and Argirova (2008a,b) below.

Tajtakova et al*.* (2006) and Radikova et al*.* (2008) reported a significantly higher thyroid volume (ThV) as detected by ultrasound, higher TSH levels, and higher thyroid peroxidase antibodies in Slovakian children from a high nitrate area (n = 324) compared with age-matched children from surrounding areas with low nitrate (n = 764). Ten- and 13-year-old children from the high nitrate area lived in an agricultural lowland where the water supply contained 51-274 mg nitrate/L. The comparison group consisted of 168 children of the same ages from a neighboring area with very low nitrate (<2 mg/L) in the water supply and 596 children from the city of Kosice, also supplied by low-nitrate water. It is not clear if researchers measuring thyroid volume were blinded to the nitrate exposure status of the children. Blood samples were obtained from 315 children of the high-nitrate area and 109 children from the low-nitrate areas. High TSH levels in the range of subclinical hypothyroidism (>4.0 mU/L) were found in 4% of the children tested from the high-nitrate area (n = 13) and in none of the children from the low-nitrate areas. Eight (2.2%) of the children from the high-nitrate area had thyroperoxidase antibodies

while these antibodies were not found in any of the children from the low-nitrate areas. Tests of statistical significance were not provided for these analyses. The mean serum total T4 in the children from the high-nitrate areas was higher than that in children from the low-nitrate area (88.9 ± 26.9 and 82.1 ± 22.6 nmol/L, respectively) although this difference was not statistically significant according to the authors (p-value not provided).

Thyroid volumes (mean ± SE) in 10-year-old (5.10 ± 0.14 ml/m^2) and 13-year-old (5.97 ± 0.11 ml/m^2) children from the high-nitrate area were significantly higher than those in the children from the neighboring low-nitrate area (4.58 ± 0.17 for 10-year-olds ($p<0.02$) and 5.23 ± 0.15 ml/m^2 ($p<0.05$) for 13-year-olds), and from those in children from the city of Kosice (4.77 ± 0.10 ml/m^2 ($p<0.05$) for 10-year-olds and 4.87 ± 0.10 ml/m^2 for 13-year-olds ($p<0.0001$). Median urinary iodine levels were higher in children from the high-nitrate area compared to children from the low-nitrate area (150 vs. 126 µg/L, respectively), and the range in urinary iodine concentrations in the high-nitrate area was somewhat large (24-320 µg/L). The high-nitrate exposures would result in estimated doses of 6.4-34 mg/kg-day for infants (0-2 years) and 3.4-18 mg/kg-day for children (2-11 years) with drinking water consumption rates of 0.125 and 0.067 L/kg-day, respectively. Overall, this study provides some evidence of an association between higher nitrate concentrations in drinking water and increased thyroid volume and increased TSH. However, weaknesses include the potential lack of blinding of thyroid volume assessment, the ecological nature of the exposure data, and the lack of information on the possible effects of other factors, like iodine and dietary nitrate, that may impact thyroid function.

Gatseva and Argirova (2008a) reported possible increases in goiter in pregnant women and children from Bulgaria exposed to high levels of nitrate in drinking water. The authors compared urinary iodine levels and findings from a clinical evaluation of the thyroid in pregnant women and children from a high-nitrate drinking water region (93 mg/L nitrate) to those for pregnant women and children from a low-nitrate region (8 mg/L). The study included 26 pregnant women (ages 17 to 37) and 50 children (ages 3 to 6) from the high-nitrate area (Chernogorovo) and 22 pregnant women and 49 children of the same ages from the low-nitrate area (Parvenez). The odds ratio for goiter in pregnant women in the higher nitrate area compared to the lower exposure area was 5.29 (95% CI, 1.00-27.9; $p = 0.045$). For the children (3 to 6 years of age), the odds ratio was 2.33 (95% CI, 0.85-6.41; $p = 0.14$). Few details were provided on how goiters were diagnosed, including whether or not those assessing the goiters were blinded to the subjects' nitrate exposure status. Low urine iodine levels (below 100 µg/L) were more common in the higher nitrate exposure area than in the lower exposure area. For example, the percentage of pregnant women with urinary iodine levels below 100 µg/L was 19.2% in the high-nitrate area and 4.5% in the low-nitrate area. Similar findings were seen in the children.

In the second study by Gatseva and Argirova (2008b), the prevalence of goiter was assessed in 156 seven- to 14-year-olds from nitrate exposed Ivailo (water nitrate concentrations of 75 mg/L) and 163 children in the same age range from lesser

exposed Parvenez (8 mg/L). A statistically significant increase in goiter frequency was seen in the higher nitrate exposed village (OR = 3.01; 95% CI, 1.29-7.03, p = 0.01). Based on a figure presented in the article (their Figure 1), the high-nitrate village seemed to have a substantially higher percentage of children with urinary iodine levels >300 µg/L and <100 µg/L than the lower nitrate village. Since both low iodine and very high iodine intakes can be associated with hypothyroidism, it is possible that the higher frequency of goiter seen in the high-nitrate village could at least in part be due to inter-village differences in iodine.

Cao et al. (2010) measured urinary concentrations of perchlorate, nitrate, iodine, thiocyanate, T4, and TSH in 92 full term infants from Pennsylvania. In analyses adjusted for age, sex, body mass index, perchlorate, iodine and thiocyanate, increasing urinary nitrate concentrations were associated with both increasing urinary TSH and T4 concentrations. However, since both TSH and T4 concentrations were increased, these results are difficult to interpret.

While associations between nitrate and thyroid toxicity have been reported in several studies (van Maanen et al., 1994; Vladeva et al., 2000; Gatseva and Argirova, 2005; Tajtakova et al., 2006; Gatseva and Argirova, 2008b; Gatseva and Argirova, 2008a; Radikova et al., 2008; Cao et al., 2010; WHO, 2011), major shortcomings were noted that could complicate the interpretation of the data. These include:

- Lack of consistency: A key aspect in evaluating causal inference is the finding of consistency across multiple studies from different areas and different researchers. Only one study assessing thyroid volume originated from a research group outside of Bulgaria, and there appears to be some overlap among the Bulgarian studies in terms of the study areas, families, and the subjects. The sample sizes tended to be rather small. In addition, changes in T4 or TSH were sometimes in the opposite direction expected based on the known mechanism of nitrate on the NIS and thyroid gland.

- Lack of adjustment for iodine status: Both very high and low iodine intakes have been linked to hypothyroidism and other thyroid effects. It is not clear that any of these studies adequately controlled for iodine intake, so iodine may have confounded some of the associations identified in these studies. In several instances, the nitrate exposed subjects had evidence of greater rates of iodine deficiency than the control subjects. As an example, several of these papers found that the subjects with the more severe goiters had especially low urinary iodine concentrations.

- Adjustment for confounders: It is not clear if any of the results were adjusted for some of the other determinants of goiter or thyroid hormone levels, such as nitrate in food, exposure to thiocyanate, diet, age, or tobacco smoke exposure.

- Possible measurement bias: Measurement of goiter can be a subjective process and the measurements done within these studies do not appear to be blinded to

the nitrate exposure status of the subjects. Inadvertent bias can lead researchers to assess goiter status differently in nitrate exposed and unexposed subjects, and this could lead to false associations between nitrate exposure and thyroid volume measurements. Because none of the studies mentioned whether the clinical measurements were done blindly, it must be assumed that they were not.

- Ecologic assessment of exposure: Although data are available for all the wells within the study area, information for wells used by the individuals and their resulting nitrate exposures was not provided. In addition, whether or not subjects may have consumed from other water sources (e.g., at school or work) and the nitrate levels of these other sources were not assessed. In general, the likely direction of this bias would be towards finding no association, but bias in either direction is possible.

Overall, these shortcomings substantially limit the interpretation of the findings presented in van Maanen et al. (1994) and in Bulgarian and Slovakian children by Vladeva et al. (2000), Tajtakova et al. (2006), Gatseva and Argirova (2008a,b), and Radikova et al. (2008). In other recent studies (Lambers et al., 2000; Hampel et al., 2003; Hunault et al., 2007; Steinmaus et al., 2007; Below et al., 2008; Blount et al., 2009; Ward et al., 2010; Aschebrook-Kilfoy et al., 2012a), clear associations between nitrate exposure and hypothyroidism were not identified.

Several studies using data from the National Health and Nutrition Examination Survey (NHANES) in the United States have evaluated possible associations between urinary nitrate levels and serum concentrations of thyroid hormones (Blount et al., 2009; Bruce et al., 2013; Suh et al., 2014). NHANES is a national representative cross-sectional survey conducted by the National Center for Health Statistics of the CDC and is designed to assess the health and nutrition status of the non-institutionalized population of the U.S. every two years. Single urine and serum samples from each subject are measured for a variety of chemical analytes. Using data from the 2001-2002 NHANES, Blount et al. (2006) reported an association between increases in the logarithm of urinary nitrate and decreases in serum T4 concentrations, but only in women with urinary iodine concentrations ≥100 µg/L (regression coefficient = -1.1215; p = 0.0249; n = 724). A somewhat similar finding was reported by Bruce et al. (2013) using the same data. Suh et al. (2014) reported that nitrate was a predictor of free T4 in non-pregnant women, regardless of urinary iodine concentration. There was no significant association between nitrate and free T4 in men or pregnant women.

In a randomized controlled human exposure study, Lambers et al. (2000) and Hunault et al. (2007) found no statistically significant effects on thyroidal ^{131}I uptake and plasma concentrations of thyroid hormones (T3, reverse triiodothyronine (rT3), T4, and TSH) in 10 adult volunteers receiving 15 mg/kg of sodium nitrate (10.9 mg/kg nitrate) for 28 days in drinking water, compared to 10 control subjects. Participants in this study were non-smoking young adults (18-35 years of age) with normal functioning thyroid glands assessed by clinical examination and hormone level measurements. The participants

were kept on an iodine-restricted diet to strengthen the nitrate effect on thyroid function. This nitrate dose is three times the WHO's ADI of 3.7 mg/kg (WHO, 2003), and is equivalent to the dose that would be received by a 70 kg adult drinking 2 L/day of water containing about 380 mg/L nitrate. However, the small sample size and relatively short duration of exposure limit the usefulness of this study when determining the effects of chronic nitrate exposure on the general population. Nonetheless, the absence of thyroidal ^{131}I uptake inhibition in human subjects directly exposed to nitrate suggests that nitrate does not adversely impact thyroid physiology.

Hampel et al. (2003) examined the relationship between nitrate exposure and goiter in Germany. Although their publication was written in German, its English abstract reported no influence of urinary nitrate excretion, used as an indicator of nitrate exposure, on the prevalence of goiter in 3,059 clinically healthy persons (ages 18-70) from several regions of Germany. Thyroid volume was measured using ultrasound and urinary nitrate by ion chromatography. The median urinary nitrate level for the population was 55.2 mg nitrate/g creatinine. The median levels were statistically significantly ($p<0.03$) higher in men (61.5 mg nitrate/g creatinine; n = 1,080) than in women (51.5 mg nitrate/g creatinine; n = 1,979). However, the authors concluded that there was no statistical connection between urinary nitrate, thyroid size, and goiter in the subjects analyzed.

Similarly, Below et al. (2008) measured the thyroid volume of 3,772 subjects (20-79 years of age) from northeast Germany who had no diagnosed thyroid disorder. The authors compared thyroid volume and goiter in individuals who had urinary nitrate levels above the 75th percentile (69.0 mg/L) to those individuals below the 75th percentile. In this area, 99.9% of the population received centrally supplied drinking water with nitrate levels ranging from 2.5 to 10 mg/L. Thyroid volume was measured using ultrasound and urinary nitrate level was determined with ion chromatography. Goiter was defined as a thyroid volume >18 ml in women and >25 ml in men. The urinary nitrate level was used as an indicator of nitrate intake and exposure. The two groups were compared with and without consideration of confounders (i.e., age, sex, smoking status, residency, and body mass index). The goiter proportion was 35.5% in the "low urinary nitrate" group (n = 2,830) and 34.7% in the "high urinary nitrate" group (n = 943), which provided no statistical difference between the two groups with or without the corrections for confounders. The analysis with the full consideration of confounders showed no association between nitrate intake and risk of goiter (OR = 1.01; 95% CI, 0.86-1.19; p = 0.90) and thyroid volume (21.4 $\pm$ 0.2 ml in the low nitrate group vs. 21.1 $\pm$ 0.4 ml in the high nitrate group; p = 0.47).

In a preliminary analysis, Steinmaus et al. (2007) also found no relationship between nitrate exposure and thyroid hormone levels. The main focus of the Steinmaus et al. (2007) study was on the relationship between perchlorate and thyroid function. However, the authors also considered the potential role of two other NIS inhibitors – thiocyanate and nitrate. Steinmaus et al. (2007) analyzed the urinary levels of perchlorate, thiocyanate, nitrate, and iodine, and serum levels of total T4, TSH, albumin, cotinine, and c-reactive protein in 2,299 men and women (≥12 years of age) who took

part in the 2001-2002 NHANES. Overall, using models adjusted for age, gender, urinary iodine concentrations, and other factors, no clear associations were seen between nitrate concentrations in urine and serum concentrations of total T4 or TSH. However, Suh et al. (2014) found an association between nitrate and free T4 levels. Additionally, urinary nitrate concentrations in men and women surveyed in NHANES have been negatively associated with serum parathyroid hormone levels, although these results have not been confirmed and an obvious mechanism for these associations is not known (Ko et al., 2014).

Ward et al. (2010) investigated the association of nitrate intake with self-reported hypothyroidism or hyperthyroidism and the risk of thyroid cancer in a cohort of 21,977 women in Iowa. Total nitrate intake was estimated based on nitrate concentrations in the women's drinking water, and dietary nitrate intake was assessed using a food frequency questionnaire and published levels of nitrate in foods. No association was found between nitrate concentrations in drinking water, including concentrations >2.46 mg/L nitrate-nitrogen, and the prevalence of hypothyroidism or hyperthyroidism. However, increasing intake of dietary nitrate was associated with the prevalence of hypothyroidism (OR = 1.24; 95% CI, 1.10–1.40 for >41.1 mg of dietary nitrate-nitrogen/day; p-trend = 0.001) but not hyperthyroidism.

In Aschebrook-Kilfoy et al. (2012a), serum concentrations of TSH were measured in 2,543 Old Order Amish men and women residing in three Pennsylvania counties during the period of 1995-2008, recruited as part of other health studies in this area. Nitrate concentrations in 3,613 wells in the study area from 1976-2006 were used to model nitrate concentrations throughout the study area. Nitrate concentrations estimated by the models ranged from 0.35 to 16.4 mg/L nitrate-nitrogen, with a median of 6.5 mg/L. Serum TSH levels of 4-10 milliinternational units per milliliter (mIU/ml) were defined as subclinical hypothyroidism, and levels above 10 mIU/ml were defined as clinical hypothyroidism. All models were adjusted for age and body mass index, and stratified by gender. No associations were seen between estimated nitrate concentrations in wells and clinical hypothyroidism in either gender, or between estimated nitrate concentrations and subclinical hypothyroidism in men. Women exposed to estimated nitrate concentrations above the median had an elevated odds ratio for subclinical hypothyroidism compared to women exposed to lower nitrate concentrations (OR = 1.60; 95% CI, 1.11-2.32). However, in analyses of subclinical hypothyroidism in women by nitrate concentration quartiles, a clear dose-response relationship was not seen. Odds ratios from the lowest to the highest quartiles in this analysis were 1.00 (reference group), 0.93 (95% CI, 0.52-1.64), 1.84 (95% CI, 1.11-3.06), and 1.28 (95% CI, 0.75-2.16) (p-trend = 0.45). Overall, while some evidence was seen of an association between estimated nitrate concentrations in wells and subclinical hypothyroidism, a clear dose-response pattern was not found.

Summary

In this review of the human studies on thyroid effects of nitrate, some associations between elevated nitrate concentrations in drinking water and adverse impacts on the

thyroid or thyroid function have been observed. The potential thyroid effects associated with in utero exposure to nitrate and other NIS inhibitors was highlighted in the US EPA Office of Inspector General's review (OIG, 2010) of US EPA's perchlorate risk assessment. The review suggested that known competitive inhibitors of the NIS may cause an imbalance in iodide distribution during pregnancy, leading to possible thyroid-related effects that could impact key neonatal developmental stages (OIG, 2010). For example, Haddow et al. (1999) reported that moderately elevated levels of TSH during pregnancy, including those in many women who did not have a clinical diagnosis of hypothyroidism, are associated with lower IQ scores in the offspring. Although hypothyroidism observed in children born to pregnant women with thyroid hormone insufficiencies (moderate or transient) may result in lower cognitive, attention, and motor performance scores (Haddow et al., 1999; Miller et al., 2009), no studies on IQ of children exposed to high levels of nitrate in drinking water have been located.

Despite the potential effects that have been pointed out, the varied results summarized above cannot be considered to present clear and consistent evidence of an association between exposure to nitrate and decreased thyroid function or IQ. Major biases or confounding factors (e.g., non-blinded assessments of thyroid size and the lack of information regarding smoking status, iodine intake, diet, and exposure to other NIS inhibitors such as perchlorate and thiocyanate) could not be ruled out.

Animal Studies

Early studies evaluating the potential of nitrate or nitrite to damage the thyroid in animals found inorganic nitrate to have a goitrogenic effect in different species. A goitrogenic effect was observed in rats by oral and parenteral application of potassium nitrate (Wyngaarden et al., 1953; Bloomfield et al., 1961). Antithyroid effects of nitrate were also found in sheep (Bloomfield et al., 1961) and in pigs administered potassium nitrate (Jahreis et al., 1986; Jahreis et al., 1987). Functional and histological changes of the thyroid gland were observed with a lowest-observed-adverse-effect level (LOAEL) of 36 mg/L nitrate (Wyngaarden et al., 1953; Bloomfield et al., 1961; Horing et al., 1986; Jahreis et al., 1986; Jahreis et al., 1987).

More recently, several investigators reported changes in the thyroid gland and thyroid activity following exposure to nitrate (Chaoui et al., 2004; Zaki et al., 2004; Eskiocak et al., 2005; Mukhopadhyay et al., 2005; Kostogrys et al., 2006a; Kostogrys et al., 2010) and nitrite (Kostogrys et al., 2006b; Kostogrys et al., 2010) . These studies are summarized in Table 5. Nitrate exposure has consistently been reported to result in increased thyroid weight and changes to follicle cells. However, thyroid hormone changes have not been as consistent. Some studies have reported increased thyroid weights with a decrease in T3 and T4 and a decrease in TSH (Eskiocak et al., 2005) or with no TSH measurement (Chaoui et al., 2004; Zaki et al., 2004). These results are not consistent with the expected outcomes of an inhibitor of thyroidal iodide uptake. Other studies, such as those of Mukhopadhyay et al. (2005) and Kostogrys et al. (2006a,b; 2010) are consistent with inhibition of iodide uptake by an NIS inhibitor.

Table 5. Summary of animal studies examining thyroid effects of nitrate or nitrite

Sex/Species	Dose/Route of Exposure	Endpoints	NOAEL/LOAEL (Thyroid Effects)	Reference
Male/Female F344/N rats (50/sex/conc.)	0, 375, 750, 1,500, 3,000, or 5,000 ppm sodium nitrite in drinking water for 10-14 weeks	No significant thyroid effects	NOAEL: 5,000 ppm sodium nitrite (310 mg/kg-day for males, 345 mg/kg-day for females)	NTP (2001)
	0, 750, 1,500, or 3,000 ppm sodium nitrite in drinking water for 105 weeks		NOAEL: 3,000 ppm sodium nitrite (130 mg/kg-day for males, 150 mg/kg-day for females)	
Male/Female B3C6F1 mice (50/sex/conc.)	0, 375, 750, 1,500, 3,000, or 5,000 ppm sodium nitrite in drinking water for 14 weeks		NOAEL: 5,000 ppm sodium nitrite (990 mg/kg-day for males, 1,230 mg/kg-day for females)	
	0, 750, 1,500, or 3,000 ppm sodium nitrite in drinking water for 104-105 weeks		NOAEL: 3,000 ppm sodium nitrite (220 mg/kg-day for males, 165 mg/kg-day for females)	
Male Wistar rats (12/conc.)	50, 100, 150, or 500 mg/L potassium nitrate in drinking water for 5 months. Control animals received tap water containing 13.55 mg/L nitrate. All animals were given food containing 22 mg/kg nitrate.	↑ absolute thyroid weight, ↑ vacuolization of the thyroid, ↓ body weight, ↓ plasma T4, ↓ plasma and serum T3	NOAEL: 50 mg/L potassium nitrate in drinking water (7.35 mg/kg-day based on a drinking rate of 0.032 L/day and body weight of 0.217 kg, from US EPA, 1988)	Zaki et al. (2004)
Female Wistar rats (12/conc.)	50, 150, or 500 mg/L potassium nitrate in drinking water for 5 months. Control animals received tap water containing 13 mg/L nitrate. All animals were given food containing 22 mg/kg nitrate.	↑ thyroid follicle size, ↑ absolute thyroid weight ↓ body weight, ↓ T3 and T4	NOAEL: 50 mg/L potassium nitrate in drinking water (8.0 mg/kg-day based on a drinking rate of 0.025 L/day and body weight of 0.156 kg, from US EPA, 1988)	Chaoui et al. (2004)
Female Wistar rats (9-10/conc.)	0, 50, 100, 250, or 500 mg/L sodium nitrate in drinking water for 30 weeks	altered iodide uptake, ↓ TSH, ↓ T4, ↓ T3, ↑ absolute and relative thyroid weight	LOAEL: 50 mg/L sodium nitrate (8.0 mg/kg-day based on a drinking rate of 0.025 L/day and body weight of 0.156 kg, from US EPA, 1988)	Eskiocak et al. (2005)

Wistar rats (sex not specified) (10/dose)	0 or 1.839 g/kg-day nitrate in diet for 4 weeks	↑ relative thyroid weight, ↑ TSH, ↑ iodine excretion, ↓ thyroid peroxidase activity, ↓serum TT4 and T3	LOAEL: 1.839 g/kg-day nitrate	Mukhopadhyay et al. (2005)
Male Wistar rats (6/dose)	0, 500, 1,500, or 3,000 mg/kg-day sodium nitrate in diet for 20 days	thyroid follicular cell hypertrophy, ↓ colloid, ↓ T4 (NS), ↑serum TSH	LOAEL: 500 mg/kg-day sodium nitrate (365 mg/kg-day nitrate)	Kostogrys et al. (2006a)
Male Wistar rats (6/dose)	0, 20, 80, or 250 mg/kg-day sodium nitrite in diet for 20 days	hypertrophy and hyperplasia of thyroid follicular epithelium, ↓ iodine absorption in the GI tract, ↓ serum T4, ↑ serum TSH (NS) ↓ serum cholesterol and high density lipoprotein	LOAEL: 20 mg/kg-day sodium nitrite (13.3 mg/kg-day nitrite)	Kostogrys et al. (2006b)
Pregnant Wistar rats (8-12 dams/conc.)	0, 17.5, 50, 450, or 900 mg/L nitrate in drinking water from gestation days 7-21	↑ plasma T4 in female fetuses (questionable due to small number of positive results)	NA[a]	Hansen et al. (2009)
Male Wistar rats (6/dose)	3 g/kg-day sodium nitrate or 250 mg/kg-day sodium nitrite in diet for 18 days	changes to thyroid follicular cells, ↓ colloid, ↓ serum T4 (NS), ↑serum TSH (nitrite)	LOAEL: 3 g/kg-day sodium nitrate and 250 mg/kg-day sodium nitrite	Kostogrys et al. (2010)

[a] Not applicable. T4 levels were below the level of detection in all samples except three fetuses in the high dose group, so the results are questionable.
NOAEL: no-observed-adverse-effect level; LOAEL: lowest-observed-adverse-effect level; NS: not significant

Structural changes to the thyroid is a significant toxicological endpoint of nitrate exposure, and has been reported in several species – rat, sheep, and pigs, although not always with statistical significance. Zaki et al. (2004) found non-statistically significant increases in thyroid weight in male Wistar rats, and decreases in body weight with exposure to 50 mg/L potassium nitrate in drinking water. However, Eskiocak et al. (2005) reported statistically significant increases in relative thyroid weight and a decrease in T3 with sodium nitrate at the same concentration in female Wistar rats. Thus, 50 mg/L sodium nitrate (23 mg nitrate/kg-day) from the Eskiocak et al. (2005) study is determined by OEHHA to be the LOAEL for thyroid effects. The enlarged thyroid reported by Zaki et al. (2004), Chaoui et al. (2004), Eskiocak et al. (2005),

Mukhopadhyay et al. (2005), and Kostogrys et al. (2006a; 2010) provide important support for the goitrogenic effects of nitrate. However, the relevance of rat thyroid toxicity studies to human health has been questioned due to substantial interspecies differences in thyroid physiology (Lewandowski et al., 2004). Comparisons of the dose-response relationships of perchlorate-related thyroidal radioactive iodide uptake (RAIU) in rodents and humans reveal that humans may be more sensitive to RAIU inhibition than rodents following subacute exposure to perchlorate for 14 days. Perchlorate is an appropriate surrogate test agent for the thyroidal effects of nitrate because, like nitrate, it also works by competitively inhibiting iodide uptake by the NIS.

The reasons why the sensitivity of rodent and human thyroids differ is unknown, but several potential mechanisms exist. NIS expression and distribution in the thyroid is more widespread in rodents than in humans. Virtually all follicular cells of the mouse and rat thyroid express NIS, whereas the distribution is uneven in human thyroid follicular cells (Josefsson et al., 2002). The rat NIS also had a higher rate of iodide uptake when expressed in vitro compared to the human NIS (Heltemes et al., 2003).

Differences in thyroid hormone protein binding between rats and humans may also affect the ability of these species to compensate for iodide uptake inhibition. In humans, most circulating thyroid hormone is bound to thyroid binding globulin (TBG), which has a very high affinity for T4, the main form of thyroid hormone. In rats, serum T4 binding is primarily to proteins that have much less T4 affinity. Because of this, the half-life of T4 in rats (12 hours) is much less than that in humans (about 5-9 days) (McClain, 1995) and the circulating reserves of thyroid hormone are less in rats than humans. Thus thyroid hormone production and serum TSH levels are markedly higher in rats than in humans (Lewandowski et al., 2004). This may explain why follicular cell tumors are much more prevalent in rats than in humans (2% incidence in rats versus 0.004% in humans (McClain, 1995).

Lewandowski et al. (2004) also evaluated differences in dose-response for perchlorate-related effects on thyroid hormone levels. Humans showed no changes in TSH at perchlorate exposures of 0.5 mg/kg-day, while rats exhibited an increase in serum TSH at perchlorate doses near 0.1 mg/kg-day. This might suggest that thyroid hormone levels in rats are more sensitive to iodide uptake disruption than those in humans. However, the human studies involved only 14 days of exposure, which is substantially shorter than the estimated time needed to exhaust thyroid hormone reserves in humans. In adult humans, thyroid hormone reserves sufficient for several months are stored in the thyroid colloid (Ahad and Ganie, 2010). Rats, however, have much less colloid, and thus most likely have less thyroid hormone reserves, which suggests that rats are less able to compensate for short-term decreases in iodide uptake or other thyroid hormone stressors compared to humans (McClain, 1995). Thus the increased rat TSH responses may be relevant to humans when thyroid hormone storage is depleted, which would presumably require longer exposure periods to iodide-uptake disrupting chemicals for humans than rodents.

In summary, these lines of evidence, including possible differences in NIS distribution and function, thyroid hormone protein binding, and short- and long-term thyroid hormone reserves, suggest significant susceptibility differences between rats (and probably other rodents) and humans. Because of these differences and the prevalence of other toxicity data, the rodent thyroid toxicity data are not selected for PHG development.

Carcinogenicity

N-Nitroso Compound Formation

A major concern regarding possible long-term effects of exposure to nitrate and nitrite is associated with formation of N-nitroso compounds, which are hepatotoxic, mutagenic, teratogenic and carcinogenic. Nitrite can be easily transformed into a nitrosating agent in an acidic environment (e.g., in the stomach) and can react with a variety of compounds such as ascorbic acid, amines, and amides to form N-nitrosamides or N-nitrosamines (IPCS, 1996; WHO, 2003). The intermediate reactions during this conversion under acidic conditions include the protonation of nitrite to nitrous acid (HNO_2), which in turn spontaneously yields dinitrogen trioxide (N_2O_3), nitric oxide (NO), nitrogen dioxide (NO_2), and nitrous oxide (N_2O). Nitrous oxide is a bioactive intermediate found to play a role in vasodilation and in defense against pathogens (Bjorne et al., 2004; Jensen, 2009; Shiva and Gladwin, 2009). Two of the other intermediates (N_2O_3 and HNO_2) can react with amides or amines to form N-nitrosamides or N-nitrosamines respectively (Hecht, 1997). At neutral pH, nitrite can be reduced by bacterial activity to form NO, which can react with molecular oxygen to form the nitrosating compounds N_2O_3 and nitrogen tetroxide (N_2O_4) (Ward et al., 2005).

The N-nitrosamines, N-nitrosoamides and N-nitrosoureas formed through the nitroso mechanism can be highly carcinogenic. According to the WHO's report, *Safety Evaluation of Certain Food Additives,* the rate of formation is often pH-dependent, and an optimum pH in the range of 2.5-3.3 is commonly observed for N-nitrosamine formation (Mirvish, 1975; Challis, 1981; Foster et al., 1981; Challis, 1985; Shephard et al., 1987 as cited in WHO, 1995). Nitrosation by nitrite is more prevalent in humans, rabbits, and guinea pigs (stomach pH 1-3) than in rats and dogs (stomach pH 3-7) (Sen et al., 1969; Rickard and Dorough, 1984). However, a recent study indicates that the gastric pH of dogs is lower than previously estimated, ranging from 1-2 in both fed and fasted dogs (Sagawa et al., 2009). This suggests that the gastric pH of dogs can be variable, and predictions of nitrate-induced nitrosation based on dog studies may not be very accurate. It has been predicted that acid-catalyzed generation of N-nitroso compounds from dietary nitrate would be maximal at the gastro-esophageal junction and cardia (Moriya et al., 2002; Suzuki et al., 2003).

As reported by WHO (1995), nitrite and nitrosatable compounds incubated with gastric fluids of animals can generate N-nitroso compounds both in vivo and in vitro (Sander et al., 1968; Alam et al., 1971a,b; Eisenbrand et al., 1974; Sen et al., 1974; Walters et al., 1979; Borzsonyi et al., 1980; Iqbal et al., 1980; Kubacki and Kupryszewski, 1980;

Ziebarth and Teichmann, 1980; Lijinsky, 1981; Ohshima and Bartsch, 1981; Rickard and Dorough, 1984; Kyrtopoulos et al., 1985a; Kyrtopoulos et al., 1985b; Yamamoto et al., 1988; Moller et al., 1989 as cited by WHO, 1995). The addition of nitrate and food products (e.g., milk, cheese, and slurried meals of fried eggs, bread, butter, cheese, biscuits, milk and luncheon meat) or tobacco smoke condensate to gastric juice mixtures has also led to N-nitroso compound formation (Walters et al., 1979; Groenen et al., 1982 as cited by WHO, 1995).

Nitrosamines have also been detected in the urine of people with urinary tract infections, but not in healthy individuals (Hicks et al., 1978; El-Merzabani et al., 1979; Eisenbrand et al., 1981, as cited by WHO, 1995).

N-nitroso compound formation by nitrites in humans has been observed by several researchers (Sander et al., 1968; Walters et al., 1979; Kubacki and Kupryszewski, 1980; Ziebarth and Teichmann, 1980 as cited by WHO, 1995). Investigators have reported the presence of N-nitroso compounds in human gastric juice following administration of meals with different nitrate contents (e.g., fish with high- and low-nitrate vegetables), nitrate, nitrite, and nitrosatable compounds (Sander and Seif, 1969; Walters et al., 1979; Groenen et al., 1982; Bellander et al., 1984 as cited by WHO, 1995). Dimethylnitrosamine and diethylnitrosamine were found in the blood of normal human subjects (Melikian et al., 1981; Lakritz et al., 1982; Yamamoto et al., 1988; Yamamoto et al., 1989 as cited by WHO, 1995) and in blood after consumption of a meal with bacon, spinach, bread and beer (Fine et al., 1977; Melikian et al., 1981 as cited by WHO, 1995). However, elevated nitrosamine levels were not always detected following nitrate or nitrate-rich meal consumption (Kowalski et al., 1980; Eisenbrand et al., 1981; Ellen et al., 1982a; Ellen et al., 1982b; Groenen et al., 1982; Lakritz et al., 1982; Yamamoto et al., 1988; Yamamoto et al., 1989 as cited by WHO, 1995).

Thus, it is clear that N-nitroso compounds can be formed in vivo after concurrent administration of nitrate or nitrite and dietary precursors (i.e., amino groups). However, whether formation of nitrosamines would pose a significant cancer risk under actual food or water intake conditions is still not clear. Confounders include the fact that there are a variety of nitrosating agents, several nitrosation pathways, and many different precursors and scavengers of N-nitroso compounds that may be present in the diet. The potential risk to human health from nitrate-derived endogenously formed N-nitroso compounds cannot be accurately estimated.

Potential Effects of N-Nitroso Compounds

For the non-carcinogenic effects of N-nitroso compounds, the National Toxicology Program (NTP) and the Agency for Toxic Substances and Disease Registry (ATSDR) reported several N-nitroso compounds to be hepatotoxic, mutagenic, and/or teratogenic. In reviews prepared for three N-nitroso compounds (N-nitroso-N-propylamine, N-nitrosodimethylamine, and N-nitrosodiphenylamine), ATSDR found that liver toxicity was associated with acute exposures to N-nitroso-N-propylamine and N-nitrosodimethylamine in animals, and chronic exposures to N-nitrosodimethylamine in

animals (ATSDR, 1989a; ATSDR, 1989b; ATSDR, 1993). Bladder toxicity in rats and mice was reported following exposure to N-nitrosodiphenylamine (ATSDR, 1993). Other N-nitroso compounds have been shown to have an allergic potential (i.e., N-nitrosodimethylamine) (ATSDR, 1989b) or are teratogenic (e.g., N-nitrosodimethylurea, ethylnitrosourea, dimethylnitrosamine) (NAS, 1981; ATSDR, 1989b; Nagao et al., 1991).

NTP has evaluated several N-nitroso compounds, with the majority of them showing positive results for genotoxicity and many producing positive results in carcinogenicity bioassays. NTP included fifteen N-nitrosamines as "reasonably anticipated to be a human carcinogen" in its Report on Carcinogens, Thirteenth Edition (NTP, 2014).

The JECFA reviewed numerous toxicological and epidemiological reports concerning the in vivo carcinogenic potential of nitrate and nitrite (WHO, 2003). Several of the N-nitroso compounds or nitrosating species have been found to be genotoxic in vitro in several tests, but the results obtained in vivo were negative. The results of carcinogenicity studies with nitrite were negative, except those in which extremely high doses of both nitrite and nitrosatable precursors were administered simultaneously or pre-mixed in the diet. The JECFA committee reviewed the epidemiological data and concluded that there was no evidence of an association between exposure to nitrite/nitrate and the risk of cancer. The committee reported no quantitative evidence of the endogenous formation of carcinogenic N-nitroso compounds at the nitrite and nitrosatable precursor intake levels achievable in the diet. As such, the JECFA decided not to conduct a quantitative risk assessment of nitrite on the basis of endogenously formed N-nitroso compounds.

Human Studies

Numerous studies evaluating the role of nitrate (and, indirectly, nitrite) in the etiology of various cancers (e.g., gastric, esophageal, urinary, brain, pancreatic, colon, and rectum) in humans have been extensively reviewed (ECETOC, 1988; Forman et al., 1988; Walker, 1990; L'hirondel and L'hirondel, 2002; WHO, 2003; IARC, 2010). Investigating associations between nitrate/nitrite exposure, N-nitroso compound formation, and cancer risk is complex due to the interplay of several variables: the amount of nitrate ingested, the concomitant ingestion of nitrosation cofactors and precursors (e.g., meats, vegetables, or vitamin C), the endogenous production and metabolism of nitrate, and specific medical conditions that increase nitrosation.

In its 2003 review, the WHO (2003) identified some studies that provided some indication of an increased risk for esophageal and gastric cancer with increasing nitrate exposure. However, other studies, including several prospective cohort studies, did not find such clear associations.

The International Agency for Research on Cancer (IARC) conducted an extensive evaluation of nitrates and nitrite and cancer (IARC, 2010), reviewing the literature through 2006. IARC concluded that there was inadequate evidence in humans for the carcinogenicity of nitrate in food or drinking water, but "limited evidence in humans for

the carcinogenicity of nitrite in food" (IARC, 2010), because "nitrite in food is associated with an increased incidence of stomach cancer." This was based primarily on the positive associations reported by six of the seven "well designed" case-control studies IARC reviewed at the time. IARC also reviewed two cohort studies of ingested nitrites and stomach cancer, one from Finland and one from the Netherlands. In the Finnish study, clear associations were not seen (Knekt et al., 1999). In the Netherlands study, a statistically significant association was seen, but was not statistically significant after adjustment for potential confounders (van Loon et al., 1998).

The following sections review cohort, case-control, and ecologic epidemiologic studies of nitrates or nitrites and cancer published since 2006. Studies are organized by organ or cancer type. Studies of nitrate in drinking water and studies of nitrate or nitrite in food were reviewed. A summary table of the studies examined here can be found in Appendix I.

Bladder cancer: Most studies of bladder cancer have either been negative (Catsburg et al., 2014; Espejo-Herrera et al., 2015) or have mostly identified associations only in particular subgroups. Ferrucci et al. (2010) reported an elevated hazard ratio (HR) of 1.28 (95% CI, 1.02-1.61; p-trend = 0.06) for subjects consuming diets with total nitrite levels in the upper quintile (median = 0.91 mg/1,000 kcal). However, the HR for high dietary nitrate intake or the HRs for nitrite from animal and plant sources separately were not elevated. A study of bladder cancer mortality in Taiwan (Chiu et al., 2007) reported a positive association (OR = 1.96; 95% CI, 1.41-2.72; p-trend <0.001) for nitrate water concentrations of 0.48-2.86 mg/L nitrate-nitrogen (median = 0.77 mg/L). Exposure data were limited to water concentrations at the residence at the time of death and there was little or no information on potentially relevant confounders like smoking, diet, or occupations. A meta-analysis of bladder cancer did not find a significant increase in relative risk (Wang et al., 2012).

Brain cancer, including glioma: In two case-control studies from Taiwan of brain cancer mortality and drinking water nitrate concentrations, an association was identified for brain cancer in children (OR = 1.40; 95% CI, 1.07-1.84 for >0.31 ppm nitrate-nitrogen) (Weng et al., 2011) but not in adults (Ho et al., 2011). Dose-response relationships were not assessed in the former (only two exposure levels were examined), and only limited information on potential confounders was available. Two cohort studies examined nitrate levels in foods and risks of glioma (Michaud et al., 2009; Dubrow et al., 2010). No association was found in either for nitrate or nitrite intake overall. In Dubrow et al. (2010) an association was observed for high nitrite intake from plant sources only (HR = 2.04; 95% CI, 1.46-2.87; p-trend = 0.0026), but not for nitrates. A meta-analysis of 6 studies revealed a significant association between dietary nitrite and adult glioma when comparing the highest vs. lowest levels of dietary nitrite intake (RR = 1.21; 95% CI, 1.03-1.42) (Xie et al., 2016).

Breast cancer: Two studies of breast cancer did not find clear evidence of associations with nitrates or nitrites in food or water (Yang et al., 2010; Inoue-Choi et al., 2012).

Some evidence of an interaction between nitrates and folate was seen in both studies, but these interactions were in opposite directions.

Colon cancer: Case-control studies from Taiwan of colon cancer mortality and residential drinking water nitrate concentrations at the time of death have produced inconsistent results (Yang et al., 2007; Chiu et al., 2010; Yang et al., 2010; Chiu et al., 2011). In a case-control study in rural Wisconsin, drinking water nitrate exposure was assessed using nitrate concentrations interpolated from a survey of 289 wells in the study area (McElroy et al., 2008). Overall, an association was seen between increasing nitrate concentrations and cancer of the proximal colon (OR = 2.76; 95% CI, 1.42-5.38) but not for cancer of the distal colon or rectum. The highest exposure category for the elevated proximal colon OR included 11 cases and 6 controls and no adjustments for diet were made. In addition, the accuracy of the interpolated exposure data is unknown. A case-control study in Pennsylvania revealed an association with the highest quintile of nitrate and nitrite in food consumption (>496.6 μg/1,000 kcals) and proximal colon cancer (OR = 1.57; 95% CI, 1.06-2.34, p-trend = 0.023) (Miller et al., 2013). Several confounders, including age, sex, body mass index, and dietary information were considered in the analysis, but nitrate and nitrite were analyzed together.

In an analysis of subjects from the large National Institutes of Health-American Association of Retired Persons (NIH-AARP) cohort (n = 300,948 men and women), Cross et al. (2010) observed an association with nitrates from processed meats (HR = 1.16; 95% CI, 1.02-1.32 for subjects in the upper quintile for consumption; p-trend = 0.001; median = 289.2 μg/1,000 kcals) for all colorectal cancers combined. However, an inverse association was seen for total dietary nitrates (HR = 0.82; 95% CI, 0.71-0.95 for subjects in the upper quintile; p-trend = 0.11). The authors noted that this may be related to the fact that the most common sources of dietary nitrates in their study were fruits and vegetables (Cross et al., 2010). In the Shanghai Women's Health Study, a prospective cohort investigation, an elevated HR for subjects in the upper quintile of dietary nitrate (median = 291.5 mg/day) was reported, but only in those who also had a low intake of vitamin C (<83.9 mg/day) (HR = 2.45; 95% CI, 1.15-5.18; p-trend = 0.02) (Dellavalle et al., 2013). Three studies of dietary nitrates or nitrites and colorectal adenomas found no clear evidence of an association (Ward et al., 2007a; Ferrucci et al., 2009; Ferrucci et al., 2012).

Esophageal cancer: Studies of nitrate in drinking water or nitrates or nitrites in food and risks of esophageal cancer have been mostly negative (Navarro et al., 2008; Ward et al., 2008; Cross et al., 2011; Liao et al., 2013). In the Netherlands Cohort Study (Keszei et al., 2013), an association with dietary nitrite and esophageal squamous cell carcinoma was seen in men (HR = 1.19; 95% CI, 1.05-1.36 for each 0.1 mg/day increase in dietary nitrite) but not in women. Zhang et al. (2012) reported a correlation between drinking water nitrate concentrations and incidence of esophageal cancer in Shexian County, China (Spearman correlation coefficient = 0.38, p = 0.01), but these data were ecologic and there was no individual level information on exposure or potential confounders. Xie et al. (2011) reported an increased risk of esophageal

carcinoma in workers exposed occupationally to sodium nitrite for 16-23 years (RR = 1.26; 95% CI, 1.08-1.46, p<0.001).

Non-Hodgkin lymphoma (NHL): Two case-control studies of NHL found associations with dietary nitrites from animal sources but not with dietary nitrates (e.g., OR = 1.35; 95% CI, 1.05-1.75 for nitrite intake from animal sources above the median, level unclear) (Kilfoy et al., 2010; Aschebrook-Kilfoy et al., 2013b). However, an analysis of the large NIH-AARP prospective cohort found no association between NHL and nitrates or nitrites from processed meats (Daniel et al., 2012). A Taiwan case-control study found no association with nitrate concentrations in drinking water, although exposure data were ecologic (Chang et al., 2010b). A small case-control study in Nebraska (Chiu et al., 2008) found an association with elevated dietary nitrite intake, but only for a specific NHL subtype and sample sizes were small (n = 60 cases for this subtype). A population case-control study in Germany revealed an increased risk of high-malignancy (OR = 2.22; 95% CI, 1.48-3.35) and low-malignancy NHL (OR = 1.45; 95% CI, 1.05-2.01) in workers occupationally exposed to nitrate, nitrite, or nitrosamines (Richardson et al., 2008).

Ovarian cancer: Aschebrook-Kilfoy et al. (2012a) studied ovarian cancer in subjects in the large NIH-AARP cohort, and identified an association with dietary nitrate (HR = 1.31; 95% CI, 1.01-1.68) in the upper exposure quintile (median = 126.5 mg/1,000 kcal), although the dose-response trend was only borderline statistically significant (p = 0.06). A similar association was seen for nitrites from animal sources. Inoue-Choi et al. (2015) examined ovarian cancer risk in postmenopausal women in the Iowa Women's Health Study, and found a significant association with drinking water nitrate concentration in the upper quartile (HR = 2.03; 95% CI, 1.22-3.38; n = 315 cases). Reduced vitamin C intake (≤190 mg/day) and increased servings of red meat (>5/week) strengthened the association. Given the novelty of their findings, the authors concluded that, "Additional confirmatory studies with a larger number of ovarian cancer cases are warranted…"

Pancreatic cancer: An ecologic case-control study of nitrate in water and a prospective cohort study of nitrates and nitrites in food did not find clear evidence of associations with pancreatic cancer (Yang et al., 2009; Aschebrook-Kilfoy et al., 2011).

Prostate cancer: An investigation of prostate cancer in the NIH-AARP cohort (n = 175,343 men; 10,313 cases; average follow-up = 9 years) found an association with high intakes of dietary nitrates (HR = 1.31; 95% CI, 1.07-1.61; p-trend = 0.03), but only for advanced prostate cancer (Sinha et al., 2009). A case-control study nested in the US Health Professionals Follow-up Study found no association between pre-diagnostic plasma concentrations of nitrate and subsequent prostate cancer (Wu et al., 2013).

Rectal cancer: Two case-control studies of rectal cancer mortality from Taiwan have reported statistically significant associations, although excess ORs were low (i.e., ORs of 1.15 and 1.36), exposure data was ecologic and based only on the residence at the time of death, and individual information on potential confounders including diet and smoking were not available (Kuo et al., 2007; Chang et al., 2010a). Colorectal cancer

was not associated with nitrate or nitrite intake (HR = 1.08; 95% CI, 0.73-1.59) in the Shanghai Women's Health Study cohort (Dellavalle et al., 2014).

Renal cancer: Studies of renal cancer have mostly been negative or have only identified associations in specific sub-analyses such as nitrite from animal sources (Dellavalle et al., 2013) or in subjects with high red meat or low vitamin C intake (Ward et al., 2007b). Similar associations in these subgroups have not been examined and confirmed in other recent studies.

Stomach cancer: A case-control study in Nebraska of nitrates in food or water involving 79 cases and 321 controls did not find an association between stomach cancer and drinking water with nitrate-nitrogen concentrations >10 mg/L for nine or more years (OR = 1.1; 95% CI, 0.5-2.3). ORs above 1.0 were seen for dietary sources of nitrates or nitrites but were not statistically significant (Ward et al., 2008). In an ecological study in Thailand, Mitacek et al. (2008) reported an association between intake of nitrate, nitrite, and nitrosamines and stomach cancer rates across the country. In a case-control study from Mexico City (257 cases and 478 controls), Hernandez-Ramirez et al. (2009) identified an association of stomach cancer with dietary nitrate from animal sources (OR = 1.92, 95% CI, 1.23-3.02; p-trend = 0.004 for intakes >3.9 mg/day). Nitrate from drinking water was not assessed. In a Netherlands Cohort Study (Keszei et al., 2013), the HR for elevated nitrite intake (upper tertile, median = 0.28 mg/day) and gastric non-cardia adenocarcinoma was 1.36 (95% CI, 1.01-1.82) with age adjustment, but was only 1.23 (95% CI, 0.89-1.70, not statistically significant) following additional adjustment for smoking, body mass index, alcohol, fruit and vegetable intake, education, and other factors. A case-control study in Taiwan found an association between nitrate-nitrogen concentration ≥0.38 ppm in water and stomach cancer (OR = 1.16; 95% CI, 1.05-1.29) (Chiu et al., 2012). Navarro et al., (2008) found an association between nitrites in food and non-cardia stomach cancer (OR = 1.88; 95% CI, 1.24-2.84). Other studies of dietary nitrate and nitrite and stomach cancer have been mostly negative (Kim et al., 2007; Cross et al., 2011). A meta-analysis of 15 gastric cancer studies revealed an inverse association between nitrate and stomach cancer, when comparing the highest vs. lowest levels of dietary nitrate intake (RR = 0.78; 95% CI, 0.67-0.91) (Xie et al., 2016).

Thyroid cancer: An investigation involving subjects from the Iowa Women's Health Study (n = 21,977 women, cases = 45; median follow-up = 9.3 years) reported a relative risk (RR) of 2.6 (95% CI, 1.1-6.2) for ≥5 years of consumption of publicly supplied drinking water with nitrate-nitrogen concentrations >5 mg/L (Ward et al., 2010). Two studies of nitrates and nitrites in food and thyroid cancer have produced mixed results (Kilfoy et al., 2011; Aschebrook-Kilfoy et al., 2013a). A meta-analysis of these three aforementioned studies showed a statistically significant association between nitrite and thyroid cancer risk (RR = 1.48, 95% CI, 1.09-2.02), but a statistically significant association with nitrate was not seen (RR = 1.36; 95% CI, 0.67-2.75) (Bahadoran et al., 2015). A meta-analysis of 2 studies revealed a significant association between dietary nitrite and thyroid cancer when comparing the highest vs. lowest levels of dietary nitrite intake (RR = 1.52; 95% CI, 1.12-2.05) (Xie et al., 2016).

Other cancers: A limited number of studies have assessed other cancer types (Mitacek et al., 2008; Freedman et al., 2010; Assesorova et al., 2011; Loh et al., 2011; Karimzadeh et al., 2012). Mitacek et al. (2008) reported an association between intake of nitrate, nitrite, and nitrosamines and liver cancer in an ecological study in Thailand, while Freedman et al. (2010) studied dietary nitrates or nitrites and liver cancer and found no association. Assesorova et al. (2011) reported a correlation between N-nitroso compounds in drinking water and digestive malignancy morbidity rates in an ecological study in Russia. Karimzadeh et al. (2012) reported in a study of dietary nitrates and nitrites in Iran a RR for lung cancer of 2.7 for elevated nitrate intake. However, few study details were provided and the confidence interval was likely incorrect. Loh et al. (2011) reported no association between dietary nitrite and any cancer type.

Summary

Overall, while a few studies have reported associations between cancer and nitrate or nitrite in water or food, consistent findings have not been seen across different studies. Several studies identified associations with nitrates or nitrates from food but not water. Some studies only identified associations in particular subgroups (e.g., low Vitamin C), but these findings have not yet been confirmed. Other studies used either ecologic or cross-sectional exposure data, or did not adequately address potential confounders. Each of these issues can potentially impact the validity of these studies.

The series of ecologic investigations of nitrate concentrations in drinking water in Taiwan and cancer mortality (Chiu et al., 2007; Kuo et al., 2007; Yang et al., 2009; Chang et al., 2010a; Chang et al., 2010b; Weng et al., 2011) have reported several positive associations. However, nitrate exposure estimates were based on the nitrate concentration of the drinking water at the residence at the time of death, without any information regarding actual exposure to the water (i.e., information on whether the subjects actually consumed tap water or for how long they may have consumed it). This is problematic since the latency between first exposure and cancer diagnosis for many chemical carcinogens can be several decades or more. Lack of information on past exposure in these studies could lead to errors in categorizing the subjects' most relevant exposures. In addition, these studies had little information on potential confounders, including smoking status, occupation, or diet. Thus it is unclear whether carcinogenesis was influenced by other factors.

Several investigations have been done in large prospective cohorts, including the NIH-AARP Diet and Health Study,[3] the US Health Professionals Follow-up Study,[4] the Iowa Women's Health Study.[5] Most of these assessed dietary nitrate and nitrite exposure

[3] http://dietandhealth.cancer.gov/
[4] https://www.hsph.harvard.edu/hpfs/
[5] http://epi.grants.cancer.gov/Consortia/members/iowawomen.html

using a food frequency questionnaire administered to subjects several years before cancer diagnosis. Most of these also collected detailed data on potential confounding factors including smoking, family history of cancer, medication use, body mass index, and various socioeconomic and dietary factors. This detailed information on confounders and the prospective design are strengths. However, potential disadvantages include the fact that diet was usually only assessed for a single point or short period of time, and information on diet from the distant past was typically not assessed. Another weakness of most of the prospective cohort studies is that information on water sources and nitrate exposures from drinking water were generally not evaluated. In OEHHA's analysis, neither the large NIH-AARP cohort study nor the large case-cohort study nested in the Netherlands Cohort Study identified clear associations between nitrites and stomach cancer (Cross et al., 2011; Keszei et al., 2013).

In conclusion, while some intriguing results from a few of these studies warrant further investigation, adequate data on nitrates and cancer with which to establish a PHG are currently not available.

Animal Studies

Several studies have evaluated the potential of nitrite to cause cancer in animals. In addition, other studies have evaluated the carcinogenic potential of concurrent administration of nitrite with other known carcinogens or tumor promoters. For the purpose of the PHG, key studies evaluating the carcinogenic potential of nitrite are reviewed and additional studies are briefly summarized. Cancer studies with positive results are summarized in Table 6.

The NTP (2001) two-year bioassays showed a marginal increase of forestomach squamous cell papilloma or carcinoma in female mice exposed to 3,000 ppm sodium nitrite in drinking water. However, no other significant neoplastic endpoints were observed in rats or mice of either sex. In fact, a decrease in the incidence of mononuclear cell leukemia in rats was observed. This phenomenon has also been observed in other studies (Maekawa et al., 1982; Lijinsky et al., 1983; Grant and Butler, 1989). NTP (2001) concluded that there was equivocal evidence of carcinogenic activity for female mice, and no evidence of it in male mice and rats of either sex.

An increase in malignant lymphomas was observed in rats exposed to sodium nitrite in food or water for up to 26 months (Newberne, 1979). However, in a blinded re-evaluation of slides from 25% of the animals diagnosed with cancer in that study (Newberne, 1979), two independent pathologists, one from the US Food and Drug Administration and one from the National Cancer Institute, disagreed substantially with the study author's diagnoses (US GAO, 1980). A significant increase in total hepatocellular neoplasms in the liver of female rats given 2,000 ppm sodium nitrite in drinking water for 104 weeks was observed (Lijinsky et al., 1983). An increase in liver tumors was observed in male rats administered sodium nitrate at 1,600 ppm in the diet for 646 days (Aoyagi et al., 1980) or at 0.16% in the diet for 480 days (Matsukura et al.,

1977). A slight increase in lung tumors and lymphomas was observed in $B6CF_1$ mice exposed to sodium nitrite in drinking water for their entire lifetime (including in utero), but the effect was not dose-dependent (Anderson et al., 1985).

Table 6. Carcinogenicity studies with positive results in experimental animals following nitrite administration

Sex/Species	Dose/Route of Exposure	Endpoints	Reference
Female B6C3F1 mice (50/sex/conc.)	0, 750, 1,500, or 3,000 ppm sodium nitrite in drinking water for 104-105 weeks	Forestomach squamous cell papilloma or carcinoma (1/50, 0/50, 1/50, 5/50)	NTP (2001)
Male $B6CF_1$ mice (51-54/dose)	0, 30.7, or 310 mg/kg-day sodium nitrite in drinking water for lifetime (including in utero exposure)	Lung tumors (30/52, 41/52, 38/54) Lymphoma (7/52, 16/52, 6/54)	Anderson et al. (1985)
Female F344 rats (24/conc.)	0 or 2,000 ppm sodium nitrite in drinking water or food for 104 weeks	Hepatocellular neoplasms in females (combined incidences for both exposure routes: 8/48, 27/48)	Lijinsky et al. (1983)
Male Wistar rats (24/conc.)	0, 800, or 1,600 ppm sodium nitrite in pellet diet for 646 days	Hepatocellular tumors (0/19, 1/22, 5/19)	Aoyagi et al. (1980)
Male and female Sprague-Dawley rats (136/conc.)	0, 250, 500, 1,000, or 2,000 ppm sodium nitrite in agar gel, drinking water, or chow diet starting in utero and continuing for up to 26 months	Increased incidences of malignant lymphomas in all treated groups (10.2% vs. 5.4% in controls)	Newberne (1979)
Male Wistar rats (5 or 10/conc.)	0 or 0.16% sodium nitrite in pellet diet for 480 days	Hepatocellular tumors (0/10, 3/4[a])	Matsukura et al. (1977)

[a] One animal died of pneumonia at 11 months without any detectable tumors

It is difficult to determine if exposure to nitrite caused cancer in these studies due to various shortcomings, including the use of a single administered dose (Matsukura et al., 1977; Lijinsky et al., 1983), non-monotonic dose-responses (Anderson et al., 1985), low sample size (Matsukura et al., 1977; Aoyagi et al., 1980), and high background cancer rates (Anderson et al., 1985). Additionally, many other studies reported no significant increases in tumor incidence in rodents following chronic exposure to nitrate or nitrite alone (Greenblatt et al., 1971; Shuval and Gruener, 1972; Greenblatt and Mirvish, 1973; Taylor and Lijinsky, 1975; Inai et al., 1979; Maekawa et al., 1982; Grant and Butler, 1989; Hawkes et al., 1992; Mascher and Marth, 1993; Yoshida et al., 1993). Thus, it is unclear whether nitrate or nitrite alone is carcinogenic in rodents as the evidence for carcinogenicity is equivocal.

An extensive series of studies at the Japanese National Institute of Health Sciences and several universities in Japan has addressed the effect of concurrent administration of sodium nitrite in drinking water and known carcinogens or tumor promoters. In general, nitrite administration in drinking water increased the incidence of forestomach tumors (Hirose et al., 1993; Kawabe et al., 1994; Yoshida et al., 1994; Miyauchi et al., 2002;

Okazaki et al., 2006; Kuroiwa et al., 2007), kidney tumors (Furukawa et al., 2000a; Furukawa et al., 2000b), and colon and Zymbal's gland tumors (Kitamura et al., 2006) in rats previously exposed to a tumor-initiating compound. According to the authors, concurrent exposure to ascorbic acid enhanced tumor promotion, possibly due to oxidative DNA damage (Okazaki et al., 2006; Kuroiwa et al., 2008b). Nitrite alone, and in the presence of antioxidants (such as ascorbic acid, alpha-tocopherol, propyl gallate, and butylated hydroxyanisole, among others) induced forestomach lesions and hyperplasia, but did not appear to drive tumorigenesis (Hirose et al., 1993; Kawabe et al., 1994; Miyauchi et al., 2002; Okazaki et al., 2006; Kuroiwa et al., 2008a). These findings provide some limited and weak evidence of the potential carcinogenicity of nitrite under certain conditions, but are inadequate for a quantitative extrapolation of possible carcinogenicity to humans.

Overall, the cancer bioassay data do not provide adequate evidence that nitrate or nitrite alone induces cancer in laboratory animals. Thus, OEHHA cannot develop a PHG based on the carcinogenicity of nitrate or nitrite in animals.

Additional Toxicities

Acute Toxicity

Human Studies

The lethal oral dose of nitrate (as potassium nitrate) for an adult human has been estimated to be between 4 and 50 g, or about 67 to 830 mg/kg (WHO, 2011). In adults, light methemoglobinemia, diarrhea, and vomiting were reported following doses of >100 mg/kg-day of nitrate. Increased metHb in blood has been observed with nitrate doses from 33 to 150 mg/kg-day (Walker, 1990). For nitrite, the lethal dose in adults was estimated to range from 33 to 250 mg/kg (WHO, 2011).

Animal Studies

Oral LD_{50} values for sodium nitrate were 2,480-6,250 mg/kg in mice, 4,860-9,000 mg/kg in rats and 1,600 mg/kg in rabbits (Corré and Breimer, 1979 as cited in OEHHA, 1997). The LD_{50} in cows following a single oral administration of sodium nitrate was estimated to be 450 mg/kg (WHO, 2011). The lethal dose of nitrate in cows appeared to be one-tenth that in non-ruminants (Gwatkin and Plummer, 1946; Emerick, 1974 as cited in OEHHA, 1997). LD_{50} values for sodium nitrite in rodents vary between 85 and 220 mg/kg (WHO, 2011).

Cardiovascular effects of nitrite and organic nitrates included a fatal drop in blood pressure in cows, dilation and thinning of the intramuscular coronary blood vessel, and vasodilating properties in rats (WHO, 2003). It was reported that intravenous administration of 36 mmol/L potassium nitrite (at 500 µg/kg) to anaesthetized rats caused transient hypotension (Vleeming et al., 1997). Nitrite induced an immediate, dose-dependent decrease in blood pressure of 10-20% with a single intravenous dose

of 30 µmol/kg (417 µg/kg). Earlier studies with sodium nitrate administered at 100 µmol/kg over 5 minutes observed no effect on mean arterial pressure. Potassium nitrite caused a decrease in mean arterial pressure and increased the heart rate, while potassium chloride had no effect (WHO, 2003). Rats given drinking water containing 50-75 mmol/L sodium nitrite for 4, 8, and 12 months (50 mg/kg-day and higher) showed a decrease in arterial blood pressure compared to controls (Haas et al., 1999). Based on the review of the data, JECFA and WHO determined that a dose of 50 mg/kg or higher, expressed as nitrite ion, lowered blood pressure by causing vasodilation (WHO, 2003). The Committee further calculated that an oral dose equivalent to 160 mg/kg of nitrate ion could induce a reduction in blood pressure of 15-20 mm Hg.

More recently, Blood and Power (2007) reported that a significant portion of nitrite is metabolized through pathways that do not produce metHb. By evaluating the in vivo and in vitro disappearance of nitrite and presence of metHb, the authors proposed that the vasodilating effects of nitrite are potentiated under hypoxic conditions due to the reduction of nitrite to nitric oxide by deoxyhemoglobin.

Subchronic Toxicity

Human Studies

There are no additional subchronic studies in human subjects identified.

Animal Studies

Studies in laboratory animals have shown that nitrate or nitrite can affect several systems following subchronic exposure. Sodium nitrate (50 or 100 mg/kg-day) administered via intraperitoneal injection caused spleen, liver, and kidney damage in mice, but the slight histopathological effects were reversible (WHO, 2011). In rats, no effects were observed at a dose of 500 mg/kg-day given for 4 weeks. Female rats exposed to 1,500 mg potassium nitrate/kg-day or higher had slightly elevated metHb levels and the male rats showed increased relative kidney weights (WHO, 2011). Increased metHb was also observed in F344 rats treated for 6 weeks with 5,000 or 10,000 mg/kg-day potassium nitrate. A 14-month study reported no metHb increases in rats given drinking water that provided a sodium nitrate dose of 400 mg/kg-day (WHO, 2011). Based on these studies, metHb appears to increase in rodents at doses above 400 or 500 mg/kg-day.

Additional subchronic toxicity studies were evaluated (Maekawa et al., 1982; Til et al., 1988; Til et al., 1997; Panesar and Chan, 2000; NTP, 2001; WHO, 2003; Sharma et al., 2011; Sharma et al., 2013), and the study results are summarized in Table 7. In general, rodents orally exposed to nitrites had higher levels of metHb, increased incidences of forestomach hyperplasia, adrenal zona glomerulosa hypertrophy, and changes in organ weights.

Table 7. Subchronic toxicity studies of nitrate and nitrite in laboratory animals

Sex/Species	Dose/Route of Exposure	Endpoint	NOAEL/ LOAEL	Reference
Male/female F344 rats (10/sex/conc.)	0, 0.06, 0.125, 0.25, 0.5, or 1% sodium nitrite in drinking water for 6 weeks	↑ metHb ↓ weight gain at highest concentration	NOAEL: 0.5% for decreased body weight	Maekawa et al. (1982)
Male/female F344 rats (10/sex/conc.)	0, 1.25, 2.5, 5, 10, or 20% sodium nitrate in diet for 6 weeks	↑ metHb ↑ mortality at highest dose ↓ weight gain (conc. of 10% for females, 20% for males)	NOAEL: 5% for decreased body weight	
Male/female Wistar rats (10/sex/conc.)	0, 100, 300, 1,000, or 3,000 ppm potassium nitrite in drinking water for 13 weeks	↑ adrenal zona glomerulosa hypertrophy ↑ metHb	NOAEL: 100 ppm (10 mg/kg-day) for adrenal zona glomerulosa hypertrophy	Til et al. (1988)
Male Wistar rats (10/conc.)	0 or 36 mmol/L potassium nitrite or potassium nitrate in drinking water for 90 days	Nitrite: ↑ adrenal zona glomerulosa hypertrophy, cyanosis ↑ metHb	LOAEL: 36 mmol/L (500 mg/kg-day) for all endpoints	Boink et al. (1996) as cited by WHO (2003)
Male/female Wistar rats (10/sex/conc.)	0, 12.5, 25, 50, 100, or 3,000 ppm of potassium nitrite and 81 or 2,432 ppm sodium nitrite in drinking water for 13 weeks	↑ adrenal zona glomerulosa hypertrophy	NOAEL: 50 ppm potassium nitrite for adrenal zona glomerulosa hypertrophy	Til et al. (1997)
Male Sprague-Dawley rats (6/conc.)	*50 mg/L sodium nitrate or sodium nitrite in distilled water for 4 weeks	↓ blood corticosteroid and testosterone levels		Panesar & Chan (2000)
Male/female B6C3F1 mice (50/sex/conc.)	0, 375, 750, 1,500, 3,000, or 5,000 mg/L sodium nitrite in drinking water for 14 weeks	↑ forestomach hyperplasia ↑ spleen hematopoiesis **Males**: ↓ body weight, ↑ relative spleen weight ↓ sperm motility ↑ testes degeneration **Females**: ↑ absolute and relative heart, liver, spleen, and kidney weight longer estrous cycle	NOAEL: 750 mg/L (240 mg/kg-day for females) for spleen hematopoiesis	NTP (2001)
Male/female F344/N rats (50/sex/conc.)	0, 375, 750, 1,500, 3,000, or 5,000 mg/L sodium nitrite in	↑ metHb ↑ erythropoietic activity ↑ forestomach hyperplasia	LOAEL: 375 mg/L (30 mg/kg-day for males) for	

	drinking water for 10-14 weeks	↑ relative kidney and spleen weight ↑ reticulocyte counts cyanosis (mouth, tongue, ears, and feet) brown discoloration in the eyes **Males**: ↓ sperm motility	metHb formation	
Rabbits (unspecified sex) (2/conc.)	*45, 100, 200, 400, or 500 mg/L nitrate in drinking water, and food was soaked in nitrate-contaminated water for 120 days	↑ respiratory epithelial hyperplasia ↑ inflammatory cells and lymphocytes in alveolar space ↑ heart rate and respiration	NOAEL: 45 mg/L for respiratory epithelial hyperplasia and inflammation	Sharma et al. (2011)
		↑ hepatic inflammation and necrosis cyanosis and lethargy ↑ portal triaditis ↑ heart rate and respiration	NOAEL: 45 mg/L for inflammation and necrosis	Sharma et al. (2013)

* There was no untreated control group in this study.

Chronic Toxicity (Non-Carcinogenic)

Human Studies

A series of epidemiological studies were published by Gupta and colleagues that examined the relationship between nitrate water concentration and various toxicity endpoints, particularly in children (Gupta et al., 1999a; Gupta et al., 1999b; Gupta et al., 2000; Gupta et al., 2001). For the studies focusing exclusively on children's health, eighty-eight randomly selected children up to eight years of age from five different villages in Rajasthan, India (with drinking water concentrations of 26, 45, 95-100, 220-222, and 449-459 mg/L nitrate) were selected for these studies. The authors reported significant correlations between metHb levels in children and recurring respiratory tract infections, and nitrate concentrations and recurrent diarrhea (Gupta et al., 2000; Gupta et al., 2001). In another study where larger populations with more extensive age ranges were examined, the authors determined a significant correlation between cytochrome b5 reductase activity and recurrent stomatitis (Gupta et al., 1999b). Although the subjects from each village were matched for age, socioeconomic status, and food habits, it is unclear if any other confounding factors were considered in these analyses. The cross-sectional nature of these studies, the lack of details of the statistical analyses, and the unusual dose-response relationships seen for several findings make these studies difficult to interpret.

Animal Studies

The non-carcinogenic effects of chronic exposure to nitrate or nitrite in experimental animals have been described in several studies. In general, decreases in body weight

Table 8. Non-carcinogenic chronic toxicity studies of nitrate and nitrite in laboratory animals

Sex/Species	Dose/Route of Exposure	Endpoints	NOAEL/LOAEL	Reference
Male/Female F344/N rats (50/sex/conc.)	0, 750, 1,500, or 3,000 ppm sodium nitrite in drinking water for 105 weeks	↓ mean body weight (3,000 ppm) ↓ water consumption ↑ forestomach epithelial hyperplasia (3,000 ppm) ↑ liver inflammation	NOAEL: 1,500 ppm (80 mg/kg-day for females) for body weight decrease and forestomach hyperplasia	NTP (2001)
Male/Female B6C3F1 mice (50/sex/conc.)	0, 750, 1,500, or 3,000 ppm sodium nitrite in drinking water for 104-105 weeks	↓ mean body weight in females (3,000 ppm) ↓ water consumption ↑ glandular stomach epithelial hyperplasia in males (3,000 ppm)	NOAEL: 1,500 ppm (120 mg/kg-day for males) for decreased body weight in females and stomach hyperplasia in males	
Female NMRI mice (100/conc.)	0, 100, or 1,000 mg/L calcium nitrate in drinking water for 18 months	**high dose**: ↓ body weight ↓ survival rate ↑ urea and ammonium concentration	NOAEL: 100 mg/L for all endpoints	Mascher and Marth (1993)
Male Sprague-Dawley rats (9-12/conc.)	0 or 2,000 mg/L sodium nitrite, or 4,000 mg/L sodium nitrate in drinking water for 14 months	↓ body weight ↑ lung lesions ↑ lung weight ↓ vitamin E in plasma **Nitrite only**: ↑ metHb ↓ liver weight ↑ GSH in red blood cells	LOAEL: 2,000 mg/L sodium nitrite and 4,000 mg/L sodium nitrate for all endpoints	Chow et al. (1980)
Male Sprague-Dawley rats (8/conc.)	0 or 200 mg/L sodium nitrite, or 400 mg/L sodium nitrate in drinking water for 16 weeks	**Nitrite only**: slight ↑ metHb range and lung weight	NOAEL: 400 mg/L sodium nitrate LOAEL: 200 mg/L sodium nitrite for all endpoints	
Male rats (strain not specified) (8/conc.)	0, 100, 1,000, 2,000, or 3,000 mg/L sodium nitrite in drinking water for 24 months	↑ metHb histopathological changes in liver, spleen, kidney, heart, and lungs ↑ EEG wave frequency and paroxysmal outbursts	NOAEL: 100 mg/L for increased metHb and lung histopathology	Shuval and Gruener (1972)
Male black 6J mice (57/conc.)	0, 100, 1,000, 1,500, 2,000 mg/L sodium nitrite in drinking water (duration not specified, but called chronic)	↑ metHb ↓ motor activity	Specific dose-response data not available	

and water consumption, increased metHb levels, and stomach epithelial hyperplasia were observed. These results are summarized in Table 8.

Genotoxicity

The genotoxicity of nitrate and nitrite have been reviewed by other authoritative bodies (IARC, 2010; Health Canada, 2013). In general, nitrate produced mostly negative results in several in vitro and in vivo genotoxicity assays although micronucleus formation in mouse erythrocytes in vivo and chromosomal aberrations in bone marrow cells in rats and mice in vivo and Chinese hamster fibroblast cells in vitro were reported following nitrate exposure (IARC, 2010). However, the chromosomal aberrations in Chinese hamster fibroblasts may have been induced by osmotic pressure and sodium ion concentration (IARC, 2010). Nitrite, on the other hand, produced more positive results in in vitro and in vivo genotoxicity assays compared to nitrate, including mutations in *Salmonella typhimurium* and *Drosophila melanogaster*, chromosomal aberrations, cell transformations, and aneuploidy (IARC, 2010).

Tsezou et al. (1996) investigated the potential genotoxicity of nitrate in drinking water. The frequencies of sister chromatid exchange and chromatid or chromosomal aberrations were studied in peripheral blood lymphocytes of 70 children ages 12-15 who were permanent residents of areas of Greece with high nitrate concentrations in drinking water (56-88 mg/L). The control group comprised 20 healthy children living in areas with a low nitrate concentration in drinking water (0.7 mg/L). The mean number of sister chromatid exchanges per cell was not increased significantly in the high-nitrate group (89 mg/L) compared to the other groups evaluated (70-73, 56 and 0.7 mg/L nitrate). A significant increase was observed in the mean number of chromatid or chromosomal breaks in children with nitrate intakes >70 mg/L ($p<0.01$), compared to controls, but no statistically significant increase was observed between the 56 mg/L group and the controls. The study implies that intake of water contaminated with >70 mg/L nitrate has the capability of inducing cytogenetic effects.

In earlier studies, nitrates under anaerobic conditions were shown to have mutagenic activity in microbial tests. In addition, nitrite was found to be mutagenic in cultured mammalian cells (Walker, 1990). Under anaerobic conditions, the active species may be nitrite, formed via reduction of nitrate (Walker, 1990). Mutagenic effects were also observed in in vivo and in vitro experiments using Syrian hamsters. In vivo assays have produced equivocal results, with both positive and negative results reported (Walker, 1990).

Genetic toxicology studies have been conducted in *S. typhimurium*, rat and mouse bone marrow, and mouse peripheral blood. NTP (2001) reported that sodium nitrite was mutagenic in *S. typhimurium* strain TA100, with and without Aroclor 1254-induced hamster and rat liver enzymes. However, no mutagenicity was observed in strain TA98. No evidence of bone marrow micronucleus formation was noted in male mice and rats following intraperitoneal injections of sodium nitrite. Also, a micronucleus test using

peripheral blood from mice treated for 14 weeks via intraperitoneal injection showed no evidence of genetic toxicity (NTP, 2001).

In a cell suspension of *E. coli* treated with either nitrate or nitrite and then exposed to simulated sunlight, an increased incidence of mutations was observed over that of sunlight exposure alone (Salih, 2006). Further research is needed to evaluate the risk associated with sun and nitrate/nitrite co-exposure.

The NTP (2001) genotoxicity studies show that sodium nitrite was mutagenic in a test for reverse mutation, but did not induce micronucleus formation in the bone marrow or peripheral erythrocytes of mice treated in vivo. These findings are consistent with earlier studies.

In summary, nitrate does not appear to be genotoxic. However, nitrite produced many more positive results both in vivo and in vitro compared to nitrate. The overall body of evidence suggests that nitrite may be genotoxic.

Developmental and Reproductive Toxicity

Human Studies

A need for further assessment of the possible effects of nitrate on human reproduction was stated in OEHHA's previous PHG document (OEHHA, 1997). The statement was based on animal studies that suggested an association between maternal nitrate exposure from drinking water and developmental effects in offspring (Fan et al., 1987; Bruning-Fann and Kaneene, 1993; Fan and Steinberg, 1996), plus limited human evidence of these effects (CDC, 1996). However, no causal link or cause-and-effect relationship was noted in the available human studies. Furthermore, OEHHA's Developmental and Reproductive Toxicant (DART) Identification Committee, an expert group of scientists, reviewed the available animal experimental and human epidemiologic studies for sodium nitrite in 2000 and concluded that sodium nitrite has not been clearly shown to cause reproductive toxicity (OEHHA, 2000).

Ward et al. (2005) presented a summary of several epidemiological studies evaluating the potential reproductive effects (e.g., spontaneous abortions, stillbirths, premature birth, or intrauterine growth retardation) associated with nitrate exposure in drinking water (Table 9). The authors reported that the reproductive outcomes were inconsistent across the various studies evaluated. However, the studies were not conducted under the same experimental conditions (i.e., exposure periods, nitrate levels, and administered cofactors), so total endpoint concordance across the studies would not be expected. Although there are shortcomings within the summarized studies, some reports did find an association between nitrate and congenital malformations (e.g., central nervous system or neural tube defects) (Dorsch et al., 1984; Arbuckle et al., 1988; Croen et al., 2001; Brender et al., 2004b). In some studies, adverse reproductive outcomes were observed with low nitrate levels in drinking water (≤5.5 mg/L)

Table 9. Studies of the relationship between drinking water nitrate[a] and reproductive outcomes (adapted from Ward et al., 2005)

Study Population; Design	Measurement of Water Nitrate	Reproductive Outcome[b]	Reported Findings	Reference
South West Africa; Cross-sectional study	Water sample taken from well used at time of home visit	Spontaneous premature labor, size of infant at birth	No association between water from high nitrate regions and prematurity or size of infant	Super et al. (1981)
South Australia; Case-control study	Address at delivery linked to sources of water and data on nitrates	Congenital malformations	Elevated ORs for congenital malformations associated with nitrate levels ≥5 mg/L relative to nitrate levels <5 mg/L in groundwater: any congenital malformation (OR = 2.8; 95% CI, 1.6-4.4); malformations of the CNS (OR = 3.5; 95% CI, 1.1-14.6); musculoskeletal system malformation (OR = 2.9; 95% CI, 1.2-8.0)	Dorsch et al. (1984)
Canada-New Brunswick; Case-control study	Collected water sample at maternal residence	Congenital CNS malformations	OR = 2.3 (95% CI, 0.73-7.29) for CNS malformations with exposure to nitrate at 26 mg/L relative to baseline of 0.1 mg/L	Arbuckle et al. (1988)
All Sweden; Case-control study	Earliest known maternal address linked to water nitrate results	NTDs	Average water nitrate similar between cases and controls. Nitrate not linked to NTD etiology.	Ericson et al. (1988)
USA-MA residents; Hospital case-control study	Matched maternal residence at pregnancy outcome to tap water nitrate	SBs through 27 weeks of gestation	OR = 0.5 (95% CI, 0.2-0.9) for SB with exposure to water nitrate levels of 0.1-5.5 mg/L relative to non-detectable levels	Aschengrau et al. (1989)
USA-MA residents; Hospital case-control study	Matched maternal residence during pregnancy or outcome to tap water nitrate	Congenital anomalies, stillbirths, neonatal deaths	Stillbirths and congenital anomalies not associated with water nitrate (0.2-4.5 mg/L); small positive association between water nitrates and neonatal deaths.	Aschengrau et al. (1993)

Study Population; Design	Measurement of Water Nitrate	Reproductive Outcome[b]	Reported Findings	Reference
USA-IN residents; Cluster investigation	Wells tested for nitrates after cluster reported	SBs	Water nitrate above US EPA MCL for women with SBs	Centers for Disease Control and Prevention (1996)
Canada-Prince Edward Island; Case-control study	Residential postal code at time of delivery linked to nitrate map	IUGR, premature birth	Dose-response relationship between nitrate level and ORs for IUGR and prematurity (OR >1 above median nitrate level of 3.1 mg/L)	Bukowski et al. (2001)
USA-CA; Case-control study	Linked periconceptional addresses to water companies and databases	NTDs	Exposure to water (groundwater, surface water, and mixed water) nitrates >45 mg/L associated with anencephaly (OR = 4.0; 95% CI, 1.0-15.4) but not with spina bifida; increased risks for anencephaly at nitrate levels below the CA MCL (45 mg/L) among groundwater drinkers only; dietary nitrate and nitrite not associated with NTDs	Croen et al. (2001)
Sweden; Retrospective cohort study	Linked periconceptional address to water supplies using a geographic information system	Any congenital cardiac defect	Weak association (OR = 1.18; 95% CI, 0.97-1.44) between water nitrate ≥2 mg/L and cardiac malformations	Cedergren et al. (2002)
USA counties along Texas-Mexico border; Case-control study	Usual periconceptional drinking water source tested for nitrates	NTDs	OR = 1.9 (95% CI, 0.8-4.6) if water nitrates ≥3.52 mg/L; higher water nitrate levels (not specified in abstract) associated with spina bifida (OR = 7.8; 95% CI, 1.6-74.6) but not with anencephaly (OR = 1.0; 95% CI, 0.3-2.7); slightly inverse relationship between dietary nitrite, total nitrite intake and NTDs	Brender et al. (2004a)

[a]Nitrate units are mg/L as nitrate.
[b]Abbreviations: CNS, central nervous system; IUGR, intrauterine growth retardation; SB, spontaneous abortion; NTDs, neural tube defects; OR, odds ratio; CI, confidence interval; MCL, Maximum Contaminant Level.

(Bukowski et al., 2001) whereas other studies reported no adverse effects with higher nitrate levels (20 mg/L) (Super et al., 1981).

Manassaram et al. (2006) studied the relationship between nitrates in drinking water and reproductive and developmental outcomes via a literature search of publications in English through January 2004 for both animal and human studies. The authors concluded that the epidemiological literature reviewed did not provide sufficient evidence of a causal relationship between exposure to nitrates in drinking water and adverse reproductive effects. The evidence for an increased risk of adverse reproductive and developmental outcomes in humans due to nitrate in drinking water was stated to be sparse and suggestive at best.

Although some epidemiological studies have suggested an association of nitrate exposure with reproductive and development effects (e.g., spontaneous abortions, intrauterine growth retardation, and various birth defects), the uncertainties in the epidemiologic studies (e.g., limited exposure information and multiple confounding factors, including other contaminants) prevent establishment of a causal relationship. OEHHA concludes that the epidemiologic evidence does not provide a sufficient exposure-response relationship between drinking water nitrate and adverse reproductive effects either to identify any specific reproductive effects caused by nitrate exposure or to quantify an effect level.

Animal Studies

Most of the attention regarding the developmental and reproductive toxicity potential of nitrite and nitrate has been directed toward nitrite. The earlier nitrate studies did not demonstrate a consistent association of levels of nitrate in drinking water with congenital malformations and cardiovascular effects (WHO, 2011). In addition, no evidence of teratogenic effects was reported by Fan et al. (1987) in their review of the reproductive toxicity data. However, increased levels of nitrite and metHb were reported in pregnant rats given 2.5-50 mg/kg sodium nitrite in drinking water or by intraperitoneal injection (Fan et al., 1987; Fan and Steinberg, 1996). Selected animal developmental and reproductive toxicity studies are summarized in Table 10.

Table 10. Developmental and reproductive toxicity studies of nitrate and nitrite in laboratory animals

Sex/Species	Dose/Route of Exposure	Endpoints	NOAEL/ LOAEL	Reference
Pregnant albino "sabra" rats (12 dams/conc.)	0, 2,000 or 3,000 mg/L sodium nitrite in drinking water (duration not specified)	**maternal:** ↑ metHb ↑ anemia **fetal:** ↑ mortality ↓ weight gain	LOAEL: 2,000 mg/L sodium nitrite	Shuval and Gruener (1977)
Pregnant Long Evans rats (5-12/conc.) and pups	**dams:** 0[a], 0.5, 1, 2, or 3 g/L sodium nitrite in drinking water throughout gestation **pups:** lactational exposure and 0, 0.5, 1, 2 or 3 g/L sodium nitrite in drinking water post-weaning	↓ body weight, ↑ mortality, ↑ anemia, ↓ hemoglobin, ↓ red blood cell count, ↓ mean corpuscular volume, iron deficiency syndrome effects in pups; no observed toxicity in dams	NOAEL: 0.5 g/L sodium nitrite for reduced mean corpuscular volume in pups LOAEL: 2 g/L sodium nitrite for signs of anemia in pups	Roth et al. (1987); Roth and Smith (1988)
Pregnant ICR mice (12-15/conc.)	0, 100 or 1,000 mg/L sodium nitrite in drinking water from GD07 to GD18	No observed effects	NOAEL: 1,000 mg/L sodium nitrite	Shimada (1989)
Male and female Swiss CD-1 mice (sample size not reported)	0, 125, 260, or 425 mg/kg-day sodium nitrite in drinking water across 2 generations	↓ pup growth to weaning in F_1 generation; pups were otherwise healthy	NOAEL: 425 mg/kg-day sodium nitrite	Chapin et al. (1997)
Swiss male mice (5/conc.)	0, 90, 200, 500, 700, or 900 ppm potassium nitrate in drinking water for 35 days	900 ppm: ↓ sperm count ↓ sperm mobility ↑ abnormal sperm ↑ testicular atrophy	NOAEL: 700 ppm potassium nitrate	Pant and Srivastava (2002)
Pregnant Wistar rats (8-12/conc.) and male pups	0, 17.5, 50, 150, 450, or 900 mg/L sodium nitrate in drinking water from GD07 to GD21	↑ plasma T4 in female fetuses at highest dose (questionable due to small number of positive results)[b]	NOAEL: 900 mg/L sodium nitrate	Hansen et al. (2009)

[a]The control consisted of tap water, presumably with 0 mg/L nitrate but was not specified in the study
[b]T4 levels were below the level of detection in all samples except three fetuses in the high dose group, so the results are questionable.
GD, gestation day

Immunotoxicity

Human Studies

No human immunotoxicity studies have been identified for nitrate or nitrite.

Animal Studies

An immunosuppressive effect was reported by Abuharfeil et al. (2001) for sodium nitrite. Sublethal doses of 0, 25, 50 or 100 mg/kg of sodium nitrite in drinking water were given to Balb/c mice for 21 days. Immune changes were determined 1 day, 1 week, and 3 weeks after the last exposure to sodium nitrite. The authors reported that sodium nitrite caused dose-dependent, reversible immunosuppressive effects, which included decreases in lymphocyte counts, natural killer cell activity, IgM and IgG titers. The maximum suppressions were obtained at the 24-hr observation point with 100 mg/kg-day sodium nitrite. Dose-dependent increases in neutrophil count (up to 71.3%) at 24 hours and phagocytic activation up to 133% at the 24-hour and 1-week evaluation points were also found (Abuharfeil et al., 2001).

DOSE-REPONSE ASSESSMENT

In several studies, a higher incidence of methemoglobinemia in infants and children was observed in areas where the water contained ≥50 mg/L nitrate. Sadeq et al. (2008) clearly showed a significant increase in metHb in children exposed to drinking water containing >50 mg/L nitrate. Several other investigators found similar results in neonates and children (Bosch et al., 1950; Walton, 1951; Vigil et al., 1965; Toussaint and Selenka, 1970; Shuval and Gruener, 1977; Ayebo et al., 1997; Knobeloch and Proctor, 2001). The Gupta et al. (1999) study is not considered for dose-response analysis because metHb values in all exposure groups at any age were significantly higher than 2% without showing any consistent dose-response relationships, and the study did not provide results for non-exposed controls. Other studies (Johnson and Kross, 1990; Kross et al., 1992; Savino et al., 2006; Abu Naser et al., 2007) did not contain enough information to use for nitrate risk assessment.

After reviewing the literature on nitrate and nitrite since the publication of the PHG in 1997, OEHHA concludes that methemoglobinemia remains the primary adverse health effect associated with human exposure to these chemicals. Thus, OEHHA is retaining this critical endpoint and its supporting studies for PHG derivation. Due to the prevalence of human data, animal studies for this endpoint are not being used for point of departure determination.

Infants under the age of 3 months are especially vulnerable to methemoglobinemia since fetal hemoglobin is more readily oxidized to metHb and metHb reductase is less effective in the reduction of metHb in the neonate. In addition to the greater physiological propensity for methemoglobinemia in infants, this age group also has the highest water ingestion proportional to body weight. The 95th percentile water intake

rate estimated by OEHHA (2012) is 0.294 L/kg-day for infants 0-3 months of age, which is considered the most sensitive population for metHb effects.

To determine a health-protective concentration for nitrate in drinking water based on metHb increase, OEHHA considered the studies of Bosch et al. (1950), Walton (1951), and Sadeq et al. (2008). In these studies, intake of drinking water containing ≥50 mg/L nitrate lead to increases in metHb levels, whereas lower levels of nitrate did not. A concentration of 45 mg/L nitrate (10 mg nitrate-nitrogen/L) was not associated with any cases of methemoglobinemia (Bosch et al., 1950; Walton, 1951). At 50-90 mg/L nitrate (11 to 20 mg nitrate-nitrogen/L), 2% of infants exposed were reported to have methemoglobinemia; at 220 mg/L nitrate (50 mg nitrate-nitrogen/L), more than 80% of infants had methemoglobinemia (Walton, 1951). Thus, the NOAEL for nitrate is identified as 45 mg/L or 13.2 mg/kg-day in infants using the water consumption rate of 0.294 L/kg-day (OEHHA, 2012).

PHG DERIVATION

For estimation of a health-protective concentration of a chemical in drinking water, an acceptable daily dose (ADD) of the chemical from all sources is first calculated. This involves incorporation of appropriate estimates of uncertainty in the extrapolation of the critical toxic dose from human or animal studies to the estimation of a lifetime ADD that is unlikely to result in any toxic effects. For this purpose, the following equation is used:

$$\text{ADD} = \frac{\text{POD}}{\text{UF}}$$

where,

ADD = acceptable daily dose, an estimate of the maximum daily dose that can be consumed by humans for an entire lifetime without toxic effects;

POD = point of departure, in units of milligrams per kilogram of body weight per day (mg/kg-day); this can be the no-observed-adverse-effect level (NOAEL), lowest-observed-adverse-effect level (LOAEL), or the lower limit of the 95% confidence interval of the benchmark dose estimated from the critical study (BMDL);

UF = uncertainty factor(s). Default uncertainty factors for PHG derivation can be found in Appendix II.

Calculation of a public health-protective concentration (C, in mg/L) for a chemical in drinking water uses the following equation for non-carcinogenic endpoints:

$$\text{C} = \frac{\text{ADD (mg/kg-day)} \times \text{RSC}}{\text{DWI}}$$

where,

RSC = relative source contribution (usually 20% to 80%, expressed as 0.20 to 0.80); 100% may be used if ingestion of water is anticipated to be the sole route of exposure.

DWI = daily water intake rate expressed as liters per kilogram of body weight per day (from OEHHA, 2012).

A NOAEL of 13.2 mg/kg-day is determined from several case studies of infant methemoglobinemia (Bosch et al., 1950; Walton, 1951; Sadeq et al., 2008), and is selected as the POD. A total uncertainty factor of 1 is applied because the critical studies examined effects on human infants, which is the most sensitive subpopulation. Thus, interspecies and intraspecies uncertainty factors of 1 are used (OEHHA, 2008). Therefore, the ADD is:

$$\text{ADD} = \frac{13.2 \text{ mg/kg-day}}{1} = 13.2 \text{ mg/kg-day}$$

The relative source contribution (RSC) is the proportion of exposures to a chemical attributed to tap water (including inhalation and dermal exposures, e.g., during showering), as part of total exposure from all sources (including food and air pollution). The RSC values typically range from 20% to 80% (expressed as 0.20 to 0.80), but may deviate from this range based on available exposure data. It is assumed that infants that are not breast-fed get all of their nutrition from formula reconstituted with tap water until around 6 months of age, when solid food is introduced into the diet. Because infants do not typically consume vegetables before 6 months of age, the primary source of nitrate exposure is contaminated drinking water. Therefore, for infants under 6 months of age, it is anticipated that 100% of their nitrate exposure will come from drinking water. Accordingly, a RSC of 100% is used for the derivation of the PHG.

Inhalation exposure to nitrate from car exhaust and cigarette smoke are possible (Health Canada, 2013), but these pathways are expected to be minor compared to ingestion of drinking water for an infant. There is minimal information about the potential of nitrate or nitrite to penetrate skin. Dermal absorption across normal skin is anticipated to be negligible. However, abraded skin may facilitate absorption into the bloodstream (Saito et al., 1997). Topical medications containing nitrates may also be absorbed dermally (ATSDR, 2013), but this exposure scenario would be a special case, and not applicable for the majority of the population. Therefore, oral ingestion of drinking water is the only exposure pathway considered for PHG development.

For oral ingestion rates, the PHG program uses age-specific water ingestion estimates (OEHHA, 2012) derived from a nationwide survey of food and beverage intake from approximately 20,000 individuals (US Department of Agriculture's Continuing Survey of Food Intake of Individuals 1994-1996, 1998 dataset). These age-specific intake rates are normalized to body weight and expressed as liters of water ingested per kilogram of body weight per day (L/kg-day). The updated water ingestion rates indicate that

drinking water ingestion per unit body weight is higher in infants than in adults. Previous PHGs using ingestion rates of 2 liters per day for adults and 1 liter per day for a 10 kg child are being updated with these more refined estimates. For non-carcinogenic endpoints, the time-weighted average daily water ingestion rate for a 70-year lifetime for the general population is generally used. However, if there is a particularly sensitive age group or other subgroup, the high end estimates (95th percentile) of the age-specific water ingestion rate for the subgroup is used in the PHG calculations (OEHHA, 2012). OEHHA is mandated to consider sensitive subgroups, such as children and infants, who may be at greater risk of adverse health effects due to exposure to drinking water contaminants than the general population. These improvements in water ingestion estimates are crucial to the assessment of risk to these sensitive subgroups as well as the general population.

In this case, the DWI is the 95th percentile infant (age 0-3 months) drinking water intake rate of 0.294 L/kg-day derived by OEHHA (OEHHA, 2012). The health-protective concentration for nitrate in drinking water is calculated as follows:

$$C = \frac{13.2 \text{ mg/kg-day} \times 1}{0.294 \text{ L/kg-day}}$$

$$= 45 \text{ mg/L or } 45 \text{ ppm (10 ppm nitrate-nitrogen)}$$

Approximately 25% of ingested nitrate is transported to the saliva, and approximately 20% of salivary nitrate is converted to nitrite in the oral cavity (about 5% of ingested nitrate) (WHO, 2011). Other researchers have estimated that 5-10% of ingested nitrate is converted to nitrite in saliva (Zeilmaker et al., 1996; ATSDR, 2013). However, these estimates refer only to conversion in the mouth. As noted above, nitrate-reducing bacteria in the mouths of neonates and infants do not convert nitrate to nitrite as efficiently as adults, with infant salivary nitrite levels being approximately 6-fold lower than adults (Kanady et al., 2012). It is not certain what total percentage of ingested nitrate is reduced to nitrite in neonates/infants (both in the mouth and the gut). Therefore, in the absence of infant-specific data, OEHHA is utilizing the adult nitrate-to-nitrite conversion of 10% for derivation of the nitrite PHG. The molecular weight ratio of nitrite/nitrate is utilized to account for the molecular weight differences of the two chemical species. The health-protective concentration for nitrite in drinking water can be calculated as follows:

$$C = 45 \text{ mg/L} \times (46 \text{ g/mol} \div 62 \text{ g/mol}) \times 10\%$$

$$= 3.3 \text{ mg/L} = 3 \text{ ppm (1 ppm nitrite-nitrogen) rounded}$$

The Public Health Goals for nitrate and nitrite are expected to be protective against methemoglobinemia in infants, as well as any other known toxicity.

RISK CHARACTERIZATION

The PHG of 45 mg/L (10 mg/L nitrate-nitrogen) for nitrate and 3 mg/L (1 mg/L nitrite-nitrogen) for nitrite are determined based on cases of methemoglobinemia in infants. Infants are particularly susceptible to methemoglobinemia due to various factors that facilitate metHb formation and prevent the conversion back to oxyhemoglobin. Methemoglobinemia was not observed in any cases where the drinking water nitrate levels were ≤45 mg/L.

Infant methemoglobinemia is a relevant public health concern. Knobeloch et al. (2000) raise the possibility that infant methemoglobinemia is more widespread than what is reported in the literature due to underreporting. Although there is strong evidence of nitrate-induced methemoglobinemia in infants, some researchers argue that bacterial contamination of well water and enteritis, as opposed to nitrates, are primarily responsible for methemoglobinemia. OEHHA acknowledges that bacterial contamination was present in some of the reported cases of methemoglobinemia, and might play a role in metHb formation, but it does not explain the observed incidences of methemoglobinemia in the absence of coliform in the drinking water. A mechanism for nitrate-induced metHb formation has been established, which supports the causative role of nitrate in methemoglobinemia induction. In summary, the body of evidence strongly suggests that nitrate plays a critical role in methemoglobinemia induction.

Other toxicity endpoints of nitrate/nitrite, such as thyroid effects, reproductive effects, and carcinogenicity due to N-nitroso compound formation, were also considered for PHG development. However, due to inconsistency among the studies, limitation of study designs, and the presence of confounding factors, OEHHA did not choose them as critical endpoints.

Several studies reported goiters in children in areas where water nitrate concentrations were >50 mg/L, but these studies lacked critical details, which lowered confidence in their results. Since the PHG is 45 mg/L nitrate, it should also protect against the goitrogenic effects of nitrate exposure.

The epidemiology studies linking nitrate/nitrite exposure to cancer did not demonstrate a strong causative association. Individual studies have reported associations with nitrates and various cancer types, but confounding factors and contradictory results from other epidemiology studies make interpretation of the cancer database difficult. The association between nitrate/nitrite exposure and cancer risk is a complex relationship that involves several variables, including the amount of nitrate ingested, the concomitant ingestion of nitrosation cofactors and precursors, and other factors that increase nitrosation, such as medical conditions. The picture is further complicated by the fact that there are endogenous sources of nitrate and nitrite, and nitrate can be converted to nitrite and vice versa in the body. Thus the findings observed in epidemiological studies seem contradictory at times. Animal studies showed equivocal results of tumor formation following chronic exposure to nitrate or nitrite, and the majority of these studies had various deficiencies that complicated the interpretation of the results.

Genotoxicity results were also mixed. IARC (2010) previously determined that the evidence of human carcinogenicity from nitrate in food and water was inadequate, and OEHHA agrees that the body of evidence is not conclusive enough to establish a causal relationship between nitrate exposure and cancer.

There is inconsistent evidence of nitrate-induced developmental and reproductive toxicity in humans. Inconsistency among studies, multiple confounding factors, and the presence of other chemical contaminants complicate the analysis of the epidemiological data. Several of the animal developmental/reproductive toxicity studies of nitrite reported no observable effects, while others reported effects only at high levels (≥900 ppm). Based on the available data, setting the PHG at levels to protect against infant methemoglobinemia would also protect against developmental and reproductive toxicity.

OTHER REGULATORY STANDARDS

The State Water Resources Control Board (SWRCB) has a MCL of 45 mg/L for nitrate as NO_3^- (equivalent to 10 mg/L for nitrate as nitrogen), 10 mg/L for nitrate plus nitrite as nitrogen, and 1 mg/L for nitrite as nitrogen, which are the same as the federal MCLs (SWRCB, 2014). The current state and federal MCLs for nitrate and nitrite are based on the occurrence of infantile methemoglobinemia resulting from ingestion of nitrate-contaminated water. The two principal studies used as the basis of these standards are the studies of Bosch et al. (1950) and Walton (1951).

The WHO provided drinking water guidelines of 50 mg/L nitrate and 3 mg/L nitrite based on epidemiology data for infant methemoglobinemia (WHO, 2011).

The Canadian Maximum Acceptable Concentrations (MACs) are 45 mg/L for nitrate and 3 mg/L for nitrite (Health Canada, 2013). These standards were based on methemoglobinemia induction in bottle-fed infants.

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APPENDIX I: EPIDEMIOLOGY STUDIES OF NITRATE/NITRITE

Table A1. Human Epidemiologic Studies of Nitrates or Nitrites and Cancer Published Since 2006

Reference	Organ	Source	Where, When	Study Type	Size	Results	Adjusted	Problems	Notes
Catsburg et al., 2014	Bladder	Food only; from FFQ	Los Angeles Bladder Cancer Study, 1987-1996	CC, I	1,660 cases; 1,586 controls (neighborhood)	No association with nitrate (up to ≥148.4 mg/day) or nitrites (up to ≥533 μg/day) Possible interaction between heme intake and nitrate	BMI, race, education, diabetes, vegetable intake, vitamins A and C, carotenoids, total servings of food/day, smoking	Past diet not assessed	Ages 25-64 years; Diet during the "reference year"; Some small associations with certain processed meats in those with high nitrate intake
Chiu et al., 2007	Bladder	Mean water NN for year 1990 from municipal records and residence at time of death	Taiwan, 1999-2003	CC, M	513 cases; 513 controls (other deaths excluding GU or cancer related)	Nitrate: OR=1.96 (95% CI, 1.41-2.72; p-trend <0.001) for 0.48-2.86 mg/L NN (median=0.77 mg/L NN)	Age, sex, urbanization	Only includes residence at time of death Ecologic exposure data	
Espejo-Herrera et al., 2015	Bladder	Water only; based on municipality of residence and year	Spain, 1998-2001	CC, I	531 cases; 556 controls	No association with nitrate levels (below regulatory limit of 50 mg/L)	Age, sex, study area, smoking status, use of non-steroidal anti-inflammatory drugs, night-time urinary frequency, agricultural activity, vitamin C intake, diagnosis of urinary tract infections	Limited number of nitrate measurements	
Ferrucci et al., 2010	Bladder	Food only; FFQ at baseline	US, NIH-AARP Diet and Health Study, 1995-6 to 2003; average F/U=7 years	Coh, I	300,933 women and men ages 50-71 at baseline; 854 cases	Nitrate: no association Nitrite: HR=1.28 (95% CI, 1.02-1.61; p-trend=0.06) for upper quintile of dietary nitrite (median=0.91 mg/1,000 kcals)	Age, sex, smoking, fruit and vegetables, beverages, calories	See notes on other NIH-AARP cohort studies; Separate HRs for nitrite from plant or animal sources are lower, not significant	No interaction with vitamin C; Excluding subjects from areas with high water nitrate levels had little impact on results

Reference	Organ	Source	Where, When	Study Type	Size	Results	Adjusted	Problems	Notes
Wang et al., 2012 Meta-analysis	Bladder	Water only; Multiple				RR=1.27 (95% CI, 0.75-2.15) in the high nitrate exposure groups	Multiple	Few studies	Heterogeneity present; RR elevated (1.56; 1.01-2.41) when Ward et al. 2003 is excluded, but marked heterogeneity still present
Ho et al., 2011	Brain	Mean water NN for year 1990 from municipal records and residence at time of death	Taiwan, 2003-2008	CC, M	787 cases; 787 controls, (other deaths excluding cancer and nervous system deaths)	No association for ≥0.38 ppm NN	Age, sex, married, urbanization	Only includes residence at time of death; Ecologic exposure data	No interaction with magnesium or calcium levels
Weng et al., 2011	Brain, childhood	Mean water NN for year 1990 from municipal records and residence at time of death	Taiwan, 1999-2008	CC, M	457 cases; 457 controls, (other deaths excluding cancer related)	OR=1.40 (95% CI, 1.07-1.84) for >0.31 ppm NN	Age, sex, urbanization	Only includes residence at time of death; Ecologic exposure data	No interaction with calcium or magnesium; Municipalities with >1 water supply excluded; 0.31 ppm seems to be median in controls
Xie et al., 2016 Meta-analysis	Brain, adult glioma	Food only				RR=1.21 (95% CI, 1.03-1.42) for nitrite			No significant heterogeneity for nitrite
Inoue-Choi et al., 2012	Breast	Water and food; Municipal water records for 1955-88; FFQ at baseline	Iowa Women's Health Study, 1986-2008	Coh, I	34,388 post-menopausal women; 2,875 cases	Nitrate: no association overall (diet or water) RR=1.40 (95% CI, 1.05-1.87; p-trend=0.04) in those with high folate (≥400 μg/day) for water nitrate in the upper quintile (≥33.5 mg/2L) Nitrite: no association	Age, BMI, waist-hip ratio, education, smoking, alcohol, family history, age of menopause and childbirth, estrogen, calories, diet	42% response rate; baseline questionnaire; Limited residential data	Nitrate levels in private wells not measured; Private well users HR=1.14 (95% CI, 0.97-1.34); Upper nitrate quintile had a median of 57.8 mg/2L
Yang et al., 2010	Breast	Food only; FFQ for 12 months three years prior to interview	Seoul, South Korea, 2004-2006	CC, I	362 cases; 362 controls (hospital)	Nitrate: OR=1.54 (95% CI, 0.88-2.70; p-trend=0.265) for highest quintile (median=716.1 mg/day); Nitrate/folate ratio OR=2.03 (95% CI, 1.16-3.54; p-trend=0.052) for upper quintile (median ratio=2.10)	Multi-vitamins, parity, breast feeding, soy, mushrooms, calories		No dose-response; Unadjusted OR for nitrate=1.05 (95% CI, 0.64-1.72; p-trend=0.767) Evidence of interaction with low folate; No nitrite data

Reference	Organ	Source	Where, When	Study Type	Size	Results	Adjusted	Problems	Notes
Chiu et al., 2010	Colon	Mean water NN for year 1990 from municipal records and residence at time of death	Taiwan, 2003-2007	CC, M	3,707 cases, 3,707 controls (other deaths excluding cancer and GI related)	OR=1.16 (95% CI, 1.04-1.30; p-trend=0.001) for >0.043 mmol/L NN (median=0.071 mmol/L); OR=1.47 (95% CI, 1.21-1.78) in the low magnesium subgroup	Age, sex, marital status, urbanization	Only includes residence at time of death; Ecologic exposure data; Small excess OR	Municipalities with >1 water supply excluded
Chiu et al., 2011	Colon	Mean water NN for year 1990 from municipal records and residence at time of death	Taiwan, 2003-2007	CC, M	3,707 cases; 3,707 controls (other deaths excluding GI and cancer related)	Nitrate RR=1.37 (95% CI, 1.11-1.69) (for nitrate NN >0.60 mg/L) in those with low calcium water concentrations (<34.6 mg/L)	Age, sex, marital status, urbanization	Only includes residence at time of death; Ecologic exposure data	P-value for interaction between nitrate and low calcium <0.05 Same data as Chiu et al., 2010
Yang et al., 2007	Colon	Mean water NN for year 1990 from municipal records and residence at time of death	Taiwan, 1999-2003	CC, M	2,234 cases, 2,234 controls (other deaths excluding cancer and GI related)	No association for 0.48-2.86 mg/L NN; median=0.74 mg/L NN)	Age, sex, calcium, urbanization	Only includes residence at time of death; Ecologic exposure data	Municipalities with >1 water supply excluded
Cross et al., 2010	Colorectal	Food only; FFQ at baseline	US, NIH-AARP Diet and Health Study, 1995-6 to 2003; average F/U=7.2 years	Coh, I	300,948 women and men ages 50-71 at baseline; 2,719 cases	Nitrate (total dietary): HR=0.82 (95% CI, 0.71-0.95; p-trend=0.11) for highest quintile; for processed meat, HR=1.16 (95% CI, 1.02-1.32; p-trend=0.001) for highest quintile (median=289.2 µg/1,000 kcals); Nitrite: no clear association	Gender, education, BMI, smoking, calories, fiber, calcium	See notes on other NIH-AARP cohort studies	Largest sources of total dietary nitrate were fruits and vegetables (spinach, broccoli, potatoes, bananas)
DellaValle et al., 2014	Colorectal	Food only; FFQ at baseline	Shanghai Women's Health Study, 1996-2000 to 2009; average F/U=11 years	Coh, I	73,118 women ages 40-70; 619 cases	Nitrate: overall no association; in those with low vitamin C (<83.9 mg/day) HR=2.45 (95% CI, 1.15-5.18; p-trend=0.02) for highest nitrate quintile (median=291.5 mg/day); Nitrite: no association	Age, calories, education, exercise, vitamin C, carotene, folate	Only a single assessment of diet	

Reference	Organ	Source	Where, When	Study Type	Size	Results	Adjusted	Problems	Notes
McElroy et al., 2008	Colorectal	From 1994 water quality survey (289 randomly selected wells), interpolated results assigned to residences	Wisconsin (rural), 1990-1992, and 1999-2001 (two studies)	CC, I	475 cases; 1,447 controls (DMV and Medicare) women ages 20-74	Colorectal OR=1.57 (95% CI, 0.97-2.52) for NN ≥10.0 ppm; Proximal colon OR=2.76 (95% CI, 1.42-5.38; equivocal dose-response, no p-trend given)	Age, interview period, family history, colorectal screening, smoking	Appears to be very limited or no data on past residences; Relatively small number of cases of proximal colon cancer	No clear association with distal colon or rectal cancer; One well measured in each one square mile section
Miller et al., 2013	Colorectal	Food only; FFQ for the year prior to diagnosis or interview (controls)	Pennsylvania, 2007-2011	CC, I	989 cases, 1,033 controls (RDD)	OR for highest quintile (>496.6 μg/1,000 kcals) =1.57 1.06-2.34; p-trend=0.023) for proximal colon cancer; no increase for distal, rectal or total colon cancer	Age, sex, calories, BMI, medications, fruit and vegetables	Combined nitrates and nitrites	
Ferrucci et al., 2009	Colorectal adenoma	Food only; FFQ – past year	US, navy and army medical centers, 2000-2002	CS, P	158 cases; 649 non-cases (negative sigmoido-scopy)	Nitrite: no association (highest category median=0.22 mg/day)	Age, education, race, exercise, smoking, BMI, study center, hormone use, family history, medications, alcohol, fiber, calcium, calories, others	Only includes nitrite from processed meat	Colorectal screening Study; Started with asymptomatic women; No nitrate data
Ferrucci et al., 2012	Colorectal adenoma	Food only; FFQ at baseline	US, PLCOCST, 1993-2001; average F/U=3-5 years	Coh, I	17,072 subjects; 1,008 cases	No association	Age, center, sex, race, education, family history, medications, BMI, exercise, smoking, alcohol, calcium, calories, others	Relatively short F/U; Results only for nitrate and nitrite combined from processed meats	
Ward et al., 2007a	Colorectal adenoma	Food only; FFQ – usual diet 12 months before sigmoido-scopy	Maryland, navy medical center, 1994-1996	CS, P	146 cases; 228 controls, negative sigmoidoscopy	OR=1.6 (95% CI, 0.8-3.2) for combined nitrate and nitrite of 0.48-2.76 mg/day; No increase for nitrite alone; No data on nitrate alone	Age, gender, smoking, calories, heterocyclic amine	Controls also had sigmoidoscopy; Only includes nitrates and nitrites from meat products	Some elevated ORs but not statistically significant; Some attenuation after adjustment for other meat components

Reference	Organ	Source	Where, When	Study Type	Size	Results	Adjusted	Problems	Notes
Cross et al., 2011	Esophagus	Food only; FFQ at baseline	US, NIH-AARP Diet and Health Study, 1995-6 to 2006; median F/U=10 years	Coh, I	494,979 women and men ages 50-71 at baseline; 215 esophageal squamous, 630 esophageal adenocarci-noma	No association with nitrates (highest quintile median=298.0 μg/1,000 kcals) or nitrites (highest quintile median=199.2 μg/1,000 kcals)	Age, sex, education, BMI, race, smoking, alcohol, exercise, fruits and vegetables, fat, calories	Nitrates and nitrites from processed meats only; See notes on other NIH-AARP cohort studies	Some association with red meat and some meat components
Keszei et al., 2013	Esophagus	Food only; FFQ at baseline (1986)	Netherlands Cohort Study, 1986 to 2002; maximum F/U=16.3 years	Case-coh, I	120,852 men and women ages 55-69, with 261 esophageal cancer cases; 4,032 controls	Nitrate: no association; Nitrite: esophageal squamous cancer HR=1.19 (95% CI, 1.05-1.36) for each 0.1 mg/day increase in men (no association in women)	Age, smoking, BMI, alcohol, fruit and vegetable intake, education, exercise	Assessed diet for only a single year	Cohort subjects are a random sample from municipal population registries; Some data available on water nitrate levels but this comprised 1% of total nitrate intake
Liao et al., 2013	Esophagus	Mean water NN for year 1990 from municipal records and residence at the time of death	Taiwan, 2006-2010	CC, M	3,024 cases; 3,024 controls (other deaths excluding cancer and GI related)	No association overall for ≥0.66 mg/L NN OR=1.27 (95% CI, 1.03-1.57) for high NN (≥0.66) and low magnesium (<9.3 mg/L)	Age, sex, married, urbanization	Only includes residence at time of death Ecologic exposure data	
Navarro et al., 2008	Esophagus	Food only; FFQ for diet 3-5 years before diagnosis or ascertainment	Connecticut, New Jersey, Washington 1993-95	CC, I	282 adeno-carcinoma, 206 squamous, 687 controls (RDD, Medicare)	Nitrites: elevated ORs for both subtypes but not statistically significant; even higher ORs for red meat consumption. No nitrate data.	Sex, site, age, race, education, income, diet, others	No nitrate data	
Ward et al., 2008	Esophagus	Water and food. Water municipal records 1965-85, water samples collected from private wells; Diet, FFQ	Nebraska, 66 counties in eastern part of the state, 1988-1993	CC, I	84 cases; 321 controls (death certificates and RDD)	Water nitrate: no association for 9+ years at >10 mg/L NN or average >4.32 mg/L NN 1965-1984; Private wells: no association; Diet: OR=2.2; 95% CI, 0.9-5.7; p-trend=0.015 for nitrate and nitrite from animal sources ≥8.3 mg/day	Age, gender, BMI, smoking, alcohol	Limited data on private wells	White men and women age 21 or older; Controls from a previous study were re-interviewed; Water analysis restricted to subjects with water nitrate data for ≥70% of person-years after 1964

Reference	Organ	Source	Where, When	Study Type	Size	Results	Adjusted	Problems	Notes
Xie et al., 2011	Esophagus	Occupational	1977-2000	Coh, I	160 subjects, 255 controls; 11 cases	Nitrite: RR=1.26 (95% CI, 1.08-1.46, p<0.001)		Gender composition difference between groups	Worker follow up rate: 98.07% Exposure ages 18-45 years Exposure period 16-23 years, mean exposure for 22.1 years
Zhang et al., 2012	Esophagus	Water samples from wells, rivers, cisterns in the study area	Shexian, China, Jan-Dec, 2010	Eco, I	Population of 54,716; 661 cases	Nitrate: correlation between nitrate and cancer (incidence=0.38, p=0.01) Nitrite: no association	Unclear	Ecologic analysis	Mean NN=4.55 to 8.08 mg/L; Squamous cell carcinoma only
Assesorova et al., 2011	GI	Water only	Tashkent, Russia, time period not given	Eco, I		Nitrate: correlation between GI malignancy, morbidity and water nitrate concentrations		Ecologic study; Correlation coefficients used	Range of water concentrations: 1.0-20.3 mg/L; Abstract only
Dubrow et al., 2010	Glioma	Food only; FFQ at baseline, and shorter FFQ on foods at ages 12-13	US, NIH-AARP Diet and Health Study, 1995-6 to 2003; average F/U=7.2 years	Coh, I	545,770 women and men ages 50-71 at baseline; 585 cases	Nitrate: no association; Nitrite: RR=2.04 (95% CI, 1.46-2.87; p-trend=0.0026) for highest quintile (median=0.68 mg/1000 kcal) from plant sources in males, not in females	Age, sex, race, calories, education, height, cancer	Unclear dose-response pattern; See notes on other NIH-AARP cohort studies	Excluding subjects from areas with high water nitrate levels had little impact on results; No interaction with vitamin C
Michaud et al., 2009	Glioma	Food only; FFQ	US, Nurses Health Study I and II, and the Health Professionals Follow-up Study, 1976-89 to 2004-5; average F/U=14-24 years	Coh, I	230,655 men and women; 335 cases	No association with nitrates or nitrites	Age and calories		Nurses Health Study I and II (initiated in 1976 and 1989, respectively); Health Professionals Follow-up Study (initiated in1986); Multiple cut-off points used – see footnotes their Table 4
Freedman et al., 2010	Liver	Food only; FFQ at baseline	US, NIH-AARP Diet and Health Study, 1995-6 to 2003	Coh, I	495,006 women and men ages 50-71 at baseline; 338 cases	No association with nitrates or nitrites	Age, sex, alcohol, BMI, race, smoking, diabetes, education, fruit and vegetable intake, calories, exercise, others	Nitrites and nitrites from processed meats only. See notes on other NIH-AARP studies.	

Reference	Organ	Source	Where, When	Study Type	Size	Results	Adjusted	Problems	Notes
Karimzadeh et al., 2012	Lung	Food only; FFQ – diet over the preceding 12 months	Mazandaran, Iran, 2007-2008	CC, I	40 cases; 40 controls (hospital based)	OR=2.7 for nitrate and nitrite from animal sources, levels unclear; No clear association with plant sources	Education, residential area, smoking, cooking method, bread consumption, family history, supplement intake, and other dietary factors	Limited information on controls; OR of 2.7, with CI given as 0.13-0.96; Nitrites and nitrates combined	
Aschebrook-Kilfoy et al., 2013b	NHL	Food only; from FFQ	Nebraska women, 1999-2002	CC, I	348 cases; 470 controls	Nitrate: no association Nitrite: OR=1.9 (95% CI, 1.0-3.4) from animal sources			No association with plant sources of nitrite
Chang et al., 2010b	NHL	Mean water NN for year 1990 from municipal records and residence at the time of death	Taiwan 2000-2006	CC, M	1,716 cases; 1,716 controls (other deaths excluding cancer related)	No association for up to 0.48-2.86 mg/L NN (median=0.77 mg/L NN)	Age, sex, urbanization	Only includes residence at time of death; Ecologic exposure data	
Chiu et al., 2008	NHL	Food only; FFQ	Nebraska, 1983-1986	CC, I	147 cases, 1,075 controls	For NHL subtype t (14;18) OR=2.8 (95% CI, 1.3-6.1) for nitrite >1 mg/day; No association for other subtypes or nitrate	Age, sex, proxy, family history, BMI	Small sample size	
Daniel et al., 2012	NHL	Food only. FFQ at baseline	US, NIH AARP Diet and Health Study 1995-6 to 2006; average F/U=9 years	Coh, I	492,186 women and men ages 50-71 at baseline; 3611 cases	No association for nitrites and nitrates combined	Age, sex, education, family history, race, BMI, smoking, exercise, calories, alcohol, fruits/vegetables, cooking methods	Nitrate and nitrite from processed meats only. See notes on other NIH AARP cohort studies	
Kilfoy et al., 2010	NHL	Food only; FFQ for the year prior to interview	Connecticut 1996-2000	CC, I	594 cases; 710 controls (RDD and Medicare)	Nitrate: no association Nitrite: OR=1.35 (95% CI, 1.05-1.75) for animal sources, no association with plant sources	Age, family history, calories, vitamin C, vitamin E, protein intake	Diet from distant past not assessed Somewhat low control participation rates (69% and 47%)	Women ages 21-84 Levels not clear ORs higher in some subtypes No interaction with vitamin C
Richardson et al., 2008	NHL	Occupational	Germany 1986-1998	CC, I	56 cases high malignancy, 81 cases low	Nitrate, nitrite and nitrosamines: OR=2.2 (95% CI, 1.48-3.35) for high	Smoking	Control participation rate of 55%	Men and women ages 15-75

Reference	Organ	Source	Where, When	Study Type	Size	Results	Adjusted	Problems	Notes
					malignancy, 1821 controls	malignancy, OR=1.45 (95% CI, 1.05-2.01) for low malignancy			Data ascertained with in-person interview
Aschebrook-Kilfoy et al., 2012c	NHL (survival)	Food only FFQ	Connecticut 1996-2000 case ascertainment, F/U to 2008	CC, S	568 cases; 250 deaths	No association with survival overall for nitrate or nitrite Levels up to ≥141.0 mg/day nitrate and ≥1.4 mg/day nitrite	Age, calories, family history, vitamin C	Survival only	Women ages 21-84 Asked about diet in the year before interview
Aschebrook-Kilfoy et al., 2012b	Ovarian	Food only; FFQ at baseline	US, NIH AARP Diet and Health Study 1995-6 to 2006; average F/U=10 years	Coh, I	151,316 women ages 50-71 at baseline; 709 cases	Nitrate: HR=1.31 (95% CI, 1.01-1.68; p-trend=0.06) for upper quintile (median= 126.5 mg/1000 kcal) Nitrite: HR=1.34 (95% CI, 1.05-1.69; p-trend=0.02) for upper quintile of animal sources (median=0.33 mg/1000 kcal)	Age, race, calories, family history, BMI, education, smoking, menopause, parity, age at menarche, vitamin C	See notes on other NIH AARP cohort studies	No association for plant nitrite No association with nitrate or nitrite from processed meats No clear interaction with vitamin C
Inoue-Choi et al., 2015	Ovarian	Water and food Municipal water records for 1955-88; FFQ at baseline	Iowa Women's Health Study 1986 to 2008	Coh, I	28,555 post-menopausal women; 315 cases	Nitrate: HR=2.03 (95% CI, 1.22-3.38; p-trend=0.003) for water nitrate in the highest quartile (2.98-25.34 mg/L NO_3-N).	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, total energy intake	42% response rate baseline questionnaire	Nitrate levels in private wells not measured Private well users HR=1.53 (95% CI, 0.93-2.54)
Aschebrook-Kilfoy et al., 2011	Pancreas	Food only; FFQ at baseline, also questions on meat intake ages 12-13	US, NIH AARP Diet and Health Study 1995-6 to 2006; average F/U=10 years	Coh, I	492,226 women and men ages 50-71 at baseline; 1728 cases	No association with nitrate or nitrite (plant or animal sources)	Age, race, calories, smoking, family history, BMI, fat intake, folate, vitamin C	See notes on other NIH AARP cohort studies	Borderline association with nitrate plus nitrite from processed meat at age 12-13
Yang et al., 2009	Pancreas	Mean water NN for year 1990 from municipal records and residence at	Taiwan 2000-6	CC, M	2412 cases; 2412 controls (deaths other than cancer)	No association for 0.48-2.86 mg/L NN; median=0.84 mg/L NN)	Age, sex, urbanization	Only includes residence at time of death Ecologic exposure data	Municipalities with >1 water supply excluded

Reference	Organ	Source	Where, When	Study Type	Size	Results	Adjusted	Problems	Notes
		the time of death							
Sinha et al., 2009	Prostate	Food only FFQ at baseline	US, NIH AARP Diet and Health Study1995-6 to 2003; average F/U=9 years	Coh, I	175,343 men; 10,313 cases (1,102 advanced, 419 fatal)	No association overall. For advanced cancer HR=1.31 (95% CI, 1.07-1.61; p-trend=0.03) for nitrate upper quintile (median=0.314 mg/1000 kcals) and HR=1.24 (95% CI, 1.02-1.51; p-trend=0.03) for nitrite upper quintile (median=0.215 mg/1000 kcal)	Age, calories, race, education, married, PSA, diabetes, BMI, smoking, exercise, alcohol, other	Only assessed nitrates and nitrites from processed meat. See notes on other NIH AARP cohort studies	Large numbers of cases Small increases in HRs for red meat and some other meat constituents Not a completely clear dose-response relationships
Wu et al., 2013	Prostate	Plasma nitrate at baseline (1994)	US, Health Professionals Follow-up Study F/U from 1997-2005	Nested CC, I	630 cases; 630 controls (randomly selected from cohort matched on age and blood draw timing)	No association overall Possible protective association for advanced stage cancer	Age, blood draw timing, family history, smoking hypertension, BMI, diabetes, exercise, calories, fasting, other diet factors	Single measure of plasma nitrate may not represent long-term exposure	Cohort of 51,529 men RRs below 1.0 seen in some of the middle quartiles
Chang et al., 2010a	Rectal	Mean water NN for year 1990 from municipal records and residence at time of death	Taiwan 2003-7	CC, M	1838 cases; 1838 controls (other deaths excluding GI-related deaths and some cancers)	OR=1.15 (95% CI, 1.01-1.32) for ≥0.38 mg/L NN	Age, sex, married, urbanization	Residence only at time of death Ecologic exposure data Only two exposure levels, no dose-response data	Nitrate OR even higher with low calcium (1.3, 95% CI, 1.05-1.6) (for nitrate ≥0.38 mg/L NN and calcium 34.7 mg/L No interaction with magnesium
Kuo et al., 2007	Rectal	Mean water NN for year 1990 from municipal records and residence at the time of death	Taiwan 1999-2003	CC, M	1118 cases; 1118 controls (deaths from other causes excluding GI and cancer)	Nitrate: OR=1.36 (95% CI, 1.08-1.70; p-trend=0.02) for water concentrations of 0.48-2.85 mg/L NN (median=0.72 mg/L NN)	Age, sex, calcium, urbanization	Only includes residence at time of death Ecologic exposure data	
Daniel et al., 2012a	Renal	Food only; FFQ at baseline	US, NIH AARP Diet and Health Study 1995-6 to 2006; average F/U=9 years	Coh, I	492,186 women and men ages 50-71 at baseline; 1814 cases	No association after adjustments: HR=1.44 (95% CI, 1.10-1.88 before adjustments and 1.03 (95% CI, 0.77-1.38) after	Age, sex, calories, BMI, diabetes, hypertension education, family history, race, alcohol, fruit and	Only includes nitrate and nitrite from processed meats. See notes on other NIH	For renal cell carcinoma Some increase in HR with red meat consumption and some meat constituents Nitrate and nitrite combined

Reference	Organ	Source	Where, When	Study Type	Size	Results	Adjusted	Problems	Notes
							vegetable intake, others	AARP cohort studies	
DellaValle et al., 2013	Renal	Food only; FFQ at baseline	US, NIH AARP Diet and Health Study 1995-6 to 2006; average F/U=9.1 years	Coh, I	491,841 women and men ages 50-71 at baseline; 1816 cases	Nitrate: no association Nitrite: HR=1.28 (95% CI, 1.10-1.49) for 0.31-2.27 g/1000 kcal) from animal sources, positive trend (p<0.01)	Age, sex, calories, smoking, race, family history, BMI, alcohol, education, hypertension, diabetes	See notes on other NIH AARP cohort studies	Stronger findings for clear cell type (HR=1.68) Renal cell carcinoma Overlap with Daniel *et al.,* 2012
Ward et al., 2007b	Renal	Water and food. Public drinking water monitoring data linked to residences from 1960 Food: FFQ	Iowa 1986-9	CC, I	201 cases, 1,244 controls (DMV and Medicare)	No association with nitrate in public water supplies (>2.78 mg/L average) or >10 years at >5 mg/L Joint effects of water nitrate and high red meat and low vitamin C intake. No association with dietary nitrate or nitrite	Age, sex, population size, BMI	No data on private well nitrate levels	Asked about lifetime residences although limited data before 1960 Limited to people with nitrate data on ≥70% person-years since 1960 Renal cell cancer
Chiu et al., 2012	Stomach	Water only; Mean water NN for year 1990 from municipal records and residence at time of death	Taiwan, 2006-2010	CC, M	2,832 cases; 2,832 controls (other deaths excluding cancer and GI related)	OR=1.16 (95% CI, 1.05-1.29) for ≥0.38 ppm NN	Age, sex, marital status, urbanization	Residence at time of death; Ecologic exposure data; Only two exposure levels, no dose-response data	Nitrate ORs (for ≥0.38 ppm NN) are higher with low calcium (<34.6 mg/L) (OR=1.70; 95% CI, 1.43-2.03) and low magnesium (<9.3 mg/L) (OR=1.49; 95% CI, 1.24-1.80)
Cross et al., 2011	Stomach	Food only; FFQ at baseline	US, NIH-AARP Diet and Health Study, 1995-6 to 2006; median F/U=10 years	Coh, I	494,979 women and men ages 50-71 at baseline; 454 gastric cardia adeno-carcinoma, 501 gastric non-cardia adeno-carcinoma	No association with nitrates (highest quintile median=298.0 μg/1,000 kcals) or nitrites (highest quintile median=199.2 μg/1,000 kcals)	Age, sex, education, BMI, race, smoking, alcohol, exercise, fruit and vegetable, fat, calories	Only includes nitrates and nitrites from processed meats; See notes on other NIH-AARP studies	Some association with red meat and some meat components
Hernandez-Ramirez et al., 2009	Stomach	Food only; FFQ for diet 3 years before diagnosis or interview (controls)	Mexico City, 2004-2005	CC, I	257 cases; 478 controls (from the Mexican National Health Survey)	Nitrate: OR=1.92 (95% CI, 1.23-3.02; p-trend=0.004) for >3.9 mg/day from animal sources; Nitrite: OR=1.56 (95% CI, 1.02-2.4; p-trend=0.030) for	Calories, age, sex, *H. pylori*, education, salt, chili, alcohol intake	Control selection and validity not well described	Possible protective association with nitrates from fruit and vegetables (OR=0.62; 95% CI, 0.40-0.97) for >134.9 mg/day

Reference	Organ	Source	Where, When	Study Type	Size	Results	Adjusted	Problems	Notes
						>0.4 mg/day from animal sources			
Keszei et al., 2013	Stomach	Food only; FFQ at baseline (1986)	Netherlands Cohort Study, 1986 to 2002; maximum F/U=16.3 years	Case-cohort, I	120,852 men and women age 55-69; 663 stomach cases; 4,032 controls	Nitrate: no association; Nitrite: no association after adjustments	Age, smoking, BMI, alcohol, fruit and vegetable intake, education, exercise	Assessed diet for only a single year	Subjects are a random sample from municipal population registries; Some data available on water nitrate levels but this comprised only about 1% of total nitrate intake
Kim et al., 2007	Stomach	Food only; FFQ for 12-month period 3 years before interview	Korea, 1997-1998	CC, I	136 cases; 136 controls (hospital based)	Nitrate: no association (up to 811 mg/day)	Age, sex, SES, family history, *H. pylori*, diet	Small sample size; Diet from distant past not assessed	No nitrite data; Possible interaction with low folate
Magalhaes et al., 2008	Stomach	Food only			70 cases; 70 controls	"Food rich in…nitrates…used more by gastric cancer patients"		Small study; No other results for nitrates	Abstract only
Navarro et al., 2008	Stomach	Food only; FFQ for diet 3-5 years before diagnosis or ascertainment	Connecticut, New Jersey, Washington, 1993-1995	CC, I	Controls (RDD, Medicare)	For nitrites and non-cardia gastric cancer OR=1.88 (95% CI, 1.24-2.84), no association with gastric cardia cancer	Sex, site, age, race, education, income, diet, others	No nitrate data	Levels unclear
Sumathi et al., 2009	Stomach	Food only; Study questionnaire	Chennai, India, 2002-2006	CC	89 cases; 89 controls (hospital)	Pickled food associated with gastric cancer risk		No quantification of nitrate/nitrite levels	100% response rate reported
Ward et al., 2008	Stomach	Water and food; Water: municipal records 1965-1985, water samples collected from private wells; Food: FFQ	Nebraska, 1988-1993	CC, I	79 cases; 321 controls (death certificates and RDD)	Water: no association for 9+ years at >10 mg/L NN or average >4.32 mg/L NN 1965-1984 Private wells: OR=5.1; 95% CI, 0.5-52) for nitrate >4.5 mg/L Diet: some elevated ORs for nitrate and nitrite but not statistically significant and no obvious trend	Age, gender, education, smoking, alcohol	Limited data on private wells	66 counties in eastern Nebraska; White men and women age 21 or older; Controls from a previous study re-interviewed; Water analysis restricted to subjects with data for ≥70% of person-years after 1964
Xie et al., 2016 Meta-analysis	Stomach	Food only				RR=0.78 (95% CI, 0.67-0.91) for nitrate			Heterogeneity present for nitrate

Reference	Organ	Source	Where, When	Study Type	Size	Results	Adjusted	Problems	Notes
Aschebrook-Kilfoy et al., 2013a	Thyroid	Food only; FFQ at baseline, intakes in the past year	Shanghai Women's Health Study, 1996-2000 to 2009; average F/U=11 years	Coh, I	73,317 women ages 40-70; 164 cases	Nitrate: no association; Nitrite: some association (RR=2.05; 95% CI, 1.20-3.51) but no dose-response (p-trend=0.36); Association with nitrite from animal sources, mostly processed meat (RR=1.96, p-trend<0.01)	Age, calories, education, thyroid disease, vitamin C, carotene, folate	Diet one year at baseline	Overall high nitrate intake in this population, (300 mg/day) although less processed meat; No interaction with vitamin C
Bahadoran et al., 2015 Meta-analysis	Thyroid	Water and food				RR=1.36 (95% CI, 0.67-2.75) for nitrate RR=1.48 (95% CI, 1.09-2.02) for nitrite		Few studies	Heterogeneity present for nitrate
Kilfoy et al., 2011	Thyroid	Food only; FFQ at baseline	US, NIH-AARP Diet and Health Study, 1995-6 to 2003; average F/U=7 years	Coh, I	490,194 men and women ages 50-71 370 cases	Nitrate: elevated RR in men but not women (RR=2.28; 95% CI, 1.29-4.04; p-trend <0.001) for highest quintile (median=94.8 mg/day); Nitrite: no association	Age, sex, smoking, calories, race, family history, education, BMI, exercise, alcohol (no change with macronutrients)	17.6% response rate; Finding only in men; See notes on other NIH-AARP cohort studies	Little change in results when people from areas with high water nitrate concentrations removed.
Ward et al., 2010	Thyroid	Water and food; Water nitrate 1955-1988 and usual source drinking water in 1989; Diet: FFQ at baseline (1986)	Iowa Women's Health Study, 1986-2004; median F/U=9.3 years	Coh, I	21,977 women ages 55-69 from Iowa DMV files; 45 cases	Water: RR=2.6 (95% CI, 1.1-6.2) (no dose-response) for water >5 mg/L NN for ≥5 years Diet: RRs by increasing intake, 1.00/1.65/1.69/ 2.85 (95% CI, 1.00-8.11) for >41.1 mg/day NN	Age, vitamin C, rural or town size	No data on private well nitrate levels; Only 8 high exposure cases and 17 low exposure; Only for usual drinking water source in 1989	Only includes those who used same water supply for >10 years; 18% private wells; No association with private well use; Excluded communities with multiple water sources
Xie et al., 2016 Meta-analysis	Thyroid	Food only				RR=1.52 (95% CI, 1.12-2.05) for nitrite		Few studies	No significant heterogeneity for nitrite

Reference	Organ	Source	Where, When	Study Type	Size	Results	Adjusted	Problems	Notes
Loh et al., 2011	Multiple	Food only; FFQ	United Kingdom, 1993-1997 recruitment, F/U through 2008	Coh, I	3,268 cancers total	Nitrite: data provided for nine different cancer sites, no associations seen with any type	Age, sex, BMI, smoking, exercise, education, alcohol, menopause	HRs only for nitrite	

BMI, body mass index; CC, case-control; Cat, categories (defined below); CI, confidence interval; Coh, cohort; CS, cross-sectional; DMV, Department of Motor Vehicles; F/U, follow-up; FFQ, food frequency questionnaire; GI, gastrointestinal; GU, genitourinary; HR, hazard ratio; Study types M/I/S/P, mortality, incidence, survival, or prevalence; NHL, non-Hodgkin lymphoma; NIH-AARP, National Institutes of Health-American Association of Retired Persons; NN, nitrate-nitrogen; OR, odds ratio; PLCOCST, Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial (subjects undergoing baseline and F/U sigmoidoscopy); RDD, random digit dialing; RR, relative risk; SES, socioeconomic status; t(14:18), translocation from chromosome 18 to chromosome 14

APPENDIX II. DEFAULT UNCERTAINTY FACTORS FOR PHG DERIVATION

This appendix describes the default uncertainty factors OEHHA generally uses to calculate the Acceptable Daily Dose when deriving PHGs. When scientific evidence is compelling these defaults are supplanted by alternative factors or modeled results. Table A6 below is adapted from OEHHA's "Technical Support Document for the Development of Noncancer Reference Exposure Levels" (OEHHA, 2008).

Table A2. Default uncertainty factors for PHG derivation, adapted from OEHHA (2008)

LOAEL uncertainty factor (UF_L)		
Values used:	10 1	LOAEL, any effect NOAEL or benchmark used
Interspecies uncertainty factor (UF_A)		
Combined interspecies uncertainty factor (UF_A):	1 √10 10	human observation animal observation in nonhuman primates where no data are available on toxicokinetic or toxicodynamic differences between humans and a non-primate test species
Toxicokinetic component (UF_{A-k}) of UF_A:	1 √10	where animal and human PBPK models are used to describe interspecies differences non-primate studies with no chemical- or species-specific kinetic data
Toxicodynamic component (UF_{A-d}) of UF_A:	1 2 √10	where animal and human mechanistic data fully describe interspecies differences. (*This is unlikely to be the case.)* for residual susceptibility differences where there are some toxicodynamic data non-primate studies with no data on toxicodynamic interspecies differences
Intraspecies uncertainty factor (UF_H)		
Toxicokinetic component (UF_{H-k}) of UF_H:	1 √10 10	human study including sensitive subpopulations (e.g., infants and children), or where a PBPK model is used and accounts for measured inter-individual variability for residual susceptibility differences where there are some toxicokinetic data (e.g., PBPK models for adults only) to allow for diversity, including infants and children, with no human kinetic data

Toxicodynamic component (UF_{H-d}) of UF_H:	1	Human study including sensitive subpopulations (e.g., infants and children)
	√10	Studies including human studies with normal adult subjects only, but no reason to suspect additional susceptibility of children
	10	Suspect additional susceptibility of children (e.g., exacerbation of asthma, neurotoxicity)
Subchronic uncertainty factor (UF_S)[1]		
Values used:	1	Study duration >12% of estimated lifetime
	√10	Study duration 8-12% of estimated lifetime
	10	Study duration <8% of estimated lifetime
Database deficiency factor (*UF_D*)		
Values used:	1	No substantial data gaps
	√10	Substantial data gaps including, but not limited to, developmental toxicity

[1]Exposure durations of 13 weeks or less are subchronic regardless of species (OEHHA, 2008)

References

OEHHA (2008). Air Toxics Hot Spots Risk Assessment Guidelines: Technical Support Document for the Derivation of Noncancer Reference Exposure Levels. Office of Environmental Health Hazard Assessment, California Environmental Protection Agency, Sacramento, CA.