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IRIS Assessment Plan for Nitrate and Nitrite

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1. INTRODUCTION

The Integrated Risk Information System (IRIS) Program is undertaking a reassessment of the health effects of nitrate and nitrite. Nitrate and nitrite were included on the December 2015 IRIS Program multi-year agenda (https://www.epa.gov/iris/iris-agenda) as chemicals having high priority for assessment development, largely because of EPA Office of Water interest in an updated health assessment.

IRIS assessments provide high quality, publicly available information on the toxicity of chemicals to which the public might be exposed. These assessments are not regulations, but provide a critical part of the scientific foundation for decisions made in EPA program and regional offices to protect public health.

Before beginning an assessment, the IRIS Program consults with EPA program and regional offices to define the scope of the assessment, including the nature of the hazard characterization needed, identification of the most important exposure pathways, and level of detail needed to inform program and regional office decisions. Based on the scope defined by EPA, the IRIS Program undertakes problem formulation activities to frame the scientific questions that will be the focus of the assessment, which is conducted using systematic review methodology.

This document presents the draft assessment plan for nitrate and nitrite, including a summary of the IRIS Program's scoping and initial problem formulation conclusions, objectives, and specific aims of the assessment; draft PECO (Population, Exposure, Comparators, and Outcomes) framework that outlines the evidence considered most pertinent to the assessment; and identification of key areas of scientific complexity. Brief background information on uses and potential for human exposure is provided for context. This document also discusses how EPA will consider recent authoritative reviews, in particular, the Agency for Toxic Substances and Disease Registry's *Toxicological Profile for Nitrate and Nitrite* (ATSDR, 2017) and the International Agency for Research on Cancer monograph on nitrate and nitrate (IARC, 2010). The assessment will address both nitrate and nitrite together, as both are chemically related and metabolically linked, and their biological effects are determined by conversion of nitrate to nitrite and vice versa. Review of the health effect literature for both chemicals in a single health assessment also follows the approach taken by other health agencies (ATSDR, 2017; WHO, 2016; Health Canada, 2013; IARC, 2010).

2. SCOPING AND INITIAL PROBLEM FORMULATION

2.1. BACKGROUND

Nitrate (NO_3^-) and nitrite (NO_2^-), naturally occurring anions in the environment, play an essential role in Earth's nitrogen cycle. Since 1950, human sources of reactive nitrogen into the environment—released either intentionally (e.g., through fertilizer application) or unintentionally (e.g., as a byproduct of fossil fuel combustion)—have increased substantially (Fields, 2004). Nitrate salts are mainly used as nitrogen fertilizers and in industrial explosives, fireworks, and glass making; nitrites are largely used as preservatives for meat and fish curing and as color fixatives (IARC, 2010; Pokorny L, 2006).

Nitrates account for most of the available total nitrogen in both ground and surface waters; nitrite levels are generally low in both (DeSimone, 2009). According to monitoring data obtained during EPA's second Six-Year Review of National Primary Drinking Water Regulations (U.S. EPA, 2009), nitrate was detected in approximately 70% of drinking water systems at a median concentration of approximately 2–3 mg nitrate-nitrogen/L; maximum concentrations in ground and surface waters were 99 and 49 mg nitrate-nitrogen/L, respectively. Nitrite was detected in approximately 22% of drinking water systems at a median concentration of 0.02 mg nitrite-nitrogen/L; maximum concentrations in ground and surface waters were 13 and 9 mg nitrate-nitrogen/L, respectively (U.S. EPA, 2009). Human activities are responsible for increased levels of nitrate in drinking water sources; DeSimone (2009) reported that nitrate concentrations greater than 1 mg/L (as N) are levels in well water considered to result from the effects of human activities in many parts of the United States. Populations served by private well water, especially shallow wells in agricultural areas, may be exposed to nitrate at levels several times higher than those served by public water systems (DeSimone, 2009; Ward, 2009).

The general population is exposed to nitrate in both drinking water and food. Vegetables are the main source of exposure to ingested nitrate, with leafy vegetables comprising nearly 80% of nitrate exposure in an average person's diet. Other sources of dietary nitrate include cured meats/fish, cereal grains, dairy products, and beer (ATSDR, 2017; IARC, 2010). In contrast to nitrates, endogenous sources account for approximately 80% of all nitrites in the human body, as 5–8% of the total nitrate intake is converted into nitrite (WHO, 2016; Mensinga et al., 2003). Almost all exogenous exposure to nitrite comes from food, with relatively higher nitrite concentrations found in cured meats (IARC, 2010). Drinking water is generally a minor source of exposure to nitrite (IARC, 2010).

The IRIS Program previously evaluated the oral health effects of nitrate and nitrite; oral reference doses (RfDs) for nitrite[1] and nitrate[2] were posted to the IRIS database in 1987 and 1991, respectively. EPA based these RfDs on surveys of clinical cases of methemoglobinemia in infants associated with ingestion of nitrate-containing drinking water conducted in the early 1950s (Walton, 1951; Bosch et al., 1950). Since 1987, a growing body of literature indicates potential associations between nitrate/nitrite exposure and other noncancer health effects. Some epidemiological studies also suggest an increased risk of cancer, especially gastric cancer, associated with dietary nitrite exposure (ATSDR, 2017). Cancer risk associated with nitrate or nitrite exposure is complicated by the fact that, under conditions of concurrent exposure to amines or amides or low levels of antioxidants, endogenous nitrosation can occur, leading to the formation of carcinogenic nitroso compounds (ATSDR, 2017; IARC, 2010). IARC (2010) concluded that ingested nitrate or nitrite under conditions that result in endogenous nitrosation is probably carcinogenic to humans (Group 2A).

2.2. SCOPING SUMMARY

During scoping, the IRIS Program met with EPA program and regional offices that had interest in an updated IRIS assessment for nitrates and nitrites to discuss specific assessment needs. Table 1 provides a summary of input from this outreach.

[1] https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0078_summary.pdf
[2] https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0076_summary.pdf

Table 1. EPA program or regional office interest in an updated nitrate/nitrite assessment

Program or regional office	Oral	Inhalation	Statutes/regulations	Description of authority/regulation	Anticipated uses/interest
Office of Water	✓		Safe Drinking Water Act (SDWA) – Section 1412	EPA must review each national primary drinking water regulation at least once every six years and revise them, if appropriate.	Six-year review of the National Primary Drinking Water regulations.
Region 5[a]	✓			At the discretion of the state, nitrate levels, not to exceed 20 mg/L, may be allowed in a non-community water system if the supplier of water demonstrates to the satisfaction of the State that: (1) Such water will not be available to children under 6 months of age; and (2) The non-community water system is meeting the public notification requirements under §141.209, including continuous posting of the fact that nitrate levels exceed 10 mg/L and the potential health effects of exposure; and (3) Local and state public health authorities will be notified annually of nitrate levels that exceed 10 mg/L; and (4) No adverse health effects shall result.	Evaluation of special provision of the NPDW regulation [40 CFR 141.11(d)] allowing, at the discretion of the state, non-community water systems to exceed the nitrate MCL.

[a]Region 5 serves Illinois, Indiana, Michigan, Minnesota, Ohio, Wisconsin, and 35 tribes.

The Office of Water regulates nitrates and nitrites under the National Primary Drinking Water Regulations (40 CFR 141, 142); the current maximum contaminant levels (MCLs) for nitrate and nitrite, promulgated in 1991, are 10 mg/L and 1 mg/L (as nitrogen), respectively (40 CFR 141.62; 56 FR 3594, January 30, 1991). An updated health assessment of nitrate and nitrite will be considered in the next Six-Year Review cycle for National Primary Drinking Water regulations. A provision of the current regulation [40 CFR 141.11(d)] allows, at the discretion of the state, noncommunity water systems to exceed the nitrate MCL up to 20 mg/L if the supplier can demonstrate that the water will not be available to children under 6 months of age and that no adverse health effects will result. The availability of more recent health effect literature published

since 1991 raises questions about whether the current MCLs for nitrate and nitrite and the provision allowing exceedance of the nitrate MCL up to 20 mg/L provide adequate health protection for the general population (all life stages).

This assessment will address inorganic forms of nitrate and nitrite and will specifically consider health effect information for the compounds included in Table 2. These salts are highly soluble in water and dissociate under environmental conditions; in solution, they exist as ions (ATSDR, 2017). Because the cations are not expected to introduce significant differences in the toxicity of the different salts, toxicity findings from all five compounds are considered relevant to an assessment of nitrate and nitrite toxicity. These five compounds listed in Table 2 are the most common nitrate and nitrite salts in the environment (ATSDR, 2017). These compounds were also the subject of two recent health assessments of nitrate and nitrite (ATSDR, 2017; IARC, 2010). The decision to develop the assessment of nitrate/nitrite using health effect information for these five compounds was also based on known general population exposure to these five compounds and availability of epidemiological or toxicological information. Specifically, ammonium nitrate is a leading nitrogen fertilizer, and for this reason, has been used in toxicological studies as a component of "California mixture" and "Iowa mixture." These two mixtures are representative of groundwater contamination by fertilizers and pesticides and used for simulations of environmental exposures to pesticides mixtures. Sodium nitrate, sodium nitrite, potassium nitrate, and potassium nitrite are used as food additives to cure meats. The National Toxicology Program (NTP) has assessed the toxicities of sodium nitrate and sodium nitrite in animal toxicology and carcinogenicity studies.

Table 2. Nitrate/nitrite compounds considered for assessment

Compound	Chemical formula	CAS Number
Ammonium nitrate	NH_4NO_3	6484-52-2
Sodium nitrate	$NaNO_3$	7631-99-4
Sodium nitrite	$NaNO_2$	7632-00-0
Potassium nitrate	KNO_3	7757-79-1
Potassium nitrite	KNO_2	7758-09-0

Assessment of the health effects of nitrate and nitrite following inhalation and dermal routes of exposure will not be included in the scope of this assessment. Inhalation and dermal exposures to nitrate or nitrite in the general population (i.e., populations not exposed occupationally, such as factory and fertilizer workers) are expected to be negligible compared to oral exposure (ATSDR, 2017). Focusing on the health effects associated with oral exposure to nitrate and nitrite is consistent with the needs of EPA programs and regional offices.

Based on input received during scoping, the IRIS assessment will include evaluation of both noncancer and cancer human health hazards associated with ingested nitrate and nitrite. Because

the association between nitrate/nitrite and methemoglobinemia has been well established (Ward et al., 2005; Walton, 1951), the assessment of this outcome will focus primarily on the quantitative relationship between nitrate/nitrite exposure and methemoglobinemia. For cancer, EPA will develop a qualitative assessment of the carcinogenic potential of nitrate and nitrite, and will explore the feasibility of developing a quantitative assessment based in part on consistency of effects observed across studies and the availability of data that support dose-response analysis. EPA anticipates that a quantitative cancer assessment will be particularly challenging, given the influence of concurrent exposure to dietary sources of nitrosatable compounds and antioxidants, conflicting results across studies, and design limitations in a number of epidemiological studies that have investigated associations between nitrate or nitrite exposure and cancer at different sites.

2.3. PROBLEM FORMULATION

A preliminary literature survey was performed using health assessments produced by other federal, state, and international health agencies (ATSDR, 2017; WHO, 2016; Health Canada, 2013; IARC, 2010; IPCS, 2005; Cal/EPA, 2000) to identify noncancer and cancer health outcomes for which possible association with exposure to nitrate/nitrite has been investigated.

In particular, EPA relied on the ATSDR (2017) *Toxicological Profile for Nitrate and Nitrite*, as the most recent authoritative health agency assessment, to identify the pertinent health effect literature through 2016.[3] Because ATSDR (2017) updated the comprehensive review of the cancer epidemiological literature provided in IARC (2010) (i.e., literature published up to approximately 2007), the IARC monograph also was used to identify the cancer literature. The numbers of animal and human studies cited in ATSDR (2017) and IARC (2010) by health effect category were tallied as a measure of the extent to which the association between a given health effect and nitrate/nitrite exposure has been investigated (see Table 3).

Table 3. Summary of the number of studies cited in ATSDR (2017)[a]

Health effect	Human studies				Animal studies					
	Occupational epidemiology studies	General population epidemiology studies	Controlled exposure studies	Case reports and case series reports	Chronic	Subchronic	Short-term	Acute	Multigenerational	Gestational
Cancer		60+[b]			18					
Hematological		25+	3	10+	4	6	3	1		
Developmental		14							2	6

[3]ATSDR conducted their literature search through May 2016.

Health effect	Human studies				Animal studies					
	Occupational epidemiology studies	General population epidemiology studies	Controlled exposure studies	Case reports and case series reports	Chronic	Subchronic	Short-term	Acute	Multigenerational	Gestational
Endocrine (thyroid)		6	1		4	3	1			
Gastrointestinal		1	1	7	5	1				
Other systemic toxicity[c]					10	2	1		1	
Neurological and sensory			2	6	1	1			1	
Metabolic disease (type 1 diabetes)		8								
Reproductive			3	1	2	3			1	
Hepatic					3			2		
Cardiovascular		1	1	3						
Dermal and ocular				1						
Renal					1					
Immunological										
Musculoskeletal										
Respiratory										

[a]The numbers represent the numbers of studies that investigated a particular health effect, not the number of studies that identified a positive association with exposure to nitrate or nitrite. If a journal article or report included, for example, a study in both rats and mice, it was counted as two studies. Health effects are listed generally in decreasing order of the number of studies that investigated that effect.

[b]More than 50 epidemiological studies that examined the association between nitrate/nitrite intake and cancer were cited in the 2010 IARC Monograph (IARC, 2010); an additional 13 selected cohort and case-control studies published after IARC conducted the literature search were identified in the ATSDR (2017) Toxicological Profile.

[c]Body weight.

Based on the preliminary literature survey (i.e., the secondary sources used to develop Table 3), EPA anticipates conducting a systematic review for the following health effect categories:

- Cancer
- Hematological effects
- Developmental effects
- Thyroid effects (endocrine effect)
- Type 1 diabetes (metabolic effect)

- Reproductive effects

For these health effect categories, the available epidemiology and experimental animal studies are likely to be sufficient for drawing conclusions about human hazard. Of the studies identified for other health effect categories (e.g., gastrointestinal, hepatic, and nervous system) positive findings generally were reported in only a few studies, some with study design deficiencies, and generally were inconsistent. Therefore, EPA anticipates that a systematic review for health effect categories other than the six identified above will not be undertaken unless additional evidence of a positive association is discovered upon review of references identified during the comprehensive literature search. Such determinations will involve evaluation of the body of evidence from both new and previously identified studies, taking into consideration factors such as study quality, directness or relevance of the experimental model, nature of the endpoints examined, and consistency across studies.

The preliminary literature survey revealed several studies reporting potential association between nitrate/nitrite exposure and beneficial cardiovascular outcomes. Because IRIS assessments focus on the adverse effects associated with exposure to environmental chemicals, a systematic review of the potential beneficial outcomes to the cardiovascular system associated with the intake of nitrate or nitrite will not be included in this assessment.

3. OVERALL OBJECTIVE, SPECIFIC AIMS AND DRAFT POPULATIONS, EXPOSURES, COMPARATORS, AND OUTCOMES (PECO) FRAMEWORK

The overall objective of this assessment is to identify adverse health effects and characterize exposure-response relationships for these effects of nitrate and nitrite to support development of toxicity values. This assessment will use systematic review methods to evaluate the epidemiological and toxicological literature for these chemicals, including consideration of relevant mechanistic evidence. The evaluations conducted in this assessment will be consistent with relevant EPA guidance.[4] The systematic review protocol will be disseminated after review of the draft assessment plan and will reflect changes made to the specific aims and PECO framework in response to public input.

3.1. SPECIFIC AIMS

- Identify epidemiological (i.e., human) and toxicological (i.e., experimental animal) literature reporting effects of exposure to nitrate and nitrite as outlined in the PECO framework. Chapter 3 (Health Effects) of ATSDR's *Toxicological Profile of Nitrate and Nitrite* (ATSDR, 2017) and the IARC monograph on nitrate and nitrate (IARC, 2010) will serve as starting points to identify PECO-relevant evidence published through 2016. A literature search update will be performed to identify new health effect references for nitrate/nitrite published since completion of the literature searches conducted by ATSDR and IARC. Health outcome studies cited in ATSDR (2017) and IARC (2010) will be combined with the literature search results from the updated database search and screened for PECO relevance.

- Use an iterative approach to determine which mechanistic studies are most important to summarize, based on factors such as robustness of the evidence in humans and animals, likelihood to impact evidence synthesis conclusions for human health, and directness or relevance of the model systems for understanding potential human health hazards. When summarizing individual mechanistic studies is not critical, this information will generally be summarized by relying on other published authoritative sources, such as public health agency reports and expert review articles.

- Conduct study evaluations (risk of bias and sensitivity) for individual epidemiological and toxicological studies. Studies with critical deficiencies will be considered uninformative and will not be considered further.

[4]EPA guidance documents: http://www.epa.gov/iris/basic-information-about-integrated-risk-information-system#guidance/

- Extract data on relevant health outcomes from epidemiological and toxicological studies included based on the study evaluation.

- Synthesize the evidence across studies, assessing similar health outcomes using a narrative approach or meta-analysis (if appropriate).

- For each health outcome, express confidence in conclusions from across studies (or sub-sets of studies) within human and animal evidence streams, evaluating each evidence stream (human and animal) separately.

- For each health outcome, integrate results across evidence streams (human and animal) to conclude whether a substance is hazardous to humans. Identify and discuss issues concerning potentially susceptible populations and life stages. Biological support provided from mechanistic studies and non-mammalian model systems will be considered based on the iterative prioritization approach outlined in the PECO framework.

- Derive toxicity values, as supported by the available data. For nitrate and nitrite anions separately, consider deriving RfDs and, if feasible, a cancer slope factor.

- Characterize uncertainties and identify key data gaps and research needs such as limitations of the evidence base, limitations of the systematic review, and consideration of dose relevance and pharmacokinetic differences when extrapolating findings from higher-dose animal studies to lower levels of human exposure.

3.2. DRAFT PECO FRAMEWORK

A PECO (Populations, Exposures, Comparators, and Outcomes) framework is used as an aid to focus the research question(s), search terms, and inclusion/exclusion criteria in a systematic review. The draft PECO framework for nitrate and nitrite (Table 4) was based on (1) nomination of the chemical for assessment, (2) discussions with scientists in EPA program and regional offices to determine the scope of the assessment that will best meet Agency needs, and (3) preliminary review of the health effects literature for nitrate and nitrite (primarily reviews and authoritative health assessment documents) to identify the major health hazards associated with exposure to nitrate and nitrite and key areas of scientific complexity.

Table 4. Draft PECO framework for the nitrate/nitrite assessment

PECO element	Evidence
Population	**Human:** Any population and life stage (e.g., children, general population, occupational, or high exposure from an environmental source). As children younger than 6 months of age appear to be a susceptible population, this population will be emphasized during review. The following study designs will be considered most informative: controlled exposure, cohort, case-control, cross-sectional, and ecological. Note: Case reports and case series will be tracked during study screening but are not the primary focus of this assessment. They may be retrieved for full-text review and subsequent evidence synthesis if no or few more informative study designs are available. Case

PECO element	Evidence
	reports also can be used as supportive information to establish biologic plausibility for some target organs and health outcomes.
	Animal: Nonhuman mammalian animal species (whole organism) of any life stage (including preconception, in utero, lactation, peripubertal, and adult stages).
	Nonmammalian model systems/in vitro/in silico: Nonmammalian model systems (e.g., fish, amphibians, birds, *Caenorhabditis elegans*); human or animal cells, tissues, or biochemical reactions (e.g., ligand-binding assays) with in vitro exposure regimens; bioinformatics pathways of disease analysis; or high-throughput screening data. These studies are tagged during title and abstract screening, and an iterative approach is used to prioritize their inclusion for full-text retrieval and evidence synthesis based on likelihood to impact evidence synthesis conclusions for human health.[a]
Exposure	Exposure to specific nitrate/nitrite compounds including (CASRN): ammonium nitrate (6484-52-5), potassium nitrate (7757-79-1), potassium nitrite (7758-09-0), sodium nitrate (7631-99-4), sodium nitrite (7632-00-0), inorganic nitrate/nitrite in drinking water and inorganic nitrate/nitrite in foods. Mixture studies for animal and in vitro studies will be included if they have an arm with a nitrate/nitrite compound only. **Human and animal:** Exposure routes to be considered are any oral exposures. Where possible, exposures will be assessed separately for drinking water and dietary nitrate/nitrite. Other exposure routes, including inhalation, dermal, or injection, will be tracked during title and abstract as "supplemental information."
	Nonmammalian model systems/in vitro/in silico: Exposure via growth or assay medium.
Comparator	**Human:** A comparison or reference population exposed to lower levels (or no exposure/exposure below detection levels) of nitrate/nitrite or to nitrate/nitrite for shorter periods.
	Animal: Quantitative exposure versus lower or no exposure with concurrent vehicle control group.
	Nonmammalian model systems/in vitro/in silico: Quantitative exposure versus lower or no exposure with concurrent vehicle control group.
Outcomes	All health outcomes (both cancer and noncancer). For methemoglobinemia, only studies that inform the quantitative relationship between nitrate/nitrite exposure and methemoglobinemia will be included. In general, endpoints related to clinical diagnostic criteria, disease outcomes, histopathological examination, or other apical/phenotypic outcomes will be prioritized for evidence synthesis over outcomes such as biochemical measures. As discussed above, EPA anticipates that a systematic review for health effect categories other than the six identified above (hematological, thyroid, type 1 diabetes, development, reproduction, and cancer) will not be undertaken unless a significant amount of new evidence is found upon review of references identified during the comprehensive literature search.

[a]**Note:** An iterative approach is used to prioritize evidence from nonmammalian model systems (e.g., fish, amphibians, birds, *C. elegans*), in vitro, in silico, and other types of mechanistic studies based on likelihood to impact evidence synthesis conclusions for human health. Evidence from these studies will be tagged preliminarily during title/abstract screening as "Other Informative Studies" or "Supplemental Information" according to hazard categories or types of mechanistic outcomes/pathways. These studies are prioritized for full-text retrieval and evidence synthesis to focus on those studies most important to summarize, based on factors such as robustness of the evidence in humans and animals, directness or relevance of the model systems, and concentrations tested. For example, if robust epidemiological or nonhuman mammalian evidence is available, the need to conduct a thorough assessment of individual nonmammalian and mechanistic studies could be diminished unless controversial issues need to be resolved, e.g., issues related to applicability of animal evidence to humans or the shape of the dose-response relationship at low exposure levels.

3.3. KEY SCIENCE ISSUES

Based on the preliminary literature survey, the following key scientific issues were identified that warrant evaluation in the assessment.

- Nitrate and nitrite are generated endogenously as part of the nitrate-nitrite-nitric oxide cycle that controls the availability of nitric oxide, which is a signaling molecule involved in the regulation of both physiological and pathological processes. The roles of endogenous versus exogenous nitrate and nitrite in toxicity, particularly methemoglobinemia in infants, have been debated in the scientific literature.

- Several susceptible populations and life stages have been identified for methemoglobinemia. These include infants under 6 months of age; individuals with higher-than-normal gastric pH; individuals with glucose-6-phosphate dehydrogenase or NADH (nicotinamide adenine dinucleotide (NAD) + hydrogen)-dependent methemoglobin reductase deficiency; individuals with diseases such as anemia, cardiovascular disease, lung disease, and sepsis; individuals with abnormal hemoglobin species including carboxyhemoglobin, sulfhemoglobin, and sickle hemoglobin.

- A physiologically based pharmacokinetic (PBPK) model for simulating the kinetics of methemoglobinemia formation after oral exposure to nitrate in adults is available (Zeilmaker et al., 2010; Zeilmaker et al., 1996) and needs to be evaluated for its potential to inform interspecies variability in the dose-response assessment.

- Previously published assessments by Health Canada (2013), ATSDR (2017), IARC (2010), and WHO (2016) and newer animal and epidemiological studies published after 2014 raise the following issues related to cancer risk:

    - Risk associated with intake of nitrates, nitrites, or both from cured meats, vegetables, and drinking water could differ because of co-occurrence with antioxidants (e.g., vitamin C, vitamin E) in vegetables, amines in fish and meats, and calcium in drinking water. Consequently, risks associated with dietary intake, intake by drinking water, and total intake may need to be assessed separately.

    - Susceptible populations, such as postmenopausal women (Inoue-Choi et al., 2015) (Jones et al., 2016), appear to display increased risk associated with intake of nitrate/nitrite.

    - Populations vary significantly in the ability to reduce salivary nitrate by oral bacteria (e.g., actinomyces and veilonella) (Bryan and Petrosino, 2017). For example, patients with migraines were shown to have higher abundance of nitrate, nitrite, and nitric oxide reductase genes in their oral bacterial metagenome (Gonzalez et al., 2016). In contrast, the use of antiseptic mouthwashes appears to deplete nitrate-reducing oral bacteria and affect some nitrite-mediated biological processes (Kapil et al., 2013). Individuals from some subgroups might be able to convert more nitrate to nitrite and consequently produce more carcinogenic N-nitroso derivatives.

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