Appendix D – Nitrate in Private Domestic Wells Outreach Toolbox

NITRATE IN PRIVATE DOMESTIC WELLS OUTREACH TOOLBOX

November 2024

Table of Contents

List of Figures

Appendices

Appendix A – [Tools and Resources](#page-26-0)

EXECUTIVE SUMMARY

This Nitrate in Private Domestic Wells Outreach Toolbox covers background information related to nitrate in groundwater in Nebraska. It also includes information related to past, current, and future efforts to address drinking water quality nitrate problems for private domestic well owners. While the Nebraska Department of Environment and Energy (NDEE) is the lead conjoiner of the effort to produce this toolbox, it must be emphasized that other agencies such as the Natural Resource Districts (NRDs), University of Nebraska (e.g. Nebraska Extension, Nebraska Water Center, and University of Nebraska Medical Center- Climate and Health Program), and state and local public health departments, have been facilitating many education and outreach efforts for the public related to water sampling, testing, and potential solutions in order to mitigate potential acute and chronic health implications related to high nitrate levels in private domestic wells. Therefore, this preliminary outreach toolbox is a first step in an ongoing process to compile as much information related to these efforts as possible to assist the public and various stakeholders in the essential first step resources to help address the nitrate issue both locally and personally. The full list of resources by agency and applicable topic are in the Appendix of this outreach toolbox document.

One of the objectives of the water quality study was to develop a risk communication-based outreach toolbox that NDEE and other partners can use to promote awareness of nitrate in private domestic drinking water supplies. The water quality study accomplished the following tasks associated with this objective:

NDEE and its partners developed an outreach toolbox and identified private domestic well owners, the medical community, well drillers, septic system installers, realtors, and mortgage lenders as target audiences to promote awareness of nitrate issues in private domestic drinking water supplies. This toolbox can be used by NDEE and its project partners to help deliver consistent messaging related to nitrate in drinking water.

Key recommendations for future nitrate related outreach in private domestic drinking water include:

- NDEE and its partners should continue to develop and refine risk communication resources developed during the study to provide a clear, unified message on nitrate in drinking water. As funding allows, NDEE and its partners could continue private well sampling and treatment programs. Coordination with partner agencies could improve the visibility of these programs for private domestic well owners.
- Continue work to increase private domestic well testing.
- Create a database of unregistered well locations and owner contact information by implementing the methodology discussed in the Outreach Toolbox. This would allow for direct and more cost-effective outreach to unregistered well owners.
- Increase well registrations by reducing obstacles for registration. This could potentially include temporarily waiving the fee for the registration of old wells that predated the well registration requirement (pre-1993). Additionally, creating a simplified registration form for old wells or modifying the existing form may help avoid discouraging owners from registering wells due to lack of detailed information currently required to register a well.

1.0 PURPOSE, SCOPE, AND BACKGROUND

During the 2023 legislative session, the Nebraska Legislature, at the request of Governor Jim Pillen, appropriated funding for the Nebraska Department of Environment and Energy (NDEE) to conduct a statewide water quality study (LB 814). The overall goal of the water quality study is to provide an analysis and recommend viable solutions for nitrate-affected drinking water, including drinking water not regulated by the Safe Drinking Water Act (SDWA) (i.e. private domestic wells). This outreach toolbox fulfills the study objective to develop a risk communication-based outreach toolbox that NDEE and other partners can use to promote awareness of nitrate in private domestic drinking water supplies.

The outreach toolbox must identify the target audience(s) impacted by nitrate-affected groundwater used for drinking water, provide methods to reach them, and resources for current and future outreach partners to use. The resources must be engaging and provide related background/summary information on the concerns with drinking nitrate-affected water. The toolbox must also have specific recommendations for proactively reaching the target audiences, risk communication-based options and templates for engaging with the target audience and contact information for NDEE and others if they choose to contact representatives from local, county, state, and/or federal environmental and health related departments/agencies.

Nitrate $(NO₃)$ is a compound that occurs naturally and has many anthropogenic sources. Nitrate is in some lakes, rivers, and groundwater in Nebraska. Because nitrate is highly soluble, contamination occurs primarily through leaching of nitrate-nitrogen through the soil profile. Primary sources of organic nitrate include human sewage and livestock manure. The primary source of inorganic nitrate is agricultural and domestic fertilizer. Protection of groundwater sources from leaching nitrate is a complex issue because leaching depends on several factors including precipitation amount and timing, soil type and depth, biological fixation of nitrate, and uptake rates and timing of nitrate by plants (Shaver, 2014).

Of the approximately 1,960,000 people (about twice the population of South Dakota) living in Nebraska, over 1,600,000 Nebraskans are served their drinking water by community water systems (CWS), including rural water districts (RWDs), leaving an estimated 360,000 Nebraskans that rely on private domestic wells for their drinking water supply (NDEE, 2023). Before September 1993, private domestic wells were not required to be registered with Nebraska. Since then, private domestic wells have been required to be registered and are listed in the Nebraska Department of Natural Resources (NDNR) Registered Well Database. The NDNR Registered Well Database listed over 34,600 active private domestic wells in the state as of November 2023. NDEE has estimated there are approximately 145,000 private domestic wells across the state, meaning over 110,000 private domestic wells are unregistered (2023).

Addressing water quality concerns regarding nitrate contamination in groundwater used for drinking water must be a collaborative and iterative process that involves public and private entities, spanning across all sectors because drinking water quality affects more than just environmental quality, it affects public health and economies at various scales. Therefore, this outreach toolbox seeks to unify the entities already working across Nebraska to inform, manage, and address water quality concerns regarding nitrate contamination in drinking water as well as to identify the challenges in providing effective outreach to the owners of registered and unregistered private domestic wells. Locally, there may be other drinking water quality concerns, but this effort is focused solely on nitrate.

1.1 Existing Partners, Resources, and Ongoing Outreach

There are multiple entities and resources that are working to inform the public, specifically private domestic well owners, about the causes, threats, and actions to take when facing nitrate contamination in groundwater used for drinking water. One outcome of this outreach toolbox is to identify those partners and existing resources available to the public.

1.1.1 NDEE

NDEE is the lead agency behind the development of the statewide water quality study and subsequential risk assessment and outreach tools. NDEE is also one of the leading governing bodies regarding surface water and groundwater quality in the State of Nebraska and regularly provides education and outreach to the public. That said, this toolbox's intent is to summarize available resources that NDEE and its partners can use for outreach related to nitrate in drinking water, specifically private domestic wells. NDEE has some existing resources tailored for outreach to private domestic well owners and has developed new resources as part of the water quality study such as a "Nitrate in Drinking Water Fact Sheet" with input from several project partners. These resources will be coupled with the outreach materials that NDEE's partners have available to present a unified and consistent message using risk-communication techniques to present the information on what nitrate is, why knowing exposure to high concentrations is important, and what a well owner can do to address these issues. Resources such as a "Nitrate in Drinking Water Fact Sheet" and information related to "Nitrate Test Kits and Treatment Units" can be found in Appendix A.

1.1.2 Natural Resources Districts

Nebraska's 23 Natural Resources Districts (NRDs) were established to provide locally led management of resource concerns. The Nebraska Legislature has identified 12 areas of responsibilities for the NRDs with programs and priorities then being established by a locally elected board of directors. This unique resource management approach has been tailored to match Nebraska's climatic, geologic, soil type, topographical, land use and hydrologic variation. Regarding groundwater quality, each NRD has developed a State-approved management plan that lays out the process for monitoring and evaluation of groundwater in a region and the steps to be taken to address declines in quantity or quality. As a result of this process, the Nebraska Legislature has found that Nebraska NRDs have the legal authority to regulate certain activities and, as local entities, are the preferred regulators of activities which may contribute to groundwater contamination in both urban and rural areas. Additionally, the Legislature has found that the powers given to the NRDs and the NDEE should be used to stabilize, reduce, and prevent the increase or spread of groundwater contamination.

As stated above, the unique nature of Nebraska allows for cropping practices and types that include corn, soybeans, alfalfa, wheat, dry edible beans, sorghum, sugar beets, potatoes, and others. In some areas, production requires supplemental irrigation where precipitation may not consistently meet the targeted crop's needs.

Annual monitoring and assessment are conducted to track groundwater quality conditions within each district to identify concerns. When concerns are detected, NRDs will delineate control areas and initiate regulatory actions. While areas of nitrate contamination have been identified, not all groundwater supplies are a health concern.

While similarities do exist in each NRD's management approach, the difference from district to district requires that management be tailored for the local conditions, and, therefore, a one-sizefits-all nitrate management approach is not practical. Each NRD provides information where patrons can navigate through and find out more information on groundwater monitoring, assessment, regulatory and cost share programs.

To find out more about your NRD go to [www.nrdnet.org/nrds/find-your-nrd.](http://www.nrdnet.org/nrds/find-your-nrd)

1.1.3 University of Nebraska

The University of Nebraska (NU) system is at the forefront of research and education on the nitrate contamination issue facing the State of Nebraska. Many entities and partners are nested under the NU umbrella. The roles and resources of each of those entities are described below. NDEE worked with two liaisons from the NU system who were on the Advisory Group for this project and helped provide information about existing resources available under the NU umbrella.

1.1.3.1 Nebraska Extension

Nebraska Extension is an organization that aims to strengthen Nebraska's agriculture and food systems, provide outreach to Nebraskans and their communities, and enhance the health and wellbeing of all Nebraskans with the resources, research, and innovation of the University of Nebraska. One of Nebraska Extension's primary focus areas is "Water and Cropping Systems". Extension employees who work within the Water and Cropping Systems focus area aim to assist all Nebraskans with issues related to water and crops, including drinking water quality, nitrate, and nitrogen fertilizer applications. Extension employees are in every county throughout the entire state, giving them the opportunity to build strong relationships with the people they serve. Nebraska Extension is closely aligned with outreach as a primary driver of operations, thus their involvement as a partner in outreach to private domestic well owners are paramount. A few resources available through Nebraska Extension that would be most pertinent to this outreach toolbox include various extension/education documents, also known as "NebGuides", a "Water Quality Network Mapping Resource" and a recent 2024 YouTube Video dedicated to private domestic well owners in Nebraska can be found in Appendix A.

Lastly, below (Figure 1) is a link and map version to the directory of all Extension employees who work within water and crops.

Figure 1. Extension Employee Map. [https://epd.unl.edu/program-area/water-integrated-cropping-systems.](https://epd.unl.edu/program-area/water-integrated-cropping-systems)

Additionally, Extension provides information called "CropWatch" which is a central resource that houses an article relating to crops, water, and pests. [CropWatch | University of Nebraska–](https://cropwatch.unl.edu/) [Lincoln \(unl.edu\)](https://cropwatch.unl.edu/)

1.1.3.2 University of Nebraska Medical Center

The University of Nebraska Medical Center (UNMC) is one of four campuses in the NU system and stands as the only public academic health science center in the state. UNMC plays a crucial role in educational outreach and engagement, leveraging its strong public relationships and integrating cutting-edge research.

Nitrate concentrations in drinking water are regulated to protect infants from methemoglobinemia (blue baby syndrome), but research suggests that ingestion of nitrate in drinking water may have other adverse health effects on both adults and children (Ward et al., 2018). Because of this, having a strong partnership with the UNMC adds credibility to the public health dimension of the outreach described in this toolbox. UNMC's deep ties with Nebraska Medicine, Children's Hospital, the Buffet Cancer Center, and the Water, Climate, and Health Program amplify its impact on public health outreach.

Nebraska Medicine and Children's Hospital, as clinical partners, bridge research with patient care, enhancing the credibility and effectiveness of public health initiatives. The Buffet Cancer Center's pioneering work in cancer research and treatment offers vital resources for community health education. UNMC's Water, Climate, and Health Program, located within the College of Public Health, conducts interdisciplinary research and disseminates information on environmental issues related to water and health.

1.1.3.3 Nebraska Water Center

The Nebraska Water Center and its Water Sciences Laboratory are part of the Daughtery Water for Food Global Institute at the University of Nebraska. The Nebraska Water Center is a water research institution that focuses on helping the University of Nebraska become an international leader in water research, teaching, extension, and outreach. The Water Sciences Laboratory is an incredible resource for private domestic well owners as it provides additional academic resources and a resource for these well owners to test their drinking water for contaminants. A few resources available through the Nebraska Water Center that are most pertinent to this outreach toolbox are located in Appendix A and include a "Nitrogen Timeline" and "Water Facts" informational flyer.

Figure 2. [Nebraska Nitrate Network • nitrate related programs / Topic • Kumu](https://kumu.io/crystalwater/nebraska-nitrate-network#nitrate-related-programs/topic) (created by UNL Water Center).

Figure 2 displays an ongoing effort to map who is doing what in Nebraska regarding water work, specifically drinking water. Network mapping is a way to visualize the connections in complex systems. With the number of academic, governmental, private, and non-governmental organization (NGO) partners involved in education, research, and incentive programs to help address nitrate, the Water Center wanted to visualize the network. This map includes the highlevel categories specific to nitrate in drinking water. A project level map is under development: [https://kumu.io/crystalwater/nebraska-nitrate-network](https://urldefense.com/v3/__https:/kumu.io/crystalwater/nebraska-nitrate-network__;!!OlGkjAV-rZfRNzw!NIB6Aag1Qxds1NT0NYlchk5d29fdalz7OJ4LugZw9la8dz2QsLx7fuj_rLd4f0-gWn6m94aSltEFQU0$).

If you have projects you want to add to the map, please contact the Nebraska Water Center.

Isotope analysis, in combination with other water quality indicators, can help identify sources of nitrate in groundwater. Ratios of nitrogen and oxygen isotopes vary between sources of nitrogen. Figure 3, from Kendall (2008), plots the relative ranges of isotope ratios from common environmental sources of nitrogen. Analyzing the ratio of isotopes allows organic and inorganic sources of nitrogen to be identified and, in turn, informs us more about potential sources impacting a specific well. Isotope testing is a valuable tool in determining the source of the nitrate impacts.

Figure 3. Nitrate Isotope Ratios by Source Type (Kendall, 2008).

1.1.4 NDHHS Public Health Environmental Lab

The Nebraska Department of Health and Human Services (NDHHS) Public Health Environmental Lab can supply nitrate test kits with instructions for the well owner to collect and return the sample for analysis. The NDHHS Lab generally calls customers if their nitrate result is over 10 mg/L to educate them about the health risks associated with drinking water with a nitrate concentration above the MCL. The NDHHS Public Health Environmental Lab is an important partner in nitrate outreach since they provide test kits and follow-up information, and often have direct communication with both registered and unregistered private domestic well owners.

1.1.5 Local Health Departments

Local Health Departments (LHDs) play an important role in raising awareness for the connection between drinking water quality and adverse health effects. To engage with the owners of private domestic wells, LHDs provide a valuable avenue to reach the primary audience. All LHDs are working on water quality, primarily focusing on encouraging private domestic well testing. Many are collaborating with NRDs in their efforts. One thing to keep in mind is that LHDs typically have different community connections than NRDs, such as with the medical community, hospitals, and underserved populations. Therefore, a continued partnership with the LHDs is very important in addressing nitrate in private domestic wells. Additionally, by partnering with

LHDs, the research and expertise of other partners like UNMC can provide guidance to these LHDs to proactively address these public health concerns, have more informed diagnoses, and provide specific guidance on addressing the public health component of drinking water quality concerns.

Some current resources include efforts by the North Central District Health Department, the Upper Elkhorn NRD, and the Lower Niobrara NRD who invited NDEE to help with a radio show in February 2024. NDEE provided them with information about the free nitrate test kits and the reverse osmosis rebate program to share during the radio show. A recording of this show is included in Appendix A. Also, as part of Appendix A, is a list of LHD contacts that can help clients who are concerned about their private domestic well drinking water quality.

1.1.6 Nebraska Department of Natural Resources

The NDNR and the NRDs are responsible for surface water and groundwater integrated management planning. The NDNR is responsible for well registrations in the state and maintains the Registered Well Database. They provided NDEE the list of mailing addresses for all registered well owners that was used to mail out the postcards as part of the free nitrate test kit effort. Because of this, they are an important partner in communicating with registered well owners. Additionally, since they manage all well registrations in the state, any potential changes in registration processes to help facilitate well registration must be done in coordination with them.

1.2 Existing Challenges

There are many existing challenges in the effort to engage and provide outreach to the owners of the estimated 110,000 unregistered private domestic wells and the 34,600 registered private domestic wells in the state. The following list of challenges is not exhaustive, but it does include a few of the principal challenges expected to be encountered during engagement of the target audiences.

1.2.1 Effective Communication Channels

The principal difficulty in outreach to the owners of private, unregistered domestic wells is the absence of any of these wells or owners being listed within the NDNR Registered Well Database. Due to this, there is no clear way to locate, contact, and inform these individuals of drinking water quality concerns regarding nitrate contamination. Many challenges facing this effort stem from the inability to effectively communicate with these well owners. Options exist for tracking these well locations such as the creation of a statewide unregistered well database or providing an opportunity for these wells to be registered in a simplified manner to help encourage the adoption of registration. However, to complicate the matter further, the parcel owner listed with the local county assessor's office for a specific parcel does not necessitate the owner of the well on a parcel to be identical to the listed parcel owner's name. Thus, directly contacting an unregistered private domestic well owner becomes even less feasible as there may be no way to digitally connect a potential unregistered private domestic well location to a specific individual. Thus, other avenues of engagement will be needed to reach the target audience aside from direct communication with the owners of these unregistered private domestic wells. Direct communication channels exist for private domestic wells registered with NDNR and listed in the Registered Well Database. When available, this channel could be and has been used. Outreach partners must understand and work to mitigate the difference in the effectiveness of communication between those with registered and unregistered private domestic wells.

1.2.2 Consistent Messaging

One issue in addressing a problem as complex as nitrate-affected groundwater used for drinking water is that each of the entities affected by nitrate contamination has their own mission, goals, and vision, as well as authority and messaging regarding the situation. NDEE is working with its partners to create consistent messaging, informed by risk-communication techniques that will provide information, solutions, and resources to owners of private domestic wells and other key stakeholders. The messages will be crafted as a coordinated and collective message from all the partners involved and create a unified stance for entities across the state to refer to when they work to address the complex issue of nitrate-affected groundwater used for drinking water.

1.2.3 Well Registration

As discussed in Section 1.0, there are an estimated 110,000 unregistered wells in Nebraska. Relative to outreach methods, this means the owners, locations and contact information are unknown which is a significant limiting factor in communicating risk to an unregistered well owner. Although having these wells registered allows for a more direct line of communication, the engagement with these individuals must transcend simply asking the unregistered private domestic well owners to register their wells. Nevertheless, providing outreach to private domestic well owners about nitrate in drinking water also provides an opportunity for NDEE and its partners to educate private domestic well owners about the benefits of registering their well. For example, if there is no formal documentation of the presence of a well in the NDNR Registered Well Database, the State is unable to protect the well when evaluating setbacks for a wastewater construction or onsite septic system permit or land application of wastewater. Additionally, any other entity, such as a well driller or septic system installer, that does a desktop review to ensure they meet well setback distances will only be aware of the presence of registered wells. The benefits of well registration and guidance on the process of well registration, although not the main priority of this outreach toolbox, is still an important message for NDEE and its partners to communicate. The benefits of registering a well extend beyond those issues associated with nitrate.

1.2.4 Cost to Target Audience

Many of the recommendations provided to the owners of private domestic wells come at some cost to the owner, and depending on the recommendation, sometimes a significant cost. One difficulty NDEE has already faced and will continue to face is apprehension to changes in private domestic well management purely from a cost-aversion stance. NDEE and its partners should strive to provide clear estimates of cost to the private domestic well owners for each of the different recommendations for addressing nitrate contamination in drinking water and, equally important, cost-share opportunities for these recommendations. NDEE and its partners occasionally have limited funding available through programs, grants, and funding sources that provide some level of financial support to private domestic well owners, but many individuals are not as aware of these financial assistance programs as those in the agencies that provide them. Pointing individuals to financial assistance opportunities, when available, will work to overcome the cost-aversion that could prevent a private domestic well owner from testing, treating, or protecting their drinking water.

1.3 Important Feedback from 2024 Well Testing

From November 29, 2023, through March 1, 2024, NDEE offered free nitrate test kits to all private domestic well owners in Nebraska regardless of well registration status. Over 29,000 postcards advertising the free kits were mailed out to all registered private domestic well owners in the state. Although there are more than 34,000 active registered domestic wells, some owners have multiple wells registered to the same mailing address. Press releases were published by Nebraska news outlets to encourage private domestic well owners to have their well tested (See Appendix A). As of May 1, 2024, 4,508 sample kits were requested, and 3,499 were returned to the NDHHS Public Health Environmental Lab for analysis. Figure 4 shows the ranges of nitrate concentrations, and the locations of wells tested during this sampling effort. As of May 2024, NDEE had called 13.7% of well owners that opted to test their wells or over 480 well owners with a nitrate level above 10 mg/L. NDEE fielded over 2,500 calls related to the free nitrate test kit effort (Figure 5). During these calls, NDEE and NRDs helped many private domestic well owners with the well registration process and the application process for the reverse osmosis rebate program. NDEE also mailed a nitrate brochure out to every well owner that participated in the free sampling effort when their results were mailed out. These phone calls and mailings have provided NDEE an avenue to provide direct, personal outreach to many Nebraskans who were previously unaware of the importance of regularly sampling their well for nitrate or the health risks associated with drinking water with a nitrate level above 10 mg/L.

3,478 Private Domestic Well Nitrate Samples from the 2023-2024 Free Nitrate Sampling Effort

Figure 4. Free Private Domestic Well Sampling Results (as of May 1, 2024).

Figure 5. Number of Overall Calls Regarding the Testing, Results, and Well Registration. Calls received were only tracked formally through March 1, 2024. These total numbers are as of May 2024 and also include calls made by NDEE to private domestic well owners.

The feedback received from the public, lessons learned by NDEE staff engaging with the public, and questions asked by the public have all been incorporated to tailor outreach tools to address these curiosities, fears, and questions and align with providing the target audiences with the information they need. Some of the topics informed by this feedback include explaining sample analysis results to the general public and communicating to a non-scientific community, being able to provide private domestic well owners an immediate recommendation to address high nitrate concentrations in their well, walking individuals through the well registration process, and the necessity for a clear and consistent message from all outreach partners. Appendix A includes the nitrate informational brochure and postcard, various press releases, and other resources related to the sampling effort including sampling instructions and how to interpret the results (both of which are products from NDHHS).

HEALTH QUESTIONS/CONCERNS:

- A caller reported that she recently had an infant that had to stay in the hospital after birth due to hemoglobin issues. They are renters and had not previously tested their drinking water. The nitrate concentration for their well was 19.2 mg/L. The doctors treating the infant never mentioned the possibility of nitrate-contaminated drinking water as a potential cause of the infant's health problems. This same NDEE employee talked to at least two other women that are currently pregnant and have been drinking water over 10 mg/L and were not aware of the risks prior to sampling.
- Many questions about potential health effects in addition to methemoglobinemia or for adults. UNL has resources that these private domestic well owners can be directed to if they want to know more about potential health effects in adults.
- Doctors may not know to talk about nitrate in drinking water or consider nitrate in drinking water as a potential cause of certain health effects.
- Concern regarding how nitrate in drinking water can affect pets and livestock.
- What they can and cannot use high nitrate water for (bathing, washing dishes, etc.)

QUESTIONS/CONCERNS RELATED TO REGISTRATION, TESTING, AND SOLUTIONS:

- Hesitation to connect to a RWD even when the nitrate concentration in their well is above 10 mg/L. Some private domestic well owners indicated they think their well water would be of higher quality than water supplied by the RWD. Others have indicated they prefer to have their own well or like the taste of their well water. For others, connecting to a RWD is cost prohibitive.
- Emotional responses if an immediate solution is not available (treatment or bottled drinking water). It is important to provide recommendations to private domestic well owners, so they know how to properly address a nitrate level above the MCL, such as bottled water for drinking and the installation of an RO unit (or other treatment device) and retesting after installation.
- Hesitation to test wells if not registered due to concerns of getting in trouble. A fear of the government having additional information about their well.
- Questions about what other contaminants they should test for in private domestic wells.
- How to find out if well is registered and how to register a well if it is not registered.
- Questions about what technologies remove nitrate from water. Many wondered if carbon filters or water softeners are effective at removing nitrate from their water, both of which are not.

2.0 TARGET AUDIENCES

The issue of nitrate contamination in drinking water is so vast, outreach could not reasonably be tailored to address every entity affected by the issue. Instead, outreach tools and materials have been specifically designed for the primary audience of homeowners and businesses that have private domestic wells which may be used for drinking water. The toolbox also includes secondary audiences, the larger medical community outside of the UNMC system, licensed well drillers, septic system installers, realtors, and mortgage lenders. The method to engage and desired outcome for each of these audiences differs, consequently this section will outline the method of which to reach each target audience along with the desired outcome for said audience.

2.1 Private Domestic Well Owners

The primary audience is individuals and businesses that have private domestic wells which may be used for drinking water, regardless of well registration. The desired outcomes for this audience are to engage the private domestic well owners so they are aware of the issue of nitrate in the groundwater they rely on for drinking water and inform these owners of the resources available to them regarding the identification of potential localized risks to their well, potential management practices to protect their source water and/or treat their drinking water, and funding sources.

The methodology to reach this population widely varies with the status of a private domestic well's registration. Those who are registered are much simpler to engage as their information is listed in NDNR's Registered Wells Database. These well owners can be contacted in direct communication (i.e. direct mailing) through the information provided to NDNR. Conversely, the owners of unregistered private domestic wells will need to be addressed through indirect channels of communication without the development of a tool to allow for a more direct channel of communication. One method to reach these unregistered private domestic well owners include the education of secondary target audiences with the outlook that these secondary audiences may have direct contact with the unregistered private well owners and would be able to communicate the resources or direct the owners to the resources that have been created or will be created. Additionally, public events such as the State Fair, public meetings, education events at local schools, and broadcasting over local news outlets or radio provide opportunities for these individuals to be engaged.

2.2 Medical Community

UNMC is a partner in the development of this outreach toolbox and is critical in communication to one of the secondary audiences: the larger medical community outside of the NU system. Extending this outreach to the broader medical community is important because drinking water quality has a direct impact on the health of those drinking it. The desired outcomes for the engagement with the larger medical community are to equip and empower LHDs and subsequently local health care providers with the knowledge and resources to have conversations with patients about the importance of drinking water quality and the effects it can have on one's health, as well as be able to identify health issues that may be an outcome of drinking high nitrate water and direct those with water quality issues to the resources available through NDEE and its partners.

To reach this sector, all partners could build on their connection with NDHHS and past work with LHDs as well as encourage partners such as UNMC to supply information to medical professionals through newsletters, education events, and continuing education credits. There are numerous associations for medical professionals that NDEE and other partners have worked with in the past and could utilize to pass along information regarding new resources and tools available and seek to get approval for new continuing education credit opportunities related to nitrate.

2.3 Licensed Well Drillers and Septic System Installers

Licensed well drillers and septic system installers across the state play a crucial role in educating private domestic well owners about how to properly maintain their well, the basics of hydrogeology and groundwater movement, and how contamination of a private domestic well can occur. Most septic systems and private domestic wells are installed in similar areas where individuals are outside of a public water system and are not served by a sanitary sewer, thus these audiences are paired as one because these professionals engage with private domestic well owners in similar fashions. The desired outcome for these professionals is to create and provide resources to assist them with having conversations and passing along materials and tools to private domestic well and septic system owners when these professionals are hired to replace, repair, inspect, or install their respective systems.

To reach these two groups of individuals, NDEE can make direct engagement by utilizing the license numbers of licensed well drillers and septic system installers as both licensure programs reside within NDEE. These professionals must complete continuing education, and NDEE, along with other organizations, provides educational courses, workshops, and conferences. Education materials can be incorporated into these training sessions to help inform owners and minimize potential impacts to private domestic wells. NDEE could also send these individuals direct information through mail and email. Furthermore, there are professional associations that NDEE engages with that could pass information along in a newsletter such as the Nebraska Well Drillers Association.

2.4 Realtors and Mortgage Lenders

Realtors and mortgage lenders are commonly involved with private domestic well owners during property transactions. Additional outreach with realtors and mortgage lenders could increase private domestic well sampling and treatment if warranted and increase awareness of the NDEE Well and Septic Loan Evaluation program [\(http://dee.ne.gov/NDEQProg.nsf/OnWeb/WSLE\)](http://dee.ne.gov/NDEQProg.nsf/OnWeb/WSLE). Realtors and mortgage lenders are familiar with radon issues and processes for testing indoor air and mitigation. General awareness outreach efforts to inform realtors and mortgage lenders of nitrate issues faced by Nebraska private domestic well owners could increase well testing, registration, and treatment. Realtor and mortgage lender associations should be targeted for additional indirect outreach and to provide education on available tools and resources.

3.0 LONG-TERM PLANNING AND PREPAREDNESS

Addressing complex issues such as nitrate-affected groundwater used for drinking water must be an iterative and ongoing process, therefore communication with the target audiences mentioned in **Section 2** must also be similarly viewed as a long-term process that continues to evolve as the situation changes, new findings arise, and as progress is made. Establishing an expectation of coordination and a framework for actions that make progress towards the outreach goals will ensure that progress is made, and efforts remain consistent and unified.

3.1 Future Coordination and Management

Although the list of existing partners is significant and casts a wide net regarding sectors involved, there are countless additional partnerships to be developed. These partnerships include furthering the relationship between NDEE, its partners and other organizations that play a role in the use and management of nitrate across all sectors of the economy of the State of Nebraska. This section aims to outline the partnerships that should be developed or enhanced. As UNMC and others continue to research the health implications of nitrate in drinking water, consistent guidance on having conversations about drinking water sources, identifying when water quality concerns are present, and being able to point patients in the direction of resources are all actions local health officials should be empowered to do. Furthermore, incorporating additional programs within the NDHHS, the entity in charge of licensing Health Care Professionals and the program for the prevention of waterborne illnesses, into the list of partners to ensure the messages provided are consistent with the other main partners. Aligning the message coming from separate state agencies would add to the credibility and confidence with which the public may perceive this issue.

The education sector provides excellent opportunities for NDEE to leverage existing relationships such as Nebraska Extension to reach Nebraska's youth and educate young generations on nitrate in drinking water. High schools, middle schools, and elementary schools all offer opportunities for NDEE and its partners to engage with the public, including both students and parents or guardians of students, and inform, educate, and provide resources to this population. For instance, the "Know Your Well" program is a joint citizen science training program that involves mostly high school students on how to sample and test well water quality. This project began in 2017 with up to at least four schools and, to date, has worked with over 30 schools and hundreds of students across Nebraska [\(https//:knowyourwell.unl.edu\).](https://knowyourwell.unl.edu/)

Two groups of professionals that directly encounter private domestic well owners are well drillers and septic system installers. These two groups of people are pivotal in protecting the drinking water sources; for example, septic system installers can protect drinking water by siting new septic systems downgradient (in terms of groundwater flow) of existing private domestic wells and well drillers by siting new private domestic wells upgradient (in terms of groundwater flow) of existing septic systems. Furthermore, these two groups of people can educate members of the public on proper usage and maintenance of their wells and septic systems. These individuals can be reached by NDEE through their respective license numbers.

Other partnerships to enhance include RWDs and associations, agronomists, and elected officials such as members of the Corn Board or Cattleman's Association. The above list of potential partnerships to develop or enhance is not exhaustive nor should the development of partnerships be limited to this list. Different entities provide various opportunities to engage with the public through different channels and using different mechanisms. The channels,

mechanisms, and entities not listed in this toolbox should be considered as viable outreach partners.

3.2 Other Potential Considerations and Activities

Addressing the challenges outlined in **Section 1.2** will require not only future coordination and partnerships between entities, but also the development of tools to close the gap in communication between the partnering entities of this outreach toolbox and the target audiences described in **Section 2**. Some of these tools include the possible creation of a potential unregistered well database. The development of this database could incorporate existing resources such as using Microsoft Building Footprints to identify potential residences anticipated to have private domestic wells while eliminating locations with registered wells.

4.0 OUTREACH IMPLEMENTATION

Engagement and outreach regarding nitrate-affected groundwater used for drinking water has been ongoing, thus, materials and tools developed for this effort are not geared towards pioneering outreach, but to reinforce the ongoing efforts of NDEE's programs and partners and unify efforts under a common message. Implementation of renewed outreach efforts begins with the development of new materials that are tailored to the primary and secondary audiences while carrying the unified message regarding nitrate contamination in drinking water. Figure 6 provides an overview of the numerous agencies contributing to outreach and the target audiences.

4.1 NDEE

NDEE has a multi-faceted team of professionals across the water quality realm. This team was charged with reaching a consensus on what new materials, tools, resources, and steps to develop to meet the objective of the outreach toolbox. All materials and tools follow riskcommunication techniques and adhere to the best available science regarding nitrate in drinking water and the implications of such. NDEE staff from various programs will utilize outreach materials through the course of normal business and will identify additional opportunities to collaborate with new and existing partners to conduct outreach to broader audiences.

4.1.1 Nitrate in Drinking Water Fact Sheet

This resource provides a high-level understanding of nitrate in drinking water and was developed to target private domestic well owners. This fact sheet provides background information about nitrate and sources of nitrate; health effects of consuming drinking water contaminated with nitrate; information on prevention, protection, and potential actions for private domestic well owners to take if their drinking water has nitrate concentrations above the 10 mg/L MCL; and additional resources to learn more or contact professionals. This fact sheet should be provided to all private domestic well owners. This fact sheet can be mailed directly to those private domestic well owners in the NDNR Registered Well Database and should be dispersed to the secondary audiences to provide to the public in their storefronts, waiting rooms, or while they are on the job at the site of a private domestic well in an effort to get this resource to unregistered private domestic well owners.

4.1.2 Frequently Asked Questions

The following questions were frequently asked by well owners during calls associated with the free nitrate test kit program, and the resources in Appendix A were compiled and developed to assist in answering these in the future:

- Q: *What could be causing high nitrate in my well?*
- A: See the Nitrate in Drinking Water fact sheet for common nitrate sources that may contributing to nitrate in your well, and the isotope testing discussed in Section 2.1.3.3. Isotope testing would need to be completed to determine the specific cause of high nitrate in your well.
- Q: *Are there any other contaminants I should be concerned about in my well water?*
- A: Yes, see the Nitrate in Drinking Water fact sheet. There are numerous other contaminants that could be in your well water. The NDHHS laboratory can assist with nitrate analysis and other contaminant analysis. At a minimum, you should test for nitrate and bacteria annually.
- Q: *Are there any health concerns associated with drinking water with a nitrate level above 10 mg/L for adults or just infants?*

A: Yes, see the Nitrate in Drinking Water fact sheet. Adults with certain health conditions may be at a higher risk of developing methemoglobinemia from drinking water with a nitrate level above 10 mg/L. These health conditions include anemia, cardiovascular disease, sepsis, and certain gastric diseases. Additionally, research is ongoing and suggests a link between drinking nitrate-contaminated water and colorectal cancer, thyroid disease, and neural tube defects in adults.

Q: *How can I address high nitrate in my well?*

- A: There are multiple options, see the Nitrate in Drinking Water fact sheet. The quickest and most common option is to install a reverse osmosis, ion exchange or distillation filtration system to remove nitrate from your drinking water. Additionally, you can use bottled water for drinking and cooking.
- Q: *Can my pets/livestock drink my well water with nitrate above 10 mg/L?*
- A: See the Nitrate in Drinking Water fact sheet. It is recommended to not allow livestock to drink water with a nitrate level above 100 mg/L. Please consult with your veterinarian if you have concerns about an acceptable nitrate level for pets, such as dogs and cats.
- Q: *I heard that drinking water with high nitrate levels can cause cancer. Can you tell me more about that?*
- A: See the Nitrate in Drinking Water fact sheet. This document includes information about potential health effects of drinking water with nitrate above the MCL of 10 mg/L. Additionally, this document includes links from the University of Nebraska-Lincoln with more information about potential health effects.
- Q: *Can I wash dishes or bathe in high nitrate water?*
- A: Nitrate does not easily absorb through the skin, so it is safe to use water with nitrate levels above 10 mg/L for bathing and laundry, as long as the water is not swallowed while bathing. Very little water clings to smooth surfaces so washing dishes with water with nitrate levels above 10 mg/L is also acceptable.
- Q: *Will my fridge filter remove nitrate?*
- A: No. See the Nitrate in Drinking Water fact sheet for types of treatment processes that will remove nitrate from your water. You should test your water after installation of a treatment process to confirm adequate removal of nitrate. Additionally, treatment systems require ongoing maintenance to remain effective.
- Q: *Will a water distiller remove nitrate?*
- A: Yes, see the Nitrate in Drinking Water fact sheet.
- Q: *Why isn't my well registered?*
- A: Wells constructed prior to September 1993 were not required to be registered. If you have questions about well registration, please contact the Nebraska Department of Natural Resources.

4.1.3 Predictive Nitrate Model

One goal of the water quality study was to evaluate the relative risk of elevated nitrate concentrations in private domestic wells. To this end, models based on available regional nitrate sample data, well construction, land use trends, soils, and geology were developed to estimate the probability that nitrate concentrations across Nebraska will exceed threshold concentrations. Model predictions were used to estimate a low, medium, or high-risk level and supplement the GIS tool for use by NDEE and key partners, which is described in the following section. Figure 5 maps these results, where green and yellow areas represent low to medium risk and orange and red areas represent medium to high risk. It is important to note these are estimated probabilities and not field-confirmed data. Although outreach should occur throughout the state, these model results help target higher risk areas for nitrate where outreach implementation is particularly important.

Predictive Nitrate Model Results: Composite Layer in Terms of Nitrate Concentration

Figure 6. Predictive Model Risk Levels. Green and yellow areas represent low to medium risk and orange and red areas represent medium to high risk.

4.1.4 Geographic Information System Groundwater Tool

NDEE and project partners coordinated during the study to develop an interactive, online GIS risk assessment tool for use by NDEE or agency partners. Users of the tool can enter the address of a well or drop a pin on the map at a well location. Then, the tool queries available information for the well location and calculates a risk index for that point. There are four different indicators included in the tool to calculate the overall risk index: predictive model results, nearby nitrate sample results, possible nearby sources of nitrate, and estimated depth to groundwater. A personalized report is created for the user detailing the overall risk for that point.

NITRATE IN PRIVATE DOMESTIC WELLS

Figure 7. Nitrate in Private Domestic Wells – Outreach Agencies and Target Audiences

5.0 CONCLUSIONS AND RECOMMENDATIONS

The effort to address nitrate-affected groundwater used for drinking water is a continuous, statewide, multi-faceted, and multi-agency endeavor. Outreach tools and materials compiled and developed out of the nitrate study are available (Appendix A) to partners to adopt and apply locally in the context of their sphere of influence. This outreach effort has:

- Compiled and modified existing resources for future outreach activities,
- Developed new tools for future outreach activities,
- Identified opportunities and methods for engaging private domestic well owners and other audiences to promote awareness and options for addressing nitrate-affected groundwater used for drinking water.

Key recommendations for future nitrate-related outreach for private domestic wells used for drinking water include:

- NDEE and its partners should continue to develop and refine risk communication resources developed during the study to provide a clear, unified message on nitrate in drinking water. As funding allows, NDEE and its partners could continue private well sampling and treatment programs. Coordination with partner agencies could improve the visibility of these programs for private domestic well owners.
- Continue work to increase private domestic well testing. Regularly testing private domestic wells for nitrate and bacteria is an important part of continued outreach to protect public health. Although 4,508 kits were requested as part of the free sampling effort, only 3,499 (74%) had been returned as of May 1, 2024. This indicates that some owners may have initially been interested in requesting the kit since it was free but lacked urgency to collect the sample and return it to the lab. Continued outreach to educate private domestic well owners of the importance of regularly testing their well for nitrate and bacteria is crucial to increase the amount of people that test their well.
- Create a database of likely unregistered well locations and owner contact information by implementing the methodology discussed in the Outreach Toolbox. This would allow for direct and more cost-effective outreach to unregistered well owners.
- Increase well registrations by reducing obstacles for registration. This could potentially include temporarily waiving the fee for the registration of old wells that predated the well registration requirement (pre-1993). Additionally, creating a simplified registration form for old wells or modifying the existing form may help avoid discouraging owners from registering wells due to lack of detailed information currently required to register a well.

6.0 REFERENCES

- Dubrovsky, N.M., K.R. Burow, G.M. Clark, J.M. Gronberg, P.A. Hamilton, K.J. Hitt, D.K. Mueller, M.D. Munn, B.T. Nolan, L.J. Puckett, M.G. Rupert, T.M. Short, N.E. Spahr, L.A. Sprague, and W.G. Wilber. 2010. The quality of our Nation's waters—Nutrients in the Nation's streams and groundwater*,* 1992–2004. U.S. Geological Survey Circular 1350.
- Nebraska Department of Environment and Energy (NDEE). 2023. 2023 Nebraska Groundwater Quality Monitoring Report. Retrieved from: [http://dee.ne.gov/Publica.nsf/PubsForm.xsp?documentId=60154D2D29AFC49486258A](http://dee.ne.gov/Publica.nsf/PubsForm.xsp?documentId=60154D2D29AFC49486258A760053F4DC&action=openDocument) [760053F4DC&action=openDocument](http://dee.ne.gov/Publica.nsf/PubsForm.xsp?documentId=60154D2D29AFC49486258A760053F4DC&action=openDocument)
- Shaver, T., R. Khosla, and D. Westfall. (2014) Evaluation of Two Crop Canopy Sensors for Nitrogen Recommendations in Irrigates Maize, Journal of Plant Nutrition, 37:3, 406-419.
- Ward, M. H., Jones, R. R., Brender, J. D., de Kok, T. M., Weyer, P. J., Nolan, B. T., Villanueva, C. M., & van Breda, S. G. (2018). Drinking Water Nitrate and Human Health: An Updated Review. International journal of environmental research and public health, 15(7), 1557.<https://doi.org/10.3390/ijerph15071557>

Appendix A – Tools and Resources

Web-Based Resources

Nebraska Department of Environment and Energy (NDEE)

[Well and Septic Loan Evaluation](https://giscat.ne.gov/portal/apps/experiencebuilder/experience/?id=8c60c82720e34f88bd28c909eb12bdcf) <http://dee.ne.gov/NDEQProg.nsf/%24%24OpenDominoDocument.xsp?documentId=5A8EB80D> [6AB5B906862583AE005AB4D3&action=OpenDocument](http://dee.ne.gov/NDEQProg.nsf/%24%24OpenDominoDocument.xsp?documentId=5A8EB80D)

Natural Resources Districts (NRD)

Natural Resources Districts <http://www.nrdnet.org/nrds/find-your-nrd>

University of Nebraska-Lincoln School of Natural Resources

CropWatch <https://cropwatch.unl.edu/>

Water and Health Resources <https://water.unl.edu/category/water-and-health>

Water and Cropping Systems Personnel <https://epd.unl.edu/program-area/water-integrated-cropping-systems>

NebGuides <https://water.unl.edu/article/drinking-water/nebguides>

Call to Action Nebraska Nitrate Conference <https://water.unl.edu/article/nitrate/nebraska-nitrate-working-groups-summary-and-call-action>

UNL Extension YouTube Nitrate Videos [https://www.youtube.com/@nebraskawaves6405.com](https://www.youtube.com/%40nebraskawaves6405.com)

Nebraska Water Center (Daugherty Water for Food Global Institute)

Nebraska Statewide Nitrate Network Graphic (real time) [https://kumu.io/crystalwater/nebraska-nitrate-network#nitrate-related-programs/topic](https://kumu.io/crystalwater/nebraska-nitrate-network%23nitrate-related-programs/topic)

Nitrate Network Map for Nebraska https://knowyourwell.unl.edu

Nebraska WAVES YouTube Channel

Private Well Testing https://www.youtube.com/watch?v=3KzHU2d_u6o

KBRX Podcast

Water Nitrate Testing Information <https://www.kbrx.com/episode/water-nitrate-testing-information-from-ncdhd-uenrd-lnnrd/>

Social Media Links

NDEE Twitter: @NebraskaDEE DHHS Twitter: @NEDHHS NE Water Center Twitter: @NebrWaterCenter UNMC Twitter: @unmc UNMC's Water, Climate, and Health Program Twitter: @UNMC_WCHP DNR Facebook: https:/[/www.facebook.com/nebraskadnr/](http://www.facebook.com/nebraskadnr/)

NITRATE IN PRIVATE DOMESTIC WELLS

3,478 Private Domestic Well Nitrate Samples from the 2023-2024 Free Nitrate Sampling Effort

Predictive Nitrate Model Results: Composite Layer in Terms of Nitrate Concentration

Credits: NDEE Drinking Water and **Groundwater Division** Spatial Reference: NAD 1983 State Plane Nebraska FIPS 2,600 Feet Projection: Lambert Conformal Conic

FREE DRINKING WATER NITRATE TESTING

Learn more about how to request your free private domestic well sample kit

Greetings,

You are receiving this postcard because you are listed as the owner of a registered domestic well in Nebraska. If your domestic well is used for drinking water, the Nebraska Department of Environment and Energy is offering to provide a free sample kit and lab analysis to test your well for nitrate for a limited time. To request a sample kit, scan the QR code on the front of this postcard, go to the website listed below, or call our office at (402) 471-2186. A sample kit, sampling instructions, and prepaid return postage will be sent to you. You will receive your sample results and the data will be used in a statewide nitrate study that NDEE is currently conducting.

Sincerely

Jim Macy, NDEE Director

To request a sample kit, visit: https://dhhs.ne.gov/Pages/Lab-Price-List.aspx The maximum contaminant level (MCL) for nitrate in drinking water is

10 mg/L

If your water sample is above this level, see the RO rebate information inside this brochure.

Contact information

Nebraska Department of Environment and Energy

Front desk — 402-471-2186 **Email** — ndee.moreinfo@nebraska.gov **website** — dee.ne.gov

P.O. Box 98922 Lincoln, Nebraska 68509-8922

NEBRASKA

Good Life. Great Resources.

DEPT. OF ENVIRONMENT AND ENERGY

incoln, Nebraska 68509-8922 Lincoln, Nebraska 68509-8922 Nebraska Department of Environment and Energy
P.O. Box 98922 Environment and Energy Nebraska Department of 84001097 3400109° P.O. Box 98922

Understanding your well sample results

Provided by NDEE's free nitrate sample kit program

You are receiving this brochure because your private drinking water well has been tested for nitrate recently. This brochure is intended to help you understand the sample results.

It is recommended that private well owners regularly test their wells to know the quality of their water. Regardless of your result, it is recommended that you test your well for nitrate each year in case your water quality changes.

What is nitrate?

Nitrate and nitrite are forms of nitrogen in the environment from both natural and man-made causes. They are used for fertilizer in agriculture and residential applications; are present near certain industrial sites, septic, and livestock waste systems; and are also found naturally occurring in the soil. Additionally, shallow wells, wells in sand aquifers, or poorly constructed wells are more vulnerable to nitrate contamination. Large amounts of nitrate in drinking water can be harmful to a person's health.

Health Concerns Associated with Nitrate

The Safe Drinking Water Act requires the Environmental Protection Agency to determine safe levels of chemicals in drinking water. The maximum contaminant level of nitrate in drinking water set by the EPA is 10 mg/L. Although private wells are not regulated by the Safe Drinking Water Act, there are safety concerns associated with consuming water with a nitrate concentration above 10 mg/L. Consuming high levels of nitrate has been linked to methemoglobinemia, or blue baby syndrome. Babies that are under six months old and are bottle fed are at risk for this disease that can cause the skin to turn a bluish color and, if left untreated, could result in serious illness or death.

Nitrate and private wells

Approximately 84% of Nebraskans get their water from community water systems that are regulated by the Safe Drinking Water Act and are protected from harmful nitrate exposure. However, private wells are not regulated by the Safe Drinking Water Act and are not required to be tested for nitrate under state or federal law. Only a portion of the private drinking water wells in Nebraska have been tested, and those that have often have a higher nitrate level than community water systems. It is imperative for Nebraskans on private drinking water wells to know if their drinking water is safe. Elevated nitrate levels are a particular concern in Nebraska.

What should I do if my test results are above 10 mg/L?

You should not use water from this well to prepare formula for a bottle-fed infant or for cooking. Do not boil the water. Boiling causes evaporation and concentrates the nitrate in the water. It is okay to bathe with this water, however, the Department recommends that you locate an alternate drinking water source or install an inhome treatment system.

Resources to treat nitrate in drinking water

If your well tested over 10 mg/L of nitrate, you may be eligible for NDEE's Reverse Osmosis (RO) Rebate Program to treat your water. This program offers up to \$4000 in rebates to well owners whose wells are registered through the Nebraska Department of Natural Resources and whose water sample is above 10 mg/L of nitrate.

Learn more about the RO Rebate Program by scanning the QR code, or visiting the website listed below:

http://dee.ne.gov/Publica.nsf/pages/22-051

IT IS IMPORTANT TO KNOW IF YOUR DRINKING WATER PUTS YOU AT RISK. NDEE CAN HELP.

DEPT. OF ENVIRONMENT AND ENERGY

NITRATEINDRINKINGWATER Nitrate is a compound that occurs naturally and has many human-made sources.

Nitrate is in some lakes, rivers, and groundwater in Nebraska. You cannot taste, smell, or see nitrate in water. Consuming too much nitrate can be harmful—especially for babies.

Background Information

Nitrate occurs naturally and at safe and healthy levels in some foods (e.g., spinach and carrots) and comes from natural processes, like plant decay. The primary source of inorganic nitrate is from fertilizers used on yards, gardens, golf courses, and crops. Certain industrial processes and leaks from fertilizer storage can also be a source of inorganic nitrate. Common sources of organic nitrate are human and animal waste.

Nitrate in Nebraska Water

Nitrate has been found in groundwater across Nebraska. While nitrate occurs naturally, levels in groundwater above 3 mg/L are considered an indicator of human-driven contamination.

Based on available data, there were 16,403 domestic well nitrate samples collected from 2003-2024. Of all the domestic wells sampled over this period, 6,468 (39.4%) of them were above 3 mg/L for nitrate and 2,775 (16.9%) of them were above 10 mg/L for nitrate. For more information about nitrate in Nebraska surface water and groundwater, see the Nebraska Department of Environment and Energy's (NDEE's) annual water program publications included in the Resources section.

Health Effects

HUMANS: The U.S. Environmental Protection Agency (EPA) established the Maximum Contaminant Level (MCL) for nitrate in drinking water at 10 milligrams of nitrate (measured as nitrogen) per liter of drinking water (mg/L NO3-N).

Drinking water with nitrate above the MCL can affect how blood carries oxygen and may cause methemoglobinemia (also known as blue baby syndrome). Bottle-fed babies under six months old are at the highest risk of getting methemoglobinemia. This illness can cause the skin to turn a bluish color and result in serious illness or death. Other symptoms connected to methemoglobinemia include decreased blood pressure, increased heart rate, headaches, stomach cramps, and vomiting.¹ Pregnant women are also a high-risk group and should not consume water with nitrate above the MCL.² The following conditions may also put people at higher risk of developing nitrateinduced methemoglobinemia: anemia, cardiovascular disease, sepsis, glucose-6-phosphate- dehydrogenase deficiency, gastrointestinal diseases and other metabolic problems. $2, \frac{3}{2}$

The EPA standard was set based on immediate health effects of consuming nitrate above 10 mg/L. There is additional research being done by others, including the University of Nebraska-Lincoln (UNL), on other potential health effects, including chronic health effects. Chronic health effects occur from ingesting a contaminant over a long period of time.

For more information about other potential health effects, visit the UNL websites located in the Resources section.

LIVESTOCK: It is recommended to not allow livestock to drink water with a nitrate level above 100 mg/L. Nitrate can affect livestock similarly to how it affects humans. ⁴ Additionally, nitrate levels above 100 mg/L may cause reproductive problems in adult cattle and reduce growth rates in replacement heifers.⁵ It is recommended that you consult with a veterinarian if you have questions about an acceptable nitrate level in drinking water for other species of animals.

How to Protect Yourself and Your Family

IF YOU AREON APUBLIC WATER SYSTEM: Your public water system regularly tests for nitrate and makes sure levels meet the EPA standard. You can find the level of nitrate detected in your public water system by reading the system's Consumer Confidence Report (CCR) which is a water quality report that is required to be provided to water customers annually. Call your water system to get a paper copy of your community's most recent report or find drinking water quality information about your system online at the Drinking Water Watch website listed in the Resources section.

IF YOU HAVE A PRIVATE WELL: The following types of wells are the most vulnerable to nitrate contamination, especially if they are near or downgradient of septic tanks and absorption/leach fields, certain industrial areas, areas with agricultural activities, or areas with known high concentrations of nitrate in groundwater:

- Shallow wells 50 feet or less in depth.
- Wells in sand aquifers.
- Dug wells or wells with casings that are not watertight due to damage or construction materials used.
- Wells in a pit.
- Improperly constructed wells.
- Wells constructed prior to the 1988 construction standards.

¹ Agency for Toxic Substances and Disease Registry (ATSDR). 2015: ToxFAQsTM for Nitrate and Nitrite

[\(https://www.atsdr.cdc.gov/toxfaqs/tfacts204.pdf\).](https://www.atsdr.cdc.gov/toxfaqs/tfacts204.pdf) Accessed April 2024.

² ATSDR. 2013. ATSDR Case Studies in Environmental Medicine Nitrate/Nitrite Toxicity [\(https://www.atsdr.cdc.gov/csem/nitrate_2013/docs/nitrite.pdf\)](https://www.atsdr.cdc.gov/csem/nitrate_2013/docs/nitrite.pdf). Page 37. Accessed April 2024

3 U.S. Environmental Protection Agency. 1991. Integrated Risk Information System (IRIS) Chemical Assessment Summary [\(https://iris.epa.gov/static/pdfs/0076_summary.pdf\)](https://iris.epa.gov/static/pdfs/0076_summary.pdf). Accessed April 2024.

4 Rasby, R. & Walz, T. 2011. Water Requirements for Beef Cattle. University of Nebraska-Lincoln Extension. [\(https://extensionpubs.unl.edu/publication/g2060/html/view\)](https://extensionpubs.unl.edu/publication/g2060/html/view). Accessed May 2024.

⁵ Kononoff, P. & Clark, K. 2017. Water Quality and Requirements for Dairy Cattle. University of Nebraska-Lincoln Extension. [\(https://extensionpubs.unl.edu/publication/g2292/html/view\)](https://extensionpubs.unl.edu/publication/g2292/html/view). Accessed May 2024.

Prevent Contamination

- **Construct your well in a safe spot.** Domestic wells constructed in Nebraska are required to adhere to setback distances and construction standards set in Nebraska Administrative Code (NAC) Title 178, Chapter 12. Ensure your installer is a licensed Water Well Professional using the NDEE website listed in the Resources section or by calling 402-471-0546
- **Keep nitrate sources away from your well**. Sources may include fertilizer application and storage, fuel storage, septic systems, wastewater treatment facilities, and livestock facilities. See NAC Title 178, Chapter 12, Chart 1 for setback distances from common sources of well contamination*.* Consult with a Certified Onsite Wastewater Treatment (OWT) Professional if you have concerns about the location or condition of your septic system in relation to your well. A link to find a Certified OWT Professional is listed in the Resources section.
- **Get your well inspected.** Work with a licensed professional to take any corrective actions that may be needed. Water Well Professionals with a current license are listed on the NDEE website listed in the Resources section.
- **Test for nitrate and bacteria every year.** You are responsible for regularly testing your well water. NDEE recommends using an accredited laboratory to test your well water. Well owners can request sample kits from the Nebraska Department of Health and Human Services (NDHHS) online at the website listed in the Resources section or by calling 402-471-3935. Additionally, the NDHHS's website has a list of other accredited laboratories. Contact the laboratory to get sample containers and instructions or ask your local Natural Resources District (NRD) or public health services if they provide well water testing services. If you need help finding your local NRD, visit the website in the Resources section.

Address Contamination

If nitrate is detected in your water at levels above 10 mg/L, follow these steps:

- **Get your drinking water from a safe source**, such as bottled water, or a public water system including rural water districts. This is especially important if babies under six months old drink the water or formula is made with the water. Pregnant or nursing mothers should consult with their doctor about how elevated nitrate levels in drinking water may affect them. Boiling water is not a solution for elevated nitrate levels as it causes evaporation and concentrates the nitrate in the water.
- **Consider testing the well for other contaminants that commonly occur with nitrate such as bacteria and uranium.** Sample test kits for other contaminants, such as bacteria and uranium may be requested from the Nebraska Department of Health and Human Services online at the website listed in the Resources section or by calling 402-471-3935. For more information about other potential contaminants in your well, visit the NebGuides link under the UNL Resources section.
- **Contact a local rural water district. Connection to** the rural water district-supplied water may be an option in your area.
- **Consider your well construction.** If your existing well is poorly constructed or is located near a contamination source such as a septic system, drilling a new well or rehabilitating your well may be an option. However, this can be costly and is not a guarantee that the new or modified well will have nitrate below 10 mg/L. Water Well Professionals with a current license that can help drill a new well or rehabilitate an existing well are listed on the NDEE website listed in the Resources section.
- **Consider a Point of Use (POU) or Point of Entry (POE) treatment system to remove nitrate from drinking water.** POU treatment systems treat water at one tap while POE treatment systems treat all the water that enters your home. Reverse osmosis, ion exchange, or distillation filtration systems are the typical types of treatment systems used to remove nitrate from drinking water. These systems require regular maintenance and testing to ensure they are working correctly and must be properly installed, operated, and maintained to be effective. You may be able to purchase a basic system from your local home improvement store. Consult with a licensed plumber for help installing a more sophisticated system. Additionally, your local NRD may have assistance available to help fund the installation of a treatment system. If you need help finding your local NRD, visit the website located in the Resources section.

Resources

- **Drinking Water Watch** https://drinkingwater.ne.gov
- **Find Your NRD** <https://www.nrdnet.org/>
- **NDEE Annual Report to the Legislature** [https://dee.nebraska.gov/forms/publications-grants](https://dee.nebraska.gov/forms/publications-grants-forms/ndee034)[forms/ndee034](https://dee.nebraska.gov/forms/publications-grants-forms/ndee034)
- **Groundwater Quality Monitoring Report** [https://dee.nebraska.gov/forms/publications-grants-forms/24-](https://dee.nebraska.gov/forms/publications-grants-forms/24-026) [026](https://dee.nebraska.gov/forms/publications-grants-forms/24-026)
- **NDEE Water Quality Integrated Report** [https://dee.nebraska.gov/forms/publications-grants-forms/23-](https://dee.nebraska.gov/forms/publications-grants-forms/23-012) 012
- NDEE Certified Onsite Wastewater Treatment Professionals Lookup [https://dee.nebraska.gov/water/wastewater/onsite-](https://dee.nebraska.gov/water/wastewater/onsite-wastewater-program/certified-installers-mound-endorsement-and-professional-engineers)

[wastewater-program/certified-installers-mound-endorsement](https://dee.nebraska.gov/water/wastewater/onsite-wastewater-program/certified-installers-mound-endorsement-and-professional-engineers)[and-professional-engineers](https://dee.nebraska.gov/water/wastewater/onsite-wastewater-program/certified-installers-mound-endorsement-and-professional-engineers)

- NDEE Water Well Professionals Licensee Lookup https://deq-iis.ne.gov/zs/wwp/main_pro.php
- NAC [Title](http://www.health.state.mn.us/communities/environment/water/wells/construction/protect) 178 (Chapter 12 Setback Distances) <https://rules.nebraska.gov/rules?agencyId=37&titleId=107>
- **NDHHS Water Sampling Test Kit Request** <https://www.nebraska.gov/dhhs/water-test-kits/private.html>
- **NDHHS Certified Labs** [https://dhhs.ne.gov/Pages/Lab-Certification-](https://dhhs.ne.gov/Pages/Lab-Certification-Requirements.aspx)[Requirements.aspx](https://dhhs.ne.gov/Pages/Lab-Certification-Requirements.aspx)
- **[EPA Fact Sheet](http://www.mda.state.mn.us/townshiptesting)** [https://archive.epa.gov/water/archive/web/pdf/archived](https://archive.epa.gov/water/archive/web/pdf/archived-consumer-fact-sheet-on-nitrates-and-or-nitrites.pdf)[consumer-fact-sheet-on-nitrates-and-or-nitrites.pdf](https://archive.epa.gov/water/archive/web/pdf/archived-consumer-fact-sheet-on-nitrates-and-or-nitrites.pdf)
- **UNL Resources:** <https://water.unl.edu/category/water-and-health> <https://water.unl.edu/category/water-and-health/resources> <https://water.unl.edu/article/drinking-water/nebguides>

Nebraska Department of Environment and Energy 402-471-2186

ndee.moreinfo@nebraska.gov

NOVEMBER 30, 2023 TO MARCH 1, 2024

5/2/24, 8:57 AM NDEE News Release

5/2/24, 9:00 AM NDEE News Release

245 Fallbrook Blvd Suite 100, Lincoln, NE 68521 -- PO Box 98922, Lincoln, NE 68509 -- phone (402) 471-2186, toll-free (877) 253-2603

5/2/24, 9:00 AM NDEE News Release

245 Fallbrook Blvd Suite 100, Lincoln, NE 68521 -- PO Box 98922, Lincoln, NE 68509 -- phone (402) 471-2186, toll-free (877) 253-2603

5/2/24, 9:01 AM NDEE News Release

SAMPLING INSTRUCTIONS FOR PRIVATE DRINKING WATER NITRATE TESTING

******PLEASE READ ALL INSTRUCTIONS BEFORE COLLECTING SAMPLES******

The enclosed sample kit(s) are being sent to you to collect for a drinking water nitrate. **Please contact the Private Well Water Program at (402) 471-0930, if you have questions about this sample collection.**

Prior to Sample Collection

The enclosed sample container(s) is for nitrate analysis. The sample container should be used within 90 days. Sample containers not used within this time frame should be returned to the laboratory. Do not use this container to collect samples for tests other than nitrate analysis.

Each kit contains the following items. If you did not get all of the items, please call 402-471-3935.

- 1. A plastic 4-ounce bottle, with a small amount of **sulfuric acid** added as a preservative
- 2. A laboratory sample submission form with return mailing label
- 3. Re-sealable bag
- 4. Sampling instructions

Caution: Sulfuric acid is highly toxic by digestion and inhalation: and a strong irritant to eyes and skin. Please wash hands thoroughly and use protective gloves to prevent chemical burns.

Sampling Instructions

- 1. Before handling the sampling container, make sure your hands are clean of dirt, grease, and oil.
- 2. Allow the cold water tap to run approximately 3 minutes.
- 3. Carefully remove the lid from the 4oz plastic container and fill with water up to the shoulder area. Avoid contamination by not touching the containers inside, lip, or inside cap surface. **Do not overfill**, as the sulfuric acid preservative would be lost and the results may then be invalid.
- 4. Be sure to recap the container securely. Place one sample barcode sticker on the bottle and one on the lid. Place the sample bottle into the re-sealable bag and seal it. Per USPS requirements the contents must be leak proof. Place this in the Styrofoam packaging.
- 5. Complete the collector, sample date, sample time, and sampling location on the submission form as well as a phone number where you can be reached if we have questions. Keep one barcode sticker for your records.
- 6. If you received multiple sampling kits, repeat steps 1 through 6 to collect the remaining samples.

(Continued on reverse side)

7. Place the completed laboratory submission form and the Styrofoam containing the bagged sample back into the box. Multiple sample kits can then be packed into a larger cardboard box if desired. USPS return mailing labels are part of the submission form for your convenience. Samples may be shipped by U.S. Mail, Federal Express or United Parcel Service. **The sample should be shipped within a business day of collection.** Avoid sending samples on Friday. Return postage is at the client's expense.

USPS Mailing Address for the sample Nebraska Health and Human Services, Public Health Environmental Laboratory 3701 S 14th St PO Box 22790 Lincoln, NE 68542-2790

Physical Address if using other delivery Service Nebraska Public Health Environmental Laboratory 3701 S 14th St Lincoln, NE 68502

Test Results Interpretation

If you have results that concern you, first consult the link below for more information. Nitrate - http://extensionpubs.unl.edu/publication/9000016365631/drinking-water/ If you still need further consultation, call Dave Miesbach at 402-471-4982. For sample interpretation, call 402-471-0930.

Understanding My Drinking Water Test Results

Coliform Results

If your results column has 0 Total Coliform and 0 *E. coli* **then your water is safe!**

What is Coliform and *E. coli*?

Coliform is a group of bacteria found in plant material, water, and soil. Coliform bacteria is also present in the digestive tracts and feces of humans and animals. Most of the time, these bacteria are not harmful. Finding coliform bacteria in a drinking water sample however, can indicate potential contamination. *E. coli* is a type of coliform bacteria. Most strains of *E. coli* are harmless, but some can cause serious illness in humans.

What should I do if Coliform is present but is *E.coli* absent?

The Department recommends conducting further testing to determine the extent of bacterial infiltration. Results less than 5 MPN/100mL indicate a potential sampling error. Flush the line and re-collect a sample. If your results are higher than 5 MPN/100mL, the Department recommends chlorination of the well by a certified well contractor.

What should I do if my test results are *E.coli* positive?

You should not drink, bathe, or cook with water from this well. We also recommend that the well be treated by shock chlorination by a certified well contractor. If the water must be used before the well is treated, you should boil it for a minimum of 1 minute before using it for cooking or drinking. Allow for cooling prior to consumption.

Guidance on treating a well using shock chlorination can be found at the link below: <http://extensionpublications.unl.edu/assets/pdf/g1761.pdf>

The Nebraska Department of Environment and Energy recommends you contact a licensed water well contractor to shock chlorinate a well. Click this link [\(https://deq-iis.ne.gov/zs/wwp/main_pro.php\)](https://deq-iis.ne.gov/zs/wwp/main_pro.php) to search for a licensed water well contractor.

What is Nitrate/Nitrite?

Nitrates are used as fertilizers for agriculture but are also present in livestock waste. They can easily contaminate both surface and groundwater when used in excess or due to runoff. Infants, pregnant women, and nursing mothers are especially vulnerable when exposed to nitrate levels above 10 mg/L**.**

What should I do if my test results are above 10 mg/L?

You should not use water from this well to prepare formula for a bottle-fed infant or for cooking. The Department recommends that you locate an alternate drinking water source or install in-home treatment. Do not boil the water. Boiling causes evaporation and concentrates the nitrate in the water. It is okay to bathe with this water.

For coliform or nitrate questions, please see the back side of this paper if you need further assistance. Locate your county of residence and contact the representative in your area.

If you need to order a sample kit, contact the Nebraska Environmental Health Lab at 402-471-3935.

Environmental Safety Programs

NEBRASKA Good Life. Great Resources DEPT. OF ENVIRONMENT AND ENERGY

Health Department

Teresa Anderson Central District Health Dept., Grand Island (308) 385-5175 tanderson@cdhd.ne.gov

Lindsay Huse, PhD Douglas County Health Dept., Omaha (402) 444-7471 lindsay.huse@douglascounty-ne.gov

Gina Uhing Elkhorn Logan Valley Public Health Dept., Wisner (402) 529-2233 gina@elvphd.ne.gov

Pat Lopez Lincoln-Lancaster County Health Dept., Lincoln (402) 441-8000 patdlopez@lincoln.ne.gov

Heidi Kuklis North Central District Health Dept., O'Neill (402) 336-2406 heidi@ncdhd.ne.gov

Kim Engel Panhandle Public Health District, Hemingford (308) 487-3600 kengel@pphd.ne.gov

Sarah Schram Sarpy/Cass Health Dept., Papillion (402) 339-4334 sschram@sarpycasshealth.com

Grant Brueggemann Southeast District Health Dept., Auburn (402) 274-3993 grant@sedhd.org

Terra Uhing Three Rivers Public Health Dept., Fremont (402) 727-5396 terra@3rphd.org

Susan Bockrath Nebraska Association of Local Health Directors (402) 904-7946 susanbockrath@nalhd.org

Theresa Grove Dakota County Health Dept., Dakota City (402) 987-2164 tgrove@dakotacounty.ne.gov

Jessica Hicks East Central District Health Dept., Columbus (402) 562-7500 jhicks@ecdhd.ne.gov

Laura McDougall Four Corners Health Dept., York (402) 362-2621 lauram@fourcorners.ne.gov

Amanda Jeffres Loup Basin Public Health Dept., Burwell (308) 346-5795 ajeffres@lbphd.org

Julie Rother Northeast Nebraska Public Health Dept., Wayne (402) 375-2200 julie@nnphd.org

Kim Showalter Public Health Solutions, Crete (402) 826-3880 kshowalter@phsneb.org

Michele Bever, PhD South Heartland District Health Dept., Hastings (402) 462-6211 michele.bever@shdhd.ne.gov

Myra Stoney Southwest Nebraska Public Health Dept., McCook (308) 345-4223 director@swhealth.ne.gov

Jeremy Eschliman Two Rivers Public Health Dept., Kearney (888) 669-7154 Jeschliman@trphd.ne.gov

Mona Zuffante Winnebago Public Health Dept., Winnebago (402) 878-2294 mona.zuffante@ihs.gov

Nebraska Extension Research-Based Information That You Can Use

G1784

NebGuide

Drinking Water Nitrate-Nitrogen

Becky Schuerman, Extension Associate Domestic Water/Wastewater Management

Bruce I. Dvorak, Extension Environmental Engineering Specialist

Nitrate-nitrogen is sometimes present in drinking water. At certain levels it can present a health risk. Properly locating and constructing wells along with regularly testing water can help to manage the risk.

Many Nebraskans have questions about the impact of nitrate in their drinking water. Water quality monitoring shows that nitrate is present in groundwater throughout much of Nebraska and concentrations are increasing in some areas.

Nitrogen is essential for all living things, as it is an essential component of protein. Nitrogen exists in the environment in many forms and changes forms as it moves through the nitrogen cycle. However, excessive concentrations of nitrate-nitrogen in drinking water can be hazardous to health, especially for infants, nursing mothers, and pregnant women.

Sources of Nitrate in Drinking Water

Nitrogen is a nutrient applied for lawn and garden care and crop production to increase productivity. Feedlots, animal yards, septic systems, and other waste treatment systems are additional sources of nitrogen that is carried in waste. Nitrogen occurs naturally in the soil in organic forms from decaying plant and animal residues.

Bacteria in the soil convert various forms of nitrogen to nitrate, a form of nitrogen and oxygen. This is desirable since the majority of the nitrogen used by plants is absorbed in the nitrate form. However, nitrate is highly soluble and readily moves with water through the soil profile. If there is excessive rainfall or over-irrigation, nitrate will drain below the plant's root zone and may eventually reach groundwater.

Nitrate in groundwater may result from point sources such as sewage disposal systems and livestock facilities, from nonpoint sources such as fertilized cropland, parks, golf courses, lawns, and gardens, or from naturally occurring sources of nitrogen. Proper site selection for the location of domestic water wells can reduce potential nitrate contamination of drinking water. Important considerations include a sufficient well depth, an adequate distance from possible contamination sources, and a location upslope from possible contamination sources. Proper well construction and maintenance also reduces the risk of drinking water contamination. See NebGuide G2050 "Protecting Private Drinking Water Supplies: Water Well Location, Construction, Condition, and Management" for additional information.

Indications of Nitrate

Nitrate in water is colorless, odorless, and tasteless, which makes it undetectable without testing.

Potential Health Effects

The U.S. Environmental Protection Agency (EPA) Maximum Contaminant Level (MCL) for nitrate-nitrogen in a public water supply is 10 milligrams per liter (mg/L), sometimes expressed as 10 parts per million (ppm) measured as nitrate-nitrogen ($\rm NO_3$ -N.) It is based on acute health effects, specifically the risk of methemoglobinemia (explained below.) Acute health effects are those that result from ingestion of a contaminant over a short period of time.

The acute health hazard associated with drinking water with elevated levels of nitrate occurs when bacteria in the digestive system transform nitrate to nitrite. The nitrite reacts with iron in the hemoglobin of red blood cells to form methemoglobin, which lacks the oxygen-carrying ability of hemoglobin. This creates the condition known as methemoglobinemia (sometimes referred to as "blue baby syndrome"), in which blood lacks the ability to carry sufficient oxygen to the individual body cells.

Infants under one year of age have the highest risk of developing methemoglobinemia from consuming water with elevated levels of nitrate. Contributing risk factors include digestive and enzyme systems that are not fully developed. Older persons who have a gastrointestinal system disorder resulting in increased bacteria growth may be at greater risk than the general population. In addition, individuals who have a genetically impaired enzyme system for metabolizing methemoglobin may be at greater risk. The general population has a low risk of developing methemoglobinemia, even when ingesting relatively high levels of nitrate/nitrite.

Historical information on infants with methemoglobinemia suggests that a number of infants with the condition also showed signs of diarrhea, inflammation, and infection of the gastrointestinal track, or protein intolerance. The significance of these factors in regard to methemoglobinemia risk, if any, is not known.

Definitive guidelines for determining susceptibility to methemoglobinemia have not been developed. The EPA has established the regulatory threshold for acute health effects based on best available science. The intake from food, drugs, and other sources also is important and must be considered.

Although the EPA standard was set at 10 mg/L based on acute health effects, questions have been raised regarding possible chronic health effects from consuming water with nitrate at various concentrations. Chronic health effects are those that can occur when a contaminant has been ingested over long periods of time. Research is limited regarding the possibility of chronic health effects

due to long-term ingestion of drinking water with nitrate at various concentrations. However, studies have shown a correlation between long-term ingestion of water with nitrate, both above and below 10mg/L, and increased incidence of certain diseases and cancers, particularly pediatric brain tumors, and colorectal and thyroid cancers in adults. Based on the available scientific evidence, the World Health Organization International Agency for Research on Cancer has stated that nitrate and nitrite are probably carcinogenic to humans when ingested under conditions favorable for endogenous nitrosation. Endogenous nitrosation is the process by which nitrate is consumed and converted by the human body into nitrite, which can morph into nitrosamines—compounds that can cause cancer. Other studies have shown a correlation between increased birth defects and consumption of drinking water with elevated nitrate while pregnant. While correlations may not prove cause and effect, the possibility of chronic health risk resulting from ingestion of nitrate-nitrogen must be considered. The connections between the level of nitrate in drinking water, volume ingested, duration of exposure, and possible chronic risks are not fully understood.

Livestock, especially cattle and hogs, are also susceptible to nitrate poisoning. In cattle, it results in lower milk production and loss of calves; in hogs, it results in loss of piglets. Dogs are also known to be susceptible to nitrate poisoning, resulting in loss of litters and decreased milk production.

Note: This publication is not a substitute for professional medical advice. If you have questions or concerns related to potential health effects from consuming water containing nitrate, consult your physician.

Testing

Testing Public Water Supplies

Public water supplies classified as either community or non-community are required to test for nitrate concentration. If water comes from a public water supply, users can contact the water utility to learn about the nitrate level in their water.

Testing Private Water Supplies

Water quality in private wells is not currently regulated by federal or state statutes; thus, the regular testing of a private water supply is not required under state or federal law. If users want to know the concentration of nitrate in a private water supply, they will need to have the water tested for a fee and on a confidential basis.

An initial test of a new water supply is recommended to determine the baseline nitrate concentration in the water source. Activities near a well potentially can contaminate the water supply, changing the nitrate concentration over time. Private drinking water wells should be tested annually to monitor changes in nitrate concentration. In addition, private drinking water wells should be tested any time an infant, pregnant woman, nursing mother, or elderly person begins to use the water supply. These groups are believed to be the most susceptible to nitrate health effects.

Tests to determine the presence of nitrate in drinking water should be done by a laboratory certified for nitrate testing. The Nebraska Department of Health and Human Services Public Health Environmental Laboratory) certifies laboratories to conduct tests for drinking water supplies. This approval means that recognized, standard test and quality control procedures are used. See *Drinking Water: Certified Water Testing Laboratories in Nebraska* (G1614) for a list of certified laboratories and contact information for each.

Some Nebraska Natural Resources Districts (NRDs) may offer assistance or cost-sharing to help well owners with water testing. Individuals can contact their NRD to find out if testing assistance is provided.

Laboratories not specifically certified to test for nitrate may use the same equipment and procedures as certified laboratories. Such laboratories may provide accurate analysis, but there is no independent information about the laboratory's ability to obtain reliable nitrate concentration results.

In addition, a variety of test kits and dip strips are available for nitrate testing outside of a laboratory environment. These might be used for preliminary "screening" and to raise awareness of nitrate issues. When using these tests, users should understand the nature of the test and the accuracy of the test results. While an estimate of nitrate concentration level might be obtained, laboratory analysis is needed for an accurate and reliable nitrate measurement.

To have water tested, private water well owners or users must select a laboratory and obtain a drinking water nitrate test kit from the laboratory. The kit will usually include a pre-preserved sample bottle, an information form, and sampling instructions. The sample bottle for nitrate testing may contain a preservative to prevent any loss of nitrate in the sample. This sample bottle should not be rinsed before filling and should only be used for samples intended for nitrate analysis. It must be used within 90 days to ensure validity of the analysis. The sampling instructions provide information on how to collect the sample. These instructions must be followed carefully to avoid contamination and to obtain a representative sample. The sample must be promptly mailed or delivered to the laboratory along with the completed information form.

Interpreting Test Results

Public Water Supply Test Results

The quality of water supplied by Public Water Systems is regulated by the EPA and the Nebraska Department of Environment and Energy(NDEE). This includes any well with 15 or more service connections or that serves 25 or more people on a regular basis.

Public drinking water standards established by EPA fall into two categories—Secondary Standards and Primary Standards. Secondary Standards are based on aesthetic factors such as taste, odor, color, corrosivity, foaming, and staining properties of water that may affect the suitability of a water supply for drinking and other domestic uses. Primary Standards are based on health considerations and are designed to protect human health. The EPA has established an enforceable Primary Standard for nitrate in public drinking water supplies.

The EPA Maximum Contaminant Level (MCL) is measured and reported as nitrate-nitrogen, $\text{(NO}_{3}\text{-N)}$, which is the amount of nitrogen in the nitrate form. The MCL for nitrate-nitrogen in a public water supply is 10 milligrams per liter (mg/L) which can also be expressed as 10 parts per million (ppm). This drinking water standard was established to protect the health of infants and is based on risk assessment using the best knowledge available.

It is worth noting that the European standard is measured and reported as total nitrate $(NO₃)$ with a maximum allowable level of 40 mg/L or 40 ppm. The two reporting systems can be compared as follows:

> 1 mg/L nitrate-nitrogen $(NO₃-N)$ = 4.4 mg/L nitrate $(NO₃)$

Therefore, the U.S. standard of 10 mg/L nitrate-nitrogen would be reported as 44 mg/L nitrate if the European reporting method was used, or the European standard of 40 mg/L nitrate would be reported as 9 mg/L nitrate-nitrogen if the U.S. reporting method was used.

Although not common, a few U.S. laboratories report total nitrate $(NO₃)$ rather than the more commonly used nitrate-nitrogen $\text{(NO}_{3}^{\text{-}}\text{N)}$ quantity. Because potential health risks are often unknown or hard to predict, many drinking water standards are set at some fraction of the level of "no observed adverse health effects." In general, the greater the uncertainty about potential health effects, the

greater the margin of safety built into the standard. In the case of nitrate, there may not be a large safety factor.

Private Water Supply Test Results

While EPA and Nebraska regulations do not apply to private drinking water wells, users of private drinking water should consider the EPA guideline of 10 ppm nitratenitrogen when considering the risk associated with their water supply. If nitrate-nitrogen concentrations are found to be above 10 ppm, private drinking water users might voluntarily try to reduce the nitrate-nitrogen concentration in the water, taking into account health risks, cost, and benefits.

Options

Options for Public Water Supplies

If a test indicates that the nitrate-nitrogen concentration of public water exceeds the standard, the public must be notified and steps must be taken by the water supplier to bring the water into compliance. Often, the treatment may be as simple as blending the water that exceeds the standard with water that has a nitrate-nitrogen concentration less than 10 mg/L such that the average concentration of the delivered water is below the EPA standard. Another option for achieving compliance is water treatment, such as with ion exchange or reverse osmosis, to reduce the nitratenitrogen concentration. Biological filtration to remove nitrate has been successfully applied by public water systems in other states that have the managerial capacity to operate and monitor this advanced system. In some cases, compliance may be achieved by offering bottled water to vulnerable consumers in conjunction with developing a source water protection plan designed to eliminate or reduce the source of contamination, which should result in the reduction of nitrate-nitrogen concentration in the water supply over time. Public water systems cannot achieve compliance by supplying bottled water as the only means of addressing high nitrate levels.

The NDEE has the responsibility for implementing the federal requirements and can take action toward public water supplies that are not in compliance. This action includes Administrative Orders, a precursor to legal action. NDEE issues a Nitrate Administrative Order to public water systems exceeding 10 ppm twice in a three quarter period. At any given time, a very small percentage of public water supplies in Nebraska may have a nitrate concentration above 10 ppm, and some systems may be under Administrative Order for noncompliance with the MCL. NDEE requires any public water system exceeding 20 ppm in any sample to discontinue the use of the well and provide alternate safe water to all consumers until the concentration of nitrate is less than 20 ppm for two consecutive quarters.

Options for Private Water Supplies

If nitrate-nitrogen exceeds 10 ppm, users should consider that their water exceeds the EPA MCL for nitratenitrogen in drinking water. Also, users might consider that NDEE takes immediate action toward public water suppliers exceeding this concentration, and voluntarily consider an alternative drinking water source or water treatment. Decisions should be based on a nitrate analysis by a certified laboratory, and after consulting with a physician to help evaluate the level of risk.

It may be possible to obtain a satisfactory alternate water supply by drilling a new well in a different location or a deeper well in a different aquifer, especially if the nitrate contamination is from a point source such as livestock or human waste. If the water supply with high nitrate is coming from a shallow aquifer, there may be an uncontaminated, deeper aquifer protected by a clay layer that prevents the downward movement of the nitrate-contaminated water. A new well should be constructed so surface contamination cannot enter the well. It should be located away from any potential sources of contamination, such as septic systems or feedlots. Consult a Nebraska-licensed water well professional regarding this option. Another alternate source of water is bottled water that can be purchased in stores or direct from bottling companies. This alternative especially might be considered if the primary concern is water for infant food and drinking.

Drinking water can be treated for nitrate-nitrogen by three treatment methods: distillation, reverse osmosis, and ion exchange. Home treatment equipment using these processes is available from several manufacturers. **Carbon filters and standard water softeners do not remove nitrate-nitrogen. Merely boiling water does not remove nitrate-nitrogen. The act of boiling water for an extended period of time results in evaporation, and a decrease in water volume. The nitrate does not evaporate with the water, resulting in an increased nitrate-nitrogen concentration in the remaining volume of water**.

The distillation process involves heating the water to boiling and collecting and condensing the steam by means of a coil. This process can remove nearly 100 percent of the nitrate-nitrogen, since the nitrate-nitrogen does not volatilize with the steam. For information on this treatment method see NebGuide 1493, *Drinking Water Treatment: Distillation*.

In the reverse osmosis process, pressure is applied to water to force it through a semipermeable membrane. As the water passes through, the membrane filters out most of the impurities. This process can remove approximately 85 percent to 95 percent of the nitrate-nitrogen. Actual removal rates may vary, depending on the initial quality of the water, the system pressure, membrane technology, and water temperature. For information on this treatment method see NebGuide 1490, *Drinking Water Treatment: Reverse Osmosis*.

Ion exchange for nitrate-nitrogen removal operates on the same principle as a household water softener. However, for the nitrate-nitrogen removal process, special anion exchange resins are used that exchange chloride ions for nitrate and sulfate ions in the water as it passes through the resin. Since most anion exchange resins have a higher selectivity for sulfate than nitrate, the level of sulfate in the water is an important factor in the efficiency of an ion exchange system for removing nitrate-nitrogen.

Summary

Nitrate can be present in some water sources, most often as a result of point or nonpoint source pollution from fertilizer or human or animal waste. Proper well location and construction are key practices to avoiding nitrate

contamination of drinking water. Management practices to reduce the risk of contamination from fertilizers and manure/sewage help keep the water supply safe. Ingesting drinking water containing nitrate-nitrogen can present an acute health risk, especially for infants. Public water supplies must comply with the EPA standard for nitratenitrogen of 10 ppm. Management of a private drinking water well for nitrate-nitrogen is a decision made by the well owner and/or water user. A water test is the only way to determine the nitrate-nitrogen concentration. If public drinking water exceeds the EPA nitrate-nitrogen standard, the utility must inform water users and must take steps to reduce the nitrate-nitrogen concentration. If private drinking water exceeds an acceptable nitrate-nitrogen concentration, choices are to use an alternate water supply or treat the water. An alternate supply may be bottled water or a new well in a different location or aquifer. Water treatment options include distillation, reverse osmosis, or ion exchange.

Acknowledgment

This revision is based on previous versions of the NebGuide by Paul J. Jasa, Extension Engineer; Sharon O. Skipton, Extension Water Quality Educator; David L. Varner, Extension Educator; DeLynn Hay, former Extension Water Resources Specialist; Wayne Woldt, former Extension Water and Environment Specialist; and Colleen Cassada, former Drinking Water Program Specialist for Nitrate, Nebraska Department of Health and Human Services

This publication has been peer reviewed. Nebraska Extension publications are available online at http://extensionpubs.unl.edu/.

Extension is a Division of the Institute of Agriculture and Natural Resources at the University of Nebraska—Lincoln cooperating with the Counties and the United States Department of Agriculture.

Nebraska Extension educational programs abide with the nondiscrimination policies of the University of Nebraska—Lincoln and the United States Department of Agriculture.

© 2022, The Board of Regents of the University of Nebraska on behalf of the Nebraska Extension. All rights reserved.

University of Nebraska–Linc In Extensio Institute f Agriculture and Natural Resou

Know how. Know w.

Tebraska-line In Extension Institute TAgriculture and Natural Resources

Know W.

Private Drinking Water Wells:

Planning for Water Use

Sharon O. Skipton, Extension Water Quality Educator

Jan R. Hygnstrom, Extension Wate

Wayne Woldt — Extension, Water and Environment Specialist

This publication is one of six in a series designed to help rural families understand and manage their private drinking water supplies.

When planning for a private drinking water supply, it is important to determine if the well capacity will meet water demands. The well capacity — the amount of water that can be produced — must be accurately determined. A Nebraskalicensed water well professional can help determine the appropriate well flow rate $\frac{z}{z}$ a measure of the gallons of water that can be produced per minute. The flow rate must be capable of providing the total quantity of water needed every day - the total daily demand. In addition, the flow rate should be sufficient to meet temporary large demands that occur throughout the day — the peak use demand. If the amount of water produced by the well is greater than the total daily demand, but the flow rate is insufficient to meet the peak use demand, intermediate storage can be used to supplement the water supply during peak use periods.

In addition, it is important to determine if the well water quality is adequate for its intended use. Drinking water does not need to be pure to be safe for consumption, hygiene, and other domestic uses. Whether drinking water is safe will depend on which substances are present and in what amounts. Water quality can be determined by having a sample tested by a certified laboratory. If water quality does not meet the minimum standard desired, water treatment may be an option.

Determining Well Capacity

A Nebraska-licensed water well professional can determine the yield of a well. The professional will pump the well continuously for an extended period. During the pumping period, measurements will be taken to determine the water level drop in the well relative to the rate at which water is being pumped out. The balance point will occur when the water level stops dropping as a given amount is pumped, providing the well yield in gallons per minute. Quantities of water produced by marginal aquifers are difficult to es-

timate by the contractor and sometimes wells do not yield as desired. There are several variables that cannot be easily accounted for when dealing with marginal aquifers. It is important to have realistic expectations when dealing with marginal aquifers.

Determining Water Quantity Needs

The quantity of water supplied by a private water supply must meet the total daily demand and all peak use demands. Both demands can be minimized with wise water use.

Total Daily Demand

The average American uses from 60 to 100 gallons of water per day. You can estimate the total daily domestic water demand by multiplying use $(100 \text{ gallons per day})$ by the number of people that might reasonably be expected to live in the rural home. This gives an estimate of the total gallons per day that will be needed for domestic use. A more specific total daily demand can be calculated by using the water use estimates in Table I. Amounts are based on long-term national averages; actual water use may vary significantly.

The well flow rate (gallons per minute, or gpm) multiplied by the number of minutes in a day that the well pump will operate provides an estimate of the total gallons of water that will be produced each day. This amount must be equal to or greater than the total daily demand. Minimizing water use will reduce the total daily demand. To reduce demand, start with the fixtures and activities that use the largest volumes of water. The toilet, shower/bath, and clothes washer account for two-thirds of the water used in an average household.

Toilets installed after 1993 are water efficient. Toilet dams or water-filled plastic containers can be installed in older toilet tanks but the reduced flow can affect the effectiveness of a flush; you may have to flush two or three times to remove the waste. In older toilets, about 3 gallons of water may be needed in the tank to flush properly. Avoid placing bricks that crumble in the tank; they may affect operation. Reduce the number of toilet flushes by not using the toilet as a waste paper basket.

Table I. Water use estimates for household appliances and fixtures

eaks acco for a g eat volume of water usage. Abou 20 percent of toilets leak. To test for a leak, put a few drops of food dye in the tank. If, after 15 minutes, color appears in the bowl, there is a leak that should be repaired. Typically, the toilet flapper needs replacing or the water level needs adjusting.

The rate that water flows through fixtures will affect the amount of water used. Showerheads manufactured after 1993 have flow rates of no more than 2.5 gpm. Faucet aerators at sinks restrict the water going through the faucet by about 50 percent, adding air to make the flow appear the same. Faucet aerators with flow rates of 1.5 gpm or lower $(\frac{1}{2} - 1)$ gpm are available.

Water-saving clothes washers use about $\frac{1}{3}$ as much water as traditional washers. When purchasing a new washer, check the label to determine water efficiency. Try to narrow your options to those that are most efficient. Features to look for include the option of reusing water from one wash cycle for another wash cycle, and the ability to adjust water levels to accommodate different size loads or different degree of washing needed. When laundering, adjust water levels to the laundry load size and degree of soil. Typically, fewer full loads use less total water than several small loads.

Peak Use Demand

Water use will not be the same over the course of a day; it will fluctuate. Water systems must meet the needs of many uses during short periods of time. These times, called peak use periods, usually occur near mealtimes, during laundry periods, and when occupants are showering or bathing. The water system should be able to produce enough water to meet the peak demand for a period of two hours. If it cannot, intermediate storage can be used to supplement the water supply during peak use periods.

The Private Water Systems Handbook, developed by Midwest Plan Services¹, recommends the following minimum flow rates for homes to meet peak demand.

A minimum of 10 gpm is recomme ded for a 2-bedroom, 2-bath home. The minimum flow rate increases with additional bedrooms and/or baths since larger homes will be likely to have more residents using more fixtures and appliances at the same time. Other recommended minimum flow rates include:

3 bedrooms, 2 baths -12 gpm 4 bedrooms, 3 baths -16 gpm 5 bedrooms, 3 baths -17 gpm

In general, add 2 gpm for each additional bedroom and 2 gpm for each additional bath.

Ideally, the yield of the well should exceed the recommended minimum flow rate. This is because the recommended minimum flow rate may not support the operation of multiple water-using devices at the same time, and some devices may require greater flow rates to operate properly. The Private Water Systems Handbook lists flow rate requirements for typical devices (Table II).

Table II. Typical flow rate requirements for household waterusing devices. MWPS (Midwest Plan Service), Iowa State University, Ames, IA, www.mwps.org. Used with permission: Jones, D. Private Water Systems Handbook.

¹CMWPS (MidWest Plan Service), Iowa Sta e Universi y, Ames, IA., www. mwps.org. Used with permission: Jones, D., Private Water Systems Handbook.

awn sprinkler s stems place add onal demands on the system. Typical lawn sprinkler irrigation systems may require a 12-gpm flow rate to operate properly. This is in addition to that required to meet normal household needs.

Peak use demand can be reduced by changing the timing of water-using activities and spreading out water use. Spread out laundry, doing only one or two loads per day. Have some family members shower at night while others shower in the morning. Install low-flow water fixtures, and encourage short showers. Run the dishwasher at night after family members have gone to bed. Compost food wastes and avoid using a garbage disposal.

Conserving water and distributing water use over an extended period has the additional benefit of extending the life of a septic system. Wastewater generated by a household should remain in the septic tank long enough — at least 24 hours for heavy solids to settle out, forming sludge, and light solids to float to the top, forming scum. Except for the period immediately after pumping, a septic tank contains was tewater to its full capacity at all times. As a gallon of wastewater flows into the tank from the house, a gallon of effluent flows out of the tank into the drainfield. If wastewater moves in and out of the tank too rapidly, due to constant flow for extended periods, or heavy water flow at any time, solids remain suspended in the wastewater. This means they may move with the effluent out of the tank and into the drainfield. Solids can clog a drainfield, decreasing its ability to treat wastewater. This can lead to costly repairs or even the need for replacement.

Determining and Protecting Water Quality

Drinking water is never pure. Water naturally contains minerals and microorganisms from the rocks, soil, and air with which it comes in contact. Human activities can add many more substances to water. Drinking water does not need to be pure to be safe, however. In fact, some dissolved minerals in water can be beneficial to health. For example, the National Research Council states that drinking water containing dissolved calcium and magnesium generally contributes a small amount toward human dietary needs. Naturally occurring fluoride in groundwater can help protect against tooth decay. Whether or not drinking water is safe will depend on which substances are present and in what amounts.

An ounce of protection is worth a pound of cure when it comes to the drinking water source. If wells are poorly located, constructed, or maintained, they can allow bacteria, nitrate, or other pollutants to contaminate the groundwater serving as the drinking water source.

Most acreages, farms, and ranches with private wells use septic systems or other onsite wastewater treatment systems for treating wastewater and returning it to the environment. If systems are poorly designed, located, constructed, or maintained, they can contribute to groundwater contamination.

Runoff is the water from rain, melting snow, or excess irrigation that moves across property. As it flows, runoff can collect and transport soil, pet waste, livestock manure, salt, pesticides, fertilizer, oil and grease, leaves, litter, and many other potential pollutants. Polluted runoff can flow down a poorly sealed or an unplugged well where it can con am nate groundwater. In areas with porous, sandy soi s, pollutants carried by runoff may percolate through the soil into groundwater.

Pesticides (herbicides, insecticides, fungicides, and rodenticides) and fertilizers (nitrate and phosphorus) play an important role in the management of rural property. If pesticides and fertilizers are not stored, handled, and applied correctly, they can seep through soil into groundwater.

Consider the variety of products used in households and on rural property — paints, solvents, oils, cleaners, wood preservatives, batteries, and adhesives. Also, consider the amount of these products that goes unused or is thrown away. Minimizing usage of these substances, along with practicing proper disposal procedures can protect groundwater, the source of drinking water.

For additional information on drinking water protection, see:

- Protecting Private Drinking Water Supplies: An Introduction, www.ianrpubs.unl.edu/sendIt/g2049.pdf
- Protecting Private Drinking Water Supplies: Water Well Location, Construction, Condition, and Management, www.ianrpubs.unl.edu/sendIt/g2050.pdf
- Protecting Private Drinking Water Supplies: Household Wastewater (Sewage) Treatment System Management, www.ianrpubs.unl.edu/sendIt/g2051.pdf
- Protecting Private Drinking Water Supplies: Hazardous Materials and Waste Management, www.ianrpubs.unl. edu/sendIt/g2053.pdf
- Protecting Private Drinking Water Supplies: Pesticide and Fertilizer Storage and Handling, www.ianrpubs.unl.edu/ sendIt/g2054.pdf
- Protecting Private Drinking Water Supplies: Runoff Management. www.ianrpubs.unl.edu/sendIt/g2052.pdf

Testing Private Drinking Water

The quality of water provided by a private drinking water well can be determined through laboratory analysis. Several Nebraska laboratories offer testing services that include drinking water analysis. Some are operated by government agencies and others are private commercial laboratories.

Since there are many potential water contaminants, it would be very costly — and in most cases unnecessary — to test a private water supply for all of them. Tests for nitrate and bacteria often are used as general indicators of the safety of private well water. Generally, private water supplies should be checked annually for these contaminants. Tests for nitrate and bacteria do not guarantee the water is safe, however, as other contaminants could be present. Tests should be done for other substances when specific contamination is suspected. Contamination might be the result of a spill, backflow, use of product near the well, the presence of industrial or commercial activities in the vicinity of the well, the presence of a contaminant in neighboring wells, or other similar situations.

The safety and quality of private drinking water in Nebraska is not subject to any federal or state regulation. It is at the discretion of the water user except in cases where water quality is regulated at the local level or when state licensing

may be required for a specific activity. Alt oug not required by federal or state regulations, the quality of private well water can be evaluated by com aring test results to water quality standards enforced for ublic drinking water sup lies.

The Federal Safe rinking Water Act directs the U.S. Environmental Protection Agency (EPA) to establis minimum national drinking water standards for public water supplies. These standards set limits on the amounts of various substances allowed in public drinking water. EPA regulations currently cover about 100 potential contaminants. Drinking water regulations established by EPA reflect the best available scientific and technical judgment. The number of contaminants regulated is increasing, and standards are re-evaluated as new data and information become available.

The maximum concentration of nitrate-nitrogen allowed in public water su plies is 10 milligrams er liter, which also can be reported as 10 parts per million. Public water supplies must be free of bacteria. While not required, users of private water supplies might strive to meet these standards.

For additional information on water qu lity, see:

- An Introduction to Drinking Water, www.ianrpubs.unl.edu/ sendIt/g1539.pdf
- Drinking Water: Arsenic, www.ianrpubs.unl.edu/sendIt/ $q1552.pdf$
- Drinking Water: B cteria, www.ianrpubs.unl.edu/sendIt/ $g1826.pdf$
- Drinking Water: Copper, www.ianrpubs.unl.edu/sendIt/ g1360.pdf
- Drinking Water: Fluoride, www.ianrpubs.unl.edu/sendIt/ $g1376.pdf$
- Drinking Water: H rd Water (C lcium nd Magnesium), www. ianrpubs.unl.edu/sendIt/g1274.pdf
- Drinking Water: Iron nd Manganese, www.ianrpubs.unl.edu/ sendIt/g1714.pdf
- Drinking Water: Lead, www.ianrpubs.unl.edu/sendIt/g1333. pdf
- Drinking Water: Nitrate-Nitrogen, www.ianrpubs.unl.edu/ sendIt/g1784.pdf
- Drinking Water: Sulfur (Sulfates and Hydrogen Sulfide), www. ianrpubs.unl.edu/sendIt/g1275.pdf
- Drinking Water: Uranium, www.ianrpubs.unl.edu/sendIt/ $g1569.pdf$

If water quality does not meet the desired minimum standard, water treatment may be an o tion. No single piece of treatment equi ment manages all contaminants. All treatment methods have limitations and a situation may require a combination of treatment processes to effectively achieve the desired water quality.

For additional information on water tre tment, see:

Drinking Water Treatment: An Overview, www.ianrpubs.unl. edu/sendIt/ec703.pdf

Drinking Water Treatment: W at You Need to Know When Selecting Water Treatment Equipment, www.ianrpubs. unl.edu/sendIt/g1488.pdf

- Drinking Water Tre tment: Sediment Filtration, www.ianrpubs. unl.edu/sendIt/g1492.pdf
- Drinking Water Treatment: Carbon Filtration, www.ianrpubs. unl.edu/sendIt/g1489.pdf
- Drinking Water Treatment: Water Softening (Ion Exchange), www.ianrpubs.unl.edu/sendIt/g1491.pdf
- Drinking Water Treatment: Reverse Osmosis, www.ianrpubs. unl.edu/sendIt/g1490.pdf
- Drinking Water Treatment: Distillation, www.ianrpubs.unl. edu/sendIt/g1493.pdf
- Drinking Water Treatment: Continuous Chlorination, www. ianrpubs.unl.edu/sendIt/g1496.pdf
- Drinking Water Tre tment: Shock Chlorination, www.ianrpubs. unl.edu/sendIt/g1761.pdf

Summary

When planning for a private drinking water supply, consider the quantity and quality of water that will be needed. The quantity of water supplied by a private water supply must meet the total daily demand and all peak use demands. Both total daily demand and peak use demands can be reduced with wise water use. The quality of a private drinking water supply is at the discretion of the user, and drinking water does not need to be pure to be safe. The quality of water can be evaluated by testing the water and com aring laboratory results to guidelines established for public water sup lies. If the water quality is less than desired, treatment may be an option.

Acknowledgments

This ublication is the result of a collaborative effort between t e University of Nebraska-Lincoln Extension, the Nebraska Department of Health and Human Services, the Nebr ska epartment of Environmental Quality, the Nebraska Well Drillers Association, and the Nebraska On-site Waste Water Association, all of whom place a high priority on protecting Nebraska's drinking water resources.

Partial funding was provided by the Nebraska Well Drillers Association, the Nebraska On-site Waste Water Association, and the Water Well Standards and Contractors' Licensing Board.

The Midwest Plan Service publication Private Water Systems Handbook provides additional information on private drinking water systems. The publication can be purchased from Midwest Plan Service, 122 Davidson Hall, Iowa State University, Ames, Iowa, 50011-3080; phone 800-562-3618; website www.mwps.org.

This publication has been peer reviewed.

UNL Extension public tions are available online at http://extension.unl.edu/publications.

> **Index: Water Management Drinking Water** Issued May 2012

xtension is a ivision of the Institute o Agriculture nd Natural Resources at the University of Nebraska-Lincoln cooperating with the Counties and the United States Department of Agriculture.

University of Nebraska-Lincoln Extension educational programs abide with the nondiscrimination policies of the University of Nebraska-Lincoln and the United States Department of Agriculture.

ONE HUNDRED YEARS OF NEBRASKA NITROGEN

GROUNDWATER

Background levels of groundwater nitrate are <1 mg/L nitrate-N

1921 - UNL Conservation and Survey established to study Nebraska water

1940s

1940 - Nitrate identified by State of Nebr. Dept. of Health as a problem in poorly built wells 1947 - Methemoglobinemia (blue baby) in infants investigated in Nebr. Medical Journal. First nitrate compound carcinogen research

1951 - Research review suggests 10-20 mg/L nitrate-N limit in water used for infant formula

1960s

1962 - U.S. Public Health Service adopts 10 mg/L nitrate-N drinking water standard 1965 - Nebraska Water Quality Survey by UNL CSD finds pockets above 10 mg/L, attributed to coarse soils, irrigation, inorganic N fertilizer, shallow groundwater, and livestock manure

1971 -NE Dept of Environmental Control (now NDEE) 1972 - Nebr. Natural Resource Districts established and widespread nitrate testing begins 1974 - Safe Drinking Water Act adopts 10 mg/L nitrate-N maximum contaminant level

NITROGEN IS AN ESSENTIAL PLANT NUTRIENT BUT INCREASINGLY FOUND IN OUR WATER. **HERE IS A BRIEF HISTORY**

FERTILIZER

Historically, nitrogen fertilizer only came from plant or animal sources

1909

Haber Bosch process developed for turning atmospheric nitrogen into inorganic ammonia

1920s

Nebr. corn: 26 bushels/acre UNL Agricultural Experiment Station begins inorganic N research

Nebr. Corn: 26 bu/ac 1944 - UNL Extension's first inorganic N fertilizer soil fertility recommendations 1946 - UNL Soil Testing Lab opened 1947 - UNL recommends minimizing leaching loss with in-season N and efficient irrigation 1949 - Adding N through irrigation water begins

1950s

Nebr. irrigated corn: 60 bu/ac; 0.5 mill acres 1952 - Center pivot manufacturing begins

Nebr. irrigated corn: 80 bu/ac; 1.5 mill acres 1964 - UNL cautions "mounting evidence that many farmers are using more fertilizer nitrogen than necessary for best economic return." Water meters and soil moisture sensors recommended for irrigation scheduling

1970s

Nebr. irrigated corn: 110 bu/ac; 1.6 lb N/bu; 2.5 mill acres; about 10% irrigated by pivot 1979 - Inorganic N fertilizer identified as main nitrate source in Central Platte. First NRD irrigation water allocations

1980s

1981 - UNL Extension Nitrate Task Force 1984 - First adverse birth outcomes research 1985 - Nebraska Natural Resources Commission study identifies growing regions impacted. Groundwater Foundation established 1988 - Children's Groundwater Festivals begin

1991 - Nitrate travel timing and amount estimated 1993 - Public Water Supplies required to provide drinking water below 10mg/L nitrate-N Creighton builds first reverse osmosis filtration 1996 - CDC Report on miscarriages from nitrate

2000s

2004 - International workgroup review of nitrate and health (updated 2018)

> 2010 - Int. Agency for Research on Cancer determines nitrate is probably carcinogenic 2013 - More than 1 out of 3 Nebr. irrigated acres have groundwater exceeding 10 mg/L

2020s

2023 - Nitrate shown to release uranium. EPA reopens evaluation of chronic health effects Nebr. irrigated corn: 125 bu/ac; 1.3 lb N/bu; 5 mill acres; about a third irrigated with center pivots 1985 - Groundwater Management & Protection Act 1987 - Central Platte NRD first quality policies 1989 - Bazile Triangle established in Northeast NE

1990s

Nebr. irrigated corn: 145 bu/ac; 1.2 lb N/bu; 0.15 inches water/bu; 5 mill acres 1992 - On-Farm Research program started 1994 - UNL Corn Nitrogen calculator 1993 - crop nitrogen sensors recommended 1999 - nitrate leaving corn rootzone 24-42 mg/L

Nebr. irrigated corn: 165 bu/ac; 71% pivot 1.0 lb N/bu; 0.14" water/bu; 4.8 mill acres 2005 - Renewable Fuel Standard for ethanol

2010s

Nebr. irrigated corn: 190 bu/ac; 86% pivot 0.9 lb N/bu; 0.13" water/bu; 5.3 mill acres 2015 - Project SENSE, sensor-based N 2017 - UNL TAPS competition for N & irrigation

Nebr. irrigated corn: 205 bu/ac; 91% pivot 2023 - nitrate leaving corn rootzone 17-22 mg/L

Author: Crystal Powers

Reviewers: Dan Snow, Richard Ferguson

NEBRASKA WATER FACTS

from the **NEBRASKA WATER CENTER**

Nebraska means "flat water" from the Omaha Sioux "ni braska" and Oto "ni brathge" describing the Platte
River. The Platte River was named by early French explorers, also meaning "flat."

SURFACE WATER

- Nearly 80,000 miles of rivers and streams drain to the Missouri River in the East.
	- Along the Niobrara and Missouri Rivers, 197 miles are designated as National Wild and Scenic.
- Tallest waterfall is Smith Falls, spilling 63 feet into the Niobrara River.
- Largest storage reservoir: Lake McConaughy
	- o When full is 1.74 million acre-feet of storage
	- o Covers 30,500 acres
	- Created by the state's largest dam, Kingsley Dam
	- Supplies irrigation directly & indirectly for 530,000 ac
- More than 2,900 dams, >25 ft tall or 50 ac-ft storage

GROUNDWATER

- Mostly from the Ogallala Aquifer, part of the High Plains Aquifer.
	- Water among mostly sand and gravel.
	- ∘ From 1 to 1000+ feet thick.
	- Poured over the surface of the state, the water would be 38 feet deep.
- Groundwater and surface water are connected. For example:
	- More than 90% of the Loup Rivers' streamflow started as groundwater.
- Nebraska has more than 192,000 registered groundwater wells.

WATER USE

Irrigated acres: 9.1 million #1

- Annual average additional crop value of \$1.5 billion statewide. Added property valuation of \$13-24 billion.
- Agriculture irrigation is 91% of Nebraska's total consumptive water use.
- From 1990 to 2014, Nebraska now grows 1.7 times more corn and 1.8 times more soybeans per gallon of water.
- From 1960 to 2016, Nebraska raises 1.8 times the amount of beef per gallon of water and 5.1 times more milk.
- Other uses of Nebraska's water: • home 5%; industrial 1%; thermoelectric 1%; livestock 1%
- 85% of Nebraskans get their home water from groundwater.
- 594 public water supply systems serve 1.69 million residents. EPA requires testing for 90 contaminants.
- More than 360,000 residents use private wells. Exempt from testing.
- Each person uses an average of 122 gallons of water each day. • Home water use has dropped by 1/3 in the last 20 years.
-

Center pivot irrigating soybeans. Photo: UNL

13 inches precipitation 5,424 feet above sea level 2.5 times more precipitation

More than 4,500 feet drop in elevation

Changes West to East

33 inches precipitation 840 feet above sea level

Smith Falls. Photo: Nebraskaland Magazine.

RECREATION

Lake McConaughy with >2 million visitors

- 7 out of top 10 Nebraska attractions involve water.
- Crane migration annual visitors' impact: \$17.2 mill.

LAND USE

- Nebraska's farms and ranches utilize 44.8 million acres, 92% of the state's total land area.
- 22 million acres of rangeland and pastureland in Nebraska, half of which are in the Sandhills.
- 1/3 of Nebraska land is annual crops.
- Nebraska's cities and town cover about 1% of the land, less than wetlands and forest.

Author: Crystal A. Powers

Reviewers: Katie Pekarek, Gary Stone |

THE DAUGHERTY **GLOBAL INSTITUTE** at the University of Nebraska

 \mathbf{I}

International Journal of *[Environmental Research](http://www.mdpi.com/journal/ijerph) and Public Health*

Review **Drinking Water Nitrate and Human Health: An Updated Review**

Mary H. Ward 1,*, Rena R. Jones ¹ [ID](https://orcid.org/0000-0003-1294-1679) , Jean D. Brender ² , Theo M. de Kok ³ , Peter J. Weyer ⁴ , Bernard T. Nolan ⁵ , Cristina M. Villanueva 6,7,8,9 [ID](https://orcid.org/0000-0002-0783-1259) and Simone G. van Breda ³

- ¹ Occupational and Environmental Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, 9609 Medical Center Dr. Room 6E138, Rockville, MD 20850, USA; rena.jones@nih.gov
- ² Department of Epidemiology and Biostatistics, Texas A&M University, School of Public Health, College Station, TX 77843, USA; jdbrender@sph.tamhsc.edu
- ³ Department of Toxicogenomics, GROW-school for Oncology and Developmental Biology, Maastricht University Medical Center, P.O Box 616, 6200 MD Maastricht, The Netherlands; t.dekok@maastrichtuniversity.nl (T.M.d.K.); s.vanbreda@maastrichtuniversity.nl (S.G.v.B.)
- ⁴ The Center for Health Effects of Environmental Contamination, The University of Iowa, 455 Van Allen Hall, Iowa City, IA 52242, USA; peter-weyer@uiowa.edu
- ⁵ U.S. Geological Survey, Water Mission Area, National Water Quality Program, 12201 Sunrise Valley Drive, Reston, VA 20192, USA; btnolan@usgs.gov
- 6 ISGlobal, 08003 Barcelona, Spain; cvillanueva@isiglobal.org
- 7 IMIM (Hospital del Mar Medical Research Institute), 08003 Barcelona, Spain
- ⁸ Department of Experimental and Health Sciences, Universitat Pompeu Fabra (UPF), 08003 Barcelona, Spain
- ⁹ CIBER Epidemiología y Salud Pública (CIBERESP), 28029 Madrid, Spain
- ***** Correspondence: wardm@mail.nih.gov

Received: 17 May 2018; Accepted: 14 July 2018; Published: 23 July 2018

Abstract: Nitrate levels in our water resources have increased in many areas of the world largely due to applications of inorganic fertilizer and animal manure in agricultural areas. The regulatory limit for nitrate in public drinking water supplies was set to protect against infant methemoglobinemia, but other health effects were not considered. Risk of specific cancers and birth defects may be increased when nitrate is ingested under conditions that increase formation of *N*-nitroso compounds. We previously reviewed epidemiologic studies before 2005 of nitrate intake from drinking water and cancer, adverse reproductive outcomes and other health effects. Since that review, more than 30 epidemiologic studies have evaluated drinking water nitrate and these outcomes. The most common endpoints studied were colorectal cancer, bladder, and breast cancer (three studies each), and thyroid disease (four studies). Considering all studies, the strongest evidence for a relationship between drinking water nitrate ingestion and adverse health outcomes (besides methemoglobinemia) is for colorectal cancer, thyroid disease, and neural tube defects. Many studies observed increased risk with ingestion of water nitrate levels that were below regulatory limits. Future studies of these and other health outcomes should include improved exposure assessment and accurate characterization of individual factors that affect endogenous nitrosation.

Keywords: drinking water; nitrate; cancer; adverse reproductive outcomes; methemoglobinemia; thyroid disease; endogenous nitrosation; *N*-nitroso compounds

1. Introduction

Since the mid-1920s, humans have doubled the natural rate at which nitrogen is deposited onto land through the production and application of nitrogen fertilizers (inorganic and manure), the combustion of fossil fuels, and replacement of natural vegetation with nitrogen-fixing crops such as soybeans [\[1,](#page-82-0)[2\]](#page-82-1). The major anthropogenic source of nitrogen in the environment is nitrogen fertilizer, the application of which increased exponentially after the development of the Haber–Bosch process in the 1920s. Most synthetic fertilizer applications to agricultural land occurred after 1980 [\[3\]](#page-82-2). Since approximately half of all applied nitrogen drains from agricultural fields to contaminate surface and groundwater, nitrate concentrations in our water resources have also increased [\[1\]](#page-82-0).

The maximum contaminant level (MCL) for nitrate in public drinking water supplies in the United States (U.S.) is 10 mg/L as nitrate-nitrogen (NO₃-N). This concentration is approximately equivalent to the World Health Organization (WHO) guideline of 50 mg/L as $NO₃$ or 11.3 mg/L $NO₃$ -N (multiply NO³ mg/L by 0.2258). The MCL was set to protect against infant methemoglobinemia; however other health effects including cancer and adverse reproductive outcomes were not considered [\[4\]](#page-82-3). Through endogenous nitrosation, nitrate is a precursor in the formation of *N*-nitroso compounds (NOC); most NOC are carcinogens and teratogens. Thus, exposure to NOC formed after ingestion of nitrate from drinking water and dietary sources may result in cancer, birth defects, or other adverse health effects. Nitrate is found in many foods, with the highest levels occurring in some green leafy and root vegetables [\[5](#page-82-4)[,6\]](#page-82-5). Average daily intakes from food are in the range of $30-130 \text{ mg/day}$ as $NO₃$ (7–29 mg/day $NO₃$ -N) [\[5\]](#page-82-4). Because NOC formation is inhibited by ascorbic acid, polyphenols, and other compounds present at high levels in most vegetables, dietary nitrate intake may not result in substantial endogenous NOC formation [\[5](#page-82-4)[,7\]](#page-82-6).

Studies of health effects related to nitrate exposure from drinking water were previously reviewed through early 2004 [\[8\]](#page-83-0). Further, an International Agency for Research on Cancer (IARC) Working Group reviewed human, animal, and mechanistic studies of cancer through mid-2006 and concluded that ingested nitrate and nitrite, under conditions that result in endogenous nitrosation, are probably carcinogenic [\[5\]](#page-82-4). Here, our objective is to provide updated information on human exposure and to review mechanistic and health effects studies since 2004. We summarize how the additional studies contribute to the overall evidence for health effects and we discuss what future research may be most informative.

2. Drinking Water Nitrate Exposures in the United States and Europe

Approximately 45 million people in the U.S. (about 14% of the population) had self-supplied water at their residence in 2010 [\[9\]](#page-83-1). Almost all (98%) were private wells, which are not regulated by the U.S. Environmental Protection Agency (EPA). The rest of the population was served by public water supplies, which use groundwater, surface water, or both. The U.S. Geological Survey's National Water Quality Assessment (USGS-NAWQA) Project [\[10\]](#page-83-2) sampled principal groundwater aquifers used as U.S. public and private drinking water supplies in 1988–2015. Nitrate levels in groundwater under agricultural land were about three times the national background level of $1 \text{ mg/L NO}_3\text{-N}$ (Figure 1) [\[11\]](#page-83-3). The mixed land use category mostly had nitrate concentrations below background levels reflecting levels in deeper private and public water supply wells. Based on the NAWQA study, it was estimated that 2% of public-supply wells and 6% of private wells exceeded the MCL; whereas, in agricultural areas, 21% of private wells exceeded the MCL [\[10\]](#page-83-2). The USGS-NAWQA study also revealed significant decadal-scale changes in groundwater nitrate concentrations among wells sampled first in 1988–2000 and again in 2001–2010 for agricultural, urban, and mixed land uses [\[12\]](#page-83-4). More sampling networks had increases in median nitrate concentration than had decreases.

A study of U.S. public water supplies (PWS) using data from EPA's Safe Drinking Water Information System estimated that the percentage of PWS violating the MCL increased from 0.28 to 0.42% during 1994–2009; most increases were for small to medium PWS (<10,000 population served) using groundwater [\[13\]](#page-83-5). As a result of increasing nitrate levels, some PWS have incurred expensive upgrades to their treatment systems to comply with the regulatory level [\[14–](#page-83-6)[16\]](#page-83-7).

Figure 1. Boxplots of nitrate concentrations in shallow groundwater beneath agricultural and urban land uses, and at depths of private and public drinking water supplies beneath mixed land use. The number of sampled wells were 1573 (agricultural land), 1054 (urban), and 3417 (mixed). agricultural and urban wells were sampled to assess land use effects, whereas the mixed category The agricultural and urban wells were sampled to assess land use effects, whereas the mixed category wells were sampled at depths of private and public supplies. Median depths of wells in the wells were sampled at depths of private and public supplies. Median depths of wells in the agricultural, urban, and mixed categories were 34, 32, and 200 feet, respectively. The height of the upper bar is 1.5 times the length of the box, and the lower bound was truncated at the nitrate detection limit of 0.05 mg/L NO₃-N.

In Europe, the Nitrates Directive was set in 1991 [17,18] to reduce or prevent nitrate pollution In Europe, the Nitrates Directive was set in 1991 [17,18] to reduce or prevent nitrate pollution from agriculture. Areas most affected by nitrate pollution are designated as 'nitrate vulnerable zones' from agriculture. Areas most affected by nitrate pollution are designated as 'nitrate vulnerable zones' and are subject to mandatory Codes of Good Agricultural Practice [18]. The results of compliance and are subject to mandatory Codes of Good Agricultural Practice [18]. The results of compliance with this directive have been reflected in the time trends of nitrate in some countries. For example, nitrate levels in groundwater in Denmark increased in 1950–1980 and decreased since the 1990s [19]. Average nitrate levels in groundwater in most other European countries have been stable at around 17.5 mg/L $NO₃$ (4 mg/L $NO₃$ -N) across Europe over a 20-year period (1992–2012), with some differences between countries both in trends and concentrations. Average concentrations are lowest in Finland (around 1 mg/L NO₃ in 1992–2012) and highest in Malta (58.1 mg/L in 2000–2012) [20]. Average annual nitrate concentrations at river monitoring stations in Europe showed a steady decline from 2.7 NO₃-N in 1992 to 2.1 mg/L in 2012 [20], with the lowest average levels in Norway (0.2 mg/L NO₃-N in 2012) and highest in Greece (6.6 mg/L NO₃-N in 2012).

Levels in finished public drinking water have been published only for a few European countries. Levels in finished public drinking water have been published only for a few European countries. Trends of nitrate in drinking water supplies from 1976 to 2012 in Denmark showed a decline in public supplies but not in private wells [21]. In Spain, median concentrations were 3.5 mg/L NO₃ 0.4−66.8) in 108 municipalities in 2012 [22], and 4.2 mg/L (range: <1−29) in 11 provinces in 2010 [23]. (range: 0.4−66.8) in 108 municipalities in 2012 [22], and 4.2 mg/L (range: <1−29) in 11 provinces in Levels in other countries included a median of 0.18 mg/L (range: <0.02−7.9) in Iceland in 2001−2012 2010 [23]. Levels in other countries included a median of 0.18 mg/L (range: <0.02−7.9) in Iceland in 2001−2012 [24], a mean of 16.1 mg/L (range: 0.05−296 mg/L) in Sicily, Italy in 2004−2005 [25] and a undetected to 63.3 mg/L in Deux-Sèvres, France in in 2005−2009 [26]. range from undetected to 63.3 mg/L in Deux-Sèvres, France in in 2005−2009 [26].

Nitrate levels in bottled water have been measured in a few areas of the EU and the U.S. and Nitrate levels in bottled water have been measured in a few areas of the EU and the U.S. and have been found to be below the MCL. In Sicily, the mean level was 15.2 mg/L NO₃(range: 1.2−31.8 mg/L) in 16 brands [25] and in Spain, the median level was 5.2 mg/L NO₃ (range: <1.0−29.0 mg/L) in 9 brands [23]. In the U.S., a survey of bottle water sold in 42 Iowa and 32 Texas communities found 9 brands [23]. In the U.S., a survey of bottle water sold in 42 Iowa and 32 Texas communities foundvarying but generally low nitrate levels. Nitrate concentrations ranged from below the limit of

 $(0.1 \text{ mg/L NO}_3\text{-N})$ to 4.9 mg/L NO₃-N for U.S. domestic spring water purchased in Texas. There are few published studies of nitrate concentrations in drinking water outside the U.S. and Europe. Nitrate concentrations in groundwater were reported for Morocco, Niger, Nigeria, Senegal, India-Pakistan, Japan, Lebanon, Philippines and Turkey with maximum levels in Senegal (median

42.9 mg/L NO₃-N) [\[5\]](#page-82-4). In India, nitrate in drinking water supplies is particularly high in rural areas, where average levels have been reported to be 45.7 mg/L NO_3 [\[27](#page-84-0)[,28\]](#page-84-1) and 66.6 mg/L NO₃ [\[28\]](#page-84-1); maximum levels in drinking water exceeded 100 mg/L $NO₃$ in several regions [\[27,](#page-84-0)[29\]](#page-84-2). Extremely high levels of nitrate have been reported in The Gaza Strip, where nitrate reached concentrations of 500 mg/L NO_3 in some areas, and more than 50% of public-supply wells had nitrate concentrations above 45 mg/L NO_3 [\[30\]](#page-84-3).

3. Exposure Assessment in Epidemiologic Studies

With the implementation of the Safe Drinking Water Act in 1974, more than 40 years of monitoring data for public water supplies in the U.S. provide a framework of measurements to support exposure assessments. Historical data for Europe are more limited, but a quadrennial nitrate reporting requirement was implemented as part of the EU Nitrates Directive [\[17,](#page-83-8)[18\]](#page-83-9). In the U.S., the frequency of sampling for nitrate in community water systems is stipulated by their sources (ground versus surface waters) and whether concentrations are below the MCL, and historically, by the size of the population served and vulnerability to nitrate contamination. Therefore, the exposure assessment for study participants who report using a public drinking water source may be based on a variable number of measurements, raising concerns about exposure misclassification. In a study of bladder cancer risk in Iowa, associations were stronger in sensitivity analyses based on more comprehensive measurement data [\[31\]](#page-84-4). Other studies have restricted analyses to subgroups with more complete or recent measurements [\[32–](#page-84-5)[35\]](#page-84-6), with implications for study power and possible selection biases. Sampling frequency also limits the extent to which temporal variation in exposure can be represented within a study population, such as the monthly or trimester-based estimates of exposure most relevant for etiologic investigations of adverse reproductive outcomes. In Denmark, limited seasonal variation in nitrate monitoring data suggested these data would sufficiently capture temporal variation for long-term exposure estimates [\[36\]](#page-84-7). Studies have often combined regulatory measurements with questionnaire and ancillary data to better characterize individual variation in nitrate exposure, such as to capture changes in water supply characteristics over time or a participant's duration at a drinking water source [\[31,](#page-84-4)[33,](#page-84-8)[37,](#page-84-9)[38\]](#page-84-10). Most case-control studies of drinking water nitrate and cancer obtained lifetime residence and drinking water source histories, whereas cohort studies typically have collected only the current water source. Many studies lacked information about study participants' water consumption, which may be an important determinant of exposure to drinking water contaminants [\[39\]](#page-84-11).

Due to sparse measurement data, exposures for individuals served by private wells are more difficult to estimate than exposures for those on public water supplies. However, advances in geographic-based modeling efforts that incorporate available measurements, nitrogen inputs, aquifer characteristics, and other data hold promise for this purpose. These models include predictor variables describing land use, nitrogen inputs (fertilizer applications, animal feeding operations), soils, geology, climate, management practices, and other factors at the scale of interest. Nolan and Hitt [\[40\]](#page-84-12) and Messier et al. [\[41\]](#page-84-13) used nonlinear regression models with terms representing nitrogen inputs at the land surface, transport in soils and groundwater, and nitrate removal by processes such as denitrification, to predict groundwater nitrate concentration at the national scale and for North Carolina, respectively. Predictor variables in the models included N fertilizer and manure, agricultural or forested land use, soils, and, in Nolan and Hitt [\[40\]](#page-84-12), water-use practices and major geology. Nolan and Hitt [\[40\]](#page-84-12) reported a training R^2 values of 0.77 for a model of groundwater used mainly for private supplies and Messier, Kane, Bolich and Serre [\[41\]](#page-84-13) reported a cross-validation testing R^2 value of 0.33 for a point-level

private well model. These and earlier regression approaches for groundwater nitrate [\[42](#page-84-14)[–46\]](#page-84-15) relied on predictor variables describing surficial soils and activities at the land surface, because conditions at depth in the aquifer typically are unknown. Redox conditions in the aquifer and the time since water entered the subsurface (i.e., groundwater age) are two of the most important factors affecting groundwater nitrate, but redox constituents typically are not analyzed, and age is difficult to measure. Even if a well has sufficient data to estimate these conditions, the data must be available for all wells in order to predict water quality in unsampled areas. In most of the above studies, well depth was used as a proxy for age and redox and set to average private or public-supply well depth for prediction.

Recent advances in groundwater nitrate exposure modeling have involved machine-learning methods such as random forest (RF) and boosted regression trees (BRT), along with improved characterization of aquifer conditions at the depth of the well screen (the perforated portion of the well where groundwater intake occurs). Tree-based models do not require data transformation, can fit nonlinear relations, and automatically incorporate interactions among predictors [\[47\]](#page-84-16). Wheeler et al. [\[48\]](#page-84-17) used RF to estimate private well nitrate levels in Iowa. In addition to land use and soil variables, predictor variables included aquifer characteristics at the depth of the well screen, such as total thickness of fine-grained glacial deposits above the well screen, average and minimum thicknesses of glacial deposits near sampled wells, and horizontal and vertical hydraulic conductivities near the wells. Well depth, landscape features, nitrogen sources, and aquifer characteristics ranked highly in the final model, which explained 77% and 38% of the variation in training and hold-out nitrate data, respectively.

Ransom et al. [\[49\]](#page-85-0) used BRT to predict nitrate concentration at the depths of private and public-supply wells for the Central Valley, California. The model used as input estimates of groundwater age at the depth of the well screen (from MODFLOW/MODPATH models) and depth-related reducing conditions in the groundwater. These estimates were generated by separate models and were available throughout the aquifer. Other MODFLOW-based predictor variables comprised depth to groundwater, and vertical water fluxes and the percent coarse material in the uppermost part of the aquifer where groundwater flow was simulated by MODFLOW. Redox variables were top-ranked in the final BRT model, which also included land use-based N leaching flux, precipitation, soil characteristics, and the MODFLOW-based variables described above. The final model retained 25 of an initial 145 predictor variables considered, had training and hold-out R^2 values of 0.83 and 0.44 respectively, and was used to produce a 3D visualization of nitrate in the aquifer. These studies show that modeling advances and improved characterization of aquifer conditions at depth are increasing our ability to predict nitrate exposure from drinking water supplied by private wells.

4. Nitrate Intake and Endogenous Formation of *N***-Nitroso Compounds**

Drinking water nitrate is readily absorbed in the upper gastrointestinal tract and distributed in the human body. When it reaches the salivary glands, it is actively transported from blood into saliva and levels may be up to 20 times higher than in the plasma $[50-53]$ $[50-53]$. In the oral cavity 6–7% of the total nitrate can be reduced to nitrite, predominantly by nitrate-reducing bacteria [\[52,](#page-85-3)[54,](#page-85-4)[55\]](#page-85-5). The secreted nitrate as well as the nitrite generated in the oral cavity re-enter the gastrointestinal tract when swallowed.

Under acidic conditions in the stomach, nitrite can be protonated to nitrous acid $(HNO₂)$, and subsequently yield dinitrogen trioxide (N_2O_3) , nitric oxide (NO) , and nitrogen dioxide (NO_2) . Since the discovery of endogenous NO formation, it has become clear that NO is involved in a wide range of NO-mediated physiological effects. These comprise the regulation of blood pressure and blood flow by mediating vasodilation [\[56](#page-85-6)[–58\]](#page-85-7), the maintenance of blood vessel tonus [\[59\]](#page-85-8), the inhibition of platelet adhesion and aggregation [\[60](#page-85-9)[,61\]](#page-85-10), modulation of mitochondrial function [\[62\]](#page-85-11) and several other processes [\[63–](#page-85-12)[66\]](#page-85-13).

On the other hand, various nitrate and nitrite derived metabolites such as nitrous acid (HNO2) are powerful nitrosating agents and known to drive the formation of NOC, which are

suggested to be the causal agents in many of the nitrate-associated adverse health outcomes. NOC comprise *N*-nitrosamines and *N*-nitrosamides, and may be formed when nitrosating agents encounter *N*-nitrosatable amino acids, which are also from dietary origin. The nitrosation process depends on the reaction mechanisms involved, on the concentration of the compounds involved, the pH of the reaction environment, and further modifying factors, including the presence of catalysts or inhibitors of *N*-nitrosation [\[66–](#page-85-13)[69\]](#page-85-14).

Endogenous nitrosation can also be inhibited, for instance by dietary compounds like vitamin C, which has the capacity to reduce $HNO₂$ to NO; and alpha-tocopherol or polyphenols, which can reduce nitrite to NO [\[54,](#page-85-4)[70–](#page-85-15)[72\]](#page-86-0). Inhibitory effects on nitrosation have also been described for dietary flavonoids such as quercetin, ferulic and caffeic acid, betel nut extracts, garlic, coffee, and green tea polyphenols [\[73](#page-86-1)[,74\]](#page-86-2). Earlier studies showed that the intake of 250 mg or 1 g ascorbic acid per day substantially inhibited *N*-nitrosodimethylamine (NDMA) excretion in 25 women consuming a fish meal rich in amines (nitrosatable precursors) for seven days, in combination with drinking water containing nitrate at the acceptable daily intake (ADI) [\[75\]](#page-86-3). In addition, strawberries, garlic juice, and kale juice were shown to inhibit NDMA excretion in humans [\[76\]](#page-86-4). The effect of these fruits and vegetables is unlikely to be due solely to ascorbic acid. Using the *N*-nitrosoproline (NPRO) test, Helser et al. [\[77\]](#page-86-5) found that ascorbic acid only inhibited nitrosamine formation by 24% compared with 41–63% following ingestion of juices (100 mL) made of green pepper, pineapple, strawberry or carrot containing an equal total amount of ascorbic acid.

The protective potential of such dietary inhibitors depends not only on the reaction rates of *N*-nitrosatable precursors and nitrosation inhibitors, but also on their biokinetics, since an effective inhibitor needs to follow gastrointestinal circulation kinetics similar to nitrate [\[78\]](#page-86-6). It has been argued that consumption of some vegetables with high nitrate content, can at least partially inhibit the formation of NOC [\[79](#page-86-7)[–81\]](#page-86-8). This might apply for green leafy vegetables such as spinach and rocket salad, celery or kale [\[77\]](#page-86-5) as well as other vegetables rich in both nitrate and natural nitrosation inhibitors. Preliminary data show that daily consumption of one bottle of beetroot juice containing 400 mg nitrate (the minimal amount advised for athletes to increase their sports performances) for one day and seven days by 29 young individuals results in an increased urinary excretion of apparent total nitroso compounds (ATNC), an effect that can only be partially inhibited by vitamin C supplements (1 g per day) [\[82\]](#page-86-9).

Also, the amount of nitrosatable precursors is a key factor in the formation of NOC. Dietary intakes of red and processed meat are of particular importance [\[83–](#page-86-10)[87\]](#page-86-11) as increased consumption of red meat (600 vs. 60 g/day), but not white meat, was found to cause a three-fold increase in fecal NOC levels [\[85\]](#page-86-12). It was demonstrated that heme iron stimulated endogenous nitrosation [\[84\]](#page-86-13), thereby providing a possible explanation for the differences in colon cancer risk between red and white meat consumption [\[88\]](#page-86-14). The link between meat consumption and colon cancer risk is even stronger for nitrite-preserved processed meat than for fresh meat leading an IARC review to conclude that processed meat is carcinogenic to humans [\[89\]](#page-86-15).

In a human feeding study [\[90\]](#page-86-16), the replacement of nitrite in processed meat products by natural antioxidants and the impact of drinking water nitrate ingestion is being evaluated in relation to fecal excretion of NOC, accounting for intakes of meat and dietary vitamin C. A pilot study demonstrated that fecal excretion of ATNC increased after participants switched from ingesting drinking water with low nitrate levels to drinking water with nitrate levels at the acceptable daily intake level of 3.7 mg/kg. The 20 volunteers were assigned to a group consuming either 3.75 g/kg body weight (maximum 300 g per day) red processed meat or fresh (unprocessed) white meat. Comparison of the two dietary groups showed that the most pronounced effect of drinking water nitrate was observed in the red processed meat group. No inhibitory effect of vitamin C intake on ATNC levels in feces was found (unpublished results).

5. Methemoglobinemia

The physiologic processes that can lead to methemoglobinemia in infants under six months of age have been described in detail previously [\[8,](#page-83-0)[91\]](#page-86-17). Ingested nitrate is reduced to nitrite by bacteria in the mouth and in the infant stomach, which is less acidic than adults. Nitrite binds to hemoglobin to form methemoglobin, which interferes with the oxygen carrying capacity of the blood. Methemoglobinemia is a life-threatening condition that occurs when methemoglobin levels exceed about 10% [\[8](#page-83-0)[,91\]](#page-86-17). Risk factors for infant methemoglobinemia include formula made with water containing high nitrate levels, foods and medications that have high nitrate levels [\[91](#page-86-17)[,92\]](#page-86-18), and enteric infections [\[93\]](#page-87-0). Methemoglobinemia related to high nitrate levels in drinking water used to make infant formula was first reported in 1945 [\[94\]](#page-87-1). The U.S. EPA limit of 10 mg/L NO₃-N was set as about one-half the level at which there were no observed cases [\[95\]](#page-87-2). The most recent U.S. cases related to nitrate in drinking water were reported by Knobeloch and colleagues in the late 1990s in Wisconsin [\[96\]](#page-87-3) and were not described in our prior review. Nitrate concentrations in the private wells were about two-times the MCL and bacterial contamination was not a factor. They also summarize another U.S. case in 1999 related to nitrate contamination of a private well and six infant deaths attributed to methemoglobinemia in the U.S. between 1979–1999 only one of which was reported in the literature [\[96,](#page-87-3)[97\]](#page-87-4). High incidence of infant methemoglobinemia in eastern Europe has also been described previously [\[98,](#page-87-5)[99\]](#page-87-6). A 2002 WHO report on water and health [\[100\]](#page-87-7) noted that there were 41 cases in Hungary annually, 2913 cases in Romania from 1985–1996 and 46 cases in Albania in 1996.

Results of several epidemiologic studies conducted before 2005 that examined the relationship between nitrate in drinking water and levels of methemoglobin or methemoglobinemia in infants have been described previously [\[8\]](#page-83-0). Briefly, nitrate levels $>10 \text{ mg/L NO}_3$ -N were usually associated with increased methemoglobin levels but clinical methemoglobinemia was not always present. Since our last review, a cross-sectional study conducted in Gaza found elevated methemoglobin levels in infants on supplemental feeding with formula made from well water in an area with the highest mean nitrate concentration of 195 mg/L NO_3 (range: 18–440) compared to an area with lower nitrate concentration (mean: 119 mg/L NO₃; range 18–244) [\[101\]](#page-87-8). A cross-sectional study in Morocco found a 22% increased risk of methemoglobinemia in infants in an area with drinking water nitrate >50 mg/L (>11 as NO₃-N) compared to infants in an area with nitrate levels <50 mg/L nitrate [\[102\]](#page-87-9). A retrospective cohort study in Iowa of persons (aged 1–60 years) consuming private well water with nitrate levels $\langle 10 \text{ mg/L} \rangle$ NO₃-N found a positive relationship between methemoglobin levels in the blood and the amount of nitrate ingestion [\[103\]](#page-87-10). Among pregnant women in rural Minnesota with drinking water supplies that were mostly \leq 3 mg/L NO₃-N, there was no relationship between water nitrate intake and women's methemoglobin levels around 36 weeks' gestation [\[104\]](#page-87-11).

6. Adverse Pregnancy Outcomes

Maternal drinking water nitrate intake during pregnancy has been investigated as a risk factor for a range of pregnancy outcomes, including spontaneous abortion, fetal deaths, prematurity, intrauterine growth retardation, low birth weight, congenital malformations, and neonatal deaths. The relation between drinking water nitrate and congenital malformations in offspring has been the most extensively studied, most likely because of the availability of birth defect surveillance systems around the world.

Our earlier review focused on studies of drinking water nitrate and adverse pregnancy outcomes published before 2005 [\[8\]](#page-83-0). In that review, we cited several studies on the relation between maternal exposure to drinking water nitrate and spontaneous abortion including a cluster investigation that suggested a positive association [\[105\]](#page-87-12) and a case-control study that found no association [\[106\]](#page-87-13). These studies were published over 20 years ago. In the present review, we were unable to identify any recently published studies on this outcome. In Table [1,](#page-71-0) we describe the findings of studies published since 2004 on the relation between drinking water nitrate and prematurity, low birthweight, and congenital malformations. We report results for nitrate in the units (mg/L $NO₃$ or $NO₃-N$) that were reported in the publications. In a historic cohort study conducted in the Deux-Sèvres district (France), Migeot et al. [\[26\]](#page-83-10) linked maternal addresses from birth records to community water system measurements of nitrate, atrazine, and other pesticides. Exposure to the second tertile of nitrate $(14–27 \text{ mg/L NO}_3)$ without detectable atrazine metabolites was associated with small-for-gestational age births (Odds Ratio (OR) 1.74, 95% CI 1.1, 2.8), but without a monotonic increase in risk with exposures. There was no association with nitrate among those with atrazine detected in their drinking water supplies. Within the same cohort, Albouy-Llaty and colleagues did not observe any association between higher water nitrate concentrations (with or without the presence of atrazine) and preterm birth [\[107\]](#page-87-14).

Stayner and colleagues also investigated the relation between atrazine and nitrate in drinking water and rates of low birth weight and preterm birth in 46 counties in four Midwestern U.S. states that were required by EPA to measure nitrate and atrazine monthly due to prior atrazine MCL violations [\[108\]](#page-87-15). The investigators developed county-level population-weighted metrics of average monthly nitrate concentrations in public drinking water supplies. When analyses were restricted to counties with less than 20% private well usage (to reduce misclassification due to unknown nitrate levels), average nitrate concentrations during the pregnancy were associated with increased rates of very low birth weight (<1.5 kg Rate Ratio $(RR)_{per 1 ppm} = 1.17, 95\%$ CI 1.08, 1.25) and very preterm births (<32 weeks $RR_{per 1 ppm} = 1.08$, 95% CI 1.02, 1.15) but not with low birth weight or preterm birth overall.

In record-based prevalence study in Perth Australia, Joyce et al. mapped births to their water distribution zone and noted positive associations between increasing tertiles of nitrate levels and prevalence of term premature rupture of membranes (PROM) adjusted for smoking and socioeconomic status [\[109\]](#page-87-16). Nitrate concentrations were low; the upper tertile cut point was 0.350 mg/L and the maximum concentration was 1.80 mg/L NO_3 -N. Preterm PROM was not associated with nitrate concentrations.

Among studies of drinking water nitrate and congenital malformations, few before 2005 included birth defects other than central nervous system defects [\[8\]](#page-83-0). More recently, Mattix et al. [\[110\]](#page-87-17) noted higher rates of abdominal wall defects (AWD) in Indiana compared to U.S. rates for specific years during the period 1990–2002. They observed a positive correlation between monthly AWD rates and monthly atrazine concentrations in surface waters but no correlation with nitrate levels. Water quality data were obtained from the USGS-NAWQA project that monitors agricultural chemicals in streams and shallow groundwater that are mostly not used as drinking water sources. A case-control study of gastroschisis (one of the two major types of AWD), in Washington State [\[111\]](#page-87-18) also used USGS-NAWQA measurements of nitrate and pesticides in surface water and determined the distance between maternal residences (zip code centroids) and the closest monitoring site with concentrations above the MCL for nitrate, nitrite, and atrazine. Gastrochisis was not associated with maternal proximity to surface water above the MCL for nitrate (>10 mg/L NO₃-N) or nitrite (>1 mg/L NO₂-N) but there was a positive relationship with proximity to sites with atrazine concentrations above the MCL. In a USA-wide study, Winchester et al. [\[112\]](#page-87-19) linked the USGS-NAWQA monthly surface water nitrate and pesticide concentrations computed for the month of the last menstrual period with monthly rates of 22 types of birth defects in 1996–2002. Rates of birth defects among women who were estimated to have conceived during April through July were higher than rates among women conceiving in other months. In multivariable models that included nitrate, atrazine, and other pesticides, atrazine (but not nitrate or other pesticides) was associated with several types of anomalies. Nitrate was associated with birth defects in the category of "other congenital anomalies" (OR 1.18, 95% CI 1.14, 1.21); the authors did not specify what defects were included in this category. None of these three studies included local or regional data to support the assumption that surface water nitrate and pesticide concentrations correlated with drinking water exposures to these contaminants.

Using a more refined exposure assessment than the aforementioned studies, Holtby et al. [\[113\]](#page-87-20) conducted a case-control study of congenital anomalies in an agricultural county in Nova Scotia,

Canada. They linked maternal addresses at delivery to municipal water supply median nitrate concentrations and used kriging of monthly measurements from a network of 140 private wells to estimate drinking water nitrate concentrations in private wells. They observed no associations between drinking water nitrate and all birth defects combined for conceptions during 1987–1997. However, the prevalence of all birth defects occurring during 1998–2006 was associated with drinking water nitrate concentrations of 1–5.56 mg/L NO₃-N (OR 2.44, 95% CI 1.05, 5.66) and \geq 5.56 mg/L (OR 2.25, 95% CI 0.92, 5.52).

None of the studies of congenital anomalies accounted for maternal consumption of bottled water or the quantity of water consumed during the first trimester, the most critical period of organ/structural morphogenesis. Attempting to overcome some of these limitations, Brender, Weyer, and colleagues [\[38,](#page-84-10)[114\]](#page-88-0) conducted a population-based, case-control study in the states of Iowa and Texas where they: (1) linked maternal addresses during the first trimester to public water utilities and respective nitrate measurements; (2) estimated nitrate intake from bottled water based on a survey of products consumed and measurement of nitrate in the major products; (3) predicted drinking water nitrate from private wells through modeling (Texas only); and (4) estimated daily nitrate ingestion from women's drinking water sources and daily consumption of water. The study populations were participants of the U.S. National Birth Defects Prevention Study [\[115\]](#page-88-1). Compared to the lowest tertile of nitrate ingestion from drinking water $(<0.91 \text{ mg/day NO}_3$), mothers of babies with spina bifida were twice as likely (95% CI 1.3, 3.2) to ingest \geq 5 mg/day NO₃ from drinking water than control mothers. Mothers of babies with limb deficiencies, cleft palate, and cleft lip were, respectively, 1.8 (95% CI 1.1, 3.1), 1.9 (95% CI 1.2, 3.1), and 1.8 (95% CI 1.1, 3.1) times more likely to ingest \geq 5.4 mg/day of water NO₃ than controls. Women were also classified by their nitrosatable drug exposure during the first trimester [\[116\]](#page-88-2) and by their daily nitrate and nitrite intake based on a food frequency questionnaire [\[117\]](#page-88-3). Higher ingestion of drinking water nitrate did not strengthen associations between maternal nitrosatable drug exposure and birth defects in offspring [\[38\]](#page-84-10). However, a pattern was observed of stronger associations between nitrosatable drug exposure and selected birth defects for women in the upper two tertiles of total nitrite ingestion that included contributions from drinking water nitrate and dietary intakes of nitrate and nitrite compared to women in the lowest tertile. Higher intake of food nitrate/nitrite was found to also modify the associations of nitrosatable drug exposure and birth defects in this study [\[118](#page-88-4)[,119\]](#page-88-5) as well as in an earlier study of neural tube defects conducted in south Texas [\[120\]](#page-88-6). Multiplicative interactions were observed between higher food nitrate/nitrite and nitrosatable drug exposures for conotruncal heart, limb deficiency, and oral cleft defects [\[118\]](#page-88-4).

In summary, five out of six studies, conducted since the 1980s of drinking water nitrate and central nervous system defects, found positive associations between higher drinking water nitrate exposure during pregnancy and neural tube defects or central nervous system defects combined [\[38,](#page-84-10)[120–](#page-88-6)[123\]](#page-88-7). The sixth study, which did not find a relationship, did not include measures of association, but compared average drinking water nitrate concentrations between mothers with and without neural tube defect-affected births, which were comparable [\[124\]](#page-88-8).

Table 1. Studies of drinking water nitrate ^a and adverse pregnancy outcomes published January 2005–March 2018.

Table 1. *Cont*.

Abbreviations: CI, 95% CI confidence interval; OR, odds ratio; RR, rate ratio; USGS-NAWQA, U. S. Geological Survey National Water Quality Assessment; ^a nitrate units are specified as reported in publications. NO₃ can be converted to NO₃-N by multiplying by 0.2258; ^b USGS-NAWQA data for 186 streams in 51 hydrological study areas; streams were not drinking water sources.
7. Cancer

Most early epidemiologic studies of cancer were ecologic studies of stomach cancer mortality that used exposure estimates concurrent with the time of death. Results were mixed, with some studies showing positive associations, many showing no association, and a few showing inverse associations. The results of ecologic studies through 1995 were reviewed by Cantor [\[125\]](#page-88-0). Our previous review included ecologic studies of the brain, esophagus, stomach, kidney, ovary, and non-Hodgkin lymphoma (NHL) published between 1999 and 2003 that were largely null [\[8\]](#page-83-0). We did not include ecologic studies or mortality case-control studies in this review due to the limitations of these study designs, especially their inability to assess individual-level exposure and dietary factors that influence the endogenous formation of NOC.

Since our review of drinking water nitrate and health in 2005 [\[8\]](#page-83-0), eight case-control studies and eight analyses in three cohorts have evaluated historical nitrate levels in PWS in relation to several cancers. Nitrate levels were largely below 10 mg/L $NO₃$ -N. Most of these studies evaluated potential confounders and factors affecting nitrosation. Table [2](#page-77-0) shows the study designs and results of studies published from 2005 through 2018, including findings from periodic follow-ups of a cohort study of postmenopausal women in Iowa (USA) [\[31,](#page-84-0)[37,](#page-84-1)[126](#page-88-1)[–129\]](#page-88-2). In the first analysis of drinking water nitrate in the Iowa cohort with follow-up through 1998, Weyer and colleagues [\[130\]](#page-88-3) reported that ovarian and bladder cancers were positively associated with the long-term average PWS nitrate levels prior to enrollment (highest quartile average 1955–1988: >2.46 mg/L NO₃-N). They observed inverse associations for uterine and rectal cancer, but no associations with cancers of the breast, colon, rectum, pancreas, kidney, lung, melanoma, non-Hodgkin lymphoma (NHL), or leukemia. Analyses of PWS nitrate concentrations and cancers of the thyroid, breast, ovary, bladder, and kidney were published after additional follow-up of the cohort. The exposure assessment was improved by: (a) the computation of average nitrate levels and years of exposure at or above 5 mg/L NO_3 -N, based on time in residence (vs. one long-term PWS average nitrate estimate used by Weyer and colleagues); and (b) by estimation of total trihalomethanes (TTHM) and dietary nitrite intake.

Thyroid cancer was evaluated for the first time after follow-up of the cohort through 2004. A total of 40 cases were identified [\[37\]](#page-84-1). Among women with >10 years on PWS with levels exceeding 5 mg/L NO₃-N for five years or more, thyroid cancer risk was 2.6 times higher than that of women whose supplies never exceeded 5 mg/L. With follow-up through 2010, the risk of ovarian cancer remained increased among women in the highest quartile of average nitrate in PWS [\[129\]](#page-88-2). Ovarian cancer risk among private well users was also elevated compared to the lowest PWS nitrate quartile. Associations were stronger when vitamin C intake was below median levels with a significant interaction for users of private wells. Overall, breast cancer risk was not associated with water nitrate levels with follow-up through 2008 [\[128\]](#page-88-4). Among women with folate intake $\geq 400 \mu g/day$, risk was increased for those in the highest average nitrate quintile (Hazard Ratio (HR) = 1.40; 95% CI: = 1.05–1.87) and among private well users (HR = 1.38; 95% CI: = 1.05–1.82), compared to those with the lowest average nitrate quintile. There was no association with nitrate exposure among women with lower folate intake. With follow-up through 2010, there were 130 bladder cancer cases among women who had used PWS >10 years. Risk remained elevated among women with the highest average nitrate levels and was 1.6 times higher among women whose drinking water concentration exceeded 5 mg/L NO_3 -N for at least four years [\[31\]](#page-84-0). Risk estimates were not changed by adjustment for TTHM, which are suspected bladder cancer risk factors. Smoking, but not vitamin C intake, modified the association with nitrate in water; increased risk was apparent only in current smokers (*p*-interaction <0.03). With follow-up through 2010, there were 125 kidney cancer cases among women using PWS; risk was increased among those in the 95th percentile of average nitrate (>5.0 mg/L NO₃-N) compared with the lowest quartile (HR = 2.2, 95% CI: 1.2–4.2) [\[127\]](#page-88-5). There was no positive trend with the average nitrate level and no increased risk for women using private wells, compared to those with low average nitrate in their public supply. An investigation of pancreatic cancer in the same population (follow-up through 2011)

found no association with average water nitrate levels in public supplies and no association among women on private wells [\[126\]](#page-88-1).

In contrast to the positive findings for bladder cancer among the cohort of Iowa women, a cohort study of men and women aged 55–69 in the Netherlands with lower nitrate levels in PWS found no association between water nitrate ingestion (median in top quintile = 2.4 mg/day NO_3 -N) and bladder cancer risk [\[131\]](#page-88-6). Dietary intake of vitamins C and E and history of cigarette smoking did not modify the association. A hospital-based case-control study of bladder cancer in multiple areas of Spain [\[33\]](#page-84-2) assessed lifetime water sources and usual intake of tap water. Nitrate levels in PWS were low, with almost all average levels below 2 mg/L $NO₃-N$. Risk of bladder cancer was not associated with the nitrate level in drinking water or with estimated nitrate ingestion from drinking water, and there was no evidence of interaction with factors affecting endogenous nitrosation.

Several case-control studies conducted in the Midwestern U.S. obtained lifetime histories of drinking water sources and estimated exposure for PWS users. In contrast to findings of an increased risk of NHL associated with nitrate levels in Nebraska PWS in an earlier study [\[132\]](#page-88-7), there was no association with similar concentrations in public water sources in a case-control study of NHL in Iowa [\[35\]](#page-84-3). A study of renal cell carcinoma in Iowa [\[34\]](#page-84-4) found no association with the level of nitrate in PWS, including the number of years that levels exceeded 5 or 10 mg/L $NO₃$ -N. However, higher nitrate levels in PWS increased risk among subgroups who reported above the median intake of red meat intake or below the median intake of vitamin C (*p*-interaction <0.05). A small case-control study of adenocarcinoma of the stomach and esophagus among men and women in Nebraska [\[133\]](#page-89-0) estimated nitrate levels among long-term users of PWS and found no association between average nitrate levels and risk.

A case-control study of colorectal cancer among rural women in Wisconsin estimated nitrate levels in private wells using spatial interpolation of nitrate concentrations from a 1994 water quality survey and found increased risk of proximal colon cancer among women estimated to have nitrate levels >10 mg/L NO₃-N compared to levels < 0.5 mg/L. Risk of distal colon cancer and rectal cancer were not associated with nitrate levels [\[134\]](#page-89-1). Water nitrate ingestion from public supplies, bottled water, and private wells and springs over the adult lifetime was estimated in analyses that pooled case-control studies of colorectal cancer in Spain and Italy [\[135\]](#page-89-2). Risk of colorectal cancer was increased among those with >2.3 mg/day NO₃-N (vs. <1.1 mg/day). There were no interactions with red meat, vitamins C and E, and fiber except for a borderline interaction (p -interaction = 0.07) for rectum cancer with fiber intake. A small hospital-based case-control study in Indonesia found that drinking water nitrate levels above the WHO standard (>11.3 mg/L as $NO₃-N$) was associated with colorectal cancer [\[136\]](#page-89-3). A national registry-based cohort study in Denmark [\[32\]](#page-84-5) evaluated average nitrate concentrations in PWS and private wells in relation to colorectal cancer incidence among those whose 35th birthday occurred during 1978–2011. The average nitrate level was computed over residential water supplies from age 20 to 35. Increased risks for colon and rectum cancer were observed in association with average nitrate levels \geq 9.25 mg/L NO₃ (\geq 2.1 as NO₃-N) and \geq 3.87 mg/L NO₃ ($>$ 0.87 as NO₃-N), respectively, with a significant positive trend. Because the study did not interview individuals, it could not evaluate individual-level risk factors that might influence endogenous nitrosation.

A case-control study of breast cancer in Cape Cod, Massachusetts (US) [\[137\]](#page-89-4) estimated nitrate concentrations in PWS over approximately 20 years as an historical proxy for wastewater contamination and potential exposure to endocrine disruption compounds. Average exposures >1.2 mg/L NO₃-N (vs. <0.3 mg/L) were not associated with risk. A hospital-based case-control study in Spain found no association between water nitrate ingestion and pre- and post-menopausal breast cancers [\[138\]](#page-89-5).

Table 2. Case-control and cohort studies of drinking water nitrate and cancer (January 2004–March 2018) by cancer site.

Table 2. *Cont*.

Table 2. *Cont*.

ND = not detected; PWS = public water supplies; ^a nitrate or nitrite levels presented in the publications as mg/L of the ion were converted to mg/L as NO₃-N or NO₂-N; ^b Odds ratios (OR) for case-control studies, incidence rate ratios (RR) and hazard ratios (HR) for cohort studies, and 95% confidence intervals (CI); ^c Factors evaluated are noted. Interaction refers to reported $p \leq 0.10$ from test of heterogeneity.

Animal studies demonstrate that in utero exposure to nitrosamides can cause brain tumors in the exposed offspring. Water nitrate and nitrite intake during pregnancy was estimated in a multi-center case-control study of childhood brain tumors in five countries based on the maternal residential water source [\[139\]](#page-89-13). Results for the California and Washington State sites were reported in our previous review [\[8,](#page-83-0)[140\]](#page-89-14). Nitrate/nitrite levels in water supplies were measured using a nitrate test strip method in four countries including these U.S. sites; most of these measurements occurred many years after the pregnancy. Measured nitrate concentrations were not associated with risk of childhood brain tumors. However, higher nitrite levels (>1.5 mg/L NO₂-N) in the drinking water were associated with increased risk of astrocytomas.

8. Thyroid Disease

Animal studies demonstrate that ingestion of nitrate at high doses can competitively inhibit iodine uptake and induce hypertrophy of the thyroid gland [\[141\]](#page-89-15). An early study of women in the Netherlands consuming water with nitrate levels at or above the MCL, found increased prevalence of thyroid hypertrophy [\[142\]](#page-89-16). Since the last review, five studies have evaluated nitrate ingestion from drinking water (the Iowa cohort study also assessed diet) and prevalence of thyroid disease. A study of school-age children in Slovakia found increased prevalence of subclinical hypothyroidism among children in an area with high nitrate levels $(51-274 \text{ mg/L NO}_3)$ in water supplies compared with children ingesting water with nitrate \leq 50 mg/L (11 mg/L NO₃-N). In Bulgarian villages with high nitrate levels (75 mg/L NO₃) and low nitrate levels (8 mg/L), clinical examinations of the thyroids of pregnant women and school children revealed an approximately four- and three-fold increased prevalence of goiter, respectively, in the high nitrate village [\[143,](#page-89-17)[144\]](#page-89-18). The iodine status of the populations in both studies was adequate. Self-reported hypothyroidism and hyperthyroidism among a cohort of post-menopausal women in Iowa was not associated with average nitrate concentrations in PWS [\[37\]](#page-84-1). However, dietary nitrate, the predominant source of intake, was associated with increased prevalence of hypothyroidism but not hyperthyroidism. Modeled estimates of nitrate concentrations in private wells among a cohort of Old Order Amish in Pennsylvania (USA) were associated with increased prevalence of subclinical hypothyroidism as determined by thyroid stimulating hormone measurements, among women but not men [\[145\]](#page-89-19).

9. Other Health Effects

Associations between nitrate in drinking water and other non-cancer health effects, including type 1 childhood diabetes (T1D), blood pressure, and acute respiratory tract infections in children were previously reviewed [\[8\]](#page-83-0). Since 2004, a small number of studies have contributed additional mixed evidence for these associations. Animal studies indicate that NOC may play a role in the pathology of T1D through damage to pancreatic beta cells [\[146\]](#page-89-20). A registry-based study in Finland [\[147\]](#page-89-21) found a positive trend in T1D incidence with levels of nitrate in drinking water. In contrast, an ecological analysis in Italy showed an inverse correlation with water nitrate levels and T1D rates [\[148\]](#page-89-22). A small T1D case-control study in Canada with 57 cases showed no association between T1D and estimated intake of nitrate from drinking water (highest quartile >2.7 mg/day NO₃-N) [\[149\]](#page-89-23). Concentrations of nitrate in drinking water (median \sim 2.1 mg/L NO₃-N) were not associated with progression to T1D in a German nested case-control study of islet autoantibody-positive children, who may be at increased risk of the disease [\[150\]](#page-89-24).

In a prospective, population-based cohort study in Wisconsin (USA), increased incidence of early and late age-related macular degeneration was positively associated with higher nitrate levels $(≥5 \text{ mg/L vs.} < 5 \text{ mg/L NO}_3-N)$ in rural private drinking water supplies [\[151\]](#page-90-0). The authors suggested several possible mechanisms, including methemoglobin-induced lipid peroxidation in the retina.

Potential benefits of nitrate ingestion include lowering of blood pressure due to production of nitric oxide in the acidic stomach and subsequent vasodilation, antithrombotic, and immunoregulatory effects [\[152\]](#page-90-1). Experimental studies in animals and controlled feeding studies in humans have

demonstrated mixed evidence of these effects and on other cardiovascular endpoints such as vascular hypertrophy, heart failure, and myocardial infarction (e.g., [\[152–](#page-90-1)[154\]](#page-90-2)). Ingested nitrite from diet has also been associated with increased blood flow in certain parts of the brain [\[155\]](#page-90-3). Epidemiologic studies of these effects are limited to estimation of dietary exposures or biomarkers that integrate exposures from nitrate from diet and drinking water. Recent findings in the Framingham Offspring Study suggested that plasma nitrate was associated with increased overall risk of death that attenuated when adjusted for glomerular function (HR: 1.16, 95% CI: 1.0–1.35) but no association was observed for incident cardiovascular disease [\[156\]](#page-90-4). No epidemiologic studies have specifically evaluated nitrate ingested from drinking water in relation to these outcomes. Another potential beneficial effect of nitrate is protection against bacterial infections via its reduction to nitrite by enteric bacteria. In an experimental inflammatory bowel disease mouse model, nitrite in drinking water was associated with both preventive and therapeutic effects [\[157\]](#page-90-5). However, there is limited epidemiologic evidence for a reduced risk of gastrointestinal disease in populations with high drinking water nitrate intake. One small, cross-sectional study in Iran found no association between nitrate levels in public water supplies with mean levels of \sim 5.6 mg/L NO₃-N and gastrointestinal disease [\[158\]](#page-90-6).

10. Discussion

Since our last review of studies through 2004 [\[8\]](#page-83-0), more than 30 epidemiologic studies have evaluated drinking water nitrate and risk of cancer, adverse reproductive outcomes, or thyroid disease. However, the number of studies of any one outcome was not large and there are still too few studies to allow firm conclusions about risk. The most common endpoints studied were colorectal cancer, bladder, and breast cancer (three studies each) and thyroid disease (four studies). Considering all studies to date, the strongest evidence for a relationship between drinking water nitrate ingestion and adverse health outcomes (besides methemoglobinemia) is for colorectal cancer, thyroid disease, and neural tube defects. Four of the five published studies of colorectal cancer found evidence of an increased risk of colorectal cancer or colon cancer associated with water nitrate levels that were mostly below the respective regulatory limits [\[32,](#page-84-5)[134,](#page-89-1)[135,](#page-89-2)[159\]](#page-90-7). In one of the four positive studies [\[159\]](#page-90-7), increased risk was only observed in subgroups likely to have increased nitrosation. Four of the five studies of thyroid disease found evidence for an increased prevalence of subclinical hypothyroidism with higher ingestion of drinking water nitrate among children, pregnant women, or women only [\[37,](#page-84-1)[144](#page-89-18)[,145](#page-89-19)[,160\]](#page-90-8). Positive associations with drinking water nitrate were observed at nitrate concentrations close to or above the MCL. The fifth study, a cohort of post-menopausal women in Iowa, had lower drinking water nitrate exposure but observed a positive association with dietary nitrate [\[37\]](#page-84-1). To date, five of six studies of neural tube defects showed increased risk with exposure to drinking water nitrate below the MCL. Thus, the evidence continues to accumulate that higher nitrate intake during the pregnancy is a risk factor for this group of birth defects.

All but one of the 17 cancer studies conducted since 2004 were in the U.S. or Europe, the majority of which were investigations of nitrate in regulated public drinking water. Thyroid cancer was studied for the first time [\[37\]](#page-84-1) with a positive finding that should be evaluated in future studies. Bladder cancer, a site for which other drinking water contaminants (arsenic, disinfection by-products [DBPs]) are established or suspected risk factors, was not associated with drinking water nitrate in three of the four studies. Most of the cancer studies since 2004 evaluated effect modification by factors known to influence endogenous nitrosation, although few observed evidence for these effects. Several studies of adverse reproductive outcomes since 2004 have indicated a positive association between maternal prenatal exposure to nitrate concentrations below the MCL and low birth weight and small for gestational age births. However, most studies did not account for co-exposure to other water contaminants, nor did they adjust for potential risk factors. The relation between drinking water nitrate and spontaneous abortion continues to be understudied. Few cases of methemoglobinemia, the health concern that lead to the regulation of nitrate in public water supplies, have been reported in the U.S. since the 1990s. However, as described by Knobeloch et al. [\[96\]](#page-87-0), cases may be underreported

and only a small proportion of cases are thoroughly investigated and described in the literature. Based on published reports, [\[100\]](#page-87-1) areas of the world of particular concern include several eastern European countries, Gaza, and Morocco, where high nitrate concentrations in water supplies have been linked to high levels of methemoglobin in children. Therefore, continued surveillance and education of physicians and parents will be important. Biological plausibility exists for relationships between nitrate ingestion from drinking water and a few other health outcomes including diabetes and beneficial effects on the cardiovascular system, but there have been only a limited number of epidemiologic studies.

Assessment of drinking water nitrate exposures in future studies should be improved by obtaining drinking water sources at home and at work, estimating the amount of water consumed from each source, and collecting information on water filtration systems that may impact exposure. These efforts are important for reducing misclassification of exposure. Since our last review, an additional decade of PWS monitoring data are available in the U.S. and European countries, which has allowed assessment of exposure over a substantial proportion of participants' lifetimes in recent studies. Future studies should estimate exposure to multiple water contaminants as has been done in recent cancer studies [\[31,](#page-84-0)[33](#page-84-2)[,127](#page-88-5)[,129\]](#page-88-2). For instance, nitrate and atrazine frequently occur together in drinking water in agricultural areas [\[161\]](#page-90-9) and animal studies have found this mixture to be teratogenic [\[162\]](#page-90-10). Regulatory monitoring data for pesticides in PWS has been available for over 20 years in the U.S.; therefore, it is now feasible to evaluate co-exposure to these contaminants. Additionally, water supplies in agricultural areas that rely on alluvial aquifers or surface water often have elevated levels of both DBPs and nitrate. Under this exposure scenario, there is the possibility of formation of the nitrogenated DBPs including the carcinogenic NDMA, especially if chloramination treatment is used for disinfection [\[163](#page-90-11)[,164\]](#page-90-12). Studies of health effects in countries outside the U.S. and Europe are also needed.

A comprehensive assessment of nitrate and nitrite from drinking water and dietary sources as well as estimation of intakes of antioxidants and other inhibitors of endogenous nitrosation including dietary polyphenols and flavonoids is needed in future studies. Heme iron from red meat, which increases fecal NOC in human feeding studies, should also be assessed as a potential effect modifier of risk from nitrate ingestion. More research is needed on the potential interaction of nitrate ingestion and nitrosatable drugs (those with secondary and tertiary amines or amides). Evidence from several studies of birth defects [\[38](#page-84-12)[,118–](#page-88-13)[120\]](#page-88-14) implicates nitrosatable drug intake during pregnancy as a risk factor for specific congenital anomalies especially in combination with nitrate. Drugs with nitrosatable groups include many over-the-counter and prescription drugs. Future studies with electronic medical records and record-linkage studies in countries like Denmark with national pharmacy data may provide opportunities for evaluation of these exposures.

Populations with the highest exposure to nitrate from their drinking water are those living in agricultural regions, especially those drinking water from shallow wells near nitrogen sources (e.g., crop fields, animal feeding operations). Estimating exposure for private well users is important because it allows assessment of risk over a greater range of nitrate exposures compared to studies focusing solely on populations using PWS. Future health studies should focus on these populations, many of which may have been exposed to elevated nitrate in drinking water from early childhood into adulthood. A major challenge in conducting studies in these regions is the high prevalence of private well use with limited nitrate measurement data for exposure assessment. Recent efforts to model nitrate concentrations in private wells have shown that it is feasible to develop predictive models where sufficient measurement data are available [\[41,](#page-84-13)[48,](#page-84-14)[49\]](#page-85-0). However, predictive models from one area are not likely to be directly translatable to other geographic regions with different aquifers, soils, and nitrogen inputs.

Controlled human feeding studies have demonstrated that endogenous nitrosation occurs after ingestion of drinking water with nitrate concentrations above the MCL of 10 mg/L NO₃-N (\sim 44 mg/L as $NO₃$). However, the extent of NOC formation after ingestion of drinking water with nitrate concentrations below the MCL has not been well characterized. Increased risks of specific cancers and central nervous system birth defects in study populations consuming nitrate below the MCL is indirect evidence that nitrate ingestion at these levels may be a risk factor under some conditions. However, confounding by other exposures or risk factors can be difficult to rule out in many studies. Controlled human studies to evaluate endogenous nitrosation at levels below the MCL are needed to understand interindividual variability and factors that affect endogenous nitrosation at drinking water nitrate levels below the MCL.

A key step in the endogenous formation of NOC is the reduction of nitrate, which has been transported from the bloodstream into the saliva, to nitrite by the nitrate-reducing bacteria that are located primarily in the crypts on the back of the tongue [\[165–](#page-90-13)[167\]](#page-90-14). Tools for measuring bacterial DNA and characterizing the oral microbiome are now available and are currently being incorporated into epidemiologic studies [\[168,](#page-90-15)[169\]](#page-91-0). Buccal cell samples that have been collected in epidemiologic studies can be used to characterize the oral microbiome and to determine the relative abundance of the nitrate-reducing bacteria. Studies are needed to characterize the stability of the nitrate-reducing capacity of the oral microbiome over time and to determine factors that may modify this capacity such as diet, oral hygiene, and periodontal disease. Interindividual variability in the oral nitrate-reducing bacteria may play an important role in modifying endogenous NOC formation. The quantification of an individual's nitrate-reducing bacteria in future epidemiologic studies is likely to improve our ability to classify participants by their intrinsic capacity for endogenous nitrosation.

In addition to characterizing the oral microbiome, future epidemiologic studies should incorporate biomarkers of NOC (e.g., urinary or fecal NOC), markers of genetic damage, and determine genetic variability in NOC metabolism. As many NOC require α-hydroxylation by CYP2E1 for bioactivation and for formation of DNA adducts, it is important to investigate the influence of polymorphisms in the gene encoding for this enzyme. Studies are also needed among populations with medical conditions that increase nitrosation such as patients with inflammatory bowel disease and periodontal disease [\[8\]](#page-83-0). Because NOC exposures induce characteristic gene expression profiles [\[170,](#page-91-1)[171\]](#page-91-2), further studies linking drinking water intake to NOC excretion and gene expression responses are relevant to our understanding of health risks associated with drinking water nitrate. The field of 'Exposome-research' [\[172,](#page-91-3)[173\]](#page-91-4) generates large numbers of genomics profiles in human population studies for which dietary exposures and biobank materials are also available. These studies provide opportunities to measure urinary levels of nitrate and NOC that could be associated with molecular markers of exposure and disease risk.

Nitrate concentrations in global water supplies are likely to increase in the future due to population growth, increases in nitrogen fertilizer use, and increasing intensity and concentration of animal agriculture. Even with increased inputs, mitigation of nitrate concentrations in water resources is possible through local, national, and global efforts. Examples of the latter are the International Nitrogen Initiative [\[174\]](#page-91-5) and the EU Nitrates Directive [\[17,](#page-83-1)[18\]](#page-83-2), which aim to quantify human effects on the nitrogen cycle and to validate and promote methods for sustainable nitrogen management. Evidence for the effectiveness of these efforts, which include the identification of vulnerable areas, establishment of codes of good agricultural practices, and national monitoring and reporting are indicated by decreasing trends in groundwater nitrate concentrations in some European countries after the implementation of the EU Nitrates Directive [\[19\]](#page-83-3). However, the effect of this initiative was variable across the EU. In the U.S., nitrogen applications to crop fields are not regulated and efforts to reduce nitrogen runoff are voluntary. Although strategies such as appropriate timing of fertilizer applications, diversified crop rotations, planting of cover crops, and reduced tillage can be effective [\[175\]](#page-91-6), concentrations in U.S. ground and surface water have continued to increase in most areas [\[10\]](#page-83-4). Climate change is expected to affect nitrogen in aquatic ecosystems and groundwater through alterations of the hydrological cycle [\[176\]](#page-91-7). Climatic factors that affect nitrate in groundwater include the amount, intensity, and timing of precipitation. Increasing rainfall intensity, especially in

the winter and spring, can lead to increases in nitrogen runoff from agricultural fields and leaching to groundwater.

11. Conclusions

In summary, most adverse health effects related to drinking water nitrate are likely due to a combination of high nitrate ingestion and factors that increase endogenous nitrosation. Some of the recent studies of cancer and some birth defects have been able to identify subgroups of the population likely to have greater potential for endogenous nitrosation. However, direct methods of assessing these individuals are needed. New methods for quantifying the nitrate-reducing bacteria in the oral microbiome and characterizing genetic variation in NOC metabolism hold promise for identifying high risk groups in epidemiologic studies.

To date, the number of well-designed studies of individual health outcomes is still too few to draw firm conclusions about risk from drinking water nitrate ingestion. Additional studies that incorporate improved exposure assessment for populations on PWS, measured or predicted exposure for private well users, quantification of nitrate-reducing bacteria, and estimates of dietary and other factors affecting nitrosation are needed. Studies of colorectal cancer, thyroid disease, and central nervous system birth defects, which show the most consistent associations with water nitrate ingestion, will be particularly useful for clarifying these risks. Future studies of other health effects with more limited evidence of increased risk are also needed including cancers of the thyroid, ovary, and kidney, and the adverse reproductive outcomes of spontaneous abortion, preterm birth, and small for gestational age births.

Acknowledgments: This work was partly supported by the Intramural Research Program of the National Cancer Institute, Division of Cancer Epidemiology and Genetics, Occupational and Environmental Epidemiology Branch. Two authors (TMdK, SvB) acknowledge financial support from the European Commission in the context of the integrated project PHYTOME financed under the Seventh Framework Programme for Research and Technology Development of the European Commission (EU-FP7 grant agreement no. 315683), investigating the possible replacement of nitrite in meat products by natural compounds. CMV notes that ISGlobal is a member of the CERCA Programme, Generalitat de Catalunya.

Conflicts of Interest: The authors declare no conflict of interest.

References

- 1. Davidson, E.A.; David, M.B.; Galloway, J.N.; Goodale, C.L.; Haeuber, R.; Harrison, J.A.; Howarth, R.W.; Jaynes, D.B.; Lowrance, R.R.; Nolan, B.T.; et al. Excess nitrogen in the U.S. environment: Trends, risks, and solutions. In *Issues in Ecology*; Ecological Society of America: Washington, DC, USA, 2012.
- 2. Vitousek, P.M.; Aber, J.D.; Howarth, R.W.; Likens, G.E.; Matson, P.A.; Schindler, D.W.; Schlesinger, W.H.; Tilman, D. Human alteration of the global nitrogen cycle: Sources and consequences. *Ecol. Appl.* **1997**, *7*, 737–750. [\[CrossRef\]](http://dx.doi.org/10.1890/1051-0761(1997)007[0737:HAOTGN]2.0.CO;2)
- 3. Howarth, R.W. Coastal nitrogen pollution: A review of sources and trends globally and regionally. *Harmful Algae* **2008**, *8*, 14–20. [\[CrossRef\]](http://dx.doi.org/10.1016/j.hal.2008.08.015)
- 4. USEPA. Regulated Drinking Water Contaminants: Inorganic Chemicals. Available online: [https://www.](https://www.epa.gov/ground-water-and-drinking-water/table-regulated-drinking-water-contaminants) [epa.gov/ground-water-and-drinking-water/table-regulated-drinking-water-contaminants](https://www.epa.gov/ground-water-and-drinking-water/table-regulated-drinking-water-contaminants) (accessed on 23 September 2017).
- 5. International Agency for Research on Cancer (IARC). *IARC Monographs on the Evaluation of Carcionogenic Risks to Humans: Ingested Nitrate and Nitrite and Cyanobacterial Peptide Toxins*; IARC: Lyon, France, 2010.
- 6. National Research Council (NRC). *The Health Effects of Nitrate, Nitrite, and N-Nitroso Compounds*; NRC: Washington, DC, USA, 1981.
- 7. Mirvish, S.S. Role of N-nitroso compounds (NOC) and N-nitrosation in etiology of gastric, esophageal, nasopharyngeal and bladder cancer and contribution to cancer of known exposures to NOC. *Cancer Lett.* **1995**, *93*, 17–48. [\[CrossRef\]](http://dx.doi.org/10.1016/0304-3835(95)03786-V)
- 8. Ward, M.H.; deKok, T.M.; Levallois, P.; Brender, J.; Gulis, G.; Nolan, B.T.; VanDerslice, J. Workgroup report: Drinking-water nitrate and health-recent findings and research needs. *Environ. Health Perspect.* **2005**, *113*, 1607–1614. [\[CrossRef\]](http://dx.doi.org/10.1289/ehp.8043) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/16263519)
- 9. Maupin, M.A.; Kenny, J.F.; Hutson, S.S.; Lovelace, J.K.; Barber, N.L.; Linsey, K.S. *Estimated Use of Water in the United States in 2010*; US Geological Survey: Reston, VA, USA, 2014; p. 56.
- 10. U.S. Geological Survey. USGS Water Data for the Nation. Available online: <https://waterdata.usgs.gov/nwis> (accessed on 1 January 2018).
- 11. Dubrovsky, N.M.; Burow, K.R.; Clark, G.M.; Gronberg, J.M.; Hamilton, P.A.; Hitt, K.J.; Mueller, D.K.; Munn, M.D.; Nolan, B.T.; Puckett, L.J.; et al. *The Quality of Our Nation's Waters—Nutrients in the Nation's Streams and Groundwater, 1992–2004*; U.S. Geological Survey: Reston, VA, USA, 2010; p. 174.
- 12. Lindsey, B.D.; Rupert, M.G. *Methods for Evaluating Temporal Groundwater Quality Data and Results of Decadal-Scale Changes in Chloride, Dissolved Solids, and Nitrate Concentrations in Groundwater in the United States, 1988–2010*; U.S. Geological Survey Scientific Investigations Report: 2012–5049; U.S. Geological Survey: Reston, VA, USA, 2012; p. 46.
- 13. Pennino, M.J.; Compton, J.E.; Leibowitz, S.G. Trends in Drinking Water Nitrate Violations across the United States. *Environ. Sci. Technol.* **2017**, *51*, 13450–13460. [\[CrossRef\]](http://dx.doi.org/10.1021/acs.est.7b04269) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/29052975)
- 14. Van Grinsven, H.J.M.; Tiktak, A.; Rougoor, C.W. Evaluation of the Dutch implementation of the nitrates directive, the water framework directive and the national emission ceilings directive. *NJAS-Wagening. J. Life Sci.* **2016**, *78*, 69–84. [\[CrossRef\]](http://dx.doi.org/10.1016/j.njas.2016.03.010)
- 15. Vock, D.C. Iowa Farmers Won a Water Pollution Lawsuit, But at What Cost? Available online: [http://www.](http://www.governing.com/topics/transportation-infrastructure/gov-des-moines-water-utility-lawsuit-farmers.html) [governing.com/topics/transportation-infrastructure/gov-des-moines-water-utility-lawsuit-farmers.html](http://www.governing.com/topics/transportation-infrastructure/gov-des-moines-water-utility-lawsuit-farmers.html) (accessed on 10 February 2018).
- 16. Des Moines Water Works. On Earth Day, Des Moines Water Works Reflects on Resources Spent to Manage Agrotoxins in Source Waters. Available online: [http://www.dmww.com/about-us/news-releases/on-earth](http://www.dmww.com/about-us/news-releases/on-earth-day-des-moines-water-works-reflects-on-resources-spent-to-manage-agrotoxins-in-source-water.aspx)[day-des-moines-water-works-reflects-on-resources-spent-to-manage-agrotoxins-in-source-water.aspx](http://www.dmww.com/about-us/news-releases/on-earth-day-des-moines-water-works-reflects-on-resources-spent-to-manage-agrotoxins-in-source-water.aspx) (accessed on 10 February 2018).
- 17. European Commission. The Nitrates Directive. Available online: [http://ec.europa.eu/environment/water/](http://ec.europa.eu/environment/water/water-nitrates/index_en.html) [water-nitrates/index_en.html](http://ec.europa.eu/environment/water/water-nitrates/index_en.html) (accessed on 10 May 2018).
- 18. European Union (EU). *Council Directive 91/676/EEC of 12 December 1991 Concerning the Protection of Waters against Pollution Caused by Nitrates from Agricultural Sources*; European Union (EU): Brussels, Belgium, 1991.
- 19. Hansen, B.; Thorling, L.; Dalgaard, T.; Erlandsen, M. Trend Reversal of Nitrate in Danish Groundwater—A Reflection of Agricultural Practices and Nitrogen Surpluses since 1950. *Environ. Sci. Technol.* **2011**, *45*, 228–234. [\[CrossRef\]](http://dx.doi.org/10.1021/es102334u) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/21138289)
- 20. European Environment Agency (EEA). Groundwater Nitrate. Available online: [https://www.eea.europa.eu/](https://www.eea.europa.eu/data-and-maps/daviz/groundwater-nitrate#tab-chart_1_filters=%7B%22rowFilters%22%3A%7B%7D%3B%22columnFilters%22%3A%7B%22pre_config_country%22%3A%5B%22Slovenia%22%5D%7D%7D) [data-and-maps/daviz/groundwater-nitrate#tab-chart_1_filters=%7B%22rowFilters%22%3A%7B%7D%](https://www.eea.europa.eu/data-and-maps/daviz/groundwater-nitrate#tab-chart_1_filters=%7B%22rowFilters%22%3A%7B%7D%3B%22columnFilters%22%3A%7B%22pre_config_country%22%3A%5B%22Slovenia%22%5D%7D%7D) [3B%22columnFilters%22%3A%7B%22pre_config_country%22%3A%5B%22Slovenia%22%5D%7D%7D](https://www.eea.europa.eu/data-and-maps/daviz/groundwater-nitrate#tab-chart_1_filters=%7B%22rowFilters%22%3A%7B%7D%3B%22columnFilters%22%3A%7B%22pre_config_country%22%3A%5B%22Slovenia%22%5D%7D%7D) (accessed on 10 February 2018).
- 21. Schullehner, J.; Hansen, B. Nitrate exposure from drinking water in Denmark over the last 35 years. *Environ. Res. Lett.* **2014**, *9*, 095001. [\[CrossRef\]](http://dx.doi.org/10.1088/1748-9326/9/9/095001)
- 22. Vitoria, I.; Maraver, F.; Sanchez-Valverde, F.; Armijo, F. Nitrate concentrations in tap water in Spain. *Gac. Sanit.* **2015**, *29*, 217–220. [\[CrossRef\]](http://dx.doi.org/10.1016/j.gaceta.2014.12.007) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/25661464)
- 23. Espejo-Herrera, N.; Kogevinas, M.; Castano-Vinyals, G.; Aragones, N.; Boldo, E.; Ardanaz, E.; Azpiroz, L.; Ulibarrena, E.; Tardon, A.; Molina, A.J.; et al. Nitrate and trace elements in municipal and bottled water in Spain. *Gac. Sanit.* **2013**, *27*, 156–160. [\[CrossRef\]](http://dx.doi.org/10.1016/j.gaceta.2012.02.002) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/22444517)
- 24. Gunnarsdottir, M.J.; Gardarsson, S.M.; Jonsson, G.S.; Bartram, J. Chemical quality and regulatory compliance of drinking water in Iceland. *Int. J. Hyg. Environ. Health* **2016**, *219*, 724–733. [\[CrossRef\]](http://dx.doi.org/10.1016/j.ijheh.2016.09.011) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/27717751)
- 25. D'Alessandro, W.; Bellomo, S.; Parello, F.; Bonfanti, P.; Brusca, L.; Longo, M.; Maugeri, R. Nitrate, sulphate and chloride contents in public drinking water supplies in Sicily, Italy. *Environ. Monit. Assess.* **2012**, *184*, 2845–2855. [\[CrossRef\]](http://dx.doi.org/10.1007/s10661-011-2155-y) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/21717203)
- 26. Migeot, V.; Albouy-Llaty, M.; Carles, C.; Limousi, F.; Strezlec, S.; Dupuis, A.; Rabouan, S. Drinking-water exposure to a mixture of nitrate and low-dose atrazine metabolites and small-for-gestational age (SGA) babies: A historic cohort study. *Environ. Res.* **2013**, *122*, 58–64. [\[CrossRef\]](http://dx.doi.org/10.1016/j.envres.2012.12.007) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/23340115)
- 27. Taneja, P.; Labhasetwar, P.; Nagarnaik, P.; Ensink, J.H.J. The risk of cancer as a result of elevated levels of nitrate in drinking water and vegetables in Central India. *J. Water Health* **2017**, *15*, 602–614. [\[CrossRef\]](http://dx.doi.org/10.2166/wh.2017.283) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/28771157)
- 28. Suthar, S.; Bishnoi, P.; Singh, S.; Mutiyar, P.K.; Nema, A.K.; Patil, N.S. Nitrate contamination in groundwater of some rural areas of Rajasthan, India. *J. Hazard. Mater.* **2009**, *171*, 189–199. [\[CrossRef\]](http://dx.doi.org/10.1016/j.jhazmat.2009.05.111) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/19545944)
- 29. Gupta, I.; Salunkhe, A.; Rohra, N.; Kumar, R. Groundwater quality in Maharashtra, India: Focus on nitrate pollution. *J. Environ. Sci. Eng.* **2011**, *53*, 453–462. [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/23505824)
- 30. Weinthal, E.; Vengosh, A.; Marei, A.; Kloppmann, W. The water crisis in the Gaza strip: Prospects for resolution. *Ground Water* **2005**, *43*, 653–660. [\[CrossRef\]](http://dx.doi.org/10.1111/j.1745-6584.2005.00064.x) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/16149960)
- 31. Jones, R.R.; Weyer, P.J.; DellaValle, C.T.; Inoue-Choi, M.; Anderson, K.E.; Cantor, K.P.; Krasner, S.; Robien, K.; Freeman, L.E.B.; Silverman, D.T.; et al. Nitrate from drinking water and diet and bladder cancer among postmenopausal women in Iowa. *Environ. Health Perspect.* **2016**, *124*, 1751–1758. [\[CrossRef\]](http://dx.doi.org/10.1289/EHP191) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/27258851)
- 32. Schullehner, J.; Hansen, B.; Thygesen, M.; Pedersen, C.B.; Sigsgaard, T. Nitrate in drinking water and colorectal cancer risk: A nationwide population-based cohort study. *Int. J. Cancer* **2018**, *1*, 73–79. [\[CrossRef\]](http://dx.doi.org/10.1002/ijc.31306) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/29435982)
- 33. Espejo-Herrera, N.; Cantor, K.P.; Malats, N.; Silverman, D.T.; Tardon, A.; Garcia-Closas, R.; Serra, C.; Kogevinas, M.; Villanueva, C.M. Nitrate in drinking water and bladder cancer risk in Spain. *Environ. Res.* **2015**, *137*, 299–307. [\[CrossRef\]](http://dx.doi.org/10.1016/j.envres.2014.10.034) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/25601732)
- 34. Ward, M.H.; Rusiecki, J.A.; Lynch, C.F.; Cantor, K.P. Nitrate in public water supplies and the risk of renal cell carcinoma. *Cancer Causes Control* **2007**, *18*, 1141–1151. [\[CrossRef\]](http://dx.doi.org/10.1007/s10552-007-9053-1) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/17717631)
- 35. Ward, M.H.; Cerhan, J.R.; Colt, J.S.; Hartge, P. Risk of non-Hodgkin lymphoma and nitrate and nitrite from drinking water and diet. *Epidemiology* **2006**, *17*, 375–382. [\[CrossRef\]](http://dx.doi.org/10.1097/01.ede.0000219675.79395.9f) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/16699473)
- 36. Schullehner, J.; Stayner, L.; Hansen, B. Nitrate, Nitrite, and Ammonium Variability in Drinking Water Distribution Systems. *Int. J. Environ. Res. Public Health* **2017**, *14*, 276. [\[CrossRef\]](http://dx.doi.org/10.3390/ijerph14030276) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/28282914)
- 37. Ward, M.H.; Kilfoy, B.A.; Weyer, P.J.; Anderson, K.E.; Folsom, A.R.; Cerhan, J.R. Nitrate intake and the risk of thyroid cancer and thyroid disease. *Epidemiology* **2010**, *21*, 389–395. [\[CrossRef\]](http://dx.doi.org/10.1097/EDE.0b013e3181d6201d) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/20335813)
- 38. Brender, J.D.; Weyer, P.J.; Romitti, P.A.; Mohanty, B.P.; Shinde, M.U.; Vuong, A.M.; Sharkey, J.R.; Dwivedi, D.; Horel, S.A.; Kantamneni, J.; et al. Prenatal nitrate intake from drinking water and selected birth defects in offspring of participants in the national birth defects prevention study. *Environ. Health Perspect.* **2013**, *121*, 1083–1089. [\[CrossRef\]](http://dx.doi.org/10.1289/ehp.1206249) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/23771435)
- 39. Baris, D.; Waddell, R.; Beane Freeman, L.E.; Schwenn, M.; Colt, J.S.; Ayotte, J.D.; Ward, M.H.; Nuckols, J.; Schned, A.; Jackson, B.; et al. Elevated Bladder Cancer in Northern New England: The Role of Drinking Water and Arsenic. *J. Natl. Cancer Inst.* **2016**, *108*. [\[CrossRef\]](http://dx.doi.org/10.1093/jnci/djw099) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/27140955)
- 40. Nolan, B.T.; Hitt, K.J. Vulnerability of shallow groundwater and drinking-water wells to nitrate in the United States. *Environ. Sci. Technol.* **2006**, *40*, 7834–7840. [\[CrossRef\]](http://dx.doi.org/10.1021/es060911u) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/17256535)
- 41. Messier, K.P.; Kane, E.; Bolich, R.; Serre, M.L. Nitrate variability in groundwater of North Carolina using monitoring and private well data models. *Environ. Sci. Technol.* **2014**, *48*, 10804–10812. [\[CrossRef\]](http://dx.doi.org/10.1021/es502725f) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/25148521)
- 42. Eckhardt, D.A.V.; Stackelberg, P.E. Relation of ground-water quality to land use on Long Island, New York. *Ground Water* **1995**, *33*, 1019–1033. [\[CrossRef\]](http://dx.doi.org/10.1111/j.1745-6584.1995.tb00047.x)
- 43. Nolan, B.T.; Hitt, K.J.; Ruddy, B.C. Probability of nitrate contamination of recently recharged groundwaters in the conterminous United States. *Environ. Sci. Technol.* **2002**, *36*, 2138–2145. [\[CrossRef\]](http://dx.doi.org/10.1021/es0113854) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/12038822)
- 44. Rupert, M.G. *Probability of Detecting Atrazine/Desethyl-Atrazine and Elevated Concentrations of Nitrate in Ground Water in Colorado*; Water-Resources Investigations Report 02-4269; U.S. Geological Survey: Denver, CO, USA, 2003; p. 35.
- 45. Tesoriero, A.J.; Voss, F.D. Predicting the probability of elevated nitrate concentrations in the Puget Sound Basin: Implications for aquifer susceptibility and vulnerability. *Ground Water* **1997**, *35*, 1029–1039. [\[CrossRef\]](http://dx.doi.org/10.1111/j.1745-6584.1997.tb00175.x)
- 46. Warner, K.L.; Arnold, T.L. *Relations that Affect the Probability and Prediction of Nitrate Concentration in Private Wells in the Glacial Aquifer System in the United States*; U.S. Geological Survey Scientific Investigations Report 2010–5100; U.S. Geological Survey: Reston, VA, USA, 2010; p. 55.
- 47. Elith, J.; Leathwick, J.R.; Hastie, T. A working guide to boosted regression trees. *J. Anim. Ecol.* **2008**, *77*, 802–813. [\[CrossRef\]](http://dx.doi.org/10.1111/j.1365-2656.2008.01390.x) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/18397250)
- 48. Wheeler, D.C.; Nolan, B.T.; Flory, A.R.; DellaValle, C.T.; Ward, M.H. Modeling groundwater nitrate concentrations in private wells in Iowa. *Sci. Total Environ.* **2015**, *536*, 481–488. [\[CrossRef\]](http://dx.doi.org/10.1016/j.scitotenv.2015.07.080) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/26232757)
- 49. Ransom, K.M.; Nolan, B.T.; Traum, J.A.; Faunt, C.C.; Bell, A.M.; Gronberg, J.A.M.; Wheeler, D.C.; Rosecrans, C.Z.; Jurgens, B.; Schwarz, G.E.; et al. A hybrid machine learning model to predict and visualize nitrate concentration throughout the Central Valley aquifer, California, USA. *Sci. Total Environ.* **2017**, *601–602*, 1160–1172. [\[CrossRef\]](http://dx.doi.org/10.1016/j.scitotenv.2017.05.192) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/28599372)
- 50. Leach, S.A.; Thompson, M.; Hill, M. Bacterially catalyzed *N*-nitrosation reactions and their relative importance in the human stomach. *Carcinogenesis* **1987**, *8*, 1907–1912. [\[CrossRef\]](http://dx.doi.org/10.1093/carcin/8.12.1907) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/3119247)
- 51. Lv, J.; Neal, B.; Ehteshami, P.; Ninomiya, T.; Woodward, M.; Rodgers, A.; Wang, H.; MacMahon, S.; Turnbull, F.; Hillis, G.; et al. Effects of intensive blood pressure lowering on cardiovascular and renal outcomes: A systematic review and meta-analysis. *PLoS Med.* **2012**, *9*, e1001293. [\[CrossRef\]](http://dx.doi.org/10.1371/journal.pmed.1001293) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/22927798)
- 52. Spiegelhalder, B.; Eisenbrand, G.; Preussmann, R. Influence of dietary nitrate on nitrite content of human saliva: Possible relevance to in vivo formation of *N*-nitroso compounds. *Food Cosmet. Toxicol.* **1976**, *14*, 545–548. [\[CrossRef\]](http://dx.doi.org/10.1016/S0015-6264(76)80005-3)
- 53. Tricker, A.R.; Kalble, T.; Preussmann, R. Increased urinary nitrosamine excretion in patients with urinary diversions. *Carcinogenesis* **1989**, *10*, 2379–2382. [\[CrossRef\]](http://dx.doi.org/10.1093/carcin/10.12.2379) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/2591028)
- 54. Eisenbrand, G.; Spiegelhalder, B.; Preussmann, R. Nitrate and nitrite in saliva. *Oncology* **1980**, *37*, 227–231. [\[CrossRef\]](http://dx.doi.org/10.1159/000225441) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/7443155)
- 55. Eisenbrand, G. *The Significance of N-Nitrosation of Drugs*; Nicolai, H.V., Eisenbrand, G., Bozler, G., Eds.; Gustav Fischer Verlag, Stuttgart: New York, NY, USA, 1990; pp. 47–69.
- 56. Ceccatelli, S.; Lundberg, J.M.; Fahrenkrug, J.; Bredt, D.S.; Snyder, S.H.; Hokfelt, T. Evidence for involvement of nitric oxide in the regulation of hypothalamic portal blood flow. *Neuroscience* **1992**, *51*, 769–772. [\[CrossRef\]](http://dx.doi.org/10.1016/0306-4522(92)90518-7)
- 57. Moncada, S.; Palmer, R.M.J.; Higgs, E.A. Nitric oxide: Physiology, pathophysiology, and pharmacology. *Pharmacol. Rev.* **1991**, *43*, 109–142. [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/1852778)
- 58. Rees, D.D.; Palmer, R.M.; Moncada, S. Role of endothelium-derived nitric oxide in the regulation of blood pressure. *Proc. Natl. Acad. Sci. USA* **1989**, *86*, 3375–3378. [\[CrossRef\]](http://dx.doi.org/10.1073/pnas.86.9.3375) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/2497467)
- 59. Palmer, R.M.; Ferrige, A.G.; Moncada, S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature* **1987**, *327*, 524–526. [\[CrossRef\]](http://dx.doi.org/10.1038/327524a0) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/3495737)
- 60. Radomski, M.W.; Palmer, R.M.; Moncada, S. Endogenous nitric oxide inhibits human platelet adhesion to vascular endothelium. *Lancet* **1987**, *2*, 1057–1058. [\[CrossRef\]](http://dx.doi.org/10.1016/S0140-6736(87)91481-4)
- 61. Radomski, M.W.; Palmer, R.M.J.; Moncada, S. The Anti-Aggregating Properties of Vascular Endothelium—Interactions between Prostacyclin and Nitric-Oxide. *Br. J. Pharmacol.* **1987**, *92*, 639–646. [\[CrossRef\]](http://dx.doi.org/10.1111/j.1476-5381.1987.tb11367.x) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/3322462)
- 62. Larsen, F.J.; Schiffer, T.A.; Weitzberg, E.; Lundberg, J.O. Regulation of mitochondrial function and energetics by reactive nitrogen oxides. *Free Radic. Biol. Med.* **2012**, *53*, 1919–1928. [\[CrossRef\]](http://dx.doi.org/10.1016/j.freeradbiomed.2012.08.580) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/22989554)
- 63. Ceccatelli, S.; Hulting, A.L.; Zhang, X.; Gustafsson, L.; Villar, M.; Hokfelt, T. Nitric oxide synthase in the rat anterior pituitary gland and the role of nitric oxide in regulation of luteinizing hormone secretion. *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 11292–11296. [\[CrossRef\]](http://dx.doi.org/10.1073/pnas.90.23.11292) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/7504302)
- 64. Green, S.J.; Scheller, L.F.; Marletta, M.A.; Seguin, M.C.; Klotz, F.W.; Slayter, M.; Nelson, B.J.; Nacy, C.A. Nitric oxide: Cytokine-regulation of nitric oxide in host resistance to intracellular pathogens. *Immunol. Lett.* **1994**, *43*, 87–94. [\[CrossRef\]](http://dx.doi.org/10.1016/0165-2478(94)00158-8)
- 65. Langrehr, J.M.; Hoffman, R.A.; Lancaster, J.R.; Simmons, R.L. Nitric oxide—A new endogenous immunomodulator. *Transplantation* **1993**, *55*, 1205–1212. [\[CrossRef\]](http://dx.doi.org/10.1097/00007890-199306000-00001) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/8516803)
- 66. Wei, X.Q.; Charles, I.G.; Smith, A.; Ure, J.; Feng, G.J.; Huang, F.P.; Xu, D.; Muller, W.; Moncada, S.; Liew, F.Y. Altered immune responses in mice lacking inducible nitric oxide synthase. *Nature* **1995**, *375*, 408–411. [\[CrossRef\]](http://dx.doi.org/10.1038/375408a0) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/7539113)
- 67. D'Ischia, M.; Napolitano, A.; Manini, P.; Panzella, L. Secondary Targets of Nitrite-Derived Reactive Nitrogen Species: Nitrosation/Nitration Pathways, Antioxidant Defense Mechanisms and Toxicological Implications. *Chem. Res. Toxicol.* **2011**, *24*, 2071–2092. [\[CrossRef\]](http://dx.doi.org/10.1021/tx2003118) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/21923154)
- 68. Mirvish, S.S. Formation of N-nitroso compounds: Chemistry, kinetics, and in vivo occurrence. *Toxicol. Appl. Pharmacol.* **1975**, *31*, 325–351. [\[CrossRef\]](http://dx.doi.org/10.1016/0041-008X(75)90255-0)
- 69. Ridd, J.H. Nitrosation, diazotisation, and deamination. *Q. Rev.* **1961**, *15*, 418–441. [\[CrossRef\]](http://dx.doi.org/10.1039/qr9611500418)
- 70. Akuta, T.; Zaki, M.H.; Yoshitake, J.; Okamoto, T.; Akaike, T. Nitrative stress through formation of 8-nitroguanosine: Insights into microbial pathogenesis. *Nitric Oxide* **2006**, *14*, 101–108. [\[CrossRef\]](http://dx.doi.org/10.1016/j.niox.2005.10.004) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/16309933)
- 71. Loeppky, R.N.; Bao, Y.T.; Bae, J.Y.; Yu, L.; Shevlin, G. Blocking nitrosamine formation—Understanding the chemistry of rapid nitrosation. In *Nitrosamines and Related N-Nitroso Compounds: Chemistry and Biochemistry*; Loeppky, R.N., Michejda, C.J., Eds.; American Chemical Society: Washington, DC, USA, 1994; Volume 553, pp. 52–65.
- 72. Qin, L.Z.; Liu, X.B.; Sun, Q.F.; Fan, Z.P.; Xia, D.S.; Ding, G.; Ong, H.L.; Adams, D.; Gahl, W.A.; Zheng, C.Y.; et al. Sialin (SLC17A5) functions as a nitrate transporter in the plasma membrane. *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 13434–13439. [\[CrossRef\]](http://dx.doi.org/10.1073/pnas.1116633109) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/22778404)
- 73. Stich, H.F.; Dunn, B.P.; Pignatelli, B.; Ohshima, H.; Bartsch, H. *Dietary Phenolics and Betel Nut Extracts as Modifiers of n Nitrosation in Rat and Man*; IARC Scientific Publications: Lyon, France, 1984; pp. 213–222.
- 74. Vermeer, I.T.; Moonen, E.J.; Dallinga, J.W.; Kleinjans, J.C.; van Maanen, J.M. Effect of ascorbic acid and green tea on endogenous formation of N-nitrosodimethylamine and *N*-nitrosopiperidine in humans. *Mutat. Res.* **1999**, *428*, 353–361. [\[CrossRef\]](http://dx.doi.org/10.1016/S1383-5742(99)00061-7)
- 75. Vermeer, I.T.; Pachen, D.M.; Dallinga, J.W.; Kleinjans, J.C.; van Maanen, J.M. Volatile N-nitrosamine formation after intake of nitrate at the ADI level in combination with an amine-rich diet. *Environ. Health Perspect.* **1998**, *106*, 459–463. [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/9681972)
- 76. Chung, M.J.; Lee, S.H.; Sung, N.J. Inhibitory effect of whole strawberries, garlic juice or kale juice on endogenous formation of *N*-nitrosodimethylamine in humans. *Cancer Lett.* **2002**, *182*, 1–10. [\[CrossRef\]](http://dx.doi.org/10.1016/S0304-3835(02)00076-9)
- 77. Helser, M.A.; Hotchkiss, J.H.; Roe, D.A. Influence of fruit and vegetable juices on the endogenous formation of N-nitrosoproline and *N*-nitrosothiazolidine-4-carboxylic acid in humans on controlled diets. *Carcinogenesis* **1992**, *13*, 2277–2280. [\[CrossRef\]](http://dx.doi.org/10.1093/carcin/13.12.2277) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/1473234)
- 78. Zeilmaker, M.J.; Bakker, M.I.; Schothorst, R.; Slob, W. Risk assessment of *N*-nitrosodimethylamine formed endogenously after fish-with-vegetable meals. *Toxicol. Sci.* **2010**, *116*, 323–335. [\[CrossRef\]](http://dx.doi.org/10.1093/toxsci/kfq093) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/20351056)
- 79. Khandelwal, N.; Abraham, S.K. Intake of anthocyanidins pelargonidin and cyanidin reduces genotoxic stress in mice induced by diepoxybutane, urethane and endogenous nitrosation. *Environ. Toxicol. Pharmacol.* **2014**, *37*, 837–843. [\[CrossRef\]](http://dx.doi.org/10.1016/j.etap.2014.02.012) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/24642102)
- 80. Conforti, F.; Menichini, F. Phenolic Compounds from Plants as Nitric Oxide Production Inhibitors. *Curr. Med. Chem.* **2011**, *18*, 1137–1145. [\[CrossRef\]](http://dx.doi.org/10.2174/092986711795029690) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/21291370)
- 81. Abraham, S.K.; Khandelwal, N. Ascorbic acid and dietary polyphenol combinations protect against genotoxic damage induced in mice by endogenous nitrosation. *Mutat. Res.* **2013**, *757*, 167–172. [\[CrossRef\]](http://dx.doi.org/10.1016/j.mrgentox.2013.08.004) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/23973768)
- 82. De Kok, T.M.; (Maastricht, The Netherlands). Unpublished work. 2018.
- 83. Haorah, J.; Zhou, L.; Wang, X.J.; Xu, G.P.; Mirvish, S.S. Determination of total *N*-nitroso compounds and their precursors in frankfurters, fresh meat, dried salted fish, sauces, tobacco, and tobacco smoke particulates. *J. Agric. Food Chem.* **2001**, *49*, 6068–6078. [\[CrossRef\]](http://dx.doi.org/10.1021/jf010602h) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/11743810)
- 84. Cross, A.J.; Pollock, J.R.; Bingham, S.A. Haem, not protein or inorganic iron, is responsible for endogenous intestinal N-nitrosation arising from red meat. *Cancer Res.* **2003**, *63*, 2358–2360. [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/12750250)
- 85. Bingham, S.A.; Pignatelli, B.; Pollock, J.R.A.; Ellul, A.; Malaveille, C.; Gross, G.; Runswick, S.; Cummings, J.H.; O'Neill, I.K. Does increased endogenous formation of *N*-nitroso compounds in the human colon explain the association between red meat and colon cancer? *Carcinogenesis* **1996**, *17*, 515–523. [\[CrossRef\]](http://dx.doi.org/10.1093/carcin/17.3.515) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/8631138)
- 86. Bingham, S.A.; Hughes, R.; Cross, A.J. Effect of white versus red meat on endogenous *N*-nitrosation in the human colon and further evidence of a dose response. *J. Nutr.* **2002**, *132*, 3522s–3525s. [\[CrossRef\]](http://dx.doi.org/10.1093/jn/132.11.3522S) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/12421881)
- 87. Bingham, S.A. High-meat diets and cancer risk. *Proc. Nutr. Soc.* **1999**, *58*, 243–248. [\[CrossRef\]](http://dx.doi.org/10.1017/S0029665199000336) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/10466162)
- 88. Bouvard, V.; Loomis, D.; Guyton, K.Z.; Grosse, Y.; Ghissassi, F.E.; Benbrahim-Tallaa, L.; Guha, N.; Mattock, H.; Straif, K. International Agency for Research on Cancer Monograph Working, G. Carcinogenicity of consumption of red and processed meat. *Lancet Oncol.* **2015**, *16*, 1599–1600. [\[CrossRef\]](http://dx.doi.org/10.1016/S1470-2045(15)00444-1)
- 89. International Agency for Research on Cancer (IARC). *IARC Monographs on the Evaluation of Carcionogenic Risks to Humans: Red Meat and Processed Meat*; IARC: Lyon, France, 2018.
- 90. Phytochemicals to Reduce Nitrite in Meat Products (PHYTOME). Available online: <www.phytome.eu> (accessed on 3 May 2018).
- 91. Greer, F.R.; Shannon, M. American Academy of Pediatrics Committee on Nutrition and the Committee on Environmental Health. Infant methemoglobinemia: The role of dietary nitrate in food and water. *Pediatrics* **2005**, *116*, 784–786. [\[CrossRef\]](http://dx.doi.org/10.1542/peds.2005-1497) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/16140723)
- 92. Sanchez-Echaniz, J.; Benito-Fernandez, J.; Mintegui-Raso, S. Methemoglobinemia and consumption of vegetables in infants. *Pediatrics* **2001**, *107*, 1024–1028. [\[CrossRef\]](http://dx.doi.org/10.1542/peds.107.5.1024) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/11331681)
- 93. Charmandari, E.; Meadows, N.; Patel, M.; Johnston, A.; Benjamin, N. Plasma nitrate concentrations in children with infectious and noninfectious diarrhea. *J. Pediatr. Gastroenterol. Nutr.* **2001**, *32*, 423–427. [\[CrossRef\]](http://dx.doi.org/10.1097/00005176-200104000-00006) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/11396807)
- 94. Comly, H.H. Landmark article 8 September 1945: Cyanosis in infants caused by nitrates in well-water. By Hunter H. Comly. *JAMA* **1987**, *257*, 2788–2792. [\[CrossRef\]](http://dx.doi.org/10.1001/jama.1987.03390200128027) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/3553637)
- 95. Walton, G. Survey of literature relating to infant methemoglobinemia due to nitrate-contaminated water. *Am. J. Public Health Nation's Health* **1951**, *41*, 986–996. [\[CrossRef\]](http://dx.doi.org/10.2105/AJPH.41.8_Pt_1.986)
- 96. Knobeloch, L.; Salna, B.; Hogan, A.; Postle, J.; Anderson, H. Blue babies and nitrate-contaminated well water. *Environ. Health Perspect.* **2000**, *108*, 675–678. [\[CrossRef\]](http://dx.doi.org/10.1289/ehp.00108675) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/10903623)
- 97. Johnson, C.J.; Bonrud, P.A.; Dosch, T.L.; Kilness, A.W.; Senger, K.A.; Busch, D.C.; Meyer, M.R. Fatal outcome of methemoglobinemia in an infant. *JAMA* **1987**, *257*, 2796–2797. [\[CrossRef\]](http://dx.doi.org/10.1001/jama.1987.03390200136029) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/3573274)
- 98. Lutynski, R.; Steczek-Wojdyla, M.; Wojdyla, Z.; Kroch, S. The concentrations of nitrates and nitrites in food products and environment and the occurrence of acute toxic methemoglobinemias. *Prz. Lek.* **1996**, *53*, 351–355. [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/8711190)
- 99. Ayebo, A.; Kross, B.C.; Vlad, M.; Sinca, A. Infant Methemoglobinemia in the Transylvania Region of Romania. *Int. J. Occup. Environ. Health* **1997**, *3*, 20–29. [\[CrossRef\]](http://dx.doi.org/10.1179/oeh.1997.3.1.20) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/9891097)
- 100. World Health Organization. *Water and Health in Europe*; World Health Organization: Geneva, Switzerland, 2002.
- 101. Abu Naser, A.A.; Ghbn, N.; Khoudary, R. Relation of nitrate contamination of groundwater with methaemoglobin level among infants in Gaza. *East Mediterr. Health J.* **2007**, *13*, 994–1004. [\[CrossRef\]](http://dx.doi.org/10.26719/2007.13.5.994) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/18290391)
- 102. Sadeq, M.; Moe, C.L.; Attarassi, B.; Cherkaoui, I.; ElAouad, R.; Idrissi, L. Drinking water nitrate and prevalence of methemoglobinemia among infants and children aged 1–7 years in Moroccan areas. *Int. J. Hyg. Environ. Health* **2008**, *211*, 546–554. [\[CrossRef\]](http://dx.doi.org/10.1016/j.ijheh.2007.09.009) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/18155958)
- 103. Zeman, C.; Beltz, L.; Linda, M.; Maddux, J.; Depken, D.; Orr, J.; Theran, P. New Questions and Insights into Nitrate/Nitrite and Human Health Effects: A Retrospective Cohort Study of Private Well Users' Immunological and Wellness Status. *J. Environ. Health* **2011**, *74*, 8–18. [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/22187853)
- 104. Manassaram, D.M.; Backer, L.C.; Messing, R.; Fleming, L.E.; Luke, B.; Monteilh, C.P. Nitrates in drinking water and methemoglobin levels in pregnancy: A longitudinal study. *Environ. Health* **2010**, *9*, 60. [\[CrossRef\]](http://dx.doi.org/10.1186/1476-069X-9-60) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/20946657)
- 105. Grant, W.; Steele, G.; Isiorho, S.A. Spontaneous abortions possibly related to ingestion of nitrate-contaminated well water: LaGrange County, Indiana, 1991–1994. *Morb. Mortal. Wkly. Rep.* **1996**, *45*, 569–572.
- 106. Aschengrau, A.; Zierler, S.; Cohen, A. Quality of community drinking water and the occurrence of spontaneous abortion. *Arch Environ. Health* **1989**, *44*, 283–290. [\[CrossRef\]](http://dx.doi.org/10.1080/00039896.1989.9935895) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/2554824)
- 107. Albouy-Llaty, M.; Limousi, F.; Carles, C.; Dupuis, A.; Rabouan, S.; Migeot, V. Association between Exposure to Endocrine Disruptors in Drinking Water and Preterm Birth, Taking Neighborhood Deprivation into Account: A Historic Cohort Study. *Int. J. Environ. Res. Public Health* **2016**, *13*, 796. [\[CrossRef\]](http://dx.doi.org/10.3390/ijerph13080796) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/27517943)
- 108. Stayner, L.T.; Almberg, K.; Jones, R.; Graber, J.; Pedersen, M.; Turyk, M. Atrazine and nitrate in drinking water and the risk of preterm delivery and low birth weight in four Midwestern states. *Environ. Res.* **2017**, *152*, 294–303. [\[CrossRef\]](http://dx.doi.org/10.1016/j.envres.2016.10.022) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/27816866)
- 109. Joyce, S.J.; Cook, A.; Newnham, J.; Brenters, M.; Ferguson, C.; Weinstein, P. Water disinfection by-products and prelabor rupture of membranes. *Am. J. Epidemiol.* **2008**, *168*, 514–521. [\[CrossRef\]](http://dx.doi.org/10.1093/aje/kwn188) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/18635574)
- 110. Mattix, K.D.; Winchester, P.D.; Scherer, L.R. Incidence of abdominal wait defects is related to surface water atrazine and nitrate levels. *J. Pediatr. Surg.* **2007**, *42*, 947–949. [\[CrossRef\]](http://dx.doi.org/10.1016/j.jpedsurg.2007.01.027) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/17560200)
- 111. Waller, S.A.; Paul, K.; Peterson, S.E.; Hitti, J.E. Agricultural-related chemical exposures, season of conception, and risk of gastroschisis in Washington State. *Am. J. Obstet. Gynecol.* **2010**, *202*, e241–e246. [\[CrossRef\]](http://dx.doi.org/10.1016/j.ajog.2010.01.023) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/20207240)
- 112. Winchester, P.D.; Huskins, J.; Ying, J. Agrichemicals in surface water and birth defects in the United States. *Acta Paediatr.* **2009**, *98*, 664–669. [\[CrossRef\]](http://dx.doi.org/10.1111/j.1651-2227.2008.01207.x) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/19183116)
- 113. Holtby, C.E.; Guernsey, J.R.; Allen, A.C.; VanLeeuwen, J.A.; Allen, V.M.; Gordon, R.J. A Population-Based Case-Control Study of Drinking-Water Nitrate and Congenital Anomalies Using Geographic Information Systems (GIS) to Develop Individual-Level Exposure Estimates. *Int. J. Environ. Res. Public Health* **2014**, *11*, 1803–1823. [\[CrossRef\]](http://dx.doi.org/10.3390/ijerph110201803) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/24503976)
- 114. Weyer, P.J.; Brender, J.D.; Romitti, P.A.; Kantamneni, J.R.; Crawford, D.; Sharkey, J.R.; Shinde, M.; Horel, S.A.; Vuong, A.M.; Langlois, P.H. Assessing bottled water nitrate concentrations to evaluate total drinking water nitrate exposure and risk of birth defects. *J. Water Health* **2014**, *12*, 755–762. [\[CrossRef\]](http://dx.doi.org/10.2166/wh.2014.237) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/25473985)
- 115. Yoon, P.W.; Rasmussen, S.A.; Lynberg, M.C.; Moore, C.A.; Anderka, M.; Carmichael, S.L.; Costa, P.; Druschel, C.; Hobbs, C.A.; Romitti, P.A.; et al. The National Birth Defects Prevention Study. *Public Health Rep.* **2001**, *116* (Suppl. 1), 32–40. [\[CrossRef\]](http://dx.doi.org/10.1093/phr/116.S1.32) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/11889273)
- 116. Brender, J.D.; Kelley, K.E.; Werler, M.M.; Langlois, P.H.; Suarez, L.; Canfield, M.A. National Birth Defects Prevention Study. Prevalence and Patterns of Nitrosatable Drug Use among U.S. Women during Early Pregnancy. *Birth Defects Res. A* **2011**, *91*, 258–264. [\[CrossRef\]](http://dx.doi.org/10.1002/bdra.20808) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/21472845)
- 117. Griesenbeck, J.S.; Brender, J.D.; Sharkey, J.R.; Steck, M.D.; Huber, J.C., Jr.; Rene, A.A.; McDonald, T.J.; Romitti, P.A.; Canfield, M.A.; Langlois, P.H.; et al. Maternal characteristics associated with the dietary intake of nitrates, nitrites, and nitrosamines in women of child-bearing age: A cross-sectional study. *Environ. Health* **2010**, *9*, 10. [\[CrossRef\]](http://dx.doi.org/10.1186/1476-069X-9-10) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/20170520)
- 118. Brender, J.D.; Werler, M.M.; Shinde, M.U.; Vuong, A.M.; Kelley, K.E.; Huber, J.C., Jr.; Sharkey, J.R.; Griesenbeck, J.S.; Romitti, P.A.; Malik, S.; et al. Nitrosatable drug exposure during the first trimester of pregnancy and selected congenital malformations. *Birth Defects Res. A* **2012**, *94*, 701–713. [\[CrossRef\]](http://dx.doi.org/10.1002/bdra.23060) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/22903972)
- 119. Brender, J.D.; Werler, M.M.; Kelley, K.E.; Vuong, A.M.; Shinde, M.U.; Zheng, Q.; Huber, J.C., Jr.; Sharkey, J.R.; Griesenbeck, J.S.; Romitti, P.A.; et al. Nitrosatable drug exposure during early pregnancy and neural tube defects in offspring: National Birth Defects Prevention Study. *Am. J. Epidemiol.* **2011**, *174*, 1286–1295. [\[CrossRef\]](http://dx.doi.org/10.1093/aje/kwr254) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/22047825)
- 120. Brender, J.D.; Olive, J.M.; Felkner, M.; Suarez, L.; Marckwardt, W.; Hendricks, K.A. Dietary nitrites and nitrates, nitrosatable drugs, and neural tube defects. *Epidemiology* **2004**, *15*, 330–336. [\[CrossRef\]](http://dx.doi.org/10.1097/01.ede.0000121381.79831.7b) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/15097014)
- 121. Dorsch, M.M.; Scragg, R.K.; McMichael, A.J.; Baghurst, P.A.; Dyer, K.F. Congenital malformations and maternal drinking water supply in rural South Australia: A case-control study. *Am. J. Epidemiol.* **1984**, *119*, 473–486. [\[CrossRef\]](http://dx.doi.org/10.1093/oxfordjournals.aje.a113764) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/6711537)
- 122. Croen, L.A.; Todoroff, K.; Shaw, G.M. Maternal exposure to nitrate from drinking water and diet and risk for neural tube defects. *Am. J. Epidemiol.* **2001**, *153*, 325–331. [\[CrossRef\]](http://dx.doi.org/10.1093/aje/153.4.325) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/11207149)
- 123. Arbuckle, T.E.; Sherman, G.J.; Corey, P.N.; Walters, D.; Lo, B. Water nitrates and CNS birth defects: A population-based case-control study. *Arch Environ. Health* **1988**, *43*, 162–167. [\[CrossRef\]](http://dx.doi.org/10.1080/00039896.1988.9935846) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/3377550)
- 124. Ericson, A.; Kallen, B.; Lofkvist, E. Environmental factors in the etiology of neural tube defects: A negative study. *Environ. Res.* **1988**, *45*, 38–47. [\[CrossRef\]](http://dx.doi.org/10.1016/S0013-9351(88)80005-7)
- 125. Cantor, K.P. Drinking water and cancer. *Cancer Causes Control* **1997**, *8*, 292–308. [\[CrossRef\]](http://dx.doi.org/10.1023/A:1018444902486) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/9498894)
- 126. Quist, A.J.L.; Inoue-Choi, M.; Weyer, P.J.; Anderson, K.E.; Cantor, K.P.; Krasner, S.; Freeman, L.E.B.; Ward, M.H.; Jones, R.R. Ingested nitrate and nitrite, disinfection by-products, and pancreatic cancer risk in postmenopausal women. *Int. J. Cancer* **2018**, *142*, 251–261. [\[CrossRef\]](http://dx.doi.org/10.1002/ijc.31055) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/28921575)
- 127. Jones, R.R.; Weyer, P.J.; DellaValle, C.T.; Robien, K.; Cantor, K.P.; Krasner, S.; Freeman, L.E.B.; Ward, M.H. Ingested nitrate, disinfection by-products, and kidney cancer risk in older women. *Epidemiology* **2017**, *28*, 703–711. [\[CrossRef\]](http://dx.doi.org/10.1097/EDE.0000000000000647) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/28252454)
- 128. Inoue-Choi, M.; Ward, M.H.; Cerhan, J.R.; Weyer, P.J.; Anderson, K.E.; Robien, K. Interaction of nitrate and folate on the risk of breast cancer among postmenopausal women. *Nutr. Cancer* **2012**, *64*, 685–694. [\[CrossRef\]](http://dx.doi.org/10.1080/01635581.2012.687427) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/22642949)
- 129. Inoue-Choi, M.; Jones, R.R.; Anderson, K.E.; Cantor, K.P.; Cerhan, J.R.; Krasner, S.; Robien, K.; Weyer, P.J.; Ward, M.H. Nitrate and nitrite ingestion and risk of ovarian cancer among postmenopausal women in Iowa. *Int. J. Cancer* **2015**, *137*, 173–182. [\[CrossRef\]](http://dx.doi.org/10.1002/ijc.29365) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/25430487)
- 130. Weyer, P.J.; Cerhan, J.R.; Kross, B.C.; Hallberg, G.R.; Kantamneni, J.; Breuer, G.; Jones, M.P.; Zheng, W.; Lynch, C.F. Municipal drinking water nitrate level and cancer risk in older women: The Iowa Women's Health Study. *Epidemiology* **2001**, *12*, 327–338. [\[CrossRef\]](http://dx.doi.org/10.1097/00001648-200105000-00013) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/11338313)
- 131. Zeegers, M.P.; Selen, R.F.; Kleinjans, J.C.; Goldbohm, R.A.; van den Brandt, P.A. Nitrate intake does not influence bladder cancer risk: The Netherlands cohort study. *Environ. Health Perspect.* **2006**, *114*, 1527–1531. [\[CrossRef\]](http://dx.doi.org/10.1289/ehp.9098) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/17035137)
- 132. Ward, M.H.; Mark, S.D.; Cantor, K.P.; Weisenburger, D.D.; Correa-Villasenor, A.; Zahm, S.H. Drinking water nitrate and the risk of non-Hodgkin's lymphoma. *Epidemiology* **1996**, *7*, 465–471. [\[CrossRef\]](http://dx.doi.org/10.1097/00001648-199609000-00003) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/8862975)
- 133. Ward, M.H.; Heineman, E.F.; Markin, R.S.; Weisenburger, D.D. Adenocarcinoma of the stomach and esophagus and drinking water and dietary sources of nitrate and nitrite. *Int. J. Occup. Environ. Health* **2008**, *14*, 193–197. [\[CrossRef\]](http://dx.doi.org/10.1179/oeh.2008.14.3.193) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/18686719)
- 134. McElroy, J.A.; Trentham-Dietz, A.; Gangnon, R.E.; Hampton, J.M.; Bersch, A.J.; Kanarek, M.S.; Newcomb, P.A. Nitrogen-nitrate exposure from drinking water and colorectal cancer risk for rural women in Wisconsin, USA. *J. Water Health* **2008**, *6*, 399–409. [\[CrossRef\]](http://dx.doi.org/10.2166/wh.2008.048) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/19108561)
- 135. Espejo-Herrera, N.; Gracia-Lavedan, E.; Boldo, E.; Aragones, N.; Perez-Gomez, B.; Pollan, M.; Molina, A.J.; Fernandez, T.; Martin, V.; La Vecchia, C.; et al. Colorectal cancer risk and nitrate exposure through drinking water and diet. *Int. J. Cancer* **2016**, *139*, 334–346. [\[CrossRef\]](http://dx.doi.org/10.1002/ijc.30083) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/26954527)
- 136. Fathmawati; Fachiroh, J.; Gravitiani, E.; Sarto; Husodo, A.H. Nitrate in drinking water and risk of colorectal cancer in Yogyakarta, Indonesia. *J. Toxicol. Environ. Health Part A* **2017**, *80*, 120–128. [\[CrossRef\]](http://dx.doi.org/10.1080/15287394.2016.1260508) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/28095125)
- 137. Brody, J.G.; Aschengrau, A.; McKelvey, W.; Swartz, C.H.; Kennedy, T.; Rudel, R.A. Breast cancer risk and drinking water contaminated by wastewater: A case control study. *Environ. Health-Glob.* **2006**, *5*, 28. [\[CrossRef\]](http://dx.doi.org/10.1186/1476-069X-5-28) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/17026759)
- 138. Espejo-Herrera, N.; Gracia-Lavedan, E.; Pollan, M.; Aragones, N.; Boldo, E.; Perez-Gomez, B.; Altzibar, J.M.; Amiano, P.; Zabala, A.J.; Ardanaz, E.; et al. Ingested Nitrate and Breast Cancer in the Spanish Multicase-Control Study on Cancer (MCC-Spain). *Environ. Health Perspect.* **2016**, *124*, 1042–1049. [\[CrossRef\]](http://dx.doi.org/10.1289/ehp.1510334) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/26942716)
- 139. Mueller, B.A.; Nielsen, S.S.; Preston-Martin, S.; Holly, E.A.; Cordier, S.; Filippini, G.; Peris-Bonet, R.; Choi, N.W. Household water source and the risk of childhood brain tumours: Results of the SEARCH International Brain Tumor Study. *Int. J. Epidemiol.* **2004**, *33*, 1209–1216. [\[CrossRef\]](http://dx.doi.org/10.1093/ije/dyh215) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/15567873)
- 140. Mueller, B.A.; Newton, K.; Holly, E.A.; Preston-Martin, S. Residential water source and the risk of childhood brain tumors. *Environ. Health Perspect.* **2001**, *109*, 551–556. [\[CrossRef\]](http://dx.doi.org/10.1289/ehp.01109551) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/11445506)
- 141. De Groef, B.; Decallonne, B.R.; Van der Geyten, S.; Darras, V.M.; Bouillon, R. Perchlorate versus other environmental sodium/iodide symporter inhibitors: Potential thyroid-related health effects. *Eur. J. Endocrinol.* **2006**, *155*, 17–25. [\[CrossRef\]](http://dx.doi.org/10.1530/eje.1.02190) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/16793945)
- 142. Van Maanen, J.M.; Welle, I.J.; Hageman, G.; Dallinga, J.W.; Mertens, P.L.; Kleinjans, J.C. Nitrate contamination of drinking water: Relationship with HPRT variant frequency in lymphocyte DNA and urinary excretion of N-nitrosamines. *Environ. Health Perspect.* **1996**, *104*, 522–528. [\[CrossRef\]](http://dx.doi.org/10.1289/ehp.96104522) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/8743440)
- 143. Radikova, Z.; Tajtakova, M.; Kocan, A.; Trnovec, T.; Sebokova, E.; Klimes, I.; Langer, P. Possible effects of environmental nitrates and toxic organochlorines on human thyroid in highly polluted areas in Slovakia. *Thyroid Off. J. Am. Thyroid Assoc.* **2008**, *18*, 353–362. [\[CrossRef\]](http://dx.doi.org/10.1089/thy.2007.0182) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/18298316)
- 144. Tajtakova, M.; Semanova, Z.; Tomkova, Z.; Szokeova, E.; Majoros, J.; Radikova, Z.; Sebokova, E.; Klimes, I.; Langer, P. Increased thyroid volume and frequency of thyroid disorders signs in schoolchildren from nitrate polluted area. *Chemosphere* **2006**, *62*, 559–564. [\[CrossRef\]](http://dx.doi.org/10.1016/j.chemosphere.2005.06.030) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/16095667)
- 145. Aschebrook-Kilfoy, B.; Heltshe, S.L.; Nuckols, J.R.; Sabra, M.M.; Shuldiner, A.R.; Mitchell, B.D.; Airola, M.; Holford, T.R.; Zhang, Y.; Ward, M.H. Modeled nitrate levels in well water supplies and prevalence of abnormal thyroid conditions among the Old Order Amish in Pennsylvania. *Environ. Health* **2012**, *11*, 6. [\[CrossRef\]](http://dx.doi.org/10.1186/1476-069X-11-6) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/22339761)
- 146. Longnecker, M.P.; Daniels, J.L. Environmental contaminants as etiologic factors for diabetes. *Environ. Health Perspect.* **2001**, *109* (Suppl. 6), 871–876. [\[CrossRef\]](http://dx.doi.org/10.1289/ehp.01109s6871) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/11744505)
- 147. Moltchanova, E.; Rytkonen, M.; Kousa, A.; Taskinen, O.; Tuomilehto, J.; Karvonen, M.; Spat Study, G. Finnish Childhood Diabetes Registry, G. Zinc and nitrate in the ground water and the incidence of Type 1 diabetes in Finland. *Diabet. Med.* **2004**, *21*, 256–261. [\[CrossRef\]](http://dx.doi.org/10.1111/j.1464-5491.2004.01125.x) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/15008836)
- 148. Muntoni, S.; Cocco, P.; Muntoni, S.; Aru, G. Nitrate in community water supplies and risk of childhood type 1 diabetes in Sardinia, Italy. *Eur. J. Epidemiol.* **2006**, *21*, 245–247. [\[CrossRef\]](http://dx.doi.org/10.1007/s10654-006-0014-x) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/16547840)
- 149. Benson, V.S.; Vanleeuwen, J.A.; Taylor, J.; Somers, G.S.; McKinney, P.A.; Van Til, L. Type 1 diabetes mellitus and components in drinking water and diet: A population-based, case-control study in Prince Edward Island, Canada. *J. Am. Coll. Nutr.* **2010**, *29*, 612–624. [\[CrossRef\]](http://dx.doi.org/10.1080/07315724.2010.10719900) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/21677125)
- 150. Winkler, C.; Mollenhauer, U.; Hummel, S.; Bonifacio, E.; Ziegler, A.G. Exposure to environmental factors in drinking water: Risk of islet autoimmunity and type 1 diabetes—The BABYDIAB study. *Horm. Metab. Res.* **2008**, *40*, 566–571. [\[CrossRef\]](http://dx.doi.org/10.1055/s-2008-1073165) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/18500677)
- 151. Klein, B.E.K.; McElroy, J.A.; Klein, R.; Howard, K.P.; Lee, K.E. Nitrate-nitrogen levels in rural drinking water: Is there an association with age-related macular degeneration? *J. Environ. Sci. Health Part A* **2013**, *48*, 1757–1763. [\[CrossRef\]](http://dx.doi.org/10.1080/10934529.2013.823323) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/24007430)
- 152. Ahluwalia, A.; Gladwin, M.; Coleman, G.D.; Hord, N.; Howard, G.; Kim-Shapiro, D.B.; Lajous, M.; Larsen, F.J.; Lefer, D.J.; McClure, L.A.; et al. Dietary Nitrate and the Epidemiology of Cardiovascular Disease: Report From a National Heart, Lung, and Blood Institute Workshop. *J. Am. Heart Assoc.* **2016**, *5*, e003402. [\[CrossRef\]](http://dx.doi.org/10.1161/JAHA.116.003402) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/27385425)
- 153. Kapil, V.; Khambata, R.S.; Robertson, A.; Caulfield, M.J.; Ahluwalia, A. Dietary nitrate provides sustained blood pressure lowering in hypertensive patients: A randomized, phase 2, double-blind, placebo-controlled study. *Hypertension* **2015**, *65*, 320–327. [\[CrossRef\]](http://dx.doi.org/10.1161/HYPERTENSIONAHA.114.04675) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/25421976)
- 154. Omar, S.A.; Webb, A.J.; Lundberg, J.O.; Weitzberg, E. Therapeutic effects of inorganic nitrate and nitrite in cardiovascular and metabolic diseases. *J. Intern. Med.* **2016**, *279*, 315–336. [\[CrossRef\]](http://dx.doi.org/10.1111/joim.12441) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/26522443)
- 155. Presley, T.D.; Morgan, A.R.; Bechtold, E.; Clodfelter, W.; Dove, R.W.; Jennings, J.M.; Kraft, R.A.; King, S.B.; Laurienti, P.J.; Rejeski, W.J.; et al. Acute effect of a high nitrate diet on brain perfusion in older adults. *Nitric Oxide* **2011**, *24*, 34–42. [\[CrossRef\]](http://dx.doi.org/10.1016/j.niox.2010.10.002) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/20951824)
- 156. Maas, R.; Schwedhelm, E.; Kahl, L.; Li, H.; Benndorf, R.; Luneburg, N.; Forstermann, U.; Boger, R.H. Simultaneous assessment of endothelial function, nitric oxide synthase activity, nitric oxide-mediated signaling, and oxidative stress in individuals with and without hypercholesterolemia. *Clin. Chem.* **2008**, *54*, 292–300. [\[CrossRef\]](http://dx.doi.org/10.1373/clinchem.2007.093575) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/18070819)
- 157. Jadert, C.; Phillipson, M.; Holm, L.; Lundberg, J.O.; Borniquel, S. Preventive and therapeutic effects of nitrite supplementation in experimental inflammatory bowel disease. *Redox Biol.* **2014**, *2*, 73–81. [\[CrossRef\]](http://dx.doi.org/10.1016/j.redox.2013.12.012) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/24494186)
- 158. Khademikia, S.; Rafiee, Z.; Amin, M.M.; Poursafa, P.; Mansourian, M.; Modaberi, A. Association of nitrate, nitrite, and total organic carbon (TOC) in drinking water and gastrointestinal disease. *J. Environ. Public Health* **2013**, *2013*, 603468. [\[CrossRef\]](http://dx.doi.org/10.1155/2013/603468) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/23690803)
- 159. De Roos, A.J.; Ward, M.H.; Lynch, C.F.; Cantor, K.P. Nitrate in public water supplies and the risk of colon and rectum cancers. *Epidemiology* **2003**, *14*, 640–649. [\[CrossRef\]](http://dx.doi.org/10.1097/01.ede.0000091605.01334.d3) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/14569178)
- 160. Gatseva, P.D.; Argirova, M.D. High-nitrate levels in drinking water may be a risk factor for thyroid dysfunction in children and pregnant women living in rural Bulgarian areas. *Int. J. Hyg. Environ. Health* **2008**, *211*, 555–559. [\[CrossRef\]](http://dx.doi.org/10.1016/j.ijheh.2007.10.002) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/18164247)
- 161. Toccalino, P.L.; Norman, J.E.; Scott, J.C. Chemical mixtures in untreated water from public-supply wells in the U.S.—Occurrence, composition, and potential toxicity. *Sci. Total Environ.* **2012**, *431*, 262–270. [\[CrossRef\]](http://dx.doi.org/10.1016/j.scitotenv.2012.05.044) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/22687436)
- 162. Joshi, N.; Rhoades, M.G.; Bennett, G.D.; Wells, S.M.; Mirvish, S.S.; Breitbach, M.J.; Shea, P.J. Developmental abnormalities in chicken embryos exposed to *N*-nitrosoatrazine. *J. Toxicol. Environ. Health Part A* **2013**, *76*, 1015–1022. [\[CrossRef\]](http://dx.doi.org/10.1080/15287394.2013.831721) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/24168037)
- 163. Mitch, W.A.; Sharp, J.O.; Rhoades Trussell, R.; Valentine, R.L.; Alvarez-Cohen, L.; DSedlak, D.L. *N*-Nitrosodimethylamine (NDMA) as a Drinking Water Contaminant: A Review. *Environ. Eng. Sci.* **2003**, *20*, 389–404. [\[CrossRef\]](http://dx.doi.org/10.1089/109287503768335896)
- 164. Krasner, S.W. The formation and control of emerging disinfection by-products of health concern. *Philos. Trans.* **2009**, *367*, 4077–4095. [\[CrossRef\]](http://dx.doi.org/10.1098/rsta.2009.0108) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/19736234)
- 165. Hezel, M.P.; Weitzberg, E. The oral microbiome and nitric oxide homoeostasis. *Oral Dis.* **2015**, *21*, 7–16. [\[CrossRef\]](http://dx.doi.org/10.1111/odi.12157) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/23837897)
- 166. Hyde, E.R.; Andrade, F.; Vaksman, Z.; Parthasarathy, K.; Jiang, H.; Parthasarathy, D.K.; Torregrossa, A.C.; Tribble, G.; Kaplan, H.B.; Petrosino, J.F.; et al. Metagenomic analysis of nitrate-reducing bacteria in the oral cavity: Implications for nitric oxide homeostasis. *PLoS ONE* **2014**, *9*, e88645. [\[CrossRef\]](http://dx.doi.org/10.1371/journal.pone.0088645) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/24670812)
- 167. Burleigh, M.C.; Liddle, L.; Monaghan, C.; Muggeridge, D.J.; Sculthorpe, N.; Butcher, J.P.; Henriquez, F.L.; Allen, J.D.; Easton, C. Salivary nitrite production is elevated in individuals with a higher abundance of oral nitrate-reducing bacteria. *Free Radic. Biol. Med.* **2018**, *120*, 80–88. [\[CrossRef\]](http://dx.doi.org/10.1016/j.freeradbiomed.2018.03.023) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/29550328)
- 168. Vogtmann, E.; Chen, J.; Amir, A.; Shi, J.; Abnet, C.C.; Nelson, H.; Knight, R.; Chia, N.; Sinha, R. Comparison of Collection Methods for Fecal Samples in Microbiome Studies. *Am. J. Epidemiol.* **2017**, *185*, 115–123. [\[CrossRef\]](http://dx.doi.org/10.1093/aje/kww177) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/27986704)
- 169. Sinha, R.; Abu-Ali, G.; Vogtmann, E.; Fodor, A.A.; Ren, B.; Amir, A.; Schwager, E.; Crabtree, J.; Ma, S.; The Microbiome Quality Control Project Consortium; et al. Assessment of variation in microbial community amplicon sequencing by the Microbiome Quality Control (MBQC) project consortium. *Nat. Biotechnol.* **2017**, *35*, 1077–1086. [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/28967885)
- 170. Hebels, D.G.; Jennen, D.G.; van Herwijnen, M.H.; Moonen, E.J.; Pedersen, M.; Knudsen, L.E.; Kleinjans, J.C.; de Kok, T.M. Whole-genome gene expression modifications associated with nitrosamine exposure and micronucleus frequency in human blood cells. *Mutagenesis* **2011**, *26*, 753–761. [\[CrossRef\]](http://dx.doi.org/10.1093/mutage/ger043) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/21724973)
- 171. Hebels, D.G.; Jennen, D.G.; Kleinjans, J.C.; de Kok, T.M. Molecular signatures of N-nitroso compounds in Caco-2 cells: Implications for colon carcinogenesis. *Toxicol. Sci.* **2009**, *108*, 290–300. [\[CrossRef\]](http://dx.doi.org/10.1093/toxsci/kfp035) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/19221148)
- 172. Vineis, P.; Chadeau-Hyam, M.; Gmuender, H.; Gulliver, J.; Herceg, Z.; Kleinjans, J.; Kogevinas, M.; Kyrtopoulos, S.; Nieuwenhuijsen, M.; Phillips, D.H.; et al. The exposome in practice: Design of the EXPOsOMICS project. *Int. J. Hyg. Environ. Health* **2017**, *220*, 142–151. [\[CrossRef\]](http://dx.doi.org/10.1016/j.ijheh.2016.08.001) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/27576363)
- 173. Hebels, D.G.; Georgiadis, P.; Keun, H.C.; Athersuch, T.J.; Vineis, P.; Vermeulen, R.; Portengen, L.; Bergdahl, I.A.; Hallmans, G.; Palli, D.; et al. Performance in omics analyses of blood samples in long-term storage: Opportunities for the exploitation of existing biobanks in environmental health research. *Environ. Health Perspect.* **2013**, *121*, 480–487. [\[CrossRef\]](http://dx.doi.org/10.1289/ehp.1205657) [\[PubMed\]](http://www.ncbi.nlm.nih.gov/pubmed/23384616)
- 174. International Nitrogen Initiative. Available online: <http://www.initrogen.org/> (accessed on 22 April 2018).
- 175. Dinnes, D.L.; Karlen, D.L.; Jaynes, D.B.; Kaspar, T.C.; Hatfield, J.L.; Colvin, T.S.; Cambardella, C.A. Nitrogen management strategies to reduce nitrate leaching in tile-drained midwestern soils. *Agron. J.* **2002**, *94*, 153–171. [\[CrossRef\]](http://dx.doi.org/10.2134/agronj2002.1530)
- 176. Baron, J.S.; Hall, E.K.; Nolan, B.T.; Finlay, J.C.; Bernhardt, E.S.; Harrison, J.A.; Chan, F.; Boyer, E.W. The interactive effects of excess reactive nitrogen and climate change on aquatic ecosystems and water resources of the United States. *Biogeochemistry* **2013**, *114*, 71–92. [\[CrossRef\]](http://dx.doi.org/10.1007/s10533-012-9788-y)

© 2018 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license [\(http://creativecommons.org/licenses/by/4.0/\)](http://creativecommons.org/licenses/by/4.0/.).

NITRATE AND HEALTH

PROTECT THE HEALTH OF YOU AND YOUR LOVED ONES BY KNOWING WHAT IS IN YOUR DRINKING WATER!

WHERE AND WHAT IS NITRATE?

Nitrate is a form of nitrogen that can sometimes be found in our drinking water. Nitrogen fertilizers used for growing crops are the largest contributor to nitrate in our drinking water. Therefore, if you live in an area where there is a lot of agricultural production, you are at risk of drinking nitrate-contaminated water.

WHAT CAN I DO TO PROTECT MYSELF AND MY FAMILY?

If you drink water from a private well, it is up to you to ensure you are drinking safe water. There are no requirements for private well owners to test or treat their water. Nitrate is colorless, odorless and tasteless. **The only way to know if you have nitrate in your drinking water is to test for it.**

Private well users should test their drinking water annually. You can order a test kit from a certified laboratory or do-it-yourself test kits are available as well. The do-it-yourself kits should be used as a screening tool only. An analysis by an approved lab is recommended for the most accurate, reliable and precise measurement.

If you find nitrate above the safe drinking water level (10 ppm) in your water, the quickest and easiest solution is to install a reverse osmosis water filtration system in your house. For more information, go to https://water.unl.edu/

HOW CAN CONSUMING NITRATE IMPACT HUMAN HEALTH?

Children and Infants

- A result in infants consuming nitrate-contaminated water is methemoglobinemia (blue-baby syndrome); bottle-fed babies under six months old are at the highest risk. This illness can cause the skin to turn a bluish color and result in serious illness or death.
- There are studies suggesting potential linkages between nitrate consumption and pediatric cancers. Nebraska has the highest rate of pediatric cancer in the Midwest and 7th highest in the entire United States. More research needs to be conducted before we can draw sure conclusions.

Pregnant Women

- During pregnancy, it is common for a woman's methemoglobin levels to increase from normal. Therefore, pregnant women are particularly susceptible to methemoglobinemia as well.
- Pregnant women exposed to too much nitrate are at greater risk of giving birth prematurely
- Maternal exposure to nitrate through drinking water has been linked to birth defects. Nebraska has double the national average rate of birth defects.

Other Adults

- The University of Nebraska Medical Center, along with researchers across the globe, continue to study linkages between consuming nitrate and human health impacts
- A growing body of studies indicate potential associations between nitrate and...

increased heart rate, nausea, headaches, thyroid disease, and other cancers such as colorectal, bladder, ovarian and kidney

*Please consult your doctor if you are experiencing any of these symptoms

WHATIS NITRATE?

First and foremost, know what you're consuming. Check labels for nitrate preservatives and TEST YOUR WATER. Nitrate is odorless, tasteless and colorless. If you find high nitrate concentrations in your water (over 10 mg/L), you need to immediately switch to a safe source of drinking water, such as bottled water. Next, take the necessary steps to ensure your home has clean water. This may include installing a reverse osmosis system or digging a new well.

WANT TO LEARN MORE?

Nitrate can be found in highly processed meats such as bacon, ham and sausage. It is often used as a preservative and can improve the color of the raw meat. A diet high in processed meats can lead to high nitrate consumption, and put you at risk for certain health effects.

DRINKING WATER

PROCCESSED MEATS

A common way you might consume nitrate is through drinking water, as nitrate can infiltrate the groundwater we rely on for drinking. While public water is tested and treated for nitrate

contamination, private well owners are responsible to test their water and make sure it is safe.

Consuming too much nitrate can interfere with the ability of blood to carry oxygen. The result in infants is methemoglobinemia, also called blue baby syndrome. Bottle-fed babies under six months old are at the highest risk of getting methemoglobinemia. This illness can cause the skin to turn a bluish color from a lack of oxygen, and result in serious illness or death.

PREGNANT WOMEN

INFANTS

During pregnancy, it is common for a woman's methemoglobin levels to be thigher than normal. Therefore, pregnant women are particularly susceptible to methemoglobinemia. Additionally, pregnant women exposed to high nitrate concentrations in their drinking water are at greater risk of pre-term births, birth

* WHAT CAN YOU DO? *

NITRATE AWARENESS **What is in Your Water?**

Nitrate occurs naturally as part of the nitrogen cycle, but it also is generated through human activity, often times through agricultural practices. Nitrogen fertilizers can breakdown into nitrate and then seep into our groundwater. The United States Enviornmental Protection Agency (EPA) mandates that public water systems keep nitrate contamination

levels under 10 mg/L due to its associated health risks.

There are certain populations that are more susceptible to negative health outcomes. These include pregnant women, infants, children, and individuals with oxygen transport issues. Scientific research, including studies carried out at the University of Nebraska Medical Center, is ongoing regarding the health effects of nitrate consumption. These studies include research on the effects of nitrate consumption on thyroid disease and cancer, specifically colorectal, bladder, ovarian, and kidney cancers. Further research is needed to fully understand these potential health impacts.

> Talk to your doctor if you have questions or concerns about the health impacts related to consuming nitrate. Additionally, visit the University of Nebraska's website on this topic for more information.

https://water.unl.edu/category/water-and-health

NEBRASKA WATER FACTS

from the **NEBRASKA WATER CENTER**

Nebraska means "flat water" from the Omaha Sioux "ni braska" and Oto "ni brathge" describing the Platte
River. The Platte River was named by early French explorers, also meaning "flat."

SURFACE WATER

- Nearly 80,000 miles of rivers and streams drain to the Missouri River in the East.
	- Along the Niobrara and Missouri Rivers, 197 miles are designated as National Wild and Scenic.
- Tallest waterfall is Smith Falls, spilling 63 feet into the Niobrara River.
- Largest storage reservoir: Lake McConaughy
	- o When full is 1.74 million acre-feet of storage
	- o Covers 30,500 acres
	- Created by the state's largest dam, Kingsley Dam
	- Supplies irrigation directly & indirectly for 530,000 ac
- More than 2,900 dams, >25 ft tall or 50 ac-ft storage

GROUNDWATER

- Mostly from the Ogallala Aquifer, part of the High Plains Aquifer.
	- Water among mostly sand and gravel.
	- ∘ From 1 to 1000+ feet thick.
	- Poured over the surface of the state, the water would be 38 feet deep.
- Groundwater and surface water are connected. For example:
	- More than 90% of the Loup Rivers' streamflow started as groundwater.
- Nebraska has more than 192,000 registered groundwater wells.

WATER USE

Irrigated acres: 9.1 million #1

- Annual average additional crop value of \$1.5 billion statewide. Added property valuation of \$13-24 billion.
- Agriculture irrigation is 91% of Nebraska's total consumptive water use.
- From 1990 to 2014, Nebraska now grows 1.7 times more corn and 1.8 times more soybeans per gallon of water.
- From 1960 to 2016, Nebraska raises 1.8 times the amount of beef per gallon of water and 5.1 times more milk.
- Other uses of Nebraska's water: • home 5%; industrial 1%; thermoelectric 1%; livestock 1%
- 85% of Nebraskans get their home water from groundwater.
- 594 public water supply systems serve 1.69 million residents. EPA requires testing for 90 contaminants.
- More than 360,000 residents use private wells. Exempt from testing.
- Each person uses an average of 122 gallons of water each day. • Home water use has dropped by 1/3 in the last 20 years.
-

Center pivot irrigating soybeans. Photo: UNL

13 inches precipitation 5,424 feet above sea level 2.5 times more precipitation

More than 4,500 feet drop in elevation

Changes West to East

33 inches precipitation 840 feet above sea level

Smith Falls. Photo: Nebraskaland Magazine.

RECREATION

Lake McConaughy with >2 million visitors

- 7 out of top 10 Nebraska attractions involve water.
- Crane migration annual visitors' impact: \$17.2 mill.

LAND USE

- Nebraska's farms and ranches utilize 44.8 million acres, 92% of the state's total land area.
- 22 million acres of rangeland and pastureland in Nebraska, half of which are in the Sandhills.
- 1/3 of Nebraska land is annual crops.
- Nebraska's cities and town cover about 1% of the land, less than wetlands and forest.

Author: Crystal A. Powers

Reviewers: Katie Pekarek, Gary Stone |

THE DAUGHERTY **GLOBAL INSTITUTE** at the University of Nebraska

 \mathbf{I}

1.Agua potable en Nebraska

¿Sabía que no es un requerimiento analizar ni tratar el agua de pozos privados? Por lo tanto, la única manera de saber si su agua es segura para beber es hacer pruebas para averiguarlo usted mismo.

2. Contaminantes comunes del agua

En gran parte de Nebraska, el monitoreo del agua muestra varios contaminantes dañinos. El contaminante más común es el nitrato el cual está relacionado con el uso de fertilizantes nitrogenados.

3. Impactos de por vida en la salud

. Hay impactos conocidos en la salud por beber agua contaminada con nitratos. Los vínculos más fuertes son: síndrome del bebé azul, problemas de parto prematuro, defectos de nacimiento, cáncer infantil y cáncer en adultos.

4. ¿Quién está en mayor riesgo?

Las poblaciones más vulnerables son las mujeres embarazadas y sus fetos, los bebés pequeños, los niños y las personas con condiciones de transporte/entrega de oxígeno.

5. ¡Hágale un análisis a su agua de pozo!

¡La única manera de estar seguro de lo que hay en su agua potable es analizándola! La forma recomendada de realizar el análisis es pedir un kit de prueba de un laboratorio oficial de Nebraska. Después de saber qué hay en su agua, puede empezar a construir un plan de tratamiento si es necesario.

Para más información:

Laura Nagengast lnagengast3@unl.edu

O ¡Escanee aquí!

DEPT. OF ENVIRONMENT AND ENERGY