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Too Much of a Good Thing? Nitrate from Nitrogen Fertilizers and Cancer:

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INTRODUCTION

Man-made nitrogen fertilizers became widely used in U.S. agriculture after WWII, providing an inexpensive source of nitrogen and other plant nutrients that resulted in huge increases in agricultural productivity. Since the 1950s, nitrogen fertilizer use increased over five-fold in the United States and many other industrialized countries /[1]/, resulting in profound changes to the global nitrogen cycle that rival human effects on the carbon cycle /[2]/. The ecological effects of too much nitrogen in the environment are well-known and include algal blooms resulting in 'dead zones' in freshwater lakes and coastal areas, such as the annual dead zone that occurs in the Gulf of Mexico. The human health effects of excess nitrogen in the environment are less well characterized.

The primary route of human exposure to nitrogen fertilizers is through ingestion of contaminated drinking water. Nitrate, the final breakdown product of nitrogen fertilizers, accumulates in ground water under agricultural land and can be several- to a hundred-fold higher than levels under natural vegetation /[3]/. Nitrate levels can also be high in streams and rivers due to runoff of excess nitrogen fertilizer from agricultural fields. Groundwater is used for drinking water by 90% of the rural population in the United States and many rural residents have private wells, which are not regulated by the Environmental Protection Agency under the Safe Drinking Water Act. In contrast, public water utilities are required to maintain nitrate levels below a Maximum Contaminant Level (MCL) of 10 mg/L nitrate-nitrogen (N) that was instituted to protect against methemoglobinemia (blue baby syndrome), to which infants are especially susceptible. In addition to methemoglobinemia, a range of other health effects have been associated with ingesting nitrate-contaminated drinking water, including various cancers, adverse reproductive outcomes (especially neural tube defects), diabetes, and thyroid conditions /[4]/. Despite suggestions of other adverse health effects and increases in environmental exposure levels over the past 50 years, there has been no comprehensive research initiative to evaluate the health effects due to nitrate ingestion and to determine whether the current regulatory limit is adequate.

U.S. POPULATION EXPOSURE TO NITRATE IN DRINKING WATER

Populations living in agricultural areas typically have the highest exposures to drinking water contaminated with nitrate, with households using private wells for their drinking water often having exposures several-fold above households using public supplies. The United States (U.S.) Geological Survey assessed available private well measurements across the U.S. between 1970 and 1992 and found that 9 percent of samples exceeded the regulatory limit for nitrate /[3]/. In agricultural areas, it is estimated that 22 percent of private wells exceed the MCL in contrast to less than five percent of public water supplies. As nitrate levels in water resources have risen, cities like Des Moines, Iowa have been forced to use

expensive energy-intensive treatment systems to remove nitrate in order to comply with the MCL. With the increasing production of corn for fuel, nitrate levels in our water resources will likely continue to increase, resulting in higher exposures. When nitrate levels in water supplies are below the MCL, the primary source of individual exposure to nitrate comes mainly from diets high in green leafy and root vegetables; average daily intakes have been estimated to be in the range 30-130 mg nitrate/day /5/. Drinking water can contribute the majority of nitrate intake when levels are near the MCL /6/.

BIOLOGICAL EFFECTS OF INGESTED NITRATE

There are three primary mechanisms by which ingested nitrate from drinking water may have detrimental effects on health. The first relates to the mechanism by which methemoglobin is formed, thereby inhibiting the oxygen-carrying capacity of the blood. In the body, the oral bacteria (and stomach bacterial flora of infants) convert nitrate to nitrite, which competes with oxygen and binds to hemoglobin. The second mechanism involves the endogenous formation of *N*-nitroso compounds, which are potent carcinogens and teratogens in animal studies. Nitrite, the reduced form of nitrate, reacts in the acidic stomach to form various nitrosating agents that then react with amines and amides from proteins or other sources such as medications, to form *N*-nitroso compounds, many of which are potent animal carcinogens. Finally, nitrate at high doses can competitively inhibit iodine uptake and induce hypertrophic changes in the thyroid, as demonstrated in animal studies. Recent studies in Eastern Europe have found higher rates of thyroid abnormalities in children ingesting drinking water with high nitrate levels (1 to 2 times the MCL) compared with children in areas with lower nitrate levels /[7],8/. The most relevant mechanism for human cancer is likely to be the endogenous formation of *N*-nitroso compounds. Human biomonitoring studies demonstrate that ingestion of nitrate via drinking increases urinary excretion of *N*-nitroso compounds /[9-11]/. *N*-nitroso compound formation is inhibited by dietary antioxidants found in vegetables and fruits /12-[14]/, which may partly account for the observed inverse (protective) association between vegetable intake and many cancers. Because antioxidants occur together with nitrate in most vegetables, dietary nitrate intake may not result in substantial *N*-nitroso compounds formation /[15]/. The endogenous formation of *N*-nitroso compounds from drinking water sources of nitrate adds to exogenous sources of exposure that include preserved meats and fish, beer, certain occupations, cosmetics, and some drugs. Although variable, it is estimated that approximately 45%-75% of human exposure to *N*-nitroso compounds comes from *in vivo* formation /[16]/.

In a 2006 review of the evidence for the carcinogenicity of ingested nitrate and nitrite, a Working Group convened by the International Agency for Research on Cancer (IARC) concluded that “ingested nitrate or nitrite under conditions that result in endogenous formation of *N*-nitroso compounds is probably carcinogenic to humans” (Group 2A) /[17]/. There was no separate evaluation for nitrate or nitrite *per se*, because nitrite is produced endogenously from nitrate and the conditions leading to endogenous formation of *N*-nitroso compounds are frequently present in the normal human stomach (nitrite and nitrosatable amines or amides in an acidic environment). Several *N*-nitroso compounds formed endogenously in humans from dietary precursors are considered probable human carcinogens (as determined by previous IARC reviews) and are transplacental neurocarcinogens in animals. Further, *N*-nitroso compounds cause cancer in every animal species that have been tested and at multiple organ sites /[18]/, so it is unlikely that humans are not affected.

EPIDEMIOLOGIC STUDIES OF DRINKING WATER NITRATE AND CANCER

Most epidemiologic studies of drinking water nitrate and cancer have been ecologic in design, linking incidence or mortality rates to drinking water nitrate levels for large groups of people at the town or county level. This study design is useful for generating hypotheses about disease risk factors, but analytic studies based on individual exposure information and disease status are needed in order to establish causality. This is particularly true for the evaluation of nitrate exposure via drinking water because of the complex process by which nitrate intake forms potentially carcinogenic N-nitroso compounds. Most ecologic studies of cancer estimated exposure using drinking water nitrate measurements concurrent with the time period of cancer incidence or mortality and results were mixed /[19]/. More recently, two ecologic studies of stomach cancer mortality used historical measurement data ranging up to several-fold above the MCL. A study in Hungary /[20]/ found elevated stomach cancer rates in areas with nitrate levels above 18 mg/L nitrate-N compared to background exposure levels. A study in 258 municipalities in Valencia, Spain found elevated stomach cancer mortality at nitrate levels over about 11 mg/L nitrate-N /[21]/.

In the past two decades, several case-control and cohort studies have evaluated historical nitrate levels in public water supplies (largely below 10 mg/L nitrate-N) and risk of several cancers Some of these individual-based studies evaluated other cancer risk factors that might act as confounders as well as factors affecting the endogenous nitrosation process such as antioxidant intake. A cohort study of older women in Iowa (U.S.) /[22]/ found an increased risk of bladder and ovarian cancers associated with higher long-term average nitrate levels in public drinking water supplies used by the women. They also observed significant inverse associations for uterine and rectal cancer and no significant exposure-response association for non-Hodgkin lymphoma, leukemia, melanoma, and cancers of the rectum, pancreas, kidney, and lung. Some analyses were limited by small numbers. Case-control studies of bladder /23/, kidney /24/, colon and rectum /25/, and pancreas cancer /[26]/ in Iowa found no overall association between the average nitrate levels in public water supplies over an almost 30-year period or the years participants used a supply with levels at or above 5 mg/L (half the MCL). Each of these studies evaluated risk among subgroups of the population likely to have higher endogenous formation of N-nitroso compounds, that is, those with both higher nitrate intake from drinking water and lower intake of vitamin C or higher intake of meat (a source of amines and amides). For colon and kidney cancer, there was a significant positive interaction between exposures of 10 or more years above 5 mg/L nitrate-N and low vitamin C and high meat intake.

A case-control study of non-Hodgkin lymphoma in Nebraska /[27]/ found a significant positive association between the average nitrate level in public water supplies over about 40 years and risk among men and women. This was not confirmed in a later study in Iowa /[28]/. Case-control studies in Nebraska /[29]/ and Germany /[30]/ found no association with long-term average nitrate levels in public water supplies and adult brain cancer. A cohort study in the Netherlands /[31]/ found no association between stomach cancer risk and quintiles of water nitrate intake determined from public supply levels and tap water intake.

A few case-control studies evaluated well water use and cancer risk. In Germany /32/, stomach cancer risk was positively associated with private well water use compared with public supply use. In Colombia, well water use in the first 10 years of life was associated with an increased risk of stomach cancer precursor lesions /33/. In California and Washington State /[34]/, there was no overall association with well water use during pregnancy and subsequent risk of brain cancer in the offspring; however, the offspring of women in western Washington State who used private wells as their drinking water source during the pregnancy had a significantly increased risk of brain cancer. Dipstick

measurements of the nitrate levels in water supplies, often occurring many years after the pregnancy, were not associated with risk overall although there was a nonsignificant two-fold increase in risk of astroglial tumors associated with the highest nitrate category(≥ 11 mg N/L).

RESEARCH NEEDS

Although the number of epidemiologic studies with individual exposure data has increased in the past decade, there are still few studies of drinking water nitrate ingestion for any single cancer site, making interpretation difficult. The recent analytic studies have generally included historical nitrate measurements from public water supplies and have evaluated potential confounders; however, several of these studies did not evaluate risk separately for the group likely to have higher rates of endogenous nitrosation /[22,30,31]/. Children have exposure starting with *in utero* exposure via maternal consumption of nitrate-contaminated tap water. The effects of early life exposures to nitrate will be an important area of research.

Several cross-sectional studies to evaluate genotoxic effects of nitrate in drinking water have been conducted for individuals drinking well water with nitrate concentrations ranging from 11-65 mg/L nitrate-N. No association was found between the frequency of peripheral lymphocyte sister chromatid exchanges and higher water nitrate levels in a study in the Netherlands /[35]/. A subsequent study employed the hypoxanthineguanine phosphoribosyltransferase (HPRT) variant frequency test in peripheral lymphocytes /[36]/ and found an increased prevalence of HPRT variants in subjects drinking medium and high levels of nitrate. An inverse correlation between the labeling index in lymphocytes and nitrate exposure was suggestive of an exposure-related immunosuppressive effect. Such cross-sectional biomonitoring studies of nitrate ingestion are important in enhancing our understanding of the adverse effects of nitrate exposure, and warrant further exploration.

Formation of N-nitroso compounds from drinking water nitrate ingestion has been demonstrated in humans at nitrate levels above the MCL of 10 mg/L nitrate-N /[9,10]/; however, further studies are needed to determine to what extent nitrosation occurs *in vivo* at the intermediate levels (5-10 mg/L) observed in many public water supplies and to clarify the role of nitrate from water as compared with food sources. Furthermore, the role of precursors such as dietary amines, amides, and nitrosatable drugs, and modulators of N-nitroso compounds formation such as dietary antioxidants, should be more fully investigated. Future biomonitoring studies should be conducted among healthy individuals as well as individuals with medical conditions, such as inflammatory bowel disease, that increase endogenous nitrosation, representing a potentially vulnerable subgroup of the population.

Because many N-nitroso compounds require α-hydroxylation by metabolizing enzymes for bioactivation and to form DNA adducts, it is important to investigate the influence of polymorphisms in the N-nitroso compounds-metabolizing genes. One study found that specific variants in *CYP*2E1 increased rectal cancer risk, particularly in subjects with high intake of red and processed meat /[37]/. Moreover, gene expression levels of human *CYP*2E1 were found to be related to cytotoxicity and DNA damage by nitrosamines in pancreatic beta-cell lines /[38]/. These promising lines of research point to a possible interaction between drinking water nitrate exposure and genetic variation in *CYP*2E1, which may influence the risk of several adverse health outcomes.

The population using private wells in agricultural areas represents the group with the highest exposures to drinking water nitrate levels. Because historical monitoring data are not usually available, this population has generally been excluded from analyses of nitrate concentrations and cancer risk. Many states have laboratories that measure nitrate levels in

household wells as part of an effort to ensure safe drinking water for their entire population. However, such measurement data is typically not available for health studies and may not be representative of exposures levels due to the self-selected nature of the samples. Several agricultural states and the U.S. Geological Survey have conducted random surveys to characterize water quality in domestic wells. More studies of this type in areas with known contamination problems would go along way towards addressing this paucity of measurement data for this population. Drinking water contaminants that may co-occur with nitrate, such as agricultural pesticides, should also be evaluated. Public water supply monitoring data includes measurement of some pesticides allowing for further detailed exposure assessment of populations using public supplies; however, pesticide measurement data are sparse for private wells. A Geographic Information System (GIS)-based modeling approach that uses information on the location of crops, nitrogen fertilizer and pesticide applications, soil characteristics, and depth to the ground water table to predict nitrate and pesticide concentrations in wells is a promising new method of exposure assessment /[39]/.

SUMMARY

Nitrate levels in water supplies have been increasing in many areas of the world; therefore, additional studies of populations with well-characterized exposures are urgently needed to further our understanding of cancer risk associated with nitrate ingestion. Future studies should assess exposure for individuals (e.g., case-control, cohort studies) in a time frame relevant to disease development, and evaluate factors affecting nitrosation. Estimating N-nitroso compounds formation via nitrate ingestion requires information on dietary and drinking water sources of nitrate, inhibitors of nitrosation (e.g., vitamin C), nitrosation precursors (e.g., red meat, nitrosatable drugs), and medical conditions that may increase nitrosation (e.g., inflammatory bowel disease). Studies should account for the potentially different effects of dietary and water sources of nitrate and should include the population using private wells for whom exposure levels are often higher than public supplies.

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